

Co-Hosted by SOT 
Hawaii 
IUTOX ICTXV 2019

SOT | Society of
Toxicology



IUTOX 15th International Congress of Toxicology

July 15–18, 2019 | Hawaii Convention Center, Honolulu, Hawaii, USA

Final Program

IUTOX President's Message

On behalf of the IUTOX Executive Committee and our 63 member societies, I am honored to welcome you to the 15th International Congress of Toxicology (ICTXV) in the beautiful city of Honolulu. Under the strong leadership of Drs. William Slikker, Jr., Ivan Rusyn, and Peter Goering, the Society of Toxicology team has worked tirelessly to plan a superb scientific program around the relevant theme, *"Toxicology Solutions for Global Public, Environmental, and Personal Health."*

In Honolulu we will gather together with our member societies at the General Assembly to report on our activities since 2016 and to undertake the important business of electing new leaders to serve on the Executive Committee and to vote on future ICT and CTDC venues. When I pass the gavel to Dr. Peter Di Marco on July 17, I will do so with gratitude to the Executive Committee, all of whom have served IUTOX with great dedication to our mission and the scientists we serve. I'm also grateful to our member society leaders who have supported the work of IUTOX to ensure its future.

I extend a special thanks to each and every Congress participant, speaker, exhibitor, and volunteer for participating in ICTXV and for making this exceptional week possible. Most notable is the *mahalo* spirit of our US hosts who have provided us with the warmest of welcomes and hospitality as is the proud tradition in the Aloha state. I thank them for creating an unforgettable Congress experience.

Finally, I invite each of you to participate in IUTOX activities and to contribute your time and talents to our work of improving human health through the science and practice of toxicology world-wide. What better way than to get acquainted with our amazing members at the Congress of Toxicology in Developing Countries (CTDC11) in Kuala Lumpur, Malaysia (June 13-16, 2021) and at the next ICT meeting (ICTXVI) in Maastricht, the Netherlands (September 18-21, 2022).

I wish all of you a week of engaging science and the company of old and new friends. I also bid you a fond farewell after ten years of service to IUTOX.

Arigato,



Jun Kanno, MD, PhD
2016-2019 IUTOX President



SOT President's Message

Dear Colleagues:

I am pleased and honored to represent the Society of Toxicology (SOT) in welcoming you to the IUTOX 15th International Congress of Toxicology (ICTXV). SOT is pleased to serve as the co-host of ICTXV as we explore "Toxicology Solutions for Global Public, Environmental, and Personal Health." Like that of IUTOX, the Society's mission is a global one: to create a safer and healthier world by advancing the science and increasing the impact of toxicology. And to further this mission, collaboration on an international scale is paramount.

IUTOX and SOT have been working for more than half a decade to ensure that the ICTXV scientific program addresses today's most pressing problems, features the best science, and serves the needs of toxicologists worldwide. The many Scientific Sessions and Continuing Education courses available to attendees, as well as the research to be presented during Poster Sessions, also have been designed to represent and celebrate the diversity of toxicological stakeholders from all backgrounds.

This year's program encompasses the expanse of the toxicological sciences, covering such topics as computational toxicology; food safety and nutrition; drug safety, reproductive, developmental, and juvenile toxicology; and much more. We invite you to immerse yourself in the science delivered at this year's meeting and to engage with the variety of tools and resources available in the Exhibit Hall throughout the week.

As a longtime IUTOX member society, SOT is proud to have the opportunity to partner with IUTOX in working toward a healthier world by harnessing the knowledge of toxicology. It is a privilege to be exploring the influence of the toxicological sciences alongside leaders in the field—and against the backdrop of stunning Honolulu, Hawaii. We anticipate a prosperous week abounding in the international collaboration that is so important in a global world.

Sincerely,



Ronald N. Hines, MS, PhD, ATS
2019–2020 SOT President



Welcome to the ICTXV

Aloha, colleagues!

We are elated to welcome you to beautiful Waikiki Beach in downtown Honolulu, Hawaii, for the 15th International Congress of Toxicology (ICTXV)—a forum for scientists in academia, government, and industry from around the world to learn about the latest developments in toxicological science and regulation. The Society of Toxicology (SOT) is pleased to partner with the International Union of Toxicology (IUTOX) to host this exciting iteration of the triennial ICT global conference. The word “aloha” is more than a greeting or farewell. One of its meanings is “connectedness.” This week ICTXV will bring together scientists from East and West, North and South, to renew existing and foster new connections in the spirit of aloha.

Years in the making, ICTXV is the first ICT held in the United States since ICTVI in 1995. The ICT has a long tradition which aligns with the SOT mission and guiding principles of fostering lifelong learning, supporting toxicologists from diverse backgrounds and circumstances, and advancing the impact and reach of toxicology. By co-hosting this congress, SOT hopes to expand the educational and capacity-building opportunities for all toxicologists by providing expertise through a scientific conference that recognizes the needs of scientists throughout our global community.

“Toxicology Solutions for Global Public, Environmental, and Personal Health” is the theme of the meeting. The Scientific Program Committee, in collaboration with IUTOX and other societies, has created an inclusive and diverse program that highlights excellence in the science and practice of toxicology around the globe, working to balance the interests of different countries and world regions. Indeed, of the almost 800 abstracts received through regular and late-breaking submission, presenters from developing countries represent nearly one-third of the presentations to be seen in Honolulu, offering an opportunity for presenters to inform their peers of toxicological challenges and solutions that are applicable to their own countries and regions.

We are pleased to offer an exciting slate of:

- 8 Continuing Education Courses,
- 4 Keynote Lectures,
- 20 Symposia,
- 8 Platform Sessions,
- 660 Posters, and
- An Exhibit Hall providing products, services, and technology benefitting the toxicological community.

These sessions will explore informative and inspiring strategies to improve human and environmental health in countries with both robust and developing research and regulatory enterprises. ICTXV will serve as a global forum for the exchange of ideas that can create lasting partnerships and measurably elevate the competency of toxicologists globally.

Sincerely,



William Slikker Jr.
ICTXV Organizing
Committee Chair



Ivan Rusyn
ICTXV Scientific Program
Committee Chair



Peter Goering
ICTXV Continuing Education
Committee Chair

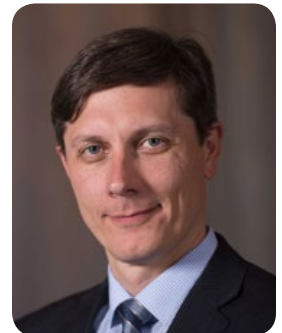
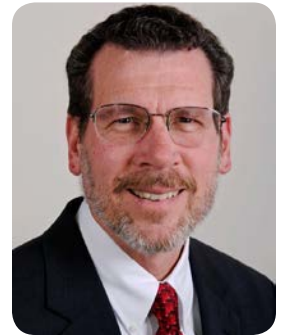


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To view session details on the go,
download the ICTXV app from the Apple or
Android stores by searching ICTXV.



Available on the
App Store



Get it on
Google play

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Program Overview

Monday, July 15, 2019

Exhibitor Set-Up 8:00 AM–4:00 PM
Continuing Education Courses 9:00 AM–11:30 AM
[Separate Registration Required](#)
Lunch and Networking Time 11:30 AM–2:30 PM
Continuing Education Courses 2:30 PM–5:00 PM
Opening Ceremony 5:00 PM–6:00 PM
Deichmann Lecture: Linda Birnbaum 6:00 PM–7:00 PM
Welcome Reception in Exhibit Hall 7:00 PM–8:30 PM

Tuesday, July 16, 2019

Keynote Lecture: Peter Warner 8:00 AM–8:50 AM
Scientific Symposia 9:00 AM–11:00 AM
Exhibitor-Hosted Sessions 11:30 AM–12:30 PM
Soft Skills Workshop 11:30 AM–1:20 PM
[Separate Registration Required](#)
Exhibit Hall Open 12:00 Noon–6:30 PM
Exhibitor-Hosted Sessions 1:00 PM–2:00 PM
Scientific Symposia 2:30 PM–4:30 PM
Poster Session 1 and Refreshments in Exhibit Hall
4:30 PM–6:30 PM

Wednesday, July 17, 2019

Keynote Lecture: Christopher Austin 8:00 AM–8:50 AM
Scientific Symposia 9:00 AM–11:00 AM
Perspectives of Women in Toxicology 11:00 AM–12:30 PM
Exhibit Hall Open 12:00 Noon–6:30 PM
IUTOX General Assembly and Lunch 12:00 Noon–5:00 PM
[Open to IUTOX Member Society Leaders Only](#)
Find Your Mentor 12:30 PM–1:20 PM
[Separate Registration Required](#)
Scientific Symposia 2:30 PM–4:30 PM
Poster Session 2 and Refreshments in Exhibit Hall
4:30 PM–6:30 PM
Gala Dinner and Reception 7:00 PM–10:00 PM
[Separate Registration Required](#)

Thursday, July 18, 2019

Keynote Lecture: MOA vs. AOP Debate 8:00 AM–9:15 AM
Platform Presentations 9:30 AM–11:00 AM
Platform Presentations 11:00 AM–12:30 PM
IUPHAR-IUTOX Exchange Session 12:30 PM–2:30 PM
Scientific Symposia 2:30 PM–4:30 PM
Closing Ceremony 4:30 PM–5:30 PM

General Scientific Sessions (Listed by type, then date and time)

CE Continuing Education Courses

PS Poster Sessions

E Exhibitor-Hosted Sessions

PL Platform Sessions

S Symposium Sessions

All events in this overview take place in the Hawaii Convention Center unless otherwise noted.

Abstract numbers are four digits. Poster boards are indicated with a "P" followed by 3 digits. See page 19 for more poster information.

Monday, July 15

Tuesday, July 16

Wednesday, July 17

Thursday, July 18

8:00 AM	Separate Registration Required to Attend Continuing Education Courses				8:00 AM	Keynote Lecture: Peter Warner Challenges in Global Health: Innovative Discovery and Translational Strategies (Kalākaua Ballroom)				8:00 AM	Keynote Lecture: Christopher Austin The National Center for Advancing Translational Sciences: Catalyzing Translational Innovation in Toxicology Research (Kalākaua Ballroom)				8:00 AM	Keynote Lecture: MOA vs. AOP Debate Mechanisms vs. AOP: Does It Matter for the Use of the Mechanistic Data in Decision-Making? (Kalākaua Ballroom)						
9:00 AM	CE AM01: Applications of Chemical Risk Assessment in Establishing Food Safety Standards (Room 313)	CE AM02: Immunotoxicology Hazard Identification Using Novel <i>In Vitro</i> Methods (Room 312)	CE AM03: Recent Developments in Adult and Developmental Neurotoxicity Testing for Regulatory Assessment (Room 315)	CE AM04: Understanding Translational Biomarkers: Science and Qualification (Room 316)	9:00 AM	SYM01: Emerging Mechanisms of Chemical Teratogenesis (Room 312)	SYM02: International Efforts to Ensure Global Food Safety: Incorporating Advanced Toxicological Sciences in Food Risk Assessment (Room 316)	SYM03: Lysosomes: Key Regulators of Inflammation and Life or Death of a Cell (Room 313)	SYM04: Systemic and Functional Impacts of Maternal Pulmonary Nanomaterial Exposures on the Offspring (Room 315)	9:00 AM	SYM09: Clinical and Mechanistic Aspects of Drug-Induced Liver Injury (Room 316)	SYM10: Effects of Environmental Mixture Exposure on Brain Development and Function Based on Epidemiological, <i>In Vivo</i> , and <i>In Vitro</i> Studies (Room 313)	SYM11: Immunotoxicity Associated with Exposure to Nanomaterials (Room 312)	SYM12: The Carcinogenicity of Hexavalent Chromium: A Global Public and Environmental Health Concern (Room 315)	9:00 AM							
10:00 AM					10:00 AM					10:00 AM					10:00 AM	PL01: Alternatives to Animal Testing (Room 312)	PL02: Immunotoxicology (Room 315)	PL03: Metals (Room 316)	PL04: Risk and Safety Evaluation (Room 313)			
11:00 AM					11:00 AM										11:00 AM	Perspectives of Women in Toxicology (Room 312)		11:00 AM	PL05: Computational Toxicology (Room 315)	PL06: Nanotoxicology (Room 312)	PL07: Neurotoxicology (Room 316)	PL08: Reproductive/ Dev Toxicology (Room 313)
12:00 PM	Lunch and Networking on Own				12:00 PM	Soft Skills Workshop (Room 312) Separate Registration Required	Exhibitor-Hosted Sessions (Room 317B, 318A)		12:00 PM	Find Your Mentor (Room 317A) Separate Registration Required	IUTOX General Assembly (Room 319) 12:00 Noon–5:00 PM See Program for Details		12:00 PM	IUPHAR IUTOX Exchange Session Precision Medicine and ADME-Targets (Room 312)								
1:00 PM					1:00 PM				Exhibitor-Hosted Sessions (Room 318B)				1:00 PM							1:00 PM		
2:00 PM					Exhibit Hall Open 12:00 Noon–6:30 PM								2:00 PM							Exhibit Hall Open 12:00 Noon–6:30 PM		
3:00 PM	CE PM05: Big Data Analytics in the Era of the 21st Century of Toxicology (Room 315)	CE PM06: Epigenetics and Toxicology: Overview and Advances in Methodology (Room 316)	CE PM07: New Risk Assessment Approaches for Reproductive and Developmental Toxicology (Room 312)	CE PM08: What Is an Adverse Effect for Human Health: Defining Adversity in Risk/Safety Assessments (Room 313)	3:00 PM	SYM05: Concerns of Herbal Product-Induced Toxicity (Room 315)	SYM06: Emerging CRISPR Applications in Environmental Health Sciences and Toxicology (Room 313)	SYM07: Epigenetic Biomarkers of Exposure to Environmental Carcinogens (Room 312)	SYM08: New Approach Methodologies: A Global Perspective (Room 316)	3:00 PM	SYM13: Acute, Chronic, and Long-Term Pesticide Effects: Mechanisms of Actions, Epidemiological Data, Priority Areas for Research (Room 313)	SYM14: Collaborative Approaches to Proactively Advance 3Rs in Nonclinical Drug Development (Room 312)	SYM15: Empowering Toxicology and Environmental Health Research to Address Disasters and Emerging Threats (Room 315)	SYM16: Out of the Box: Contribution of the Immune System to Organ Toxicity (Room 316)	3:00 PM	SYM17: Applying the Key Characteristics Paradigm in Hazard Identification and Risk Assessment (Room 313)	SYM18: Biomarker Qualification: Accelerating Drug Development by Enabling the Use of Novel Diagnostic and Safety Biomarkers (Room 312)	SYM19: Leveraging Zebrafish to Support Global Toxicology Challenges (Room 316)	SYM20: Recent Trends in Research on Arsenic Toxicity (Room 315)			
4:00 PM					4:00 PM										4:00 PM							
5:00 PM	Opening Ceremony (Kalākaua Ballroom)				5:00 PM	Poster Session 1 and Refreshments (Exhibit Hall)				5:00 PM	Poster Session 2 and Refreshments (Exhibit Hall)				5:00 PM	Closing Ceremony (Kalākaua Ballroom)						
6:00 PM	Deichmann Lecture: Linda Birnbaum Human Health and the Environment: Global <i>is</i> Local <i>is</i> Personal (Kalākaua Ballroom)				6:00 PM					6:00 PM					6:00 PM	CE Continuing Education	SYM Symposium	PL Platform Session				
7:00 PM	Welcome Reception (Exhibit Hall)				7:00 PM									7:00 PM								
8:00 PM					8:00 PM									8:00 PM	Gala Dinner and Reception (Convention Center Rooftop Garden) Separate Registration Required				8:00 PM			
9:00 PM					9:00 PM									9:00 PM								

Oahu

Sometimes called “The Gathering Place,” Oahu certainly lives up to its name. The third largest Hawaiian island is home to the majority of Hawaii’s diverse population, a fusion of East and West cultures rooted in the values and traditions of the Native Hawaiian people. It’s this fundamental contrast between the ancient and the modern that makes discovering Oahu—from bustling city life to laidback surf towns—so enjoyable. You’ll find plenty of different options for exploring Oahu’s different regions, the island’s many activities, its eclectic restaurants and accommodations.

Hilton Hawaiian Village Waikiki Beach Resort

Located in the heart of Waikiki, the headquarters hotel is a 10-minute walk from the Hawaii Convention Center (the location of the ICTXV meeting) and 25 minutes from Daniel K. Inouye International Airport (HNL). The largest shopping mall in Hawaii—Ala Moana Center—is a 15-minute walk giving you access to more than 340 shops, services, and entertainment.

Climate

Oahu is generally drier on the Leeward Coast (west) and wetter and greener on the Windward Coast (east). The resort area of Waikiki and the surfing mecca of the North Shore offer pleasant weather year round. The average temperature is between 75°-85° F. Summer is warmer and drier and the big waves of the North Shore subside for ideal beach going conditions.

Ground Transportation from the Airport

Ground Transportation is located curbside outside the baggage claim area.

Shuttle Services

SpeediShuttle provides shared ride airport transportation in Mercedes shuttles between Daniel K. Inouye International Airport (HNL) and Hilton Hawaiian Village at the rate of \$16.00 one way, \$32.00 round trip.

All Airlines EXCEPT International arrivals should remain in the baggage claim area where you will be met by a SpeediShuttle greeter holding a SPEEDISHUTTLE sign. DO NOT EXIT TO THE STREET.

International arrivals will need to exit Customs to the street and wait by the information counter. You will be met and assisted by a SpeediShuttle greeter holding a SPEEDISHUTTLE sign, who will then escort you to your pickup area where the driver will load your luggage into a SpeediShuttle vehicle.

- Online Reservations: <http://hgts.co/t/MZA1XJ>
- Phone Reservations: 1.877.242.5777

Taxis

AMPCO Express is the managing contractor of the Airport’s Open Taxi System, established by the state. This system allows taxi drivers from all taxi companies the ability to pick up passengers at the airport.

Taxi Service is available on the center median in front of the terminal baggage claim areas. See the taxi dispatchers (yellow shirts with black lettering and the wording “TAXI DISPATCHER”) for service.

The fare from the airport to Waikiki during non-rush hour periods is approximately \$40.00-\$45.00, plus a baggage charge of 50 cents per bag. Fare is by meter only.

Car Rental Companies

Daniel K. Inouye International Airport (HNL) provides six different car rental companies located on airport grounds and four different off-airport companies.

Rental counters are located in the Baggage Claim area. Airport rental car offices are located on the ground level of the Terminal 2 Parking Garage.

Car rental shuttles stop in designated areas along the center median on the ground level outside of baggage claim areas. For more information on car rental companies, please visit the [HNL-Official State of Hawaii Website](#).

Getting Around Town and the Island

You can get around by car rental (advance reservations recommended), shuttle, taxi, or Oahu’s public transportation system. Many visitors also enjoy taking bus tours to conveniently explore the Island. Others rent mopeds and motorcycles.

Honolulu’s “The Bus” system provides convenient access around the Island every 30 minutes depending on the route number. For more information, visit www.thebus.org. Looking to visit favorite local attractions, shopping, and dining destinations, the Waikiki Trolley offers a convenient, safe and fun way to explore what Honolulu has to offer. www.waikikitrolley.com.

Taxis are a great way to travel when in a group and the cost can be shared. You can find taxi stands at most major shopping malls, in the business district, and at the airport. Note that rather than hailing a cab, finding taxi stands at major shopping malls and businesses as well as calling for pick-ups via hotel concierge or phone is common practice in Hawaii.

Agricultural Restrictions

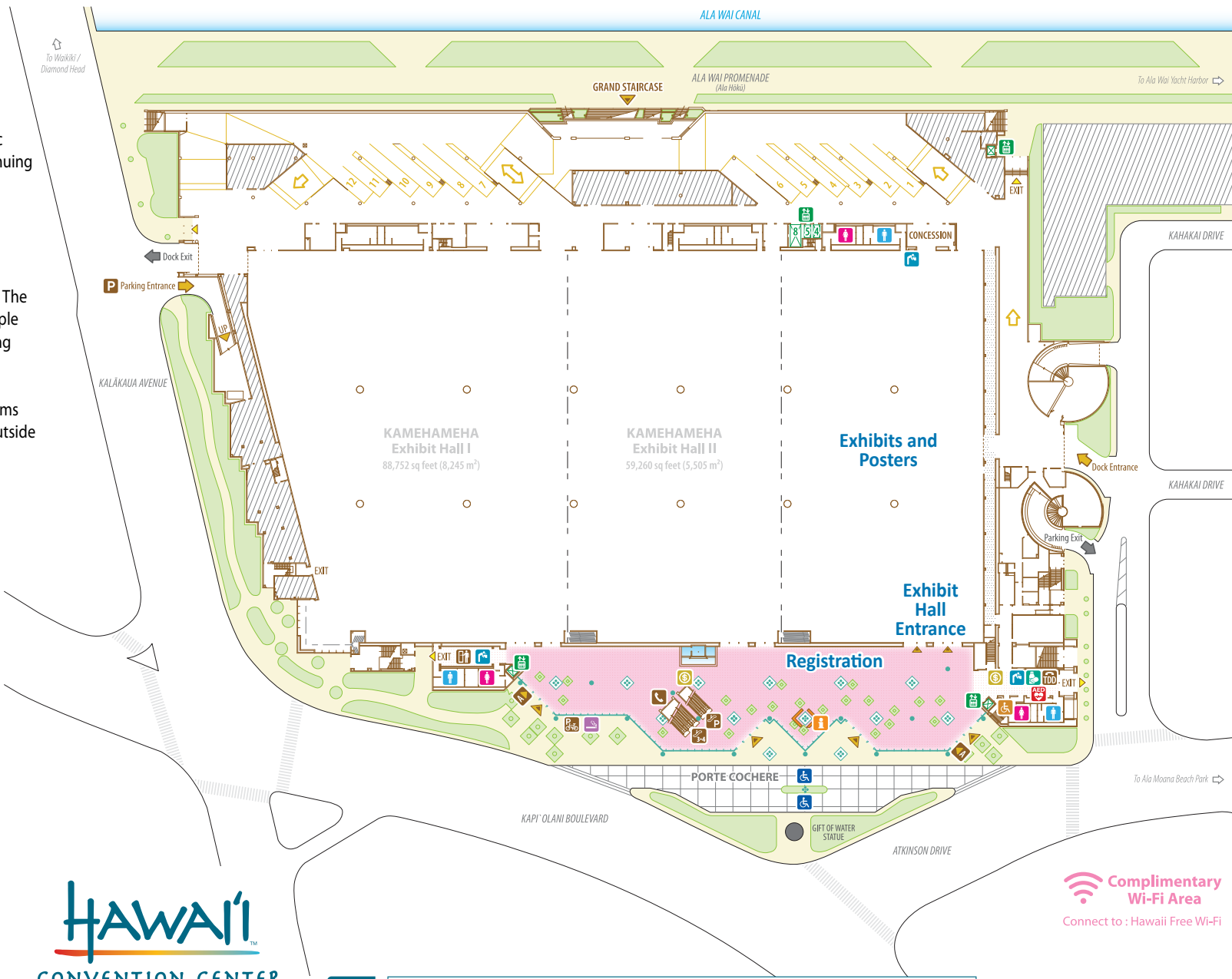
Flying to Hawaii is different than traveling to many other destinations. According to the Hawaii Biological Survey, Hawaii is the “endangered species capital of the world,” with more endangered or threatened species per square mile than any other place on the planet. Bringing non-native species of plants and animals into The Hawaiian Islands can cause harmful effects on human health and well-being, agriculture, and Hawaii’s fragile native ecosystem.

As a result, the State of Hawaii Department of Agriculture requires passengers arriving with plants, food, seeds, animals, and any other agricultural materials to declare them on the Agricultural Declaration Form distributed aboard your flight to Hawaii. Plant quarantine inspectors will be stationed in the baggage claim area to examine baggage and agricultural items. There also is an optional survey passengers may fill out on the back side of the form.

Hawaii Convention Center Map—Level 1

Hawaii Convention Center

The ICTXV Scientific Sessions and Continuing Education courses will be held in the Hawaii Convention Center located at 1801 Kalakaua Ave, Honolulu, HI 96815. The center provides ample space for networking opportunities both inside the air-conditioned rooms and on the many outside verandas.



HAWAII
CONVENTION CENTER
Where Business and Aloha Meet

1 EXHIBIT HALL / LOBBY

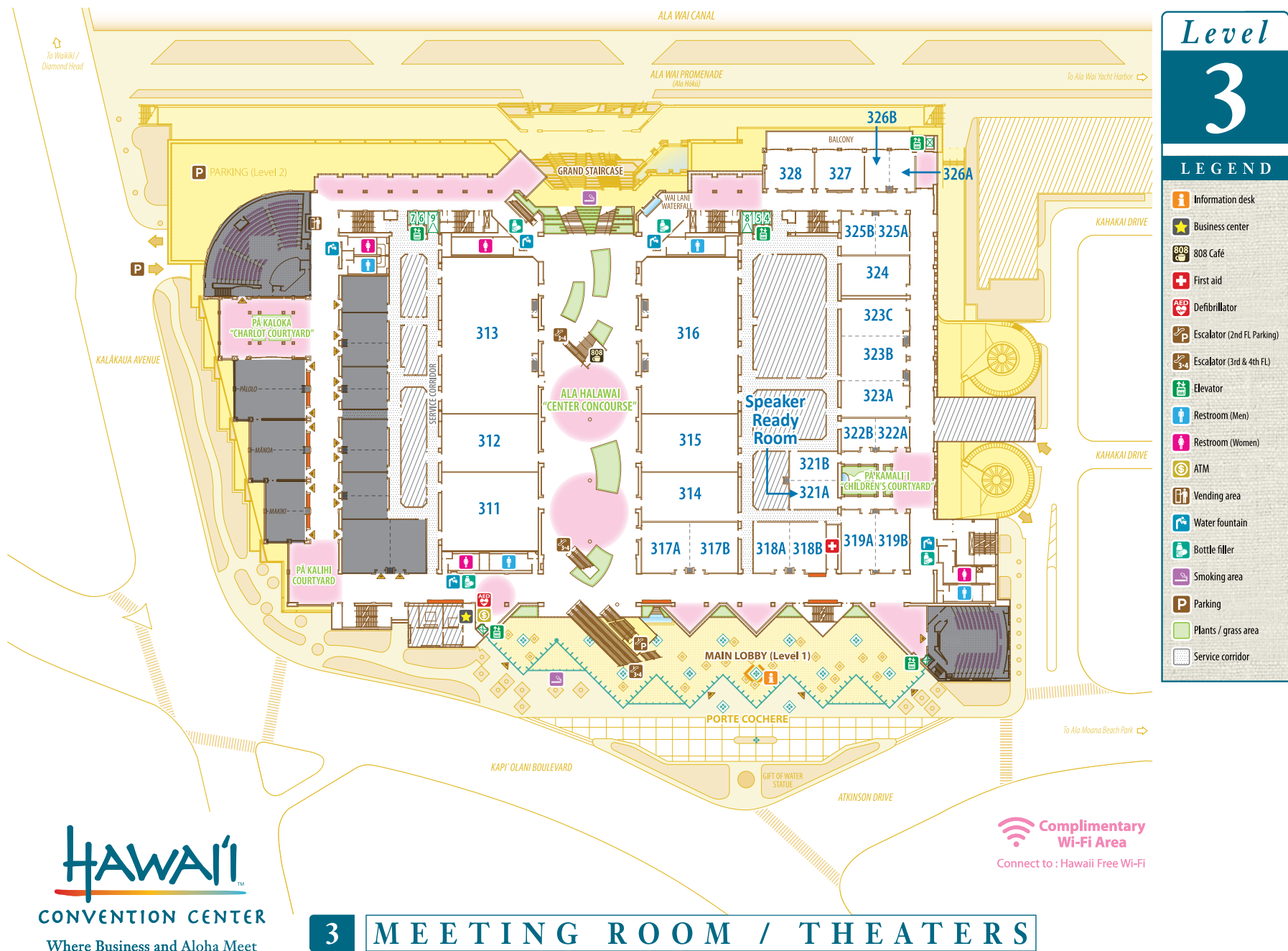
Level
1

LEGEND

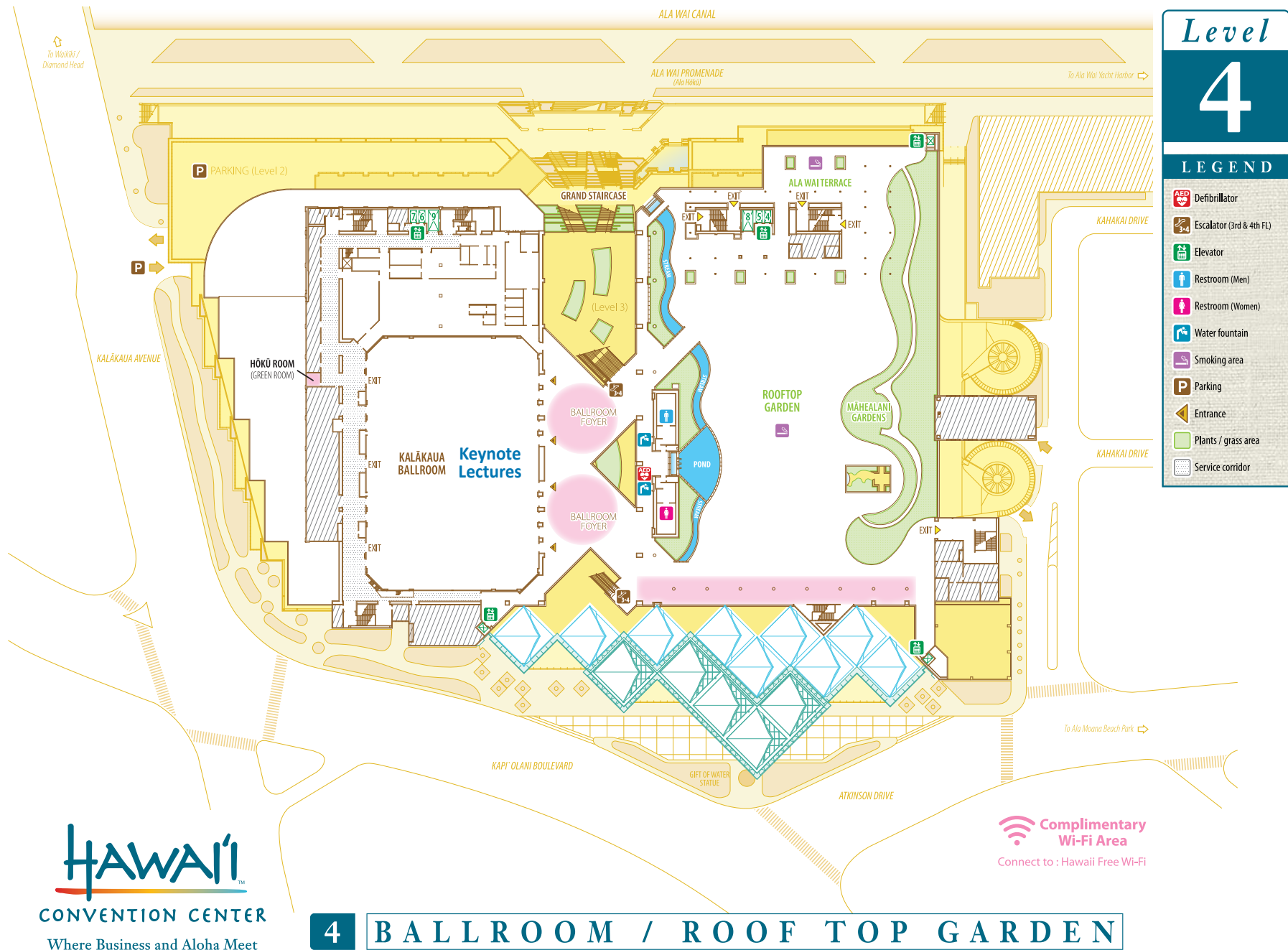
- Information desk
- Defibrillator
- Escalator (2nd FL Parking)
- Escalator (3rd & 4th FL)
- Elevator
- Restroom (Men)
- Restroom (Women)
- Restroom (Universal)
- Pay phone
- TDD / Pay phone
- ATM
- Vending area
- Water fountain
- Bottle filler
- Smoking area
- Parking
- Bicycle Parking
- Entrance
- Automatic entry door
- Plants / grass area
- Walkway
- Crosswalk
- Service corridor
- Accessible Passenger Loading Zone

Complimentary Wi-Fi Area
Connect to : Hawaii Free Wi-Fi

Hawaii Convention Center Map—Level 3



Hawaii Convention Center Map—Level 4



Registration

The ICTXV Registration Desk is located in the main lobby of the Hawaii Convention Center near the Exhibit Hall entrance.

🕒 Registration Hours

Sunday.....	4:00 PM–6:00 PM
Monday.....	8:00 AM–5:00 PM
Tuesday.....	7:00 AM–5:00 PM
Wednesday.....	7:00 AM–5:00 PM
Thursday.....	7:00 AM–12:30 PM

Early Badge Pick-Up

Early registration badge pick-up will be available for attendees staying at the Hilton Hawaiian Village Waikiki Beach Resort and Ala Moana by Mantra hotels.

Early pick-up will be on Sunday, July 14 from 11:00 AM–1:00 PM in the Kona Tower Lobby (Ala Moana) and near the front desk (Hilton).

Speaker Ready Room

The Speaker Ready Room is located in Convention Center room 321. Once in Honolulu, all presenters should visit the Speaker Ready Room to upload and view their presentation to ensure that everything is working properly. The Speaker Ready Room is staffed with experts available to answer any presentation-related questions and address any presentation concerns. Presentations should be loaded and previewed in the Speaker Ready Room—not in the Scientific Session rooms—at least 30 minutes prior to the session start times.

🕒 Speaker Ready Room Hours

Sunday.....	3:00 PM–5:00 PM
Monday.....	8:00 AM–5:00 PM
Tuesday.....	7:00 AM–5:00 PM
Wednesday.....	7:00 AM–5:00 PM
Thursday.....	7:00 AM–12:30 PM

Photography Policy During Scientific Sessions

Photography of scientific presentations is prohibited without advance specific consent of the presenter(s)/author(s). Session Chairs are asked to strictly enforce this policy and individuals who do not comply will be asked to leave the session. In addition, cameras and recording devices are prohibited in the Exhibit Hall.

First Aid and Security

The Hawaii Convention Center has equipped each meeting room with a beige-colored house phone for use in case of an emergency. If you need medical or security assistance, pick-up the house phone and dial “0” for Security-base and report your emergency.

Food Services

The 808 Coffees Café is located on the 3rd floor meeting level of the Convention Center. 808 Coffees Cafe features snacks and a selection of coffees from Hawaii (home to the only major coffee-growing region in the US.) This includes a variety of popular Kona coffees, as well as the wonderful flavors of coffees grown on Kauai, Maui, and the North Shore of Oahu.

Child Care Services

The congress headquarters hotel (Hilton Hawaiian Village) offers child care services in-house for the convenience of the attendees through [Camp Penguin](#), featuring a program of Hawaii-centric children’s activities.

Other child care options include [Kama’aina Kids](#) and [Keiki Sitters](#). [GreatAuPair](#) is an international organization serving the Honolulu area with staff who can care for children or adults.

For adult care, one of the closest facilities to the Convention Center is [Malama Adult Day Care](#), while the [Hawaii Elderly Affairs Division](#) also provides resources on their website.

Nursing Pod

The Hawaii Convention Center (HCC) offers a private, comfortable, and clean ADA compliant Mamava lactation pod. The pod is spacious, secure and offers a 60” turnaround and grab bars for easy wheelchair access. The pod includes the following features:

- Secure access with Mamava app or access code
- Two benches
- Dual outlet and USB port
- Shelf, mirror, and coat hook
- Ceiling vents and exhaust fan
- Occupancy-activated lighting

Look for the nursing pod located at the Lobby Level near Exhibit Hall 1.

To unlock, users may download the Mamava app or use HCC’s house phone (located beside the pod) and dial “0” to request for the access code. For more information about the Mamava app, click [HERE](#).

Currency Exchanges

The American Dollar is the only acceptable form of payment in the United States. The Hawaii Convention Center does not offer Currency Exchange. The nearest bank locations to the convention center are:

First Hawaiian Bank (Kapiolani Branch)	American Savings Bank (Kapiolani Branch)	Bank of Hawaii (Kapiolani Branch)
1580 Kapiolani Blvd. Honolulu, HI 96814 808.943.4437	1600 Kapiolani Blvd. Honolulu, HI 96814 808.946.2802	1441 Kapiolani Blvd. Honolulu, HI 96814 808.942.6111

Credit Cards & Travelers Checks

Visa, Mastercard, American Express, Diners Club, Discover Card, and Traveler’s Checks are widely accepted at hotels, department stores, shops, and restaurants.

ATM

There are ATMs located in the convention center lobby near the Kamehameha Exhibition Hall 3 and along-side the escalator fronting Kamehameha Exhibition Hall 2. An alternative ATM is located on Level 3 next to the Business Center.

Insurance

Participants are advised to provide their own personal insurance as the ICTXV cannot assume responsibility on behalf of participants for personal accidents, sickness, theft, or property damage.

Electrical Appliances

North American outlets require appliances with 110V compatibility.

Tipping

Restaurants: Restaurant service is not usually included in restaurant checks. The average tip is 15% of the total check.

Taxis: Average tip is 15% of the fare.

Airport and Hotel Portage: Tipping is expected for all baggage handling. Airport portage is usually \$1 per bag.

Tax

A non-refundable 4.5% general excise tax must be paid on every item. This tax will not be refunded at the airport upon departure.

Educational Resources and Webinars Available
Visit SOT Website www.toxicology.org

Continuing Education Courses Online

*Update Your Skills with CEd-Tox:
SOT Continuing Education Online*

Benefits of Continuing Education

Stay competitive and keep your knowledge up-to-date in your field



Access presentations by top experts



Diverse course offerings cover a range of topics



Why CEd-Tox Online?

Learn anywhere, anytime



Earn hours for certification



View in a group setting



CEd-Tox

For a listing of courses and to register, visit www.toxicology.org/cedtox.asp. Graduate Student and Postdoctoral SOT members, as well as individuals from developing countries, receive complimentary access to all online courses.

Opening Ceremony

The Opening Ceremony will be held on Monday, July 15 from 5:00 pm–6:00 pm in the Kalākaua Ballroom. Please join us as we open the meeting in the local tradition and enjoy a performance from the keiki members of the Halau Hula Olan dance troupe.



The word “keiki” is Hawaiian for “baby” or “child,” literally “the little one”. On a Phalaenopsis, a keiki is a small plant growing from one node along the flower stem.

Welcome Reception in Exhibit Hall

The Welcome Reception will be held on Monday evening from 7:00 PM–8:30 PM immediately following the Deichmann Lecture presentation. A cash bar will be available along with entertainment.

Gala Dinner and Reception

The Gala Dinner and Reception will be held on the Hawaii Convention Center Rooftop Garden on Wednesday, July 17 from 7:00 pm–10:00 pm.

Entertainment will include a performance by the wahine members of the Halau Hula Olan dance troupe and instrumental trio.



The word “wahine” is used for “woman” in Hawaiian. It came into the English language in the late 18th century from Maori, the language of a Polynesian people native to New Zealand; it was originally used for a Maori woman, especially a wife.

Gala Registration: The Gala is NOT included in the Registration fee. You must pay an additional fee to attend the Gala. (Guest registration includes access to the Welcome Reception and Gala for non-scientists.) Visit the ICXTV Registration Desk by Tuesday at 12:00 noon to register to attend the banquet.

General Assembly

Open to Leaders of IUTOX Member Societies in Good Standing

The General Assembly will be held in Room 319 on Wednesday, July 17 from 12:00 noon–5:00 pm.

Every three years, during the International Congress of Toxicology, the General Assembly of IUTOX votes for the site of the International Congress of Toxicology along with future site of the Congress of Toxicology in Developing Countries (CTDC), and future Executive and Nominating Committee members. In the General Assembly, all national and regional IUTOX Member Societies in good standing have the right to vote.

Closing Ceremony

The Closing Ceremony will be held in the Kalākaua Ballroom on Thursday, July 18 from 4:30 pm–5:30 pm.

Ancillary Meetings

Tuesday, July 16, 4:30 PM–6:30 PM, Hilton Hawaiian Village Waikiki Beach Resort Hibiscus 1 & 2

AOP Hands-on Training: Building the Foundation for Predictive Toxicology

AOPs were originally conceived as a practical solution to a practical problem: how to integrate molecular and cellular information into chemical safety assessments. But in reality they are so much more: they are a transparent, highly curated, living document representing current biological knowledge and are useful in assay and integrated testing strategy design, supporting weight-of-evidence interpretations and decisions, hypothesis generation, developing predictive models and focusing and directing research. This seminar invites you to deepen your understanding of the Adverse Outcome Pathway framework. Following an introduction and overview of the OECD AOP framework and presentation of a real-life case study of AOP development and application, participants will be guided through an example of AOP-building in the AOP Wiki.

Please bring a computer and register in advance to kwillett@hsi.org. Registration is appreciated, but not required.

Agenda:

Introductory presentations

- 20 min Introduction and overview of the OECD AOP framework
Catherine Willett, HTPC/Humane Society International
- 20 min Building AOPs for Neurotoxicity: Perspective from an Academic
Prof. Dr. Ellen Fritsche, IUF –Leibniz Research Institute for Environmental
- 20 min Application of the AOP framework to regulatory decisions:
Early case studies
Kristie Sullivan, Physicians Committee for Responsible Medicine

1-hour AOP Wiki demonstration and hands-on activity

Finding AOPs and AOP info in the Wiki | Entering information into the Wiki | Adding a diagram

Wednesday, July 17, 2019, 1:00 PM–2:20 PM, Hilton Hawaiian Village Waikiki Beach Resort, Hibiscus 1 & 2

Training on New Approach Methodologies: Alternatives for Acute and Contact Toxicity Assessment

The American Society for Cellular and Computational Toxicology invites you to attend this training to get up to date on the latest *in vitro* and *in silico* models and approaches for acute and contact toxicology. Presenters from EPA, NIH, and more will discuss scientific and regulatory progress in the development and acceptance of new approaches that are replacing and reducing *in vivo* toxicology tests in the chemical, pesticide, and personal care sectors and provide practical advice on implementing them.

Light snacks will be served. View program at www.ascctox.org.

Whether this is your first visit or a repeat visit to Hawaii, there are many exciting ways to explore the islands. The concierge at the Hilton Hawaiian Village Waikiki Beach Resort can assist you and your accompanying guests find an activity taking place on and off of their property. A variety of water activities (rentals and tours) and site seeing adventures can be arranged. Below are a few that may be of interest to you.

Hele Mai Ho'olaule'a! (come celebrate!)

Waikiki's only outdoor Luau features extravagant Lei greetings, Hula kahiko performers, 4 acrobatic fire dancers and authentic Hawaiian dining in a memorable setting beneath the starlit, mid-Pacific skies.

Hilton Hawaiian Village's Waikiki Starlight Luau is available from Sunday to Thursday at 5:30 pm (weather-permitting) on the rooftop of the Mid-Pacific Conference Center (schedule subject to change).

Compete in the Conch Shell Blowing Challenge. Join the Group Hula Lesson. Or simply unwind with authentic Hawaiian cuisine and a refreshing cocktail with a backdrop of lilting island music and dance.

Discover Hawaii's diverse array of culinary influences in an incredible rooftop setting. Enjoy exotic flavors and locally sourced ingredients in Kalua Pig, ocean-fresh Island Fish or grilled Huli Huli Chicken with Sweet Potatoes and Hawaiian Fried Rice.

Waikiki Starlight Luau includes live native Hawaiian music and Polynesian chants, dances from Hawaii, Polynesia and the Pacific and acrobatic Fire Knife Dancers under the stars.

Visit the [Hilton Hawaiian Village's website](#) for more information or to make a reservation.

Pearl Harbor

The Pearl Harbor National Memorial is located in Joint Base Pearl Harbor-Hickam. The Pearl Harbor area was designated a national historic landmark in 1964 for its strategic importance related to the United States' annexation of Hawaii, and for the December 7, 1941, Japanese attack during World War II. The Pearl Harbor Visitor Center is part of the Pearl Harbor National Memorial, a National Park Service site. Pearl Harbor consistently ranks among the top three most heavily-visited tourist destinations in Hawaii.

Admission to the Pearl Harbor Visitor Center is free. Tickets are available on a first-come, first-served basis and are issued to each individual visitor. Therefore, all individuals in your party must be present at the ticket counter at the time of issue. For more information and to reserve a ticket, visit the [National Park Service website](#).

Fireworks

If you are extending your stay, the Hilton Hawaiian Village offers a fireworks show every Friday beginning at 8:00 pm. The best view can be seen from the Hau Tree Cantina and the Tropics Bar & Grill's outdoor seating.

Iolani Palace

Built in 1882 by King Kalakaua, Iolani Palace was the home of Hawaii's last reigning monarchs and served as the official royal residence and the residence of the Kingdom's political and social life until the overthrow of the monarchy in 1893.

Registered as a National Historic Landmark since 1962 and the only official royal residence in the United States, the Palace is one of the most recognizable buildings in Hawaii. Meticulously restored to its former grandeur, Iolani Palace tells of a time when their Majesties, King Kalakaua and his sister and successor, Queen Liliuokalani walked the grand halls. Open to the public for guided tours, the first floor consists of the public reception areas—the Grand Hall, State Dining Room, Blue Room, and Throne Room—while the second floors showcase the private suites, including the King and Queen's suites, Music Room, and the Imprisonment Room. Book a tour or learn more about the [Iolani Palace](#).

Diamond Head State Monument

Diamond Head State Monument offers views overlooking the Pacific Ocean and Honolulu. Originally used by the US military as a post to prevent attacks against Honolulu, the trail offers views of the 300,000 year old crater.

Overall the hike takes about 1.5–2 hours, round trip. The 0.8 mile hike from the trailhead to the summit is steep and strenuous, gaining 650 feet in elevation with 99 steep steps near the end of the hike. The last entrance to hike the trail is at 4:30 PM and the gates lock daily at 6:00 pm.

Dole Plantation

The Dole Plantation is one of Oahu's most popular visitor attractions, welcoming over one million visitors a year. The plantation offers family friendly options including the Pineapple Garden Maze and the Pineapple Express Train Tour.

Admission to the Dole Plantation grounds is free and activities take place daily from 9:30 am–5:00 pm.

Manoa Falls

Manoa Falls Trail may look familiar to fans of Lost and Jurassic Park—many of the jungle screens were filmed along this trail. The 30-minute hike rewards hikers a 150-foot waterfall at the end.

Because Manoa Valley receives the most rainfall on Oahu, the trail can be muddy and closed-toe hiking shoes are strongly recommended. The last 100 yards are challenging and involve climbing up and over boulders to reach the 150-foot waterfall.

Travel Award Reception

Tuesday, July 16, 1:30 PM–2:30 PM

Sponsored by the American College of Toxicology
Third Floor Middle Concourse, Ala Wai Terrace
By Invitation Only



IUTOX Merit Award

Elaine M. Faustman

Institute for Risk Analysis & Risk Communication, University of Washington

Dr. Faustman holds three prestigious positions at the University of Washington. She serves as a Professor in the Department of Environmental and Occupational Health Sciences, an adjunct professor in the Daniel J. Evans School of Public Policy and Governance, and the Director of the Institute for Risk Analysis and Risk Communication. Dr. Faustman has also been an adjunct professor in the Department of Engineering and Public Policy at Carnegie-Mellon University. She received her PhD at Michigan State University in the School of Pharmacology and Toxicology.

Dr. Faustman's research interests lie in developmental toxicology, risk assessment methodologies and molecular mechanisms of metals and pesticides. The long-range objective of her research is two-fold: To identify biochemical mechanisms of developmental toxicity and to develop new methods for the evaluation of health risks posed by environmental agents. Dr. Faustman has over 285 peer-reviewed publications including 217 research publications, 68 book chapters and reviews, technical and book reports, comments and short articles and 426 abstracts.

Through her exceptional commitment to train junior and senior scientists around the world, Dr. Faustman has led the IUTOX risk assessment training efforts in Africa, Asia, Europe, and South America. She created a relationship with the International Science Council (ISC) to procure funding for a series of water security courses and partnered with IUTOX member societies to develop and deliver training courses responsive to local and regional needs. Dr. Faustman also has been devoted to training the next generation of risk assessors by mentoring over 60 MS and PhD students, 8 postdoctoral fellows, and 16 visiting scientists from 13 countries in her lab and institute.

The global toxicology community has been well served by Dr. Faustman. She has been instrumental in assisting scientists in South Africa and Nigeria form their own toxicology societies and encouraging further efforts in other parts of Africa. Dr. Faustman has generously shared her time and talents with IUTOX by serving as Secretary-General (2010-2016), Director (2007-2010) and Chair of the Education Committee (2007-2016). She also has been invited to serve on and often lead numerous committees associated with the International Science Council, the World Health Organization (WHO), Food and Agricultural Organization, US Environmental Protection Agency, the National Toxicology Program, The National Institute of Environmental Health Sciences, Teratology Society, and the Health Professionals Advisory Board among others. Of special note, Dr. Faustman served on 10 SOT committees and served on SOT Council (2004-2006).

Dr. Faustman's body of work and exemplary dedication to the global toxicology community embodies the IUTOX mission *to improve human health through the science and practice of toxicology world-wide*. IUTOX is proud to honor Elaine Faustman, global citizen-scientist, with the 2019 Merit Award in recognition of excellence in her field and her tireless efforts to bring toxicology research and education to scientists throughout the world.



TEF Dixon Award

Lenny Kamelia, PhD Candidate,

Wageningen University & Research, The Netherlands

The Toxicology Education Foundation has provided the Robert L. Dixon International Travel Award to support a graduate student in attending the 15th International Congress of Toxicology.

The Robert L. Dixon Award was established in memory of Robert L. Dixon (SOT President 1982–1983) as a tribute to his dedication to the field of toxicology and student training.

IUTOX Travel Awards

IUTOX is pleased to provide travel awards to scientists attending the ICTXV meeting. Recipients are junior and senior scientists residing in a country where toxicology is underrepresented.

IUTOX ICTXV Travel Award Recipients



Katarina Baralić
Serbia



Anca Oana Docea
Romania



Danijela Đukić-Ćosić
Serbia



María García-Espiñeira
Colombia



Andrey Kukharensky
Russia



Jinhua Li
China



Kamakshi Mehta
India



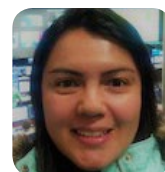
Umar Muhammed Bello
Nigeria



Francis Okonkwo
Nigeria



Kelly Olympio
Brazil



Yuliana Parra-Forero
Mexico



Betzabet Quintanilla-Vega
Mexico



Sheikh Raisuddin
India



Natasha Sanabria
South Africa



Monruedee Sukprasansap
Thailand



Hulya Turkan
Turkey



Chinyere Usoro
Nigeria



M. Wahajuddin
India



Quanjun Wang
China



Yuanyuan Xu
China



Victor Yariwake
Brazil



Itzel Zavala-Guevara
Mexico

IUTOX ICTXV Travel Award Sponsors



SOT Endowment Fund Travel Awards

SOT is pleased to provide travel awards to junior and senior scientists made available through the SOT Endowment Fund. Recipients are ICTXV Poster and Platform presenting authors from countries where toxicology is underrepresented.



SOT Endowment Fund Travel Award Recipients



**Stephen
Abiola Akinola**
South Africa



**Chand
Davuljigari Basha**
India



**Alejandro
Déciga-Alcaráz**
Mexico



**Claudia
Escudero-Lourdes**
Mexico



Alicia Faletti
Argentina



Keyu He
China



Lori Hjar
Mexico



Eui-Bae Jeung
Korea



Neelakshi Kar
India



Man Long Kwok
China



Kwun Kwan Lo
China



Qun Lou
China



**Alonso Andres
Doria Manzur**
Colombia



**Wanchanok
Nantacharoen**
Thailand



Mahesh Nepal
Nepal



**María de los
Angeles Andrade
Oliva**
Mexico



**Orish
Ebere Orisakwe**
Nigeria



**Manolo
Ortega-Romero**
Mexico



**Silvia Carolina
Moreno Rivas**
Mexico



Nalinee Sripaung
Thailand



**Lesly
Tejeda-Benitez**
Colombia



Qi Wang
China



Tingting Wei
China



Hongyu Yang
China



Tingjie Zhan
China

Scientific Poster Preparation

Poster presenters should have their posters printed prior to travel and carry the printed poster to the meeting. If you would like to have your poster printed in Honolulu, you will need to take advantage of local printing options outside of the Convention Center, such as [FedEx](#).

Poster Mounting Information

The presenting author of each poster abstract is responsible for the proper assembly, mounting, and presentation of their poster. Presenters will be allowed to begin mounting their display on their assigned board per the following schedule:

Date	Poster Display Times	Begin Mounting Posters	Location	Entrance for Mounting
Tuesday, Wednesday	4:30 PM–6:30 PM	12:00 Noon	Exhibit Hall	Main Lobby

Poster presentations have unique identifying numbers known as the abstract number. Abstract numbers do not specify poster board locations. Presentations have separate poster board numbers. Poster presentations are mounted on pre-numbered boards throughout the Exhibit Hall. See page 125 for the listing of Poster Session 1 Abstracts, and page 206 for Poster Session 2 Abstracts. Poster board numbers are preceded by a "P." Example: P101–0279, reflecting Abstract #0279 and Poster Board Number P101.

Presenters should display posters **ONLY** on the assigned date. Posters must be removed immediately at the end of each day, so that the boards may be prepared for the next day's Poster Sessions.

Presentation Times and Expectations

Presenters are required to stand by their posters during their assigned 120-minute attendance time block. The Poster Sessions are:

Poster Session 1: Tuesday, July 16, 4:30 PM–6:30 PM, Exhibit Hall

Category	Poster Board #s
Alternatives to Animal Models	P101–P131
Computational Toxicology, Modeling, and Kinetics	P132–P148
Ecotoxicology	P149–P166
Epidemiology and Public Health	P167–P187
Exposure Assessment	P188–P205
Food Safety/Nutrition	P206–P241
Metals	P242–P276
Mixtures	P277–P284
Nanotoxicology	P285–P324
Other	P325–P348
Persistent Organic Pollutants	P349–P368
Pesticides	P369–P390
Risk Assessment	P391–P415
Safety assessment	P416–P430

Poster Session 2: Wednesday, July 17, 4:30 PM–6:30 PM, Exhibit Hall

Category	Poster Board #s
Air Pollution	P101–P126
Biomarkers	P127–P154
Carcinogenesis	P155–P173
Cardiovascular Toxicology	P174–P191
Clinical and Translational Toxicology	P192–P206
Endocrine Active Compounds	P207–P225
Epigenetics	P226–P241
Genotoxicity and Mutagenesis	P242–P259
Immunotoxicology	P260–P279
Kidney	P280–P288
Liver	P289–P321
Metabolism of Drugs and Chemicals	P322–P341
Neurotoxicology	P342–P387
Reproductive, Developmental, and Juvenile Toxicology	P388–P414
Respiratory Toxicology	P415–P427
Skin	P428–P436

See page 125 for a listing of Poster Session 1 Abstracts.

See page 206 for a listing of Poster Session 2 Abstracts.

The presenting author, you (or an assigned co-author), are asked to be physically present by your poster for the duration of the Poster Session. Presenters are expected to discuss their work with scientists visiting their poster. Should circumstances prevent you from making your presentation, you must arrange for a co-author or colleague to present your poster and you must notify SOT Headquarters.

Monday Program Schedule

Continuing Education Courses

The ICTXV Continuing Education Committee has worked diligently with IUTOX and other societies to offer quality learning opportunities intended to provide fundamental concepts and knowledge of toxicology and present information that can serve as a foundation upon which to improve human and environmental health in countries with both robust and developing research and regulatory enterprises.

See page 94 for a listing of CE presentation abstracts.

Monday, July 15, 9:00 AM to 11:30 AM, Room 313

AM01 | MORNING COURSE

➡ Separate registration required.



Continuing Education Course: Applications of Chemical Risk Assessment in Establishing Food Safety Standards

Chairperson(s): Angelika Tritscher, Food Safety Director Specialised Nutrition, Danone; and Yu Janet Zang, US Food and Drug Administration.

With changing climate and agricultural practices, continuous growth of international food trade and increasing integration of developing countries, food standards are of critical importance for global food security, public health and economy. There is urgent need to promote the understanding of food risk assessment and standard development process among toxicologists and risk assessment professionals from different geographic and economic regions of the world. This basic-level training course provides essential knowledge on food chemical risk assessment methodologies used by Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) in assessing health risks and recommending safe exposure levels as basis for global food standards for pesticides, additives and contaminants. The training will give a short overview of the review process applied by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), followed by lectures that elaborate hazard identification, hazard characterization including dose-response analysis, and exposure assessment. Case examples will be provided, including a hands-on exercise to apply the risk assessment procedures.

Abstract

AM01-01	9:00	Risk Assessment of Food Additives and Chemical Contaminants. Gordon Shephard, Cape Peninsula University of Technology, South Africa.
AM01-02	9:30	Risk Assessment of Veterinary Drug and Pesticide Residues in Food. Alan Boobis, Imperial College London, United Kingdom.
	10:00	Refreshment Break
AM01-03	10:30	The Key to Risk Assessment: Dose-Response Analysis. Matthew Wheeler, Centers for Disease Control and Prevention, The United States of America.
AM01-04	11:00	Dietary Exposure Assessment of Chemicals in Food. Peter Cressey, Institute of Environmental Science and Research Limited, New Zealand.

Monday, July 15, 9:00 AM to 11:30 AM, Room 312

AM02 | MORNING COURSE

➡ Separate registration required.



Continuing Education Course: Immunotoxicology Hazard Identification Using Novel *In Vitro* Methods

Chairperson(s): Dori Germolec, National Institute of Environmental Health Sciences; and Emanuela Corsini, Università degli Studi di Milano.

Significant progress has been made regarding the use of *in vitro* assessments to evaluate immunotoxicity. This session will focus on current strategies that may transform immunotoxicity assessments from a system based on animal testing to one founded primarily on *in vitro* methods that evaluate changes in immunologic processes using primary cells, cell lines, or cellular components. The human whole-blood cytokine release assay measures the chemical induced modulation of interleukin (IL)-1 β and IL-4 release in human blood samples in response to lipopolysaccharide (LPS) or staphylococcal enterotoxin B (SEB). The Multi-ImmunoTox assay, which uses reporter cell lines derived from Jurkat and THP-1 cells to examine cytokine changes following chemical treatment, has shown promising results in early validation efforts. Machine learning analyses suggest that various integrated approaches based on the adverse outcome pathway for skin sensitization are more accurate in identifying potential skin sensitizers than *in vitro*, *in chemico*, or *in silico* methods by themselves, and can show a high degree of accuracy in predicting known human skin sensitizers. *In vitro* methods that assess signaling pathways associated with immune function allow the ability to explore indirect mechanisms of immunosuppression. Each of these tools contribute to the identification of compounds that modify immune function.

Monday Program Schedule

Abstract

- AM02-01 9:00 **Approaches and Challenges for Assessing Unintended Cytokine Release by Novel Protein Therapeutics.** *Madeline Fort, Amgen Inc., The United States of America.*
- AM02-02 9:30 **Immunotoxicological Profiling of Chemicals Using Novel *In Vitro* Assays.** *Setsuya Aiba, Tohoku University Graduate School of Medicine, Japan.*
- 10:00 **Refreshment Break**
- AM02-03 10:30 **NTP Approaches to *In Vitro* Assessment of Dermal Hypersensitivity.** *Dori Germolec, NIEHS, The United States of America.*
- AM02-04 11:00 **Indirect Immunotoxicity: *In Vitro* Opportunities.** *Emanuela Corsini, Università degli Studi di Milano, Italy.*

Monday, July 15, 9:00 AM to 11:30 AM, Room 315

AM03 | MORNING COURSE

➡ Separate registration required.



Continuing Education Course: Recent Developments in Adult and Developmental Neurotoxicity Testing for Regulatory Assessment

Chairperson(s): Abby Li, Exponent, Inc.; and Kristen Ryan, National Toxicology Program, National Institute of Environmental Health Sciences.

New efforts have been made to improve conduct and evaluation of adult and developmental neurotoxicity (DNT) studies for regulatory assessment. In 2017, Health Canada and US EPA published the North American Free Trade Agreement (NAFTA) internal guidance document for regulatory evaluators on how data from OECD and US EPA DNT studies should be reviewed. This, plus new recommendations recognized by Society of Toxicologic Pathology on brain processing and quantitative analysis have relevance for adult and DNT studies. Additionally, adverse outcome pathways (AOP) relevant to adult and developmental neurotoxicity have been published for potential use in regulatory frameworks by the European Food Safety Authority. This continuing education (CE) course will provide participants with basic understanding of state-of-the-art approaches for conduct, analysis, reporting and synthesis of neurotoxicology data. The course will begin with a review of current global guideline studies for adult and developmental neurotoxicology, followed by review of recent recommendations on evaluation and reporting of behavioral and neuropathology data. The third speaker will illustrate synthesis of toxicokinetic, neuropharmacologic, additional histological, and stereology approaches that are not required but recommended for further investigation by US EPA and OECD guidelines. The final speaker will describe the potential use of *in vitro* neurotoxicity studies in regulatory frameworks and critical areas for consideration such as biological/human relevance, assay validation, and systematic review.

Abstract

- AM03-01 9:00 **Introduction to Adult and Developmental Neurotoxicity Testing: History, Guidelines, and Use in Risk Assessment.** *Hiroaki Aoyama, The Institute of Environmental Toxicology, Japan.*
- AM03-02 9:30 **Neurotoxicity Testing: From Benchtop to Regulatory Evaluation.** *Abby Li, Exponent, Inc., The United States of America.*
- 10:00 **Refreshment Break**
- AM03-03 10:30 **Integrated Approaches to Testing and Assessment: Aggregating Lines of Evidence from Toxicokinetics and Advanced *In Vivo* Neuropathology and Neurochemistry Studies.** *Daniel Minnema, Syngenta Crop Protection, LLC, United Kingdom.*
- AM03-04 11:00 **Introduction to and Quality Criteria for *In Vitro* Neurotoxicity Assays and Alternative Methods for Use in Regulatory Frameworks.** *Kristen Ryan, National Toxicology Program, National Institute of Environmental Health Sciences, The United States of America.*

Monday, July 15, 9:00 AM to 11:30 AM, Room 316

AM04 | MORNING COURSE

➡ Separate registration required.



Continuing Education Course: Understanding Translational Biomarkers: Science and Qualification

Chairperson(s): Vishal Vaidya, Pfizer, Inc.; and Warren Glaab, Merck and Co.

Biomarkers can serve as variety of functions, such as monitoring safety, provide quantitative measures of chemical exposures and biologically effective doses, predict outcome in a patient with disease, and identify who will respond to an intervention and whether the intervention is working. This course will provide an overview of biomarker science that goes from discovery to evaluation and qualification of biomarkers for safety assessment. After a brief introduction to the pipeline of biomarker development and qualification, the first talk will present past successes and current state of biomarkers for early detection of kidney injury in nonclinical and clinical studies. Lack of reliable biomarkers for detection of pancreatic as well as skeletal muscle damage remains to be unmet medical need and will be the focus of the next two talks. The fourth talk will describe the significant progress that has been made towards identification of mechanistic biomarkers for liver damage at the interface of novel proteins and microRNAs. Amongst several consortia working on advancing biomarker science the Innovative Medicines Initiative has devoted significant resources towards biomarker discovery and qualification. The final talk will not only summarize learning's from past initiatives but also discuss opportunities around their latest initiative called TransBioLine.

Monday Program Schedule

Abstract

- AM04-01 9:00 **Clinical Biomarkers for Skeletal Muscle Injury and Neuromuscular Diseases.** Vishal Vaidya, Pfizer, Inc., The United States of America.
- AM04-02 9:30 **Translational Biomarkers of Kidney Damage.** Mira Pavkovic, Bayer AG, Germany.
- 10:00 **Refreshment Break**
- AM04-03 10:30 **Advancing Biomarkers for Pancreatic Toxicity and Pancreatitis.** Warren Glaab, Merck and Co., The United States of America.
- AM04-04 11:00 **Translational and Mechanistic Biomarkers of Liver Injury.** Chris Goldring, MRC Centre for Drug Safety Science, University of Liverpool, United Kingdom.

Monday, July 15, 2:30 PM to 5:00 PM, Room 315

PM05 | AFTERNOON COURSE

➡ Separate registration required.



Continuing Education Course: Big Data Analytics in the Era of the 21st Century of Toxicology

Chairperson(s): Weida Tong, US Food and Drug Administration, National Center for Toxicological Research; and Richard Paules, National Toxicology Program/National Institute of Environmental Health Sciences.

Toxicology in the 21st century is marked with increasing incorporation of high throughput technologies to improve our molecular-level understanding of toxicity, moving towards a data-driven regulatory decision-making paradigm. These technologies generate vast amounts of data, representing a truly big data scenario in modern toxicology. Efforts in this field include the Tox21 and ToxCast programs in US, and several EU-based projects motivated by the EU REACH regulation and guided by the 3Rs principle (Replacement, Reduction and Refinement) of animal use. The lack of advanced data mining and knowledge discovery tools powered by big data analytics threatens progress in this new approach to toxicological science. Thus, this era is characterized by significant focus on computational and automational approaches to extract useful information from big toxicology data. This session will introduce some big data methodologies for analysis of large toxicogenomics data sets, particularly by considering designs involving dose-response and time-dependent features that are essential for advancing toxicology. In addition, the read-across of integrating chemical structure information and *in vitro* data will be also discussed. The focus will be on the analytics to turn data into knowledge, which is the next innovation cycle in this area of big data science.

Abstract

- PM05-01 2:30 **Transcriptional Responses to Chemicals in Hepatocytes *In Vivo* and *In Vitro*.** Benjamin Haibe-Kains, Princess Margaret Cancer Centre and University of Toronto, Canada.
- PM05-02 3:00 **Assessing the Potential Liver Toxicity of Compounds Using TG-GATEs Database: A Connectivity Map Approach.** Xiaohui Fan, College of Pharmaceutical Sciences, Zhejiang University, China.
- 3:30 **Refreshment Break**
- PM05-03 4:00 **Genomic Dose Response Analysis: Identifying Biological Potency at a Genomic Level.** Scott Auerbach, Division of the National Toxicology Program at NIEHS, The United States of America.
- PM05-04 4:30 **Transitioning towards Objective Read-Across Approaches: Landscape, Research, and Practical Application.** Grace Patlewicz, National Center for Computational Toxicology, US EPA, The United States of America.

Monday, July 15, 2:30 PM to 5:00 PM, Room 316

PM06 | AFTERNOON COURSE

➡ Separate registration required.



Continuing Education Course: Epigenetics and Toxicology: Overview and Advances in Methodology

Chairperson(s): Aline de Conti, US Food and Drug Administration, National Center for Toxicological Research; and Alexandra Antunes, ULisboa, Instituto Superior Técnico.

Recently epigenetics has become one of the fastest-growing areas of science and due its impact on global, environmental and personal health it is also rapidly expanding in the toxicology field. This course will provide an overview of the current knowledge and a variety of perspectives, challenges and application of epigenetics in toxicological sciences. Initially, the course will cover the basic concepts of epigenetics and its practical application in toxicology. The following presentations will provide recent discoveries on how epigenetic mechanisms are related to adverse health outcomes caused by toxicant exposures early in life or to development of noncommunicable diseases, as cancer. Moreover, the course will bring information on the potential application of epigenetic biomarkers for safety assessment, as well as, the development of epigenetic therapies. The basic concepts will be always be accompanied by studies illustrating the key role of epigenetics for toxicology and safety assessment. The course will also emphasize the experimental methodologies used for epigenetics analysis providing to participants an overall idea of the more accurate experimental methodologies to assess epigenetic modifications. Participants can expect to have an understanding of the role of epigenetics events in toxicology and the specific methodologies used in epigenetic studies.

Monday Program Schedule

Abstract

- PM06-01 2:30 **Epigenetics Overview and Advances.** *Aline de Conti, National Center for Toxicological Research, US Food and Drug Administration, The United States of America.*
- PM06-02 3:00 **Longitudinal Effects of Developmental Exposures on Age-Related DNA Methylation and Hydroxymethylation.** *Dana C. Dolinoy, University of Michigan School of Public Health, The United States of America.*
- 3:30 **Refreshment Break**
- PM06-03 4:00 **Epigenetics and Environmental Origins of Cancer.** *Zdenko Herceg, International Agency for Research on Cancer (IARC), France.*
- PM06-04 4:30 **Importance of Investigating Epigenetic Alterations for Industry and Regulators.** *Jonathan Moggs, Novartis Institutes for BioMedical Research, Switzerland.*

Monday, July 15, 2:30 PM to 5:00 PM, Room 312

PM07 | AFTERNOON COURSE

 Separate registration required.



Continuing Education Course: New Risk Assessment Approaches for Reproductive and Developmental Toxicology

Chairperson(s): *Barbara F. Hales, McGill University; and Thomas Hartung, Center for Alternative Animal Testing (CAAT), Johns Hopkins University.*

New advances in the area of reproductive and developmental toxicology require updated educational opportunities for both current experts in the field as well as new graduate students. The purpose of this educational session will be to share these updates within an international context. Emphasis on *in vitro* models for both reproductive as well as developmental toxicology endpoints will be presented. Methods of integrating *in vivo* (rodent and human) with *in vitro* output will be presented and linkage with quantitative values. The first speaker will illustrate how *in silico*, *in vitro* and *in vivo* assessments of developmental neurotoxicity can be integrated in Adverse Outcome Pathways (AOPs). The second speaker will use case studies to demonstrate how high-throughput screening data, based on embryonic stem cell assays, and developmentally conserved cell signaling pathways, in simulations with agent-based models, contribute to the risk assessment for environmental chemicals. The third speaker will use the plasticizer diethylhexyl phthalate (DEHP) as an example to present a four-step approach for the design, characterization and toxicological assessment of alternative responsible replacements for chemicals with endocrine disrupting effects. The fourth speaker will describe new *in vitro* approaches based on the evaluation of specific androgen based endpoints and 3D cultures for use in the early identification of chemicals with an adverse impact on male reproductive outcomes. The experts chosen for this session are from a variety of agencies and organizations and have global experience.

Abstract

- PM07-01 2:30 **Alternative Approaches for Developmental Neurotoxicity (DNT) Evaluations.** *Anna Bal-Price, European Commission–DG Joint Research Centre (JRC), Italy.*
- PM07-02 3:00 **Computational Systems Biology: Translational Tools for Data Integration and Modeling.** *Thomas B. Knudsen, National Center for Computational Toxicology (NCCT), US Environmental Protection Agency, The United States of America.*
- 3:30 **Refreshment Break**
- PM07-03 4:00 **Developing a Responsible Approach for Replacing Endocrine Disrupting Chemicals.** *Bernard Robaire, McGill University, Canada.*
- PM07-04 4:30 **Linking *In Vivo* and *In Vitro* Male Reproductive Toxicity Risk Assessment.** *Elaine M. Faustman, University of Washington, The United States of America.*

Monday, July 15, 2:30 PM to 5:00 PM, Room 313

PM08 | AFTERNOON COURSE

 Separate registration required.



Continuing Education Course: What Is an Adverse Effect for Human Health: Defining Adversity in Risk/Safety Assessments

Chairperson(s): *Maria Lucia Zaidan Dagli, University of São Paulo, School of Veterinary Medicine and Animal Science; and Robert R. Maronpot, Maronpot Consulting, LLC.*

Defining adversity is a central question in toxicology. Regulatory agencies require information on adverse effects from animal toxicological studies to approve the release of commercial products destined for the human population. Establishing what is adverse contributes substantially to risk/safety assessment, helping to define the hazard and allowable exposures to a particular substance ('no-observed-adverse-effect-level' (NOAEL) and margin of exposure) and other toxicological parameters. The definition of "adverse" depends on the complete pathological evaluation and interpretation of findings in animal studies. That is why it is important to define whether the relevant pathological alterations are related to the test material, are adaptive and reversible, relevant and translatable to humans. In current toxicology practice, it is

Monday Program Schedule

often challenging to reliably determine if a particular pathological alteration qualifies as an adverse effect. This course aims to present the most important tools to help toxicologists to define adversity in risk/safety assessments. General pathology and toxicologic pathology concepts will be presented and the audience will learn from specific examples, including theoretical and practical aspects of adverse effects on the liver, endocrine (thyroid) and immune/lymphoid systems.

Abstract

- PM08-01 2:30 **Defining Adverse, Non-Adverse, and Adaptive Responses in Safety/Risk Assessments.** *Robert R. Maronpot*, Maronpot Consulting, LLC, The United States of America.
- PM08-02 3:00 **Importance of Defining Adverse Effects for Toxicologic Evaluations Risk/Safety Assessment: The Case of Octamethylcyclotetrasiloxane and Decamethylcyclopentasiloxane.** *Wolfgang Dekant*, University of Wuerzburg, Germany.
- 3:30 **Refreshment Break**
- PM08-03 4:00 **The Application of Adversity and Adverse Outcome Pathway Analysis to Rodent Thyroid Hormone Perturbation.** *John R. Foster*, ToxPath Sciences Ltd, United Kingdom.
- PM08-04 4:30 **Fundamental Concepts to Understand Adverse Effects in the Immune/Lymphoid System.** *Maria Lucia Zaidan Dagli*, University of São Paulo, School of Veterinary Medicine and Animal Science, Brazil.

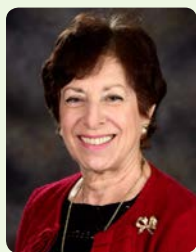
Monday, July 15, 5:00 PM to 6:00 PM, Kalākaua Ballroom

Opening Ceremony

Monday, July 15, 6:00 PM to 7:00 PM, Kalākaua Ballroom

Deichmann Plenary Lecture: Human Health and the Environment: Global is Local is Personal

Lecturer: *Linda S. Birnbaum, PhD, DABT, ATS, National Institute of Environmental Health Sciences and National Toxicology Program.*



Human health is inextricably linked to the environments in which we live. Global trends in environmental health are an aggregate of environmental health at the local or community level, and those trends and impacts we see at the local community level are the result of personal health outcomes. By researching opportunities to increase the health and wellness of the individuals, and engagement with communities, we can affect global shifts in health outcomes. To be successful the toxicology community must look for new ways to investigate the cumulative effects of exposures from air, water, chemical toxins, radiation, pest control. In addition, we must look more broadly and partner with other disciplines to address cross-cutting areas, including such topics as building design, urban and regional planning, energy, transportation, disaster preparedness and response, climate change, and environmental stress. To fulfill our mission of reducing the burden of human illness and disability associated with environmental exposures, scientific research must lead to public health action. This means that we have an obligation not only to produce the best science possible, including the exploration of precision approaches, but also to communicate science in ways that further public health protection.

Linda S. Birnbaum, PhD, is director of the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health, and the National Toxicology Program (NTP). A board-certified toxicologist, Birnbaum has served as a federal scientist for nearly 39 years. Prior to her appointment as NIEHS and NTP Director in 2009, she spent 19 years at the US Environmental Protection Agency (US EPA), where she directed the largest division focusing on environmental health research.

Dr. Birnbaum has received many awards and recognitions. In 2016, she was awarded the North Carolina Award in Science. She was elected to the Institute of Medicine of the National Academies, one of the highest honors in the fields of medicine and health. She was also elected to the Collegium Ramazzini and to the Institute of Medicine of the National Academies of Science, and received an honorary Doctor of Science from the University of Rochester and a Distinguished Alumna Award from the University of Illinois. She also received an Honorary Doctorate from Ben-Gurion University, Israel; the Surgeon General's Medallion 2014; and 14 Scientific and Technological Achievement Awards, which reflect the recommendations of US EPA's external Science Advisory Board, for specific publications.

Dr. Birnbaum is an active member of the scientific community. She was vice president of the International Union of Toxicology, the umbrella organization for toxicology societies in more than 50 countries, and former president of the Society of Toxicology, the largest professional organization of toxicologists in the world. She is the author of more than 800 peer-reviewed publications, book chapters, and reports. Birnbaum's own research focuses on the pharmacokinetic behavior of environmental chemicals, mechanisms of action of toxicants including endocrine disruption, and linking of real-world exposures to health effects. She is also an adjunct professor in the Gillings School of Global Public Health, the Curriculum in Toxicology, and the Department of Environmental Sciences and Engineering at the University of North Carolina at Chapel Hill, as well as in the Integrated Toxicology and Environmental Health Program at Duke University.

A native of New Jersey, Birnbaum received her MS and PhD in microbiology from the University of Illinois at Urbana-Champaign.

Monday, July 15, 7:00 PM to 8:30 PM, Exhibit Hall

Welcome Reception

Join attendees in the Exhibit Hall for networking and food. A cash bar will be available.

Tuesday Program Schedule

Tuesday, July 16, 8:00 AM to 8:50 AM, Kalākaua Ballroom

Keynote Lecture: Challenges in Global Health: Innovative Discovery and Translational Strategies

Lecturer: Peter Warner, MA, DPhil, Bill & Melinda Gates Foundation.



Millions of people suffer and die from diseases that disproportionately affect the poor. These include malaria and tuberculosis as well as a long list of other tropical diseases such as onchocerciasis and lymphatic filariasis. The Bill & Melinda Gates foundation (BMGF) is driven by the belief that every life has equal value. Providing the tools to improve the health of the poorest is a critical aspect of our work and this includes the discovery and development of drugs which will have the most impact on the lives of the poor. Often pharmaceutical companies cannot easily justify the significant investments needed to discover and develop drugs for these diseases. The BMGF can help to build partnerships and provide resources and expertise to de-risk drug discovery and development projects. The BMGF works with Product Development Partners including Medicines for Malaria Venture, The TB Alliance and Drugs for Neglected Diseases Initiative to harness the best academic research and couple it with research institute and pharmaceutical company partners to generate new drugs. A network led by the BMGF is the TB Drug Accelerator and this is as an example of what can be achieved when world leading disease knowledge and biology is coupled with excellence in drug discovery. This lecture will provide examples of how the challenges in developing drugs for neglected diseases can be overcome.

Peter Warner, MA, DPhil is the Senior Program Officer in Discovery and Translational Sciences. Peter joined the Bill and Melinda Gates Foundation (BMGF) in 2013 after a 28-year career in the Pharmaceutical industry. Trained as a medicinal chemist, he has worked across a broad range of therapy areas delivering drug candidates to the clinic. Immediately prior to joining the BMGF he led the AstraZeneca Neglected Diseases Research Unit in Bangalore. Within the BMGF he manages a portfolio of drug discovery grants, projects and partnerships focused primarily on tuberculosis, malaria and filarial disease drug discovery. He is the co-leader of the TB Drug Accelerator consortium which brings together multiple partners to discovery new TB drugs and treatment regimens through a unique collaborative model.

See page 100 for a listing of Symposium presentation abstracts.

Tuesday, July 16, 9:00 AM to 11:00 AM, Room 312



SYM01: Emerging Mechanisms of Chemical Teratogenesis

Chairperson(s): Peter G. Wells, University of Toronto; and Neil Vargesson, University of Aberdeen.

By age 5, 12–14% of children show problems of developmental origin (teratogenesis) including structural malformations, infant mortality and functional deficits like neurodevelopmental disorders. In utero exposure to drugs and environmental chemicals (xenobiotics) is estimated to cause about 5% of malformations detected at birth, and a higher percentage of postnatal functional disorders. Current associations are likely underestimates due to detection limitations and dependence upon interactions with genetic/epigenetic factors. This symposium presents emerging data from animal models and humans revealing mechanisms that underlie these disorders and determine individual risk. The first speaker will discuss the involvement of reactive oxygen species, DNA oxidation and repair in neurodevelopmental deficits initiated by fetal exposure to methamphetamine and alcohol (ethanol). The second speaker will discuss the role of stress response signaling pathways, and particularly the P53 pathway, in abnormal embryonic development caused by cyclophosphamide and hydroxyurea. The third speaker will focus upon epigenetic mechanisms, and particularly microRNAs (miRNAs), in humans and human trophoblast cell lines, in ethanol teratogenesis. The final speaker will discuss the vascular basis of teratogenesis exemplified by thalidomide. Insights from these studies may facilitate the development of diagnostic biomarkers for high-risk individuals, and novel strategies for therapeutic mitigation.

Abstract

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| S01-01 | 9:00 | Oxidative DNA Damage and Repair in Teratogenesis. <i>Peter G. Wells, University of Toronto, Canada.</i> |
| S01-02 | 9:30 | Stress-Response Signaling Pathways in the Organogenesis-Stage Embryo. <i>Barbara F. Hales, McGill University, Canada.</i> |
| S01-03 | 10:00 | Assessing the Function of Endocrine miRNAs in Pregnant Women That Predict FASD Infant Outcomes. <i>Rajesh C. Miranda, Texas A&M Health Science Center, The United States of America.</i> |
| S01-04 | 10:30 | Vascular Basis of Teratogenesis. <i>Neil Vargesson, University of Aberdeen, United Kingdom.</i> |

Tuesday Program Schedule

Tuesday, July 16, 9:00 AM to 11:00 AM, Room 316

SYM02: International Efforts to Ensure Global Food Safety: Incorporating Advanced Toxicological Sciences in Food Risk Assessment

Chairperson(s): Angelika Tritscher, World Health Organization; and Yongxiang Fan, China National Centre for Food Safety Risk Assessment.

Science-based food standards are essential to protecting public health and facilitating international food trade. To assist the Codex Alimentarius Commission to establish international standards for food additives, chemical contaminants, veterinary drug residues and pesticide residues, the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) regularly convene joint expert committees on Food Additives (JECFA) and joint meetings on pesticide residues (JMPR) to assess the health risk of exposures to these chemicals from food. Another important element of this work is the update and harmonization of risk assessment methods and principles as science evolves, and the development of interactive tools and databases to assist food chemical risk assessment and management globally. In this symposium, WHO and experts involved in this international work will present recent efforts and new developments initiated by JECFA and JMPR that reflect the implementing of current science. Toxicologists who have served on these international committees will give their perspectives and share examples that showcase how advances in toxicological sciences improve the quality and rigor of food chemical risk assessments. This symposium intends to enhance awareness, share knowledge and encourage participation among the international toxicology and risk assessment community.

Abstract

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| S02-01 | 9:00 | Risk Assessment Methodologies As Applied by JECFA and JMPR. Angelika Tritscher, World Health Organization, Switzerland. |
| S02-02 | 9:30 | Applications of Evolving Science in Assessing the Safety of Food Additives and Chemical Contaminants by JECFA. Yu Janet Zang, US Food and Drug Administration, The United States of America. |
| S02-03 | 10:00 | Incorporation of Duration of Exposure into The Risk Assessment of Residues of Pesticides and Veterinary Drugs in Food. Alan Boobis, Imperial College London, United Kingdom. |
| S02-04 | 10:30 | Updated WHO Guidance on the Evaluation of Genotoxicity of Compounds in Food. Virunya Bhat, NSF International, The United States of America. |

Tuesday, July 16, 9:00 AM to 11:00 AM, Room 313

SYM03: Lysosomes: Key Regulators of Inflammation and Life or Death of a Cell

Chairperson(s): Andrij Holian, The University of Montana; and Srikanth Nadadur, National Institute of Environmental Health Sciences.

Lysosomes are important organelles involved in the maintenance of cellular homeostasis and degrading extracellular pathogens and antigen presentation. It is now recognized that lysosomes also play a critical role in fundamental cellular processes, such as protein secretion, endocytic receptor recycling, energy metabolism, and cell signaling. Lysosomes are also involved in the degradation of cellular components through the process of autophagy as homeostatic pathways for normal cells. The lysosomal membrane is protected from the acidic hydrolases by lysosome specific expression of membrane proteins such as Lamp-1 and Lamp-2. However, in response to certain toxic stimuli, lysosomal membrane permeabilization (LMP) may occur causing NLRP3 inflammasome activation, alteration of normal autophagy, etc., leading to the initiation of intracellular pathways resulting in inflammation, fibrosis and cancer. Therefore, an understanding of the regulation of lysosomal membrane integrity is therefore essential in preventing the release of hydrolytic enzymes to the cytosol which would affect normal cell function and likely involved in a number of chronic diseases. This symposium will review current information on the role of this important organelle in normal cellular function, as well as, potential mechanisms leading to LMP resulting in cell death and inflammation.

Abstract

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| S03-01 | 9:00 | Mechanistic Insights in Lysosome-Mediated Cell Death. Karin Öllinger, Linköping University, Sweden. |
| S03-02 | 9:30 | Lysosomal Alterations in Health and Disease. Patricia Boya, Centro de Investigaciones Biológicas, CIB-CSIC, Spain. |
| S03-03 | 10:00 | Impact of Lipids and Particles on Lysosomal Membrane Permeability (LMP). Andrij Holian, University of Montana, The United States of America. |
| S03-04 | 10:30 | Why Lysosomal Membrane Permeabilization (LMP) Could Be Considered a Key Event (KE) in Adverse Outcome Pathway (AOP) to Particle-Induced Inflammation and Fibrosis of the Lung. Mary Gulumian, Toxicology Research Projects NIOH, South Africa. |

Tuesday, July 16, 9:00 AM to 11:00 AM, Room 315

SYM04: Systemic and Functional Impacts of Maternal Pulmonary Nanomaterial Exposures on the Offspring

Chairperson(s): Phoebe Stapleton, Rutgers University; and Karin Sørig Hougaard, National Research Centre for the Working Environment.

Toxicological exposures during gestation often results in outward teratogenicity, malformations, and fetal death. Recently xenobiotic exposures have been found to induce subtle teratogenic responses in the fetus and long lasting effects in the offspring. The effects of nanomaterial exposure are still poorly understood, and even more so for the shaping of fetal physiology. Recent studies point towards fetal multi-organ sensitivity to maternal particle exposure during pregnancy. This symposium will focus

Tuesday Program Schedule

on how xenobiotic engineered nanomaterial exposures during susceptible gestational time points can affect the health of the developing offspring. These presentations will demonstrate exposure to engineered nanomaterials during gestation may compromise the in utero environment. Fetal impairments may be associated with particle translocation and direct placental toxicity. Maternal exposures have led to severe consequences on the fetal and adult physiological function of the pulmonary, neurological, reproductive, and cardiovascular systems. Lastly, maternal exposures can lead to epigenetic alterations in the fetal genome leading to modifications of gene expression. In summary, this symposium seeks to address the significant threats that engineered nanomaterial exposure during gestation may impose to alter fetal developmental trajectory towards suboptimal physiological phenotypes and identify how future research may enlighten our insight within the field of developmental nanotoxicology.

Abstract

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| S04-01 | 9:00 | Inhalation of Nanomaterials Can Result in Systemic Effects Leading to Pre- and Postnatal Effects. <i>Flemming R. Cassee, National Institute for Public Health and the Environment (RIVM), The Netherlands.</i> |
| S04-02 | 9:30 | Deposition of Nanomaterials at the Feto-Maternal Interphase and Their Effects on the Fetus. <i>Luisa Campagnolo, University of Rome "Tor Vergata", Italy.</i> |
| S04-03 | 10:00 | Effects of Maternal Lung Exposure to Nanomaterials on the Nervous and Male Reproductive Systems. <i>Karin Sørig Hougaard, National Research Centre for the Working Environment, Denmark.</i> |
| S04-04 | 10:30 | Cardiovascular, Metabolic, and Epigenetic Susceptibility in Offspring after Maternal Engineered Nanomaterial Exposure. <i>Phoebe Stapleton, Rutgers University, The United States of America.</i> |

Tuesday, July 16, 11:30 AM to 1:20 PM, Room 312

Soft Skills Workshop: A Key Differentiator in the Workplace

➡ Separate registration required for limited participants. Box Lunch provided.

Chairpersons: Brinda Mahadevan, Abbot Laboratories, India; and Patrick Allard, University of California Los Angeles, The United States of America.

This session will focus on few essential must have soft skills such as Listening, Accountability & Taking Initiative, Creative thinking, Emotional Awareness & Empathy. An introduction to the soft skills that will speak to personal branding will be presented. This will be followed by three speakers who will illuminate through personal experience and testimonials on how the furnishing of the soft skills is important for smooth communication and key differentiator in the workplace leading to success. There will be a panel discussion and a Q&A session at the end as a conclusion to the workshop.

Tuesday, July 16, 12:00 Noon to 6:30 PM, Exhibit Hall

Exhibit Hall Open

Tuesday, July 16, 11:30 AM to 12:30 PM, Room 318A



Exhibitor-Hosted Session: Global Initiatives for the Replacement, Reduction, and Refinement of Animal Use in Toxicology

Presented by: NC3Rs

This session will highlight work of the UK's National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs). The NC3Rs brings together international consortia of scientists from industry, academia and regulatory agencies to promote best practice within existing regulations and provide evidence supporting regulatory change where appropriate.

Tuesday, July 16, 11:30 AM to 12:30 PM, Room 317B



Exhibitor-Hosted Session: miRNA and Protein Biomarkers of Drug-Induced Organ Toxicity using FirePlex®

Presented by: Abcam

This session will highlight multiomic approaches for screening biomarkers of organ toxicity using the FirePlex® Platform. We will present data from preclinical animal models assessed for both miRNA and protein urinary biomarkers of drug-induced kidney injury (DIKI), and highlight candidate miRNAs that may serve as translational biomarkers of DIKI.

Tuesday Program Schedule

Tuesday, July 16, 1:00 PM to 2:00 PM, Room 318B

E Exhibitor-Hosted Session: *In Silico* Toxicology: From Fragment-Based QSAR to Deep Learning Networks

Presented by: MultiCASE

This session will cover the methodological changes in QSAR systems, developed by MultiCASE Inc. in the last 20 years. The advantages and disadvantages of used approaches will be presented along with examples of QSAR assessments, which can be performed with modern systems and cover broad aspects of *in silico* toxicology.

Tuesday, July 16, 2:30 PM to 4:30 PM, Room 315

S SYM05: Concerns of Herbal Product-Induced Toxicity

Chairperson(s): Nan Mei, US FDA, National Center for Toxicological Research; and Amy C. Brown, John A. Burns School of Medicine, University of Hawaii at Manoa.

Global interest in botanicals or derivative products is growing because people often believe that “natural” sources may be beneficial to health. Herbal products made from botanicals are usually used to maintain or improve health. A group of chemicals can be isolated from a botanical and sold as a botanical dietary supplement. Thousands of herbal products are sold in many forms and millions of people worldwide regularly consume herbal products. Herbal products are expected to be safe, effective, and of appropriate quality. However, the complex chemical nature of herbal products makes it difficult for efficacy and safety evaluation. Herbal products often exhibit great variability in quality because of problems in authentication, adulteration and substitution, along with numerous variable factors during growth, harvest, and postharvest processing. Reported adverse effects have raised public health risk concerns regarding the concentration, composition, and individual contaminants of dietary supplements. Recently, the IARC has assessed the carcinogenicity of some herbal products, and part of them are classified as Group 2B (volume 108 of the IARC Monographs). Herbal products also have been recognized in certain cases of drug-induced liver injury. Therefore, testing of herbal products for efficacy and safety is important and needs to be conducted scientifically.

Abstract

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| S05-01 | 2:30 | Safety Evaluation of Herbal Products: Current Standards to a Proposed More Comprehensive Evaluation. Ahmet Aydin, Yeditepe University, Turkey. |
| S05-02 | 3:00 | Hepatotoxicity of Usnic Acid and Its Mechanisms. Si Chen, National Center for Toxicological Research, US Food and Drug Administration, The United States of America. |
| S05-03 | 3:30 | Neurotoxicity of Ginkgo biloba Seeds and Its Mechanisms. Daisuke Kobayashi, Health Sciences University of Hokkaido, Japan. |
| S05-04 | 4:00 | Cardiotoxicity of Aconitum Plants and Its Mechanisms. Xianju Huang, South-Central University for Nationalities, China. |

Tuesday, July 16, 2:30 PM to 4:30 PM, Room 313

S SYM06: Emerging CRISPR Applications in Environmental Health Sciences and Toxicology

Chairperson(s): Luoping Zhang, University of California, Berkeley; and Chris Vulpe, University of Florida.

Advanced genome editing technology, most notably the CRISPR-Cas system, has been rapidly applied to numerous fields, including gene-therapy for treatment of human diseases, drug screening and development, and plant engineering to improve food security. Contrary to its widespread interdisciplinary use, CRISPR application in environmental health science and toxicology has remained limited. Similar to drug screens, toxicity of environmental chemicals can be readily tested in a cell-based assay and their mechanistic pathways can be further elucidated through genome-wide functional testing. Previously, this approach was applied in a yeast screening system, and findings were validated in human cells *in vitro* and in population studies. To date, the novel CRISPR technology, including genome-wide CRISPRi (interference, loss-of-function) and CRISPRa (activation, gain-of-function) can be employed to fully examine genomic responses to a tested chemical. Recently, a targeted approach, Tox-CRISPR, is developed to focus on testing genes frequently involved in toxicity caused by environmental exposures. Applying both genome-wide and targeted approaches, we can harness the power and flexibility of the CRISPR functional screening to gain mechanistic insight in modern toxicology for an unprecedented protection of human and environmental health. This symposium aims to guild and encourage ICT community to apply CRISPR in environmental health research.

Abstract

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| S06-01 | 2:30 | Genome-Wide and Targeted CRISPR Screening to Understand Gene-Environment Interactions. Chris Vulpe, University of Florida, The United States of America. |
| S06-02 | 3:00 | CRISPR Screening to Identify Suppressors of Cellular Stress Response to Proteo-Toxicants. Quan Lu, School of Public Health, Harvard University, The United States of America. |
| S06-03 | 3:30 | CRISPR Application to Uncover Redox Biology. Navdeep Chandel, School of Medicine, Northwestern University, The United States of America. |
| S06-04 | 4:00 | A Vision for CRISPR Applications in Toxicology and Environmental Health. Luoping Zhang, School of Public Health, University of California, Berkeley, The United States of America. |

Tuesday Program Schedule

Tuesday, July 16, 2:30 PM to 4:30 PM, Room 312

SYM07: Epigenetic Biomarkers of Exposure to Environmental Carcinogens

Chairperson(s): Igor Pogribny, US FDA, National Center for Toxicological Research.

Cancer development in humans is determined by the interaction of two key etiological factors, genetics and environmental exposures. There is increasing evidence, however, that genetic factors might not be the principal cause of cancer development, but that exposure to natural and man-made chemical and physical carcinogens may have a more profound impact. Extensive and constantly growing data indicate that epigenome is a major target for environmental carcinogens and is greatly affected by their exposure. This suggests that carcinogen-induced epigenetic changes might be useful in safety assessment of carcinogens, including pharmaceutical products and food additives and contaminants. Furthermore, the transition from using epigenetic signatures of exposure as potential biomarkers only to identifying their mechanistic role in cancer development allows for better characterization and fundamental understanding of the biology of how environmental insults are involved in the carcinogenic process. This symposium will present current knowledge on the linkages among exposure, genome, epigenome and cutting-edge research on the role of the epigenome as a central component linking environmental exposures to cancer development. Additionally, it will illustrate that the evaluation of carcinogen-induced epigenomic alterations may not only enhance the carcinogen assessment process but also provide opportunity for the development of better cancer preventive strategies.

Abstract

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| S07-01 | 2:30 | Epigenetic Mechanisms and Cancer: An Interface between the Environment and the Genome. Zdenko Herceg, International Agency for Research on Cancer, France. |
| S07-02 | 3:00 | Epigenomics and Drug-Induced Carcinogenicity. Jonathan Moggs, Novartis Institutes for BioMedical Research, Switzerland. |
| S07-03 | 3:30 | The Role of Epigenetic Science in Carcinogen Detection. Igor Pogribny, National Center for Toxicological Research, The United States of America. |
| S07-04 | 4:00 | Epigenetic Mechanism of Chromium-Induced Cell Transformation and Tumorigenesis. Chengfeng Yang, Department of Toxicology and Cancer Biology, University of Kentucky, The United States of America. |

Tuesday, July 16, 2:30 PM to 4:30 PM, Room 316

SYM08: New Approach Methodologies: A Global Perspective

Chairperson(s): Charu Chandrasekera, Canadian Centre for Alternatives to Animal Methods/Validation of Alternative Methods (CaCVAM); and Nicole Kleinstreuer, The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods.

From the Americas to the Far East, countries across the globe have established national Centers for the Validation of Alternatives Methods (CVAMs) dedicated to the development, validation, and promotion of non-animal alternative methods that can reduce and replace the current—predominantly animal based—modes of toxicological risk assessment. These integrative new approach methodologies offer rapid, human-relevant predictions, enabling research and regulatory enterprises to streamline safety and risk assessment. Promising new technologies across the spectrum—from molecular and cellular *in vitro* alternatives to microphysiological systems to systems biology and computational toxicology—are poised to improve the reliability, reproducibility, sensitivity, and human relevance of assessing both toxicity and efficacy. In keeping with the theme for IUTOX 2019—to create diverse sessions highlighting excellence in science and toxicology from across the globe—here we present the latest 21st century toxicology and regulatory testing efforts from three continents: Americas (USA and Canada), European Union, and Asia (Japan). These members of the International Cooperation on Alternative Test Methods (ICATM) representing academic, government, and regulatory sectors will discuss their respective strategies, projects, and global harmonization efforts.

Abstract

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| S08-01 | 2:30 | Beyond the Silver Anniversary: The Next 25 Years of Alternatives in the European Union. Maurice Whelan, European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), European Commission Joint Research Centre, Italy. |
| S08-02 | 3:00 | Adopting New Approach Methodologies: The US Strategic Roadmap and Implementation Activities. Nicole Kleinstreuer, The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, The United States of America. |
| S08-03 | 3:30 | 21st Century Toxicology and Regulatory Testing: An Update from East Asia. Hajime Kojima, Japanese Center for the Validation of Alternative Methods (JaCVAM), National Institute of Health Sciences, Japan. |
| S08-04 | 4:00 | O Canada: A Strategic Vision toward Replacement in the True North. Charu Chandrasekera, Canadian Centre for Alternatives to Animal Methods/Validation of Alternative Methods (CaCVAM), Canada. |

Tuesday, July 16, 4:30 PM to 6:30 PM, Exhibit Hall

Poster Session 1 and Refreshments

See next page for the Poster Session 1 Index.

- P101-0073** ***In Vitro* Buccal Membrane Absorption (IVBMA) Model for Tobacco Constituent Testing: Membrane Integrity Assays.** Margaret Kraeling¹, Rebecca Justiniano¹, Cory Vaught¹, Dana Lauterstein², Juan Crespo-Barreto², Robert Sprando¹, Roxana Weil², Raymond Yeager², Jeffrey Yourick¹. ¹US FDA/CFSA, Center for Food Safety and Applied Nutrition, Office of Applied Research and Safety Assessment, Laurel, MD, The United States of America. ²US FDA, Center for Tobacco Products, Office of Science, White Oak, MD, The United States of America
- P102-0162** **The Interchangeability of *In Vitro* Key Event–Based Skin Sensitization Assays—Impact on Defined Approaches.** Donna Macmillan, Martyn Chilton. *Lhasa Limited, Granary Wharf House, Leeds, United Kingdom*
- P103-0219** **Transcriptional Profiling of Cytochrome P450 Genes in Adult Liver and in Whole Embryo Fry of Zebrafish in Some Developmental Stages.** Hiroki Teraoka¹, Natsumi Yamashita¹, Yusuke Kawai², Shuangyi Zhang¹, Takio Kitazawa¹, Akira Kubota². ¹Rakuno Gakuen University, School of Veterinary Medicine, Ebetsu, Japan. ²Obihiro University of Agriculture and Veterinary Medicine, Department of Veterinary Medicine, Obihiro, Japan
- P104-0253** **Evaluation of Skin Barrier Protection Effect of *Lactobacillus rhamnosus* GG Using. ³D Reconstructed Skin Model.** Ye-On Jung¹, Kyung-Min Lim¹, Haengdueng Jeong², Yejin Cho², Kitaek Nam². ¹Ewha Womans University, College of Pharmacy, Seoul, The Republic of Korea. ²Yonsei University, College of Medicine, Seodaemungu, The Republic of Korea
- P105-0274** **Using *C. elegans* for Predictive Oral Toxicity Assessments.** Piper Hunt, Robert Sprando. *US FDA/CFSA, Center for Food Safety and Applied Nutrition, Office of Applied Research and Safety Assessment, Division of Toxicology, Laurel, MD, The United States of America*
- P106-0353** **Cytotoxicity Differences of Troglitazone on HepG2 and CD133 Liver Cancer Stem Cells.** Quanjun Wang. *Beijing Institute of Pharmacology and Toxicology, Beijing Institute of Pharmacology and Toxicology, Beijing, China*
- P107-0367** **Novel Deep Learning Methodology to Enable Drug-Induced Abnormality Detection Using Cultured Neurons on Microelectrode Arrays.** Norimasa Miyamoto¹, Atsuko Ojima², Tetsuo Kitamura³, Tomoharu Osada⁴, Ikuro Suzuki⁵, Takashi Yoshinaga¹. ¹Eisai Co., Ltd., Global Cardiovascular Assessment, Tsukuba, Japan. ²TechnoPro R&D, Company, TechnoPro, Inc., Tsukuba Branch, Tsuchiura, Japan. ³LSI Medience Corporation, Safety Assessment Department, Nonclinical Research Center, Kamisu, Japan. ⁴LSI Medience Corporation, Advanced Medical Business Development Department, Chiyoda, Japan. ⁵Tohoku Institute of Technology, Department of Electronics, Sendai, Japan
- P108-0369** **Tris(1,3-Dichloro-2-Propyl) Phosphate Exposure during Early Development Alters the Normal Trajectory of Zebrafish Embryogenesis.** Subham Dasgupta, Vanessa Cheng, Sara Frie Vliet, Constance Mitchell, David Volz. *University of California Riverside, Environmental Sciences, Riverside, CA, The United States of America*
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- P184-0557** **Impact of Tobacco Use and Environmental Tobacco Smoke Exposure in Perinatal Outcomes in a Portuguese Population.** Ana Inês Silva¹, Alexandra Camelo¹, Joana Madureira^{1,2}, Ana Teresa Reis^{1,2}, Fernando Barbosa Jr³, João Paulo Teixeira^{1,2}, Carla Costa^{1,2}. ¹National Institute of Health, Environmental Health Department, Porto, Portugal. ²EPIUnit, Instituto de Saúde Pública, Universidade do Porto, Porto, Portugal. ³School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, São Paulo, Brazil
- P185-0610** **FDALabel Database: Enabling Insights from US FDA Drug Labeling to Advancement of Adverse Drug Reaction Study.** Hong Fang, LeiHong Wu, Taylor Ingle, Zhichao Liu, Shraddha Thakkar, Joshua Xu, Weida Tong. *US FDA/NCTR, Jefferson, AR, The United States of America*
- P186-0838** **Scoping Environmental Chemical Exposures Associated with Parkinson's Disease: An Evidence Map.** Windy Boyd¹, Ana Antonic-Baker², Nisha Sipes¹, Vickie Walker¹, Courtney Skuce³, Kristina Thayer⁴, Andrew Rooney¹. ¹NIEHS/NTP, Division of the National Toxicology Program, Research Triangle Park, NC, The United States of America. ²Monash University, Department of Neuroscience, Melbourne, Australia. ³ICF International Inc., Durham, NC, The United States of America. ⁴US EPA, Integrated Risk Information System Division, Research Triangle Park, NC, The United States of America
- P187-0901** **PI3K/AKT Pathway-Mediated Cyclin D1 Expression Participates in Low Dose Arsenic-Induced Transformation in Human Hepatocyte L-02.** Qun Lou, Mei-chen Zhang, Fanshuo Yin, Hua-zhu Yan, Bingyang Li, Fuxun Chen, Yanhui Gao, Yan-mei Yang. *Center for Endemic Disease Control, Chinese Center for Disease Control and Prevention, Harbin, China*
- P188-0059** **Effect of Octyl-phenol and Bisphenol A on Cardiomyocytes Differentiation of Mouse Embryonic Stem Cells.** Jae-Hwan Lee, Dinh Nam Tran, Bo-Hui Jeon, Eui-Man Jung, Eui-Bae Jeung. *Chungbuk National University, College of Veterinary Medicine, Cheongju, The Republic of Korea*
- P189-0090** **Status of Urinary Metals/Metalloids of School-Age Children in Xuyi, Jiangsu Province, China.** Ying Du¹, Chao Zhao¹, Yang Xie², Ying Zhang¹, Hu Zhang¹, Ran Liu¹, Yuepu Pu¹, Lihong Yin¹. ¹Southeast University, Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Nanjing, China. ²Xuyi Center for Disease Prevention and Control, Xuyi, China
- P190-0127** **Research on the Effects of Heart Injury in Rats Induced by Short Wave Radiation.** Jing Zhang, Ping Xu, Hui Wang, Chao Yu, Wei Yao, Ji Dong, Yu Wang, Li Zhao, Ruiyun Peng. *Beijing Institute of Radiation Medicine, Beijing, China*
- P191-0293** **Knowledge Awareness and Practice Regarding Arsenicosis among the Community Health Care Providers of Bangladesh.** Mobin UL Islam. *Shaheed Ziaur Rahman Medical College, Bogra, Bangladesh*
- P192-0331** **Effect of Low-Dose of Ethanol on Zebrafish Embryogenesis.** Neelakshi Kar, Jayesh Bellare. *Indian Institute of Technology, Bombay, Department of Chemical Engineering, Mumbai, India*
- P193-0451** **Home-Based and Informal Jewelry Production Increases the Working Families' Exposure to Cadmium.** Fernanda Salles¹, Ana Paula Ferreira¹, Bruna Freire², Allan Oliveira¹, Bruno Batista², Kelly Olympio¹. ¹University of São Paulo School of Public Health, Environmental Health, São Paulo, Brazil. ²Federal University of ABC, Center for Natural and Human Sciences, Santo Andre, Brazil
- P194-0462** **Brazilian Preschool Children Attending Day Care Centers Located Near Chemicals Hot Spots and in High Vehicle Traffic Density Sites Present High Blood Lead Levels.** Allan Oliveira¹, Eric Costa², Kelly Olympio¹. ¹University of São Paulo School of Public Health, Environmental Health, São Paulo, Brazil. ²Federal University of ABC, UFABC, Risk Management Laboratory (LabGRis), São Paulo, Brazil
- P195-0511** **Integrated Risk Assessment of Seven Carcinogenic Metals through Oral, Dermal, and Inhalation.** Changwoo Yu, Hoonjeong Kwon. *Seoul National University, Seoul, The Republic of Korea*
- P196-0638** **Tableware Plastic Utensils Supposed to Be Used for Children and Sold in the Brazilian Commerce Present High Lead and Cadmium Concentrations.** Isabelle Leroux, Elizeu Pereira, Kelly Olympio. *University of São Paulo School of Public Health, Department of Environmental Health, São Paulo, Brazil*

- P197-0649** **Assessment of Renal Toxicity and Biochemical Effects of Detergent-Processed Cassava on Wistar Rats (*Rattus norvegicus*).** Gloria Oghobase, Titilayo Aladesanmi, Rufus Akomolafe. *Obafemi Awolowo University, Ile-Ife, Nigeria*
- P198-0693** ***In Vivo* Toxicity Assessment of Metal Contaminated Wind Blown Particulate Matter from Abandoned Uranium Mines Surrounding Arizona and New Mexico American Indian Reservations.** Jessica Begay¹, Yoselin Ordóñez¹, Selita Lucas¹, Bethany Sanchez¹, Abigail Wheeler¹, Floyd Baldwin, Jr.², Katherine Zychowski¹, Guy Herbert¹, Chris Shuey³, Jack Harkema⁴, James Wagner⁴, Masako Morishita⁴, Barry Bleske¹, Matthew Campen¹. ¹*University of New Mexico, College of Pharmacy, Albuquerque, NM, The United States of America.* ²*Dine College, Tsale, AZ, The United States of America.* ³*University of New Mexico, Southwest Research and Information Center, Albuquerque, NM, The United States of America.* ⁴*Michigan State University, East Lansing, MI, The United States of America*
- P199-0708** **Organ-Specific Changes in Gene Expression in Microminipigs following a Single Exposure to a Mixture of Perfluoroalkyl Acids (PFAAs).** Akiko Sakuma¹, Haruyo Wasada-Ochi², Miyako Yoshioka³, Noriko Yamanaka³, Mitsutaka Ikezawa³, Keerthi S. Guruge³. ¹*Miyagi Prefectural Sendai Livestock Hygiene Service Center, Sendai, Japan.* ²*Kumamoto Prefectural Central Livestock Hygiene Service Center, Kumamoto, Japan.* ³*National Institute of Animal Health, NARO, Tsukuba, Japan*
- P200-0774** **Toxicological Effects of Donepezilo, Eutebrol, and Medazepam on *Caenorhabditis elegans*.** Liset Mallarino-Miranda, Mario Alvear-Alayon, Barbara Arroyo-Salgado. *Universidad de Cartagena, Cartagena, Colombia*
- P201-0853** **Are Al, Cd, and Pb Contents in Tofu a Risk?** Carmen Rubio-Arméndariz, Soraya Paz, Arturo Hardisson, Ángel Gutiérrez, Dailos González¹. *Universidad de La Laguna, Toxicology Department, La Laguna, Spain*
- P202-0884** **Mitochondrial DNA as Quantitative Tools for Evaluating RT-PCR for Cell Therapy.** Young-Woo Cho, Ji-Ho Ryu, Soo-Han Lee. *Osong Medical Innovation Foundation, New Drug Development Center, Cheongju-si, The Republic of Korea*
- P203-0894** **Are Canned Food Containers Safe? Exposure to Nine Bisphenol Analogues in a Duplicate Diet Study.** Neus González¹, Sara Cunha², José Fernandes², Montse Marqués¹, José Luis Domingo¹, Martí Nadal¹. ¹*Universitat Rovira i Virgili - Institut d'Investigació Sanitària Pere Virgili, Laboratory of Toxicology and Environmental Health, School of Medicine, Reus, Spain.* ²*LAQV-REQUIMTE, Universidade do Porto, Department of Bromatology, Porto, Portugal*
- P204-0925** **Reproducibility of Collision Cross Section Measurements with Ion Mobility Mass Spectrometry-Mass Spectrometry.** Noor Aly¹, Luo Yu-Syuan¹, Alina Roman-Hubers¹, Ivan Rusyn¹, Weihsueh Chiu¹, Yina Liu¹, Xueyun Zheng¹, Erin Baker². ¹*Texas A&M University, College Station, TX, The United States of America.* ²*NC State University, Chemistry, Raleigh, NC, The United States of America*
- P205-0941** **Migration Test for Seven Phthalates from Paper Cups.** Jun Sang Yu, Young Seok Ji, Jeong Hoon Park, Eun Gyu Lee, Hye Hyun Yoo. *Hanyang University, College of Pharmacy, Ansan, The Republic of Korea*
- P206-0035** **7,10-epoxy Octadeca 7,9-dienoic Acid as a Synergistic Antibacterial Agent against Multidrug-Resistant *Staphylococcus aureus*.** Hak-Ryul Kim, Ji-Sun Moon, Yeon-Jung Lee. *Kyungpook National University, School of Food Science and Biotechnology, Daegu, The Republic of Korea*
- P207-0043** **4-methylimidazole Induces Skin Aging through Sirtuin 6 Activation.** Yu-ting Hsiao¹, Shing-Hwa Liu², Chiung-Jung Wen³, Jui-Zhi Loh², Chen-Ning Chang¹, Wan-Ru Liao¹, Kuo-Ching Wen⁴, Hsiu-Mei Chiang Chiang⁴, Chen-Yuan Chiu¹. ¹*Institute of Food Safety and Health, National Taiwan University, Taipei, Taiwan.* ²*Institute of Toxicology, National Taiwan University, Taipei, Taiwan.* ³*National Taiwan University Hospital Department of Geriatrics and Gerontology, Taipei, Taiwan.* ⁴*China Medical University Department of Cosmeceutics, Taipei, Taiwan*
- P208-0044** **4-methylimidazole, a Maillard Reaction Intermediate in Caramel Colors, Promotes Breast Cancer Stemness Characteristics via Activation of RAGE/SIRT6 Axis.** Chen-Ning Chang¹, Shing-Hwa Liu², Wan-Ru Liao¹, Yu-ting Hsiao¹, Chiung-Jung Wen³, Jui-Zhi Loh², Chen-Yuan Chiu¹. ¹*Institute of Food Safety and Health, National Taiwan University, Taipei, Taiwan.* ²*Institute of Toxicology, National Taiwan University, Taipei, Taiwan.* ³*National Taiwan University Hospital Department of Geriatrics and Gerontology, Taipei, Taiwan*
- P209-0045** **The Esculent Dose of 4-methylimidazole, a Caramel Coloring Ingredient, Promotes Cancer Stemness Characteristics via SIRT6 Activation in Osteosarcoma Cells and Chondrosarcoma Cells.** Wan-Ru Liao¹, Shing-Hwa Liu², Chiung-Jung Wen³, Yu-ting Hsiao¹, Chen-Ning Chang¹, Jui-Zhi Loh², Chen-Yuan Chiu¹. ¹*Institute of Food Safety and Health, National Taiwan University, Taipei, Taiwan.* ²*Institute of Toxicology, National Taiwan University, Taipei, Taiwan.* ³*National Taiwan University Hospital Department of Geriatrics and Gerontology, Taipei, Taiwan*

- P210-0135** **Toxin Enterosorbents for Inclusion in Food and Water during Emergencies and Natural Disasters.** Meichen Wang, Sara Hearon, Timothy Phillips. *Texas A&M University, Veterinary Integrative Biosciences, College Station, TX, The United States of America*
- P211-0159** **Pancreatic Toxicity Induced by Excess Selenoprotein P.** Yoshiro Saito^{1,2}. ¹Tohoku University, Graduate School of Pharmaceutical Sciences, Sendai, Japan. ²Doshisha University, Faculty of Life and Medical Sciences, Kyotanabe, Japan
- P212-0195** **Shikonin Augments Hyperthermia-Induced Apoptosis through Enhancement of Oxidative Stress.** Zheng-Guo Cui, Yu-Jie Jin, S. A. Zakki, Lu Sun, Meng-Ling Li, Qian-Wen Feng, Hidekuni Inadera. *University of Toyama, Department of Public Health, Graduate School of Medicine and Pharmaceutical Sciences, Toyama, Japan*
- P213-0236** **Estimation on Safety of Newly Developed Sugar Substitute, Maltobionic Acid, through Gastrointestinal Dynamics by Prolonged Feeding in Mice.** Kenichi Tanabe¹, Asuka Okuda¹, Ken Fukami², Sadako Nakamura³, Tsuneyuki Oku³. ¹Nagoya Women's University, Nagoya, Japan. ²San-ei Sucochemical Co., Ltd., Chita-City, Japan. ³Jumonju University, Institute of International Nutrition and Health, Niza, Japan
- P214-0243** **Elucidation of the Molecular Mechanisms Underlying Proapoptotic Effect of *Trans*-fatty Acid in Response to DNA Damage.** Miki Takahashi, Yusuke Hirata, Saki Suzuki, Ryosuke Matsui, Takuya Noguchi, Atsushi Matsuzawa. *Tohoku University Graduate School of Pharmaceutical Sciences Laboratory of Health Chemistry, Sendai, Japan*
- P215-0272** **Research on Toxicological Characterization of Marine Biotoxins in New Zealand.** Jeane Nicolas¹, Sarah Finch². ¹Ministry for Primary Industries, Science & Risk Assessment Directorate, Wellington, New Zealand. ²AgResearch Limited, Ruakura Research Centre, Hamilton, New Zealand
- P216-0288** **Safety and Efficacy Assessment of Novel Blood Glucose Regulation Supplement: SugarOut.** Zoe Tse¹, Liao Po-Lin², George Hsiao³, Yu-Wen Cheng⁴. ¹Taipei Medical University, School of Pharmacy, College of Pharmacy, Taipei, Taiwan. ²National Yang-Ming University Institute of Food Safety and Health Risk Assessment, School of Pharmaceutical Sciences, Taipei, Taiwan. ³Taipei Medical University, Department of Pharmacology, School of Medicine, College of Medicine, Taipei, Taiwan. ⁴Taipei Medical University, School of Pharmacy, College of Pharmacy, Taipei, Taiwan
- P217-0296** **Potential Health Risk from Heavy Metals via Consumption of Leafy Vegetables in the Vicinity of Warri Refining and Petrochemical Company, Delta State, Nigeria.** Kingsley Patrick-Iwuanyanwu¹, Uchenna Nwokeji². ¹University of Port Harcourt, Biochemistry (Toxicology Unit), Port Harcourt, Nigeria. ²Madonna University, Biochemistry, Elele, Nigeria
- P218-0300** **Heavy Metals Contamination and Potential Human Health Risk via Consumption of Vegetables from Selected Communities in ONELGA, Rivers State, Nigeria.** Kingsley Patrick-Iwuanyanwu, Ahiakwo Ogbo. *University of Port Harcourt, Biochemistry, Port Harcourt, Nigeria*
- P219-0308** **Nutrition and Toxicity Relationship: Which Is Responsible?** Nazih Ramadan. *Cairo University, Cairo, Egypt*
- P220-0319** **Development of a Sensitive ELISA and Rapid Immunochromatographic Strip for Detecting Chloramphenicol in Milk.** Shih-Wei Wu, Feng-Yih Yu. *Chung Shan Medical University, Taichung, Taiwan*
- P221-0320** **Disruption of Brain and Vascular Development by Ochratoxin A in Zebrafish Embryos.** Ting-Shuan Wu, Yu-Ting Lin, Biing-Hui Liu. *National Taiwan University, Graduate Institute of Toxicology, Taipei, Taiwan*
- P222-0332** **Identification of Compound Causing Yellow Bone Discoloration Following *alpha*-Glycosyl Isoquercitrin Exposure.** Jeffrey Davis¹, Mihoko Koyanagi², Robert Maronpot³, Leslie Recio¹, Shim-mo Hayashi². ¹Integrated Laboratory Systems, Inc., Research Triangle Park, NC, The United States of America. ²San-Ei Gen F.F.I., Inc., Toyonaka, Japan. ³Maronpot Consulting, LLC, Raleigh, NC, The United States of America
- P223-0350** **Effects of Excessive Ingestion of 1, 5-Anhydroglucitol on Growth and Metabolism in Rats, and the Suppression of Blood Glucose Elevation through Inhibitory Effect on Disaccharidases.** Tsuneyuki Oku¹, Tanabe Kenichi², Akemi Miyahara³, Yuko Yamasaki¹, Kazuhiro Yoshinaga⁴, Sadako Nakamura¹. ¹Jumonju University, Niiza, Japan. ²Nagoya Women's University, Department of Food Science and Nutrition, Nagoya, Japan. ³University of Nagasaki, Department of Nursing and Nutrition, Nagayo, Japan. ⁴SUNUS Co. Ltd., Institute of Food, Kagoshima, Japan
- P224-0403** **Protective Effect of Honokiol against Oxidative Stress-Induced DNA Damage and Apoptosis of C2C12 Myoblasts.** Yung Hyun Choi. *Dong-eui University College of Korean Medicine, Department of Biochemistry, Busan, The Republic of Korea*

- P225-0418** **Effects of Newly Developed Dietary Fiber Materials on Gastrointestinal Morphology and Function by Consecutive Feeding in Rats.** Sadako Nakamura¹, Yuri Kintaka¹, Kenichi Tanabe², Toshiyuki Nakayama³, Sanae Osada⁴, Tsuneyuki Oku¹. ¹Jumonji University, Institute of International Nutrition and Health, Niza, Japan. ²Nagoya Women's University, Department of Food Science and Nutrition, Nagoya, Japan. ³University of Occupational and Environmental Health, Department of Pathology, Kitakyushu-City, Japan. ⁴Junior College, Kagawa Education Institute of Nutrition, Department of Food and Nutrition, Toshima-Ku, Japan
- P226-0440** **Influence of Heating Temperature on the Toxicity of Olive and Coconut Oil on *Caenorhabditis elegans*.** Marcela Lopez, Liset Mallarino-Miranda, Lesly Tejeda-Benitez. *Universidad de Cartagena, Facultad de Ingeniería, Cartagena, Colombia*
- P227-0455** **Ethanol Suppresses *In Vitro* Osteoblastogenesis, Chondrogenesis, and Chondrocyte Function via Different Sources of Reactive Oxidative Species.** James Watt, Kyle Meredith, Martin Ronis. *Louisiana State University Health Sciences Center, New Orleans, LA, The United States of America*
- P228-0457** **Molecular Characterization and Evaluation of Aflatoxin Production Potentials of Fungi Isolated from Animal Feedlots Kept for Food Production.** Stephen Abiola Akinola, Collins Ateba, Mulunda Mwanza. *North-West University South Africa, Mmabatho, South Africa*
- P229-0520** **Acrolein Levels following Dietary Exposure and in Stroke Patients.** Tse-Wen Wang¹, Tsung-Yun Liu¹, Hsiang-Tsui Wang², Han-Hsing Tsou³. ¹National Yang-Ming University Institute of Food Safety and Health Risk Assessment, School of Pharmaceutical Sciences, Taipei, Taiwan. ²National Yang-Ming University, Department of Pharmacology, Taipei, Taiwan. ³National Yang-Ming University Institute of Environmental and Occupational Health Sciences, Institute of Environmental and Occupational Health Sciences, Taipei, Taiwan
- P230-0575** **Green Synthesis, Characterization, and Potential Biological Application of Silver Nanoparticles by Using Leaves Extract of *Acmella oleracea*.** Sweta Bhardwaj. *Rayat Bahra University, Department of Chemistry University School of Sciences, Mohali, India*
- P231-0615** **Food Grade Titanium Dioxide Diet Reduces Hematocrit and Increases Lymphocyte Count and Phagocytic Cells in Mice Serum.** Carolina Rodríguez Ibarra, Yolanda Chirino, Norma Laura Delgado Buenrostro. *Laboratorio de Carcinogenesis y Toxicología, Tlalnepantla de Baz, Mexico*
- P232-0647** **Modulation of Gut Microbiome and Metabolome by Carcinogenic Mycotoxin, Aflatoxin B₁, in F344 Rats .** Jia-Sheng Wang, Jun Zhou, Kathy Xue, Lili Tang. *University of Georgia, Environmental Health Science, Athens, GA, The United States of America*
- P233-0736** **Inhibition of Neurotoxicity of the *Spirulina maxima* Extract from Ultrasonic Pretreatment.** Hyeon Lee¹, Woon Choi², Do Kang³. ¹Seowon University, Cheongju, The Republic of Korea. ²Kangwon National University, Chuncheon, The Republic of Korea. ³Jeju Research Center Marine Science Laboratory, Jeju, The Republic of Korea
- P234-0785** **Histopathological and Toxicological Effects of Long-Term Consumption of Energy Drinks on Some Organs of Wistar Rats.** Toyin Sherifat Ojedinan¹, Ishiaq Omotosho². ¹University College Hospital, Department of Histopathology, Ibadan, Nigeria. ²University of Ibadan, Department of Chemical Pathology, Ibadan, Nigeria
- P235-0799** **Ginsenoside Rg3, an Active Ingredient of Ginseng, Can Induce Hemolysis-Dependent Phosphatidylserine Exposure and Thrombosis.** Yiying Bian, Jin-Ho Chung, Byung Hoon Lee. *Seoul National University, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul, The Republic of Korea*
- P236-0830** **Daily Intake of Iodine of Adult Residents in the Coastal Fishery Area in Iwate Prefecture, Japan.** Keiko Chiba¹, Hideyuki Sawatari², Haruo Nakatsuka³, Takao Watanabe⁴. ¹Iwate Prefectural University, Research and Regional Cooperation Head Office, Takizawa, Japan. ²Miyagi University of Education, Faculty of Education, Sendai, Japan. ³Kanazawa Gakuin College, Kanazawa, Japan. ⁴Tohoku Bunkyo College, Faculty of Human Science, Yamagata, Japan
- P237-0848** **Sugar Substitutes Increase the Lipid Storage in *Caenorhabditis elegans*.** Barbara Arroyo-Salgado, Maria C. García-Espiñeira, Lesly Tejeda-Benitez. *University of Cartagena, Biotoxam Research Group, Cartagena, Colombia*
- P238-0896** **Docosahexaenoic Acid (DHA)–Derived Oxylipins Are Decreased in the Heart by Dietary Exposure to 2-monochloro-1,3-propanediol.** Lucien Cayer¹, Jennifer Roberts², Don Caldwell², Jayadev Raju², Harold Aukema¹. ¹University of Manitoba, Food and Human Nutritional Sciences, Winnipeg, Canada. ²Health Canada, Bureau of Chemical Safety, Ottawa, Canada

- P239-0897** **Effects of Ochratoxin A and Aristolochic Acid on the Kidney in Diabetic Animal Model.** Hyun Jung Lee¹, Felicia Wu², Dojin Ryu¹. ¹University of Idaho, School of Food Science, Moscow, ID, The United States of America. ²Michigan State University, Department of Agricultural, Food, and Resource Economics, East Lansing, MI, The United States of America
- P240-0899** **The Role of Oxidative Stress in the Ochratoxin A–Mediated Toxicity in Liver and Kidney Cell Lines.** Hyun Jung Lee¹, Enrique García-Pérez², Dojin Ryu¹. ¹University of Idaho, School of Food Science, Moscow, ID, The United States of America. ²Washington State University, School of Food Science, Pullman, WA, The United States of America
- P241-0958** **Effect of Silicon-Rich Water Intake on the Thymocyte Apoptosis and Peritoneal Inflammation of Rats with Chronic Low Levels of Aluminum Ingestion.** Zoran Radovanovic, Boris Djindjic. Faculty of Medicine, University of Niš, Nis, Serbia
- P242-0014** **Presenting in Metals Platform Session (PL03)**
- P243-0050** **HER2 and Src Co-regulate and Mediate Proliferation, Migration, and Transformation by Multiple Downstream Signaling Pathways in Arsenite-Treated Human Uroepithelial Cells.** Shuhua Xi, Qing Zhou. China Medical University, School of Public Health, Shenyang, China
- P244-0102** **Persulfide and Polysulfide Species in Plants to Detoxify Methylmercury.** Yumi Abiko, Yusuke Katayama, Yoshito Kumagai. University of Tsukuba Tsukuba, Japan
- P245-0117** **LncRNA Expression Profiling and Its Relationship with DNA Damage in Cr(VI)-Treated 16HBE Cells.** Guiping Hu, Guang Jia. Peking University, Department of Occupational and Environmental Health Sciences, Beijing, China
- P246-0118** **Search for Splicing Factors That Maintain Normal Splicing of Human Arsenic (+3 Oxidation State) Methyltransferase (AS3MT) mRNA.** Daigo Sumi, Seiichiro Himeno. Tokushima Bunri University, Faculty of Pharmaceutical Sciences, Tokushima, Japan
- P247-0136** **Induction Mechanism of TNF- α Expression by Methylmercury in Microglial BV-2 Cells.** Takayuki Hoshi, Takashi Toyama, Akira Naganuma, Gi-Wook Hwang. Tohoku University, Graduate School of Pharmaceutical Sciences, Sendai, Japan
- P248-0138** **Elucidation of the Mechanisms Involved in Methylmercury Toxicity Enhancement Shown by the Novel Transcription Factor tmRT1.** Takashi Toyama, Katsuhiko Osaki, Gi-Wook Hwang, Akira Naganuma. Tohoku University, Graduate School of Pharmaceutical Sciences, Sendai, Japan
- P249-0144** **Limit Values for Metals: Do We Need Interspecies Extrapolation Factors?** Dirk Pallapies, Peter Welge, Thomas Bruening. Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA) Institute of the Ruhr-Universitaet Bochum, Bochum, Germany
- P250-0154** **Altered Differentiation of Mouse Stem Cells after Chronic Low-Level Arsenic Exposure.** Benjamin McMichael, Sarah Coleman, Lisa Bain. Clemson University, Department of Biological Sciences, Clemson, SC, The United States of America
- P251-0157** **BIRC Family Gene Expression Changes by Toxic Metal(loid)s in Neuroblastoma, Kidney, and Hepatic Cells.** Jin-Yong Lee, Maki Tokumoto, Masahiko Satoh. Aichi Gakuin University, School of Pharmacy, Nagoya, Japan
- P252-0160** **New Findings on the Genes Involved in Cadmium Toxicity in Human Proximal Tubular Cells.** Masahiko Satoh, Jin-Yong Lee, Maki Tokumoto. Aichi Gakuin University, School of Pharmacy, Nagoya, Japan
- P253-0175** **Potential Adverse Effect of Low Cadmium Intake to Lactation Load against Bone Metabolism and Renal Function of Mother Rat.** Hisayoshi Ohta¹, Kenichi Ohba². ¹Kitasato University, Graduate School of Medical Sciences, Department of Environmental, Occupational Health and Toxicology, Sagamihara, Japan. ²Kitasato University School of Allied Health Sciences, Department of Environmental, Occupational Health and Toxicology, Sagamihara, Japan
- P254-0189** **Cadmium Induces Autophagy and Blocks Autophagosome Fusion with Lysosomes in AML12 Cells.** Hui Zou^{1,2}, Tao Wang¹, Jiaqiao Zhu^{1,2}, Yan Yuan^{1,2}, Jianhong Gu^{1,2}, Jianchun Bian^{1,2}, Zongping Liu^{1,2}. ¹Yangzhou University College of Veterinary Medicine, Yangzhou, China. ²Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou, China
- P255-0206** **Drinking Water Treatment Using Cucumber Peel Immobilized on Sodium Alginate: Multi-ion Biosorption of Arsenic and Heavy Metals.** Risha Singh, Rhonda Rosengren, Candace Martin. University of Otago, Dunedin, New Zealand

- P256-0283** **Arsenite Exerts Cytotoxic Effect through Inhibition of Synthesis of Ribose-5-Phosphate in Human Acute Monocytic Leukemia THP-1 Cells.** Tsutomu Takahashi, Tsuyoshi Nakano, Yasuyuki Fujiwara. *Tokyo University of Pharmacy and Life Sciences, School of Pharmacy, Hachioji, Japan*
- P257-0284** **Analysis of Various Renal Injury Biomarkers Using *In Vitro* Evaluation System.** Hitomi Fujishiro, Seiichiro Himeno. *Tokushima Bunri University, Faculty of Pharmaceutical Sciences, Tokushima, Japan*
- P258-0304** **Sodium Arsenite Inhibits Tissue Plasminogen Activator Release from Cultured Human Vascular Endothelial EA.hy926 Cells.** Yasuyuki Fujiwara¹, Tsuyoshi Nakano¹, Tsutomu Takahashi¹, Yayoi Tsuneoka¹, Yo Shinoda¹, Chika Yamamoto², Toshiyuki Kaji³. ¹*Tokyo University of Pharmacy and Life Sciences, School of Pharmacy, Hachioji, Japan.* ²*Toho University, Faculty of Pharmaceutical Sciences, Funabashi, Japan.* ³*Tokyo University of Science, Faculty of Pharmaceutical Sciences, Noda, Japan*
- P259-0327** **Protective Effect of Aqueous Leaf Extract of *Costus afer* (Ker Gawl) on Testicular Injuries Induced by Low Dose Heavy Metal Mixture (Lead, Cadmium, and Mercury) in Wistar Albino Rats.** Brilliance Anyanwu, Anthonet Ezejiofor, Ify Nwaogazie, Orish Ebere Orisakwe. *University of Port Harcourt, World Bank Africa Centre of Excellence in Oilfield Chemicals Research, Port Harcourt, Nigeria*
- P260-0374** **Biological and Toxicological Significance of Selenocyanate as a Selenium Metabolite.** Yasumitsu Ogra¹, Yasumi Anan². ¹*Chiba University, Graduate School of Pharmaceutical Sciences, Chiba, Japan.* ²*Showa Pharmaceutical University, Machida, Japan*
- P261-0391** **Mercury Reduces Adipocyte Size and Impairs Lipid and Glucose Metabolism in White Adipose Tissue of Rats.** Marta Miguel¹, Danize Rizzetti², Patricia Corrales³, Janaina Piagette², Jose Uranga⁴, Gema Medina-Gomez⁵, Franck Peçanha⁶, Dalton Vassallo⁷, Giulia Wiggers². ¹*Spanish National Research Council, INSTITUTE OF FOOD SCIENCE RESEARCH, Madrid, Spain.* ²*Universidade Federal do Pampa, Cardiovascular Physiology Laboratory, Uruguaiana, Brazil.* ³*Universidad Rey Juan Carlos, Department of Basic Health Sciences, Alcorcón, Spain.* ⁴*Universidad Rey Juan Carlos, Histology Laboratory, Alcorcon, Spain.* ⁵*Universidad Rey Juan Carlos, Department of Basic Health Sciences, Alcorcon, Spain* ⁶*Universidade Federal do Pampa, Cardiovascular Physiology Laboratory, Uruguaiana, Brazil* ⁷*Cardiac Electromechanical and Vascular Reactivity Laboratory, Universidade Federal do Espírito Santo, Vitória, Brazil*
- P262-0406** **Inhibition of Uptake of Iron by Chronic Exposure to Cadmium.** Maki Tokumoto¹, Jin-Yong Lee¹, Yasuyuki Fujiwara², Masahiko Satoh¹. ¹*Aichi Gakuin University, School of Pharmacy, Nagoya, Japan.* ²*Tokyo University of Pharmacy and Life Sciences, School of Pharmacy, Hachioji, Japan*
- P263-0442** **Arsenic Exposure, Gut Microbiome and Human Health.** Samendra Sherchan^{1,2}. ¹*Tulane University, GEHS, New Orleans, LA, The United States of America.* ²*Tribhuvan University, Environmental Science, Kathmandu, Nepal*
- P264-0482** **Distribution, Metabolism, and Excretion of Arsenolipids in Mice after Oral Administration.** Yayoi Kobayashi¹, Noriyuki Suzuki², Yasumitsu Ogra², Seishiro Hirano¹. ¹*National Institute for Environmental Studies, Centre for Health and Environmental Risk Research, Tsukuba, Japan.* ²*Chiba University, Graduate School of Pharmaceutical Sciences, Chiba, Japan*
- P265-0489** **Cadmium Induces Not Only Apoptotic and Necrotic Cell Death but also Necroptotic Cell Death in Human Aortic Endothelial Cells.** Yayoi Tsuneoka, Tsuyoshi Nakano, Tsutomu Takahashi, Yo Shinoda, Yasuyuki Fujiwara. *Tokyo University of Pharmacy and Life Sciences, School of Pharmacy, Hachioji, Japan*
- P266-0522** **Development of Fluorescent Probe for Hydrogen Selenide, Important Selenium Metabolic Intermediate.** Noriyuki Suzuki, Arata Shinohara, Haruka Kimura, Yasumitsu Ogra. *Chiba University, Graduate School of Pharmaceutical Sciences, Chiba, Japan*
- P267-0589** **The Effects of Whole Life, Low-Dose Cadmium Exposure on High Fat Diet-Induced Metabolic Disease and Its Modulation by Zinc.** Jamie Young¹, Bin Zhou², Zhou Li³, Lu Cai². ¹*University of Louisville, Pharmacology and Toxicology, Louisville, KY, The United States of America.* ²*University of Louisville School of Medicine, Pediatric Research Institute, Louisville, KY, The United States of America.* ³*University of Louisville School of Medicine, Pediatric Research Institute, Louisville, KY, The United States of America*

- P268-0605** **An Alginate-Yeast-Biocomposite System (AY-BY) for the Simultaneous Biosorption of Cadmium, Lead, and Arsenate from Aqueous Solutions.** Silvia Carolina Moreno Rivas¹, Jose Andre-i Sarabia Sainz², Jaqueline García Hernández³, Luz Vázquez Moreno¹, Alma Rosa Islas Rubio⁴, Gabriela Ramos Clamont Montfort¹. ¹*Centro de Investigación en Alimentación y Desarrollo, A.C., Ciencia de los Alimentos, Hermosillo, Mexico.* ²*Universidad de Sonora, Departamento de Investigación en Física, Hermosillo, Mexico.* ³*Centro de Investigación en Alimentación y Desarrollo, A.C., Unidad Guaymas, Aseguramiento de Calidad y Aprovechamiento Sustentable de Recursos Naturales, Guaymas, Mexico.* ⁴*Centro de Investigación en Alimentación y Desarrollo, A.C., Tecnología de Alimentos de Origen Vegetal, Hermosillo, Mexico*
- P269-0662** **Manifestation of Endocrine-Disrupting and Other Effects of Metals in Pregnant Women.** James Gomes¹, Premkumari Kumarathan². ¹*University of Ottawa, Interdisciplinary School of Health Sciences, Ottawa, Canada.* ²*Health Canada, Mechanistic Studies Division, Ottawa, Canada*
- P270-0676** **Cadmium Induced Nephrotoxicity via Modulating the Mitochondrial Unfolded Protein Response and Nrf2 Mediated Antioxidant Defense.** Jing Ge, Cong Zhang, Jinlong Li. *Northeast Agricultural University, Harbin, China*
- P271-0682** **Withdrawn**
- P272-0766** **Effects of an Egg White Hydrolysate on the Development of Hypertension and Vascular Dysfunction after Aluminum Exposure in Rats.** Marta Garcés Rimón¹, Caroline Silveira Martinez², Alyne Escobar³, Janaina Piagette⁴, Dalton Vassallo⁵, Franck Peçanha⁶, Marta Miguel⁷, Giulia Wiggers⁸. ¹*Universidad Francisco de Vitoria Food Biotechnology Research Group, Food Biotechnology Research Group, Madrid, Spain.* ²*Universidade Federal do Pampa, Uruguiana, Brazil.* ³*Universidade Federal do Pampa, Uruguiana, Brazil.* ⁴*Universidade Federal do Pampa, Uruguiana, Brazil.* ⁵*Universidade Federal do Espírito Santo, Marechal Campos, Vitória, Brazil* ⁶*Frank M. Peçanha, Uruguiana, Brazil* ⁷*Instituto de Investigación en Ciencias de la Alimentación, Madrid, Spain* ⁸*Universidade Federal do Pampa, Uruguiana, Brazil*
- P273-0782** **Involvement of Metallothionein-III in Mercury-Induced Chemokine Gene Expression.** Minoru Yoshida¹, Jin-Yong Lee², Maki Tokumoto², Masahiko Satoh². ¹*Hachinohe Gakuin University, Faculty of Health and Medical Care, Hachinohe, Japan.* ²*Aichi Gakuin University, School of Pharmacy, Nagoya, Japan*
- P274-0867** **Dietary Intake of Trace Elements for a Population Highly Exposed to Canned Food.** Montse Marquès, Neus González, José Luis Domingo, Martí Nadal. *URV-IISPV, Laboratory of Toxicology and Environmental Health, School of Medicine, Reus, Spain*
- P275-0872** **Do Dysregulated Cellular Energetics Play a Role in Hexavalent Chromium-Induced Human Lung Carcinogenesis?** James Wise, Lei Wang, Michael Alstott, Ntube Ngalame, John Andrew, Zhuo Zhang, Xianglin Shi. *University of Kentucky, Lexington, KY, The United States of America*
- P276-0959** **Slc30a10-Deficient Mice, a Model of Inherited Manganese Excess, Develop Hcpidin Deficiency and Increased Manganese Absorption.** Courtney Mercadante, Milan Prajapati, Heather Conboy, Thomas Barntnikas. *Brown University, Pathology and Laboratory Medicine, Providence, RI, The United States of America*
- P277-0245** **Phytoremediation and Mutual Toxicological Effects of Nickel (Ni²⁺) and Cadmium (Cd²⁺) in Coexistence with Zinc Oxide Nanoparticles Using Sorghum (*S. bicolor*) and Alfalfa (*M. sativa*).** Alonso Andres Doria Manzur, Daniela Vargas Vasquez, Yuliana Hernandez Montalvo, Lesly Tejeda-Benitez. *Universidad de Cartagena, Facultad de Ingeniería, Cartagena, Colombia*
- P278-0377** **Cigarette Smoke Extract Produces Superoxide in Aqueous Solution by Reacting with Bicarbonate.** Haerin Jeong, Jung-Min Park, Yoon-Seok Seo, Moo-Yeol Lee. *Dongguk University College of Pharmacy, Toxicology, Goyang, The Republic of Korea*
- P279-0441** **An Integrated Approach to Evaluate the Environmental Impact of Mixtures of Herbicide Formulations Using Zebrafish Embryonic Stages.** Gessyca Costa, Lara Brito, Laís Rodrigues, Thaís Santos, Marize Valadares, Gisele Oliveira. *Federal University of Goiás, Faculty of Pharmacy, Goiânia, Brazil*
- P280-0550** **Toxicogenomics Analysis of Phthalates and Bisphenol A Mixture: Lung Cancer.** Katarina Baralić¹, Katarina Živančević¹, Marijana Ćurčić¹, Zorica Bulat¹, Aleksandra Buha Đorđević¹, Vesna Matović¹, Dragana Jovanović², Danijela Đukić-Čosić¹. ¹*University of Belgrade Faculty of Pharmacy, Department of Toxicology "Akademik Danilo Soldatović", Belgrade, Serbia.* ²*Institute of Public Health of Serbia, Dr Subotića Starijeg⁵, Belgrade, Serbia*

- P281-0636** **Rat Liver and Kidney Post-mitochondrial Dysfunction by Addition of Chronic Mixed-Metal Intoxication and Hepatorenal Wellness Mediated by Phenolic Components from *Croton zambiscus* Leaves.** Akintunde Jacob¹, Seun Ayeni², Modupe Adeoye³, Abideen Shittu⁴. ¹Federal University of Agriculture, Abeokuta, Biochemistry, Abeokuta, Nigeria. ²Kwara State University, Biochemistry, Malete, Nigeria. ³Kwara State University, Biochemistry, Malete, Nigeria. ⁴Kwara State University, Biochemistry, Malete, Nigeria
- P282-0666** **Application of Generalized Concentration Addition to Predict Mixture Effects of Glucocorticoid Receptor Ligands.** Rosemarie de la Rosa¹, Thomas Webster², Jennifer Schlezinger², Martyn Smith¹. ¹University of California Berkeley, School of Public Health, Division of Environmental Health Sciences, Berkeley, CA, The United States of America. ²Boston University, School of Public Health, Department of Environmental Health, Boston, MA, The United States of America
- P283-0678** **Sex-Dependent Effect of a Cocktail of 13 Chemicals on Reproductive System in Rats.** Anca Docea¹, Konstantinos Nikolouzakis Taxiarchis², Daniela Calina³, Alexandra Kalogeraki⁴, Polychronis Stivaktakis⁵, Ovidiu Zlatian⁶, Aristidis Tsatsakis⁵. ¹University of Medicine and Pharmacy of Craiova, Department of Toxicology, Craiova, Romania. ²University of Crete Medical School, Laboratory of Anatomy-Histology-Embryology, Heraklion, Greece. ³University of Medicine and Pharmacy of Craiova, Clinical Pharmacy, Craiova, Romania. ⁴University of Crete Medical School, Department of Pathology-Cytopathology, Heraklion, Greece. ⁵University of Crete Medical School, Laboratory of Toxicology, Heraklion, Greece ⁶University of Medicine and Pharmacy of Craiova, Department of Microbiology, Craiova, Romania
- P284-0701** **Low Dose Relative Potency Factors for Non-parallel Dose-Response Curves.** Thomas Webster, Jennifer Schlezinger. Boston University School of Public Health, Dept Environmental Health, Boston, MA, The United States of America
- P285-0015** **Pulmonary Toxicity of Organomodified Nanoclay along Its Life Cycle: Integrated Exposure and *In Vitro/In Vivo* Approaches.** Todd Stueckle¹, Alixandra Wagner², Jake Jensen¹, Aliakbar Afshari¹, Eun Lee¹, Jiwoon Kwon³, Jayme Coyle¹, Raymond Derk¹, Sherri Friend¹, Sushant Agarwal², Rakesh Gupta², Cerasela Dinu². ¹NIOSH, Allergy and Clinical Immunology Branch, Morgantown, WV, The United States of America. ²West Virginia University, Biomedical and Chemical Engineering, Morgantown, WV, The United States of America. ³KOSHA, Ulsan, The Republic of Korea
- P286-0075** **The Dispersion State of Carbon Nanomaterials Affects Cellular Responses.** Hisao Haniu¹, Chika Kuroda¹, Katsuya Ueda¹, Yoshikazu Matsuda², Naoto Saito¹. ¹Shinshu University, Institute for Biomedical Sciences, Matsumoto, Japan. ²Nihon Pharmaceutical University, Clinical Pharmacology Educational Center, Ina-machi, Japan
- P287-0078** **Enhanced SIRT1 Protein via JNK Pathway in Silica Nanoparticle-Induced Pulmonary Damage.** Gang Chen, Zhao Xinyuan, Wu Yifan. Nantong University, Department of Occupational Medicine and Environmental Toxicology, Nantong, China
- P288-0164** **Comparing. 3D-Lung Cell Models in an Aerosol Exposure System with BaSO₄ Nanoparticles.** Berit Schumann¹, Felix Glahn¹, Merve Bacanlı², Diana Schneider¹, Tina Röder¹, Patrick Mai³, Andreas Schober³, Heidi Foth¹. ¹Martin-Luther-University, Institute of Environmental Toxicology, Halle (Saale), Germany. ²Hacettepe University, Department of Pharmaceutical Toxicology, Ankara, Turkey. ³Technische Universität Ilmenau, Nano-biosystem Technology, Ilmenau, Germany
- P289-0203** **Reproductive Toxicity of Combusted Diesel Additive Containing Nano-ceria: *In Vitro* Approach.** Martina Cotena^{1,2}, Melanie Auffan², Virginie Tassistro¹, Jerome Rose², Jeanne Perrin^{1,3}. ¹Faculty of Medicine, Aix Marseille Université, Equipe Biomarqueurs, Environnement, Santé - Institut Méditerranéen de Biodiversité et d'Ecologie UMR 7263, IRD 237, Biomarkers, Environment and Health, Marseille, France. ²European Centre Research And Teaching In Geosciences of Environment, ED-Environnement Durable, Marseille, France. ³AP-HM La Conception, Centre Clinico-Biologique d'Assistance Médicale à la Procréation - CECOS., Marseille, France
- P290-0205** **Comparative Mouse Lung Injury by Nickel Nanoparticles with Differential Surface Modification.** Yiqun Mo, Yue Zhang, Qunwei Zhang. University of Louisville, Department of Environmental and Occupational Health Sciences, Louisville, KY, The United States of America
- P291-0208** **Amorphous Silica Nanoparticles Would Worsen Their Hepatic Damage through the Acquired Immune System.** Shun-ichi Eto^{1,2}, Kazuma Higashisaka^{1,3}, Aoi Koshida¹, Kenta Sato¹, Mao Ogura¹, Hirofumi Tsujino¹, Kazuya Nagano^{1,3}, Yasuo Tsutsumi^{1,3,4}. ¹Osaka University Graduate School of Pharmaceutical Sciences, Suita, Japan. ²Osaka University Interdisciplinary Program for Biomedical Sciences, Suita, Japan. ³Osaka University Graduate School of Medicine, Suita, Japan. ⁴Osaka University Global Center for Biomedical Engineering and Informatics, Suita, Japan

- P292-0231** **Differential Nanoceria Distribution and Effects in Th1- and Th2-Prone Mice.** Robert Yokel¹, Michael Tseng², D. Allan Butterfield¹, Matthew Hancock¹, Eric Grulke¹, Jason Unrine¹, Uschi Graham^{1,3}. ¹University of Kentucky, Lexington, KY, The United States of America. ²University of Louisville, Louisville, KY, The United States of America. ³CDC, NIOSH, Cincinnati, OH, The United States of America
- P293-0234** **Remodeling of the Cytoskeleton and Generation of Vacuoles-Like Structures Caused by Tin Dioxide Nanoparticles in Murine Macrophages.** Octavio Ispanixtlahuatl-Meráz^{1,2}, Norma Delgado-Buenrostro¹, Alejandro Déciga-Alcaráz^{1,2}, Edgar López-Villegas³, Damaris Ilhuicatzí-Alvarado⁴, Leticia Moreno-Fierros⁴, Yolanda Chirino¹. ¹Laboratorio de Carcinogénesis y Toxicología, Unidad de Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla de Baz, Mexico. ²Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México, Coyoacán, Mexico. ³Escuela Nacional de Ciencias Biológicas, Miguel Hidalgo, Mexico. ⁴Laboratorio de Inmunidad en Mucosas, Unidad de Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla de Baz, Mexico
- P294-0263** **Nanoparticle Interactions with the Mitochondrial Genome in Primary Human Bronchial Epithelial Cells.** Ryan Snyder, Kirsten Verhein, Heather Vellers, Adam Burkholder, Stavros Garantziotis, Steven Kleeberger. NIEHS, DIR, Durham, NC, The United States of America
- P295-0289** **Repeated-Dose 28-Day and 90-Day Oral Toxicity of Titanium Dioxide Nanoparticles in Rats.** Min Beom Heo, Minjeong Kwak, Tae Geol Lee. Korea Research Institute of Standards and Science, Center for Nano-Bio Measurement, Daejeon, The Republic of Korea
- P296-0305** **Effects of Nanoparticle Core on Cellular Activities under the Controlled Shape, Size, and Surface Chemistries.** Bing Yan. Shandong University, School of Environmental Science and Technology, Jinan, China
- P297-0307** **Effects of Antioxidant and Inhibitor of γ -Glutamylcysteine Synthetase on Acute Toxicity of Silver Nanoparticles Intraperitoneally Administered in BALB/c Mice.** Young-Man Cho, Jun-ichi Akagi, Yasuko Mizuta, Tetsuya Ide, Takeshi Toyoda, Kumiko Ogawa. National Institute of Health Sciences, Division of Pathology, Kawasaki, Japan
- P298-0317** **The Effects of Exogenous Nanoparticles on Neurological Disorders and Evaluation of Its Induction Mechanism Based on ADMET Analysis.** Kazuma Higashisaka, Junpei Kasahara, Miyuki Sakurai, Shun-ichi Eto, Hirofumi Tsujino, Kazuya Nagano, Hiroshi Matsumoto, Yasuo Tsutsumi. Osaka University, Suita, Japan
- P299-0321** **High Resolution Melt (HRM) in RT-qPCR to Detect the Presence of Gold Nanoparticles (AuNPs) in RNA/DNA Samples.** Natasha Sanabria, Charlene Andraos, Mary Gulumian. National Institute for Occupational Health, Johannesburg, South Africa
- P300-0361** **Development and Evaluation of Analysis System for the Simultaneous Quantification and Physical Property Analyses of Nanoparticles in Biological Samples Using Single Particle Inductively Coupled Plasma-Mass Spectrometry.** Kazuya Nagano¹, Takuya Ishizaka¹, Ikkei Tasaki¹, Tao Hong¹, Kazuo Harada^{1,2}, Kazumasa Hirata¹, Shigeru Saito^{3,4}, Hirofumi Tsujino¹, Kazuma Higashisaka^{1,2}, Yasuo Tsutsumi^{1,2,5}. ¹Osaka University, Graduate School of Pharmaceutical Sciences, Suita, Japan. ²Osaka University, Graduate School of Medicine, Suita, Japan. ³University of Toyama, Graduate School of Medicine and Pharmaceutical Science, Toyama, Japan. ⁴University of Toyama, Toyama University Hospital, Toyama, Japan. ⁵Osaka University, Global Center for Medical Engineering and Informatics, Suita, Japan
- P301-0378** **Titanium Dioxide Nanoparticles Disrupted Cytoskeletons and Autophagy in Germ Cell Lines and Disturbed Glucose Homeostasis in Pregnant Rats.** Yankai Xia, Zhilei Mao, Yuqing Zhang, Yaqi Li, Bo Xu, Xiumei Han. Nanjing Medical University, School of Public Health, Nanjing, China
- P302-0393** **Ubiquitin C-Terminal Hydrolase L1 Mitigates Neurotoxicity Induced by Zinc Oxide Particles through Regulation of NF- κ B Signaling.** Tie Han, Lei Tian, Hu Yang, Jie Han, Zhen Fang, Xiaohua Liu. Tianjin Institute of Environmental and Operational Medicine, Department of Toxicology, Tianjin, China
- P303-0411** **Zebrafish, a Multi-Endpoint Approach for Nanoecotoxicology: Does Shape Matter?** Tarryn Lee Botha, Victor Wepener. North-West University, Water Research Group; Unit for Environmental Sciences and Management, Potchefstroom, South Africa

- P304-0448** **Naked Fe₃O₄ Magnetic Nanoparticles Are Internalized by Rat Brain Microvascular Endothelial Cells by Endocytosis Causing Toxic Effects.** Claudia Escudero-Lourdes¹, Lorena Garate-Velez², Daniela Salado-Leza¹, José Rodríguez-Lopez². ¹Universidad Autónoma de San Luis Potosí, Facultad de Ciencias Químicas, Laboratorio de Inmunotoxicología, San Luis Potosí, Mexico. ²Instituto Potosino de Investigación Científica y Tecnológica, Laboratorio de Nanoestructuras para Aplicaciones Ambientales y Biomedicas, San Luis Potosí, Mexico
- P305-0504** **Biological Responses of Human Lymphatic Endothelial Cells to Carbon Nanomaterials.** Mahoko Sano, Hisao Haniu, Katsuya Ueda, Chika Kuroda, Kaoru Aoki, Naoto Saito. *Shinshu University, Matsumoto, Japan*
- P306-0523** **Evaluation of Metal Nanoparticles on Skin Sensitization.** Seonghyun Kim, Donghan Lee, Hyun-gyung Gu, Jin Hee Lee, Jun-young Yang, Hyun-kyoung Kim, Ji Hyun Seok, Jong Kwon Lee. *Ministry of Food and Drug Safety, Cheongju-si, The Republic of Korea*
- P307-0542** **Short- and Long-Term Administration of Synthetic Amorphous Silica (NM-203) Nanoparticle and Histopathological Effects on Rat Spleen.** Roberta Tassinari, Laura Narciso, Sabrina Tait, Mauro Valeri, Andrea Martinelli, Francesca Maranghi. *Istituto Superiore di Sanità, Rome, Italy*
- P308-0543** **Improved Aerosol Generation Method and Newly Designed Whole Body Rodent Inhalation Apparatus for the Testing of Nanomaterials in Human-Relevant Exposure Scenario.** Yuhji Taquahashi¹, Satoshi Yokota¹, Koichi Morita¹, Masaki Tsuji¹, Akihiko Hirose², Jun Kanno^{2,3}. ¹National Institute of Health Sciences, Division of Cellular and Molecular Toxicology, BSRC, Kawasaki, Japan. ²National Institute of Health Sciences, Division of Risk Assessment, BSRC, Kawasaki, Japan. ³Japan Bioassay Research Center, Japan Organization of Occupational Health and Safety, Hadano, Japan
- P309-0555** **MPA-Capped CdTe Quantum Dots Exposure Causes Toxic Effects in BV2 Cells via MyD88-Dependent Toll-Like Receptor Pathway and NLRP3 Inflammasome Activation.** Xue Liang, Tianshu Wu, Meng Tang. *Southeast University, Nanjing, China*
- P310-0556** **Effect of Carbon Nanotubes on Atherosclerosis Progression in Animal and Culture Models of Atherosclerosis.** Sahoko Ichihara¹, Yuka Suzuki², Kiyora Izuoka², Akihiko Ikegami¹, Cai Zong³, Gaku Ichihara³. ¹Jichi Medical University, Department of Environmental and Preventive Medicine, Shimotsuke, Japan. ²Mie University, Graduate School of Regional Innovation Studies, Tsu, Japan. ³Tokyo University of Science, Department of Occupational and Environmental Health, Noda, Japan
- P311-0582** **Effects of Pharyngeal Aspiration Exposure to Multi-Walled Carbon Nanotubes on Pulmonary Inflammation in Mice.** Gaku Ichihara¹, Yuka Suzuki², Wenting Wu², Kiyora Izuoka², Sahoko Ichihara³. ¹Tokyo University of Science, Department of Occupational and Environmental Health, Noda, Japan. ²Mie University, Graduate School of Regional Innovation Studies, Tsu, Japan. ³Jichi Medical University, Department of Environmental and Preventive Medicine, Shimotsuke, Japan
- P312-0621** **Leveraging the Developmental Zebrafish to Evaluate the Hazard Potential of Metal Oxide Nanomaterials.** Kimberly Diane Hayward, Lisa Truong, Michael Simonich, Robert Tanguay. *Oregon State University, Department of Environmental and Molecular Toxicology, Corvallis, OR, The United States of America*
- P313-0644** **Silicon Dioxide and Titanium Dioxide Nanoparticles Induce Cytoskeleton Disruption and Inhibit Cell Migration.** Alejandro Déciga-Alcaráz¹, Norma Delgado-Buenrostro¹, Jose O. Flores-Flores¹, Adriana Ganem-Rondero¹, Yesennia Sánchez-Pérez², Claudia M. García-Cuéllar², Yolanda Chirino¹. ¹Universidad Nacional Autónoma de México, Estado de México, Mexico. ²Instituto Nacional de Cancerología, Subdirección de Investigación Básica, Ciudad de México, Mexico
- P314-0646** **Relating Nano-metal Oxide Physicochemical Properties to Developmental Toxicity: An Approach to Safer Nanomaterial Design.** Claudia Santillan, Sabrina Edwards, Michael Simonich, Lisa Truong, Robert Tanguay. *Oregon State University, Department of Environmental and Molecular Toxicology, Corvallis, OR, The United States of America*
- P315-0705** **Zinc Oxide and Silver but Not Titanium Dioxide Nanoparticles Induce Innate and Adaptive Immune Response in THP-1 Macrophages.** Hani El-Nezami¹, Wing Lam Poon², Piia Karisola³, Harri Alenius⁴. ¹The University of Hong Kong, School of Biological Sciences, Hong Kong, Hong Kong. ²The University of Hong Kong, School of Biological Sciences, Hong Kong, China. ³University of Helsinki, Medicum, Bacteriology and immunology, Helsinki, Finland. ⁴Karolinska Institutet, Institute Environmental Medicine, Stockholm, Sweden

- P316-0735** **Molecular Signature of Asthma-Enhanced Sensitivity to Aerosols of Pristine and Carboxylated CuO Nanoparticles, Identified in 3D Cell Models.** Ingeborg Kooter¹, Marit Ilves², Mariska Gröllers-Mulderij¹, Evert Duistermaat³, Peter Tromp¹, Frieke Kuper¹, Pia Kinaret^{4, 5}, Kai Savolainen⁶, Dario Greco^{4, 5}, Piia Karisola², Joseph Ndika², Harri Alenius^{2, 7}. ¹Netherlands Organisation for Applied Scientific Research, Utrecht, The Netherlands. ²University of Helsinki, Department of Bacteriology and Immunology, Medicum, Helsinki, Finland. ³Triskelion B.V., Zeist, The Netherlands. ⁴University of Tampere, Faculty of Medicine and Life Sciences, Tampere, Finland. ⁵University of Helsinki, Institute of Biotechnology, Helsinki, Finland. ⁶Finnish Institute of Occupational Health, Helsinki, Finland. ⁷Karolinska Institutet, Institute of Environmental Medicine, Stockholm, Sweden
- P317-0742** **(Un-)Supervised Learning Methods Predict and Characterize the Association between Nanomaterial-Induced Inflammatory Markers and Pathological Outcomes in the Lungs.** Naveena Yanamala^{1, 2}, Ishika Desai^{1, 3}, William Miller⁴, Vamsi Kodali¹, Girija Syamlal⁴, Jenny Roberts¹, Aaron Erdely¹. ¹NIOSH, HELD, Morgantown, WV, The United States of America. ²Carnegie Mellon University, Institute for Software Research, Pittsburgh, PA, The United States of America. ³Ohio State University, Columbus, OH, The United States of America. ⁴NIOSH, Morgantown, WV, The United States of America
- P318-0771** **Iron Oxide Nanoparticles Aggravate Neuroinflammation in a Murine Model of Experimental Autoimmune Encephalomyelitis.** Yai-ping Hsiao¹, Chung-Hsiung Huang², Tong-rong Jan¹. ¹National Taiwan University, Department of Veterinary Medicine, Taipei, Taiwan. ²National Taiwan Ocean University, Department of Food Science, Keelung, Taiwan
- P319-0802** **Genetic and Hepatic Damage Induced by TiO₂ (Anatase), ZnO Nanoparticles, and Their Mixture in Mice: A Morphological and Mechanistic Approach.** Opeoluwa Fadoju¹, Olusegun Ogunsuyi¹, Olubukola Akanni¹, Oluwatosin Adaramoye¹, Sébastien Cambier², Santhana Eswara², Arno Gutleb², Adekunle Bakare¹. ¹University of Ibadan, Ibadan, Nigeria. ²Luxembourg Institute of Science and Technology, Belvaux, Luxembourg
- P320-0803** **Withdrawn**
- P321-0831** **Graphene-Based Nanomaterials Directly Induce Epithelial-Mesenchymal Transition *In Vitro*.** Yanyan Liao¹, Peng Cai^{1, 2}. ¹Institute of Urban Environment, Chinese Academy of Sciences, Key Lab of Urban Environment and Health, Xiamen, China. ²Shanghai Institute of Nutrition and Health, Chinese Academy of Sciences, Shanghai, China
- P322-0843** **Comparative *In Vitro* Toxicity Assessment of Carbon Dots with Positive and Negative Surface Charge.** Tana Zavodna^{1, 2}, Katerina Polakova³, Tomas Malina³, Jan Belza³, Alena Milcova¹, Jolana Vankova¹, Katerina Hola³, Pavel Rossner¹, Jan Topinka¹, Radek Zboril³. ¹Institute of Experimental Medicine, Czech Academy of Sciences, Department of Genetic Toxicology and Nanotoxicology, Prague, The Czech Republic. ²Faculty of Safety Engineering, VSB - Technical University of Ostrava, Department of Occupational and Process Safety, Ostrava, The Czech Republic. ³Regional Centre of Advanced Technologies and Materials, Palacky University, Department of Physical Chemistry and Experimental Physics, Olomouc, The Czech Republic
- P323-0878** **Halloysite Nanotube Toxicity Is Predicted by Surface Area.** Kenneth Barfod¹, Katja Bendtsen¹, Trine Berthing¹, Joonas Koivisto¹, Søs Poulsen¹, Ester Segal², Andreas Holländer³, Keld Jensen¹, Karin Sørig Hougaard¹, Ulla Vogel¹. ¹National Research Centre for the Working Environment, Nanosafety Centre, Copenhagen, Denmark. ²Technion Israel Institute of Technology, Department of Biotechnology and Food Engineering, Haifa, Israel. ³Fraunhofer Institute for Applied Polymer Research IAP, Research Division Functional Polymer Systems, Potsdam, Germany
- P324-0885** **Comparative Study of Cytotoxic and Genotoxic Effects of Uncoated and Polyethylene-Glycol-Coated Gold Nanoparticles on Human Kidney (HK-2) Cells.** Paul Tchounwou, Christian Rogers, Anita Patlolla. Jackson State University, Center for Environmental Health, Jackson, MS, The United States of America
- P325-0165** **Inhibitory Effect of Kahweol on PMA-Induced MMP-9 Expression through Suppressing Akt/JNK1/2/p38 MAPK Signal Transduction and NF-κB-Dependent Transcriptional Activity in Human Fibrosarcoma Cells.** Jae Ho Choi¹, Yong Pil Hwang², Sun Woo Jin¹, Gi Ho Lee¹, Hyung Gyun Kim³, Eun Hee Han⁴, Young Chul Chung⁵, Hye Gwang Jeong¹. ¹Chungnam National University, Department of Toxicology, College of Pharmacy, Daejeon, The Republic of Korea. ²International University of Korea, Department of Pharmaceutical Engineering, Jinju, The Republic of Korea. ³Mokpo Marine Food-industry Research Center, Department Research Planning Team, Mokpo, The Republic of Korea. ⁴Korea Basic Science Institute (KBSI), Drug & Disease Target Research Team, Division of Bioconvergence Analysis, Cheongju, The Republic of Korea. ⁵International University of Korea, Department of Food and Medicine, College of Public Health and Natural Science, Jinju, The Republic of Korea

- P326-0166** **Stimulatory Effect of Platycodin D on Osteoblast Differentiation via SIRT1/ β -Catenin Pathways.** Youn Ho Han¹, Sun Woo Jin², Gi Ho Lee², Jae Ho Choi², Han Gun Kim³, Young Chul Chung⁴, Kwang Youl Lee⁵, Hye Gwang Jeong². ¹Wonkwang University, Department of Oral Pharmacology, College of Dentistry, Iksan-si, The Republic of Korea. ²Chungnam National University, Department of Toxicology, College of Pharmacy, Daejeon, The Republic of Korea. ³Sunchon National University, Department of Pharmacy, Suncheon, The Republic of Korea. ⁴International University of Korea, Department of Food and Medicine, College of Public Health and Natural Science, Jinju, The Republic of Korea. ⁵Chonnam National University, College of Pharmacy and Research Institute of Drug Development, Gwangju, The Republic of Korea
- P327-0199** **IGF-1 Promotes Platelet Recovery in Mice by Thrombopoiesis Facilitation and Radioprotective Effect after Radiation.** Shilei Chen¹, Mengjia Hu¹, Mingqing Shen¹, Fang Chen¹, Song Wang¹, Mo Chen¹, Cheng Wang¹, Dongfeng Zeng², Fengchao Wang¹, Tianmin Cheng¹, Yongping Su¹, Jinghong Zhao³, Junping Wang¹. ¹State Key Laboratory of Trauma, Burns and Combined Injury, Third Military Medical University, Chongqing, China. ²Daping Hospital, Third Military Medical University, Chongqing, China. ³Xinqiao Hospital, Third Military Medical University, Chongqing, China
- P328-0225** **AHR-Related Microbial Pattern Changes in the Murine Gut Measured by High-Resolution Flow Cytometry.** Katrin Hochrath¹, Nicole Dauzenroth², Jonathan Rathjens², Katja Ickstadt², Charlotte Esser¹. ¹IUF Leibniz Research Institute for Environmental Medicine, Immunology, Düsseldorf, Germany. ²TU Dortmund University, Mathematical Statistics with Applications in Biometrics, Dortmund, Germany
- P329-0297** **Protective Effect of 12AC3O against High PhosphateInduced Vascular Calcification.** Naoko Takase, Masatoshi Inden, Shunsuke Hirai, Yumeka Yamada, Hisaka Kurita, Eiji Yamaguchi, Akichika Itoh, Isao Hozumi. *Gifu Pharmaceutical University, Gifu, Japan*
- P330-0348** **Effect of the Conserved Residues on Reactive Oxygen Species Scavenging in Cytoglobin.** Shen Jingkai¹, Hirofumi Tsujino¹, Ryo Torii¹, Takumi Tanaka¹, Taku Yamashita², Kazuma Higashisaka^{1,3}, Kazuya Nagano^{1,3,4}, Yasuo Tsutsumi^{1,3,4}. ¹Osaka University, Graduate School of Pharmaceutical Sciences, Suita, Japan. ²Mukogawa Woman's University, School of Pharmaceutical Sciences, Nishinomiya, Japan. ³Osaka University, Graduate School of Medicine, Suita, Japan. ⁴Osaka University, The Center for Advanced Medical Engineering and Informatics, Suita, Japan
- P331-0505** **Toxicity of Metformin as an Adjunct Chemotherapeutic Agent.** Abigail Bland, Nensi Shrestha, Ivan Sammut, Rhonda Rosengren, John Ashton. *University of Otago, Dunedin, New Zealand*
- P332-0526** **Genome-Wide Screening of Formaldehyde Toxicity Using CRISPR-Cas9.** Yun Zhao¹, Abderrahmane Tagmount², Alex Loguinov², Nima Hejazi¹, Alan Hubbard¹, Chris Vulpe², Luoping Zhang¹. ¹University of California Berkeley, School of Public Health, Berkeley, CA, The United States of America. ²University of Florida, College of Veterinary Medicine, Gainesville, FL, The United States of America
- P333-0627** **Inhibition of Pyruvate Kinase M2 Reduces Ovarian Cancer Cells Growth and Migration.** Kyeong Seok Kim, Jae Hyeon Park, Ji Yeon Son, Ji Su Kim, Hyung Sik Kim. *Sungkyunkwan University, Suwon, The Republic of Korea*
- P334-0633** **Characterization of a Novel Oxygenating Therapeutic.** Amjad Dabi, Grace Aquino, Fan Zhang, Erica Bruce. *Baylor University, Waco, TX, The United States of America*
- P335-0677** **Knockdown of PKM2 Induces Autophagic Cell Death via Akt/mTOR Pathway in Human Prostate Cancer Cells.** Prasanta Dey, Amit Kundu, Richa Sachan, Su Hyun Lee, Byung-Mu Lee, Hyung Sik Kim. *Sungkyunkwan University, College of Pharmacy, Suwon, The Republic of Korea*
- P336-0717** **Establishment of Ionizing Radiation–Induced Human Umbilical Vein Endothelial Cells Aging Model.** Jiahui Sun¹, Sai Hu^{2,3}, Xiaodan Liu¹, Hua Guan¹, Pingkun Zhou¹. ¹Beijing Institute of Radiation Medicine, Department of Radiation Toxicology and Oncology, Beijing Key Laboratory for Radiobiology, Beijing, China. ²University of South China, Hengyang, Institute for Environmental Medicine and Radiation Hygiene, School of Public Health, Hengyang, China. ³Beijing Institute of Radiation Medicine, Department of Radiation Toxicology and Oncology, Beijing, China
- P337-0780** **Role of Advanced Glycation End-Products in Diabetes-Associated Pancreatic Islet Endothelial Cell Fibrosis.** Pei-Shan Tsai, Chen-Yuan Chiu, Shing-Hwa Liu. *National Taiwan University, Taipei, Taiwan*
- P338-0798** **Determination of the Antimicrobial Activity of Nine Brands of Toothpaste on *Streptococcus mutans* Isolated from the Mouth of Students in Veritas University, Abuja, Nigeria.** Theresa Ozoude. *Veritas University, Abuja, Biological Sciences, Bwari, Nigeria*

- P339-0814** **Nanofibers as Membrane for Extraction β -Lactam Antibiotics in Urban River Followed by Determination with Capillary Electrophoresis.** Hongmei Shi, Xinghua Li. *Hebei Medical University, School of Public Health, Shijiazhuang, China*
- P340-0888** **The Use of Complementary and Alternative Medicine by Cancer Patients in Libya: Beliefs versus Chemotherapy.** Hanin Hussin¹, Rehab Shembish², Sara Alwsafally³, Basma Abd elraheem⁴. ¹Univeristy of Benghazi, Pharmacology and Toxicology, Benghazi, Libya. ²Benghazi Medical Center, Oncology, Benghazi, Libya. ³Libyan International Medical University, PharmD, Benghazi, Libya. ⁴University of Benghazi, Pharmacology and Toxicology, Benghazi, Libya
- P341-0890** **Microplastics (MPs): Occurrence and Characterization Using Fourier Transform Infrared Spectroscopic Method Coupled with Attenuated Total Reflectance (FTIR-ATR).** Olushola Abiodun¹, Fidelia Osuala², Adebayo Otitoloju³, Derek Ndinteh⁴, Carine Fotsing⁴. ¹Nigerian Institute for Oceanography and Marine Research, Department of Biological Oceanography, Lagos, Nigeria. ²University of Lagos, ZOOLOGY, Akoko, Nigeria. ³University of Lagos, ZOOLOGY, Akoko, Nigeria. ⁴University of Johannesburg, APPLIED CHEMISTRY, Doornfontein, South Africa
- P342-0891** **Expression of miRNA Related to Polyhexamethylene Guanidine Phosphate–Induced Epithelial-Mesenchymal Transition (EMT) in A549.** Hyeong Tae Yu, Mi Ho Jeong, Jun Woo Kim, YuBin Han, Kyu Hyuck Chung. *Sungkyunkwan University, College of Pharmacy, Suwon, The Republic of Korea*
- P343-0902** **4 α ,5 α -Oxidoecdysm-11-en-3-one, a Potential Selective Estrogen Receptor Modulator from *Cyperus rotundus*.** Young Joo Park, In Jae Bang, Hailing Zheng, Jong Hwan Kwak, Kyu Hyuck Chung. *Sungkyunkwan University, College of Pharmacy, Suwon, The Republic of Korea*
- P344-0912** **High-Throughput System for Profiling of Epithelial Damage Induced by Fibrotic Agents.** In Jae Bang¹, Mi Ho Jeong¹, Jun Woo Kim¹, YuBin Han¹, Ha Ryong Kim². ¹Sungkyunkwan University, College of Pharmacy, Suwon, The Republic of Korea. ²Daegu Catholic University, College of Pharmacy, Daegu, The Republic of Korea
- P345-0923** **Inhibitory Effects of *Euphorbia tirucalli* lineu (Euphorbiaceae) Diluted Latex on Human and Canine Melanoma Cells.** Diego Paz, Marcia Nagamine, Maria Lucia Zaidan Dagli. *University of São Paulo, School of Veterinary Medicine and Animal Science, Department of Pathology, São Paulo, Brazil*
- P346-0926** **Exosomes Purified from Bronchoalveolar Lavage Fluid of Mice Instilled with Polyhexamethylene Guanidine Phosphate Induce Inflammation in Naive Macrophages.** Hyeong Tae Yu, Mi Ho Jeong. *Sungkyunkwan University, College of Pharmacy, Suwon, The Republic of Korea*
- P347-0927** **The Role of miR-6126 Upregulated by Polyhexamethylene Guanidine Phosphate in Lung Fibrosis via Targeting PCDH9.** YuBin Han, Mi Ho Jeong, Hyeong Tae Yu, Dong Min Kim, Kyu Hyuck Chung. *Sungkyunkwan University, College of Pharmacy, Suwon, The Republic of Korea*
- P348-0929** **Polyhexamethylene Guanidine Phosphate Induces Endothelial-Mesenchymal Transition in EA.hy926 Cells.** YuBin Han¹, Jun Woo Kim¹, Mi Ho Jeong¹. ¹Sungkyunkwan University, College of Pharmacy, Suwon, The Republic of Korea
- P349-0115** **Development of Software for Facilitating Quality Control of POPs Detection in Food and Animal Feeds.** Wenjing Guo¹, Jeffrey Archer², Morgan Moore², Jeffrey Bruce², Michelle McLain², Sina Shojaee², Wen Zou¹, Russell Fairchild², Huixiao Hong¹. ¹US FDA/NCTR, National Center for Toxicological Research, Jefferson, AR, The United States of America. ²US FDA, Arkansas Laboratory, Jefferson, AR, The United States of America
- P350-0214** **Using Gene-Network Analysis and Cell Model to Investigate the Effect of Dioxin Exposure on Inflammasome Activation and Lymphomagenesis.** Chun-Yu Chuang¹, Yi Wang¹, Yin-Kai Chen². ¹National Tsing Hua University, Department of Biomedical Engineering and Environmental Sciences, Hsinchu, Taiwan. ²National Taiwan University Hospital, Division of Hematology & Oncology, Taipei, Taiwan
- P351-0222** **Chronic Exposure to Low-Level Organochlorine Pesticides (OCPs) Mixture Causes Type 2 Diabetes–Related Metabolic Dysregulation in Zebrafish Model.** Hyojin Lee¹, Moonsung Choi¹, Sangkyu Lee², Youngja Park³, Ki-Tae Kim¹. ¹Seoul National University of Science and Technology, Seoul, The Republic of Korea. ²Kyungpook National University, College of Pharmacy, Daegu, The Republic of Korea. ³Korea University, College of Pharmacy, Sejong, The Republic of Korea
- P352-0401** **Decabromodiphenyl Ether Impairs Glucose Homeostasis in Mice Fed a High-Fat Diet.** Rie Yanagisawa, Eiko Koike, Tin-Tin Win-Shwe. *National Institute for Environmental Studies, Center for Health and Environmental Risk Research, Tsukuba, Japan*

- P353-0453** **Assessment of Polychlorinated Biphenyls (PCBs) Levels in Surface Water, Sediment, Parasitic Fauna, and Bagrid Catfish, *Chrysichthys nigrodigitatus*, as an Index of Pollution of a Tropical Lagoon.** Minasu Kuton, Bamidele Akinsanya, Amii Usese. *University of Lagos, Lagos, Nigeria*
- P354-0463** **Comparative Assessment of Per- and Polyfluoroalkyl Substances (PFASs) on Pancreatic Toxicity.** Barbara Hocevar, Jessica Hocevar, Allison Clarke, Lisa Kamendulis. *Indiana University, Environmental and Occupational Health, Bloomington, IN, The United States of America*
- P355-0465** **Coupling Transcriptomics and Developmental Toxicity in Zebrafish to Classify Polycyclic Aromatic Hydrocarbons (PAHs).** Prarthana Shankar¹, Mitra Geier¹, Ryan McClure², Lisa Truong¹, Paritosh Pande², Jane LaDu¹, Katrina Waters², Robert Tanguay¹. ¹*Oregon State University, Environmental and Molecular Toxicology, Corvallis, OR, The United States of America.* ²*Pacific Northwest National Laboratory, Health Effects and Exposure Science, Richland, WA, The United States of America*
- P356-0467** **Decolorization of Some Synthetic Dyes Using Partially Purified Peroxidase from Cucumber (*Cucumis sativus*).** Obiora Okonkwo, Tunde Muftau, Larry Barde. *Plateau State University, Boko, Nigeria*
- P357-0480** **The Study of Molecular Mechanism of Dioxin-Induced Neurotoxicity: Targeting the Cholinergic System.** Yangsheng Chen, Li Xu, Heidi Xie, Bin Zhao. *RCEES, CAS, Beijing, China*
- P358-0502** **Halogenated Marine Indoles Compete with High Affinity Ligands for Aryl Hydrocarbon Receptor Binding.** Jessie King¹, Helen Woolner², Peter Northcote², Robert Keyzers², Rhonda Rosengren¹. ¹*University of Otago, Pharmacology and Toxicology Department, Dunedin, New Zealand.* ²*Victoria University of Wellington, Wellington, New Zealand*
- P359-0533** **Decabromodiphenyl Ether Affects Immune Response in Obese Mice and in Adipocyte-Macrophage Coculture System.** Eiko Koike¹, Rie Yanagisawa¹, Tin-Tin Win-Shwe¹, Hirohisa Takano². ¹*National Institute for Environmental Studies, Center for Health and Environmental Risk Research, Tsukuba, Japan.* ²*Kyoto University, Graduate School of Engineering, Kyoto, Japan*
- P360-0590** **Low-Dose Chronic Exposure of OCPs Mixture in C57BL/6J Mice Induces the Impairment of Glucose Metabolism.** Chul-Min Park¹, Ki-Tae Kim², Dong-Young Rhyu³. ¹*Environmental Technology Research Institute, Seoul National University of Science and Technology, Seoul, The Republic of Korea.* ²*Seoul National University of Science and Technology, Seoul, The Republic of Korea.* ³*Mokpo National University Department of Oriental Medicine Resources, Muan-gun, The Republic of Korea*
- P361-0639** **Concentrations of Environmental PCBs in Breast Milk and Serum of Lactating North Carolina Women.** Erin Hines¹, Sue Fenton². ¹*US EPA/NCEA, US EPA, National Center for Environmental Assessment, Environmental Media Assessment Group, Research Triangle Park, NC, The United States of America.* ²*NIEHS/NTP/Reproductive Endocrinology Group, Research Triangle Park, NC, The United States of America*
- P362-0669** **DEHP Triggers Developmental Abnormality of Ovary and Oviduct in Quails (*Coturnix japonica*) via Disruption of Hypothalamo-pituitary-ovarian Axis Response.** Xue-Nan Li, Jinlong Li. *Northeast Agricultural University, Harbin, China*
- P363-0797** **PFAS Clearance in Cattle.** Tarah Hagen¹, Roger Drew¹, David Champness², Amelie Sellier³. ¹*ToxConsult Pty Ltd, Darling South, Australia.* ²*Agriculture Victoria, Department of Jobs, Precincts and Regions, Hamilton, Australia.* ³*AsureQuality, Wellington, New Zealand*
- P364-0919** **Applying the Concept of Essential Use to Determine Where and When PFAS Can Be Phased Out.** Mark Miller¹, Ian Cousins², Gretta Goldenman³, Dorte Herzke⁴, Andrew Lindstrom⁵, Rainer Lohmann⁶, Carla Ng⁷, Martin Scheringer⁸, Xenia Trier⁹, Zhanyun Wang⁸, Jamie DeWitt¹⁰. ¹*NIEHS and US Public Health Service, Research Triangle Park, NC, The United States of America.* ²*Stockholm University, Department of Environmental Science and Analytical Chemistry, Stockholm, Sweden.* ³*Milieu, Brussels, Belgium.* ⁴*NILU in Fram Centre, Tromsø, Norway.* ⁵*US EPA/NCCT, Research Triangle Park, NC, The United States of America* ⁶*University of Rhode Island, Kingston, RI, The United States of America* ⁷*University of Pittsburgh, Drug Discovery Institute, Pittsburgh, PA, The United States of America* ⁸*ETH Zurich, Zurich, Switzerland* ⁹*European Environment Agency, Copenhagen, Denmark* ¹⁰*East Carolina University, Greenville, NC, The United States of America*
- P365-0931** **PFAS Tissue Distribution in Cattle.** Roger Drew¹, Tarah Hagen¹, David Champness², Amelie Sellier³. ¹*ToxConsult Pty Ltd, Darling South, Australia.* ²*Agriculture Victoria, Department of Jobs, Precincts and Regions, Hamilton, Australia.* ³*AsureQuality, Wellington, New Zealand*

- P366-0932 PFAS Clearance and Tissue Distribution in Sheep.** Roger Drew¹, Tarah Hagen¹, David Champness², John Ryan³, Lisa Graham⁴. ¹ToxConsult Pty Ltd, Darling South, Australia. ²Agriculture Victoria, Department of Jobs, Precincts and Regions, Hamilton, Australia. ³Agriculture Victoria, Department of Economic Development, Jobs, Transport and Resources, Wangaratta, Australia. ⁴AsureQuality, Wellington, New Zealand
- P367-0933 PFAS Assimilation from Water by Cattle and Sheep.** Tarah Hagen¹, Roger Drew¹, Lisa Graham². ¹ToxConsult Pty Ltd, Darling South, Australia. ²AsureQuality, Wellington, New Zealand
- P368-0948 An Evaluation of the Utility of Human Cell Models for Characterizing Relative Potency for Dioxin-Like Compounds.** Jonathan Urban¹, Daniele Wikoff², Seneca Fitch³, Caroline Ring¹, Laurie Haws¹, Mark Harris³. ¹ToxStrategies, Inc., Austin, TX, The United States of America. ²ToxStrategies, Inc., Asheville, NC, The United States of America. ³ToxStrategies, Inc., Katy, TX, The United States of America
- P369-0092 A Case of Delayed Organophosphate Poisoning Caused by Fenthion Ingestion.** Ivan Chua¹, R Ponampalam², Boon Kiat Kenneth Tan³. ¹Chua Si Yong Ivan, Emergency Medicine, Singapore, Singapore. ²R Ponampalam, DEM, Singapore, Singapore. ³Tan Boon Kiat Kenneth, DEM, Singapore, Singapore
- P370-0143 Developmental Exposure to Glyphosate-Based Herbicide Causes Dysregulation of Dynorphins in the Brain of Adult Rats.** Daiane Cattani^{1,2}, Vivien Steffensen¹, Nona Struyf¹, Ariane Zamoner Pacheco de Souza², Malin Andersson¹, Eva Brittebo¹. ¹Uppsala University, Department of Pharmaceutical Biosciences, Uppsala, Sweden. ²Federal University of Santa Catarina, Department of Biochemistry, Florianopolis, Brazil
- P371-0153 Study on Systemic and Reproductive Toxicity of Acetochlor in Male Mice.** Song Xianping, Feng Zhang, Dongya Chen, Qian Bian, Hengdong Zhang, Xin Liu, Baoli Zhu. Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China
- P372-0181 Effects of Acephate, Carbendazim, and Mancozeb Isolated or in Mixture on DNA Damages and Hepatic and Renal Histopathology.** Juliana Perobelli, Deborah Cavalcante, Maria Aranha, André Almeida, Mariana Simões-Garcia. Laboratory of Experimental Toxicology - LATOEX/UNIFESP, Santos, Brazil
- P373-0184 Understanding the Role of Tyrosine in Toxicity Associated with HPPD Inhibitors and Impact on Human Health Risk Assessment.** Angela Hofstra¹, David Cowie², Elizabeth McInnes², Daniel Minnema³, Richard Lewis², Jane Botham². ¹Syngenta Canada Inc., Toxicology and Health Sciences, Guelph, Canada. ²Syngenta Ltd Jealott's Hill, Toxicology and Health Sciences, Bracknell, United Kingdom. ³Syngenta Crop Protection LLC, Toxicology and Health Sciences, Greensboro, NC, The United States of America
- P374-0207 In Vivo and In Vitro Effects of a Neonicotinoid Pesticide, Clothianidin, on Mammalian Nervous Function.** Tetsushi Hirano¹, Satsuki Minagawa¹, Yukihiro Furusawa², Tatsuya Yunoki¹, Toshifumi Yokoyama³, Nobuhiko Hoshi³, Yoshiaki Tabuchi¹. ¹University of Toyama, Toyama, Japan. ²Toyama Prefectural University, Imizu, Japan. ³Kobe University, Kobe, Japan
- P375-0301 Organophosphate-Induced ADHD-Like Behavior in Adolescent Rats.** Yuki Ito¹, Motohiro Tomizawa², Kazutaka Suzuki³, Yuichi Shirakawa³, Hiromasa Ono³, Keishi Adachi³, Himiko Suzuki³, Michihiro Kamijima³. ¹Nagoya City University Graduate School of Medical Sciences, Occupational and Environmental Health, Nagoya, Japan. ²Faculty of Life Sciences, Tokyo University of Agriculture, Chemistry, Setagaya, Japan. ³Nagoya City University Graduate School of Medical Sciences, Occupational and Environmental Health, Nagoya, Japan
- P376-0311 Serum and Caecal Metabolomics in Rats Exposed Subchronically to Glyphosate and Roundup.** Robin Mesnage¹, Laura Falcioni², Daniele Mandrioli², Fiorella Belpoggi², Michael Antoniou¹. ¹King's College London, London, United Kingdom. ²Ramazzini Institute, Bologna, Italy
- P377-0312 The Experimental Study of the Effects of a Carboxamide Derivative on the Migration-Water Hazard Indicator.** Valerii Rakitskii, Tatiana Sinitskaya, Gromova Irina. FBES "FSCH Named after F.F. Erisman" of the Rospotrebnadzor, Mytischchi, The Russian Federation
- P378-0326 The Isolated and Combined Effects of 2,4-D and Methylmercury on the Morphology of Xerogel of Rat Serum.** Valerii Rakitskii, Tatiana Sinitskaya, Gromova Irina, Gleb Masaltsev. FBES "FSCH Named after F.F. Erisman" of the Rospotrebnadzor, Mytischchi, The Russian Federation
- P379-0392 Aluminum Phosphide Poisoning: Rare Survival Case.** Nazih Ramadan. Cairo University, Cairo, Egypt
- P380-0396 Hydrogen Cyanamide Poisoning: Rare, but Serious, Case Study.** Nazih Ramadan. Cairo University, Cairo, Egypt

- P381-0430** **Endpoints as Biomarkers to Assess the Occupational Exposure to Triazoles.** Simone Machado, Isarita Martins. *Federal University of Alfenas, Alfenas, Brazil*
- P382-0439** **Withdrawn**
- P383-0475** **Development of Data-Derived Extrapolation Factors for Herbicides That Inhibit. ⁴-hydroxyphenyl Pyruvate Dioxygenase (HPPD).** Angela Hofstra¹, David Geter², David Cowie³, Jane Botham³, Alex Stevens³, Sarah Whalley³, Virunya Bhat⁴, Paul Hinderliter². ¹*Syngenta Canada Inc., Toxicology and Health Sciences, Guelph, Canada.* ²*Syngenta Crop Protection LLC, Toxicology and Health Sciences, Greensboro, NC, The United States of America.* ³*Syngenta Ltd Jealott's Hill, Toxicology and Health Sciences, Bracknell, United Kingdom.* ⁴*NSF International, Ann Arbor, MI, The United States of America*
- P384-0577** **Vitamin E (α-Tocopherol) Improves Mitochondrial Energy Metabolism in Rat Liver following Acute Aluminum Phosphide Exposure.** Leila Shahrivary¹, Bahareh Bigdeli¹, Fariba Khodagholi², Shahin Shadnia³. ¹*Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Islamic Republic of Iran.* ²*Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Islamic Republic of Iran.* ³*Toxicological Research Center, Shahid Beheshti University of Medical Sciences, Toxicological Research Center, Excellent Center of Clinical Toxicology, Department of Clinical Toxicology, Loghman Hakim Hospital Poison Center, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Islamic Republic of Iran*
- P385-0620** **Effects of Repeated Doses of the Organophosphorus Pesticide Temephos on Sperm Quality and Acetylcholinesterase Activity in Rats.** Israel Camacho-Hernández¹, Adolfo Sierra Santyo¹, Francisco Verdin Betancourt¹, Maria Solís Heredia¹, Alberto Duana Moreno¹, Aurora Rojas-García², Betzabet Quintanilla Vega¹. ¹*Center of Research and Advanced Studies (Cinvestav), Department of Toxicology, Mexico City, Mexico.* ²*Laboratorio de Contaminación y Toxicología Ambiental, Universidad Autónoma de Nayarit, Tepic Nayarit, Mexico*
- P386-0656** **A Novel Mitochondrial Dysfunction Mechanism of Atrazine-Induced Nephrotoxicity in Quail (*Coturnix C. coturnix*) via Hindering Cross Talk between Detoxification Response and Nrf2-Mediated Antioxidant Defense.** Cong Zhang, Jing Ge, Qi Zhang, Jinlong Li. *Northeast Agricultural University, Harbin, China*
- P387-0679** **Synthesis, Determination, and the Neurotoxic Potential Effect of Temephos Oxidized-Metabolites.** Francisco Verdin Betancourt¹, Mario Figueroa², Ma. de López-González¹, Elizabeth Gómez³, Yael Bernal-Hernández⁴, Aurora Rojas-García⁴, Adolfo Sierra-Santoyo¹. ¹*Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Toxicología, Ciudad de México, Mexico.* ²*Universidad Nacional Autónoma de México, Facultad de Química, Farmacia, Ciudad de México, Mexico.* ³*Universidad Nacional Autónoma de México, Instituto de Química, Ciudad de México, Mexico.* ⁴*Universidad Autónoma de Nayarit, Laboratorio de Contaminación y Toxicología Ambiental, Tepic, Mexico*
- P388-0767** **Stereoselective Copper-Dependent Hydrolysis of Thrichloronate by Turkey Serum Albumin.** Damianys Almenares¹, Antonio Monroy N.². ¹*Universidad Popular de la Chontalpa, División de Ingenierías y Ciencias Agropecuarias, Villahermosa, Mexico.* ²*Universidad Autonoma del Estado de Morelos, Facultad de Farmacia, Cuernavaca, Mexico*
- P389-0812** **Influence of an Intervention Program in the Concentrations of Organochlorinated Pesticides in Adolescents of an Agricultural Community of Mexico.** Andrea Rodriguez, Rebeca Mejia, Leticia Yañez, Vanessa Labrada. *Universidad Autónoma de San Luis Potosí, San Luis Potosí, Mexico*
- P390-0911** **Assessment of the Influence of Unutilized and Prohibited to Use Pesticides on Health of the Almaty Region Population.** Elmira Khussainova¹, Akerke Seisenbaeva¹, Oksana Cherednichenko², Gulnara Baigushikova², Oraz Sapargali¹, Unzira Kapyshova³, Sholpan Bakhtiyarova³, Leyla Djansugurova¹, Bakytzhan Bekmanov¹. ¹*Institute of General Genetics and Cytology, Laboratory of Molecular Genetics, Almaty, Kazakhstan.* ²*Institute of General Genetics and Cytology, Laboratory of Genetical Monitoring, Almaty, Kazakhstan.* ³*Institute of Human and Animal Physiology, Almaty, Kazakhstan*
- P391-0036** **Retrospective Analysis of HIV Cure Applications at the US FDA: 2008–2018.** Laine Myers, Damon Deming, Hanan Ghantous. *US FDA/CDER, CDER / OND / OAP / Division of Antiviral Products, Silver Spring, MD, The United States of America*
- P392-0232** **Use of Read-Across to Characterize the Health Effects of Substances in the Anthraquinones Group in a Screening Assessment of the Chemicals Management Plan in Canada.** Joan Wong, Shayesta Seenundun, Pamela Cebrowski, Adam Griffiths. *Health Canada, Ottawa, Canada*

- P393-0271** **Prospective Application of Human Health Risk Assessment for Rodent Eradication Program on an Inhabited Island.** Robert DeMott¹, Belinda Goldsworthy², Thomas Sendor³, Edward Jansson⁴. ¹Ramboll, Health Sciences, Tampa, FL, The United States of America. ²Ramboll, Health Sciences, The Junction, Australia. ³Ramboll, Health Sciences, Munich, Germany. ⁴Office of the Chief Scientist and Engineer, NSW Dept. of Industry, Sydney, Australia
- P394-0333** **Risk Assessment Study on Inhalation Exposure to Benzene among Gasoline Station Workers: Impact on Human Health.** Nitin Verma. Baddi University of Emerging Sciences & Technology, Himachal Pradesh, India
- P395-0363** **Probabilistic Risk Assessment of Perfluorooctanesulfonate (PFOS) by Integrating *In Vitro* and *In Vivo* Toxicity with Physiologically Based Pharmacokinetic Models in Multiple Species.** Wei-Chun Chou, Zhoumeng Lin. Kansas State University, Institute of Computational Comparative Medicine (ICCM), Department of Anatomy and Physiology, College of Veterinary Medicine, Manhattan, KS, The United States of America
- P396-0417** **Cancer Risk Estimation of Smoking Filter Cigarettes and Risk Ranking of Human Carcinogens in Active and Passive Smokers.** Seong Lim¹, Min Kim¹, Duck Lim¹, Hyung Sik Kim¹, Sam Kacew², Byung-Mu Lee¹. ¹Sungkyunkwan University, College of Pharmacy, Suwon, The Republic of Korea. ²University of Ottawa, McLaughlin Centre for Population Health Risk Assessment, Ottawa, Canada
- P397-0436** **Estimation of Cancer Risk Imposed by Arsenic Exposure through Rice Consumption in Chinese Urban Population.** Kan Shao¹, Zheng Zhou², Yijin Kang³, Huanhong Li⁴, Suzhen Cao⁵, Jianhua Xu⁶, Xiaoli Duan⁷. ¹Department of Environmental and Occupational Health, Bloomington, IN, The United States of America. ²Department of Environmental and Occupational Health, Bloomington, IN, The United States of America. ³School of Energy and Environmental Engineering, Beijing, China. ⁴College of Environmental Sciences & Engineering, Beijing, China. ⁵School of Energy and Environmental Engineering, Beijing, China. ⁶College of Environmental Sciences & Engineering, Beijing, China. ⁷School of Energy and Environmental Engineering, Beijing, China
- P398-0460** **Risk Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) Levels in Surface Water, Sediment, and Two Edible Fish Species from a Tropical Creek, Nigeria.** Amii Usese, Joel Benneth, Lucian Chukwu. University of Lagos, Department of Marine Sciences, Lagos, Nigeria
- P399-0479** **Risk Assessment of Some Heavy Metals in Cow Hides Processed by Different Methods in Nnewi and Environs.** Onyenmechi Afonne, Onyeka Okoro, Emeka Ifediba. Nnamdi Azikiwe University, Nnewi, Nigeria
- P400-0485** **Exploratory Genetic Association of Type 2 Diabetes Occurrence Using a Pharmacogenetic Panel.** Russell Fankhouser, Derek Murrell, Allen Cofer, David Hurley, Sam Harirforoosh. East Tennessee State University, Department of Pharmaceutical Sciences, Johnson City, TN, The United States of America
- P401-0510** **Analysis of Personal Genomic Susceptibility for Atopic Dermatitis Disease by Using Cord Blood DNA in Small-Scale Birth Cohort.** Seung Yong Hwang^{1, 2}, Seung Jun Kim², Moon Ju Oh², Jeong Jin Ahn², Jungeun Yang¹, Eun Jung Koh¹. ¹Hanyang University, Department of Bio-nanotechnology, Ansan-Si, The Republic of Korea. ²Bio-Core, Seoul, The Republic of Korea
- P402-0515** **The *In Vitro* Metabolism and Toxic Effects of Emerging Contaminants 2-Chlorothioxanthone Involved in Human CYP1A2 and CYP3A4 Enzymes.** Tingjie Zhan, Jiayan Chen, Shulin Zhuang. Zhejiang University College of Environmental and Resource Sciences, Hangzhou, China
- P403-0549** **Repeated-Dose *In Vivo* Oral Toxicity Study to Test Long-Term Effects of the Mycotoxin Enniatin B in Male and Female CD-1 Mice: Focus on Histopathological Data for the NOAEL Definition.** Roberta Tassinari¹, Laura Narciso¹, Sabrina Tait¹, Cinzia La Rocca¹, Gabriella Di Felice², Cinzia Butteroni², Silvia Corinti. ², Bianca Barletta², Eugenia Cordelli³, Francesca Pacchierotti³, Patrizia Eleuteri³, Paola Villani³, Ludovic Le Hegarat⁴, Valérie Fessard⁴, Océane Reale⁴, Francesca Maranghi¹. ¹Istituto Superiore di Sanità, Center for Gender-Specific Medicine, Rome, Italy. ²Istituto Superiore di Sanità, National Center for Drug Research and Evaluation, Roma, Italy. ³Italian National Agency for New Technologies, Energy and Sustainable Economic Development, Laboratory of Biosafety and Risk Assessment SSPT-TECS BIORISC, Rome, Italy. ⁴French Agency for Food, Environmental and Occupational Health and Safety, Fougères, France
- P404-0624** **Gaining Insights to Molecular Mechanisms of Combined Air Pollutants Exposures Using PBPK Models and Systems Biology Approaches.** Patricia Ruiz¹, Claude Emond², Eva McLanahan¹, Moiz Mumtaz¹. ¹CDC/ATSDR, Centers for Disease Control and Prevention (CDC), Atlanta, GA, The United States of America. ²BioSimulation Consulting Inc, Newark, DE, The United States of America

Poster Session 1 Index

- P405-0694** **Risk Assessment for Benz[a]pyrene Carcinogenicity in the Chilean Population.** Andrea Salas, [Fernanda Cavieres](#). *Universidad de Valparaíso School of Chemistry and Pharmacy, Faculty of Pharmacy, Valparaíso, Chile*
- P406-0706** **Withdrawn**
- P407-0740** **Quantitative Evaluation of Cytochrome P450 3A4 Inhibition and Hepatotoxicity in HepaRG 3D Spheroids.** [Dae-Seop Shin](#)¹, Hyewon Seo¹, Jung Yoon Yang¹, Jeongmin Joo¹, So Hee Im¹, Seong Soon Kim¹, Sang Kyum Kim², Myung Ae Bae^{1,3}. ¹*Korea Research Institute of Chemical Technology, Bio & Drug Discovery Division, Daejeon, The Republic of Korea.* ²*Chungnam National University, College of Pharmacy, Daejeon, The Republic of Korea.* ³*University of Science & Technology, Department of Medicinal Chemistry and Pharmacology, Daejeon, The Republic of Korea*
- P408-0783** **Concentration-Time Extrapolation of Short-Term Inhalation Exposure Levels for Dimethyl Sulfide.** [Eugene Demchuk](#), Shannon Ball, San Le, Andrew Prussia. *CDC/ATSDR, Division of Toxicology and Human Health Sciences, Atlanta, GA, The United States of America*
- P409-0786** **Risk Assessment of Heavy Metals in *Carica papaya* (Pawpaw) from Three Refuse Dump Sites in Rivers State, Nigeria.** [Ietan Karikpo](#), Ikem Ekweozor, Moslen Muebaka. *Rivers State University, Animal and Environmental Biology, Port Harcourt, Nigeria*
- P410-0822** ***Aspergillus* Section *Nigri* from Agricultural Products and Cultivated Soil and Their Mycotoxin Production.** [Suppara Aukka](#). *Postharvest and Processing Research and Development Division, Department of Agriculture, Chatuchak, Thailand*
- P411-0866** **Nonclinical Development of Cedazuridine, a Novel Cytidine Deaminase Inhibitor for Use in Combination with Decitabine to Enable Oral Administration to Patients with Myelodysplastic Syndromes (MDS).** [Aram Oganesian](#)¹, Richard Seals², Bradley Saville³, Mohammad Azab¹. ¹*Astex Pharmaceuticals, Inc., Pleasanton, CA, The United States of America.* ²*BASI, Mount Vernon, IN, The United States of America.* ³*Charles River Laboratories, Reno, NV, The United States of America*
- P412-0892** **Use of *In Vitro* to *In Vivo* Extrapolation (IVIVE) to Support Risk Assessment for Early Life Stages: Life-Stage Physiologically Based Pharmacokinetic (PBPK) Modeling for Pyrethroids.** [Marjory Moreau](#)¹, Pankajini Mallick¹, Gina Song^{1,2}, Salil Pendse¹, Moire Creek³, Harvey Clewell⁴, Brian Lake⁵, Paul Hinderliter⁶, Miyoung Yoon^{1,2}, Thomas Osimitz⁷. ¹*ScitoVation, PMPK division, Durham, NC, The United States of America.* ²*ToxStrategies, Inc., Cary, NC, The United States of America.* ³*Toxicology Consulting Services, Lincoln, The United States of America.* ⁴*Ramboll, Research Triangle Park, NC, The United States of America.* ⁵*Concept Life Sciences, Dundee, United Kingdom* ⁶*Syngenta, Greensboro, The United States of America* ⁷*Science Strategies, LLC, Charlottesville, The United States of America*
- P413-0955** **Risk Assessment of Lead Contamination Outdoor of Lead Recycling Company.** [Laura Börgel](#)^{1,2}, Melissa Schulthess^{1,2}, Germán Corey¹, Adolfo Börgel¹, Yerko Jeldes¹. ¹*Dra. Laura Börgel y Cía. Ltda, Recoleta, Chile.* ²*University of Chile, Legal Medicine and Anatomy Department, Independencia, Chile*
- P414-0957** **Systematic Update of the Mammalian Relative Potency Estimate (REP) Database for Dioxin-Like Compounds (DLCs).** Seneca Fitch¹, Daniele Wikoff², Kara Franke², Caroline Ring³, Mark Harris¹, [Laurie Haws](#)³. ¹*ToxStrategies, Inc., Katy, TX, The United States of America.* ²*ToxStrategies, Inc., Asheville, NC, The United States of America.* ³*ToxStrategies, Inc., Austin, TX, The United States of America*
- P415-0960** **Environmental Contamination: The Toxic Legacy of a Native American Tribe Continues.** Katherine Fetcie¹, [Oyemwenosa Avenbuan](#)¹, Dennis DeFreese², Mianhua Zhong¹, Jason Blum¹, Judith Zelikoff¹. ¹*NYU School of Medicine, Environmental Medicine, New York, NY, The United States of America.* ²*Ramapough Lenape Nation, New Jersey, The United States of America*
- P416-0005** **Oral Ingestion of a Novel Oxygenating Compound, Ox66™, Is Nontoxic and Results in Increased Oxygenation.** [Fan Zhang](#)¹, Grace Aquino², Amjad Dabi², William Nugent³, Bjorn Song³, Erica Bruce^{2,4,5}. ¹*Baylor University, Department of Environmental Science, Waco, TX, The United States of America.* ²*Baylor University, Department of Environmental Science, Waco, TX, The United States of America.* ³*Song Biotechnologies, LLC, Baltimore, MD, The United States of America.* ⁴*Baylor University, Department of Biology, Waco, TX, The United States of America.* ⁵*Baylor University, Institute of Biomedical Studies, Waco, TX, The United States of America*
- P417-0155** **A Review for the Best Practices for the Application and Preparation of SEND Datasets.** [Sue McPherson](#), Bei Zhou, Cameron McPherson. *WuXi AppTec (Suzhou), Suzhou, China*

- P418-0158** **Pioneering Better Science to Advance the 3Rs in Pharmaceutical Toxicity Testing.** Fiona Sewell, Nikki Gellatly, Helen Prior. *National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), Toxicology and Regulatory Sciences, London, United Kingdom*
- P419-0177** **A Review of Procedure-Induced Lesions in Sprague Dawley Rats and Cynomolgus Monkeys When Utilizing Continuous Intravenous Infusion as the Route of Administration.** Caibiao Hu, Leo Pan, Sue McPherson. *WuXi AppTec (Suzhou), Suzhou, China*
- P420-0269** **Preclinical Toxicology Testing Data for Alignment with the Regulatory Update of the Eurasian Economic Union.** Andrey Kukhare¹, Abhijit Vichare², Sushant Kamath², Brinda Mahadevan². ¹Abbott Laboratories, Global Preclinical Development, Moscow, The Russian Federation. ²Abbott Healthcare Pvt Ltd, Global Preclinical Development, Mumbai, India
- P421-0435** **The Needleless Injection Site Swabbable Connector (NISSC): A Practical and Reliable Tool That Permits Dogs to be Exercised on Continuous Intravenous Infusion Studies.** Arsen Zakaryan, Joseph Younan, Djamal Bennamane, Adrian Bocan. *ITR Laboratories Canada Inc., Baie-D'Urfe, Canada*
- P422-0536** **Development of QSAR Models to Predict Potential Nephrotoxic Ingredients in Traditional Chinese Medicines.** Yuqing Sun, Shaoze Shi, Yaqiu Li, Qi Wang. *Peking University, School of Public Health, Beijing, China*
- P423-0569** **Toxicological Safety Assessment of Novel 5,6-Dihydropyridazine-1(4H)-Carbohydrazide: An Antitubercular Agent.** Ravinesh Mishra¹, Anees Siddiqui². ¹Baddi University of Emerging Sciences and Technology, School of Pharmacy and Emerging Sciences, Baddi, India. ²Jamia Hamdard, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, New Delhi, India
- P424-0609** **Prediction of Seizure Liability in Cultured Human iPSC-Derived Neurons Using MEA System.** Aoi Odawara, Naoki Matsuda, Yuto Ishibashi, Ikuro Suzuki. *Tohoku Institute of Technology, Department of electronics, Sendai, Japan*
- P425-0815** **A 10-Week Telemetered Electroencephalographic and Cardiovascular Comparison between Two Sodium Channel Blockers in Sprague-Dawley Rats.** Rob Angus¹, Joan Lane², Alison Easter³, Ken Loveday¹, Annick Prefontaine¹, Claudia Harper⁴, Dale Morris¹, Patricia Schroeder⁵, Kalyan Vasudevan⁶, Mike Rooney⁷, Kris King⁷, Simon Authier⁸. ¹Biogen, Preclinical Safety, Cambridge, MA, The United States of America. ²Amgen, Comparative Biology and Safety Sciences, Cambridge, MA, The United States of America. ³Praxis Precision Medicines, Cambridge, The United States of America. ⁴AVROBIO, Cambridge, MA, The United States of America. ⁵Forma Therapeutics, Watertown, MA, The United States of America. ⁶Celgene, Summit, The United States of America. ⁷Biogen, DMPK, Cambridge, MA, The United States of America. ⁸Citoxlab, Laval, Canada
- P426-0824** **Safety Evaluation of Drugs Using PDX (Patient-Derived Xenograft) and Solid Cancer-Derived Organoid Culture Systems.** Mie Naruse, Masako Ochiai, Hirokazu Taniguchi, Nobuyoshi Hiraoka, Toshio Ima¹. *National Cancer Center Research Institute, Tokyo, Japan*
- P427-0863** **Acute and Subacute Oral Toxicity Study of KCFC0051 in Rats.** Jeong Doo Heo, Kwang Hyun Hwang, Yeon Gyu Moon, Jeheon Kim, Na Hyun Kim, Woong Soo Kim, Ju Hong Lee, Tae Kil Tak, Jungil Choi. *Korea Institute of Toxicology, Jinju-si, The Republic of Korea*
- P428-0887** **Assessment of a 28-Day Oral Exposure to the Dietary Supplements Nattokinase and Lumbrokinase Alone or in Combination with Aspirin in Sprague-Dawley Rats.** Luisa Camacho¹, Camila Silva¹, Michelle Vanlandingham¹, Ralph Patton², Greg Olson², R. Paul Felton¹, Sherry Ferguson¹, Charles Law¹, Sibyl Swift³, Ali Abdel-Rahman³, Suzanne Fitzpatrick³. ¹US FDA/NCTR, NCTR, Jefferson, AR, The United States of America. ²TPA, Jefferson, AR, The United States of America. ³US FDA/CFSAN, CFSAN, College Park, MD, The United States of America
- P429-0905** **A Collaborative Effort to Establish Nonanimal Cosmetic Safety Assessment Globally by 2023.** Catherine Willett. *Humane Society International, Research and Toxicology, Washington, DC, The United States of America*
- P430-0949** **JSOH Evaluation of 1-Bromopropane Related to Setting an OEL.** Carr Smith. *Albemarle Corporation, Toxicology, Mobile, The United States of America*

Wednesday Program Schedule

Wednesday, July 17, 8:00 AM to 8:50 AM, Kalākaua Ballroom

Keynote Lecture: The National Center for Advancing Translational Sciences: Catalyzing Translational Innovation in Toxicology Research

Lecturer: Christopher P. Austin, MD, National Center for Advancing Translational Sciences.



The process by which observations in the laboratory or the clinic are transformed into useful interventions that tangibly improve human health is frequently termed “translation.” This multi-stage and multifaceted process is poorly understood scientifically, and the current research ecosystem is operationally not well suited to the distinct needs of translation. As a result, biomedical science is in an era of unprecedented accomplishment without a corresponding improvement in meaningful health outcomes.

To meet the opportunities and needs in translational science, NCATS was created as NIH’s newest component in December 2011. NCATS is scientifically and organizationally different from other NIH Institutes and Centers. It focuses on what is common to diseases and the translational process, and acts as a catalyst to bring together the teams necessary to develop new technologies and paradigms to improve the efficiency and effectiveness of the translational process. This talk will provide an overview of NCATS mission and programs, with a view toward future developments in toxicology research.

Christopher Austin is Director of the National Center for Advancing Translational Sciences (NCATS) at the US National Institutes of Health. NCATS’ mission is to catalyze the generation of innovative methods and technologies that will enhance the development, testing and implementation of diagnostics and therapeutics across a wide range of human diseases and conditions. Before becoming NCATS Director in September 2012, he was Director of the NCATS Division of Preclinical Innovation, which focuses on translating basic science discoveries into new treatments, particularly for rare and neglected diseases, and developing new technologies and paradigms to improve the efficiency of therapeutic and diagnostic development. In this role, he founded and directed numerous initiatives including the NIH Chemical Genomics Center (NCGC), the Therapeutics for Rare and Neglected Diseases (TRND) program, and the Toxicology in the 21st Century (Tox21) program. In 2016, Dr. Austin was elected chair of the International Rare Disease Research Consortium (IRDRC). Before joining NIH in 2002, Dr. Austin directed research programs genomics-based target discovery, pharmacogenomics, and neuropsychiatric drug development at Merck, with a particular focus on schizophrenia. Dr. Austin earned an AB in biology from Princeton University and an MD from Harvard Medical School. He completed clinical training in internal medicine and neurology at Massachusetts General Hospital, and a research fellowship in genetics at Harvard.

Wednesday, July 17, 9:00 AM to 11:00 AM, Room 316



SYM09: Clinical and Mechanistic Aspects of Drug-Induced Liver Injury

Chairperson(s): José E. Manautou, University of Connecticut; and Ann K. Daly, Institute of Cellular Medicine.

Drug-induced liver injury (DILI) continues to be prominent problem in drug development and patient care. Although our knowledge of DILI mechanisms is incomplete, multiple cellular events and their molecular mediators contributing to DILI have been defined. Despite multiple efforts, preclinical animal models do not always predict liver injury in humans. This has prompted investigators to develop new and better screening tools and to identify new genetic determinants of DILI earlier in the drug discovery process. This symposium will cover the various DILI risk factors relating to immune response, including HLA alleles, followed by the impact of drug transporters on compensatory hepatocellular regeneration following liver injury and the establishment of new animal models to study DILI by acyl glucuronide intermediates. We will conclude the symposium with a description of efforts by world-wide registries and multicenter networks in identifying mechanistic-based DILI biomarkers and the use of ‘omics approaches to the study DILI.

Abstract

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| S09-01 | 9:00 | Genetic Aspects of DILI: Recent Advances on Predicting Risk. Ann K. Daly, Institute of Cellular Medicine, Newcastle University, United Kingdom. |
| S09-02 | 9:30 | Application of Circulating MicroRNA to Diagnose Early Stage of Liver Injury and Its Pathogenesis in Rat DILI Model. Tsuyoshi Yokoi, Nagoya University Graduate School of Medicine, Japan. |
| S09-03 | 10:00 | Impact of Multidrug Resistance Protein 4 (MRP4/ABCC4) Function in Compensatory Hepatocellular Proliferation. José E. Manautou, University of Connecticut, The United States of America. |
| S09-04 | 10:30 | Clinical and Mechanistic Aspects of Drug-Induced Liver Injury. Fernando Bessone, University of Rosario School of Medicine, Argentina. |

Wednesday Program Schedule

Wednesday, July 17, 9:00 AM to 11:00 AM, Room 313

SYM10: Effects of Environmental Mixture Exposure on Brain Development and Function Based on Epidemiological, *In Vivo*, and *In Vitro* Studies

Chairperson(s): Anna Bal-Price, European Commission Joint Research Centre (JRC); and Oddvar Myhre, Norwegian Institute of Public Health, Department of Toxicology and Risk Assessment.

Epidemiological and experimental data suggest that pre- and postnatal exposure towards chemical mixtures affects brain development. In addition to genetic factors, such exposure might be causally linked to the recently increased prevalence of neurodevelopmental disorders (NDDs) like attention deficit/hyperactivity disorder, autism spectrum disorders and learning disabilities. Yet, there is a profound knowledge gap in understanding the chemical-related pathogenesis of NDDs. Therefore, novel approaches in human exposure assessment, *in vitro* and *in vivo* studies are needed to identify such causative factors. Speakers of this session will present from the scientific and regulatory point of views the most recent approaches (a) on evaluation and description of an alternative developmental neurotoxicity testing battery permitting (b) evaluation of an impact of mixture exposure on brain development using *in vitro* approaches, driven by key events identified in relevant adverse outcome pathways, as well as (c) a mixture risk assessment of combined human exposures to PBDEs and (d) identification of possible mechanisms of chemical mixtures as risk factors for NDDs based on epidemiological and *in vivo* studies. As NDDs have profound consequences for families and society, the identification of preventable risk factors such as environmental pollution exposure should be of high priority.

Abstract

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| S10-01 | 9:00 | Methods for Stage-Specific Developmental Neurotoxicity Testing with Human Stem-/Progenitor Cell-Based Models for Mixture Evaluation. Ellen Fritsche, IUF, Leibniz Research Institute for Environmental Medicine, Germany. |
| S10-02 | 9:30 | Adverse Outcome Pathways (AOPs)-Driven Evaluation of Developmental Neurotoxicity Induced by Mixture of Environmental Chemicals. Anna Bal-Price, European Commission Joint Research Centre (JRC), Italy. |
| S10-03 | 10:00 | Mechanisms of Environmental Pollutants Mixtures As Risk Factor for Attention Deficit/Hyperactivity Disorder and Autism Pathophysiology Based on Epidemiological and <i>In Vivo</i> Studies. Oddvar Myhre, Norwegian Institute of Public Health, Norway. |
| S10-04 | 10:30 | A Human Mixture Risk Assessment for Neurodevelopmental Toxicity Associated with Polybrominated Diphenyl Ethers Used as Flame Retardants. Andreas Kortenkamp, Brunel University London, College of Health and Life Sciences, United Kingdom. |

Wednesday, July 17, 9:00 AM to 11:00 AM, Room 312

SYM11: Immunotoxicity Associated with Exposure to Nanomaterials

Chairperson(s): Carolyn Vickers, World Health Organization; and Henk van Loveren, Maastricht University.

Due to their properties, nanomaterials have a propensity to interact with components of the immune system. In addition to direct effects on the immune system, interaction with the immune system may be relevant in the facilitation of cancer. The World Health Organization in 2018 released a new Environmental Health Criteria Document, titled Principles and methods for assessing the risk of immunotoxicity associated with exposure to nanomaterials. The symposium will present the WHO document, and review information on the mechanisms of immunotoxicity due to exposure to nanomaterials. Information on carbon nanotubes in a range of products will be provided as examples.

Abstract

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| S11-01 | 9:00 | WHO Principles and Methods for Assessing the Risk of Immunotoxicity Associated with Exposure to Nanomaterials. Henk van Loveren, Maastricht University, The Netherlands. |
| S11-02 | 9:30 | Occurrence of TiO₂ in Food, Supplements and Toothpaste, Resulting Exposure and Toxicokinetics. Minne Heringa, Centre for Safety of Substances and Products (VSP), The Netherlands. |
| S11-03 | 10:00 | Mechanisms and Consequences of Immune Interactions of Graphene-Based Materials. Bengt Fadeel, Karolinska Institutet, Sweden. |
| S11-04 | 10:30 | Immunotoxicity and Susceptibility Issues following Pulmonary Exposure to Carbon Nanotubes. James Bonner, North Carolina State University, The United States of America. |

Wednesday, July 17, 9:00 AM to 11:00 AM, Room 315

SYM12: The Carcinogenicity of Hexavalent Chromium: A Global Public and Environmental Health Concern

Chairperson(s): John Pierce Wise Sr., University of Louisville; and Kazuya Kondo, Tokushima University.

Hexavalent chromium [Cr(VI)] is a global environmental pollutant and a major public health concern. It is an established human lung carcinogen, particularly after inhalation exposure, and lung cancer remains the leading cause of cancer death around the world. It is clear that, while cigarette smoking is the most familiar cause of lung cancer, other agents cause a significant amount of the disease. For example, in the United States, of those diagnosed with lung cancer, 1 in 5 women and 1 in 12 men will never have smoked. Thus, understanding how agents other than cigarettes cause lung cancer is a significant need and a key aspect to combating the disease. While Cr(VI)

Wednesday Program Schedule

has been known to cause lung cancer for decades, its carcinogenic mechanisms are still poorly understood. Impacts on DNA repair, chromosome structure and number, cellular metabolism, inflammation, apoptosis, cell signaling, centrosome amplification, among others have all been implicated in its carcinogenicity along with epigenetic, mutagenic and aneugenic changes. This symposium will present current perspectives that provide deeper understanding of Cr(VI) carcinogenesis and directions towards solutions with presentations spanning a spectrum of ideas ranging from clinical to laboratory to risk assessment-based paradigms in order to better understand this important carcinogen.

Abstract

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| S12-01 | 9:00 | Genetic and Epigenetic Alterations of Lung Cancer in Workers with Chromate Exposure. <i>Kazuya Kondo, Tokushima University, Japan.</i> |
| S12-02 | 9:30 | Inflammation-Driven Cancer Stem Cells Formation as Promoter of Hexavalent Chromium Carcinogenesis. <i>Carmen Alpoim, University of Coimbra, Portugal.</i> |
| S12-03 | 10:00 | Mechanisms of Hexavalent Chromium-Induced Genomic Instability: How a Lung Carcinogen Breaks DNA and Inhibits Repair. <i>John Pierce Wise Sr., University of Louisville, The United States of America.</i> |
| S12-04 | 10:30 | Using the Latest Science in Cancer Risk Assessment for Hexavalent Chromium: Is It Time to Step Away from the Default Regulatory Approaches? <i>Deborah Proctor, ToxStrategies, Inc., The United States of America.</i> |

Wednesday, July 17, 11:00 AM to 12:30 PM, Room 312

Perspectives of Women in Toxicology: Promoting Career Development and Enhancing the Status of Women Toxicologists Around the World

Chairpersons: *Tao Wang, Coherus BioSciences, The United States of America; and Nursen Basaran, Hacettepe University, Turkey.*

Panelists: *Nursen Basaran, Hacettepe University, Turkey, Betzabet Quintanilla-Vega, CINVESTAV IPN, Mexico, Hanan Ghantous, US Food and Drug Administration, The United States of America, Mary Gulumian, University of Witwatersrand, South Africa, Brinda Mahadevan, Abbott Healthcare Pvt, India, Tao Wang, Achaogen, Inc, The United States of America.*

Although the global share of women in research has increased in the last several decades, women remain underrepresented in many areas of science including toxicology. This is the case not only in developing countries but also in many developed nations. The panel speakers will share the status of women scientists in the field of toxicology in different regions around the globe; describe the challenges faced and opportunities undertaken for career advancement; and highlight successful stories on enhancing recognition of women toxicologists. In addition, available resources for women toxicologists to advance in the field of toxicology will be shared. The panel is aimed to increase awareness of the successes and advances of women in the field of toxicology and impart their experiences on how to further enhance the status of women toxicologists in the different societies.

Wednesday, July 17, 12:30 PM to 1:20 PM, Room 317A

Find Your Mentor

 *Separate registration required for limited participants.*

Chairpersons: *Brinda Mahadevan, Abbot Laboratories, India; and Patrick Allard, University of California Los Angeles, The United States of America.*

This activity seeks to provide attendees with the chance to secure a virtual mentor. Understanding that a large number of participants reside in areas other than continental US, it is critical that this venue serves as a conduit to connect individuals of different countries with mentors who could guide and advise in multiple areas of their professional/personal development.

Wednesday, July 17, 12:00 Noon to 6:30 PM, Exhibit Hall

Exhibit Hall Open

Wednesday Program Schedule

Wednesday, July 17, 2:30 PM to 4:30 PM, Room 313

SYM13: Acute, Chronic, and Long-Term Pesticide Effects: Mechanisms of Actions, Epidemiological Data, Priority Areas for Research

Chairperson(s): Aristidis Tsatsakis, University Crete; and Emanuela Corsini, University of Milano.

Pesticides have been, wrongly or reasonably, associated with increased prevalence of several pathological conditions. Among these, behaviour, dementia and Parkinson's disease, and cancer, represent particularly relevant public health issues and specific group of pesticide, in particular organ chlorinated compounds, are considered as high concern group. Acute pesticide poisoning still remain an outstanding issue, but data collection, evaluation and interpretation is quite difficult due to a significant underreporting. This workshop aims at critically evaluating the existing evidence on these issues and at identifying priority areas and approaches for research. Each speaker will develop a systematic review on the assigned topic, will show the results and will highlight evidence and doubts. A 15-minute slot at the end of the symposium will be addressed at the discussion of the presentations. The authors have committed themselves to prepare a paper on these topics, to be published in an international journal.

Abstract

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| S13-01 | 2:30 | Pesticide Exposure, Human Behavior, Parkinson's Disease and Dementia: Evidences, Uncertainties, Open Points. Claudio Colosio, Department of Health Sciences of the University of Milano and International Centre for Rural Health of the San Paolo Hospital of Milano, Italy. |
| S13-02 | 3:00 | Pesticides and Cancer: Current Knowledge and Epidemiological Evidence. Martin Wilks, Swiss Centre for Applied Human Toxicology, Switzerland. |
| S13-03 | 3:30 | Challenges in Monitoring and Biomonitoring of Pesticides. Aristidis Tsatsakis, University Crete, Greece. |
| S13-04 | 4:00 | Acute Pesticide Poisoning and the Effect of Pesticide Regulation. Won Jin Lee, Korea University College of Medicine, The Republic of Korea. |

Wednesday, July 17, 2:30 PM to 4:30 PM, Room 312

SYM14: Collaborative Approaches to Proactively Advance 3Rs in Nonclinical Drug Development

Chairperson(s): Thomas Monticello, Amgen; and Ian Pyrah, Seattle Genetics.

Animal toxicology studies are conducted during drug development to ensure human safety, identify potential organs of toxicity and aid in establishing a clinical starting dose. The conduct of animal toxicology studies is based on historical precedence and regulatory guidance, centered on the assumption that the animal model of choice and the toxicology study provide value in identifying potential human hazards and help ensure patient safety. The 3R approaches are designed to advocate for the replacement of animals with alternative models, the reduction of animal use by improvements in study designs that minimize animal numbers and optimize the information gained per animal, and refinement to minimize distress or harm in situations where the use of animal testing is unavoidable. This session will present proactive considerations of implementing the 3Rs in nonclinical safety assessment that will begin with the overview of the current state of the predictive value of animal studies, including safety pharmacology studies. Opportunities to optimize study designs to incorporate other regulatory mandated endpoints, considerations in species selection and inclusion of recovery animals, approaches to an 'animal free' drug development, and finally, opportunities to replace animal testing by implementing microphysiological system technologies (aka 'organ on a chip'), will also be presented.

Abstract

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| S14-01 | 2:30 | Setting the Bar for Positive and Negative Animal Predictive Values: The IQ Translational Database. Thomas Monticello, Amgen, The United States of America. |
| S14-02 | 3:00 | Applying the 3Rs within Regulatory Toxicology Studies in Drug Development. Helen Prior, National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), United Kingdom. |
| S14-03 | 3:30 | Animal-Free INDs and CTAs: A Reality or Dream? Michael W. Leach, Pfizer, The United States of America. |
| S14-04 | 4:00 | Strategic Application of Microphysiological Systems in Safety Assessment to Decrease Dependence on Animal Studies. Brian Berridge, National Toxicology Program, National Institute of Environmental Health Sciences, The United States of America. |

Wednesday, July 17, 2:30 PM to 4:30 PM, Room 315

SYM15: Empowering Toxicology and Environmental Health Research to Address Disasters and Emerging Threats

Chairperson(s): Scott Masten, National Institute of Environmental Health Sciences; and Shoji F. Nakayama, National Institute for Environmental Studies.

Disasters including the World Trade Center attack, Great Eastern Japan Earthquake, Gulf Oil Spill, and recent tropical storms, wildfires and volcanic eruptions have revealed the dire need for improved ability to perform rapid data collection and research to address health-related concerns. The collection of human exposure and health data in the immediate aftermath of public health emergencies and disasters is essential for ensuring situational awareness, shaping the public health response, identifying health research needs, supporting recovery efforts and enhancing future preparedness. Yet the initiation of public health research and response is often delayed by a host of

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long-standing logistical, process, and regulatory barriers. This session will provide an overview of the critical importance and need for timely toxicology and environmental health research in response to disasters. Speakers will discuss challenges in understanding human health implications and addressing public concerns for recent disasters, highlight research responses to specific large-scale disasters and describe evolving programs to develop improved disaster research response capacity. The session will encourage discussion of the challenges and potential strategies that will enable the conduct of time-critical human health assessments to support decision-making.

Abstract

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| S15-01 | 2:30 | Addressing Toxicology and Human Health Concerns with the NIH Disaster Research Response (DR2) Program. <i>Linda Birnbaum</i> , National Institute of Environmental Health Sciences and National Toxicology Program, The United States of America. |
| S15-02 | 3:00 | Disaster Response Research Development in Japan. <i>Shoji F. Nakayama</i> , National Institute for Environmental Studies, Japan. |
| S15-03 | 3:30 | When Disaster Strikes: Response, Research, and Recovery. <i>Ivan Rusyn</i> , Texas A&M University, The United States of America. |
| S15-04 | 4:00 | Addressing Public Health Hazards Arising from Recent Threats and Disasters in California. <i>Lauren Zeise</i> , Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, The United States of America. |

Wednesday, July 17, 2:30 PM to 4:30 PM, Room 316



SYM16: Out of the Box: Contribution of the Immune System to Organ Toxicity

Chairperson(s): *Emanuela Corsini, Università degli Studi di Milano; and Nurşen Başaran, Hacettepe University.*

Inflammation is known to play a prominent role in several human diseases and is a common response to many stressors, including chemicals. Under normal conditions, inflammation is an adaptive process that contrast infections and is involved in tissue damage repair. However, chemicals can elicit prolonged, severe, and inappropriate inflammatory responses that play a central role in a variety of target organ toxicities. The purpose of this symposium is to link immunotoxicity to target organ toxicity, showing the Janus face of the inflammatory reaction and of the immune system.

Abstract

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| S16-01 | 2:30 | Early-Life Exposure to Immunotoxic Compounds and Later-in-Life Diseases. <i>Rodney Reynolds Dietert</i> , Cornell University, The United States of America. |
| S16-02 | 3:00 | Role of the Immune System in Drug-Induced Liver Injury. <i>Tsuyoshi Yokoi</i> , Nagoya University Graduate School of Medicine, Japan. |
| S16-03 | 3:30 | Role of Inflammation in Chemical-Induced Kidney Damage. <i>David J. Nikolic Paterson</i> , Department of Nephrology, Australia. |
| S16-04 | 4:00 | Particles, Fibres, and Lung Toxicity: Effects Beyond the Lung. <i>Flemming R. Cassee</i> , Institute for Risk Assessment Sciences, Utrecht University, The Netherlands. |

Wednesday, July 17, 4:30 PM to 6:30 PM, Exhibit Hall

Poster Session 2 and Refreshments

See next page for the Poster Session 2 Index.

Wednesday, July 17, 7:00 PM to 10:00 PM, Convention Center Rooftop Garden

 *Separate registration required.*

Gala Dinner and Reception

Enjoy a Luau feast and a performance by the Halau Hula Olan dance troupe and instrumental trio.

- P101-0046** **Trace Element Concentrations in Atmospheric (PM₁₀) Particles from la Habana, Cuba.** Yoagne Traperero Quintana¹, Braulio Jimenez², Michel Manduca³. ¹*Instituto de Farmacia y Alimentos, La Lisa, La Habana, Cuba.* ²*Center of Environmental and Toxicological Research, San Juan, Puerto Rico.* ³*Instituto Superior de Tecnologías y Ciencias Aplicadas, Plaza, Cuba*
- P102-0053** **Effect of the Exposure to Ambient Particulate Matter on Striatal Dopamine Transmission and Motor Activity.** María de los Angeles Andrade Oliva, Yamin Debray García, Juan Escamilla Sanchez, Marisela Uribe Ramírez, Benjamin Floran, José Antonio Arias Montañó, Andrea De Vizcaya Ruiz. *Center for Research and Advanced Studies (Cinvestav), Mexico City, Mexico*
- P103-0097** **Serum Clara Cell Protein as a Pulmonary Damage Biomarker for Fine Particulate Matter Exposure in Chinese Population.** Yanhua Wang¹, Zhenjie Wang¹, Ting Wang¹, Nan Wu², Huawei Duan¹. ¹*National Institute for Occupational Health and Poison Control, Beijing, China.* ²*China Medical University School of Public Health, Shenyang, Liaoning, China*
- P104-0141** **Estimates of the Global Burden of Meteorological Factors and Air Pollution on Hand, Foot, and Mouth Disease (HFMD) Infectious among Children.** Ruixue Huang, Li He. *Central South University, Changsha, Hunan Province, China*
- P105-0239** **AhR-Mediated Regulation of SOCS3-STAT3 Axis Is Involved in Downregulation of B[a]P-Induced Retinal Lesion.** Chi-Hao Tsai¹, Yi Lee¹, Yu-Wen Cheng², Jaw-Jou Kang³. ¹*National Taiwan University, Taipei, Taiwan.* ²*Taipei Medical University, Taipei, Taiwan.* ³*National Yang-Ming University, Taipei, Taiwan*
- P106-0268** **Cytotoxicity of Ultrafine Diesel Exhaust Particles in Endothelial and Microglial Cell Monocultures and Mixed Coculture.** Grace Aquino, Erica Bruce, Amjad Dabi. *Baylor University, Waco, TX, The United States of America*
- P107-0380** **Ambient Fine Particulate Matter Induced Pulmonary Vascular Lesions in Mice and Its Cellular Mechanisms.** Pinpin Lin¹, Chia-Chi Ho¹, Yu-Cheng Chen¹, Ming-Hsien Tsai¹, Hui-Ti Tsai¹, Chen-Yi Weng¹. ¹*National Health Research Institutes, Zhunan, Taiwan*
- P108-0419** **The Hematological Effects of Airborne Fine Particles via the Interaction of the Kallikrein-Kinin, Complement, and Coagulation Systems** Xiaoting Jin, Qunfang Zhou, Guibin Jiang. *Chinese Academy of Sciences, Beijing, China*
- P109-0429** **Evaluation of Long-Term Exposure to Air Pollution in the Development of Systemic Lupus Erythematosus in NZBWF1 Mice.** Victor Yariwake¹, Janaina Torres¹, Amandda Silva², Sylvia Farhat¹, Niels Camara^{1,2}, Mariana Veras¹. ¹*University of São Paulo, São Paulo, Brazil.* ²*Federal University of São Paulo, São Paulo, Brazil*
- P110-0446** **Health Effects and Climate Change Caused by Fossil Fuels Compared with Other Energy Sources.** John Whysner. *Washington Occupational Health Associates, Inc., Toxicology, Santa Fe, NM, The United States of America*
- P111-0496** **Comparative Study on Genotoxic Activity of Different Components of Fine Particulate Matter (PM_{2.5}) Using In Vitro Bioassays.** Jing Yang, Xiaoqian Chen, Haowen Yin, Ying Zhang, Min Liu, Lijuan Jia, Yingzhi Wang. *Bioassay and Safety Assessment Laboratory, Shanghai Academy of Public Measurement, Shanghai, China*
- P112-0497** **Role of Calcium-Related Signaling Pathway in Endothelial Cell Permeability Induced by PM_{2.5}.** Yan Wang, Meng Tang. *Southeast University, Nanjing, Jiangsu, China*
- P113-0524** **Sulfur Dioxide Monitoring Associated with the. ²018 Kilauea Lower East Rift Zone Eruption.** Diana Felton¹, G. Grange¹, David Damby², Alvin Bronstein³, Dan Spyker⁴. ¹*Hawaii Department of Health, Honolulu, HI, The United States of America.* ²*United States Geological Survey, Menlo Park, CA, The United States of America.* ³*Hawaii Department of Health, Honolulu, HI, The United States of America.* ⁴*Oregon Health and Science University, Portland, OR, The United States of America*
- P114-0552** **Transcriptomic Alterations Induced by Air Pollution-Derived PM_{2.5} Reflect the Shift from Healthy to COPD-Diseased Human Bronchial Epithelium.** Sébastien Anthérieu¹, Bérénice Leclercq¹, Laurent Yves Alleman², Esperanza Perdrix², Patrice Coddeville², Jean-Marc Lo-Guidice¹, Guillaume Garçon¹. ¹*Univ. Lille, CHU Lille, Institut Pasteur de Lille, EA4483-IMPECS (IMPact of Environmental Chemicals on human health), Lille, France.* ²*IMT Lille Douai, Univ. Lille, SAGE, Lille, France*
- P115-0573** **Time Course of Olfactory Epithelium Damages after Cessation of Inhalation Exposure to. ²-Ethyl-1-Hexanol in Mice.** Mio Miyake¹, Yuki Ito, Naoko Oya, Hirotaka Sato, Takanari Wakayama, Michihiro Kamijima. *Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan*

- P116-0607** **Naphthalene-Increased CYP2F1 Expression Is Mediated via Activation of NF- κ B Signaling Pathway in Retina Pigment Epithelium Cell.** Yi-Shiou Wei¹, Chi-Hao Tsai¹, Yu-Wen Cheng², Jaw-Jou Kang³. ¹National Taiwan University, Taipei, Taiwan. ²Taipei Medical University, Taipei, Taiwan. ³National Yang-Ming University, Taipei, Taiwan
- P117-0622** **Dynamic Ozone-Induced Gene Expression Changes in Lung, Liver, and Adrenals during Early Time Point and Adaptation: The Influence of Long-Term Glucocorticoid Pretreatment.** Andres Henriquez¹, Samantha Snow², John House³, Mette Schladweiler², Colette Miller², Urmila Kodavanti². ¹Oak Ridge Institute for Science and Education, Oak Ridge, TN, The United States of America. ²US EPA/NHEERL, Research Triangle Park, NC, The United States of America. ³North Carolina State University Bioinformatics Research Center, Raleigh, NC, The United States of America
- P118-0661** **Mitochondrial Oxidative Stress and Energy Metabolism Disruption Are Involved in COF-Induced Hepatotoxicity.** Youqiong Xu, Xiaoyang Zhang, Meiling Zhou. Fuzhou Center for Disease Control and Prevention, Fuzhou, Fujian, China
- P119-0758** **Hazard Identification, Exposure Assessment and *In Vitro* Toxicity Assessment of Dust Emissions from South African Gold Mine Tailings Sites.** Charlene Andraos¹, Mary Gulumian^{1,2}. ¹National Institute for Occupational Health, Johannesburg, Gauteng, South Africa. ²University of the Witwatersrand, Johannesburg, Gauteng, South Africa
- P120-0769** **Prenatal Urinary Polycyclic Aromatic Hydrocarbon Metabolites, Global DNA Hydroxymethylation, and Neurodevelopment at 2–3 Years of Age.** Jisheng Nie¹, Jinyu Li¹, Qiao Niu¹, Deliang Tang². ¹Shanxi Medical University, Taiyuan, Shanxi, China. ²Columbia University, New York, NY, The United States of America
- P121-0811** **Organic Particulate Matter 10 (PM₁₀) in Transported African Dust at an Urban and Rural Town in Puerto Rico: *In Vitro* Implications to the Etiological and Geographical Assessment of Asthma** Fernando Isart. University of Puerto Rico, San Juan, PR, The United States of America
- P122-0875** **Military Working Dogs: Unlocking the Mystery of Deployment-Related Exposures and Health.** Terra Vincent¹, Erin Dursa², Sheldon Waugh³, Sara Mullaney³. ¹Environmental Health Program, Post-Deployment Health Services, Department of Veterans Affairs, Washington, DC, The United States of America. ²Epidemiology Program, Post-Deployment Health Services, Department of Veterans Affairs, Washington, DC, The United States of America. ³One Health Division, Veterinary Services and Public Health Sanitation Directorate, US Army Public Health Center, Aberdeen Proving Ground, MD, The United States of America
- P123-0893** **Levels of Trace Elements, PCDD/Fs, and PCBs in the Surroundings of a Municipal Solid Waste Incinerator: Assessing Health Risks.** Montse Marquès, Joaquim Rovira, José Luis Domingo, Martí Nadal. URV-IISPV, Reus, Spain
- P124-0915** **Traffic Road Dust–Derived Particulate Matter Aggravates Allergic Inflammation.** Hyun Soo Lee¹, Sehyun Han², Ki-Joon Jeon². ¹Catholic University of Korea College of Medicine, Seoul, The Republic of Korea. ²Inha University, Incheon, The Republic of Korea
- P125-0916** **Methylation Profiles in Newborns from Diverse Air Polluted Localities in the Czech Republic.** Katerina Honkova, Kristyna Vrbova, Andrea Rossnerova, Radim Sram, Jan Topinka. Institute of Experimental Medicine CAS, Prague, The Czech Republic
- P126-0917** **Outdoor Physical Exercise and Health Implications.** Joana Madureira^{1,2}, Emerson Antonio Brancher^{3,4}, Ricardo Aurino de Pinho⁵, João Paulo Teixeira^{1,2}. ¹EIPUnit, Instituto de Saúde Pública, Universidade do Porto, Porto, Portugal. ²National Institute of Health, Porto, Portugal. ³Universidade do Extremo Sul Catarinense, Criciúma, Brazil. ⁴Universidade Regional de Blumenau, Blumenau, Santa Catarina, Brazil. ⁵School of Medicine, Pontifícia Universidade Católica do Paraná, Curitiba, Paraná, Brazil
- P127-0082** **Ether-Phosphatidylcholine Characterized by Consolidated Plasma and Liver Lipidomics Is a Predictive Biomarker for Valproic Acid–Induced Hepatic Steatosis.** Keisuke Goda¹, Kosuke Saito², Kyotaka Muta¹, Akio Kobayashi¹, Yoshiro Saito², Shoichiro Sugai¹. ¹Japan Tobacco Inc., Hadano, Kanagawa, Japan. ²National Institute of Health Sciences, Kawasaki, Kanagawa, Japan
- P128-0083** **Evaluation of Exosomes as Toxic Biomarkers.** Ryuichi Ono¹, Yusuke Yoshioka², Yusuke Furukawa¹, Takahiro Ochiya^{2,3}, Satoshi Kitajima¹, Yoko Hirabayashi¹. ¹National Institute of Health Sciences, Kawasaki, Kanagawa, Japan. ²National Cancer Center Research Institute, Tokyo, Japan. ³Tokyo Medical University, Tokyo, Tokyo, Japan

- P129-0201** **Comparison of Modeled Internal Exposures to Di(2-ethylhexyl)phthalate from Foods and Cosmetics with Urinary Metabolite Concentrations—Results from the EuroMix Biomonitoring Study.** Trine Husøy¹, Maria Martínez², Vikas Kumar², Monica Andreassen¹, Friederike Sonnet¹, Amrit Sakhi¹, Hubert Dirven¹. ¹Norwegian Institute of Public Health, Oslo, Norway. ²Universitat Rovira i Virgili, Tarragona, Spain
- P130-0202** **Exposure to a Mixture of Phthalates as Measured in. ²⁴-Hour Urine and Associations with Food Intake and Use of Personal Care Products—Results from the EuroMix Biomonitoring Study.** Trine Husøy¹, Monica Andreassen¹, Hege Hjertholm¹, Monica Carlsen², Amrit Sakhi¹, Azemira Sabaredzovic¹, Hubert Dirven¹. ¹Norwegian Institute of Public Health, Oslo, Norway. ²University of Oslo, Oslo, Norway
- P131-0241** **P21 Might Be a Potential Early Toxicity Biomarker of DNA Damage and Hematopoietic Depression by Benzene Exposure.** Bosheng Wang¹, Hong Zhang¹, Xiaoqin Li², Yanhua Zhou¹, Tong Wang¹, Mengying Zhang¹, Rongli Sun¹, Kun Wang¹, Wu Jin², Lihong Yin¹, Yuepu Pu¹, Juan Zhang¹. ¹Southeast University, Nanjing, Jiangsu, China. ²Yangzhou Center for Disease Control and Prevention, Yangzhou, Jiangsu, China
- P132-0282** **Molecular Mechanism of Low Expression of miR-486-5p in Lung Squamous Cell Carcinoma.** Sheng Yang, Geyu Liang, Wenjuan Wu, Jing Sui, Tong Liu, Yanping Cheng, Lihong Yin, Yuepu Pu. Key Laboratory of Environmental Medicine Engineering, Ministry of Education, Southeast University, Nanjing, Jiangsu, China
- P133-0292** **8-Hydroxyguanine as an Oxidative Stress Biomarker in Saliva.** Kazuaki Kawai, Yuya Kawasaki, Yun-Shan Li, Shintaro Watanabe, Hiroshi Kasai. University of Occupational and Environmental Health, Japan, Kitakyushu, Fukuoka, Japan
- P134-0338** **Biomarkers of Acute Toxicity by Carbon Monoxide in a Cohort Study of Patients in Argentina.** Analia Cortez^{1,2}, Rocío Galarza³, Carlos Damin^{1,2}, Alicia Faletti^{1,3}. ¹Primera Cátedra de Toxicología, Ciudad A. de Buenos Aires, Buenos Aires, Argentina. ²Hospital General de Agudos Juan A. Fernández, Ciudad A. de Buenos Aires, Buenos Aires, Argentina. ³Centro de Estudios Farmacológicos y Botánicos (CEFyBO)-CONICET, Facultad de Medicina-Universidad de Buenos Aires, Buenos Aires, Argentina
- P135-0351** **The Potential Mechanisms of Cytotoxicity Induced by Halobenzoquinone Water Disinfection Byproducts.** Jinhua Li¹, Xing-Fang Li². ¹School of Public Health, Jilin University, Changchun, Jilin, China. ²University of Alberta, Edmonton, Alberta, Canada
- P136-0371** **Subclinical Nephrotoxicity Associated with Chelation Therapy in Young Thalassemia Patients: The Role of Urinary Kidney Injury Molecule.** Ola Nafea, Marwa Zakaria, Tamer Hassan, Sherif Elgebaly, Hosam Salah. Zagazig University, Zagazig, Egypt
- P137-0394** **The Effects of Indirect Toxicity to Vascular Endothelial Dysfunction by Respiratory Exposure to Single-Walled Carbon Nanotubes.** Zhuge Xi, Jun Yan, Tie Han, Xiaohua Liu, Bencheng Lin. Tianjin Institute of Environmental and Operational Medicine, Tianjin, China
- P138-0424** **Effect of Estrogen on Tumor Progression in Gastric Cancer Cells—Derived Xenograft Models.** Joohee Jung, Kyoung Mee Kim, Sun Yi Lee. Duksung Women's University, Seoul, The Republic of Korea
- P139-0428** **AdductomicsPT: A New Mass Spectrometry–Based Adductomics Pipeline for Toxicology Studies.** Alexandra Antunes¹, João Nunes¹, Catarina Charneira¹, Sofia Gouveia-Fernandes^{2,3}, Jacinta Serpa^{2,3}, Judit Morello¹. ¹Instituto Superior Técnico, ULisboa, Lisboa, Portugal. ²Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal. ³Instituto Português de Oncologia de Lisboa Francisco Gentil, Lisboa, Portugal
- P140-0494** **ASB15 and RBM45, the Novel Target Genes of HIF-1α Involved in Benzene Hematopoietic Toxicity.** Zhaodi Man, Fengxia Sun, Yunqiu Pu, Shuangbin Ji, Kai Xu, Rongli Sun, Lihong Yin, Juan Zhang, Yuepu Pu. Southeast University, Nanjing, Jiangsu, China
- P141-0530** **The Concept of “Signal Toxicity” for the Mechanistic Analysis of So-Called Low Dose Effect and Delayed Effect after Perinatal Exposure.** Jun Kanno^{1,2}, Ken-ichi Aisaki², Satoshi Kitajima², Kentaro Tanemura³. ¹Japan Bioassay Research Center, Japan Organization of Occupational Health and Safety, Hadano, Kanagawa, Japan. ²National Institute of Health Sciences, Kawasaki, Kanagawa, Japan. ³Tohoku University, Sendai, Miyagi, Japan
- P142-0600** **Physiological Biomarkers of Low-Level VX Exposure in the New Zealand White Rabbit.** Michael Horsmon¹, Nicole Vincelli¹, Chelsea Kotlowski², Shelby Roe², Theodore Moran¹. ¹US Army Chemical Biological Research and Development Center, Gunpowder, MD, The United States of America. ²Excet Inc., Springfield, VA, The United States of America

- P143-0603** **aProximate Proximal Tubule Model as a Predictive Model of Aminoglycoside Nephrotoxicity Using Clinical Relevant Biomarkers of Toxicity.** Colin Brown¹, Mike Nicholds¹, Git Chung¹, Keith Pye¹, Lyle Armstrong¹. ¹*Newcells Biotech, Newcastle upon Tyne, United Kingdom*
- P144-0712** **2-Alkenal Modification of Hemoglobin: Identification of a Novel Hemoglobin-Specific Alkanoic Acid-Histidine Adduct.** Jun Yoshitake¹, Takahiro Shibata¹, Koji Uchida². ¹*Nagoya University, Nagoya, Japan.* ²*The University of Tokyo, Tokyo, Japan*
- P145-0755** **Antioxidant Enzymes and BDNF as Biomarkers of Oxidative Stress–Induced Neurodegeneration Such as Amnesia.** Mee Ree Kim. *Chungnam National University, Daejeon, The Republic of Korea*
- P146-0776** **The Effect of Betel Quid Chewing on Smoking-Induced Oxidative Stress.** Hsiao Tung Ko¹, Guor-Jien Wei¹, Hsiang-Tsui Wang², Han-Hsing Tsou³, Tsung-Yun Liu¹. ¹*National Yang-Ming University Institute of Food Safety and Health Risk Assessment, Taipei, Taiwan.* ²*National Yang-Ming University Institute of Pharmacology, Taipei, Taiwan.* ³*National Yang-Ming University Institute of Environmental and Occupational Health Science, Taipei, Taiwan*
- P147-0850** **A Toxicology MOOC (Massive Online Open Course) for Different Learning Needs.** Rosa Laura Vicente Vicente^{1,2}, Marta Prieto^{1,2}, Moises Pescador¹, Alfredo Casanova^{1,2}, María Teresa Hernández-Sánchez^{1,2}, Ana Isabel Morales-Martín^{1,2}. ¹*University of Salamanca, Salamanca, Spain.* ²*Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain*
- P148-0856** **Identification of Protein Markers Predictive of Adverse Effects Due to Chemical Mixture Exposures.** Yue Ge, Maribel Bruno, Aimen Farraj, Brian Chorley. *US EPA, Durham, NC, The United States of America*
- P149-0868** **A New Biomarker Capable to Predict Nephrotoxicity Caused by Contrast Media in Cardiological Patients.** María Teresa Hernández-Sánchez^{1,2}, Ignacio Cruz-González^{2,3}, Rosa Laura Vicente Vicente^{1,2}, Marta Prieto^{1,2}, Alfredo Casanova^{1,2}, Moises Pescador¹, Francisco Lopez-Hernandez^{1,2}, Ana Isabel Morales-Martín^{1,2}. ¹*University of Salamanca, Salamanca, Spain.* ²*Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain.* ³*University Hospital of Salamanca, Salamanca, Spain*
- P150-0869** **Correlation between Tobacco Use and Biomarkers of Early Kidney Damage.** Alfredo Casanova^{1,2}, Francisco J. Sanz-Parras³, Marta Prieto^{1,2}, Rosa Laura Vicente Vicente^{1,2}, María Teresa Hernández-Sánchez^{1,2}, Moises Pescador¹, Ana Isabel Morales-Martín^{1,2}. ¹*University of Salamanca, Salamanca, Spain.* ²*Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain.* ⁴*Servicio Extremeño de Salud, Cáceres, Spain*
- P151-0874** **Toxicity of CMIT/MIT, a Humidifier Disinfectant on Eye-Dryness and Metabolomic Profile Using ¹H-NMR Metabolomics in Rats.** Jung Dae Lee¹, Jin ju Park², Jueng Eun Im², Hyang Yeon Kim², Soo bean Oh², Hyeyoon Goo², Kyong Jin Cho², Suhkmann Kim³, Byung-Mu Lee¹, Kyu-Bong Kim². ¹*Sungkyunkwan University, Suwon, Gyeonggi-do, The Republic of Korea.* ²*Dankook University, Cheonan, Chungnam, The Republic of Korea.* ³*Dankook University, Cheonan, Chungnam, The Republic of Korea.* ⁴*Pusan National University, Busan, The Republic of Korea*
- P152-0900** **An In Vitro Analysis of PPARα and CYP4A as Potential Biomarkers for Perfluoroalkyl Acid Exposure in Cetacean Kidney Cells.** Kaylee Kautz¹, Jennette VanderJagt¹, Jennifer Lynch^{2,3}, Brenda Jensen¹, Yongli Chen¹. ¹*Hawaii Pacific University, Kaneohe, HI, The United States of America.* ²*National Institute of Standards and Technology, Waimanalo, HI, The United States of America.* ³*Hawaii Pacific University, Waimanalo, HI, The United States of America*
- P153-0920** **Betel Quid Containing Safrole Enhances Metabolic Activation of Tobacco-Specific. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK).** Hsiang-Tsui Wang¹, Han-Hsing Tsou², Hsiao Tung Ko³, Chia-Tzu Chen², Tse-Wen Wang³, Chien-Hung Lee⁴, Tsung-Yun Liu³. ¹*National Yang-Ming University, Taipei, Taiwan.* ²*National Yang-Ming University Institute of Environmental and Occupational Health Sciences, Taipei, Taiwan.* ³*National Yang-Ming University Institute of Food Safety and Health Risk Assessment, Taipei, Taiwan.* ⁴*Kaohsiung Medical University, Kaohsiung, Taiwan*
- P154-0947** **Urine Metabolomics of a Standardised *Andrographis paniculata* Aqueous Extract in a Rat Model of Neuroinflammation.** Dahiru Sani¹, Johnson Stanslas², Hamidon Basri², Brian Kirby³. ¹*Ahmadu Bello University, Zaria, Kaduna, Nigeria.* ²*Universiti Putra Malaysia, Serdang, Selangor, Malaysia.* ³*Royal College of Surgeons in Ireland, Dublin, Ireland*
- P155-0017** **Epigenetic Mechanism of Hexavalent Chromium–Induced Cell Transformation, Cancer Stem Cell–Like Property, and Tumorigenesis.** Chengfeng Yang, Zhishan Wang. *University of Kentucky, Lexington, KY, The United States of America*
- P156-0019** **Arsenic and Benzo(a)pyrene Co-exposure Synergizes in Inducing Cancer Stem Cell–Like Property, Cell Malignant Transformation, and Lung Tumorigenesis in Mice.** Zhishan Wang, Chengfeng Yang. ¹*University of Kentucky, Lexington, KY, The United States of America*

- P157-0042** **Ne-carboxymethyl-lysine, a Major Member of Advanced Glycation End Products, Promotes Tumor Malignancy of Breast Cancers via Activation of RAGE/SIRT6 Axis *In Vitro* and *In Vivo*.** Chen-Yuan Chiu¹, Chung-Jung Wen², Shing-Hwa Liu³. ¹*Institute of Food Safety and Health, National Taiwan University, Taipei, Taiwan.* ²*National Taiwan University Hospital Department of Geriatrics and Gerontology, Taipei, Taiwan.* ³*Institute of Toxicology, National Taiwan University, Taipei, Taiwan*
- P158-0119** **Seed Oils of *Centranthus ruber* and *Valeriana officinalis* Contain Conjugated Linolenic Acids and Have a Cytotoxic Effect on Cancer Cells.** Taro Honma¹, Ikumi Sato¹, Nobuhiro Shiratani², Yuki Banno², Tsutomu Takayanagi², Kayoko Kita¹, Toshihide Suzuki¹. ¹*Teikyo University, Itabashi-ku, Tokyo, Japan.* ²*Tokyo University of Technology, Hachioji, Tokyo, Japan*
- P159-0156** **Inhibitory Effect of Sesamin on CYP1A1 and CYP1B1 Expression in Breast Cancer Cells via Prevention of Aryl Hydrocarbon Receptor.** Thi Hoa Pham, Sun Woo Jin, Hye Gwang Jeong. *Chungnam National University, Daejeon, The Republic of Korea*
- P160-0196** **Adaptive Response of Cadmium with Decreasing Concentrations to Apoptosis in Human Myelomonocytic Lymphoma U937 Cells and Its Molecular Mechanism.** Hidekuni Inadera, S. A. Zakki, Lu Sun, Qian-Wen Feng, Meng-Ling Li, Zheng-Guo Cui. *University of Toyama, Toyama, Toyama, Japan*
- P161-0210** **Nrf2 Accumulation and PI3K Akt Activation upon Exposure to Arsenate Bring about Autophagy Deficiency through Novel Signal Transduction Pathway in Human Keratinocyte Transformation.** Qianlei Yang¹, Jing Wu¹, Yiping Ni¹, Jiayuan Mao¹, Yasuyo Shimoda², Xuerui Zhu¹, Shasha Tao¹, Koichi Kato², Kenzo Yamanaka², Jie Zhang¹, Yan An¹. ¹*School of Public Health, Medical College of Soochow University, Suzhou, Jiangsu, China.* ²*Nihon University School of Pharmacy, Funabashi, Chiba, Japan*
- P162-0215** **CYP1B1 Induces XIAP Stability and Activity through Phosphorylation of PKC-ε in Human Breast Cancer Cells.** Hyoung-Seok Baek, Yeo-Jung Kwon, Dong-Jin Ye, Tae-Uk Kwon, Young-Jin Chun. *Chung-Ang University, Seoul, The Republic of Korea*
- P163-0264** **Improving Formalin-Fixed Paraffin-Embedded (FFPE) Clinical Samples for Variant Calling and Mutant Detection.** Leah Wehmas¹, Charles Wood², Ping Guan³, Helen Moore³, Carole Yauk⁴, Lauren Peel⁵, Mark Gosink⁶, Susan Hester¹. ¹*US EPA, Durham, NC, The United States of America.* ²*Boehringer Ingelheim, Ridgefield, CT, The United States of America.* ³*National Cancer Institute, Bethesda, MD, The United States of America.* ⁴*Health Canada, Ottawa, Ontario, Canada.* ⁵*HESI, Washington, DC, The United States of America.* ⁶*Pfizer, Groton, CT, The United States of America*
- P164-0355** **Zearalenone Promotes Colon Cancer Cell Proliferation via GPER.** Kwun Kwan Lo, Jetty Chung-Yung Lee, Hani El-Nezami. *The University of Hong Kong, Hong Kong, China*
- P165-0454** **The Role of NF-κB and AhR in Pb-Mediated Immune Toxic Effects on A549 Lung Cancer Cells.** Attafi Ibraheem Mohammed¹, Saleh Albakheet², Hesham Korashy³. ¹*Ministry of Health, Jazan, Saudi Arabia.* ²*King Saud University, Riyadh, Saudi Arabia.* ³*Qatar University, Doha, Qatar*
- P166-0501** **Pterostilbene Triggers Endoplasmic Reticulum Stress–Mediated Autophagy and Apoptosis in Human Hepatocellular Carcinoma Cells.** Hui-Ling Chiou, Chen-Lin Yu, Yi-Hsien Hsieh. ¹*Chung Shan Medical University, Taichung, Taiwan*
- P167-0703** **Bioactive Fractions of *Rhus trilobata* Induce Apoptosis on Colon Cancer Cells (CaCo-2) by Activation of Caspase-9.** Lori Hajar¹, Carmen Gonzalez-Horta¹, David Chavez-Flores¹, Blanca Sánchez-Ramírez¹, Patricia Talamás-Rohana². ¹*Universidad Autónoma de Chihuahua, Chihuahua, Mexico.* ²*Cinvestav, Mexico City, Mexico*
- P168-0718** **LncRNA MALAT1 Mediates PM2.5 Organic Extract Induced-Cells Epithelial-Mesenchymal Transition via miR-204/ZEB1 Pathway in Lung Bronchial Epithelial Cells.** Fei Luo, Yan Feng, Yan Wang. *Shanghai Jiao Tong University, Shanghai, China*
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- P250-0324** **Toward *In Vitro* Approaches Linking Chemical Exposure to Mutagenesis Risk.** Cécile Mingard¹, Maureen McKeague², Mirjam Schneider¹, Nathalie Ziegler¹, Seiichiro Kizaki¹, Vera Hürlimann¹, Yang Jiang¹, Mohamed Amin Choukrallah³, Nicolas Sierro³, Nikolai Ivanov³, Julia Hoeng³, Shana Sturla¹. ¹*ETH Zürich, Zürich, Switzerland.* ²*McGill, Montreal, Québec, Canada.* ³*Philip Morris Products S.A., Neuchâtel, Switzerland*
- P251-0334** **The Impact of *OPRM1* A118G Polymorphism on Addiction in Turkish Population.** Hulya Turkan¹, Benu Karahalil², Ela Kadioglu², Kenan Eren³, Defne Gürol³, AliEsat Karakaya². ¹*Bozok University, Ankara, Turkey.* ²*Gazi University, Ankara, Turkey* ³*Bakirköy State Hospital for Mental and Neurological Diseases, Istanbul, Turkey*
- P252-0423** **A Comparative Study of the Genotoxic Effects of the Neonicotinoids Nitenpyram and Imidacloprid in Human Cell Line U937 Utilizing a Novel Statistical Approach to Evaluate Comet Data.** Erik Bivehed¹, Björn Hellman¹, Anton Gustafsson¹, Anders Berglund². ¹*Uppsala University, Uppsala, Sweden.* ²*Statistikakademin AB, Uppsala, Uppsala, Sweden*
- P253-0500** **The Reproductive Toxicity of ZishenYutai Pill on Rats and Offspring in Perinatal Period.** Li Zhou, Juan Jiang, Yang Yang, Li Xu, Liming Chong, Zuyue Sun. *National Evaluation Centre for the Toxicology of Fertility Regulating Drug, Shanghai, Shanghai, China*
- P254-0518** **Repeated-Dose Toxicity Study and Explore for Safety Assessment of *Thesium chinense* Particles in Juvenile Rats.** Yang Yang¹, Liming Chong¹, Yinwen Hou¹, Congcong Shao¹, Fen Wang¹, Li Xu¹, Li Zhou¹, Zuyue Sun¹, Qinxia Wang². ¹*National Evaluation Centre for the Toxicology of Fertility Regulating Drug, Shanghai, Shanghai, China.* ²*Shanghai Institute of Planned Parenthood Research, Xuhui Qu, Shanghai Shi, China*
- P255-0827** **CRISPR-Cas9 Generated Appb Zebrafish Mutants with Early Developmental Defects and Behavioral Alterations in Adulthood.** Rakesh Kumar Banote¹, Jasmine Chebli¹, Tuğçe Şatır¹, Johan Ledin², Shawn Burgess³, Alexandra Abramsson¹, Henrik Zetterberg^{1,4,5,6}. ¹*Institute of Neuroscience and Physiology, University of Gothenburg, Gothenburg, Sweden.* ²*Uppsala University, Uppsala, Sweden.* ³*National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, The United States of America.* ⁴*Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden.* ⁵*UCL Institute of Neurology, London, United Kingdom.* ⁶*UK Dementia Research Institute, London, United Kingdom*
- P256-0877** **Withdrawn**
- P257-0879** **Withdrawn**
- P258-0906** **Evaluation of Black Cohosh Extract for Genotoxicity and Epigenetic Alterations Using the CometChip Assay.** Manju Manjanatha¹, Ji-Eun Seo¹, Xiaoqing Guo¹, Sharon Shelton¹, Ying Chen¹, Volodymyr Tryndyak¹, Kristine Witt², Staphanie Smith-Roe². ¹*US FDA/NCTR, Jefferson, AR, The United States of America.* ²*NIEHS/NTP, Research Triangle Park, NC, The United States of America*
- P259-0922** **The Comparative Evaluation of the Genotoxicity of Different Titanium Dioxide Particles Using *In Vitro* Comet Assay.** In Jae Bang. *Sungkyunkwan University, Suwon, Gyeonggi-do, The Republic of Korea*
- P260-0032** **Either IL-4 Administration or Zinc Supplementation Mitigates Aggravated Inflammatory Response Associated with Increased White Blood Cells in Zinc-Deficient Rats.** Takamasa Kido¹, Machi Suka¹, Masashi Tsunoda², Hiroyuki Yanagisawa¹. ¹*The Jikei University School of Medicine, Tokyo, Japan.* ²*National Defense Medical College, Saitama, Japan*
- P261-0072** **Development of Th1-Related Diseases during Omalizumab Treatment: A Case Series of Severe Asthma.** Hiroaki Hayashi, Yuma Fukutomi, Kentaro Watai, Kiyoshi Sekiya, Norihiro Fujita, Maki Iwata, Kisako Nagayama, Yuto Nakamura, Yuto Hamada, Kai Ryu, Yasuhiro Tomita, Yosuke Kamide, Akio Mori, Masami Taniguchi. *National Hospital Organization Sagamihara Hospital, Sagamihara, Kanagawa, Japan*
- P262-0087** **Evaluating the Pharmacodynamics and Protective Efficacy of Catalytic Bioscavenger following Subcutaneous Administration in Guinea Pigs.** Thuy Dao¹, Sandra DeBus¹, Michael Boeri², Zachary Canter², Douglas Cerasoli¹, Shane Kasten¹. ¹*US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD, The United States of America.* ²*Oak Ridge Institute for Science and Education, Aberdeen Proving Ground, MD, The United States of America*

- P263-0088** **Assessing the Skin Irritation and Sensitizing Potential of Concentrates of Water Chlorinated in the Presence of Iodinated X-ray Contrast Media.** David Lehmann¹, Mikayla Armstrong², Jane Ellen Simmon¹. ¹US EPA/NHEERL, Durham, NC, The United States of America. ²University of North Carolina at Chapel Hill, Chapel Hill, NC, The United States of America
- P264-0194** **Mercury and Arsenic Disrupt Inflammatory Responses by Inhibition of Inflammation Activation.** Huijeong Ahn, Geun-Shik Lee. Kangwon National University, Chuncheon, Gangwon, The Republic of Korea
- P265-0270** **lncRNA MALAT1 Interacts with Nrf2 to Regulate Septic Shock in Mouse: Molecular Mechanism and Potential Therapeutic Application** Jingshu Chen, Sui Ke, Benjamine Morpurgo, Andrei Golovko, Yannan Tian. Texas A&M University, College Station, TX, The United States of America
- P266-0294** **Obovatol Attenuates NLRP3, AIM2, and Caspase-11 Inflammasome Activation.** Jeongeun Kim, Geun-Shik Lee. Kangwon National University, Chuncheon, Gangwon, The Republic of Korea
- P267-0316** **Red Ginseng Extracts Inhibit Cytokine Expression via TLR4-MyD88-NF-kappaB Signaling.** SangJung Yu, Geun-Shik Lee. Kangwon National University, Chuncheon, Gangwon, The Republic of Korea
- P268-0365** **Gefitinib, an Anticancer Drug Inhibiting EGFR, Stimulates IL-1 β Secretion by Activating Drp1 and p38 MAPK.** Yuki Kudoh, Rio Naganuma, Yusuke Hirata, Takuya Noguchi, Atsushi Matsuzawa. Tohoku University Graduate School of Pharmaceutical Sciences Laboratory of Health Chemistry, Sendai, Miyagi, Japan
- P269-0413** **Whole Body Microwave Irradiation and Rat Peritoneal Macrophages.** Mirjana Matausic-Pisl, Ivan Pavicic, Ana Marija Marjanovic-Cermak. Institute for Medical Research and Occupational Health, Zagreb, Zagreb County, Croatia
- P270-0570** **Effect of Pesticide Exposure on Immune Parameters in Agricultural Workers: The Twenty Years Experience at the International Centre for Rural Health.** Emanuela Corsini, Valentina Galbiati, Stefan Mandic-Rajcevic, Claudio Colosio. Università degli Studi di Milano, Milan, Italy
- P271-0594** **Study of Generic Tebuconazole Hematotoxic Activity after Acute Intoxication on Wistar Hannover Male Rats.** Mykola Prodanchuk, Tetiana Usenko, Volodymyr Bubalo, Yana Kolianchuk, Valentyna Shulyak, Nadiia Nedopytanska. L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical Safety, Ministry of Health, Ukraine, Kyiv, Ukraine
- P272-0596** **Sex-Specific Influenza Pathology with Concurrent Chronic Arsenic Exposure in Adult C57BL/6 Mice.** Sarah Attreed, Rachelle Liu, Chloe Kashiwagi, Kristal Rychlik, Emily Illingworth, Han Zhang, Tyrone Howard, Sabra Klein, Christopher Heaney, Fenna Sillé. Johns Hopkins University, Baltimore, MD, The United States of America
- P273-0659** **Investigating the Role of Metallothionein and Gene Expression as Predictors of Immune Response in *Hypsibius dujardini* following Exposure to Cadmium.** Damola Olatoregun. Texas Southern University, Houston, TX, The United States of America
- P274-0674** **Role of the Aryl Hydrocarbon Receptor (AhR) in Inflammatory Responses Induced by Dioxin and Persistent Organochlorinated Pesticides.** W.L. William Channg, Sarah Kado, Yasuhiro Ishihara, Christoph Vogel. University of California Davis, Davis, CA, The United States of America
- P275-0733** **Allergenic Oxidized Fragrances in Consumer Goods: Understanding the Sensitizing Potential and Related Oxidative Stress in an Epidermal Skin Environment.** Fatma Sahli¹, Marta Silva e Sousa², Bertrand Vilen³, Jutta Lichter², Brunhilde Blömeke², Elena Gimenez-Arnau¹. ¹University of Strasbourg, Strasbourg, France. ²University of Trier, Trier, Germany. ³University of Strasbourg, Strasbourg, France
- P276-0734** **Formation of Free Radicals from the Skin Sensitizer Ascaridole in a 3D Epidermis Model and Activation of the Innate Immune Response.** Fatma Sahli¹, Bertrand Vilen¹, Marta Silva e Sousa², Jutta Lichter², Brunhilde Blömeke², Elena Gimenez-Arnau¹. ¹University of Strasbourg, Strasbourg, France. ²University of Trier, Trier, Germany
- P277-0743** **Effect of Immunosuppressive Drugs on Viral Proliferation.** Cha-Gyun Shin, Ga-Eun Lee. Chung-Ang University, Ansong, Kyungki, The Republic of Korea
- P278-0789** **Ovalicin Alleviated Pruritic and Inflammatory Signaling through Inhibition of IL-31 and ROS in DH82 Cells.** Yongbaek Kim^{1,2}, Sung-Hyun Hwang^{1,3}. ¹Seoul National University, Seoul, The Republic of Korea. ²Research Institute for Veterinary Science, Seoul, The Republic of Korea. ³Seoul National University, BK21 PLUS Program for Creative Veterinary Science Research, Seoul, The Republic of Korea

- P279-0810** **Cholera Toxin Can Exert Mucosal Adjuvanticity via ERdj5 in Innate Immune Cells.** Hyun-Jeong Ko¹, Mee-Sun Kim², Sunyoung Chang². ¹Kangwon National University, Chuncheon, Kanwon-do, The Republic of Korea. ²Ajou University, College of Pharmacy, and Research Institute of Pharmaceutical Science and Technology (RIPST), Suwon, Gyeonggi-do, The Republic of Korea
- P280-0224** **Prompt Elimination of Uremic Toxin Alleviates AKI to CKD Transition.** Jia-Rong Jheng¹, Jia-Huang Chen¹, Yuan-Siao Chen¹, Chih-Kang Chiang^{1,2}. ¹National Taiwan University, Taipei, Taiwan. ²National Taiwan University Hospital, Taipei, Taiwan
- P281-0328** **Effect of Moon Seed Vine (*Triclisia gilentii* Staner) on Ethane-1,2-Diol-Induced Urolithiasis and Its Renotoxicity in Wistar Albino Rats.** Olaveriju Olanrewaju^{1,2}, Olusola Elekofehinti¹, Tolulope Olaleye¹, Akintunde Akindahunsi¹. ¹Federal University of Technology Akure, Akure, Ondo, Nigeria. ²University of Pavia, Pavia, Lombardy, Italy
- P282-0349** **Acute Kidney Injury Associated with Ingestion of Star Fruit.** Thajudeen Mohammed Zuhary, R Ponampalam. Singapore General Hospital, Singapore, Singapore
- P283-0469** **Characterization of Early Kidney Damage in a Child Population Exposed to a Mixture of Environmental Pollutants from Apizaco, Tlaxcala, Mexico.** Manolo Ortega-Romero¹, Mara Medeiros-Domingo², Pablo Méndez-Hernández³, Guadalupe Aguilar-Madrid^{4,5}, Luz Maria Del Razo-Jiménez¹, Mónica Jiménez-Córdova¹, Juana Narváez-Morales¹, Ana María Hernández-Sánchez², Olivier Barbier¹. ¹Center for Research and Advanced Studies (Cinvestav), Mexico City, Mexico. ²Hospital Infantil de México Federico Gómez, Mexico City, Mexico. ³Secretaría de Salud de Tlaxcala, Tlaxcala, Mexico. ⁴Facultad de Medicina UNAM, Mexico City, Mexico. ⁵Claustro Universitario de Chihuahua, Chihuahua, Mexico
- P284-0474** **Cross-Species Assessment of Amphotericin B–Induced Renal Tubular Injury and Related Profile Changes in miRNAs, Genes, and Proteins.** James McDuffie¹, Manisha Sonee², Justin Kanerva¹, Elnaz Atabakhsh³, Harry Reese³, Michael Tackett³, Yunhai Zhang⁴, Ryan Meng⁴, Yafei Chen¹. ¹Janssen Research & Development, San Diego, CA, The United States of America. ²Janssen Research & Development, Spring House, PA, The United States of America. ³Abcam Inc, Cambridge, MA, The United States of America. ⁴Janssen Research & Development, Shanghai, China
- P285-0617** **Role of NGAL/24p3 Receptor in the Handling of CdMT-FITC along the Distal Nephron with Decreased Endocytic Capacity.** Itzel Pamela Zavala Guevara¹, Manolo Ortega-Romero¹, Juana Narváez-Morales¹, Laura Arreola Mendoza², Olivier Barbier¹. ¹Center for Research and Advanced Studies (Cinvestav), Mexico City, Mexico. ²Centro Interdisciplinario de Investigaciones y Estudios Sobre Medio Ambiente y Desarrollo del Instituto Politécnico Nacional, Mexico City, Mexico
- P286-0681** ***Dendropanax moribifera* Attenuates Nephropathy via SIRT1/AMPK Pathways in Streptozotocin-Induced Diabetic Rats.** Richa Sachan, Amit Kundu, Kyeong Seok Kim, Jong Seung Lim, In Su Kim, Byung-Mu Lee, Jong Hwan Kwak, Hyung Sik Kim. ¹Sungkyunkwan University, Suwon, Gyeonggi-do, The Republic of Korea
- P287-0754** **The Role of the Inducible Calbindin-D28k Protein in Advanced Glycation End Products–Associated Renal Proximal Tubule Fibrosis.** Cheng-Tien Wu¹, Shing-Hwa Liu^{2,3,4}, Wei-Han Lin², Chih-Kang Chiang^{2,5}, Yen-Hau Su¹, Shin-An Chen¹. ¹Shuang Ho Hospital, Taipei, Taiwan. ²Institute of Toxicology, National Taiwan University, Taipei, Taiwan. ³National Taiwan University Hospital, Taipei, Taiwan. ⁴China Medical University Hospital, Taipei, Taiwan. ⁵College of Medicine and Hospital, Taipei, Taiwan
- P288-0914** **Association of Genetic Polymorphisms in Multispecific Transporters with Mercury Nephrotoxicity in Artisanal and Small-Scale Gold Mining.** Luz Helena Sanchez Rodriguez¹, Olga Medina¹, Linda Rocha², Giovanna Rincón¹, Fernando Rondon¹, Oscar Flórez³. ¹Industrial University of Santander, Bucaramanga, Santander, Colombia. ²University of Santander, Bucaramanga, Santander, Colombia. ³National Cancer Institute, Bethesda, MD, The United States of America
- P289-0063** **Connexin Hemichannels and Pannexin Channels as Drug Targets in Liver Toxicity and Disease.** Mathieu Vinken¹, Bruno Cogliati². ¹Vrije Universiteit Brussel, Brussels, Belgium. ²University of São Paulo, São Paulo, Brazil
- P290-0111** **Mechanistic Investigation of Anti-retroviral Related Liver Injury through the Use of Metabolomics and Lipidomics Techniques.** Thomas Kralj, Dovile Anderson, Amanda De Paoli, Anubhav Srivastava, Darren Creek. Monash University, Parkville, Victoria, Australia
- P291-0116** **Population Variability in the Severity of Alcoholic Hepatitis in a Mouse Model.** Hisataka Fukushima, Luo Yu-Syuan, Lauren Lewis, David Threadgill, Ivan Rusyn. Texas A&M University, College Station, TX, The United States of America
- P292-0123** **The Role OATP2A1 in Hepatoprotection against CCl₄-Induced Liver Injury.** Hiroaki Shimada, Ryota Hashimoto, Aya Aoki, Saya Yamada, Atsushi Kawase, Masahiro Iwaki. Kindai University, Higashi-osaka, Osaka, Japan

- P293-0142** **Hepatoprotective Effect of Polyphenolic Rich Fractions from *Lauridia tetragona* (L.f.) R.H. Archer on Acetaminophen-Induced Liver Injury.** Samuel Odeyemi, John Dewar. *University of South Africa, Johannesburg, Gauteng, South Africa*
- P294-0168** **Suppressive Effect of Sesamin on Lipogenesis through Activation of CaMKK/AMPK in Hepatocytes.** Thi Hoa Pham, Sun Woo Jin, Hye Gwang Jeong. *Chungnam National University, Daejeon, The Republic of Korea*
- P295-0170** **Suppressive Effects of Platyconic Acid A on TGF-Beta1-Induced Hepatic Stellate Cell Activation through Blocking Smad-Dependent and Smad-Independent Signal Transduction Pathways.** Jae Ho Choi¹, Gi Ho Lee¹, Sun Woo Jin¹, Young Chul Chung², Hye Gwang Jeong¹. ¹*Chungnam National University, Daejeon, The Republic of Korea.* ²*International University of Korea, Jinju, Gyeongsangnam-do, The Republic of Korea*
- P296-0171** **Inhibitory Effects of Rutaecarpine on Acetaminophen-Induced Hepatotoxicity through Enhancing of Nrf2-Mediated Antioxidants Enzymes in Mice.** Gi Ho Lee¹, Jae Ho Choi¹, Han Gun Kim², Kwang Youl Lee³, Hye Gwang Jeong¹. ¹*Chungnam National University, Daejeon, The Republic of Korea.* ²*Sunchon National University, Suncheon, Jeollanam-do, The Republic of Korea.* ³*Chonnam National University, Gwangju, The Republic of Korea*
- P297-0279** **An Inhibitor of the Protein Kinases IKK ϵ and TBK1 Ameliorates Acetaminophen-Induced Oxidative Stress in Mice.** Jing Qi, Jong-Won Kim, Zixiong Zhou, Chae Woong Lim, Hyeneui Jeong, Daram Yang, Bumseok Kim. *Chonbuk National University, Iksan, Jeonbuk, The Republic of Korea*
- P298-0286** **Amlexanox Attenuates the Severity of Hepatotoxin-Induced Liver Fibrosis and Biliary Fibrosis in Mice.** Zixiong Zhou, Jing Qi, Jong-Won Kim, Hyeneui Jeong, Daram Yang, Chae Woong Lim, Bumseok Kim. *Chonbuk National University, Iksan, Jeonbuk, The Republic of Korea*
- P299-0337** **Cyp2b-Null Male Mice Are Susceptible to High-Fat-Diet-Induced Obesity Due to Changes in PUFA Metabolism and Response to Hepatic Lipids as Measured by RNAseq.** Melissa Heintz, Ramiya Kumar, William Baldwin. *Clemson University, Clemson, SC, The United States of America*
- P300-0359** **The Toxicological Profiles of 1,3-Dichloro-2-Propanol Determined by a Repeated-Dose-28-Day Oral Toxicity Study in F344 Rats.** Kohei Matsushita¹, Takeshi Toyoda¹, Tomomi Morikawa¹, Takanori Yamada^{1,2}, Kumiko Ogawa¹. ¹*National Institute of Health Sciences, Kawasaki, Kanagawa, Japan.* ²*Tokyo University of Agriculture and Technology, Fuchu, Japan*
- P301-0364** **CTR1 Overexpression Enhances Copper Toxicity in Zebrafish Liver Cell Line ZFL.** Man Long Kwok, King Ming Chan. *Chinese University of Hong Kong, Hong Kong, Hong Kong*
- P302-0461** **Early-Life Activation of mTOR Pathway by Environmental Xenobiotics Permanently Reprograms Metabolism of Lipids and Carbohydrates by Liver.** Alexander Suvorov. *University of Massachusetts Amherst, Amherst, MA, The United States of America*
- P303-0468** **Withdrawn**
- P304-0470** **How Similar among Different Toxicogenomics Study Designs?** Weida Tong¹, Zhichao Liu¹, Ruth Roberts². ¹*US FDA/NCTR, Jefferson, AR, The United States of America.* ²*University of Birmingham, Edgbaston, United Kingdom*
- P305-0486** **The Regulatory Mechanism of Hepatic PGE₂ Disposition in Carbamazepine-Induced Liver Injury.** Masahiro Iwaki, Hiroaki Shimada, Ryota Hashimoto, Ken-ichi Oba, Atsushi Kawase. *Kindai University, Higashi-osaka, Osaka, Japan*
- P306-0487** **Diclofenac Induces Lipid Accumulation in Liver by Inhibiting Chaperone-Mediated Autophagy.** Wonseok Lee, Seung-Hwan Jung, Byung Hoon Lee. *Seoul National University, Seoul, The Republic of Korea*
- P307-0490** **Diclofenac Inhibits Autophagic Flux and Sensitizes Hepatocarcinoma Cells to Sorafenib-Induced Cell Death.** Seung-Hwan Jung, Seung-Hyeon Park, Byung Hoon Lee. *Seoul National University, Seoul, The Republic of Korea*
- P308-0613** **Regorafenib Is Far More Toxic Than Its Pharmacologically Active Metabolites in Hepatocytes and Cardiomyocytes.** Qiang Shi¹, Lijun Ren¹, James Greenhaw¹, Xi Yang², William Mattes¹. ¹*US FDA/NCTR, Jefferson, AR, The United States of America.* ²*US FDA/CDER, Silver Spring, MD, The United States of America*
- P309-0640** **Human CYP2B6 is an Anti-obesity Enzyme Involved in Unsaturated Fatty Acid Metabolism.** Emily Olack, Melissa Heintz, William Baldwin. *Clemson University, Clemson, SC, The United States of America*

- P310-0680** **Estrogen Deficiency Potentiates Thioacetamide-Induced Hepatic Fibrosis.** Yong Hee Lee, Jae Hyeon Park, Yu Jung Park, Kyeong Seok Kim, Hyung Sik Kim. *Sungkyunkwan University, Suwon, Gyeonggi-do, The Republic of Korea*
- P311-0696** **Adipose FGFR1 Regulates Binge Alcohol-Induced Adipolipolysis and Fat Accumulation in Liver in Mice.** Xue Chen, Yongke Lu. *East Tennessee State University, Johnson City, TN, The United States of America*
- P312-0792** **The Influence of Alantolactone on Cholesterol Metabolism in Liver Cells.** Tomáš Zárybnický, Veronika Skalická, Terézia Kamasová, Martin Ambrož, Petra Matoušková, Lenka Skálová, Iva Boušová. *Univerzita Karlova, Hradec Králové, The Czech Republic*.
- P313-0800** **Docosahexaenoic Acid Inhibits SREBP1-Mediated Lipogenic Enzyme Expression via G-Protein-Coupled Receptor. ⁴⁰ in Primary Hepatocytes.** Hyun Young Kim, Keon Wook Kang, Jeongwoo Park, Seungtae On. *Soeul National University, Seoul, The Republic of Korea*
- P314-0855** **A Human Hepatocellular Steatosis Model for *In Vitro* Toxicity Assessment.** Gail Nelson¹, Nyssa Tucker^{1,2}, Alan Tennant¹, Brian Chorley¹. ¹*US EPA/NHEERL, Research Triangle Park, NC, The United States of America*. ²*Oak Ridge Institute for Science and Education, Research Triangle Park, NC, The United States of America*
- P315-0860** **Interactions between Epigallocatechin-3-Gallate (EGCG) and Hydroxy Citric Acid Potentiate EGCG Hepatotoxicity.** Rachael Farrington¹, Roger Byard², Ian Musgrave¹. ¹*University of Adelaide, Adelaide, Australia*. ²*Forensic Science SA, Adelaide, Australia*
- P316-0861** **Herbal Phytochemical Interactions Produce Synergistic Toxicity: Implications for Polyherbacy.** Susan Britza¹, Ian Musgrave¹, Roger Byard². ¹*University of Adelaide, Adelaide, Australia*. ²*Forensic Science SA, Adelaide, Australia*
- P317-0870** **Lichen Secondary Metabolite (Atranorin) Ameliorates Adriamycin-Induced Hepatotoxicity: Targeting the Proinflammatory Cytokine, Oxidative Stress, Apoptosis, and TGF- β 1/ STAT-3 Signaling.** Ila Shukla, Lubna Azmi, C Rao. *CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh, India*
- P318-0903** **Transcriptomic Landscape in Murine Plasma of Hepatocellular Liver Injury for Biomarker Discovery.** Shingo Oda, Ken Itoh, Tsuyoshi Yokoi. *Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan*
- P319-0904** **Carbon Monoxide-Releasing Molecule-3 Protects Oxidative Stress-Induced Cell Death in HepG2 Cells: Role of ERK-Mediated Nrf2 Activation.** Hyun Jung Choi¹, Hye Min Hwang¹, Eun Kyung Kim¹, Soyeon Jeong¹, Ami Choi¹, Keon Wook Kang², Young-Mi Kim¹. ¹*Hanyang University, Ansan, Gyeonggi-do, The Republic of Korea*. ²*Seoul National University, Seoul, The Republic of Korea*
- P320-0908** **Effect of Molybdate on Hepatic Steatosis in Mice Given a Choline-Deficient High-Fat Diet.** Seungwoo Lee, Doug-Young Ryu. *Seoul National University, Seoul, The Republic of Korea*
- P321-0961** **4-Methylpyrazole Is a New Therapy for Massive Acetaminophen Poisoning in Mice and Human.** Jephthe Y. Akakpo¹, Anup Ramachandran¹, Luqi Duan¹, Matt Schaich¹, Matthew Jaeschke¹, Brett Freudenthal¹, Wen xing Ding¹, Barry Rumack², Hartmut Jaeschke¹. ¹*University of Kansas Medical Center, Kansas City, KS, The United States of America*. ²*University of Colorado Denver, Aurora, CO, The United States of America*
- P322-0009** **The Role of Hepatic Cytochrome P450s in the Cytotoxicity of Dronedarone.** Si Chen, Qiangen Wu, Baitang Ning, Matthew Bryant, Lei Guo. *US FDA/NCTR, Jefferson, AR, The United States of America*
- P323-0100** **Toxicokinetic Characteristics of Fusarenon-X and Its Major Metabolites in Goats.** Amnart Poapolathep¹, Wanchalerm Phruksawan¹, Saranya Poapolathep¹, Kanjana Imsilp¹, Mario Giorgi², Zbynex Dzuman³, Jana Hajslova³. ¹*Kasetsart University, Bangkok, Thailand*. ²*University of Pisa, Pisa, Italy*. ³*University of Chemistry and Technology, Prague, The Czech Republic*
- P324-0106** **Withdrawn**
- P325-0126** **Withdrawn**
- P326-0132** **Quatitative Metabolism of Senecionine—A Carcinogenic Pyrrolizidine Alkaloid by LC/MS/MS Analysis.** Qingsu Xia¹, Xiaobo He¹, Ge Lin², Peter Fu¹. ¹*US FDA/NCTR, Jefferson, AR, The United States of America*. ²*Chinese University of Hong Kong, Shatin, Hong Kong, China*

- P327-0172** **Buprenorphine and Metabolites Concentrations in Neonate Cord Blood: An Exploratory Pharmacogenetic Analysis.** Lauren Dickerson, Derek Murrell, Darshan Shah, Stacy Brown, Cara Carter, Sam Harirforoosh. *East Tennessee State University, Johnson City, TN, The United States of America*
- P328-0174** **Changes in Hepatic Expression of Cytochrome P450 and Glutathione S-transferase in Mice Exposed to Cigarette Smoke.** Jieun Yu, Young Jae Choi, Chang Seon Ryu, Hyo Seon Nam, Jang su Jeon, Teayoon Jung, Ji-Eun Park, Sang Kyum Kim. *Chungnam National University, Yuseong-gu, Daejeon, The Republic of Korea*
- P329-0254** **Characterization of Role of Cytochrome P450 in Hepatic Metabolism of Fimasartan.** Ha Gyeong Kim, Young Jae Choi, Ji-Yoon Lee, Chang Seon Ryu, Sang Kyum Kim. *Chungnam National University, Daehak-ro, Yuseong-gu, Daejeon, The Republic of Korea*
- P330-0257** **Metabolism of [³H]-BADGE in Rodents: A Spotlight on the Complex *In Vivo* Fate of the Diglycidyl Ether Analogue of Bisphenol A.** Elodie Person, Isabelle Jouanin, Emilien Jamin, Florence Blas-Y-Estrada, Sandrine Bruel, Jean-Pierre Cravedi, Nicolas Cabaton, Daniel Zalko. *INRA, Toulouse, France*
- P331-0362** **Investigation of Mechanism of Rodenticide Resistance with Closed Colonies of Rodenticide-Resistant Rats from Tokyo.** Kazuki Takeda¹, Yoshinori Ikenaka^{1,2}, Kazuyuki D³, Shouta Nakayama¹, Tsutomu Tanikawa³, Mayumi Ishizuka¹. ¹*Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan.* ²*School of Environmental Sciences and Development, North-West University South Africa, Potchefstroom, North West, South Africa.* ³*Ikari Shodoku Co., Ltd., Narashino, Chiba, Japan*
- P332-0386** **Role of Intestinal Microbiota in Metabolism of Voglibose *In Vitro* and *In Vivo*.** Geon Ho Kim, Mahesh Nepal, Dong Ho Cha, Mi Jeong Kang, Tae Cheon Jeong. *Yeungnam University, Gyeongsan, The Republic of Korea*
- P333-0506** **Glutathione Conjugation of the Reactive Metabolite of Acetaminophen Catalyzed by Human Glutathione S-transferase Isoforms.** Ohnuma Tomokazu, Hiroshi Kogure, Takahito Nishiyama, Kenichiro Ogura, Hiratsuka Akira. *Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo, Japan*
- P334-0572** **Withdrawn**
- P335-0687** **Toward Understanding Metabolism of Synthetic Cannabinoids and Model Compounds by Using *In Vitro* Oxidation by Microsomes and Chemical Oxidant Reagent.** Oluseyi Vanderpuye¹, Alexius Lampkin², Alexis Bailey¹. ¹*Albany State University, Albany, GA, The United States of America* ²*University of Wisconsin–Madison, Madison, WI, The United States of America*
- P336-0698** **Cytochromes P450 Bioactivate Lamisil (Terbinafine) through Multiple N-dealkylation Pathways.** Dustyne Barnette¹, Mary Davis¹, Na Dang², Anirudh Pidugu³, Noah Flynn², Tyler Hughes², S Swamidass², Gunnar Boysen¹, Grover Miller¹. ¹*University of Arkansas for Medical Sciences, Little Rock, AR, The United States of America.* ²*Washington University, St. Louis, MO, The United States of America.* ³*Emory University, Atlanta, GA, The United States of America*
- P337-0760** **Functional Characterization of Five CYP2A13 Allelic Variations.** Vitchan Kim, Sora Yeom, Harim Kim, Donghak Kim. *Konkuk University, Seoul, The Republic of Korea*
- P338-0840** **Barcoded DNA Measurements Yield Unexpected Interference with High-Throughput CYP-Mediated Cytotoxicity Assay.** Alice Woolard¹, Steven Simmons², Brian Chorley³. ¹*Oak Ridge Institute for Science and Education, Durham, NC, The United States of America.* ²*US EPA/NCCT, Durham, NC, The United States of America.* ³*US EPA/NHEERL, Durham, NC, The United States of America*
- P339-0864** **Toxicokinetics of QiLin Pills following Single Oral Administrations in SD Rats.** Qinxia Wang, Sichong Xu, Qi Pang, Li Zhou, Zuyue Sun. *National Evaluation Centre for the Toxicology of Fertility Regulating Drug, Shanghai, Shanghai, China*
- P340-0909** **Edaravone Free Radical Metabolite Formation and Its Consequences.** Lindsey Suh, Dinesh Babu, Lusine Tonoyan, Béla Reiz, S. Amirhossein Tabatabaei-Dakhili, Andrew Morgan, Carlos Velazquez, Arno Siraki. *University of Alberta, Edmonton, Alberta, Canada*
- P341-0936** **Pharmacokinetics and Metabolism of Acetyl Triethyl Citrate in Rats.** Young Seok Ji, Jun Sang Yu, Jeong Hoon Park, Eun Gyu Lee, Hye Hyun Yoo. *Hanyang University, Ansan, The Republic of Korea*

- P342-0022** **Damaging Effect on Spatial Cognition Behavior by Bioengineered ZnO Nanoparticles of *Trianthema portulacastrum* Linn. in Wistar Rats.** Eakta Yadav, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India
- P343-0023** **Quercetin Attenuates Metronidazole-Induced Neurotoxicity through Downregulating Nitric Oxide Release and Regulation of NF- κ B Pathway.** Wahaj Uddin, Swati Chaturvedi, Mohd Malik. CSIR Central Drug Research Institute, Lucknow, Uttar Pradesh, India
- P344-0084** **Behavioral Disorders Caused by Acute Carbon Monoxide Poisoning and Its Relationship with Prognostic Biomarkers.** Analía Cortez¹, Rocío Galarza¹, Sonia Molina¹, María Meneghini¹, Analía Karadayian², Alicia Faletti¹. ¹Centro de Estudios Farmacológicos y Botánicos (CEFyBO)-CONICET, Facultad de Medicina-Universidad de Buenos Aires, Argentina, CABA, Argentina. ²Instituto de Bioquímica y Medicina Molecular (IBIMOL)-CONICET, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina, CABA, Argentina
- P345-0110** **Immunohistological Analysis of Peripheral Nerve Injury in Methylmercury-Exposed Rats.** Yo Shinoda¹, Shunsuke Ehara¹, Eiko Yoshida², Tsutomu Takahashi¹, Toshiyuki Kaji², Yasuyuki Fujiwara¹. ¹Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo, Japan. ²Tokyo University of Science, Noda, Chiba, Japan
- P346-0148** **Cellular Recovery and Resilience—A New Angle to Look at Toxicity Testing.** Lena Smirnova¹, Georgina Harris¹, Helena Hogberg¹, Alexandra Maertens¹, Andre Kleensang¹, Thomas Hartung^{1,2}. ¹Johns Hopkins University, Baltimore, MD, The United States of America. ²University of Konstanz, Konstanz, Germany
- P347-0176** **Molecular Mechanism of Methylmercury-Induced Neurotoxicity via Endoplasmic Reticulum Stress.** Hideki Hiraoka¹, Kengo Nakahara¹, Nobumasa Takasugi¹, Masatake Fujimura², Takao Iwawaki³, Yoshito Kumagai⁴, Takashi Uehara¹. ¹Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan. ²National Institute for Minamata Disease Department of Basic Medical Sciences, Minamata, Japan. ³Kanazawa Medical University, Division of Cell Medicine, Department of Life Science, Medical Research Institute, Uchinada, Kahoku, Japan. ⁴University of Tsukuba Environmental Biology Laboratory, Faculty of Medicine, Tsukuba, Ibaraki, Japan
- P348-0204** **Mitigating Effects of Resveratrol Supplementation on Arsenic Trioxide Induced Neurotoxicity in Mouse Hippocampus.** Kamakshi Mehta, Balpreet Kaur, Kamlesh Kumar, Pushpa Dhar. All India Institute of Medical Sciences, New Delhi, Delhi, India
- P349-0251** **Age-Dependent Dopaminergic Dysfunction following Early-Life Exposure to Atrazine in SD Rats.** Yanshu Li. Haerbin Medical University, Harbin, Heilongjiang, China
- P350-0258** **An Innovative Tool for Detecting Alcohol Withdrawal Neurotoxicity: Focus on Limbic Memory in Rats.** Anna Brancato¹, Carla Cannizzaro¹, Marco Diana². ¹University of Palermo, Palermo, Italy. ²University of Sassari, Sassari, Italy
- P351-0273** **Metabolomic Changes Associated with Genetic and Toxicological Perturbation of the Vesicular Monoamine Transporter (VMAT) in *Caenorhabditis elegans*.** Vrinda Kalia¹, Joshua Bradner¹, Merry Chen², Douglas Walker³, Dean Jones², Gary Miller¹. ¹Columbia University, New York, NY, The United States of America. ²Emory University, Atlanta, GA, The United States of America. ³Icahn School of Medicine at Mount Sinai, New York, NY, The United States of America
- P352-0285** **Autophagic Cell Death via Inhibition of Akt/mTOR Signaling Pathway Contributed to Antimony-Induced Neuronal Damage.** Zhao Xinyuan, Zhu Piaoyu, Wang Xiaoke, Qiyun Wu. Nantong University, Nantong, Jiangsu, China
- P353-0329** **The Attenuation of Glutamate-Induced Oxidative Toxicity and Induction of Antioxidant Genes by Resveratrol in HT22 Murine Hippocampal Neuronal Cells.** Wanchanok Nantacharoen¹, Tewin Tencomnao², Monruedee Sukprasansap³. ¹Clinical Biochemistry and Molecular Medicine, Department of Clinical Chemistry, Faculty of Allied Health Sciences, Bangkok, Thailand. ²Age-Related Inflammation and Degeneration Research Unit, Department of Clinical Chemistry, Faculty of Allied Health Sciences, Bangkok, Thailand. ³Food Toxicology Unit, Institute of Nutrition, Salaya, Thailand
- P354-0343** **ER Stress Inhibitor Salubrinal Attenuates Deltamethrin-Induced Reduction of Hippocampal Neurogenesis in Adult Mice.** Muhammad Hossain, Abdelmadjid Belkadi. Florida International University, Miami, FL, The United States of America
- P355-0372** **Regulation of Ubiquitin-Proteasome System in ERAD via S-nitrosylation of UBE2D1.** Kana Fujikawa¹, Kengo Nakahara¹, Nobumasa Takasugi¹, Akihiro Ito², Takashi Uehara¹. ¹Okayama University, Department of Medicinal Pharmacology, Graduate School Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan. ²Laboratory of Cell Signaling, Tokyo University of Pharmacy and Life Science, Tokyo, Japan

- P356-0376** **Atypical Antipsychotic Olanzapine Induces Oxidative Stress in the Hypothalamus.** Natsumi Hattori, Asuka Kaizaki-Mitsumoto, Satoshi Numazawa. *Showa University School of Pharmacy, Tokyo, Japan*
- P357-0379** **Possible Role of Endogenous S-persulfidation in the Enzymatic Activity of ER Protein.** Honami Ushirokawa, Kenichi Yamaji, Sho Okuda, Eisuke Sugino, Nobumasa Takasugi, Takashi Uehara. ¹Okayama University, Department of Medicinal Pharmacology, Graduate School Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan
- P358-0414** **Utilization of Highly Sensitive LC-MS/MS to Detect Changes in the Central Nervous System (CNS): Comprehensive Profiling of Neurotransmitters and Proteins in Cerebrospinal Fluid in Rats.** Motohiro Shiotani, Kanta Horie, Sanae Omi, Aya Goto, Kyoko Nakano, Shoji Asakura. *Eisai Co., Ltd., Tsukuba, Ibaraki, Japan*
- P359-0426** **Dimethylglycine Alleviates Behavioral Disturbances Induced by Adolescent Toluene Exposure.** Ming-Huan Chan¹, Chung-Pineh Hsieh², Hao Chen³, Hwei-Hsien Chen². ¹National Chengchi University, Taipei, Taiwan. ²National Health Research Institutes, Miaoli, Taiwan. ³National Tsing Hua University, Hsinchu, Taiwan
- P360-0491** **Psychopharmacological Effects of Pyrolysates of Synthetic Cannabinoids.** Kyoko Hataoka¹, Asuka Kaizaki-Mitsumoto¹, Mika Ohsawa², Masahiko Funada², Satoshi Numazawa¹. *Showa University School of Pharmacy, Tokyo, Japan.* ²NCNP, Tokyo, Japan
- P361-0499** **Prescription Drugs Interrupt Cholesterol Biosynthesis in Primary Cultures of Neurons.** Phillip Wages¹, Amy Palubinsky², Beth Ann McLaughlin², Ned Porter¹. ¹Vanderbilt University, Nashville, TN, The United States of America. ²Vanderbilt University Medical Center, Nashville, TN, The United States of America
- P362-0534** **Alteration of Social Behavior and Neuroimmune Biomarkers in Fragile X Mental Retardation 1 Knockout and Valproic Acid-Induced Autism Rat Models.** Tin-Tin Win-Shwe, Hidehiro Watanabe. *National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan*
- P363-0537** **Metabolomic Approach Uncovered Impaired Amino Acid Metabolism in Human Astrocytoma Cells Induced by Manganese Exposure.** Yoshinori Okamoto, Saki Kato, Akira Aoki, Koji Ueda, Hideto Jinno. *Meijo University, Nagoya, Aichi, Japan*
- P364-0539** **Optical Recording Methods with Voltage-Sensitive Dye (VSD) Is Useful to Evaluate the Impact of Chemicals on Brain Activities in Acute and Late Effects.** Yoko Tominaga¹, Makiko Taketoshi¹, Kentaro Tanemura², Takashi Tominaga¹. ¹Tokushima Bunri University, Sanuki, Kagawa, Japan. ²Tohoku University, Sendai, Miyagi, Japan
- P365-0544** **Anti-neurotoxic Effect of *Passiflora edulis* Extracts against Glutamate-Induced Oxidative Damage in Cultured HT-22 Cells.** Shugin Cao¹, Tawarit Sarachana¹, Chalinee Ronpirin², Tewin Tencomnao¹. ¹Chulalongkorn University, Bangkok, Thailand. ²Thammasat University, Khlong Luang, Pathum Thani, Thailand
- P366-0558** **Cadmium Effects on Memory Impairment and on Brain Neurochemistry: Modulation by *Lepidium sativum* L.** Ed-Day Soumia¹, Fatima-Zahra Azzaoui¹, Samira Boulbaroud², Ahmed Omar Touhami Ahami¹, Latifa Didou¹. ¹Faculty of Science, Ibn Tofail University, Kenitra, Morocco. ²Sultan Moulay Slimane University, Beni Mellal, Béni Mellal-Khénifra, Morocco
- P367-0562** **Developing a QSAR Model for Predicting Neurotoxic Agents.** Hung-Lin Kan¹, Chun-Wei Tung^{1,2}, Ying-Chi Lin^{1,2}, Chia-Chi Wang^{1,2}. ¹Kaohsiung Medical University, Kaohsiung, Taiwan. ²Kaohsiung Medical University School of Pharmacy, Kaohsiung, Taiwan
- P368-0566** **Comparative Effects of Two Pyrethroids on Behavioral Reactions in Developmental Neurotoxicity Studies.** Nadiia Nedopytanska, Mykola Prodanchuk, Oleksandr Kravchuk, Natalia Kornuta, Yana Kolianchuk, Inna Rashkivska. *L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical Safety, Ministry of Health, Ukraine, Kyiv, Ukraine*
- P369-0571** **Early-Life Exposure to Low Levels of Permethrin Exerts Impairments in Learning and Memory Associated with Glial Cell Disturbance in Adult Male Mice.** Hirokatsu Saito¹, Kenshiro Hara¹, Takashi Tominaga², Kinichi Nakashima³, Kentaro Tanemura¹. ¹Tohoku University, Sendai, Miyagi, Japan. ²Tokushima Bunri University, Sanuki, Kagawa, Japan. ³Kyushu University, Fukuoka, Fukuoka, Japan
- P370-0580** **The Transcription Factor REST/NRSF Mediates Protective Effects of 17 β -Estradiol and Tamoxifen against Manganese-Induced Toxicity in Astrocytes.** Edward Pajarillo¹, Michael Aschner², Eunsook Lee¹. ¹Florida A&M University, Tallahassee, FL, The United States of America. ²Albert Einstein College of Medicine, New York, NY, The United States of America

- P371-0583** **Pharmacological Responses in Cultured Human iPSC-Derived Sensory Neurons Using MEA System.** Aoi Odawara, Naoki Matsuda, Ikuro Suzuki. *Tohoku Institute of Technology, Sendai, Miyagi, Japan*
- P372-0595** **The Toxic Effects of MnCl₂ on Motor Function Are Reduced in Mice Using a Contaminated Water Sediment Exposure Model.** Dana Freeman, Rachel O'Neal, Qiang Zhang, Dan Lou, Zhibin Wang. *Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, The United States of America*
- P373-0645** **Analysis of Manganese Accumulation in the Pituitary Gland, Olfactory Bulb, and Hippocampus of Smelter Workers Using High-Resolution. ³D T1-Weighted MRI.** Zeinab Aly¹, Molly Cromer¹, Alison Jeffries¹, Eric Cameron^{1,2}, Ulrike Dydak^{1,3}. ¹Purdue University, West Lafayette, IN, The United States of America. ²Indiana University School of Medicine, Indianapolis, IN, The United States of America. ³Indiana University School of Medicine, Indianapolis, IN, The United States of America
- P374-0737** **Biphasic Effect of the Gliotoxin L-α-Aminoadipate on Astrocytes to Impact on Mouse Hippocampal Synaptic Plasticity.** Marlene Pereira¹, Inês Amaral¹, Daniela Madeira¹, João Pedro Lopes¹, Francisco Gonçalves¹, Rodrigo Cunha^{1,2}, Paula Agostinho^{1,2}. ¹University of Coimbra, Centre for Neuroscience and Cell Biology, Coimbra, Portugal. ²University of Coimbra, Coimbra, Portugal
- P375-0741** **Paternal Factors in Neurodevelopmental Toxicology: THC Exposure of Male Rats Causes Long-Lasting Neurobehavioral Effects in Their Offspring.** Edward Levin, Andrew Hawkey, Brandon Hall, Marty Cauley, Susan Slade, Elisa Yazdani, Bruny Kenou, Hanna White, Corinne Wells, Amir Rezvani, Susan Murphy. *Duke University, Durham, NC, The United States of America*
- P376-0751** **Adolescent Toluene Exposure Increases DOI-Induced Behavioral, Molecular, and Electrophysiological Responses in Adult Mice: Effects of Antipsychotic Treatment.** Hwei-Hsien Chen¹, Mei-Yi Lee¹, Bih-Fen Lin², Ming-Huan Chan³. ¹National Health Research Institutes, Zhunan, Miaoli County, Taiwan. ²Tzu Chi University, Hualien, Hualien, Taiwan. ³National Chengchi University, Taipei, Taiwan
- P377-0775** **Evaluation of Anticonvulsant Activity of Methanolic Leaf Extract of *Solanum nigrum* Plant and Its Fractions in Mice.** Nneka Chuka-Imarhia. *Nnamdi Azikiwe University, Nnewi, Anambra, Nigeria*
- P378-0777** **A Toxicological Profile of Valproic Acid in Experimental Model of Autism Spectrum Disorder: Developmental, Behavioral, Biochemical, and Histopathological Evidences.** Bikash Medhi, Abhishek Mishra, Phulen Sarma, Rohit Rajput, Rubal Singla, Rupa Joshi. *PGIMER Chandigarh, Chandigarh, India*
- P379-0796** **Environmental Toxicants and the Melatonin System.** Paula Pierozan^{1,2}, Daiane Cattani^{1,2}, Eleftheria Theodoropoulou^{1,2}, Ariane Zamoner Pacheco de Souza³, Eva Brittebo⁴, Oskar Karlsson^{1,2}. ¹Stockholm University, Stockholm, Sweden. ²Science for Life Laboratory, Solna, Stockholm, Sweden. ³Federal University of Santa Catarina, Florianopolis, Santa Catarina, Brazil. ⁴Uppsala University, Uppsala, Sweden
- P380-0821** ***Magnoliae* Cortex Extract Protects PC12 Cells from Oxidative Stress through Induction of Drug-Metabolizing and Antioxidant Enzymes.** Takahito Nishiyama, Ohnuma Tomokazu, Ogura Kenichiro, Hiratsuka Akira. *Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan*
- P381-0846** **Protective Effect of the Imidazoline I2 Receptor Agonist 2-BFI on Oxidative Cytotoxicity in Astrocytes.** Jiheee Yun¹, Jongmin Lee², Dong-Hee Choi². ¹Konkuk University, College of Medicine, Seoul, The Republic of Korea. ²Konkuk University School of Medicine, Konkuk University, Seoul, The Republic of Korea
- P382-0849** **Behavioral and Immunohistological Analysis of Peripheral Nerve Injury in Methylmercury-Exposed Rats.** Mari Kikuta¹, Yuta Yamada², Momoko Sakamoto², Shunsuke Ehara², Eiko Yoshida³, Tsutomu Takahashi², Toshiyuki Kaji³, Yo Shinoda², Yasuyuki Fujiwara². ¹Tokyo Gakugei University International Secondary School, Tokyo, Japan. ²Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan. ³Tokyo University of Science, Tokyo, Japan
- P383-0854** **Comparison of the Neurotoxicity Associated with Cobalt Nanoparticles and Cobalt Chloride in Wistar Rats.** Fuli Zheng^{1,2}, Zhou Song Luo¹, Chunyan Zheng¹, Jing Li¹, Jingwen Zeng¹, Hongyu Yang¹, Michael Aschner², Siying Wu¹, Qunwei Zhang³, Huangyuan Li¹. ¹Fujian Medical University, Fuzhou, Fujian, China. ²Albert Einstein College of Medicine, Bronx, NY, The United States of America. ³University of Louisville, Louisville, KY, The United States of America
- P384-0865** ***In Vitro* and *In Vivo* Toxicity of Poly-L-arginine.** Jieun Kim¹, Sungeun Lee¹, Trang H. T. Trinh¹, Hakmin Lee¹, Junwu Shin¹, Jaehyeon Kim¹, Mireong Yoo¹, A-ran Kim¹, Taeyeon Kim², Chongsuk Ryou¹. ¹Hanyang University, Ansan-si, The Republic of Korea. ²Sungshin University, Seoul, The Republic of Korea

- P385-0883** ***In Vitro and In Vivo Assessment of SGI-1027 in Toxicological Perspectives.*** Jieun Kim¹, Sungeun Lee¹, Trang H. T. Trinh¹, Hakmin Lee¹, Junwu Shin¹, Jaehyeon Kim¹, Mireong Yoo¹, Dae-Hwan Kim², Jiaojie Li³, A-ran Kim¹, Taeyeon Kim⁴, Chongsuk Ryou¹. ¹Hanyang University, Ansan-si, The Republic of Korea. ²Institute of Science and Technology, Daegu, Gyeongbuk, The Republic of Korea. ³Gwangju Institute of Science and Technology, Gwangju, The Republic of Korea. ⁴Sungshin University, Seoul, The Republic of Korea
- P386-0889** **Manganese Induced Neurotoxicity Cross-Species due to Protein Biosynthesis Impairment with Putative Implications for Neurodegeneration.** Raul Hernandez^{1, 2}, Cristina Suñol³, Montserrat Carrascal⁴, Joaquin Abian⁴, Bernhard Michalke⁵, Yasmilde González⁶, Nadja Souza-Pinto⁷, Jos Kleinjans⁸, Marcel van Herwijnen⁸, Jolanda Piepers⁸, Daniel Burnside², Houman Motesharei², Ashkan Golshani². ¹Universidade Federal de São Paulo, Diadema, São Paulo, Brazil. ²Carleton University, Ottawa, Ontario, Canada. ³Institut d'Investigacions Biomèdiques de Barcelona, IIBB-CSIC, Barcelona, Catalonia, Spain. ⁴Institut d'Investigacions Biomèdiques de Barcelona, IIBB-CSIC, Barcelona, Catalonia, Spain. ⁵Helmholtz Zentrum München GmbH, German Research Center for Environmental Health, Munich, Bavaria, Germany. ⁶University of Ottawa, Ottawa, Ontario, Canada. ⁷University of São Paulo, São Paulo, Brazil ⁸Maastricht University, Maastricht, Limburgo, The Netherlands
- P387-0924** **Association of Initial Troponin I Level with the Development of Delayed Neurologic Sequelae in Patients with Acute Carbon Monoxide Poisoning.** Namseong Baek, Jong Seung Lee. Asan Medical Center, Seoul, The Republic of Korea
- P388-0058** **Validation Study of Developmental Toxicity Test Using Embryoid Body's Area.** Jae-Hwan Lee, Dinh Nam Tran, Bo-Hui Jeon, Eui-Man Jung, Eui-Bae Jeung. Chungbuk National University, Cheongju, Chungbuk, The Republic of Korea
- P389-0071** **Quercetin Ameliorates Cadmium Chloride-Induced Hepatotoxicity in Wistar Rats.** Eneji Egbung¹, Victor Nna¹, Item Atangwho¹, Magdalene Obi-Abang². ¹University of Calabar, Calabar, Cross River, Nigeria. ²Cross River University of Technology, Calabar, Cross River, Nigeria
- P390-0093** **Treatment of Human Choriocarcinoma Cells with Acrylonitrile-Induced Cell Cycle Arrest and Apoptosis by Promoting the Formation of Reactive Oxygen Species.** Soo-Min Kim, Kyung-Chul Choi. Chungbuk National University, Cheongju, Chungbuk, The Republic of Korea
- P391-0095** **Cigarette Smoke Extracts Induced Apoptosis and Oxidative Stress in Mouse Embryonic Stem Cells via Regulating Apoptotic and Oxidative Stress Related Genes.** Cho-Won Kim, Kyung-Chul Choi. Chungbuk National University, Cheongju, Chungbuk, The Republic of Korea
- P392-0122** **Exposure to Embalment Fluid and Changes in Biomarkers of Oxidative Stress, Reproductive Hormones, and Renal Function in Embalmers in Calabar, Nigeria.** Augusta Nsonwu-Anyanwu¹, Chinyere Usoro¹, Raymond Eworo¹, Sunday Offor¹, Magnus Nsonwu². ¹University of Calabar, Calabar, Cross River, Nigeria. ²Imo State University, Owerri, Imo State, Nigeria
- P393-0124** **Cadmium Exposure of Female Mice Impairs the Meiotic Maturation of Oocytes and Subsequent Embryonic Development.** Jiaqiao Zhu^{1, 2, 3}, Hui Zou¹, Jianchun Bian^{1, 3}, Zongping Liu^{1, 2, 3}. ¹Yangzhou University College of Veterinary Medicine, Yangzhou, Jiangsu, China. ²Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou, Jiangsu, China. ³Joint International Research Laboratory of Agriculture and Agri-Product Safety, the Ministry of Education of China, Yangzhou University, Yangzhou, Jiangsu, China
- P394-0137** **Antidepressant Sertraline Induces Cytotoxicity in Human Placental Cells.** Tomohiro Nabekura, Tatsuya Kawasaki, Yuichi Uwai. Aichi Gakuin University, Nagoya, Aichi, Japan
- P395-0145** **BMAA Alters Striatal Neural Stem Cell Proliferation and Differentiation through Glutamatergic Mechanisms.** Paula Pierozan, Liselott Kallsten, Oskar Karlsson. Stockholm University, Stockholm, Sweden
- P396-0146** **The Role of Autism Risk Gene, CHD8, in Chlorpyrifos-Induced Neurotoxicity in iPSC-Derived BrainSphere Model.** Lena Smirnova¹, Xiali Zhong^{1, 2}, Francesca Fagiani^{1, 3}, David Pamies^{1, 4}, Helena Hogberg¹, Herbert Lachman⁵, Thomas Hartung^{1, 6}. ¹Johns Hopkins University, Baltimore, MD, The United States of America. ²Sun Yat-sen University, Guangzhou, Guangdong, China. ³Institute for Advanced Study, Pavia, Italy. ⁴University of Lausanne, Lausanne, Switzerland. ⁵Albert Einstein College of Medicine, New York, NY, The United States of America. ⁶University of Konstanz, Konstanz, Germany
- P397-0152** **The Combined Effects of Cadmium and Methylene Blue in Zebrafish Embryos.** Zuzana Magdolen, Andrew Rempel, Brodie Buchner-Duby, Carolyn Kapron. Trent University, Peterborough, Ontario, Canada
- P398-0211** **Teratogenicity of Multiwall Carbon Nanotubes and Asbestos Intratracheally Administered to Mice.** Tomoko Fujitani. Tokyo Metropolitan Institute of Public Health, Shinjuku, Tokyo, Japan

- P399-0242** **p, p'-DDT Induces Apoptosis in Human Endometrial Stromal Cells through Oxidative Stress.** Yeon Jean Cho, Myoungseok Han, Youngmin Kwon. *Dong-A University, College of Medicine, Busan, The Republic of Korea*
- P400-0275** **Gestational and Lactational Exposure to an Environmentally Relevant Mixture of Brominated Flame Retardants Dysregulates Cell-Cell Interactions, Thyroid Homeostasis, and the Proliferation-Apoptosis Balance in Rat Mammary Glands at Puberty.** Rita-Josiane Gouesse¹, Mélanie Lavoie¹, Elham Dianati¹, Michael Wade², Barbara Hales³, Bernard Robaire⁴, Isabelle Plante¹. ¹*INRS, Institut-Armand Frappier, Laval, Québec, Canada.* ²*Health Canada, Ottawa, Ontario, Canada.* ³*McGill University, Faculty of Medicine, Montreal, Québec, Canada.* ⁴*McGill University, Faculty of Medicine, Montreal, Québec, Canada*
- P401-0298** **Analysis of Epigenetic Effect of Low-Level Methylmercury on Neuronal Differentiation by Using an *In Vitro* Model.** Suzuna Go, Kana Matsumoto, Manami Hatano, Hisaka Kurita, Masatoshi Inden, Isao Hozumi. *Gifu Pharmaceutical University, Gifu, Japan*
- P402-0466** **Differential Gene Expression Analysis in Mouse Placentae Reveals Association between Preterm Birth Linked Genes and PM_{2.5} Exposure.** Ariana Schanzer¹, Jason Blum¹, Lung-Chi Chen¹, Maya Deyssenroth², Jia Chen², Judith Zelikoff¹. ¹*NYU School of Medicine, New York, NY, The United States of America.* ²*Icahn School of Medicine at Mount Sinai, New York, NY, The United States of America*
- P403-0471** **Overabundance of microRNA 133b Induces Oil-Like Cardiotoxicity in Developing Zebrafish (*Danio rerio*) Embryos.** Justin Greer¹, Subham Dasgupta¹, Daniela Pampanin², David Volz¹, Daniel Schlenk¹. ¹*University of California Riverside, Riverside, CA, The United States of America.* ²*University of Stavanger, Stavanger, Norway*
- P404-0473** **Maternal Exposure during Pregnancy to Electronic Cigarette Aerosols Modifies Behavior of the Adult Offspring: Learning Neurodevelopment from a Mouse Model.** Judith Zelikoff¹, Jason Blum², Jared Schwartz³, Jill Ratner¹, Jamie Church³. ¹*NYU School of Medicine, New York, NY, The United States of America.* ²*Product Safety Labs, Dayton, NJ, The United States of America.* ³*Mt Holyoke College, South Hadley, MA, The United States of America*
- P405-0554** **Poly(ADP-ribose) Polymerase-1 Inhibitor Restores Neuroimmune Function through ROR γ t and Stat3 Transcription Factors Signaling in BTBR Mice.** Saleh Albakheet, Sheikh Ahmad, Mushtaq Ansari, Sabry Attia. *King Saud University, Riyadh, Saudi Arabia*
- P406-0565** **Investigation of Gonado- and Reproductive Toxicity of Carbendazim on Male and Female Wistar Hannover Rats.** Oleksandr Kravchuk, Mykola Prodanchuk, Nadiia Nedopytanska, Inna Rashkivska, Yana Kolianchuk. *L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical Safety, Ministry of Health, Ukraine, Kyiv, Ukraine*
- P407-0612** **Comparative Analysis of Zebrafish and Planarian Model Systems for Developmental Neurotoxicity Screens Using an 87-Compound Library.** Lisa Truong¹, Danielle Hagstrom², Siqi Zhang², Eva-Maria Collins³, Robert Tanguay¹. ¹*Oregon State University, Corvallis, OR, The United States of America.* ²*University of California San Diego, La Jolla, CA, The United States of America.* ³*Swarthmore College, Department of Biology, Swarthmore, PA, The United States of America*
- P408-0631** **Occupational Exposure to n-Hexane Is Associated with Reduced FSH Levels and Also with Prolonged Menstrual Cycles in Mexican Workers of Reproductive Age.** Liliana Ruiz García¹, Octavio Jiménez¹, Nicté Figueroa¹, Juan M Malacara¹, Mariella Carrieri², Fabiola Salamon². ¹*Universidad de Guanajuato, León, Guanajuato, Mexico.* ²*Universidad de Padua, Padua, Veneto, Italy*
- P409-0648** **Exposure to Di(2-Ethylhexyl) Phthalate (DEHP) Affects the Maturation and Quality of Blastocysts.** Lyda Parra-Forero¹, Angelica Mojica-Villegas², Elim Alfaro-Pedraza³, María Hernández-Ochoa³. ¹*Center of Research and Advanced Studies (Cinvestav)-IPN, La Laguna Ticoman, Ciudad de México, Mexico.* ²*Escuela Nacional de Ciencias Biológicas-IPN, Santo Tomás, Ciudad de México, Mexico.* ³*Center for Research and Advanced Studies (Cinvestav), Mexico City, Mexico*
- P410-0650** **Effect of Pre-pubertal Exposure to Di(n-butyl) Phthalate (DBP) on Ultrastructural and Immunohistochemical Changes in Peritubular Myoid Cells (PTMC) of Adult Japanese Quail Testes.** Umar M. Bello¹, Tom A. Aire², Imam Jibrin¹, Jimoh Abdulazeez¹, Casmir Igbokwe³. ¹*Ahmadu Bello University, Zaria, Kaduna, Nigeria.* ²*St. George's University, True Blue, Grenada.* ³*University of Nigeria, Nsukka, Enugu State, Nigeria*
- P411-0704** **Sodium Arsenite Induces Inflammation and Reduces Functionality of Ovaries of Wistar Rats.** Zaira Gómez, María González, María Ballinas, Margarita Levorio, Blanca Sánchez-Ramírez. *Universidad Autónoma de Chihuahua, Chihuahua, Chihuahua, Mexico*

- P412-0719** **Maternal PCB Exposure and Disinhibitory Behaviors among Adolescent Offspring.** Yusuf Ransome¹, Aisha Dickerson², Oskar Karlsson³. ¹*Yale School of Public Health, New Haven, CT, The United States of America.* ²*Harvard School of Public Health, Boston, MA, The United States of America.* ³*Stockholm University, Stockholm, Sweden*
- P413-0732** **Withdrawn**
- P414-0829** **Enhanced Histone H3K9 Tri-methylation Suppresses Steroidogenesis in Rat Testis Chronically Exposed to Arsenic.** Qingyu Huang¹, Ambreen Alamdar². ¹*Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, China.* ²*COMSATS University, Islamabad, Pakistan*
- P415-0179** **Involvement of PI3K-Akt Signaling in Antiapoptotic Effect Evoked by 1,2-Naphthoquinone** Kengo Nakahara¹, Hideki Hiraoka¹, Kyohei Hamada¹, Yoshito Kumagai², Takashi Uehara¹. ¹*Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan.* ²*University of Tsukuba Environmental Biology Laboratory, Faculty of Medicine, Tsukuba, Ibaraki, Japan*
- P416-0233** **Chemical Characterization and Toxicological Effects of Emissions Generated from New Tobacco- and Smoking-Related Products: Comparison with Conventional Cigarette Smoke.** Romain Dusautoir¹, Gianni Zarcone¹, Marie Verrielle², Guillaume Garçon¹, Isabelle Fronval², Nicolas Beauval¹, Delphine Allorge¹, Véronique Riffault², Nadine Locoge², Jean-Marc Lo-Guidice¹, Sébastien Anthérieu¹. ¹*Univ. Lille, CHU Lille, Institut Pasteur de Lille, EA4483-IMPECS (IMPact of Environmental Chemicals on human health), Lille, France.* ²*IMT Lille Douai, Univ. Lille, SAGE, Lille, France*
- P417-0240** **TGF-Beta Induced CTGF Expression and Epithelial-Mesenchymal Transition in Human Lung Epithelial Cells through ERK, ADAM17, RSK1, and C/EBPbeta Pathways.** Bing-Chang Chen, Shu-Ching Ou. *Taipei Medical University, Taipei, Taiwan*
- P418-0277** **Sex Differences in Respiratory Toxicity Induced by Acute Ozone Inhalation and Sensitive Indicators in Rats.** Xiaohua Liu, Ning Li, Hu Yang, Tie Han, Zhuge Xi. *Tianjin Institute of Environmental and Operational Medicine, Tianjin, China*
- P419-0354** **Effects of Cigarette Smoking on the Health Status of Workers in Suburban Area of Tokyo.** Tomoko Fujitani. *Tokyo Metropolitan Institute of Public Health, Shinjuku, Tokyo, Japan*
- P420-0560** **Adverse Effects of Real-Time Gasoline Engine Emissions Exposure at Air-Liquid Interface.** Tereza Cervena^{1,2}, Vit Beranek³, Michal Vojtisek³, Pavel Rossner¹. ¹*Institute of Experimental Medicine CAS, Prague, The Czech Republic.* ²*Charles University in Prague, Faculty of Science, Prague, The Czech Republic.* ³*Czech Technical University of Prague, Faculty of Mechanical Engineering, Prague, The Czech Republic*
- P421-0623** **Sex Differences in the Respiratory Response to Multi-Walled Carbon Nanotubes.** Jessica Ray, Andrij Holian. *University of Montana, Missoula, MT, The United States of America*
- P422-0667** **The Role of Thrombospondin Type 1 Repeat (TSR) Domain in Multiwalled Carbon Nanotube-Induced Endothelial Dysfunction and Inflammation.** Tamara Young¹, Guy Herbert¹, Selita Lucas¹, Andrew Ottens², Aaron Erdely³, Matthew Campen¹. ¹*University of New Mexico, Albuquerque, NM, The United States of America.* ²*Virginia Commonwealth University, Richmond, VA, The United States of America.* ³*NIOSH, Morgantown, WV, The United States of America*
- P423-0686** **Impacts of Inhaled Ozone on Uterine and Placental Function and Maternal Cardiovascular Health.** Marcus Garcia¹, Raul Salazar¹, Brian Villalva¹, Selita Lucas¹, Guy Herbert¹, Tamara Young¹, Jessica Begay¹, Barry Bleske¹, Ryan Ashley², Stacia Prosser², Andrew Ottens³, Matthew Campen¹. ¹*University of New Mexico College of Pharmacy, Albuquerque, NM, The United States of America.* ²*New Mexico State University, Las Cruces, NM, The United States of America.* ³*Virginia Commonwealth University, Richmond, VA, The United States of America*
- P424-0805** **Breathing beyond the Barrier: The Dynamics of Oxidative Stress and Pro-inflammatory Signaling within a Novel Organotypic Airway Model.** Samantha Faber¹, Nicole McNabb², Shaun McCullough³. ¹*University of North Carolina at Chapel Hill, Chapel Hill, NC, The United States of America.* ²*University of California, Davis, Davis, CA, The United States of America.* ³*US EPA/NHEERL, Durham, NC, The United States of America*
- P425-0813** **Cellular and Epigenetic Effects of Cannabinoid Exposure on Bronchial Epithelial Cells.** Megan Doldron, Zhenquan Jia, Ramji Bhandari. *UNC Greensboro, Greensboro, NC, The United States of America*
- P426-0871** **Subchronic Inhalation Toxicity Study of Mono Methyl Formamide in F344 Rats.** Ka-young Park, Mi-Ju Lee, Cheol-Hong Lim, Yong-Soon Kim. *Occupational Safety and Health Research Institute, KOSHA, Daejeon, The Republic of Korea*

- P427-0880** ***In Vitro* Testing for Respiratory Sensitizers Using a Genomic Biomarker Signature and Machine Learning.** Andy Forreryd¹, Henrik Johansson¹, Joshua Schmidt². ¹SenzaGen AB, Lund, Sweden. ²SenzaGen Inc, Chapel Hill, NC, The United States of America
- P428-0131** **Analysis on the Trend of the Preservative System in Commercial Infant Cosmetics and Anti-sensitive Cosmetics.** Xiufang Yan, Zhitao Chen, Zhenjuan Jiang, Ying Wang, Jing Hong, Tian Chen, Ping Cao. *Jahwa United Co., Ltd, Shanghai, China*
- P429-0163** **Suppression of TARC Expression by Impressic Acid through Blockade of ERK1/2/STAT1 and Improvement of p62/Nrf2/HO-1 in Human Keratinocyte.** Jae Ho Choi, Sun Woo Jin, Young Ho Kim, Hye Gwang Jeong. *Chungnam National University, Daejeon, The Republic of Korea*
- P430-0314** **Aggravation of Acute Dermatitis Symptoms by Cosmetic Colorants Mediated through the Production of TSLP in Skin.** Gabsik Yang¹, Hye Lee¹, Kyung-Min Lim², Yong-Kyu Choi³, Kyu-Bong Kim⁴, Byung-Mu Lee⁵, Joo Lee¹. ¹Catholic University of Korea, Bucheon, The Republic of Korea. ²Ewha Womans University, Seoul, The Republic of Korea. ³National Institute of Food and Drug Safety Evaluation, Osong, The Republic of Korea. ⁴Dankook University, Cheonan, The Republic of Korea. ⁵Sungkyunkwan University, Suwon, The Republic of Korea
- P431-0688** **Molecular Pathogenesis Underlying Exposure to Cutaneous Warfare Vesicants, Arsenicals.** Mohammad Athar, Ritesh Srivastava, Changzhao Li. *University of Alabama at Birmingham Research Center of Excellence in Arsenicals, Birmingham, AL, The United States of America*
- P432-0791** **Mannosylerythritol Lipids Ameliorate UVA-Induced Aquaporin-3 Downregulation by Suppressing c-Jun N-Terminal Kinase Phosphorylation in Cultured Human Keratinocytes.** Il-Hong Bae¹, Jae Won Yoo¹, Dae-Yong Kim². ¹Amorepacific Corporation, Yongin-si, The Republic of Korea. ²Seoul National University, Seoul, The Republic of Korea
- P433-0847** **Human Steroid Sulfatase Regulates Keratinization through Inducing the Expression of E-cadherin.** Tae-Uk Kwon, Dong-Jin Ye, Hong Gyu Ahn, Young-Jin Chun. *Chung-Ang University, Seoul, The Republic of Korea*
- P434-0852** **An Ingredient of Cosmetic Hair Dye, Para-phenylenediamine, Increases TSLP and Inflammatory Cytokines and Causes Acute Dermatitis Symptoms.** Jae Kwon Lee, Hye Lee, Gabsik Yang, Joo Lee. *BK21plus Team, Catholic University of Korea, Bucheon, Gyeonggi, The Republic of Korea*
- P435-0945** **Toxicity and Mechanisms of Vesicating Chemical Warfare Agent Skin Exposure.** Dinesh Goswami¹, William Sosna², Richard Tuttle², Poojya Anantharam², Julie Roseman², B Malique Jones³, Holly Wright³, Joshua Klein³, Claire Crutch², Rajesh Agarwal¹, Neera Tewari-Singh³. ¹University of Colorado Denver, Aurora, CO, The United States of America. ²MRIGlobal, Kansas City, MO, The United States of America. ³Michigan State University, East Lansing, MI, The United States of America
- P436-0525** **Correlation of Epithelial-to-Mesenchymal Transition-Related Genes Produces Sex-Biased Differences Specifically in Liver Cancer Cell Lines.** Sun Young Kim¹, Hyesol Lim¹, Seungeun Lee¹, Ji Yoon Shin¹, Eunhye Lee¹, Joohee Jung¹, Sang Geon Kim², Aree Moon¹. ¹Duhsung Women's University, Seoul, The Republic of Korea. ²Seoul National University, Seoul, The Republic of Korea

Thursday Program Schedule

Thursday, July 18, 8:00 AM to 9:15 AM, Kalākaua Ballroom

Keynote Lecture: Mechanisms vs. AOP: Does It Matter for the Use of the Mechanistic Data in Decision-Making?

Lecturer: Kate Z. Guyton, International Agency for Research on Cancer, Lyon, France; and Maurice P. Whelan, European Commission Joint Research Centre (JRC), Ispra (VA), Italy.

Chairperson: Ivan Rusyn, Texas A&M University, College Station, TX, The United States of America.

Following in a spirit of very successful sessions that occur annually at several major national toxicology meetings since the early 1990s in which leading toxicologists advocate opposing sides of an issue of significant toxicological importance, the ICT XV Scientific Program will include a debate that will address the question: Mechanisms vs. AOP: Does it matter for the use of the mechanistic data in decision-making?

This session will debate the future prospects for using mechanistic data to bridge important gaps in assessing new and existing chemicals. Attention will be given to lessons learned from the various approaches developed over the years by toxicologists and risk assessors for identifying, evaluating and applying mechanistic knowledge and data in a variety of assessment contexts. Prominent examples include pathway-based approaches such as the Mode of Action (MoA) framework developed by the WHO's International Program on Chemical Safety (IPSC), and the Adverse Outcome Pathway (AOP) framework, initially described by the ecotoxicology community and now brought forward by the Organisation for Economic Co-operation and Development (OECD). Another recent approach has been to interrogate, assemble and evaluate the relevant evidence on various cancer mechanisms, facilitated by the definition of the Key Characteristics (KC) of carcinogens by the WHO's International Agency for Research on Cancer (IARC). All of these approaches are designed to facilitate the optimal use of mechanistic knowledge and data in decision-making, but they differ in many ways, including how the underpinning data and knowledge are assembled and how they are applied in practice. But do these differences really matter? Are there better ways out there? Should different approaches develop independently or is there a need for more consensus and convergence? What gaps in evidence and other challenges have these approaches revealed so far? Moreover, irrespective of the actual approach one might favor, how far can we actually go with basing hazard and risk conclusions on mechanistic data?

Regardless of the differences and personal convictions, each scientific debate delegate will present relevant evidence and compelling scientific arguments to persuade and appeal to the response of the audience in order to obtain the approval or refusal of the motion.



Dr. Kate Guyton is a Senior Toxicologist at the International Agency for Research on Cancer (IARC), World Health Organization in Lyon, France. Prior to joining IARC, she served as a Toxicologist in the Office of Research and Development at the US Environmental Protection Agency (2005–2014). She also has experience, as the Director of Scientific Affairs at CCS Associates (1998–2005), working with the US National Cancer Institute in cancer screening, prevention, treatment and imaging. Dr. Guyton received her BA (cum laude) from Johns Hopkins University, her PhD from the Johns Hopkins Bloomberg School of Public Health, and her postdoctoral training at the US National Institutes of Health. Dr. Guyton has been certified as a Diplomate of the American Board of Toxicology since 1998. She has authored more than 75 scientific articles in her area of expertise.



Prof. Maurice Whelan is head of the Chemical Safety and Alternative Methods Unit of the Directorate for Health, Consumers and Reference Materials of the European Commission's Joint Research Centre (JRC), based in Ispra, Italy. He also heads the JRC's EU Reference Laboratory for alternatives to animal testing (EURL ECVAM). Priorities of his work include the advancement of *in vitro* and computational methods for regulatory safety assessment of chemicals and for application in basic and applied research. Prof. Whelan is the EU co-chair of the OECD Advisory Group on Molecular Screening and Toxicogenomics that is responsible for the OECD programme on Adverse Outcome Pathways, and he is a member of the Steering Committee of the European Partnership for Alternative Approaches to Animal Testing (EPAA). In 1993 he was awarded his PhD by the University of Limerick (Ireland) in Mechanical Engineering based on his research into the design of orthopaedic knee prostheses. Publications include over 200 scientific papers and a recent book on the validation of alternative methods for toxicity testing. He holds a number of external appointments including the 2017–2018 Francqui Chair for alternative methods at the Vrije Universiteit Brussel (VUB, Belgium) and visiting Professor of Bioengineering at the University of Liverpool (UK).

See page 113 for a listing of Platform Presentation Abstracts.

Thursday, July 18, 9:30 AM to 11:00 AM, Room 312



PL01: Alternatives to Animal Testing

Abstract

0330 9:30

California Cruelty-Free Cosmetics Act Establishes First Law in North America That Bans the Sale of Animal-Tested Cosmetics and Ingredients. Elizabeth Baker, Kristie Sullivan. Physicians Committee for Responsible Medicine, Washington, DC, The United States of America.

Thursday Program Schedule

- 0237 9:45 **Practical Implementation of New Approach Methodologies (NAMs): A Corporate Perspective for Diverse Toxicology Applications.** *Lawrence Milchak*, David Brandwein, Colin Owens. 3M, St Paul, MN, The United States of America.
- 0230 10:00 **Hepatocyte-Like Cells Derived from Human Induced Pluripotent Cells Using Small Molecules: Implications of a Transcriptomic Study.** *Xiugong Gao*, Jeffrey Yourick, Robert Sprando. US FDA/CFSAN, Laurel, MD, The United States of America.
- 0150 10:15 **High-Throughput Transcriptomics Using Hepatocyte Spheroids to Identify Chemical-Induced Biological-Response Pathways and Associated Toxicity.** *Sreenivasa Ramaiahgari¹*, Scott Auerbach¹, Trey Saddler¹, Nisha Sipes¹, Michael DeVito¹, Pierre Bushel², Alex Merrick¹, Paules Richard¹, Stephen Ferguson¹. ¹NIEHS/NTP, Durham, NC, The United States of America ²NIEHS, Durham, NC, The United States of America.
- 0585 10:30 **The Cross-Laboratory Testing and Evaluation of Two Liver Microphysiological Systems.** *Courtney Sakolish¹*, Yizhong Liu¹, Celeste Reese², Richard DeBiasio², Felipe Lee-Montiel³, Caleb Lee³, Lawrence Vernetti², Lansing Taylor², Kevin Healy³, Ivan Rusyn¹. ¹Texas A&M University, College Station, TX, The United States of America ²University of Pittsburgh, Pittsburgh, PA, The United States of America ³University of California Berkeley, Berkeley, CA, The United States of America.
- 0564 10:45 **In vitro Model for the Prediction of Respiratory Sensitization.** *Aline Chary^{1,2}*, Charlotte Stoffels¹, Tommaso Serchi¹, Sébastien Cambier¹, Elisa Moschini¹, Servane Contal¹, Jennifer Hennen², Janine Ezendam³, Brunhilde Blömeke², Arno Gutleb¹. ¹Luxembourg Institute of Science and Technology, Belvaux, Luxembourg ²University Trier, Trier, Germany ³Centre for Health Protection, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.

Thursday, July 18, 9:30 AM to 11:00 AM, Room 315



PL02: Immunotoxicology

Abstract

- 0407 9:30 **Aryl Hydrocarbon Receptor (AhR) Signaling Regulates the Inflammatory Potential of Dendritic Epidermal T Cells.** *Katja Merches*, Charlotte Esser, Kathrin Hochrath, Nadine Teichweyde. IUF - Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany.
- 0472 9:45 **Cadmium Telluride Quantum Dots Exposure Causes Functional Impairments in Monocyte-Derived Dendritic Cells and Alters CD4⁺ T Cells Differentiation.** *Tingting Wei*, Tianshu Wu, Meng Tang. Southeast University, Nanjing, Jiangsu, China.
- 0643 10:00 **Docosahexaenoic Acid as a Prophylactic Treatment for Chronic Particle-Exposed Balb/c Mice.** *Paige Fletcher¹*, Ray Hamilton¹, James Pestka², Andrij Holian¹. ¹University of Montana, Missoula, MT, The United States of America ²Michigan State University, East Lansing, MI, The United States of America.
- 0080 10:15 **Lung Function and Immune Effects in Resident Lung Macrophages following Prenatal Exposure to Sodium Arsenite.** *Kristal Rychlik*, Emily Illingworth, Sarah Attreed, Han Zhang, Jeffrey Loube, Wayne Mitzner, Fenna Sillé. Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, The United States of America.
- 0655 10:30 **Protective Effect of Resveratrol on the Integrity of Alveolar and Intestinal Epithelial Barrier in Staphylococcal Enterotoxin B-Induced Acute Lung Injury.** *Hasan Alghetaa*, Amira Mohammed, Mitzi Nagarkatti, Prakash Nagarkatti. University of South Carolina School of Medicine, Columbia, SC, The United States of America.
- 0405 10:45 **Comprehensive Analysis for Immunological Characteristics of Patients with Malignant Mesothelioma and Diffuse Pleural Thickening.** *Yasumitsu Nishimura¹*, Suni Lee¹, Naoko Kumagai-Takei¹, Hidenori Matsuzaki², Kei Yoshitome¹, Kenzo Okamoto³, Takumi Kishimoto⁴, Takemi Otsuki¹. ¹Kawasaki Medical School, Kurashiki, Japan ²Prefectural University of Hiroshima, Shobara, Japan ³Hokkaido Chuo Rosai Hospital, Iwamizawa, Japan ⁴Okayama Rosai Hospital, Okayama, Japan.

Thursday, July 18, 9:30 AM to 11:00 AM, Room 316



PL03: Metals

Abstract

- 0684 9:30 **The Sex-Specific Effects of Arsenic on Immune Function, Infectious Disease Risk, and Vaccine Outcomes.** *Fenna Sillé*, Sarah Attreed, Kristal Rychlik, Emily Illingworth, Chloe Kashiwagi, Ashley Fink, Han Zhang, Ian Sanchez, Tyrone Howard, Christopher Heaney, Sabra Klein. Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, The United States of America.
- 0014 9:45 **Developmental Exposure to Manganese Causes Cognitive Deficits in Rats: Protective Effect of Nutrient Metal Mixture Supplementation.** *Chand Basha Davuljigari*, Srinivasulu Reddy, Rajarami Reddy. Sri Venkateswara University, Tirupati, Andhra Pradesh, India.
- 0051 10:00 **The Emerging Dark Side of Nuclear Factor Erythroid 2-related Factor 2 in Chemical Carcinogenesis: Taking Arsenic as an Example.** *Ruirui Wu¹*, Ru Sun¹, Xiafang Wu¹, Huihui Wang¹, Jingqi Fu¹, Jingbo Pi², Yuanyuan Xu¹. ¹China Medical University, Shenyang, Liaoning, China ²China Medical University School of Public Health, Shenyang, Liaoning, China.
- 0121 10:15 **Heavy Metal Contamination of Drinking Water Sources and Hepato-renal Toxicities among Residents of a Crude Oil-Contaminated Area in Bayelsa State, Nigeria.** *Chinyere Usoro¹*, Caroline Thomas², Augusta Nsonwu-Anyanwu¹. ¹University of Calabar, Calabar, Cross River, Nigeria ²Federal Medical Centre, Yenogoa, Bayelsa State, Nigeria.

Thursday Program Schedule

- 0197 10:30 **Determination of Lead (Pb) Exposure on Infants Living around Pb Mining Area in Kabwe, Zambia.** Haruya Toyomaki¹, John Yabe², Shouta Nakayama¹, Yared Yohannes^{1,3}, Kaampwe Muzandu², Hazuki Mizukawa⁴, Yoshinori Ikenaka^{1,5}, Hokuto Nakata¹, Takeshi Kuritani⁶, Mitsuhiro Nakagawa⁶, Mayumi Ishizuka¹. ¹Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan ²The University of Zambia, Lusaka, Central, Zambia ³College of Natural and Computational Science, University of Gondar, Gondar, Begemder, Ethiopia ⁴Graduate School of Agriculture, Ehime University, Matsuyama, Ehime, Japan ⁵School of Environmental Sciences and Development, North-West University South Africa, Potchefstroom, North West, South Africa ⁶Graduate School of Science, Hokkaido University, Sapporo, Hokkaido, Japan.
- 0593 10:45 **Prenatal Exposure to Toxic Metals Modulates Global and Specific DNA Methylation and Expression of Repair Genes.** Nereida Montes-Castro¹, Isabel Alvarado-Cruz¹, Luisa Torres-Sánchez², Israel García-Aguilar¹, Angel Barrera-Hernández¹, Consuelo Escamilla-Núñez², Luz Maria Del Razo-Jiménez¹, Betzabet Quintanilla Vega¹. ¹Center for Research and Advanced Studies (Cinvestav), Mexico City, Mexico ²National Institute of Public Health, Cuernavaca, Morelos, Mexico.

Thursday, July 18, 9:30 AM to 11:00 AM, Room 313



PL04: Risk and Safety Evaluation

Abstract

- 0347 9:30 **Evaluating Mechanistic Data in Hazard Assessment: Integration of Key Characteristics and Mode of Action.** Bette Meek. University of Ottawa, Ottawa, Ontario, Canada.
- 0057 9:45 **A New Method to Evaluate Toxicological Data Reliability in Food Safety Risk Assessments.** Qian Bian¹, Ping Yu¹, Jun Wu¹, Zhongming Lyn¹, Yan Song², Lei Zhang², Zhaoping Liu². ¹Jiangsu Provincial Center for Disease Control and Prevention, Nanjing ²China National Center for Food Safety Risk Assessment, Beijing, China.
- 0527 10:00 **Development of New Risk Assessment Guideline of Food Contact Materials Based on the TTC Concept in Food Safety Commission of Japan.** Atsushi Ono¹, Masahiro Nakamoto², Naofumi Iizuka², Makiko Isozaki². ¹Okayama University, Okayama, Japan ²Food Safety Commission of Japan, Tokyo, Japan.
- 0599 10:15 **Establishment of a Reference Chemical Database to Evaluate In Vitro and In Silico Approaches to Assess Respiratory Sensitization to Facilitate Development of IATA.** Kristie Sullivan¹, Nancy Baker², Stella Cochrane³, Steven Enoch⁴, Janine Ezendam⁵, Grace Patlewicz⁶, Ramya Rajagopal³, Erwin Roggen⁷, Raja Settivari⁸, Katherina Sewald⁹. ¹Physicians Committee for Responsible Medicine, Washington, DC, The United States of America ²Leidos, Research Triangle Park, NC, The United States of America ³Unilever Safety and Environmental Assurance Centre, Bedford, United Kingdom ⁴Liverpool John Moores University, Liverpool, United Kingdom ⁵RIVM, Bilthoven, The Netherlands ⁶US EPA/NCCT, Research Triangle Park, NC, The United States of America ⁷3RSMC ApS, Lyngby, Denmark ⁸Corteva Agriscience, Newark, DE, The United States of America ⁹Fraunhofer ITEM, Hannover, Germany.
- 0699 10:30 **The Research of Health Risk Factors in the Field of Occupational Health and Environmental Toxicology in Laboratory Workplaces.** Nalinee Sripaung, Areepit Promrat, Satit Namwicha. Bureau of Occupational and Environmental Diseases, Department of Disease Control, Ministry of Public Health, Muang District, Nonthaburi, Thailand.
- 0061 10:45 **Do the REACH Derived No-Effect Levels Extend the Landscape of Occupational Exposure Guidance Values?** Linda Schenk^{1,2}, Gunnar Johanson¹. ¹Karolinska Institutet, Stockholm, Sweden ²KTH - Royal Institute of Technology, Stockholm, Sweden.

Thursday, July 18, 11:00 AM–12:30 PM, Room 315



PL05: Computational Toxicology

Abstract

- 0062 11:00 **How to Choose the Right Cancer Cell Line for Your Research: A Transcriptomes-Guided Approach.** Xin Shao, Yang Hu, Jie Liao, Xuechun Chen, Yunru Yu, Lingqi Yu, Xiaoyan Lu, Ni Ai, Yi Wang, Xiaohui Fan. Zhejiang University College of Pharmaceutical Sciences, Hangzhou, Zhejiang, China.
- 0030 11:15 **Fit-for-Purpose Use of High-Throughput Toxicokinetics for Predicting Potential Human Health Risk.** Nisha Sipes. NIEHS, Research Triangle Park, NC, The United States of America.
- 0031 11:30 **In Vivo Toxicity Prediction Modeling with Tox²¹ Data to Aid Regulatory Sciences.** Ruili Huang. NIH/NCATS, Rockville, MD, The United States of America.
- 0047 11:45 **A Case Study in Quantitative Generalized Read-Across (GenRA) Predictions Using Acute Oral Toxicity.** George Helman^{1,2}, Imran Shah², Grace Patlewicz². ¹Oak Ridge Institute for Science and Education, Oak Ridge, TN, The United States of America ²US EPA/NCCT, Research Triangle Park, NC, The United States of America.
- 0033 12:00 **Using (Q)SAR Models to Inform Drug Safety Assessment.** Naomi Kruhlak, Marlene Kim, Christopher Ellis, Lidiya Stavitskaya. US FDA/CDER, Silver Spring, MD, The United States of America.
- 0029 12:15 **Reproducibility of Computational Toxicology: The Prerequisite for Regulatory Applications.** Huixiao Hong. US FDA/NCTR, Jefferson, AR, The United States of America.

Thursday Program Schedule

Thursday, July 18, 11:00 AM–12:30 PM, Room 312



PL06: Nanotoxicology

Abstract

- 0190 11:00 **Neurological Risks of Inhaled Incidental Particulates and Engineered Nanomaterials.** *Krishnan Sriram*, Amy Jefferson, Gary Lin, Samuel Stone, Aliakbar Afshari, Walter McKinney, James Antonini, Michael Wolfarth, Dale Porter. NIOSH, Morgantown, WV, The United States of America.
- 0415 11:15 **Mitochondrial Respiration and Metal Bioaccumulation in Zebrafish Developmental Stages (*Danio rerio*) Evaluating Three Functionalized Groups of CdTe Quantum Dot Nanoparticles.** *Suanne Bosch*, Tarryn Botha, Victor Wepener. Water Research Group, Potchefstroom, South Africa.
- 0227 11:30 **Development of Physiologically Relevant *In Vitro* Models for Assessment of Nanoparticle-Induced Physicochemical Property-Dependent Cytotoxicity of Human Lung Cells.** *Liying Rojanasakul*¹, Raymond Derk¹, Jhy-Charm Soo¹, Philip Demokritou², Dilpreet Singh², Tiffany Kornberg^{1,3}, Todd Stueckle¹, Yon Rojanasakul³, Jayme Coyle¹. ¹NIOSH, Morgantown, WV, The United States of America ²Harvard University, Boston, MA, The United States of America ³West Virginia University, Morgantown, WV, The United States of America.
- 0481 11:45 **Nano ZnO Exposure Alters *In Vitro* Lipidomic Profiles of Human Immune Cells.** Mala Jayamanne¹, *Paul Wright*², Terence Turney³, Philip Marriott¹. ¹Monash University, Clayton, Melbourne, Victoria, Australia ²RMIT University, Bundoora, Melbourne, Victoria, Australia ³Monash University, Clayton, Melbourne, Victoria, Australia.
- 0498 12:00 **Silver Nanoparticles Promote Procoagulant Activity of Red Blood Cells: A Potential Risk of Thrombosis in Susceptible Population.** *Yiyin Bian*, Jin-Ho Chung, Byung Hoon Lee. Seoul National University, Seoul, The Republic of Korea.
- 0422 12:15 **CdTe Quantum Dots Increase Microglial Polarization in Mice and Trigger Damage to Hippocampal Neurons.** *Keyu He*, Tianshu Wu, Meng Tang. Southeast University, Nanjing, Jiangsu, China.

Thursday, July 18, 11:00 AM–12:30 PM, Room 316



PL07: Neurotoxicology

Abstract

- 0091 11:00 **K-variant BCHE and Pesticide Exposure: Interactive Genome and Parkinson's Disease.** *Mohamed Mosaad Salama*¹, Thomas Roesler², Ali Shalash³, Ulrich Müller⁴, Astrid Dempfle⁵, Gregor Kuhlenbäumer⁵, Gunter Hoeglinger², ENND Consortium¹. ¹Mansoura University, Mansoura, Egypt ²Technical University of Munich, Munich, Germany ³Ain Shams University, Cairo, Egypt ⁴Justus Liebig University Giessen, Giessen, Germany ⁵Kiel University, Kiel, Germany.
- 0685 11:15 **THC Treatment Improved Neuronal Functions by Decreasing HIV gp120-Mediated Neurotoxicity.** *Amira Mohammed*, Hasan Alghetaa, Prakash Nagarkatti, Mitzi Nagarkatti. University of South Carolina School of Medicine, Columbia, SC, The United States of America.
- 0335 11:30 **Manganese-*Mycobacterium tuberculosis* Interactions: Central and Peripheral Effects.** *Nick Filipov*, Kaori Sakamoto, Tuhina Gupta, Ryan Mote, Robert Hogan, Davis Reardon. University of Georgia, Athens, GA, The United States of America.
- 0383 11:45 **Function of LncRNA NR_030777 in Affecting Cell Proliferation and Apoptosis and Protection of Motor Function by Regulating Zfp326/Cpne5 in Nerve Cell Damage Induced by Paraquat.** *Hongyu Yang*¹, Qingxia Lin¹, Nengzhou Chen¹, Zhou Song Luo¹, Jing Li¹, Fuli Zheng¹, Qunwei Zhang², Siying Wu¹, Huangyuan Li¹. ¹Fujian Medical University, Fuzhou, Fujian, China ²University of Louisville, Louisville, KY, The United States of America.
- 0597 12:00 **Effect of Silver Nanoparticles on Neural Differentiation in Human iPS Cells.** *Yasunari Kanda*, Shigeru Yamada. National Institute of Health Sciences, Kawasaki, Kanagawa, Japan.
- 0700 12:15 **Effect of MLL Modified H3K4me3 on Aluminum Induced Cognitive Impairment—Both Population and Animal Epigenetic Studies.** *Qiao Niu*. Shanxi Medical University, Taiyuan, Shanxi, China.

Thursday, July 18, 11:00 AM–12:30 PM, Room 313



PL08: Reproductive and Developmental Toxicology

Abstract

- 0056 11:00 **Determination of Epigenetic Modifications in Fetal Brain Exposed to Methylmercury during Development.** *Hisaka Kurita*, Manami Hatano, Suzuna Go, Kana Matsumoto, Masatoshi Inden, Isao Hozumi. Gifu Pharmaceutical University, Gifu, Japan.
- 0183 11:15 **Exposure to Low Doses of 3-Methylcholanthrene Impacts on the Oocyte Integrity without Causing Systemic Cytotoxicity.** Eric Rhon Calderón, Rocío Galarza, *Alicia Faletti*. CEFYBO-CONICET, School of Medicine, University of Buenos Aires, CABA, Argentina.
- 0340 11:30 **Downregulation of SDF1/CXCR4 Signaling Mediates Ethanol-Induced Craniofacial Anomalies and Cranial Nerve Defects in Zebrafish Embryos by Disrupting Neural Crest Cell-Placode Interaction.** Huadong Fan, Fuqiang Yuan, Jie Liu, *Shao-yu Chen*. University of Louisville Health Sciences Center, Louisville, KY, The United States of America.

Thursday Program Schedule

- 0368 11:45 **Teratogen Assay Using Gene Expression Profiles of Developmental Regulators in Morphogenetic Aggregates of Human Embryonic Stem Cells.** Yusuke Marikawa, Hong-Ru Chen, Vernadeth Alarcon. University of Hawaii John A. Burns School of Medicine, Honolulu, HI, The United States of America.
- 0452 12:00 **3- to 7-Ring Polycyclic Aromatic Hydrocarbons Are the Main Inducers of the *In Vitro* Prenatal Developmental Toxicity Observed with Some Petroleum Substances.** Lenny Kamelia¹, Laura de Haan¹, Hans Ketelslegers², Ivonne Rietjens¹, Peter Boogaard^{1,3}. ¹Wageningen University and Research, Wageningen, Gelderland, The Netherlands ²European Petroleum Refiners Association, Brussels, Belgium ³Shell International BV, The Hague, The Netherlands.
- 0588 12:15 **Gestational and Lactational Exposure to the Di(2-Ethylhexyl) Phthalate (DEHP) Increases Mammary Gland Weight in Adult Female Rats Potentially by the Peroxisome Proliferator-Activated Receptor (PPAR) Pathway Activation.** Béline Crobeddu¹, Elise Kolasa¹, Bernard Robaire², Isabelle Plante¹. ¹INRS - Institut Armand-Frappier, Laval, Quebec, Canada ²McGill University, Montréal, Quebec, Canada.

Thursday, July 18, 12:30 PM to 2:30 PM, Room 312

IUPHAR IUTOX Exchange Session: Precision Medicine and ADME-Targets

Chairpersons: Andrew Somogyi, University of Adelaide, Australia; and Nuala Helsby, University of Auckland, New Zealand.

ADME targets have been a major example through which Precision Medicine has been shown to be clinically important and useful, with resultant Guidelines development and promotion. Although much has been based on classical pharmacogenetic investigations, new directions into epigenetic factors are slowly evolving with mixed results for clinical relevance. In this symposium, speakers will address a) the role of germline ADME variants in providing a greater understanding of the efficacy and life-threatening toxicity to widely-used chemotherapy drugs; b) the role of genetic and epigenetic factors in drug uptake transporters and their potential contribution to Precision Medicine; c) the importance of recognizing that ethnicity can be a major factor through investigations of the pharmacogenetics of the Australian Aborigine and, d) the increasingly recognized concept of reverse translational studies in identifying previously unknown mechanisms of drug-induced toxicity. The audience will gain a greater understanding of how far Precision Medicine has evolved over the past decades and its future with respect to ADME Targets and clinical relevance.

Talk Titles

Pharmacogenomics of Chemotherapy: Personalizing Old Drugs. Nuala Helsby, University of Auckland, New Zealand.

New Vistas in Prediction of Nongenetic Variability in UGT2B17 for Applications in Precision Medicine. Bhagwat Prasad, University of Washington, The United States of America.

Precision Medicine for First Nations People: Lessons from Aboriginal Australians. Andrew Somogyi, University of Adelaide, Australia.

Reverse Translational Studies to Identify Novel Mechanisms of Drug-Induced Toxicity. Deanna Kroetz, University of California San Francisco, The United States of America.

Thursday, July 18, 2:30 PM to 4:30 PM, Room 313



SYM17: Applying the Key Characteristics Paradigm in Hazard Identification and Risk Assessment

Chairperson(s): Martyn Smith, University of California at Berkeley; and Kathryn Guyton, Monographs Programme, International Agency for Research on Cancer, World Health Organization.

This symposium will highlight the potential application of the key characteristics paradigm to the identification of human carcinogens and other health risks from chemical exposures and to suggest consensus solutions and new approaches. Smith et al. (*Env. Health Perspect.* 124: 713, 2016) identified 10 key characteristics, one or more of which are commonly exhibited by established human carcinogens. The key characteristics are distinct from the hallmarks of cancer, which are the properties of tumors. The key characteristics instead reflect the properties of a cancer-causing agent, such as 'is genotoxic,' 'is immunosuppressive,' or 'modulates receptor-mediated effects.' In the cancer hazard identification process, the key characteristics have been used to guide a systematic literature search focused on relevant end points. Further, the National Academy of Sciences recently suggested development of key characteristics for other hazards, such as cardiovascular and reproductive toxicity. The presentations will first describe the scientific basis of key characteristics of carcinogens, discuss how biomarkers of the key characteristics and new assays for their measurement could be applied to assess human cancer risk in drug development, and describe solutions based on the key characteristics to advance cancer research and hazard identification as being applied in national and international settings.

Abstract

- S17-01 2:30 **The Key Characteristics of Carcinogens.** Martyn T. Smith, University of California at Berkeley, The United States of America.
- S17-02 3:00 **Applying the Key Characteristics of Carcinogens to Assess Human Cancer Risk in Drug Development.** Mark Fielden, Amgen Inc, Comparative Biology and Safety Sciences, The United States of America.
- S17-03 3:30 **Air Pollution Exhibits Multiple Key Characteristics of Carcinogens.** Hideko Sone, Yokohama University of Pharmacy, National Institute for Environmental Studies, Japan.
- S17-04 4:00 **Application of the Key Characteristics in the IARC Monographs Programme.** Kathryn Guyton, Monographs Programme, International Agency for Research on Cancer, World Health Organization, France.

Thursday Program Schedule

Thursday, July 18, 2:30 PM to 4:30 PM, Room 312

SYM18: Biomarker Qualification: Accelerating Drug Development by Enabling the Use of Novel Diagnostic and Safety Biomarkers

Chairperson(s): Jiri Aubrecht, Takeda; and John-Michael Sauer, Critical Path Institute.

The application of novel biomarkers in drug development is needed to accelerate the approval of new therapeutic modalities and improve diagnosis of diseases in clinical practice. However, the development and qualification of biomarkers is a costly and time-consuming process. Therefore, new innovative scientific approaches and analytical technologies, as well as access to appropriate human samples for biomarker qualification and assay validation, are required. Recently consortia including Critical Path Institute, the Predictive Safety Testing Consortium (PSTC), Innovative Medicines Initiative Safer and Faster Evidence Based Translation Consortium (IMI SAFE-T), International Life Sciences Institute Health and Environmental Sciences Institute (ILSI HESI), and Foundations for the National Institutes of Health Biomarker Consortium (FNIH BC), have identified several promising advances to support biomarker development including scientific advances in analysis and quantification of circulating microRNA and toxicogenomics. In addition, the recent progress in defining the scientific and regulatory expectations for biomarker qualification, led by C-Path and FNIH BC, provides a blueprint for the conduct of regulatory qualification of biomarkers. This symposium will provide insights into innovative approaches and state-of-the-art science involved in the development and world-wide regulatory acceptance of biomarkers for use in drug development, environmental risk assessment, and diagnosis of disease.

Abstract

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| S18-01 | 2:30 | How Much Evidence Is Enough to Accept a Biomarker for Regulatory Decision-Making? Development of Evidentiary Considerations. John-Michael Sauer, Critical Path Institute, The United States of America. |
| S18-02 | 3:00 | GLDH as an Example of Regulatory Biomarker Qualification: A Liver-Specific Biomarker of Liver Injury in Drug Development and Medical Care. Jiri Aubrecht, Takeda, The United States of America. |
| S18-03 | 3:30 | Case Studies on Integration of Toxicogenomic Biomarkers in Genetic Toxicology Assessment. Carole Yauk, Health Canada, Environmental Health Centre, Canada. |
| S18-04 | 4:00 | Serum MicroRNA Signatures as "Liquid Biopsies" for Interrogating Hepatotoxic Mechanisms and Liver Pathogenesis in Human. Julian Krauskopf, University of Maastricht, The Netherlands. |

Thursday, July 18, 2:30 PM to 4:30 PM, Room 316

SYM19: Leveraging Zebrafish to Support Global Toxicology Challenges

Chairperson(s): Jennifer Freeman, Purdue University; and David Volz, Department of Environmental Sciences, University of California, Riverside.

Over the past 20 years, adoption and integration of the application of zebrafish as a toxicological model system has magnified in most areas of toxicology-based research. As a well-recognized biomedical research model, zebrafish presents numerous strengths that have been leveraged in many toxicity studies. Rapid *ex vivo* development of a small, near-transparent singular embryo permits ease for assessing chemical perturbations at all stages of early development as well as use in high-throughput chemical screens and automated phenotyping. In addition, a complete genome sequence, array of tools for manipulating gene function, and availability of several thousand mutant and transgenic lines provides, similar to mouse models, readily available resources for comprehensive mechanistic studies of toxicity. Furthermore, maturation at three months of age and a shorter lifespan allow for multi- and transgenerational studies and efficient identification and evaluation of developmental origins of health and disease. As researchers continue to expand the use of the zebrafish in toxicology, limitations of this animal model are also being identified. In this session, speakers will highlight what has been learned from over two decades of using zebrafish as a model for toxicology, spanning mechanistic studies to current applications in high-throughput screening of chemicals and chemical mixtures.

Abstract

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| S19-01 | 2:30 | Transcriptomics Coupled with High-Throughput Phenotypic Screening to Accelerate Discovery of Toxicity Pathways. Robert Tanguay, Department of Environmental and Molecular Toxicology, Oregon State University, The United States of America. |
| S19-02 | 3:00 | Methylmercury-Induced Epigenetic Transgenerational Inheritance of Abnormal Neurobehavior Is Correlated with Sperm Epimutations in Zebrafish. Michael Carvan, School of Freshwater Sciences, University of Wisconsin-Milwaukee, The United States of America. |
| S19-03 | 3:30 | Lessons Learned on the Toxicokinetics upon Waterborne Chemical Exposure in Zebrafish Larvae. Krishna Tulasi Kirla, Department of Forensic Pharmacology and Toxicology, Eawag, Switzerland. |
| S19-04 | 4:00 | The Fish Embryo Model: Update and Challenges for Its Potential Regulatory Applications. Marc Léonard, L'OREAL Research and Innovation, Environmental Research Department, France. |

Thursday Program Schedule

Thursday, July 18, 2:30 PM to 4:30 PM, Room 315

SYM20: Recent Trends in Research on Arsenic Toxicity

Chairperson(s): Yoshito Kumagai, University of Tsukuba; and Jin-Ho Chung, Seoul National University.

Arsenic is ubiquitously distributed in nature throughout Earth's crust is also reported to modulate a variety of cellular signal transduction pathways. Speakers in the symposium will introduce recent findings found by each laboratory on the basis of molecular biology or chemical biology. For example, Prof. Zhang introduces disruption of formation of the STX17-SNAP29-VAMP8 SNARE complex, leading to inhibition of the autophagy pathway. Prof. Pi introduces importance of Nrf2/1 in β -cells and adipocytes and provide insight into the effects of inorganic arsenic exposure on insulin secretion and action. Prof. Kumagai introduces (E)-2-alkenals with an α,β -unsaturated aldehyde moiety, which is a common substituent in phytochemicals isolated from *C. sativum* leaves, activate the Keap1/Nrf2 pathway associated with cellular protection against arsenic. Prof. Chung introduces prometastatic effects of arsenic exposure can indeed contribute to cancer-related mortality.

Abstract

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| S20-01 | 2:30 | Arsenic in Oxidative Stress vs. Proteotoxic Stress. Donna Zhang, University of Arizona, The United States of America. |
| S20-02 | 3:00 | Paradoxical Roles of CNC-bZIP Proteins NRF2 and NRF1 in Arsenic-Induced Dysfunction in Pancreatic β-Cells and Adipocytes. Jingbo Pi, China Medical University, China. |
| S20-03 | 3:30 | Activation of Nrf2 through Covalent Modification of Keap1 by 2-alkenal Group of Aliphatic Electrophiles in <i>Coriandrum sativum</i> L. Diminishes Arsenic-Mediated Cytotoxicity. Yoshito Kumagai, University of Tsukuba, Japan. |
| S20-04 | 4:00 | Arsenic May Act As a Pro-Metastatic Carcinogen through Promoting Tumor Cell-Platelet Aggregation. Jin-Ho Chung, Seoul National University, The Republic of Korea. |

Thursday, July 18, 4:30 PM to 5:30 PM, Kalākaua Ballroom

Closing Ceremony



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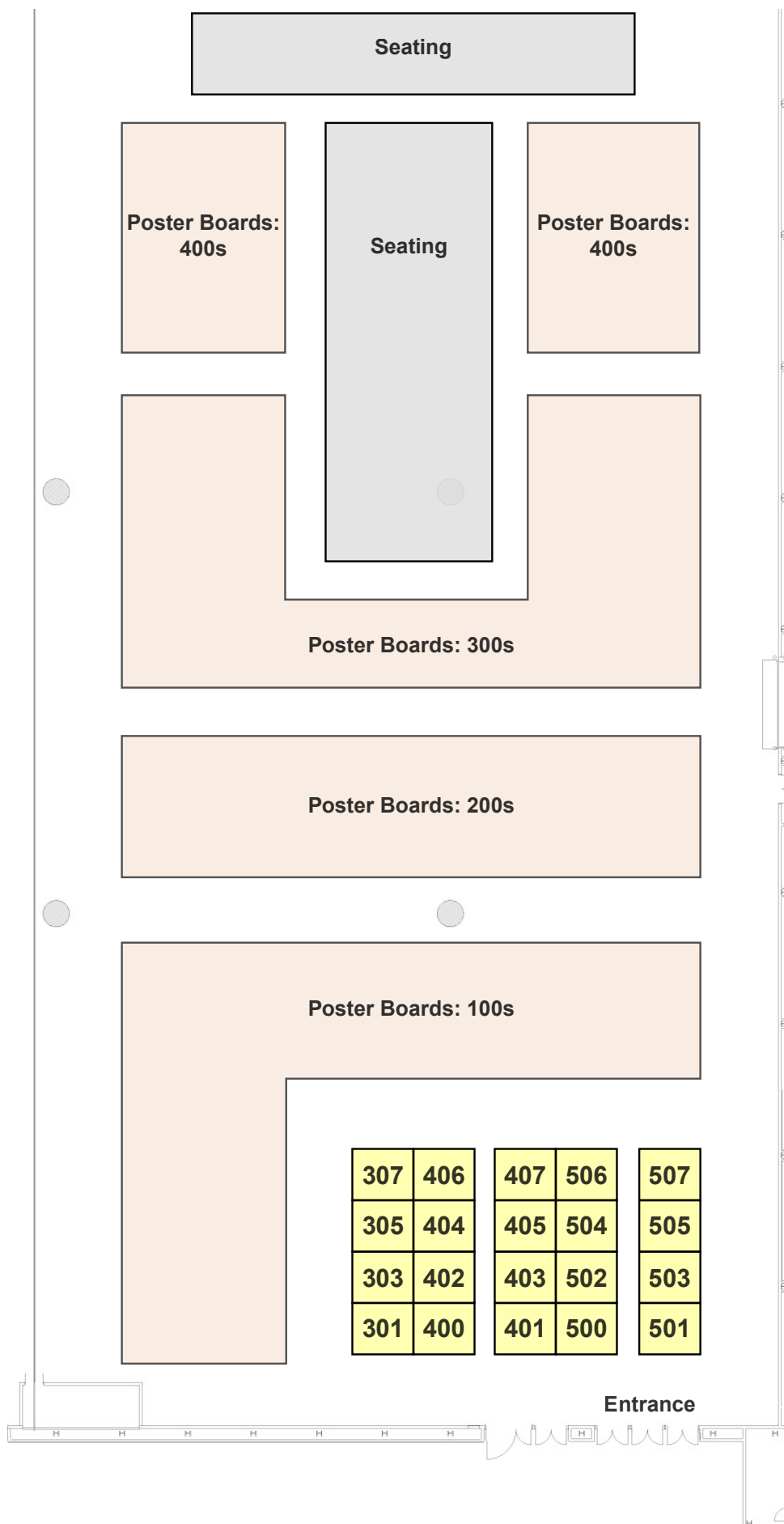
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The SOT Endowment Fund provided travel award support for 25 scientists to attend.

Abstracts



Abstracts Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and Scientific Sessions of the IUTOX 15th International Congress of Toxicology, Co-Hosted by SOT, held at the Hawaii Convention Center, Honolulu, Hawaii, USA, July 15–18, 2019.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 286.

The abstracts are reproduced as accepted by the ICTXV Scientific Program Committee and appear by session type and presentation number. Author names that are underlined in the author block indicate the presenting author.

Scientific Session Types:



Continuing Education Courses



Poster Sessions



Platform Sessions



Symposium Sessions

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AM01: Applications of Chemical Risk Assessment in Establishing Food Safety Standards

AM01-01 Risk Assessment of Food Additives and Chemical Contaminants

Gordon Shephard. *Cape Peninsula University of Technology, Cape Town, South Africa.*

Based on the Principles and Methods for the Risk Assessment of Chemicals in Food (published as Environmental Health Criteria 240), JECFA performs safety evaluations for additives and chemical contaminants in food. The acceptable daily intakes (ADIs) for food additives and tolerable intakes (TIs) for chemical contaminants established by JECFA are used by CODEX Alimentarius Commission to set health-based food standards. Thus, the processes of JECFA evaluations are required to be science-oriented, consistent and transparent. This lecture aims to provide the audience a comprehensive understanding on how JECFA establishes guidance values and makes safety recommendations for risk management. Specifically, the lecture will describe how compound-specific toxicological data are reviewed, how pivotal studies are identified and how uncertainty factors are selected. Special considerations on certain types of additives, such as flavors and enzymes, will be discussed. For chemical contaminants, safety data mainly come from published literature. Epidemiological data are often utilized for hazard identification and sometimes for hazard characterization. In the absence of potency estimates, the margin of exposure approach can be used for carcinogenic chemicals. Recent examples will be given to demonstrate how risk assessment principles are applied by JECFA in conducting safety evaluations and making safety recommendations.

AM01-02 Risk Assessment of Veterinary Drug and Pesticide Residues in Food

Alan R. Boobis. *Imperial College London, London, United Kingdom.*

When setting maximum residue limits (MRLs) for residues of veterinary drugs and pesticides in food, the Codex Alimentarius Commission relies upon risk assessments performed by JECFA and JMPR, respectively. Such assessments are performed using the established paradigm of hazard identification, hazard characterization, exposure assessment and risk characterization. Much of the necessary data are provided by sponsors, but all relevant published information and publicly generated data (e.g. on consumption), are also assessed. Whilst toxicological hazards are most often characterized from studies in experimental animals, submission of information from non-animal methods, although limited to date, is encouraged. Hazard characterization includes consideration of any available mechanistic data and is based on a weight of evidence approach. In addition to toxicological hazards, JECFA assesses potential microbiological hazards and this is now also being routinely undertaken by JMPR. Both JMPR and JECFA characterize acute and chronic toxicological and microbiological risks and is introducing methodology for assessing risks from less than lifetime exposures. In some instances, a pesticide or a veterinary drug is no longer supported by a commercial sponsor, but it still fulfills an important agricultural function. JECFA and JMPR have developed decision trees enabling such situations to be addressed.

AM01-03 The Key to Risk Assessment: Dose-Response Analysis

Matthew Wheeler. *Centers for Disease Control and Prevention, Cincinnati, OH, The United States of America.*

Dose-response analysis is a central to establishing food safety standards for chemical contaminants, and a systematic approach is necessary to ensure transparency and reproducibility in JECFA risk assessments. This presentation describes the updated dose-response standards given by the recently commissioned WHO expert committee on dose-response analysis. Rather than speak the technical details of the report, this lecture will be a series of case studies describing the specific choices a risk assessor must make, and the expert committee's recommendations on these choices. The talk will use both epidemiological and toxicological data sets used in past JECFA assessments to point out similarities and differences between the dose-response analyses when determining acceptable daily intakes (ADIs) and tolerable intakes (TIs). This talk will outline the expert committees' recommendations for the use of modern dose-response methods, including model averaging, Bayesian approaches, and the available software for these methodologies. An attendee can expect to

develop a broad understanding of the JECFA dose-response recommendations and a solid foundation for the use and application of using modern dose-response methodologies in establishing food safety standards.

AM01-04 Dietary Exposure Assessment of Chemicals in Food

Peter Cressey. *Institute of Environmental Science and Research Limited, Christchurch, New Zealand.*

Within the risk assessment paradigm (hazard identification, hazard characterization, exposure assessment and risk characterization), exposure assessment is often the main situation-specific aspect. Dietary exposure assessment combines information on food consumption and residue/additive/contaminant concentrations in those foods. Both of these aspects can vary considerably between countries and even between different regions of the same country. Dietary exposure assessment may also consider acute exposures, where the concern is the coincidence of high chemical concentrations with high food consumption levels, chronic exposure, where the concern is the ongoing, habitual patterns of chemical concentrations and food consumption. The work of JECFA and JMPR covers the range of chemicals added to foods or present in foods as undesirable contaminants and is concerned with acute and chronic dietary exposure at a country or sub-country level, as well as at the broader international level. This lecture will review the data sources and techniques that underpin dietary exposure assessment at JECFA and JMPR and will highlight the pivotal role of exposure assessment in connecting health-based guidance values, such as acceptable daily intakes, to food standards, such as maximum residue levels.

AM02: Immunotoxicology Hazard Identification Using Novel In Vitro Methods

AM02-01 Approaches and Challenges for Assessing Unintended Cytokine Release by Novel Protein Therapeutics

Madeline Fort. *Amgen Inc., Thousand Oaks, CA, The United States of America.*

The TGN1412 cytokine storm incident in 2006 highlighted the need for accurate prediction of unintended immunostimulation by novel protein therapeutics prior to first administration in humans, particularly for monoclonal antibodies (mAb) that target receptors expressed on immune cells. A variety of *in vitro* cytokine release assays (CRA) have been developed to screen for hazard identification of unintended cytokine release. Typically these assays use either human whole blood or peripheral blood mononuclear cells (PBMC) to measure the release of inflammatory cytokines. While several different CRA platforms are commonly chosen for screening approaches, no single approach has been identified a "gold standard" or preferred assay platform. The mechanism of action of a therapeutic, the expression pattern of its target, the cell types involved, and environmental signals can all impact the suitability of a CRA platform for the prediction cytokine release risk for a particular therapeutic mAb. In this session, we will review the key aspects of development of a CRA and the challenges for the translation of *in vitro* cytokine detection to cytokine release potential in humans.

AM02-02 Immunotoxicological Profiling of Chemicals Using Novel In Vitro Assays

Setsuya Aiba. *Tohoku University Graduate School of Medicine, Sendai, Japan.*

There is no gold standard to predict immunotoxicity of chemicals. However, there is accumulating evidence of immunotoxicity in humans following exposure to immunosuppressive drugs or anti-cytokine antibodies. We established a luciferase reporter assay system, the Multi-ImmunoTox Assay (MITA), which evaluates the effects of chemicals on the promoter activities of 4 cytokines: IL-2 and IFN- γ in Jurkat T cells (2H4), IL-1 β and IL-8 in THP-1 monocyte cells (THP-G1b and THP-G8). We previously reported that MITA correctly reflected the change in mRNA of human whole blood cells treated with immunosuppressive drugs. We have combined the MITA with the IL-8 Luc assay that can detect skin sensitizers (OECD442E) (modified MITA: mMITA) and analyzed 60 chemicals including well-known immunosuppressive drugs using this method. Cluster analysis of the 60 chemicals based on the LOEL for their effects on IL-2 and IL-8 promoter activities and the prediction from the IL-8 Luc could classify them into a 6-cluster solution with unique immunotoxic properties. Since each clus-

ter contains at least one immunosuppressive drug with well-known human immunotoxicity, we can deduce the immunotoxic effects of chemicals from those of immunosuppressive drugs belonging to the same cluster. This approach may present a potential procedure to predict the immunotoxicity of chemicals.

AM02-03 NTP Approaches to *In Vitro* Assessment of Dermal Hypersensitivity

Dori Germolec, NIEHS, Research Triangle Park, NC, The United States of America.

Allergic diseases are the result of aberrant immune responses directed against antigens that are innocuous in most individuals. Assessment of the potential to induce skin sensitization is a regulatory requirement for industrial chemicals, pesticides, and cosmetics and has been at the center of concerted efforts to replace animal testing in recent years. Several *in vitro* testing methods for assessment of hypersensitivity have been validated and have associated OECD test guidelines, or are in the process of validation and international interlaboratory ring-trials. It is apparent that no individual *in vitro* test can recapitulate the hypersensitivity immune response in its entirety. Machine learning approaches suggest that various integrated approaches can show up to 92% accuracy in predicting known human skin sensitizers. To date a majority of chemicals that have been evaluated have been cosmetic products. To further investigate the utility of *in vitro* approaches, the NTP solicited non-cosmetic chemical nominations for which there were existing LLNA data but limited or no *in vitro* evaluation. Data from approximately 235 chemicals (pesticides, formulations, excipients, personal care product ingredients, and industrial agents) will be presented to elucidate which combinations of *in vitro*, *in silico* and physico-chemical properties compare favorably with existing animal data.

AM02-04 Indirect Immunotoxicity: *In Vitro* Opportunities

Emanuela Corsini, Università degli Studi di Milano, Milan, Italy.

Most immunotoxic compounds directly interact with immunocompetent cells, resulting in alteration of immune functions. The close connection of immune system with other systems, in particular the nervous and endocrine systems, also puts this system at risk from secondary effects. While it is well established that hormonal homeostasis is important for keeping a competent and healthy immune system, the impact of endocrine disrupting compounds (EDC) on immunity is a matter of growing interest. Steroid hormones can modulate the function of immune cells, influencing the initiation of the immune response and the maintenance of peripheral tolerance to self-antigens. For example, a complex hormonal balance, between cortisol, and androgens, in the control of RACK1 expression and immune cells activation exists, in addition this scaffolding protein can also be targeted by EDCs. This presentation will focus on evaluation of compounds that exert their immune effects through signalling pathways that do not directly promote immune activation, and the possibility to use *in vitro* methods to highlight such effects. The evidence that EDC can affect the immune system, possibly resulting in adverse reactions, including immunosuppression, cancer, enhanced immunostimulation and autoimmunity will be discussed.

AM03: Recent Developments in Adult and Developmental Neurotoxicity Testing for Regulatory Assessment

AM03-01 Introduction to Adult and Developmental Neurotoxicity Testing: History, Guidelines, and Use in Risk Assessment

Hiroaki Aoyama, The Institute of Environmental Toxicology, Joso, Ibaraki, Japan.

This talk reviews current testing guidelines for evaluating adult and developmental neurotoxicity of xenobiotics and triggers for testing by different regulatory authorities in different countries in Asia, North America and Europe. In many countries, applicants are required to submit neurotoxicity data sets for regulatory evaluation when a chemical of interest is suspected of having the potential neurotoxicity in acute and/or repeated dose studies. Neurotoxicity on adult animals is generally thought to be a part of systemic toxicities. It can be evaluated either by conducting a 90-day neurotoxicity study according to the standard guidelines (USEPA OPPTS 870.6200, OECD TG424) or by adding necessary endpoints related to the toxicities of chemicals on nervous systems in acute, sub-chronic and chronic toxicity studies. Contrastingly, developmental neurotoxicity (DNT) has historically been recognized as a sort of delayed terato-

genicity, so exposures are during gestation until postnatal day 11 or 21. There is concern that the developing central nervous system (CNS) may be more sensitive to the exposure to certain chemicals than adult CNS. This presentation will compare and contrast different DNT related guidelines from USEPA, OECD 426 and 443.

AM03-02 Neurotoxicity Testing: From Benchtop to Regulatory Evaluation

Abby A. Li, Exponent, Inc., San Francisco, CA, The United States of America.

The standards for conducting rodent adult neurotoxicity and DNT studies have developed considerably over the years. Recently, internal guidance on how government reviewers should evaluate behavioral methods used and analysis of neurobehavioral data was made publically available by US EPA and Health Canada. These recommendations have direct application to adult neurotoxicity studies. In addition, an international panel of neuropathologists published best practices recommendations for conducting and evaluating neuropathology and morphometry measurements that has been recognized by the Society of Toxicologic Pathology. This presentation highlights the best practices in the conduct, statistical analysis and reporting of behavioral (e.g. motor activity, learning and memory, auditory startle) and neuropathology data for regulatory submission. Practical operational guidance for testing procedures as well as recommendations for quantitative (e.g. benchmark dose) and qualitative weight-of-evidence evaluation of the data will also be discussed.

AM03-03 Integrated Approaches to Testing and Assessment: Aggregating Lines of Evidence from Toxicokinetics and Advanced *In Vivo* Neuropathology and Neurochemistry Studies

Daniel Minnema, Syngenta Crop Protection, LLC, Greensboro, NC, The United States of America.

The EPA and OECD adult and developmental neurotoxicity guidelines are considered to be basic approaches for evaluating changes to the nervous system following chemical exposure. OECD guidance document for neurotoxicity testing recommends that if there are other data to indicate specific types of neurotoxic effects, then more specialized tests should be considered. In addition, OECD TG443 and EPA DNT guidelines indicate that optional TK data can be important in decisions on study design such as the route of administration and selection of dosages (e.g. evaluation for potential dose-dependent saturation of kinetic processes in comparison with estimated exposure levels). US EPA Office of Pesticide Program promotes a hypothesis based, systematic, integrative use of exposure and hazard information in considering need for further data (2013 Guiding Principles for Data Requirements). This presentation will emphasize generalizable principles using paraquat as an illustration for an integrated approach to systematic testing and assessment based on exposure, TK data, advanced neuropathology (stereology and specialized stains) and mechanistic data.

AM03-04 Introduction to and Quality Criteria for *In Vitro* Neurotoxicity Assays and Alternative Methods for Use in Regulatory Frameworks

Kristen Ryan, National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC, The United States of America.

There is increasing need to understand how to evaluate neurotoxicity data from *in vitro* assays and non-vertebrate animal models for regulatory purposes. European REACH regulation encourages the use of data from *in vitro* or alternatives to invertebrate animal models. The European Chemicals Agency indicates that registrants must justify existence or nonexistence of the triggers for the DNT cohort for the extended one-generation reproductive toxicity study (OECD 443) by evaluating neurotoxicity data including validated non-animal approaches. The European Food Safety Agency recently published a conceptual adverse outcome pathway framework for Parkinson's disease that incorporated *in vitro* neurotoxicity mechanistic assays. This presentation will provide participants with basic understanding of the more established *in vitro* neurotoxicity assays, as well as the associated challenges including assay relevance, sensitivity, or reproducibility. Current tools (e.g., Klimish score and risk-of-bias) available for assessment of internal study quality and reporting will be demon-

strated by examples. The limitations of these tools including applicability and external validity will also be discussed as they relate to non-vertebrate neuro-toxicity assays.

AM04: Understanding Translational Biomarkers: Science and Qualification

AM04-01 Clinical Biomarkers for Skeletal Muscle Injury and Neuromuscular Diseases

Vishal Vaidya. Pfizer, Inc., Cambridge, MA, The United States of America.

Skeletal muscle (SKM) injury/weakness has been reported as an adverse event as a result of number of medications such as statins, peroxisome proliferator-activated receptor alpha, etc. Conventional markers such as CK and AST lack the sensitivity and specificity to predict SKM injury. New circulating biomarkers of SKM injury would be used as effective drug development tools to not only assess and predict the safety of compounds in non clinical studies but to also monitor the toxicity and reversibility in early clinical development. Yet another benefit of SKM injury/degeneration biomarkers is that they can also be used to monitor disease progression and therefore be indicators of drug efficacy in many of the neuromuscular disorders that underlie SKM necrosis. The talk will summarize the data generated towards evaluating the performance of 4 SKM biomarkers - Skeletal troponin I (sTnI), Myosin light chain 3 (Myl3), Fatty-acid binding protein 3 (Fabp3), and Creatine kinase muscle type (Ckm) as biomarkers of drug-induced SKM injury. The clinical studies include cross sectional as well as longitudinal cohorts of either healthy volunteers or patients with drug-induced skeletal muscle injury or muscular dystrophies such as Duchenne muscular dystrophy or myotonic dystrophy type 1 or spinal muscular atrophy.

AM04-02 Translational Biomarkers of Kidney Damage

Mira Pavkovic. Bayer AG, Wuppertal, Germany.

Acute kidney injury (AKI) is a severe complication in hospitalized patients associated with higher probabilities of developing chronic kidney disease (CKD) or end-stage renal diseases. Although AKI and CKD are defined terms in nephrology their etiologies can be diverse and multifactorial including genetic predisposition, surgery, medication as well as high-risk life style and exposure environmental toxicants. Moreover, there is a high unmet medical need for both AKI and CKD not only with respect to therapeutics but also in terms of biomarkers and diagnostics. One approach to improve development of therapeutics for kidney diseases is the evaluation of biomarkers and their subsequent implementation into the trial design. Those biomarkers could improve preclinical safety assessment as well as accelerate duration and efficiency of clinical studies through appropriate patient stratification. Starting at the pre-clinical phase, protein biomarkers like KIM-1 and NGAL have made their way into the list of biomarkers qualified by the regulatory authorities and are under investigation for clinical studies, as well. In parallel, microRNAs in urine and blood have emerged as a new class of translational biomarkers for AKI and CKD. Therefore, this presentation will cover a comprehensive summary of previous work on translational kidney biomarkers as well as highlight latest results from ongoing initiatives like IMI BEAT-DKD.

AM04-03 Advancing Biomarkers for Pancreatic Toxicity and Pancreatitis

Warren Glaab. Merck and Co., West Point, PA, The United States of America.

Nonclinical drug-induced exocrine pancreatic injury can have significant negative impact on the development potential of a test article. The lack of reliable, noninvasive translational biomarkers for clinical detection, monitoring or prediction of the toxicity can preclude advancing the product to clinical testing. This talk will outline the issue from the non-clinical and clinical perspective and discuss current efforts within the pharmaceutical industry and the Critical Path's Predictive Safety Testing Consortium (PSTC) and Innovative Medicines Initiative (IMI) to identify and qualify novel translational biomarkers of acinar pancreatic toxicity. The presentation will include study results of exploratory biomarkers tested in a broad range of rodent models with a variety of testing methodologies. These exploratory biomarkers include tissue-specific microRNAs, proteins and enzyme activities measured in readily accessible matrices. Tissue localization results and histologic correlations to understand the robustness of the markers' specificity and sensitivity will be covered.

AM04-04 Translational and Mechanistic Biomarkers of Liver Injury

Chris Goldring. MRC Centre for Drug Safety Science, University of Liverpool, Liverpool, United Kingdom.

Drug-induced liver injury (DILI) is a leading cause of pharmaceutical attrition and acute liver failure. Since current clinically-used biomarkers of DILI, such as ALT, suffer from a lack of specificity and sensitivity, there exists therefore an opportunity to improve preclinical testing strategies through refinement of clinically-relevant markers which are potentially translatable from the patient back to the lab. Considerable worldwide efforts are now directed to improve the liver biomarker status quo, for example evaluating the application of markers of liver degeneration (e.g. CK18, GLDH and HMGB-1) and regeneration (e.g. CSF-1). In parallel with the protein biomarkers, the application of miRs as biomarkers of liver damage will be particularly explored in this talk, building on current knowledge of the strengths and weaknesses of miR-122 as a relatively selective biomarker of liver damage, its measurement, variability and relationship to tissue levels in *in vitro* and *in vivo* models and humans. This talk will also explore the broader miR field by assessing other liver miRs, including the role of zonation in expression of liver miRs as well as non-hepatocyte miRs, such as biliary epithelial cell miRs, as this will broaden our thinking of miR biomarkers beyond hepatocyte damage in the liver.

PM05: Big Data Analytics in the Era of the 21st Century of Toxicology

PM05-01 Transcriptional Responses to Chemicals in Hepatocytes *In Vivo* and *In Vitro*

Benjamin Haibe-Kains. Princess Margaret Cancer Centre and University of Toronto, Toronto, ON, Canada.

Genome-wide expression profiling is increasingly being used to identify transcriptional changes induced by drugs and environmental stressors. In this context, the Toxicogenomics Project-Genomics Assisted Toxicity Evaluation system (TG-GATEs) project generated transcriptional profiles from rat liver samples and human/rat cultured primary hepatocytes exposed to more than 100 different chemicals. To assess the capacity of the cell culture models to recapitulate pathways induced by chemicals *in vivo*, we show how one can leverage the TG-GATEs data set to compare the early transcriptional responses observed in the liver of rats treated with a large set of chemicals with those of cultured rat and human primary hepatocytes challenged with the same compounds *in vitro*. We describe a pathway-based computational pipeline that efficiently combines gene set enrichment analysis (GSEA) using pathways from the Reactome database with biclustering to identify common modules of pathways that are modulated by several chemicals *in vivo* and *in vitro* across species. We discuss how some chemicals induced conserved patterns of early transcriptional responses in *in vitro* and *in vivo* settings, and across human and rat genomes. The presented integrative analysis of toxicogenomics data provides a comprehensive overview of biochemical perturbations affected by a large panel of chemicals. Our analysis shows that the early toxicological response occurring in animals is recapitulated in human and rat primary hepatocyte cultures at the molecular level, indicating that these models reproduce key pathways in response to chemical stress. Reference: El-Hachem N, Grossmann P, Blanchet-Cohen A, Bateman AR, Bouchard N, Archambault J, Aerts HJ, Haibe-Kains B. 2016. Characterization of conserved toxicogenomic responses in chemically exposed hepatocytes across species and platforms. *Environ Health Perspect* 124:313–320; <http://dx.doi.org/10.1289/ehp.1409157/>.

PM05-02 Assessing the Potential Liver Toxicity of Compounds Using TG-GATEs Database: A Connectivity Map Approach

Xiaohui Fan. College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, China.

Liver toxicity is one of the main reasons for the failure of drugs during clinical trials and being withdrawn from the market. Toxicogenomics (TGx) has been extensively used to interrogate underlying mechanisms and prediction of hepatotoxicity by utilizing the large-scale preclinical TGx datasets such as TG-GATEs from Japanese Toxicogenomics Project. We developed a web server, namely LTMap, for hepatotoxicity analysis with genome-wide transcriptomic data from TG-GATEs. In LTMap, researchers could compare signatures of query compounds against a pregenerated signature database of 20,123 Affymetrix

arrays associated with about 170 compounds available from TG-GATEs. We validated our computational approach for similarity comparison using three example drugs. Internal compound of WY-14643 verified the analysis technique. External data of Thioacetamide, Nafenopin were tested to evaluate the performance of LTMMap. The result showed that identified compounds with high scoring cause similar toxicological changes, which demonstrated the utility of LTMMap in revealing the connection of chemicals according to similar toxicological behaviors. In this presentation, I will describe and demonstrate LTMMap with real examples to illustrate its potential utility to provide new insights into the risk assessment of potential drug-induced liver toxicity.

PM05-03 Genomic Dose Response Analysis: Identifying Biological Potency at a Genomic Level

Scott S. Auerbach. Division of the National Toxicology Program at NIEHS, Research Triangle Park, NC, The United States of America.

There is increasing interest in the toxicology testing community for the use of functional genomics to identify safe levels of exposure (i.e. those with minimal biological effect levels). The appeal for employing genomic tools in this manner relates to their broad biological space coverage and their ability to identify sensitive biological effects in studies of short duration. In this talk a methodology employed by the NTP and elsewhere for performing genomic dose response analysis will be described. A three step process is undertaken to identify gene set benchmark dose (BMD) levels. In the first step dose-responsive genomic features are identified using a trend test. Second, each responsive feature is fit using parametric models derived from the US EPA BMDS software Third, genomic features are grouped into gene sets and a composite BMD for each adequately populated gene set is determined. A GUI-based open-source software package, BMDExpress 2 (<https://github.com/auerbachs/BMDExpress-2/releases>), has been formulated to facilitate the democratization of the NTP's proposed genomic dose response analysis approach. Example analyses using a variety of chemicals will be used to illustrate the relationship between points of departure from apical toxicological assessments and genomic-based biological potency estimates from both *in vivo* and *in vitro* studies.

PM05-04 Transitioning towards Objective Read-Across Approaches: Landscape, Research, and Practical Application

Grace Patlewicz. National Center for Computational Toxicology, US EPA, Research Triangle Park, NC, The United States of America.

Read-across is a data gap filling technique used in regulatory programmes worldwide. The typical read-across workflow begins by assessing data gaps of a substance (target), identifying and evaluating (source) analogues for suitability for the endpoint of concern and decision context. Source analogues are often identified using structure but evaluating suitability relies upon other considerations including bioavailability, metabolism, and data quality. Constructing and assessing read-across predictions requires expert judgement which can lead to inconsistent predictions, offers little insight into overall read-across performance and is not scalable to large numbers of chemicals. In this talk we will provide an overview of the landscape of read-across in terms of the available guidance, workflows and tools. We will outline a harmonised hybrid workflow and discuss ways in which aspects of the steps lend themselves to objective approaches. We will discuss a methodology employed by the EPA (and similar to others elsewhere) for deriving systematic read-across predictions. The approach is known as generalised read-across (GenRA), which uses chemical structure information and/or high throughput screening data (from ToxCast and Tox21) to derive predictions of *in vivo* repeat-dose toxicity with quantitative measures of uncertainty. Ongoing work is investigating the impact of other contexts of similarity in improving GenRA performance. GenRA is being implemented as a workflow into a web-based tool on the EPA's Chemistry Dashboard to enable users to make read-across predictions. Practical examples of how to use and interpret GenRA will be presented to highlight the emerging transition from expert driven approaches.

PM06: Epigenetics and Toxicology: Overview and Advances in Methodology

PM06-01 Epigenetics Overview and Advances

Aline de Conti. National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR, The United States of America.

Identifying and understanding epigenetic alterations that are related to toxic effects and diseases is vital for protecting public health. Major epigenetic mechanisms including, cytosine DNA methylation, post-translational histone modifications, chromatin structure regulators and non-coding RNAs, act simultaneously to regulate various cellular processes such as gene expression and cell fate and are involved in diseases initiation and progression. The incomplete understanding of the normal state and dynamic variation of the epigenome, makes the determination of the causality of epigenetic alterations in the development of toxicity and diseases very challenging. However, currently several epigenetic enzymes have been identified as druggable, and a diversity of epigenetic targets have been proposed for risk assessment. Specifically, the past decades have witnessed an exponential advance in technologies that allowed the development of more precise and informative studies that are increasing the knowledge related to epigenetic alterations and toxic effects. Breakthroughs in several high-throughput technologies, for example next-generation sequencing of epigenome, identified toxicant-induced alterations in genome-wide DNA methylation. In addition, the development of various high-resolution technologies to determine histones post-translational modifications allowed developing new insights into the mechanisms of chemical carcinogenesis/toxicity. The advance in these methodologies will help to identify epigenetic biomarkers for clinical prediction, diagnosis, and development of therapeutically and preventive strategies.

PM06-02 Longitudinal Effects of Developmental Exposures on Age-Related DNA Methylation and Hydroxymethylation

Dana C. Dolinoy. University of Michigan School of Public Health, Ann Arbor, MI, The United States of America.

Toxicant exposures early in life adversely affect health outcomes in both animals and humans, in part due to epigenetic mechanisms (e.g. DNA methylation). Studies also indicate that exposure impact on the epigenome can be tissue and even cell specific. Yet, epigenetic epidemiology analysis of toxicants, is often limited to biologically available or "surrogate" (e.g. blood, saliva) samples, which serve as proxies for epigenetic status in tissues targeted by exposures. Using lead (Pb), bisphenol A (BPA), and phthalates (e.g. DEHP) as representative toxicants, we evaluate tissue- and cell-specific epigenetic alterations associated with perinatal exposures and disease outcomes. These analyses include a multi-omics integration of DNA methylation and hydroxymethylation, chromatin accessibility, and gene expression data in an effort to inform epigenetic epidemiology studies with an environmental focus. Our approach evaluates sex differences and conducts analyses immediately following perinatal exposure in target tissues (e.g. liver and brain) and in surrogate tissues of human relevance (e.g. blood leukocytes), as well as longitudinally into adulthood to identify persistent exposure-dependent epigenetic changes. Ultimately, we seek to identify a subset of tissue-independent labile genes, which represent regions of the epigenome that are vulnerable early in life, and may be interrogated with the use of surrogate tissues.

PM06-03 Epigenetics and Environmental Origins of Cancer

Zdenko Herceg. International Agency for Research on Cancer (IARC), Lyon, France.

Recent years have witnessed a remarkable pace of discoveries in epigenetics which have revolutionized our understanding of complex human diseases. The spectacular advances in epigenomics that allow the analysis of the epigenome with unprecedented resolution in high throughput and genome-wide settings have further accelerated investigations in this area. The challenge posed by major international sequencing efforts is to identify changes in the (epi)genome that precede and promote tumour development, and to differentiate functionally important ("drivers") from non-functional "passenger" events. In addition, there is little understanding about whether epigenetic changes can be used as biomarkers for exposure assessment, risk stratification, and early

detection. The epigenome has been proposed to function as an interface between environmental factors and the genome, therefore, the identification and functional characterization of epigenetic events deregulated by specific environmental and lifestyle stressors should enhance our understanding of mechanisms of carcinogenesis linked to risk-factor exposures. The intrinsic reversibility of epigenetic changes represents a tremendous opportunity for the development of novel strategies for cancer treatment and prevention. I will discuss recent conceptual and technological advances in epigenetics and ongoing efforts aiming to identify epigenetic targets that could be exploited in cancer prevention and therapy as well as molecular epidemiology.

PM06-04 Importance of Investigating Epigenetic Alterations for Industry and Regulators

Jonathan Moggs. *Novartis Institutes for BioMedical Research, Basel, Switzerland.*

Over the past 10 years there has been extensive debate within the toxicological sciences community regarding the relevance of epigenetic mechanisms and biomarkers for safety assessment. Whilst the current consensus is that more knowledge and experience is required prior to the routine incorporation of epigenetic evaluations into safety assessment, the increasing body of literature on diverse epigenetic mechanisms of toxicity, combined with the current trend towards therapeutic targeting of chromatin regulatory proteins, provides a strong stimulus for careful consideration of potentially unique safety assessment concerns associated with xenobiotic epigenome modulators, in particular with respect to latent toxicity phenotypes (Moggs and Terranova, 2018). Important insights have been gained through the targeted perturbation of the epigenome by defined xenobiotic exposures and through genetic modification of chromatin regulatory proteins in cellular and animal models. This presentation will highlight how epigenomic profiling methodologies are becoming an important asset for the safety assessment of therapeutic modulators of epigenetic targets, as well recent progress within the Innovative Medicines Initiative MARCAR consortium in identifying early epigenetic biomarkers of xenobiotic-induced non-genotoxic carcinogenesis.

PM07: New Risk Assessment Approaches for Reproductive and Developmental Toxicology

PM07-01 Alternative Approaches for Developmental Neurotoxicity (DNT) Evaluations

Anna Bal-Price. *European Commission–DG Joint Research Centre (JRC), Ispra, Italy.*

Multiple activities are underway in the EC to integrate developmental endpoints. These include expanded use of *in vitro* approaches, alternative disease models, and use of Alternative Outcome pathways (AOPs) for integrating *in silico*, *in vitro* and *in vivo* assessments. Examples from developmental neurotoxicity will be used in this session to illustrate these methods. An upcoming DNT special issue of the journal of Toxicology and Applied Pharmacology will highlight these methods and serve as a fantastic, current resource for this talk.

PM07-02 Computational Systems Biology: Translational Tools for Data Integration and Modeling

Thomas B. Knudsen. *National Center for Computational Toxicology (NCCT), US Environmental Protection Agency, Research Triangle Park, NC, The United States of America.*

New understanding of common signaling pathways for reproduction and developmental biology has provided a mechanistic framework for risk assessment of these endpoints. Building upon the ToxCast database and computer simulations of biological systems, this presentation will discuss two case studies illustrating the power of this new information and data integration for informing risk assessment. The case studies focus on lessons learned with data from embryonic stem cell assays and simulations with agent-based models to address developmental effects. They will illustrate how assessment of toxicity based endpoints versus specific receptor based assays can provide a useful hierarchy for risk assessment for environmental chemicals, and how high-throughput screening data can inform construction of Adverse Outcome Pathways (AOPs)

by evaluating developmental endpoints and the predictive power of developmentally conserved cell signaling pathways. *This abstract does not necessarily represent the views or policies of the US Environmental Protection Agency.*

PM07-03 Developing a Responsible Approach for Replacing Endocrine Disrupting Chemicals

Bernard Robaire. *McGill University, Montreal, QC, Canada.*

Numerous chemicals are now recognized as acting as disruptors of the endocrine system; some of these have an impact on human health as well as that of many animal species. These chemical families include plasticizers such as phthalates, epoxy resins such as bisphenol A, and flame retardants such as polybrominated diphenyl ethers. As individual chemicals become regulated or are voluntarily withdrawn because of demonstrated or suspected toxicity, a market gap develops and replacement chemicals are introduced. However, there are limited requirements for manufacturers to test these chemicals and no requirement to determine whether the replacement chemicals have similar endocrine disrupting effects as those of the legacy compounds they are replacing. A four-step approach for the design, characterization and toxicological assessment for identifying responsible alternative chemicals will be presented using replacements for the plasticizer diethylhexyl phthalate (DEHP) as an illustration.

PM07-04 Linking *In Vivo* and *In Vitro* Male Reproductive Toxicity Risk Assessment

Elaine M. Faustman. *University of Washington, Seattle, WA, The United States of America.*

A continuing challenge to our risk assessment approaches has been the early identification of chemicals that impact male reproduction. These challenges include drugs where discovery of adverse effects have occurred after marketing. New *in vitro* methods now allow for the assessment of multiple endpoints and these methods will be presented and described. Receptor based assays allow for evaluation of specific androgen based endpoints and 3 D culture methods allow for assessment of new disease relevant endpoints. Using integrated systems based approaches framed by AOPs this new information can be integrated and prove a new basis for our evolving risk assessment methods to identify adverse male reproductive outcomes.

PM08: What Is an Adverse Effect for Human Health: Defining Adversity in Risk/Safety Assessments

PM08-01 Defining Adverse, Non-Adverse, and Adaptive Responses in Safety/Risk Assessments

Robert R. Maronpot. *Maronpot Consulting, LLC, Raleigh, NC, The United States of America.*

Finding a universally agreed-upon definition of 'adverse' in the context of preclinical studies is challenging, and communicating what is 'adverse' versus 'non-adverse' or 'adaptive' to regulatory authorities and the public is even more challenging. In assessing potential risk to human health, we attempt to define adverse within the context of a given method of exposure and dose, using the concept of 'no-observed-adverse-effect-level' (NOAEL). In many cases, whether a change is adverse (pathological) or non-adverse (physiological) is subject to personal and often conflicting opinions. Some apical responses, such as death and cancer, are readily accepted as adverse. A mild change in organ morphology and function that is reversible, such as hepatocellular hypertrophy and enzyme induction, is generally regarded as non-adverse or adaptive. Adversity will need to be examined as we shift from conventional animal studies to new technologies and alternative analytical approaches. This presentation provides background for the following presentations and will present a practical approach to defining, assessing, and communicating adverse, adaptive, and non-adverse responses in preclinical studies, taking into consideration whether the response is temporary and reversible.

PM08-02 Importance of Defining Adverse Effects for Toxicologic Evaluations Risk/Safety Assessment: The Case of Octamethylcyclotetrasiloxane and Decamethylcyclopentasiloxane

Wolfgang Dekant. *University of Wuerzburg, Wuerzburg, Germany.*

Octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) are synthetic intermediates and may be present in consumer products. Repeated dose inhalation and oral toxicity studies with D4 and D5 in rats for up to 90 days show increases in liver weight, hepatomegaly, and centrilobular hypertrophy accompanied by induction of specific cytochromes P450 enzymes as major systemic effects. Besides liver weight changes, consistent changes of sufficient magnitude in clinical chemistry indicative of liver function and histopathologic changes in liver were not observed in the many available studies. D4 and D5 are functional ligands for CAR/PXR resulting in induction of a specific induction profile of cytochrome P450 enzymes in liver identical to that of phenobarbital. The liver changes seen are also very similar to those induced by phenobarbital, but D4 and D5 are weaker P450 enzyme inducers compared to phenobarbital. Neither liver enlargement nor liver tumors were seen in rats in two-year inhalation toxicity studies with D4 and D5. The absence of liver effects after long-term exposure to D4 and D5 and the absence of tumors in the two-year inhalation toxicity study with D4 and D5 further support the conclusion that the liver weight changes represent an adaptive response due to interaction with CAR/PXR.

PM08-03 The Application of Adversity and Adverse Outcome Pathway Analysis to Rodent Thyroid Hormone Perturbation

John R. Foster. *ToxPath Sciences Ltd, Congleton, Cheshire, United Kingdom.*

Disruption of circulating thyroid hormones (TH) is known to have severe consequences in the human population. In pregnancy, in particular, TH deficiencies can induce severe neurodevelopmental defects in the human fetus that cannot be rectified postnatally. Chemically-induced TH disruption in rodents can also result in thyroid cancer and may occur through a variety of different modes of action, which all result in decreased circulating TH that triggers a compensatory release of thyroid stimulating hormone (TSH) from the pituitary to increase TH production by the follicular cells. There are several critical differences in TH homeostasis between humans and rodents, which explains why they respond differently to TH disruption. Presentation describes the approach taken, using appropriate case examples, in defining no-observed-adverse-effect-levels (NOAELs), exploring the mode of action (MoA) and formulating an adverse outcome pathway (AOP) for rodent TH disruption, and clarifying the human relevance of the established MoA. Attendees will be shown how the application of adverse outcome pathway analysis (AOP) aids in defining thresholds for the molecular initiating, and for each of the other key events, occurring in the process leading to both thyroid cancer and to TH-induced neurodevelopmental abnormalities.

PM08-04 Fundamental Concepts to Understand Adverse Effects in the Immune/Lymphoid System

Maria Lucia Zaidan Dagli. *University of São Paulo, School of Veterinary Medicine and Animal Science, São Paulo, Brazil.*

Guidelines from regulatory agencies in many countries state that evaluation of the lymphoid/immune system should be performed on new products. Evaluation of the immune system is necessary in safety/risk assessment to determine mechanism of action but also target and off-target effects. Examination of the immune system includes clinical pathological evaluation of blood and bone marrow, lymphoid organs (thymus, spleen, lymph nodes) relative and absolute weights, semiquantitative histopathological changes in lymphoid organs and their compartments (B and T lymphoid areas), and immunostaining of corresponding cells. Changes observed for a given lymphoid organ should be interpreted in the context of the interaction of different lymphoid organs and/or other systems in experimental animals. For example, alterations in the lymphoid organs must be differentiated from stress, mostly when considering effects in high-dose animals. The evaluation of lymphoid tissues and immunotoxicological reactions is also important in juvenile studies. Since the development of lymphoid organs occurs in early life, immunotoxic alterations in juvenile animals may predict further immunological impairment in adults. Presentation covers what an adverse reaction in the immune system is, using theoretical and practical examples.

SYM01: Emerging Mechanisms of Chemical Teratogenesis

S01-01 Oxidative DNA Damage and Repair in Teratogenesis

Peter G. Wells. *University of Toronto, Toronto, ON, Canada.*

Birth defects and postnatal abnormalities in brain function may be caused in part by normal or drug-enhanced embryonic and fetal levels of reactive oxygen species (ROS), which oxidatively damage cellular macromolecules and alter signal transduction. Normal ROS production alone can be developmentally toxic in genetically altered mouse embryos and fetuses deficient in either antioxidant enzymes or DNA repair enzymes/proteins like oxoguanine glycosylase 1 (OGG1) and breast cancer 1 (BRCA1). The enhanced developmental abnormalities in DNA repair-deficient progeny reveals the particular pathogenic contribution of oxidative DNA damage, likely via non-mutagenic mechanisms, as distinct from damage to other cellular macromolecules, or ROS-mediated signaling. These results may be relevant to developmental abnormalities of uncertain origin, possibly including autism spectrum disorders. ROS formation and teratogenesis are increased via embryonic/fetal prostaglandin H synthase-catalyzed teratogen bioactivation, or teratogen-initiated activation of fetal ROS-forming NADPH oxidases. Ogg1 and Brca1 knockout (KO) progeny are more susceptible to embryonic/fetal brain DNA oxidation, embryopathies and/or neurodevelopmental deficits caused by ROS-initiating teratogens like ethanol, possibly relevant to fetal alcohol spectrum disorders. Antioxidative and DNA repair proteins are upregulated by fetal nuclear factor-E2-related factor 2 (Nrf2), which is regulated by BRCA1, and neurodevelopmental deficits are enhanced in Nrf2 KO progeny.

S01-02 Stress-Response Signaling Pathways in the Organogenesis-Stage Embryo

Barbara F. Hales. *McGill University, Montreal, QC, Canada.*

The exposure of mammalian embryos to insult during organogenesis can lead to malformations and/or embryo lethality. Some teratogenic chemicals disrupt signaling pathways that regulate pattern formation and differentiation; others activate stress response signalling pathways. DNA damaging exposures, such as benzo[a]pyrene, gamma radiation, cyclophosphamide and hydroxyurea, activate the P53 signaling pathway. P53 activation upregulates the expression of downstream targets involved in cell cycle arrest (Cdkn1a, Rb1) or apoptosis (Fas, Trp53inp1, Pmaip1), and induces caspase-3 cleavage. P53 has two family members, P63 and P73, with important roles during development. P63 is essential for proper limb and epithelial development, while P73 is involved in the development of the nervous and immune systems. The levels and phosphorylation of P53 are increased in embryos exposed to an insult in the absence of effects on P63 and P73. Furthermore, neither P63 nor P73 are able to compensate for the absence of P53 in embryos after a teratogenic exposure. Thus, P53 is the principal family member that responds to stress in the organogenesis-stage embryo.

S01-03 Assessing the Function of Endocrine miRNAs in Pregnant Women That Predict FASD Infant Outcomes

Rajesh C. Miranda. *Texas A&M Health Science Center, Bryan, TX, The United States of America.*

Several epigenetic changes, including changes in expression of non-protein-coding regulatory RNAs like miRNAs, have recently been implicated in teratogenesis due to prenatal alcohol exposure (PAE). We previously reported, in a study of pregnant women in Ukraine, that 11 microRNAs were significantly elevated in plasma of alcohol-exposed mothers whose infants were affected (HEA), compared to mothers who subsequently gave birth to apparently unaffected or unexposed infants. Pathway overrepresentation analysis predicted that these maternal HEA-predictive miRNAs (HeamiRNAs) influenced epithelial-mesenchymal transition (EMT) which controls placental trophoblast growth and maturation. Moreover, PAE in a mouse model also resulted in impairment of placental EMT. We investigated effects of these HeamiRNAs on human trophoblast cell lines. Overexpression of HeamiRNAs significantly reduced growth, retarded cell cycle progression, and reduced the migratory/invasive capacity in the context of alcohol exposure. Overexpression of HeamiRNAs also resulted in repression of EMT pathway genes, and reduced E-cadherin expression following forskolin induced syncytialization without interfering with syncytialization

dependent increases in human chorionic gonadotropin or IGF-1 expression. Taken together, these data suggest that pathogenic levels of maternal circulating miRNAs may affect placental growth and invasion, and selectively interfere with the EMT pathway, thereby contributing to the pathology of Fetal Alcohol Spectrum Disorders (FASD).

S01-04 Vascular Basis of Teratogenesis

Neil Vargesson. *University of Aberdeen, Aberdeen, United Kingdom.*

The developing vascular system of the embryo is essential to allow growth and maturation of tissues. Vascular development is a carefully regulated process and is one of the first functioning tissues in the embryo. Indeed, malformations of the vascular system can result in embryo lethality or deformity. Many teratogens have been shown to be anti-angiogenic in embryos, these include therapeutic drugs like thalidomide and valproate, and the antiangiogenic action of these drugs has been linked to causing the malformations. Recent work through studying antiangiogenic, anticancer drugs such as sunitinib, indicates such drugs themselves are also likely to have the potential to be teratogenic. Such work, together with studies on teratogens, like thalidomide, are shedding light on how a loss of vessels or inhibition of vascular outgrowth can result in embryonic malformations, in particular to the developing limb. This work is further shedding light on drug safety and improved drug efficacy.

SYM02: International Efforts to Ensure Global Food Safety: Incorporating Advanced Toxicological Sciences in Food Risk Assessment

S02-01 Risk Assessment Methodologies as Applied by JECFA and JMPR

Angelika Tritscher. *World Health Organization, Geneva, Switzerland.*

The Joint FAO/WHO Expert Committee in Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticides Residues (JMPR) are independent international expert committees that evaluate the health risk to consumers of chemicals in food. Such chemicals are added intentionally such as food additives, leading to residues as a result of certain processes such as residues of pesticides or of veterinary drug, or are the result of contamination, such as heavy metals or mycotoxins. To assess the potential health risks toxicological, mechanistic, epidemiological as well as chemical data are being evaluated according to a transparent and scientific process, as laid out in the Environmental Health Criteria document 240 (FAO/WHO 2009) and subsequent updates. Recent advances in exposure assessment methodologies and efforts for international collection of contamination and food consumption data are given. This presentation gives an overall overview of the work of these international Committees and how new scientific developments are incorporated in the risk assessment process. Detailed examples are given in the subsequent presentations.

S02-02 Applications of Evolving Science in Assessing the Safety of Food Additives and Chemical Contaminants by JECFA

Yu Janet Zang. *US Food and Drug Administration, College Park, MD, The United States of America.*

JECFA is tasked by the CODEX Alimentarius Commission to evaluate the safety of food additives and chemical contaminants using current and sound science. As science evolves, JECFA is often requested to re-evaluate the safe intake levels using newly available safety information. In addition, the committee also continuously face advanced scientific concept, approaches and updated risk assessment strategies. This presentation will use recent examples to demonstrate how JECFA committees process, evaluate and apply evolving scientific knowledge and concepts into the safety assessments. The acceptable daily intake of food additives and tolerable intakes for chemical contaminants may be withdrawn, sustained, or re-established based on new data or the revisit of old data using current scientific standards. JECFA also considers new concepts in safety and risk assessment and incorporates them into its principles and procedures. Examples and updates will be provided in this talk, such as the recent revisions of the application of threshold for toxicological concern (TTC) approach for flavoring compounds, the application of new dose-response analysis methodology, and the recent exercises in the integration of epidemiological and animal data in the risk assessments for chemical contaminants.

S02-03 Incorporation of Duration of Exposure into the Risk Assessment of Residues of Pesticides and Veterinary Drugs in Food

Alan Boobis. Imperial College London, London, United Kingdom.

Traditionally, JECFA has used a single health based guidance value, the ADI, for the risk assessment of residues of veterinary drugs in food. Until recently, this was compared with an estimate of exposure based on a model diet, designed to provide a conservative estimate of chronic dietary exposure. However, the increasing availability of representative consumption data has enabled JECFA to develop more refined estimates of dietary exposure, the global estimate of chronic dietary exposure (GECDE) and the global estimate of acute dietary exposure (GEADE), respectively. In parallel, JECFA has developed methodology to enable the characterization of the acute risks (acute reference dose, ARfD), both toxicological and microbiological, from exposure to veterinary drug residues. Whilst enabling more accurate characterization of both acute and chronic risks, the risks from less than lifetime excursions above the ADI, but below the ARfD, remained unassessed. JECFA has now addressed this gap by developing methodology enabling appropriate exposure scenarios to be compared with the hazard relevant to different durations of exposure. Whilst the methodology was initially evaluated for the risk assessment of veterinary drug residues, it should be more widely applicable, for example to residues of pesticides.

S02-04 Updated WHO Guidance on the Evaluation of Genotoxicity of Compounds in Food

Virunya Bhat. NSF International, Ann Arbor, MI, The United States of America.

The Food and Agriculture Organization of the United Nations jointly with the World Health Organization have published Principles and Methods for the Risk Assessment of Chemicals in Food (2009). The guidance is being updated to incorporate global advances in genotoxicity evaluation and tools to assist a wide range of decision contexts, from use of the Threshold of Toxicological Concern, read-across, and structure activity relationships for data-poor chemicals, such as pesticide metabolites, to weighting schemes that consider, for example, data quality, reproducibility, consistency, and biological or phylogenetic relevance for data-rich substances, including those with carcinogenicity data. This presentation will include case studies illustrating how the number, quality and relevance of genotoxicity endpoints and studies can differ widely, thus necessitating somewhat different evaluation approaches. The updated guidance will also address exposure context (e.g., whether the observed genotoxicity would be likely to occur in humans exposed to low levels of the pesticide as residues in food) and other factors that warrant additional consideration, such as enzyme preparations (mixtures) used in food production. The updated guidance should facilitate and simplify the genotoxicity evaluation of complex and sometimes contradictory datasets, and provide consistency in evaluations across risk assessments and regulators.

SYM03: Lysosomes: Key Regulators of Inflammation and Life or Death of a Cell

S03-01 Mechanistic Insights in Lysosome-Mediated Cell Death

Karin Öllinger. Linköping University, Linköping, Sweden.

A critical step in cell death is the release of cathepsins from the lysosomal lumen to the cytosol from lysosomal membrane permeabilization (LMP). Once released, cathepsins often mediate apoptosis upstream of mitochondrial events and proteolytic activation of Bid has been identified as one possible mechanism of action. Although LMP is poorly understood, but seems to be heavily dependent on the composition of the lysosomal membrane. LMP is studied using methyl-serine dodecylamine hydrochloride (MSDH), which is a lysomotropic detergent that selectively ruptures lysosomes, but is otherwise biochemically inactive. High cholesterol content prevents LMP whereas reduction of the cholesterol content by treatment with methyl- β -cyclodextrin sensitizes the cells to cell death-inducing stimuli. Reduction of the amount of LAMP-2 protein is shown to sensitize cells to LMP-mediated cell death. We have shown that during LMP small but significant amounts of LAMP-2 are released to the cytosol. The release is dependent on the serine carboxypeptidase cathepsin A that cleaves LAMP-2 between the luminal and transmembrane domains. Importantly, lysosomal dysfunction contributes to diseases, such as neurodegenerative disorders and cancer. Moreover, restoration of lysosomal

acidification by therapeutic intervention could represent an efficient way to promote the degradation and clearance of accumulating macromolecules in neurodegenerative diseases.

S03-02 Lysosomal Alterations in Health and Disease

Patricia Boya. Centro de Investigaciones Biológicas, CIB-CSIC, Madrid, Spain.

Alterations in lysosomal function have important consequences at the cellular level. Oxidative and other types of stress damage these organelles causing lysosomal membrane permeabilization (LMP). LMP causes the release of lysosomal proteases into the cytoplasm and triggers cell death, which can be attenuated using cathepsin inhibitors. Lysosomal dysfunction results in the blockade of autophagy; the final phase of autophagy, whereby cell components are degraded within the lysosome, is impaired. We have identified alterations in lysosomal function in several animal models of neurodegenerative diseases that results in a blockage of autophagy resulting in increased cell death. Our research underscores the importance of correct autophagy and lysosomal function to maintain nervous system homeostasis, and demonstrate how alterations in these processes may underlie multiple neurodegenerative diseases. In this symposium, I will show our latest results allowing us to understand how lysosomes can be protected in order to restore autophagy and prevent cell death.

S03-03 Impact of Lipids and Particles on Lysosomal Membrane Permeability (LMP)

Andrij Holian. University of Montana, Missoula, MT, The United States of America.

Normal function of lysosomes is critical in macrophages for a wide variety of functions including host defence, antigen processing, and removal of damaged organelles. After extracellular material is phagocytosed it is contained within a phagosome that fuses with a lysosome for degradation. The stability of the phagolysosome is critical to prevent release of degradative hydrolases into the cytoplasm and is dependent on a combination of unique proteins (e.g., LAMP 1, LAMP2, LIMP1 and LIMP2) as well as unique phospholipids (e.g., bis (monoacylglycerol)phosphate) and cholesterol that are critical to maintain membrane integrity. Changes in lipid composition will affect membrane properties and could lead to increased or decreased potential for pore formation (LMP). Membrane integrity can be assessed to evaluate lipid motion and order. Using fluorescent probes with intact membranes we show that silica and nanoparticles impact membrane properties and pore formation that could explain LMP leading to cell death. Furthermore, modulation of lipid composition (e.g., cholesterol or polyunsaturated phospholipids) can modulate pore formation and downstream events. This information can provide a mechanistic explanation for particle-induced cell death and recommendations for potential therapeutic intervention.

S03-04 Why Lysosomal Membrane Permeabilization (LMP) Could Be Considered a Key Event (KE) in Adverse Outcome Pathway (AOP) to Particle-Induced Inflammation and Fibrosis of the Lung

Mary Gulumian. Toxicology Research Projects NIOH, Johannesburg, South Africa.

To assist in the environmental risk assessment of chemicals, adverse outcome pathway (AOP) was proposed as a conceptual representation of an up-to-date knowledge concerning the relationship between a direct molecular initiating event and an adverse outcome at a biological level relevant to risk assessment. The AOP has been defined as a sequence of events from the exposure of an individual to a chemical through to an understanding of the adverse effect at the individual level (for human health) or population level (for ecotoxicology). During the said pathways, it is also essential of understanding of the critical processes or key events (KEs) measured along the pathway. Particles can have detrimental effects on the lysosomal pathways and autophagy and inflammation activation resulting in toxicological consequences including inflammation. At this symposium, it will therefore be shown as why it will be possible to propose that lysosomal dysfunction and LMP may be considered as a KE for particle-induced inflammation and fibrosis.

SYM04: Systemic and Functional Impacts of Maternal Pulmonary Nanomaterial Exposures on the Offspring

S04-01 Inhalation of Nanomaterials Can Result in Systemic Effects Leading to Pre- and Postnatal Effects

Flemming R. Cassee. *National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.*

Nanomaterials can have very different surface characteristics that change their biodistribution in biological systems. Moreover, their small dimensions may be a reason why a biological system does not adequately recognize these materials as being of xenobiotic origin. Nanomaterials have been shown to cross biological barriers such as in the lung with the potential to cause systemic effect. In addition, even responses in the lung such as oxidative stress and inflammation may lead to effects in organs beyond the lung.

S04-02 Deposition of Nanomaterials at the Feto-maternal Interphase and Their Effects on the Fetus

Luisa Campagnolo. *University of Rome "Tor Vergata," Rome, Italy.*

Recent evidences demonstrate the ability of nanomaterials to reach the placenta, while their translocation across the placental barrier is questioned. However potential developmental adverse effects to the fetus following maternal exposure to nanomaterials are demonstrated. Interestingly, placental biodistribution occurs independently of the route of exposure (e.g., inhalation, intravenous, oral). Whether nanomaterial toxicity to the fetus is driven by direct and/or indirect actions is an open question. In this respect, data will be presented and discussed on placental localization and possible translocation for different nanomaterials. A newly developed simplified *in vitro* model to investigate the potential placental translocation of nanomaterials will also be presented.

S04-03 Effects of Maternal Lung Exposure to Nanomaterials on the Nervous and Male Reproductive Systems

Karin Sørig Hougaard. *National Research Centre for the Working Environment, Copenhagen, Denmark.*

Testing of nanomaterials (NM) for effects on pregnancy and fetal development has been a latecomer within nanotoxicology, but as more and more studies emerge so do the indications that NM may interfere with developmental events. Developmental studies applying the maternal airway route of exposure report that the central nervous and the male reproductive systems may be especially prone to disruption. Existing data suffice for generation of hypotheses, but the tremendous variation in study designs and tested particles hampers generalization and risk assessment. This presentation will sum up findings for offspring effects on the central nervous system (behavior and histology) and male reproductive systems. It will focus on the replicability of study findings, within and between laboratories, to outline how future research may contribute to delineation of developmental susceptibility to maternal exposure to engineered and ambient nanosized particles.

S04-04 Cardiovascular, Metabolic, and Epigenetic Susceptibility in Offspring after Maternal Engineered Nanomaterial Exposure

Phoebe Stapleton. *Rutgers University, Piscataway, NJ, The United States of America.*

Cardiovascular disease (CVD) is the leading cause of death worldwide and associated with many genetic and lifestyle risk factors. The maternal health and fetal milieu during gestation has been explored as a contributing factor to progeny health. Maternal exposure to engineered nanomaterials during gestation has been associated with the disruption of fetal homeostasis during development. This presentation will focus on the cardiovascular health Sprague-Dawley rat offspring, from late-stage fetus to adulthood, after maternal exposure to nano-sized titanium dioxide aerosols during pregnancy. Overall data pertaining

to increased cardiovascular disease susceptibility over time including progeny health, microvascular reactivity, mitochondrial dysfunction, and epigenetic modifications will be presented.

SYM05: Concerns of Herbal Product-Induced Toxicity

S05-01 Safety Evaluation of Herbal Products: Current Standards to a Proposed More Comprehensive Evaluation

Ahmet Aydın. *Yeditepe University, Istanbul, Turkey.*

Herbal products have been used by the world's traditional medicines for various health benefits. However, their long-term use is not a guaranty of their safety. According to the European Medicine Agency (EMA), if an herbal product has been used in the world for 30 years and at least 15 years in the Europe, there is no need to carry out general toxicity tests except for developmental and reproductive toxicity, genotoxicity, carcinogenicity, and toxicokinetic data. Genotoxicity test data should be available for many herbal products due to the direct relationship between genotoxicity and carcinogenesis development. Even if they are used in the world for a long time, is this data reliable? Published regulatory documents, mainly EMA documents, were reviewed and summarized. Missing information on herbal products should be completed to further fully evaluate herbal products.

S05-02 Hepatotoxicity of Usnic Acid and Its Mechanisms

Si Chen. *US FDA/NCTR, Jefferson, AR, The United States of America.*

Usnic acid-containing dietary supplements have been marketed as weight loss agents, although severe hepatotoxicity and acute liver failure were associated with their use. Our previous mechanistic studies revealed that autophagy, disturbance of calcium homeostasis, and ER stress are involved in usnic acid-induced toxicity. Recently, we investigated the role of oxidative stress and the Nrf2 signaling pathway in usnic acid-induced toxicity in HepG2 cells. We found that a 24-h treatment with usnic acid caused DNA damage and S-phase cell cycle arrest in a concentration-dependent manner. Usnic acid also triggered oxidative stress as demonstrated by increased reactive oxygen species generation and glutathione depletion. Short-term treatment (6 h) with usnic acid significantly increased the protein level for Nrf2 (nuclear factor erythroid 2-related factor 2), promoted Nrf2 translocation to the nucleus, up-regulated antioxidant response element (ARE)-luciferase reporter activity, and induced the expression of Nrf2-regulated targets, including glutathione reductase, glutathione S-transferase, and NAD(P)H quinone oxidoreductase-1 (NQO1). Furthermore, knockdown of Nrf2 with shRNA potentiated usnic acid-induced DNA damage and cytotoxicity. These results indicate that usnic acid causes cell cycle dysregulation, DNA damage, and oxidative stress and that the Nrf2 signaling pathway is activated in usnic acid-induced cytotoxicity.

S05-03 Neurotoxicity of Ginkgo biloba Seeds and Its Mechanisms

Daisuke Kobayashi. *Health Sciences University of Hokkaido, Hokkaido, Japan.*

Ginkgo biloba seeds and leaves have been used as a traditional herbal remedy for thousands of years. *Ginkgo biloba* seeds were also cooked in traditional Japanese and Chinese food preparations. Ginkgo seed poisoning is primarily due to the neurotoxic compound 4'-O-methylpyridoxine (MPN, also known as ginkgotoxin) and MPN glucoside in ginkgo seeds, resulting in decreased γ -aminobutyric acid (GABA) concentrations in the brain. We have characterized the plasma concentration profile of MPN and intrinsic vitamin B6 concentrations (pyridoxal [PL], PL-5'-phosphate [PLP], and 4-pyridoxic acid) using a rat model. A significant decrease in the plasma concentration of PLP, an active form of vitamin B6 after MPN administration, and an increase in plasma PL and 4-pyridoxic acid concentrations were observed. The increase in PL concentration may be caused by either metabolism of MPN to PL or by MPN-mediated inhibition of PL kinase. Our study was the first *in vivo* study showing relatively rapid elimination of MPN in rats and a decrease in plasma PLP concentration caused by MPN. Characteristics of ginkgo seed poisoning and our current research data will be reported in this symposium.

S05-04 Cardiotoxicity of Aconitum Plants and Its Mechanisms

Xianju Huang. *South-Central University for Nationalities, Wuhan, China.*

Aconitum plants have been used as food or medicine in China for thousands of years. The active principles are aconitine and related alkaloids. Toxicity mainly affects cardiovascular and central nervous systems. Refractory ventricular arrhythmias and hypertension may lead to death. The sodium and calcium channels are important targets of the herbs. Lower doses of the extracts (10-1,000 µg/ml) could dose-dependently suppress TTX-S voltage-gated sodium currents in DRG neurons, leading to analgesic effects. However, higher dosage of the extract led to an opposite effect on the ion channels and induce neural excitation. Moreover, our studies revealed that the aconitine-induced toxicity in H9c2 cells is characterized by increased oxidative stress, increasing cell autophagy/apoptosis, disturbing mitochondrion potential, and triggering Ca²⁺ overload. Aconitine increases the accumulation of intracellular Na⁺ by up-regulation of the expression of voltage-gate sodium channels, and inhibits SERCA2 expression on the sarcoplasmic reticulum, leading to membrane depolarization. Such depolarization is expected to open DHPs, resulting in Ca²⁺ influx into the cells, which then activates the RyR2 channels, leading to massive release of Ca²⁺ from the sarcoplasmic reticulum, and even Ca²⁺ overload. The above mechanisms are suggested as key factors in the toxicity of aconitum herbs.

SYM06: Emerging CRISPR Applications in Environmental Health Sciences and Toxicology

S06-01 Genome-Wide and Targeted CRISPR Screening to Understand Gene-Environment Interactions

Chris Vulpe. *University of Florida, Gainesville, FL, The United States of America.*

Mechanisms of toxicity remain unclear for many chemicals. Functional screening approaches using genome wide and toxicology targeted CRISPR screening provides an approach to identify cellular adverse outcome pathways. We have utilized both genome wide and custom toxicology specific CRISPR libraries (ToxCRISPR) to identify genes and cellular processes involved in cellular response to both well studied toxicants, such as Arsenic and Acetaldehyde, as well as toxicants for which there is limited information. We generated and screened pooled genome-wide KO libraries of mutant K562 human erythroleukemic cells to identify genes whose loss-of-function alters sensitivity to acetaldehyde and arsenic trioxide. Our study on arsenic trioxide suggested a predominant role of reactive oxygen species (ROS) in acute arsenic trioxide toxicity, identified key arsenic transporters, and cellular damage response pathways involved in modulating toxicity. Unexpectedly, pathway analysis and confirmatory studies showed a novel link between arsenic trioxide toxicity and selenocysteine biosynthesis/incorporation into selenoproteins. Our study on acetaldehyde identified DNA damage response as a key cellular process and identified a gene of unknown function, OVCA2, which may play a role in DNA repair or DNA damage response. We similarly carried out targeted studies on selected ToxCast toxicants using the ToxCRISPR library. We identified common and unique cellular process involved in response to these toxicants. Our work further demonstrates the strength of high throughput screening using the CRISPR-Cas9 system in deciphering mechanisms of toxicity.

S06-02 CRISPR Screening to Identify Suppressors of Cellular Stress Response to Proteo-Toxicants

Quan Lu. *School of Public Health, Harvard University, Cambridge, MA, The United States of America.*

We report the identification and characterization of multiple cellular suppressors of ER stress response and the associated apoptosis that is induced by environmental proteo-toxicants such as arsenic. Using a genome-wide CRISPR library targeting both protein-coding genes and microRNAs, we screen for genes whose inactivation further increased ER stress-induced upregulation of C/EBP homologous protein 10 (CHOP)—the transcription factor central to ER stress-associated apoptosis. Among the top validated hits are components of the polycomb repressive complex and microRNAs. Our study reveals previously unrecognized mechanisms by which cells suppress the ER stress response and its associated cell death, and may lead to the development of new therapeutic strategies aimed at restoring cellular homeostasis.

S06-03 CRISPR Application to Uncover Redox Biology

Navdeep Chandel. *School of Medicine, Northwestern University, The United States of America.*

The major function of mitochondria in cellular homeostasis has historically been the generation of ATP through oxidative phosphorylation. However, we have previously demonstrated that mitochondria can serve as signaling organelles during hypoxia by releasing low levels of reactive oxygen species (ROS) from mitochondria are essential for physiological functions. However, the details of ROS dependent signaling pathways are not fully understood. I will present our findings in the use of CRISPR to uncover new ROS dependent pathways.

S06-04 A Vision for CRISPR Applications in Toxicology and Environmental Health

Luoping Zhang. *School of Public Health, University of California, Berkeley, Berkeley, CA, The United States of America.*

CRISPR (clustered regularly interspaced short palindromic repeats), a recently developed RNA-guided gene editing technology, has taken biomedical research by storm and provided scientists with the ultimate toolbox for genetic manipulation. Many new CRISPR applications are now being investigated and established, including fundamental disease research, drug screening and therapy development, and rapid diagnostics. Beyond medicine, CRISPR is catalyzing upstream public health intervention practices through gene disruption in critical disease-transmitting organisms, such as the Zika-carrying mosquitos. CRISPR is also revolutionizing agriculture through ecologically sustainable plant genome modification and reduced reliance on pesticides. Until recently, contrary to its widespread interdisciplinary use, CRISPR application in environmental health and toxicology has remained limited. Similar to use in drug screening, this novel technology can be readily employed to screen toxic environmental chemicals and to elucidate mechanistic pathways through genome-wide functional testing in cellular systems. Findings can be further tested and confirmed in cells then applied into human population studies. Thus, we can harness the power and flexibility of the CRISPR functional genomic screening approach to gain mechanistic insight in modern toxicology for an unprecedented protection of human and environmental health.

SYM07: Epigenetic Biomarkers of Exposure to Environmental Carcinogens

S07-01 Epigenetic Mechanisms and Cancer: An Interface between the Environment and the Genome

Zdenko Herceg. *International Agency for Research on Cancer, Lyon, France.*

Cancer, including hepatocellular carcinoma (HCC), is characterized by the presence of genetic and epigenetic alterations. These include DNA methylation, modification of histone proteins, and altered expression of several classes of non-coding RNAs. Contrary to genetic mutations, epimutations are reversible, which presents a unique opportunity for restoring the normal epigenetic pattern by drugs and bioactive food compounds (BFCs). Evidence indicates that the nutritional status is an important factor in the etiology and pathogenesis of cancer, leading or increasing its development, as well as preventing or inhibiting it. BFCs can prevent the HCC development either by correcting cancer-related epigenetic abnormalities or preventing their formation caused by a carcinogenic exposure. Using an experimental model of chemical hepatocarcinogenesis in rats, we demonstrate that tributyrin, a butyric acid pro-drug and HDAC inhibitor, administered alone or in combination with vitamin A or folic acid during the initiation or even promotion carcinogenesis stages, prevents or inhibits the carcinogenic process by correcting gene-specific epigenetic abnormalities. Similar epigenetically-mediated chemopreventive effect on liver carcinogenesis was exhibited also by butyrate-containing structured lipids. In summary, the results of our study illustrate that timely correction of carcinogen-induced epigenomic alterations by BFCs provide a unique opportunity for the development of better cancer preventive strategies.

S07-02 Epigenomics and Drug-Induced Carcinogenicity

Jonathan Moggs. Novartis Institutes for BioMedical Research, Basel, Switzerland.

Assessing the carcinogenic potential of innovative drugs spanning diverse therapeutic modalities and target biology represents a major challenge during drug development. The discovery that aberrant epigenetic events frequently accompany genetic mutations in human cancers has stimulated efforts to characterize drug-induced non-genotoxic carcinogenesis at the molecular level in animal models. Integrated genome-wide epigenomic and transcriptomic profiling of rodent models for drug-induced nuclear-receptor dependent liver tumor promotion has led to the identification of novel mechanistic insights and early biomarkers including dynamic changes in the DNA methylome, non-coding RNAs, chromatin accessibility, histone modifications and transcription factor regulatory networks. In particular, increased expression of *Dlk1-Dio3* imprinted gene cluster noncoding RNAs may represent the early stages of Constitutive Androstane Receptor activator-induced hepatocyte de-differentiation and a return to pluripotency. Recent advances in the comprehensive mapping and functional characterization of mammalian tissue-specific epigenomes also provides new opportunities to characterize the cross-strain and cross-species chromatin architecture of non-genotoxic carcinogen effector genes and to predict their potential for modulation by xenobiotics in human tissue.

S07-03 The Role of Epigenetic Science in Carcinogen Detection

Igor Pogribny. US FDA/NCTR, National Center for Toxicological Research, Jefferson, Arkansas, USA.

Human exposure to certain natural and man-made chemical carcinogens is one of the major risk factors for cancer development. In a broad sense, the carcinogenic process may be induced through either genotoxic (agents that interact with DNA), or non-genotoxic (chemicals causing tumor formation by mechanisms other than directly damaging DNA) carcinogens. Currently, the identification of carcinogen-induced genetic changes such as DNA or protein adducts often is a first step in elucidating the potential role of a genotoxic chemical in the etiology of human cancer and is widely accepted that detection of DNA or protein adducts caused by exposure is an end-point indicator in cancer risk assessment. In contrast, there are no regulatory accepted short- or medium-term tests that can predict non-genotoxic carcinogenicity. The lack of short- or medium-term tests that predict non-genotoxic carcinogens has meant that confirmation of their carcinogenic potential requires rodent lifetime bioassays, which are laborious, expensive, and time consuming. Through examples of epigenetic alterations caused by the exposure to model non-genotoxic chemicals, including, arsenic, furan, and methapyrilene, this presentation will highlight the role of epigenetic events, including cytosine DNA methylation, histone modifications, and microRNA expression, as the earliest events in the carcinogenic process.

S07-04 Epigenetic Mechanism of Chromium-Induced Cell Transformation and Tumorigenesis

Chengfeng Yang. Department of Toxicology and Cancer Biology, University of Kentucky, Lexington, Kentucky, USA.

It is now known that many environmental toxicants associated with carcinogenesis are not genotoxic and do not cause mutations or chromosomal rearrangements. Instead, they exert their actions through epigenetic modifications of the genome, altering the gene expression patterns of the susceptible cells or organs. A prime example is the plasticizer, bisphenol A (BPA). Early-life exposure of rats to BPA altered their prostate epigenome and increased prostate cancer risk in adult life, in a non-linear dose-dependent manner. The exposure induces permanent and/or dormant dysregulations in gene expression programs of target cells. Specially, the stem cells in the prostatic epithelium are affected with regard to cell fate, pluripotency, signaling mechanisms and differentiation in this animal model. A similar scenario was also observed for mammary cancer and likely endometrial cancer. Although DNA methylation was found to be a significant epigenomic mechanism for reprogramming of cancer risk, histone modifications, alteration of non-coding RNA expression, and other higher order chromatin changes play equally important roles. Future research focusing on translating environmental epigenomic findings from experimental models to population-based/clinical studies will help advance the field of personalized cancer prevention.

SYM08: New Approach Methodologies: A Global Perspective

S08-01 Beyond the Silver Anniversary: The Next 25 Years of Alternatives in the European Union

Maurice Whelan. European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), European Commission Joint Research Centre, Ispra, Italy.

The European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) is an integral part of the European Commission's Joint Research Centre. Over the past 25 years, ECVAM has played a central role in the development, validation, and acceptance of alternative methods and approaches, activities which are now mandated under the European Union Directive 2010/63/EU to replace, reduce, and refine the use of animals for scientific purposes. This presentation will highlight the major milestones achieved over the last 25 years and provide an overview of the current efforts and future strategic directions to develop, validate, and promote the regulatory acceptance and international adoption of new approach methodologies.

S08-02 Adopting New Approach Methodologies: The US Strategic Roadmap and Implementation Activities

Nicole Kleinstreuer. The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, Durham, NC, The United States of America.

The United States Interagency Coordinating Committee on Validation of Alternative Methods (ICCVAM) recently revealed an overarching national strategy addressing the development, validation, and implementation of new approach methodologies for assessing the safety of chemicals and medical products. Individual ICCVAM agencies and regulatory program offices have released detailed roadmaps addressing specific chemical sectors or legislative mandates (e.g. EPA-TSCA and FDA). Acceptance and utilization of alternatives has increased markedly in areas such as endocrine disruptor screening, topical hypersensitivity testing, and acute systemic toxicity prediction, both from an industry perspective and in regulatory decision-making. Progress in the US parallels global efforts being coordinated via the Organization for Economic Cooperation and Development (OECD) Chemical Test Guidelines Program, and both will be discussed herein.

S08-03 21st-Century Toxicology and Regulatory Testing: An Update from East Asia

Hajime Kojima. Japanese Center for the Validation of Alternative Methods (JaCVAM), National Institute of Health Sciences, Tokyo, Japan.

The Japanese Center for the Validation of Alternative Methods (JaCVAM) has a dual purpose: to promote the Replacement, Reduction, and Refinement in animal experiments for the evaluation of chemical substance safety in Japan, and to establish guidelines for new alternative experimental methods through international collaboration. Many areas of toxicity testing, such as genotoxicity, pyrogenicity, phototoxicity, and irritation/corrosion, have ongoing test method validations being coordinated by JaCVAM. These validation studies include international experts on every management team, and are in various stages of development, where some have recently led to regulatory adoption via the Organization for Economic Cooperation and Development Test Guidelines and/or government recommendations. This presentation will cover JaCVAM's efforts both within Asia and on the global stage.

S08-04 O Canada: A Strategic Vision toward Replacement in the True North

Charu Chandrasekera. Canadian Centre for Alternatives to Animal Methods/Validation of Alternative Methods (CaCVAM), Windsor, ON, Canada.

Canadian Centre for Alternatives to Animal Methods (CCAAM) and its subsidiary, Canadian Centre for the Validation of Alternative Methods (CaCVAM), were established in 2017, with the overarching vision to promote the replacement of animals in Canadian biomedical research, education, and regulatory testing

through 21st century science, innovation, and ethics. CCAAM/CaVAM serve as the leader and nexus to bring together national and international academic, industry, government, and public stakeholders to develop, validate, and promote the acceptance of new approach methodologies that yield the most relevant and reliable science-based information to make the best possible decisions about protecting Canadians and their natural heritage. This presentation will provide an overview of the nationally coordinated research, education, and regulatory testing efforts as well as how these efforts are extended to the global alternatives arena in a uniquely Canadian way.

SYM09: Clinical and Mechanistic Aspects of Drug-Induced Liver Injury

S09-01 Genetic Aspects of DILI: Recent Advances on Predicting Risk

Ann K. Daly. *Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom.*

A variety of genetic risk factors relevant to DILI have been identified. Up to the present, the strongest reported associations involve particular HLA alleles which are mainly individual drug-specific. However, more recently evidence for a more general risk factor relating to immune response has been obtained. Not all forms of DILI show immune-related risk factors and a contribution to risk from drug metabolism remains. The possibility of using genetic factors to predict risk will be described.

S09-02 Application of Circulating MicroRNA to Diagnose Early Stage of Liver Injury and Its Pathogenesis in Rat DILI Model

Tsuyoshi Yokoi. *Nagoya University Graduate School of Medicine, Nagoya, Japan.*

Recently, studies on circulating microRNAs (miRNAs) as potential biomarkers of drug-induced liver injury (DILI) have received increasing attention. It has been demonstrated that miR-122 and miR-192, which are liver enriched, could be potential biomarkers of DILI; however, these miRNAs cannot discern types of injuries. We characterized miRNA expression in LSECs and hepatocytes and investigated whether cell type specific miRNAs in plasma can discern pathogenesis of liver injuries in rats. Comprehensive miRNA expression analyses found that several miRNAs were highly and specifically expressed in LSECs and hepatocytes isolated from nontreated and/or SOS (sinusoidal obstruction syndrome) rat model, then we identified a miRNA in plasma as a specific biomarker for LSEC damage. In addition, we identified and characterized specific miRNA biomarkers in early stage of hepatocellular injury, cholestasis, and steatosis using representative rat models. Comprehensive miRNA sequence analyses using NGS and further analyses suggested the utility of miRNAs as specific biomarkers for the early detection of DILI.

S09-03 Impact of Multidrug Resistance Protein 4 (MRP4/ABCC4) Function in Compensatory Hepatocellular Proliferation

José E. Manautou. *University of Connecticut, Storrs, CT, The United States of America.*

Hepatobiliary transporters are key genetic determinants of drug pharmacokinetics, pharmacodynamics and important contributors of DILI outcome. DILI is also known to alter the expression and function of drug transporters. We have demonstrated that DILI resulting from acetaminophen (APAP) treatment leads to tolerance to subsequent hepatotoxicant exposure and that this adaptation is associated with compensatory hepatocellular proliferation. We have further investigated whether changes in the expression and function of drug transporters contribute to the development of tolerance to APAP hepatotoxicity, with emphasis on the multidrug resistance protein 4 (Mrp4, ABCC4). Research work to be discussed include studies investigating the role of ABCC4 in compensatory cell proliferation following DILI and other models of liver tissue loss and regeneration.

S09-04 Clinical and Mechanistic Aspects of Drug-Induced Liver Injury

Fernando Bessone. *University of Rosario School of Medicine, Rosario, Argentina.*

DILI case definition and characterization using liver biochemistry and histology is crucial for appropriate phenotyping. The incidence of DILI is probably higher than expected by the cases identified in clinical practice, due to misdiagnosis and underreporting. Existing databases have enabled to better predict immediate and long-term DILI prognosis. However, an under-reported and still poorly explored field is that related to the outcome of long-term assessment of DILI associated with fatality, i.e., death or liver transplantation. To overcome these limitations, country-based registries and multicenter research networks have been created in Europe, North America and Latin America. The recent discovery of various mechanistic-based biomarker candidates and the advent of omics technologies offering new approaches to the study of DILI will be discussed.

SYM10: Effects of Environmental Mixture Exposure on Brain Development and Function Based on Epidemiological, In Vivo, and In Vitro Studies

S10-01 Methods for Stage-Specific Developmental Neurotoxicity Testing with Human Stem-/Progenitor Cell-Based Models for Mixture Evaluation

Ellen Fritsche. *IUF, Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany.*

It is now agreed that an *in vitro* testing battery for developmental neurotoxicity (DNT) evaluation is needed that covers different neurodevelopmental endpoints at different developmental stages. Here, an overview over the available test systems will be given, which are based on human stem-/progenitor cells as well as the model organism zebrafish, suitable for both single compounds and mixture-induced DNT evaluation. Performance evaluation and application domains of the different assays with gap analyses for covering the endpoint DNT will be presented. As examples we show data from two assays covering parts of embryonic and fetal development by using hiPSC- and primary human neurospheres, respectively. Differentiation of hiPSC-derived neurospheres thereby mimics early embryonic neuronal migration and differentiation. Development of primary human neurospheres, which are derived from fetal brains, represent their fetal period of origin. The latter are capable of proliferating in 3D in culture and radially migrate and differentiate into neurons and glia cells, i.e. radial glia, astrocytes and oligodendrocytes, thus mimicking basic processes of cortical development, potential targets of chemical mixtures. Such a testing battery is currently applied for single compound toxicity testing however, there is a strong need in testing mixtures effects to such systems.

S10-02 Adverse Outcome Pathways (AOPs)-Driven Evaluation of Developmental Neurotoxicity Induced by Mixture of Environmental Chemicals

Anna Bal-Price. *European Commission Joint Research Centre (JRC), Ispra, Italy.*

Environmental chemicals known to trigger developmental neurotoxicity (DNT) effects, belong to different silos including pharmaceuticals, industrial chemicals, persistent organic pollutants (POPs), metals and pesticides. Therefore, their related toxic effects are differently regulated. Combined effects of these chemicals across different regulatory domains are not currently considered even it is well documented that "mixture effects" can be greater than effects triggered by the most potent single chemicals, and moreover, the mixture effects may be additive or in some cases even synergistic. In this study, the effects induced by mixture of heterogeneous classes of chemicals, acting through similar and dissimilar mode of action have been evaluated in comparison to single chemicals using human neurodevelopmental models, derived from induced pluripotent stem cells and assays anchored to common key events of adverse outcome pathways (AOPs) resulting in learning and memory impairment in children. Based on the obtained results a battery of *in vitro* assays relevant to DNT evaluation of single chemicals or in mixture is proposed to facilitate an application of mechanistic knowledge to increase scientific confidence in decision making process, and speeding up evaluation of thousands of chemicals present in our environment that lack safety data on DNT potential.

S10-03 Mechanisms of Environmental Pollutants Mixtures as Risk Factor for Attention Deficit/Hyperactivity Disorder and Autism Pathophysiology Based on Epidemiological and *In Vivo* Studies

Oddvar Myhre. Norwegian Institute of Public Health, Department of Toxicology and Risk Assessment, Oslo, Norway.

Animal and human data suggest that environmental mixtures like air pollution and persistent organic pollutants (POPs) may lead to developmental neurotoxicity (DNT). In recent years, a pattern of increasing prevalence of attention deficit/hyperactivity disorder (ADHD) and autism spectrum disorders (ASD) has been observed, which is hypothesized to be explained by early-life exposure to pollutants. However, mechanisms explaining how exposure to pollutants during early development may contribute to increased risk of neurodevelopmental disorders are lacking. Our experiments show that inhalation of biodiesel exhaust in rats and *in utero* feeding exposure of a POP mixture in mice resulted in cognitive deficits and gene expression changes related to oxidative stress, inflammation, disturbance of endocrine signalling and cognitive pathways. Epidemiological studies propose associations between air pollution and ASD. Epidemiological studies at our institute using the ADHD and ASD sub-cohorts of the Norwegian Mother and Child Cohort Study (MoBa) suggest associations between exposure to pollutants and neurodevelopmental deficits, and mechanistic data suggest associations to inflammation markers and disturbance of thyroid signalling. Although more advanced animal disease models are needed to complement epidemiological findings, these mechanistic data strengthen the hypothesis that environmental mixtures may be part of the causalities for neurodevelopmental pathophysiology.

S10-04 A Human Mixture Risk Assessment for Neurodevelopmental Toxicity Associated with Polybrominated Diphenyl Ethers Used as Flame Retardants

Andreas Kortenkamp. Brunel University London, Department of Life Sciences, College of Health and Life Sciences, Uxbridge, United Kingdom.

The exposure of small children (aged 1-3 years) to the brominated diphenyl ether (BDE)-99 may exceed acceptable levels defined in relation to neurodevelopmental toxicity in rodents. All age groups are exposed not only to BDE-209 and -99, but to a cocktail of BDE congeners with evidence of neurodevelopmental toxicity. The possible risks from combined exposures to these substances have not been evaluated. A congener-specific mixture risk assessment of human exposure to combinations of BDE-209 and other BDEs was based on estimated exposures via diet and dust intake, and on measured levels in biologic samples. We employed the Hazard Index method by using BDE congener-specific reference doses for neurodevelopmental toxicity. The results suggest that combined exposures to PBDEs may exceed acceptable levels in breast-feeding infants (aged 0-3 months) and small children (1-3 years old), even for moderate (vs. high) exposure scenarios. Small children had the highest combined exposures, with some estimated body burdens similar to body burdens associated with developmental neurotoxicity in rodents. Our estimates support the inclusion of BDE-209 in the POP convention and strategies to reduce exposures to PBDE mixtures, including maximum residue limits for PBDE in food and measures for limiting the release of PBDE from consumer waste.

SYM11: Immunotoxicity Associated with Exposure to Nanomaterials

S11-01 WHO Principles and Methods for Assessing the Risk of Immunotoxicity Associated with Exposure to Nanomaterials

Henk van Loveren. Maastricht University, Maastricht, The Netherlands.

New WHO guidance on Principles and Methods for assessing the risk of immunotoxicity associated with exposure to nanomaterials will be presented. The document addresses the current state of the art of risk assessment for assessing the health risk of immunotoxicity associated with exposure to engineered nanomaterials (ENMs). While the technology has progressed rapidly and thousands of consumer products containing ENMs are appearing on the market, occupational and general population exposure and toxicology of these materials is less understood. Extensive research conducted in the past 20 years shows that not only the chemistry but also size surface and shape are among

the important properties that influence the interaction of ENMs with biological systems. Considering the rapid development of technology, sheer number of ENMs with diverse properties and the constraints associated with conventional toxicity assessment methods, it will be impossible for toxicologists and risk assessors to keep pace with the rapidly evolving technology. In addition to applicable conventional methodologies, novel approaches for exposure estimation, hazard identification and strategies for assessing the risk, integrating the physical, chemical, and biological sciences to address the challenges presented by ENMs are needed.

S11-02 Occurrence of TiO₂ in Food, Supplements and Toothpaste, Resulting Exposure and Toxicokinetics

Minne Heringa. Centre for Safety of Substances and Products (VSP), Bilthoven, The Netherlands.

Titanium dioxide (TiO₂) is commonly applied as white pigment in toothpaste and food products such as chewing gum. A small fraction of the pigment is known to be present as nanoparticles (NPs). The oral intake of TiO₂ and its NPs from food, food supplements and toothpaste in the Dutch population aged 2 to over 70 years was estimated. The estimated mean intake of TiO₂ NPs ranges from 0.19 µg/kg bw/day in elderly, 0.55 µg/kg bw/day for 7-69 year old people, to 2.16 µg/kg bw/day in young children. This intake was based on actual measurements in products. As a consequence, the estimated intakes are lower than most other studies have found. The products contributing most to the TiO₂ intake are toothpaste (in young children only), candy, coffee creamer, fine bakery wares and sauces. After oral intake, only a very small fraction (< 1%) is absorbed by the gut, and any particles reaching the blood are immediately cleared by macrophages, mainly in the liver and spleen, where studies vary in how well they are found to be eliminated again. Accumulation is nevertheless likely, which has been shown to necessitate the inclusion of kinetic modelling in the risk assessment of these nanoparticles.

S11-03 Mechanisms and Consequences of Immune Interactions of Graphene-Based Materials

Bengt Fadeel. Karolinska Institutet, Stockholm, Sweden.

Graphene-based materials (GBMs) including graphene oxide (GO) are potential candidates for various biomedical applications (Bhattacharya et al. *Nanomedicine*. 2016;12:333-51). However, successful clinical use of nanomaterials requires a detailed understanding of their biological interactions. In particular, interactions with macrophages, neutrophils, and other cells of the innate immune system, our main defense against foreign intrusion, are of key importance. In this context, it is important to assess for endotoxin contamination as this may skew the interpretation of results when dealing with immune-competent cells. Furthermore, the bio-corona of proteins and other biomolecules adsorbed onto the surface of the nanomaterials should not be ignored. Enzymatic degradation of GBMs serves as a special case of nano-bio-interactions with important toxicological implications. Here, recent studies on the immune interactions of GO conducted in the frame of the EU-funded Graphene Flagship project are presented. In addition, inhalation exposure is of major concern in the occupational setting, and studies on pulmonary inflammatory responses to GO using both *in vitro* and *in vivo* models are also discussed.

S11-04 Immunotoxicity and Susceptibility Issues following Pulmonary Exposure to Carbon Nanotubes

James Bonner. North Carolina State University, Raleigh, NC, The United States of America.

Carbon nanotubes (CNTs) are engineered nanomaterials with numerous applications. However, they could pose a risk to human health from occupational or consumer exposures. Rodent models demonstrate that pulmonary exposure to CNTs results in lung fibrosis, exacerbation of allergic airway disease and in some cases lung cancer or mesothelioma, all of which involve altered immunity. Disease severity is determined by various physicochemical properties of the CNTs such as metal catalyst content, rigidity, length, aggregation, and surface charge. Recent studies highlight the ability of CNTs to not only exacerbate allergen-induced lung disease in mice, but also to directly cause allergic disease leading to airway mucus production, elevated serum IgE, and increased TH2 cytokines, all key indicators of asthma. CNTs are increasingly

functionalized with organic or inorganic agents to modify or enhance surface properties. The mechanisms of CNT-induced immunotoxicity include oxidative stress, macrophage inflammasome activation, dendritic cell-mediated polarization of T-lymphocytes, and systemic immunosuppression or immunostimulation, depending on the exposure scenario. A comprehensive understanding of how physicochemical properties determine the immunotoxicity of CNTs will be discussed along with genetic variability and gain or loss of function of genes encoding secreted cytokines, enzymes, or intracellular cell signaling molecules.

SYM12: The Carcinogenicity of Hexavalent Chromium: A Global Public and Environmental Health Concern

S12-01 Genetic and Epigenetic Alterations of Lung Cancer in Workers with Chromate Exposure

Kazuya Kondo. Tokushima University, Tokushima, Japan.

It is well known that hexavalent chromium [Cr(VI)] is inhaled carcinogen for lung cancer. However, their genetic effects in humans are only partially understood. We collected 38 lung cancer samples from 31 industrial workers with chromate exposure (12-38, 23±7 years) in Japan (chromate LC). We examined genetic and epigenetic alterations of chromate LC. Chromate LC had a few of TP53 mutation and no K-, H-ras mutation. The frequency of 3p, 5q, 9p and 17p LOH in chromate LC was similar to that of non-chromate LC. However, the frequency of replication error in chromate LC was higher than that of non-chromate LC (79% versus 15%). In chromate LC, the repression of hMLH1 protein, DNA mismatch repair gene, was frequent (56% vs 20%), and the inactivation of hMLH1 expression strongly correlated with the microsatellite high instability phenotype. The DNA methylation of the promoter region of hMLH1 gene in chromate LC was significantly higher than that of non-chromate LC (24.2% vs 3.8%). We speculate that chromium attacks "caretaker genes" (gene that maintain the integrity of the genome) including DNA mismatch repair gene (hMLH1), inactivation of which leads to genetic instabilities, resulting in increased mutation of cancer-related genes.

S12-02 Inflammation-Driven Cancer Stem Cells Formation as Promoter of Hexavalent Chromium Carcinogenesis

Carmen Alpoim. University of Coimbra, Coimbra, Portugal.

Intercellular communication in cancer is a deceiving and extremely efficient process. The extensive crosstalk mediated by cytokines and chemokines overcome the inefficiency of the invasion-metastasis cascade, thus allowing the development of the often-fatal metastatic disease. After observing that Cr(VI) malignantly transformed human bronchial epithelial RenG2 cells dedifferentiated following culture in the subcutaneous mouse lumbar region, co-cultures of surgically isolated mice lumbar stromal cells with RenG2 cells were established and the conditioned media studied by multiplex and ELISA. Consequently, Interleukin-6 (IL-6), Granulocyte colony-stimulating factor (G-CSF) and Activin-A were identified as the paracrine mediators of the intercellular communication process. ELISA showed the three cytokines were present inside fibroblasts-secreted exosomes. Moreover, whenever exosomes' release was blocked, dedifferentiation was abrogated, further proving the role of the aforementioned cytokines and of exosomes in the dedifferentiation process. Additionally, cytokine-blocking experiments revealed that only IL-6 and Activin-A were endowed with the potential to orchestrate dedifferentiation, while G-CSF appeared to be decisive in maintaining the undifferentiated phenotype. Altogether the attained results implicated IL-6 and Activin-A in the formation of cancer stem cells by dedifferentiation, and G-CSF as a potent keeper of the dedifferentiation status. *Work sponsored by FEDER, POFC-COMPETE and the FCT grants PTDC/BBB-BQB/2450/2012 and SFRH/BD/33884/2009.*

S12-03 Mechanisms of Hexavalent Chromium-Induced Genomic Instability: How a Lung Carcinogen Breaks DNA and Inhibits Repair

John Pierce Wise Sr. University of Louisville, Louisville, KY, The United States of America.

Particulate hexavalent chromium [Cr(VI)] is a known human lung carcinogen, but how it causes cancer is unknown. We hypothesize the carcinogenic mechanism involves the onset of chromosome instability mediated in part by the induction of DNA double strand breaks combined with the loss of DNA double

strand break repair. Consistent with our hypothesis, we discovered particulate Cr(VI) both induces DNA double strand breaks and inhibits homologous recombination repair (HR) in human lung cells. For example, zinc chromate induces concentration-dependent increases in DSBs and chromosome aberrations, which persist with longer exposure times; however, after 48 h zinc chromate simultaneously targets Rad51 and inhibits HR repair. We found zinc chromate inhibited Rad51 expression and repair foci formation, while also inducing cytoplasmic accumulation of Rad51 and loss of Rad51 filament formation on the DNA. Moreover, we found the loss of Rad51 response was a permanent phenotypic change; and successfully developed monoclonal cell lines expressing particulate Cr(VI)-induced loss of Rad51 response. These outcomes support our hypothesis that the mechanism of particulate Cr(VI)-induced lung cancer involves loss of DNA repair and chromosome instability, which has important implications for Cr(VI)-induced cancer and lung cancer in general. *This work was supported by NIEHS grant ES016893 (J.P.W.).*

S12-04 Using the Latest Science in Cancer Risk Assessment for Hexavalent Chromium: Is it Time to Step Away from the Default Regulatory Approaches?

Deborah Proctor. ToxStrategies, Inc., Mission Viejo, CA, The United States of America.

Hexavalent chromium [Cr(VI)] is recognized as a human carcinogen based on lung cancer observed among workers of certain industries, and has been shown to cause small intestinal tumors in mice and oral cavity tumors in rats by drinking water. As such, occupational epidemiology and toxicology data are commonly used to generate measures of carcinogenic potency and develop exposure limits. Traditionally, cancer risk assessment strategies for Cr(VI) have relied on linear-extrapolations of risk observed at extreme exposure levels, to estimate risk at the relatively low levels of exposure currently experienced by people in the workplace and from Cr(VI) in the environment. However, with the enhanced understanding of cancer biology and molecular mechanisms, refined risk assessment approaches for Cr(VI) are now being considered in risk assessment. The carcinogenic modes of action (MOAs) for Cr(VI)-induced tumors from oral inhalation intakes has recently been assessed in several studies, and the weight of evidence from both toxicokinetic and mechanistic data support that Cr(VI) acts by non-mutagenic MOAs including cytotoxicity, inflammation and epigenetic modifications. This presentation will review current MOA research and how these findings, along with pharmacokinetic modeling, are used in quantitative risk assessment approaches for Cr(VI).

SYM13: Acute, Chronic, and Long-Term Pesticide Effects: Mechanisms of Actions, Epidemiological Data, Priority Areas for Research

S13-01 Pesticide Exposure, Human Behavior, Parkinson's Disease, and Dementia: Evidences, Uncertainties, Open Points

Claudio Colosio. Department of Health Sciences of the University of Milano and International Centre for Rural Health of the San Paolo Hospital of Milano, Milan, Italy.

Several studies have been published on the relationship between pesticide exposure and central nervous system effects. Main focus is on neurobehavioral impairment, Parkinson's disease and dementia. In this presentation, jointly prepared by three Authors, will focus on the strengths and limitations of epidemiological studies on pesticide exposure and central nervous system effects and highlight the levels of evidence, the uncertainties and the open points for further research with specific attention to mechanism-based approach.

S13-02 Pesticides and Cancer: Current Knowledge and Epidemiological Evidence

Martin Wilks. Swiss Centre for Applied Human Toxicology, Basel, Switzerland.

All pesticide active ingredients undergo detailed toxicological testing before being authorized for use. This includes both *in vitro* and *in vivo* mutagenicity studies, as well as carcinogenicity studies in two species. Nevertheless, concerns have been expressed on the basis of epidemiological surveys such as the Agricultural Health Study which demonstrate an association of exposure to

some pesticides and certain types of cancer such as non-Hodgkin's lymphoma or prostate cancer. This presentation will focus on the strengths and limitations of epidemiological studies on pesticide exposure and cancer outcomes and highlight the need for appropriate exposure assessments and a mechanism-based approach.

S13-03 Challenges in Monitoring and Biomonitoring of Pesticides

Aristidis Tsatsakis. *University Crete, Crete, Greece.*

Persistent organic pollutants comprised organochlorinated pesticides which can be subject to long range transboundary air pollution transport, bioaccumulation and biomagnification in the food chain, and very long biological persistence in human organisms. Among possible health effects include cancer, reproductive defects, neurobehavioral abnormalities, endocrine and immunological toxicity. The Author will update a review already conducted in the past bringing new data, assessing strengths and limitations of epidemiological studies and highlighting the levels of evidence, the uncertainties and the open points for further research with specific attention to mechanism-based approach.

S13-04 Acute Pesticide Poisoning and the Effect of Pesticide Regulation

Won Jin Lee. *Korea University College of Medicine, Seoul, The Republic of Korea.*

Acute pesticide poisoning is a major public health concern worldwide. Several interventional approaches such as safe storage and formulation change have been conducted to reduce intentional and unintentional pesticide poisoning. Among them, pesticide legislative regulation have presented the most effective findings in several countries. This presentation will focus on the effects of regulatory interventions on acute pesticide poisoning and highlight the evidence on intentional pesticide poisoning with the experience of paraquat, and discussion for the magnitude of acute pesticide poisoning and future direction of pesticide management.

SYM14: Collaborative Approaches to Proactively Advance 3Rs in Nonclinical Drug Development

S14-01 Setting the Bar for Positive and Negative Animal Predictive Values: The IQ Translational Database

Thomas Monticello. *Amgen, Thousand Oaks, CA, The United States of America.*

An industry-wide nonclinical to clinical translational database was created and analyzed to determine how animal testing for safety assessment may translate to clinical risk. This effort was undertaken in part to better understand the potential value of animal testing and interrogate opportunities to better apply the 3Rs such as a reduction in animal use or provide context to replace animal testing by alternate models. A database of 182 molecules contains animal toxicology data coupled with clinical observations from phase I human studies. Concordance statistics were performed by organ system and test species. Our results indicate that while animal studies can demonstrate great value in the positive predictive values for certain species and organ categories, the negative predictive value was the more impactful measure across test species and target organs. Overall, an absence of toxicity in animal studies strongly predicts a similar outcome in the clinic. Concordance of the dedicated safety pharmacology animal study dataset will also be presented to evaluate opportunities to either include similar endpoints onto general toxicology studies or eliminate such studies if they do not provide predictive value for human safety, both of which support the 3Rs.

S14-02 Applying the 3Rs within Regulatory Toxicology Studies in Drug Development

Helen Prior. *National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), London, United Kingdom.*

The UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) convenes international consortia to discuss opportunities to apply the 3Rs within toxicology programmes. Expert working groups of industry scientists, academics and regulators take an evidence-based approach to recommend efficiencies in study designs and promote best practices to minimise and refine the use of animal testing. Information will be presented for two current projects with the potential to reduce animal use in regulatory toxicology studies: the need for two species for regulatory studies and use of recovery animal data. Themes include fuller exploitation of existing opportunities to use a single species for large molecules within ICH S6 and whether these principles could be expanded to a wider range of molecule types (e.g. small molecules) or therapeutic areas (e.g. oncology). Following a recent review of industry and regulatory use of recovery animal data, recommendations were made that recovery phase animals should not be included in FIH nonclinical studies by default and that when required for scientific reasons, consideration should be given to whether recovery assessment is necessary in multiple species and sexes. Additional work will highlight future opportunities for minimising recovery animal use.

S14-03 Animal-Free INDs and CTAs: A Reality or Dream?

Michael W. Leach. *Pfizer, Andover, MA, The United States of America.*

A monoclonal antibody (mAb) biosimilar expert working group (BEWG) was formed to scientifically evaluate the need of animal studies in support of biosimilar development and registration. Nonclinical requirements for mAb biosimilars vary in their requirements, and currently there is a lack of harmonization. The BEWG assessed current efforts regarding animal testing in the development of biosimilars, evaluated regulatory and ethical committee interactions with manufacturers, determined reasons why manufacturers were conducting animal studies, and made recommendations for a data-driven approach to assess the safety of biosimilars. While at least one animal study had been conducted for recent biosimilar programs, it was determined that the animal studies did not provide any additional safety information and that comparability to the innovator drug could have been completed without any animal testing. The BEWG believes that animal studies are usually not warranted when there is a strong analytical package, including *in vitro* functional assessments, which support a high degree of similarity between the proposed biosimilar and the originator product. Examples will be given where an animal free IND for a biosimilar would still ensure safety in the clinic.

S14-04 Strategic Application of Microphysiological Systems in Safety Assessment to Decrease Dependence on Animal Studies

Brian Berridge. *National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC, The United States of America.*

The reliance on animal toxicology studies in drug development relates to our recognition that pathobiology is complex, most often characterized phenotypically and difficult to replicate in simple cell-based *in vitro* systems. Microphysiological systems (MPS) are biologically complex *in vitro* systems that more closely mimic *in vivo* biology at the tissue and organ level. Use of human cells in MPS has also prompted optimism of improved human relevance and clinical predictivity. This presentation will explore the current evolution and state of MPS in the context of contemporary challenges in safety assessment, highlight a near term interdependence between MPS and animal studies as well as the future potential for improving current paradigms and decreasing animal use.

SYM15: Empowering Toxicology and Environmental Health Research to Address Disasters and Emerging Threats

S15-01 Addressing Toxicology and Human Health Concerns with the NIH Disaster Research Response (DR2) Program

Linda Birnbaum. *National Institute of Environmental Health Sciences (NIEHS) and National Toxicology Program, Research Triangle Park, NC, The United States of America.*

The US National Institutes of Health (NIH) and the US Department of Health and Human Services have recognized a need to improve our understanding of and capacity for performance of time-critical science and research in response to disasters and other emerging threats around the world. New efforts to improve the timeliness and capacity for performing vital disaster research, spearheaded by the National Institute for Environmental Health Sciences, partnering with the National Library of Medicine, has led to the development of the NIH Disaster Research Response (DR2) program. The genesis, accomplishments, and future of the DR2 program, which is helping to drive vitally important environmental health disaster research capacity in the US and internationally, will be highlighted. DR2 capabilities include publicly accessible surveys, tools, and support materials, pre-approved research protocols, IRB workshops, training exercises, and networks of researchers and centers. Responses to recent disasters including 2017 Hurricanes, Flint, Michigan lead poisoning, and Deepwater Horizon oil spill will be used to illustrate the value and remaining challenges for implementing needed research to support response, recovery, and future preparedness.

S15-02 Disaster Response Research Development in Japan

Shoji F. Nakayama. *National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan.*

After the 11 March 2011 Great Eastern Japan Earthquake and Tsunami, the Japanese government was urged to develop public health support capability on top of the existing emergency medical assistance capacity. Government and academic societies have been coordinating emergency surveillance and occupational health support systems. At the same time, recognized is the necessity of long-term epidemiological survey and research tools in order to prepare for future unavoidable disasters. Such tools should be composed of not only rapid public health surveillance instruments but also long-term public health monitoring systems that can be used for recovering and revitalizing affected communities, supporting and resettling evacuated people, and building resilient nations. The National Institute for Environmental Studies of Japan has been collaborating with the US National Institute of Environmental Health Sciences to exchange emergency epidemiology instruments developed in each institute. This discussion will highlight the Japanese challenges and environmental research needs in the aftermath, and new efforts to better prepare for future disasters.

S15-03 When Disaster Strikes: Response, Research, and Recovery

Ivan Rusyn. *Texas A&M University, College Station, TX, The United States of America.*

Due in large part to the vast industrial complexes, on- and off-shore gas and oil exploration, the fisheries, and proximity to the Gulf, coastal communities are increasingly at risk of disaster due to rising water, storm surges, and tropical weather. This discussion will focus on recent adaptation of NIH Disaster Research Response (DR2) tools to conduct disaster research in Gulf Coast communities impacted by Hurricane Harvey. The purpose of this study is to determine health effects of living in a home flooded and subsequently remediated of mold and contamination from flood waters. Living and working in buildings affected by flood damage has been associated with adverse health outcomes, specifically respiratory symptoms and infections, including exacerbation and new onset of asthma. Likely causative agents include biological and chemical factors linked to aerosolized fungal spores and cellular fragments, their metabolites, and mycotoxins. No standard yet exists for acceptable concentrations of molds, nor is there sufficient evidence linking health symptoms to specific fungal aeroallergens and/or their removal. This study involves collecting bio-

logical samples, health and exposure data, and environmental sampling in subject residences at multiple time points. Given that up to 80% of those affected by Hurricane Harvey did not have flood insurance, many thousands of Texans were forced into temporary housing, prior to remediation and reoccupation of their homes. The majority of study procedures were previously reviewed and approved under the UTMB Rapid Acquisition of Pre- and Post-Incident Disaster Data Protocol.

S15-04 Addressing Public Health Hazards Arising from Recent Threats and Disasters in California

Lauren Zeise. *Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Berkeley, CA, The United States of America.*

Emergency responders and communities turn to state and local public health officials for guidance in the face of large accidental releases and natural and man-made disasters. Yet the nature of the chemical exposures and their extent can be poorly or incompletely understood. Recent threats and disasters in California include large releases of natural gas October 2015 to February 2016 from failed well at an underground storage facility, situated adjacent to a community; domoic acid seafood contamination from an unprecedented large harmful algal bloom that resulted in fishery closures and sickening and death of sea mammals; oil spills; devastating wildfires; refinery fires and other upsets. The discussion will cover challenges of providing time critical health advice during ongoing events as the nature and extent of exposures are being evaluated, and health data to evaluate exposure impacts are not fully developed. Also discussed will be research needs and challenges to support answering questions within affected communities in the aftermath, such as whether the exposures experienced could lead to serious, latent health effects or whether health effects currently experienced are attributable to the event.

SYM16: Out of the Box: Contribution of the Immune System to Organ Toxicity

S16-01 Early-Life Exposure to Immunotoxic Compounds and Later-in-Life Diseases

Rodney Reynolds Dietert. *Cornell University, Ithaca, NY, The United States of America.*

The developing immune system is subject to a series of critical maturational processes that occur not only in primary lymphoid organs but also in secondary sites such as mucosal tissues. Chemical disruption of these events, whether direct or indirect via microbiota-immune interactions, can block subsequent immune homeostasis, necessary repertoire distinctions, and immune regulation. This presentation will describe key drug and chemical mediators of developmental immunotoxicity (DIT) that predispose for later life noncommunicable diseases and conditions (NCDs) (i.e., allergic, autoimmune, metabolic and inflammatory diseases). NCDs are the leading cause of death globally. While most develop later in life, biomarkers of these diseases often occur prior to adulthood. A description will be provided for the range of early-life immunotoxic alterations observed for model compounds (e.g., heavy metals, plasticizers, PAHs, industrial and agricultural chemicals). Additionally, a systems biology perspective will be used to categorize and rank immune-development-chemical interactions that appear to contribute to the most prevalent later life human diseases.

S16-02 Role of the Immune System in Drug-Induced Liver Injury

Tsuyoshi Yokoi. *Nagoya University Graduate School of Medicine, Nagoya, Japan.*

Unpredictable drug-induced liver injury (DILI) is currently a major reason why: 1) new drug candidates are discontinued or 2) existing drugs are withdrawn from the market. However, it has been difficult to predict the risk of human DILI based on existing preclinical safety evaluations conducted in animals. Recent evidence suggests that the immune system and, more specifically, imbalances among differentiated T cells, is implicated in DILI and its resulting hepatic pathology. For example, using an established mouse model of DILI, we reported on the relationship between exposure to halothane, phenytoin, dicloxacillin, diclofenac, carbamazepine, flutamide, and methimazole, changes in T helper (Th) cell differentiation, and the production of inflammation-related factors. Of

note is the role of microRNAs (miRNAs) in this relationship. This presentation will focus on role of miRNAs as regulators of toxicological signal cascades and the possible use of miRNAs as predictive biomarkers of DILI.

S16-03 Role of Inflammation in Chemical-Induced Kidney Damage

David J. Nikolic Paterson. Monash University, Clayton, Victoria, Australia.

Inflammation plays a central role in many forms of chemical-induced acute and chronic kidney damage. The kidney is highly susceptible to chemical-induced inflammation with podocytes and tubular epithelial cells the primary targets. Chemical agents such as chemotherapeutics (e.g. cisplatin), plant toxins (aristolochic acid), antibiotics (gentamycin) and analgesics (NSAID), as well as nanoparticles, can induce tubular damage leading to kidney inflammation. Functional studies in animal models have established key roles for specific leukocyte populations in chemical-induced kidney injury, such as for macrophages in aristolochic acid nephropathy and for neutrophils in cisplatin-induced acute tubular necrosis. Strategies to prevent chemical-induced acute kidney injury, such as blocking JNK signalling or cyclophilin-D mediated cell death, have been effective in animal models, but such studies have not yet been translated to the clinical situation in which treatment can only be initiated once chemical-induced kidney injury is already evident. This remains a major medical challenge in which early diagnosis of acute kidney injury is of key importance. Chemical-induced kidney injury remains a major clinical challenge although strategies to target the immune response have potential if they can be implemented in a timely fashion.

S16-04 Particles, Fibres, and Lung Toxicity: Effects beyond the Lung

Flemming R. Cassee. Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands.

Lung has been the predominantly investigated target organ and lung inflammation is the most reported outcome following exposure to particles and fibres, both at the workplace as well as due to air pollution. While lung inflammation itself may not be considered as immunotoxicity, prolonged stimulation of the various components of the inflammatory system including opsonization and complement activation may incite pathological conditions such as asthma. Thus, solid substances that are immune system stimulants should be investigated carefully for their potential to induce immunotoxicity. Several studies have shown that nano-sized particles as well as fibres can translocate from lungs to other immune responsive organs such as spleen, liver and lymph nodes, depending on the nanomaterial properties. Thus, in a scenario where lung exposure to these substances results in lung inflammation as well translocate to immune responsive organs, those particles and fibres should be prioritized for a comprehensive investigation by other immunotoxicity specific assays.

SYM17: Applying the Key Characteristics Paradigm in Hazard Identification and Risk Assessment

S17-01 The Key Characteristics of Carcinogens

Martyn T. Smith. University of California at Berkeley, Berkeley, CA, The United States of America.

An international collaboration between scientists from IARC, US EPA, the NTP and academia has developed a set of 10 key characteristics of human carcinogens that was published recently in Environmental Health Perspectives (Vol.124; 713-721, 2016). These 10 key characteristics can be used to organize mechanistic data during the evaluation of the carcinogenic hazard of a chemical. They are currently being used by IARC in their evaluations and other authoritative bodies use similar search tools and criteria. It has been proposed that scientists should develop key characteristics for other endpoints such as endocrine disruption and reproductive toxicity as suggested in the recent National Academies report on "Using 21st Century Science to Improve Risk-Related Evaluations." However, what is really lacking is a set of standardized tests and biomarkers that evaluate each of the key characteristics of carcinogens and other toxicants. The current Tox21 and Toxcast repertoire are lacking in this regard as a most clinical biomarkers. Approaches to developing such a set of tests and biomarkers through a consortium will be described along with

a discussion of the use of the key characteristics approach in hazard identification and risk assessment instead of, or as well as, the current MOA/AOP approach.

S17-02 Applying the Key Characteristics of Carcinogens to Assess Human Cancer Risk in Drug Development

Mark Fielden. Amgen Inc, Comparative Biology and Safety Sciences, Thousand Oaks, CA, The United States of America.

Assessing cancer risk of novel therapeutic drugs largely relies on results from a standard genotoxicity testing battery and lifetime rodent bioassays. While much has been written on the shortcomings of this approach for predicting human risk, alternative methods have not significantly impacted the current paradigm and studies in animals continue to challenge our ability to translate findings to humans. The key characteristics of human carcinogens provides a blueprint to develop mechanism-based tests based on these characteristics in order to begin assessing new drugs for their ability to impact known cancer risk factors. While no single test or endpoint alone is anticipated to replace the rodent bioassay, an assessment of known risk factors in human-based models can collectively inform a weight of evidence based argument to inform human risk and reduce the challenges of translating effects across species. This talk will outline how current nonclinical evaluations can leverage the key characteristics to improve how we assess human cancer risk, and also outline gaps in our toolbox in order to inform new assay development. Finally, considerations for how these key characteristics can be evaluated directly in humans will also be discussed.

S17-03 Air Pollution Exhibits Multiple Key Characteristics of Carcinogens

Hideko Sone. Yokohama University of Pharmacy, National Institute for Environmental Studies, Yokohama, Japan.

Air pollution is a human carcinogen with widespread exposures throughout the world. Exposures continue to increase in East Asia. Recent investigations in experimental systems have explored the ability of air pollution to affect endpoints related to various key characteristics of carcinogens. Prolonged exposure of Wistar rats to roadside air contaminated mainly with automobile emissions in Kawasaki City, Japan increased DNA adduct levels in the lung, nasal mucosa, and livers. Further, diesel engine exhaust increased mutation frequency in the lung of Big Blue rat. On the other hand, *in vitro* studies showed that particulate matter ($\leq 2.5\mu\text{m}$) collected in an urban area in China (Shenyang) induces inflammation and oxidative stress in macrophages via distinct pathways. In all these studies indicate that air pollution may exhibit multiple key characteristics of carcinogens, and support biological monitoring using endpoints such as DNA-adduct formation to improve understanding of the effects of ambient air on human health.

S17-04 Application of the Key Characteristics in the IARC Monographs Programme

Kathryn Guyton. Monographs Programme, International Agency for Research on Cancer, World Health Organization, Lyon, France.

Monographs identify the causes of human cancer, focusing on agents with a suspicion of carcinogenicity, evidence of human exposure, and relevance in low- and middle- income countries. Recently, IARC has applied a systematic search, screening and evaluation procedure for mechanistic data based on the key characteristics of carcinogens. This objective approach identified a broad literature encompassing multiple key characteristics for most Group 1 or 2A carcinogens evaluated since 2015. Five carcinogens are genotoxic and induce oxidative stress, including pentachlorophenol. Four others, including welding fumes, are immunosuppressive. For only two agents, tetrabromobisphenol A and tetrachloroazobenzene, did the mechanistic data result in an upgrade the overall evaluation to Group 2A. Both carcinogens modulate receptor-mediated effects in combination with other key characteristics. Fewer studies were identified for Group 2B or 3 agents, with the vast majority (17/18) showing only one or no key characteristics. Further development and mapping of toxicological and biomarker endpoints, assays and pathways relevant to the key characteristics can advance systematic search and evaluation of mechanistic

data in carcinogen hazard identification. These developments lay groundwork for future evaluations where such data may fill important gaps in evidence of carcinogenicity.

SYM18: Biomarker Qualification: Accelerating Drug Development by Enabling the Use of Novel Diagnostic and Safety Biomarkers

S 18-01 How Much Evidence Is Enough to Accept a Biomarker for Regulatory Decision-Making? Development of Evidentiary Considerations

John-Michael Sauer. *Critical Path Institute, Tucson, AZ, The United States of America.*

Biomarkers can facilitate all aspects of the drug development process. However, biomarker qualification—the use of a biomarker that is accepted by the US Food and Drug Administration—needs a clear, predictable process. We describe a multi-stakeholder effort including government, industry, and academia that proposes a framework for defining the amount of evidence needed for biomarker qualification. This framework is intended for broad application across multiple biomarker categories and uses. Although the biomarker qualification process is similar across regulatory agencies (US FDA, EMA and PMDA), there are regional difference that must be addressed. The goal of this presentation is to discuss similarities and differences in the biomarker qualification across regions by using specific examples from Critical Path Institute's Predictive Testing Safety Consortium.

S 18-02 GLDH as an Example of Regulatory Biomarker Qualification: A Liver-Specific Biomarker of Liver Injury in Drug Development and Medical Care

Jiri Aubrecht. *Takeda, Cambridge, MA, The United States of America.*

Availability of sensitive and specific biomarkers for monitoring hepatic health is critical in drug development and diagnosis of disease. Glutamate dehydrogenase (GLDH) is a promising new biomarker of liver injury that closely reflects hepatocellular necrosis and, unlike the gold standard marker alanine transaminase (ALT), is not elevated because of muscle disease and injury. To qualify GLDH as biomarker of liver injury, we evaluated GLDH in a large cohorts of healthy subjects and subjects with a broad range of acute and chronic liver impairments including subjects with muscle diseases. Our data indicate that GLDH is a sensitive and liver specific biomarker of liver injury applicable in drug development and also in medical practice for diagnosis of liver injury in subjects with concurrent muscle diseases.

S 18-03 Case Studies on Integration of Toxicogenomic Biomarkers in Genetic Toxicology Assessment

Carole Yauk. *Health Canada, Environmental Health Centre, Ottawa, ON, Canada.*

Toxicogenomic is proposed to be a useful tool in human health risk assessment. However, a systematic comparison of traditional risk assessment approaches with those applying toxicogenomic is important for achieving a wide acceptance of toxicogenomic methods. The presentation will introduce several case studies describing application of genomic biomarker TGx-DDI in genetic toxicity assessment of environmental chemicals, included application of high-throughput integrated testing approaches. Overall, our studies support the use of toxicogenomic biomarkers as a relatively fast and cost-effective tool for hazard identification, preliminary evaluation of potential carcinogens and carcinogenic potency, in addition to identifying current limitation and future directions.

S 18-04 Serum MicroRNA Signatures as "Liquid Biopsies" for Interrogating Hepatotoxic Mechanisms and Liver Pathogenesis in Human

Julian Krauskopf. *University of Maastricht, Maastricht, The Netherlands.*

MicroRNAs (miRNAs) released into the peripheral circulation upon cellular injury have shown a promise as a new class of tissue-specific biomarkers. Using next generation sequencing, we examined profiles of serum circulating miRNAs in subjects with accidental acetaminophen overdose, hepatitis B infection, liver cirrhosis and type 2 diabetes as well as gender- and age-matched healthy subjects with no evidence of liver disease. We are first to show that different types of hepatic liver impairments feature distinct signatures of circulating miRNAs and that this approach might be useful as minimally invasive diagnostic "liquid biopsies" enabling the interrogation of underlying molecular mechanisms of injury in distant tissues. Furthermore, the bioinformatics analysis of miRNA signatures revealed relevant molecular pathways associated with the mechanisms of toxicity and or pathogenesis of disease. Our study demonstrates for the first time that signatures of circulating miRNAs show specificity for liver injury phenotypes and, once validated, might become useful for diagnosis of organ pathologies as "liquid biopsies" for studying effects of drug candidates and environmental chemicals.

SYM19: Leveraging Zebrafish to Support Global Toxicology Challenges

S 19-01 Transcriptomics Coupled with High-Throughput Phenotypic Screening to Accelerate Discovery of Toxicity Pathways

Robert Tanguay. *Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR, The United States of America.*

Multi-dimensional zebrafish assays are useful to discover chemical bioactivity. From screening thousands of chemicals in the developmental zebrafish, it is evident that chemicals with different, but specific modes of action produce a narrow suite of common endpoints. This is advantageous for rapid sorting of chemicals with and without biological activity, but mapping chemical structures onto specific biological responses is challenging. To resolve this challenge, we implemented non-targeted whole animal transcriptomics to identify expression patterns that precede the development of early life-stage phenotypes. We hypothesized that transcripts and networks will provide a better diagnostic signature for chemical activity. As proof of concept, this presentation describes the use of a morphological and behavioral platform to compare the bioactivity of a diverse collection of 61 flame retardant chemicals (FRCs) in the developing zebrafish. FRC exposures produced distinct patterns of biological activity. We also collected larval transcriptomic responses from a representative set of FRCs and determined the consequences of developmental exposures on adult behaviors (social, fear, anxiety, and learning). We provide further evidence that zebrafish neurodevelopment is highly sensitive to many current FRCs and can be used to discover adverse outcome pathways for understanding potential human health vulnerabilities.

S 19-02 Methylmercury-Induced Epigenetic Transgenerational Inheritance of Abnormal Neurobehavior Is Correlated with Sperm Epimutations in Zebrafish

Michael Carvan. *School of Freshwater Sciences, University of Wisconsin-Milwaukee, The United States of America.*

The tools that originally made zebrafish a powerful model were developed to investigate cancer genetics—developmental biologists took advantage of these tools and the NIH continues to support their development. Toxicologists have been redirecting these tools toward understanding interactions between environmental chemicals, molecular pathways, and endpoints relevant to disease and dysfunction. My laboratory has focused on the effects of developmental exposures—most recently with methylmercury (MeHg)—on neurobiology and behavior in exposed eleutheroembryos, juveniles, and adults, with the potential for transgenerational effects. We have shown that developmental MeHg at low levels leads to dysfunction in all life stages, many of which can be linked to biochemical irregularities or structural abnormalities in specific neuronal subtypes. RNAseq in single cell-type isolates allows us to explore molecular pathways at the cellular level and the consequences for their dysregulation. Concurrent epigenome analysis allows for exploring mechanisms for

developmental origin of adult disease and dysfunction. This talk will discuss research findings by our laboratory and others using zebrafish to investigate the direct effects of developmental toxicants, and effects in adults and subsequent generations. More than two decades of research has revealed the benefits and weaknesses of the zebrafish as a tool for mechanistic toxicology.

S19-03 Lessons Learned on the Toxicokinetics upon Waterborne Chemical Exposure in Zebrafish Larvae

Krishna Tulasi Kirla. *Department of Forensic Pharmacology and Toxicology, Eawag, Switzerland.*

The applicability of zebrafish embryos and larvae in rapidly assessing the impact of chemicals has been expanding in toxicology and biomedical research. Yet, details of the toxicokinetic processes occurring upon waterborne exposure, although crucial to understanding the organism- and tissue-specific exposure and thus the resulting chemical effects, are thus far little explored. To better understand the contribution of toxicokinetics in the observed actions of chemicals and to evaluate the concordance of responses in zebrafish larvae and mammals, we characterized the uptake, distribution, biotransformation and elimination of different classes of psychoactive chemicals using mass spectrometry-based quantification and imaging together with toxicokinetic modelling alongside toxicity and behavioral analyses. For all chemicals thus far studied, rapid uptake, and partly very high tissue concentrations were observed. For several chemicals, we found evidence for active uptake mechanisms. Further, basic chemicals strongly accumulated in the eye, likely due to binding to melanin. The rate of chemical elimination depended on the affinity to melanin as well. Biotransformation patterns for the analyzed chemicals were largely similar to mammals. However, the overall capacity was comparatively low. Using different examples from our research, we highlight the importance of considering toxicokinetic processes when using zebrafish larvae for assessing effects of chemicals.

S19-04 The Fish Embryo Model: Update and Challenges for Its Potential Regulatory Applications

Marc Léonard. *L'OREAL Research and Innovation, Environmental Research Department, Aulnay sous Bois, France.*

There is a worldwide trend to promote the use of alternatives to animal testing for the regulatory human and environmental safety assessment of chemicals. Fish embryos (mostly zebrafish and medaka) are ethically acceptable alternative models in compliance with international animal welfare regulations. The (zebra)fish embryo test [(Z)FET], has been adopted as an international guideline (OECD TG n°236). Its regulatory acceptance has been matter of debate, but used in a weight of evidence approach it allows to predict most chemicals that present a significant acute toxicity in fish. Exploring developmental toxicity with the fish embryo model covers both domains of environmental fish chronic toxicity assessment and human teratogenicity screening of chemicals. In complement to lethality and malformations, additional endpoints to the FET focusing on endocrine disruption and behavior analysis are likely to improve its predictive capacity to identify fish toxicants and human teratogenic agents as well. Here we report the progress that have been made at enlarging the scope of applications of the fish embryo model, with their potential regulatory recognitions: from the acute and chronic fish toxicity evaluation of chemicals, to their impact assessment on the sexual steroids and thyroid axis and their potential adverse consequences on (neuro)development.

SYM20: Recent Trends in Research on Arsenic Toxicity

S20-01 Arsenic in Oxidative Stress versus Proteotoxic Stress

Donna Zhang. *University of Arizona, Tucson, AZ, The United States of America.*

Prolonged exposure to arsenic has been shown to increase the risk of developing a number of diseases, including cancer and type II diabetes. Arsenic is present throughout the environment in its inorganic forms, and the level of exposure varies greatly by geographical location. One of the most important steps in developing treatment options for arsenic-linked pathologies is to understand the cellular pathways affected by environmentally relevant concentrations of arsenic. In this talk, I will present our most recently work demonstrating that envi-

ronmentally relevant arsenite exposure does not exhibit an increase in oxidative stress, but inhibits the autophagy pathway, and results in prolonged activation of the NRF2 stress response. Specifically, arsenic disrupts formation of the STX17-SNAP29-VAMP8 SNARE complex, where SNAP29 mediates vesicle fusion through bridging STX17-containing autophagosomes to VAMP8-bearing Lysosomes. Our study challenges the existing paradigm that oxidative stress is the main underlying cause of pathologies associated with environmental arsenic exposure.

S20-02 Paradoxical Roles of CNC-bZIP Proteins NRF2 and NRF1 in Arsenic-Induced Dysfunction in Pancreatic β -cells and Adipocytes

Jingbo Pi. *China Medical University, Shenyang, Liaoning, China.*

Chronic exposure to inorganic arsenic (iAs), a potent oxidative stressor, is associated with the pathogenesis of type 2 diabetes (T2D). Nuclear factor E2-related factor 2 (NRF2) and NRF1 are CNC-bZIP transcription factors that are master regulators in the cellular adaptive response to various stress insults, in particular oxidative stress. We propose that NRF2/1 plays paradoxical roles in β -cell function and insulin signaling transduction: (1) It protects the cells from oxidative damage and cell death, thus minimizing oxidative damage-related impairment in insulin secretion and action; (2) Since ROS signaling triggered by glucose and insulin could be an important component involved in insulin secretion and action, the induction of endogenous antioxidants in the presence of iAs may blunt the signals, resulting in reduced insulin secretion and insulin resistance. Our studies highlight the importance of NRF2/1 in β -cells and adipocytes and provide insight into the effects of iAs exposure on insulin secretion and action and thus the mechanism of iAs-induced T2D.

S20-03 Activation of Nrf2 through Covalent Modification of Keap1 by 2-alkenal Group of Aliphatic Electrophiles in *Coriandrum sativum* L. Diminishes Arsenic-Mediated Cytotoxicity

Yoshito Kumagai. *University of Tsukuba, Tsukuba, Ibaraki, Japan.*

Phytochemicals able to activate the transcription factor NF-E2-related factor 2 (Nrf2) were isolated from an extract of *Coriandrum sativum* L. (*C. sativum*) leaves by preparative octadecyl silica column chromatography. Ultra-performance liquid chromatography and liquid chromatography-tandem mass spectrometry analysis of the isolated components after derivatization with 2-diphenylacetyl-1,3-indandione-1-hydrazone and experiments with HepG2 cells revealed that (E)-2-alkenals with different carbon numbers play a role in Nrf2 activation in these cells. Such Nrf2 activation appears to be attributable to S-alkylation of Kelch-like ECH-associated protein 1 (Keap1), the negative regulator for Nrf2, as determined by a biotin-PEAC5-maleimide assay. Interestingly, (E)-2-butenal caused Keap1 modification and Nrf2 activation, whereas butanal did not. Furthermore, pre-treatment with (E)-2-alkenals repressed arsenic cytotoxicity through facilitation of detoxification and excretion of arsenic into extracellular space. These results suggest that (E)-2-alkenals with an α,β -unsaturated aldehyde moiety, which is a common substituent in phytochemicals isolated from *C. sativum* leaves, activate the Keap1/Nrf2 pathway associated with cellular protection against arsenic.

S20-04 Arsenic May Act as a Pro-metastatic Carcinogen through Promoting Tumor Cell-Platelet Aggregation

Jin-Ho Chung. *Seoul National University, Gwanak-gu, Seoul, The Republic of Korea.*

Arsenic-associated carcinogenesis and related mortality are a major public health concern worldwide; however, the underlying mechanism of action remains unclear. Here, we demonstrated that arsenic exposure promotes tumor metastasis by stimulating tumor cell-platelet aggregation (TCPA), which can ultimately increase cancer-related mortality. Arsenic potentiated TCPA prompted by diverse cancer cell lines, which was attributable to increased platelet responsiveness to TCPA with respect to thrombin generation and P-selectin, GPIIb/IIIa expression. Consistently, the co-existence of platelets and arsenic significantly enhanced tumor cell adhesion, extravasation and invasion *in vitro*, which was attenuated by platelet activation blockers. Importantly, the exposure to arsenic-contaminated drinking water in mice significantly increased the metastasis of intravenously injected melanoma cells into lung. Furthermore, the exposure to arsenic-contaminated drinking water significantly reduced the survival of melanoma cell-injected mice *in vivo*, indicating the prometastatic effects of arsenic exposure can indeed contribute to cancer-related mortality.

PL 0014 Developmental Exposure to Manganese Causes Cognitive Deficits in Rats: Protective Effect of Nutrient Metal Mixture Supplementation

Chand Basha Davuljigari, Srinivasulu Reddy, Rajarami Reddy, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Manganese (Mn) is an essential element but neurotoxic at higher exposures; however, the mechanisms underlying its action on cognitive functions are not elucidated clearly. Therefore, we investigated the deficits in cognitive functions as well as impairments in hippocampus cholinergic system following exposure to Mn. Further, we evaluated the protective effect of essential nutrient metals calcium (Ca), Zinc (Zn), and Iron (Fe) against Mn-induced neurotoxicity. Male rats were exposed to Mn (6mg/Kg body weight) through intraperitoneal injection for a period of two weeks from PND 15 to PND 28. Nutrient metal mixture (Ca, Zn, and Fe) as 0.02% by a single gavage together with Mn injection was separately given to rats from PND 15 to PND 28. Studies conducted on different age groups of rats (PND 29, PND 60, and 3 months) showed decreased levels in synaptosomal acetylcholine (ACh) and acetylcholinesterase activity (AChE) in the hippocampus region following Mn exposure. Histopathological studies of the hippocampus showed degeneration in the neurons with atrophy in subiculum, and nuclear pyknosis was detected in neurons of the fascia dentate following Mn exposure. Exposure to Mn exhibited significant deficits in spatial reference memory acquisition, reversal, and working memory performance in the Morris water maze test in PND 29, PND 60, and 3 months age groups of rats. However, supplementation of nutrient metal mixture together with Mn exposure showed significant recovery in Mn-induced impairments in the cholinergic system as well as spatial learning and memory behavior. In conclusion, data indicate that the developmental exposure to Mn.

PL 0029 Reproducibility of Computational Toxicology: The Prerequisite for Regulatory Applications

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Concern on reproducibility in scientific research is arising in recent years. In 2016, the journal *Nature* conducted a survey entitled "Is there a reproducibility crisis?" Of more than 1,500 scientists who responded to the survey, 90% agreed that there is a reproducibility crisis, slight or significant. Reproducibility issues impede the translation of scientific findings to regulatory applications. Computational toxicology attracts not only scientists in scientific research but also regulatory scientists to apply it in regulation due to its low cost and high speed compared with the traditional *in vivo* and *in vitro* methods. Many *in silico* models have been developed, and some have been considered to assist regulatory science. Reproducibility of *in silico* models in computational toxicology is the prerequisite for their applications in regulatory science. Therefore, to promote applications of computational toxicology in regulatory science, reproducibility of computational toxicology needs to be studied and good practices should be explored to improve reproducibility of computational toxicology. This presentation will look over the diverse sources that cause reproducibility issues in computational toxicology and at different steps of development of *in silico* models. It will also give some examples for the reproducibility issues in computational toxicology and discuss some efforts that have been taken to improve reproducibility. It will also explore some possible practices to improve reproducibility of computational toxicology for promoting regulatory applications of *in silico* models. *The views in the presentation are my personal opinions and do not represent US FDA position or policy.*

PL 0030 Fit-for-Purpose Use of High-Throughput Toxicokinetics for Predicting Potential Human Health Risk

Nisha Sipes, NIEHS, Research Triangle Park, NC, The United States of America.

With advancing technologies, we can amass large amounts of exposure and hazard screening data on traditionally data-limited chemicals of public health concern. Computational approaches are needed to translate these data into understandable and useful units for providing context toward risk assessments. Here, we provide an intuitive framework to estimate the plausibility of *in vivo* human occurrence from *in vitro* data. Specifically, we use a generalized, freely available high-throughput toxicokinetics (HTTK) model with parameters obtained from *in silico* and *in vitro* methods to estimate human daily dose exposures required to elicit internal plasma concentrations based on *in vitro* screening concentrations. To this end, we used the *in vitro* screening data from the Tox21 collaboration, a partnership among the National Institute of

Environmental Health Sciences, National Center for Advancing Translational Sciences, US Environmental Protection Agency, and US Food and Drug Administration, and data from the US EPA's separate ToxCast program, in total encompassing *in vitro* hazard screening data on >8,000 chemicals (e.g., pesticides, commercial and industrial use) in >800 HTS assays. Using HTTK, equivalent doses were estimated for all active chemical-assay pairs and compared with estimated nonpharmaceutical exposures for risk-based prioritization. For the active chemical-assay pairs, 22,375 were active at $\leq 10\mu\text{M}$. After taking kinetics into consideration, 23,319 were active at $\leq 10\text{mg/kg/day}$. The chemical-assay pair overlap between the two evaluations, however, was only 60%, indicating *in vitro* potency should not be the only consideration for prioritization. Additional comparison of converted *in vitro* activity doses to estimated exposure doses revealed 14 and 451 chemical-assay pairs with <1 and <100 activity-to-exposure ratios (AERs), respectively, with smaller numbers indicating an increased likelihood of *in vivo* interaction. HTTK model uncertainties exist but can be quantified and provide value when no other data exist. Overall, this approach provides a framework to rapidly and quantitatively relate *in vitro* bioactivity data to chemical exposures, with the novelty of taking into consideration *in vitro* response efficacies, concentration-response fit quality, and extends the approach for a first time to the entire Tox21 10k chemical dataset. As these data and models are publicly available; researchers and risk assessors can freely use these approaches for current risk evaluation.

PL 0031 In Vivo Toxicity Prediction Modeling with Tox21 Data to Aid Regulatory Sciences

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Target-specific, mechanism-oriented *in vitro* assays post a promising alternative to traditional animal toxicology studies. The Tox21 program, a large-scale *in vitro* chemical toxicity screening effort, has tested approximately 10,000 environmental chemicals and drugs in triplicates at 15 concentrations against a panel of nuclear receptor and stress response pathway assays, producing more than 85 million data points to date that form a rich set of compound *in vitro* activity profiles. Compound activity data were applied in combination with structural information to build predictive models for a number of *in vivo* toxicity endpoints including adverse drug effects (ADE) observed in humans. Models built with human *in vitro* assay data achieved performance close to models based on animal *in vivo* toxicity data in predicting human ADEs. Combining structure and activity data resulted in better models than using structure or activity data alone. Furthermore, a small set of additional targets, when added to the current suite of Tox21 assays, resulted in models that greatly outperformed those built with the existing animal toxicity data. These models can be applied to efficiently screen large sets of chemicals and prioritize those with toxic potential for further in-depth toxicity evaluation. Strategies will be discussed on using the models to identify predictive *in vitro* assays for regulatory purposes.

PL 0033 Using (Q)SAR Models to Inform Drug Safety Assessment

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The US FDA Center for Drug Evaluation and Research (CDER) maintains a robust regulatory research program supporting the development and application of (quantitative) structure-activity relationship ((Q)SAR) models for regulatory decision-making. (Q)SAR models can make predictions of toxicity based solely on a chemical structure and are used by CDER to provide predictions for chemical substances under review in the absence of adequate experimental data. For example, (Q)SAR models are routinely used to predict the mutagenicity of drug impurities as an alternative to traditional testing in accordance with the International Council on Harmonisation (ICH) M7(R1) Guideline. Additionally, (Q)SAR models and other chemical informatics techniques are used to provide supplemental toxicity predictions—such as for carcinogenicity or liver damage—during regulatory safety review when conventional testing data are limited or conflicting. More recently, molecular docking models that predict whether a synthetic street drug binds to opioid receptors have been developed to support the legal classification of newly identified substances. This presentation will provide an overview of (Q)SAR modeling and chemical database development efforts at CDER, with a focus on drug substance impurities and drugs of abuse as two priority areas of regulatory application.

PL 0047 A Case Study in Quantitative Generalized Read-Across (GenRA) Predictions Using Acute Oral Toxicity

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Computational approaches have recently gained a lot of traction in the field of read-across. Previously we developed our generalized read-across (GenRA) approach, which utilizes *in vitro* bioactivity data in conjunction with chemical descriptor information to derive local validity domains to facilitate read-across prediction. Predictions are made using the similarity weighted average of nearest neighbors. GenRA has been used to predict up to 574 different apical effects as a binary call from repeat-dose toxicity studies available in ToxRefDB. Here we evaluate the application of GenRA for quantitative predictions, specifically using a large dataset of rat oral acute LD50 toxicity data. These data were constructed by mining and merging a number of publicly available resources and comprise 21,210 unique LD50 values for 15,698 discrete chemicals. We used 9293 chemicals for which structures were available in the US EPA CompTox Chemicals Dashboard to make GenRA predictions of LD50 values. GenRA predictions used Morgan chemical fingerprints with a minimum Jaccard similarity threshold of 0.5 and a maximum of 10 nearest neighbors over the entire dataset. We evaluated confidence in GenRA predictions using four-fold cross-validation testing producing r^2 values 0.56 ± 0.3 . These findings show the utility of GenRA for making quantitative predictions of toxicity. *This abstract does not necessarily reflect EPA policy.*

PL 0051 The Emerging Dark Side of Nuclear Factor Erythroid 2-related Factor 2 in Chemical Carcinogenesis: Taking Arsenic as an Example

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Nuclear factor erythroid 2-related factor 2 (Nrf2) is a master transcription factor in antioxidant defense. As the research on Nrf2 goes deeper, Nrf2 is reported to play a dual function in cancer. In this study, we explore the role of Nrf2 in arsenic carcinogenesis. *In vitro* and *in vivo* models were applied. Human keratinocytes (HaCaT) were chronically exposed to an environment relevant dose of arsenite (0.1 μ M) for up to 30 weeks. Unexposed passage-matched cells were used as control. Lentivirus was used to construct cells with target genes knocked down. Expression of Nrf2 and its downstream genes were detected with real-time PCR and western blot. Reactive oxygen species were detected with DCFH-DA. Cell invasion and anchor-independent growth were used to assess malignant transformation. An early-life arsenic exposure mouse model was established in C57BL/6 mice. In some cases, Nrf2-knockout mice were used. Breeders were provided *ad libitum* with water containing none (control), 0.5 ppm, 5 ppm of arsenic (arsenite: arsenate = 1:1) from 3 weeks before mating until weaning of pups. Pups were injected *i.p.* with diethylnitrosamine (DEN, 25 mg/kg) or saline at 2 weeks of age. Tumors were assessed at 40 weeks of age. Hematoxylin-eosin staining was used to observe histocytic morphology. Real-time PCR and western blot were used to detect gene expression. *In vitro* study showed that after 30-week arsenic exposure, the invasiveness of HaCaT cells was 4 times that of control; arsenic-exposed cells formed a colony with a diameter larger than 0.1 mm, whereas no colonies larger than 0.1 mm in diameter were formed in control. Arsenic-transformed cells showed significantly elevated expression of Nrf2 downstream genes (AKR1C1 and NQO1) but decreased intracellular ROS levels. When Nrf2 or its positive regulator p62 was stably silenced, increased invasive and colony-forming capacity in chronic arsenic-exposed cell were attenuated. *In vivo* study found that early-life arsenic exposure led to increased expression of Nrf2 and its downstream genes in the liver of pups. DEN treatment gave rise to live adenomas at 40 weeks of age. Early-life arsenic exposure did not affect pup growth or cause obvious liver disease or liver adenomas, whereas aggravated live tumor multiplicity and tumor burden in mice with DEN treatment. In addition, Nrf2 deficiency protected mice from DEN-induced liver adenomas with or without early-life arsenic exposure. Both *in vitro* and *in vivo* evidence suggests that chronic arsenic exposure induces constitutive Nrf2 activation, which mediates reductive stress and contributes to arsenic carcinogenesis.

PL 0056 Determination of Epigenetic Modifications in Fetal Brain Exposed to Methylmercury during Development

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Methylmercury (MeHg) is a well-known developmental neurotoxicant. Recently, adverse effects of relatively low-level MeHg have become an important issue in the toxicological study of MeHg. Epidemiological studies have reported that low-level prenatal MeHg exposure may be associated with the poorer psychomotor development. Experimental studies have also shown that low-level MeHg exposure adversely affects developing cranial nervous system. However, the underlying toxicological mechanisms remain to be elucidated. Epigenetics is important to consider the molecular mechanisms of effects of MeHg exposure in embryonic period. This study focused on toxicological mechanisms of MeHg exposure on neuronal differentiation from the view of epigenetics, using fetus exposed to MeHg during development. Pregnant C57BL/6J mice were orally administered with a single dose of MeHg (3 mg/kg/day) from embryonic day (E) 12.5 to E14.5, which is the experimental condition to cause cognitive disorder in fetus later in adulthood from the previous studies. Fetal brains were sampled at E18.5 to perform examinations. Fetal body weight and fetal brain weight were not changed by fetal MeHg exposure. The length of neurite in the cerebral cortex was significantly reduced by fetal MeHg exposure. We determined the level of histone modifications and DNA methylation, which are typical epigenetic modifications. Acetylated histone H3 (AcH3) levels, related to transcriptional activation, were decreased by MeHg exposure in the fetal cerebral cortex. In addition, increase of DNA methylation was observed by fetal MeHg exposure in the fetal cerebral cortex. Next, the level of histone deacetylase (HDAC) was examined, because significant reduction of AcH3 was observed. As a result, the levels of HDAC3 and HDAC6 were significantly increased by fetal MeHg exposure. Therefore, it was thought that neurological effect such as decrease of neurite extension by fetal MeHg exposure was caused by decrease of AcH3 level via HDAC; however, we have to perform further study to investigate the causal relationship between neurological phenotype and epigenetic changes by fetal MeHg exposure.

PL 0057 A New Method to Evaluate Toxicological Data Reliability in Food Safety Risk Assessments

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Toxicological data used for risk assessments must undergo an evaluation of reliability. Nevertheless, a reliability evaluation scheme for such data has yet to be established in China. A method termed TRAM (Toxicological data Reliability Assessment Method) was developed to evaluate toxicological data reliability. A questionnaire survey was performed to screen out the most important criteria to reliability and was divided into three parts: laboratory animal data (LAD), *in vitro* data (IVD), and human data (HD). The criteria selected in the questionnaire survey and numerical score categorization finally reached a consensus after two conferences. TRAM comprises 22 criteria for LAD, 15 for IVD, and 12 for HD. Furthermore, a combination of a quick and detailed evaluation approaches for LAD and IVD was proposed to improve evaluation efficiency. Different weights (1–4) were assigned to each criteria, and 0–4 points were marked by whether each criterion was met, then a Quality score was calculated. TRAM uses three reliability categories: high (quality score: 80–100 or met a quick evaluation approach), moderate (60–80), and low (<60). This study provides a transparent, easy-to-use, and quantitative method for evaluating toxicological data reliability.

PL 0061 Do the REACH Derived No-Effect Levels Extend the Landscape of Occupational Exposure Guidance Values?

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Derived No-Effect Levels for workers (wDNELs), an element of the European chemical legislation REACH, have many aspects in common with the traditional occupational exposure limits (OELs). In a first attempt to examine under which circumstances wDNELs might be used as occupational exposure guidance values outside their intended application under REACH, we have investigated similarities and differences between wDNELs and OELs. We reviewed deriva-

tion methods and principles, as well as the coverage of substances. We further compared the numerical values of wDNELs against OELs from two sets of regulations (from the European Commission and Sweden) and three sets of recommendations (European SCOEL, German MAK, and American ACGIH-TLV). Finally, we looked closer at wDNELs where the SCOEL concluded that data were insufficient to derive an OEL. There are several major differences between wDNELs and OELs, including regulatory background, intended use, actors involved, substance selection criteria, transparency and procedure of derivation, and operationalization in terms of risk management measures. As of the third registration deadline (mid-2018), the number of substances with wDNELs is manyfold that of the lists of OELs examined herein, individually (7x to 38x) and combined (5x). Meanwhile, many airborne pollutants relevant for workers' health are so far not covered by the REACH procedures; for instance, one third of entries in the Swedish list of OELs lack a wDNEL. We note that the wDNEL and OEL for the same substance may vary considerably, sometimes by one or several orders of magnitude. Additionally, although 67%–88% (depending on OEL list) of wDNELs for substances also having an OEL were equal or lower to the corresponding OELs, we identified some examples of excessively high wDNELs. We also note that for these excessively high wDNELs, as well as for the investigated wDNELs for substances with insufficient data, route-to-route extrapolation is a common practice that may be grossly misleading when the effect of concern is local, such as sensitization. In conclusion, as a readily available source of exposure guidance values with extensive substance coverage, wDNELs extend the landscape beyond the OELs. That said, important limitations are that a considerable number of air pollutants relevant from a workers' health perspective are not covered by REACH, and concerns for inconsistencies in the derivation of wDNELs and in their level of protection.

PL 0062 How to Choose the Right Cancer Cell Line for Your Research: A Transcriptomes-Guided Approach

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Cancer cell lines are widely used model systems in a variety of important researches, from carcinogenesis elucidation to drug evaluation, due to obvious advantage on availability and expense. However, increasing evidences have indicated phenotypic responses vary between tissue samples and cancer cell lines, which are related to intrinsic heterogeneity, e.g., mutations, copy number variations, gene expressions, of tumors. Therefore, two linked questions rise concerning if a cancer can be studied by one cancer cell line and how to find this proper one. The accumulating data on transcriptional profiles of tumor samples are now available for systemic analysis, as well as for cancer cell lines through several well-known projects. Here a comprehensive comparison of tumors samples with cancer cell lines on genomic characteristics is carried out. First, high concordance on genomic signatures of cancer cell lines was observed among the NCI60 Project, the Cancer Cell Line Encyclopedia, and the COSMIC Cell Lines Project. We observed a few cancer cell lines transcriptionally correlated with the corresponding tissue samples across 22 types; however, the fact does hold for a large part of them. Thus, a computational method is proposed to prioritize cell lines that resemble the corresponding cancer type based on gene expression pattern. Validation experiments on predicted top cell line with the closest resemblance to a certain tumor group were conducted using US FDA-approved drugs or phase III or IV drugs on breast, prostate, and thyroid carcinoma. Additionally, a web tool is under development to allow the on-the-fly evaluation, which offers good insight for drug screening or mechanism research. Our study bridges the gap between tumors and cell lines and presents a helpful guide of selecting the most suitable cell lines model for cancer studies.

PL 0080 Lung Function and Immune Effects in Resident Lung Macrophages following Prenatal Exposure to Sodium Arsenite

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Exposure to inorganic arsenic during pregnancy is known to increase severity of infectious disease in early life; however, the mechanism driving this susceptibility is unknown. Arsenic levels in drinking water around the world exceed the WHO guideline of 10 µg/L, leading to exposure in more than 140 million people. It is well established that prenatal arsenic exposure has cell-specific effects on components of the immune system, resulting in reduced immune efficacy,

particularly in the lungs. However, specific effects of prenatal arsenic exposure on resident (interstitial) macrophages in the lungs are not well understood. To study these cells and their influence on the overall immune response, we utilize C57Bl/6 mice exposed from gestation day 9 to birth to either 0 or 1000 µg/L sodium (meta) arsenite in drinking water. Male and female offspring were assessed for airway hyperresponsiveness (AHR) to methacholine challenge. AHR was altered more by prenatal arsenic exposure in females ($p = 0.0987$ at 10 mg/mL and $p = 0.0241$ at 30 mg/mL methacholine) than in males ($p = 0.0508$ at 10 mg/mL and $p = 0.0512$ at 30 mg/mL methacholine). Our data confirm findings from other groups indicating that sex and arsenic influence lung response. We also isolated interstitial macrophages (CD11b+) from whole lungs of the offspring. Cells were plated and stimulated with LPS and IFN γ , and cell supernatant was collected at 24 hours and analyzed for cytokines. In males and females G-CSF, IL-6, IL-13, LIX, MIP-1a and 1b, MIP-2, and TNF were reduced in cells from animals with prenatal arsenic exposure. In males only, IP-10 was decreased and IFN γ increased. Contrarily, IFN γ decreased in females. We are further investigating the effect of prenatal arsenic on interstitial macrophage function through analysis of gene expression, flow cytometric assessment of activation markers, and antigen presentation capacity assessment. In addition, we are investigating how arsenic-induced alterations in interstitial macrophages influence the immune response to infectious disease challenge with *Mycobacterium tuberculosis*. Results from the current research could reveal novel mechanisms of detrimental immune outcomes following prenatal arsenic exposure. Translation of these findings to the clinical setting could lead to effective intervention techniques to reduce the long-term impact of prenatal immunotoxicant exposure. Supported by NIEHS R00ES024808 (FS) and T32ES07141 (KR, EI, SA).

PL 0091 K-variant BCHE and Pesticide Exposure: Interactive Genome and Parkinson's Disease

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Pesticide exposure is associated with increased risk of Parkinson's disease (PD). We investigated in Egypt whether common variants in genes involved in pesticide detoxification or transport might modify the risk of PD evoked by pesticide exposure. We recruited 416 PD patients and 445 controls. Information on environmental factors was collected by questionnaire-based structured interviews. Candidate single-nucleotide polymorphisms (SNPs) in 15 pesticide-related genes were genotyped. We analyzed the influence of environmental factors and SNPs as well as the interaction of pesticide exposure and SNPs on the risk of PD. The risk of PD was reduced by coffee consumption [OR = 0.63, 95% CI: 0.43–0.90, $P = 0.013$] and increased by pesticide exposure [OR = 7.09, 95% CI: 1.12–44.01, $P = 0.036$]. The SNP rs1126680 in the butyrylcholinesterase gene BCHE reduced the risk of PD irrespective of pesticide exposure [OR = 0.38, 95% CI: 0.20–0.70, $P = 0.002$]. The SNP rs1803274, defining K-variant BCHE, interacted significantly with pesticide exposure ($P = 0.007$) and increased the risk of PD only in pesticide-exposed individuals [OR = 2.49, 95% CI: 1.50–4.19, $P = 0.0005$]. The K-variant BCHE reduces serum activity of butyrylcholinesterase, a known bioscavenger for pesticides. Individuals with K-variant BCHE appear to have an increased risk for PD when exposed to pesticides.

PL 0121 Heavy Metal Contamination of Drinking Water Sources and Hepato-renal Toxicities among Residents of a Crude Oil-Contaminated Area in Bayelsa State, Nigeria

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Crude oil contamination of surface and borehole water sources is associated with deleterious health effects, including cancer. Sixty water samples (20 surface water samples from 4 oil wells and 20 borehole water samples) from a crude oil-contaminated area and 20 water samples (10 surface and 10 borehole water samples) from non-crude-oil-contaminated area were collected for estimation of heavy metal content. Two hundred and forty adults comprising 120 residents of crude oil-contaminated area and 120 from non-crude-oil-contaminated area were recruited for estimation of liver and renal functions. Heavy metal (lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As)) content of the water samples, liver functions (aspartate and alanine aminotransferases (AST and ALT), alkaline phosphatase (ALP), gammaglutaryl transferase (GGT), total protein (TP), albumin (Alb), and total and conjugated bilirubin (TB and CB)), and

renal functions (urea, creatinine, sodium, chloride, potassium, bicarbonate, and kidney injury molecule-1 (KIM-1)) were determined using atomic absorption spectrometry, enzyme kinetics, colorimetry, and enzyme linked immunosorbent assay, respectively. Heavy metal content of all water samples studied was above the WHO limit. Heavy metal content of water samples from crude oil-contaminated area was significantly higher than those from non-crude-oil-contaminated area ($p < 0.05$). Heavy metal contents of surface water samples from crude oil-contaminated and non-crude-oil-contaminated areas were significantly higher than borehole water samples from both areas ($p < 0.05$). Higher levels of ALT, AST, ALP, GGT, TB, CB, urea, sodium, chloride, bicarbonate, and KIM-1 and lower albumin and TP were observed in residents from crude oil-contaminated area compared with residents from non-crude-oil-contaminated area ($p < 0.05$). Crude oil contamination of surface and borehole water appears to be associated with increase in their heavy metal content, while consumption of crude oil-contaminated water may be associated with perturbations in liver and renal functions with potential for development of hepatotoxic and nephrotoxic diseases in the absence of any preventive and remedial intervention.

PL 0150 High-Throughput Transcriptomics Using Hepatocyte Spheroids to Identify Chemical-Induced Biological-Response Pathways and Associated Toxicity

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Current high-throughput *in vitro* assays employ two-dimensional (2D) tissue culture models that poorly translate to xenobiotic exposure effects on humans. This is principally due to their poor differentiation status resulting in aberrant xenobiotic metabolism and cellular response. To overcome these challenges, we are developing new approaches to investigate chemical exposure effects on human health. Initial efforts have focused on developing organotypic three-dimensional (3D) *in vitro* liver screening models and high-throughput transcriptomics (HTT) assays with S1500+ gene set (a panel of ~3000 transcripts). Here, we describe development of metabolically competent 3D HepaRG spheroid model for high-throughput transcriptomic studies. When cultured as spheroids in a 384-well plate (2000 cells per well), HepaRG spheroids show several hallmarks of polarized hepatocytes with distinct apico-basal domains. Assessment of xenobiotic metabolism competence with clinical substrates of CYP1A2, CYP2B6, and CYP3A4 produced robust levels of liver enzymes that are within the ranges observed in primary human hepatocyte (PHH) suspensions (e.g., CYP3A4 69.4 ± 21.1 pmol/min-million cells) and 2- to 20-fold higher than median activities observed with 2D cultures of PHHs. HTT (Tempo-Seq) analysis of 3D HepaRG spheroids was performed using cell lysate samples. Our results demonstrate the effectiveness of HTT approach to generate high-quality transcriptomic data with sufficient throughput for concentration-response modeling to enable identification and resolution of "pharmacological," "off-target," and cellular stress/death pathways. Incorporation of these physiologically relevant *in vitro* models and data-rich approaches are promising new tools in predicting chemical-biological interactions and human liver toxicity potential.

PL 0183 Exposure to Low Doses of 3-Methylcholanthrene Impacts on the Oocyte Integrity without Causing Systemic Cytotoxicity

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Previously, we showed that daily exposure to 3-methylcholanthrene (3MC), a polyaromatic hydrocarbon, at 0.1–1.0 mg/kg alters the ovarian function by affecting follicle growth and ovulation in parallel with DNA damage in bone marrow cells and oocytes. Thus, the aim of this work was to study the effects of nondaily exposure to 3MC (lower doses than in our previous work) at gonadal and systemic level and the ability of α -naphthoflavone (α NF) to prevent this action. To this end, immature rats were exposed to a combination of 3MC 0.1 mg/kg and α NF 80 mg/kg three times a week for three weeks. Follicular growth was examined by histology, oocyte integrity by morphological parameters, oocyte viability by fluorescent dyes, meiotic spindle by immunostaining, and apoptosis by the TUNEL assay. Compared with control animals (C), 3MC exposure (i) decreased the percentage of immature and antral follicles (15%–25%, $P < 0.05$), the ovulation rate, expressed as the number of oocytes within oviducts (C 13.7 ± 0.3 ; 3MC 11.1 ± 0.4 , $P < 0.01$), and the percentage of health oocytes (C 86 ± 2 ; 3MC 76 ± 1 ; $P < 0.01$); (ii) increased the oocyte area, expressed as

μm^2 (C 12.1 ± 0.2 ; 3MC 12.7 ± 0.1), the perimeter expressed as μm (C 79 ± 1 ; 3MC 85 ± 2 ; $P < 0.01$), and the perivitelline space expressed as μm (C 1.57 ± 0.08 ; 3MC 1.94 ± 0.03 ; $P < 0.01$); (iii) decreased the thickness of the zona pellucida expressed as μm (C 1.7 ± 0.1 ; 3MC 1.5 ± 0.1 ; $P < 0.01$), the percentage of oocytes with normal meiotic spindle (C 97 ± 1 ; 3MC 91 ± 1 ; $P < 0.01$); and (iv) increased the number of apoptotic follicles (C 3.6 ± 0.2 ; 3MC 4.8 ± 0.3 ; $P < 0.05$). All these changes were prevented by the co-treatment with α NF. In addition, this 3MC exposure caused DNA damage in oocytes but not in peripheral blood or bone marrow cells. In conclusion, exposure to low doses of 3MC was able to destroy the follicular pool, alter the oocyte integrity, and induce apoptosis, while α NF prevented all 3MC-induced toxic effects. It is worthy to note that 3MC, at low doses, may be a potent ovotoxicant without causing systemic toxicity.

PL 0190 Neurological Risks of Inhaled Incidental Particulates and Engineered Nanomaterials

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Environmental and occupational exposures to incidental- or engineered-particulate aerosols can result in their deposition in the nose and/or lung. Direct translocation to the brain via the olfactory sensory neurons or indirectly through systemic circulation is plausible. During welding, a complex aerosol of fine- and ultrafine-metal particulates is generated that is rich in iron (Fe) and manganese (Mn). The presence of Mn is cause for concern, as it causes a neurological dysfunction akin to Parkinson's disease (PD). Likewise, an apprehension that exposure to engineered nanomaterials (ENM) can cause adverse health effects also exists. The unique physicochemical properties of ENM influences their ability to be aerosolized and thus can be inhaled. Investigation of the neurotoxic potential of such particulates is therefore critical for hazard identification and risk characterization. Using experimental models, evidence is provided to demonstrate neurotoxic outcomes associated with incidental (welding fumes) and engineered (multi-walled carbon nanotubes, MWCNT) particulates. In rats exposed to welding particulates generated by gas-metal arc-stainless steel welding (GMA-SS; 40 mg/m³ $\times$ 10 d), increased Mn, but not Fe, was seen in the olfactory bulb (OB), as well as in the dopaminergic brain areas, striatum (STR) and midbrain (MB). An inflammatory response followed (increased *Ccl2*, *Tnfa*, *Nos2* mRNAs; 1.5–3.9 fold; $P < 0.05$) as a consequence of microglial activation in response to neuronal perturbation. Astrogliosis (increased GFAP protein; 59%; $P < 0.05$) and reduced expression of specific PD-related proteins (TH, PARK5, PARK7; 18%–47% decrease; $P < 0.05$) was seen in the STR and MB. The early loss of PARK proteins and ensuing disruption of the proteasome pathway may underlie the dopaminergic pathology of Mn-containing welding fumes. Similarly, mice exposed to MWCNT (10–80 μg ; single dose) by pulmonary exposure exhibited neuroinflammation (increased *Ccl2*, *Tnfa*, *Il1b* mRNAs; 2–10 fold, $P < 0.05$) in the OB and hippocampus (HIP). Levels of b-amyloid₍₁₋₄₂₎ increased (7–38 fold; $P < 0.001$) in the HIP, aggregation and accumulation of which is a pathological hallmark of Alzheimer's disease. The persistence of MWCNT-related neurotoxic effects two months after cessation of exposure is suggestive of progressive neuronal injury. Collectively, our studies suggest olfactory and central neurotoxicity can be elicited by incidental and engineered particulates. This calls for extensive neurological hazard evaluation of environmental and occupational particulate exposures to avert adverse neurodegenerative health risks.

PL 0197 Determination of Lead (Pb) Exposure on Infants Living around Pb Mining Area in Kabwe, Zambia

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Lead (Pb) is known as one of the dangerous metals, which causes death in the worst cases. Kabwe, capital of the Zambia's Central Province, is one of the worst polluted places in the world. A previous study from Kabwe showed very high Blood Lead Levels (BLLs) in children near the mining area at which all samples were higher than the CDC recommended level (5 $\mu\text{g}/\text{dL}$). Those chil-

dren, especially infants, who are more vulnerable to Pb, should be protected from Pb exposure for their future lives. Their mothers, who breastfeed them, could be also exposed to Pb and their breast milk is potentially contaminated. However, Pb exposure on mothers has not been reported yet in the area. The present study was undertaken to assess Pb exposure on paired mothers and infants and investigate the relationship of Pb exposure between them in Kabwe, Zambia. Blood from mothers and infants, feces from infants, and breast milk samples were collected in 5 clinics around the mine. Pb concentrations and stable Pb isotope in the samples were measured using ICP-MS and Multi Collector ICP-MS, respectively. In total, 426 mothers and 414 infants participated in this study. The means BLLs in mothers and infants were 11.3 µg/dL and 18.1 µg/dL, respectively. Most of them (63% mothers and 67% infants) exceeded the CDC recommended level. Furthermore, BLLs in 5 mothers and 38 children were above the level that is necessary to be treated (45 µg/dL). The maximum BLLs in this study were 93.4 µg/dL in an infant, comparable to fatal level for children (100 µg/dL). BLLs in infants were significantly higher than those in paired mothers ($p < 0.01$), and there was a significant positive correlation between BLLs of paired mothers and infants ($p < 0.01$, Spearman's $\rho = 0.7$). Pb was detected in breast milk and fecal samples also. However, higher Pb concentrations and the stable isotope ratio in fecal samples suggested that the main source of Pb exposure on infants were contaminated soil and/or dust, especially high BLLs cases. In the present study, high BLLs were found in both of mothers and infants. High BLLs in mothers could be related with Pb exposure on their children including fetuses at not only lactating period but also pregnant period through placenta. Thus, to control Pb exposure, soil remediation and reduction of dust in the residential areas are highly recommended. The decrease of BLLs in mothers could contribute to the reduction of the Pb exposure on infants through breastfeeding. However, it usually takes time to carry out environmental remediation programs. Therefore, the education to reduce the contact and ingestion of contaminated soil to local people must be necessary in parallel.

0227 Development of Physiologically Relevant *In Vitro* Models for Assessment of Nanoparticle-Induced Physicochemical Property-Dependent Cytotoxicity of Human Lung Cells

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With a rapid increase in nanotechnology development and nanomaterial (NM) utilization, human health risk along a NMs' lifecycle has become a public health concern. However, risk assessment of emerging NMs has been limited due to several technical challenges, including the lack of appropriate *in vitro* models that mimic real-life exposure scenarios and reliably predict *in vivo* responses. For example, the administered dose under normal cell culture conditions does not reflect the actual delivered dose due to NM's slow settling rate. In addition, the artificial dissolution and/or instability of NMs under submerged conditions does not represent the real exposure condition of inhaled "dry" NMs. To address those issues, the objective of this study was to develop a physiologically relevant *in vitro* model for toxicity assessment of NMs with specific delivered dose of each NM, or using a 3D air-liquid interface (ALI) in combination with aerosol delivery system. These models allow us to evaluate and compare bio- and toxic- potential of metal oxide nanoparticles (MONPs) and by-products from thermally decomposed nanocomposites at similar delivered doses *in vitro*. Following identification of targeted lung cells at the site of inhaled NM accumulation *in vivo*, specific lung cell types were selected for our *in vitro* models. The ALI model was constructed using the VitroCell system with cultured primary human bronchial epithelial cells. Cells were allowed to differentiate on ALI for 28 days, which exhibited a pseudostratified architecture with mucous production, as well as tight junction formation and ciliogenesis, resembling airway microenvironment. Delivered doses were calculated based on the sedimentation rate of each NM under each cell culture medium, and the dose/time course known to induce pulmonary toxicity *in vivo*. Well-characterized MONPs and two pairs of thermally decomposed pristine and carbon nanotube-containing polycarbonate (PC/PC-CNT) and polyurethane (PU/PU-CNT) ultrafine particles were evaluated for their cytotoxicity in this test model. Acute and sub-chronic exposure to MONPs induced property- and time-dependent cellular alterations, consistent with *in vivo* observations. NM type-dependent cytotoxicity was also observed with PC and PU, with CNT incorporation (PC-CNT and PU/CNT) enhancing their bioactivity. Thermal decomposition of the nanocomposites resulted in the formation of particulate-bound polycyclic aromatic hydrocarbon (PAH), leading to enhanced cytotoxicity and mechanistic profiling. Data from this study will be used for structure-activity relationship and comparative toxicity studies to assess the health risks of NMs.

0230 Hepatocyte-Like Cells Derived from Human Induced Pluripotent Cells Using Small Molecules: Implications of a Transcriptomic Study

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Hepatocyte-like cells (HLCs) derived from human induced pluripotent cells (iPSCs) hold great promise in toxicological applications as well as in regenerative medicine. Previous efforts on hepatocyte differentiation have mostly relied on the use of growth factors to recapitulate developmental signals under *in vitro* conditions. Recently, the use of small molecules (SMs) has emerged as an attractive tool to induce cell fate transition due to its superiority in terms of both quality and cost. In the current study, we differentiated HLCs from iPSCs using a protocol that only involves SMs and characterized gene expression changes during the course of the SM-driven differentiation using whole genome microarrays. Transcriptomic analysis of the SM-driven differentiation defined a hepatocyte differentiation track and identified several key genes in major stages of hepatocyte differentiation. In addition, the HLCs derived using the SM protocol (SM-HLCs) were scored with CellNet, an online tool for quantifying how closely engineered cell populations resemble their target cell type, and compared to primary human hepatocytes (PHHs), adult liver tissue, fetal liver tissue, HLCs differentiated using growth factors (GF-HLCs), and commercially available HLCs. Similar to GF-HLCs, SM-HLCs displayed a mixed phenotype of fetal and adult hepatocytes and had relatively low expression of metabolic enzymes, transporters, and nuclear receptors compared to PHHs. Overall, the present study demonstrated the usefulness of the SM-based hepatocyte differentiation method, offered new insights into the molecular basis of hepatogenesis and associated gene regulation, and suggested ways for further improvements in hepatocyte differentiation in order to obtain more mature HLCs that could be used in toxicological studies.

0237 Practical Implementation of New Approach Methodologies (NAMs): A Corporate Perspective for Diverse Toxicology Applications

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3M has always been firmly committed to animal use reduction, and over the last ten years, significant progress has been made at utilizing new approach methodologies (NAMs) for evaluating human health hazard potential of raw materials and products. A particular challenge is finding methods to effectively work across a broad product portfolio, including industrial, consumer, and health care products, while having cost effective utility for product development. These efforts at 3M have primarily been focused on building an internal computational toxicology program and developing alternative test method capabilities within the 3M Strategic Toxicology Laboratory (STL), an internal resource providing toxicology support to 3M businesses. A focus in the STL has been on use of reconstructed human three-dimensional tissues, which are much more effective for evaluating mixtures, formulations, and finished products for irritation and toxicity potential, compared with standard two-dimensional cell culture methods, particularly when the test materials are insoluble in cell culture media. Numerous 3D human tissue types have been successfully used in the STL, including dermal, ocular, oral, and vaginal models, while also utilizing unique delivery systems for vapor/gas exposures to airway tissues. Standardized OECD assays have been utilized for a variety of purposes, including hazard identification and safety assessments, hazard communication, new chemical registrations, and transportation classifications, while customized studies have also proven very useful for formulation comparison and product development purposes. The computational program utilizes multiple approaches in evaluations for read-across, structural alerts, and (Q)SAR analysis to build a weight of evidence approach for preparing toxicity estimates. Customized (Q)SAR models utilizing historical test data have been built for specific endpoints of interest, such as inhalation toxicity. A database has also been built to store the computational results that is searchable by chemical structure/similarity and currently contains over 1,000 chemicals that have been assessed. The results of these alternative efforts have provided valuable information for safety and risk assessment purposes for highly diverse products, particularly during product development, while allowing significant reductions in animal use. A key learning has been ensuring that the NAMs incorporated have practical utility to the business needs and the product types to be evaluated. Integration and coordination of the alternative assessment processes have also been found to be critical to maximize their effectiveness.

PL 0330 California Cruelty-Free Cosmetics Act Establishes First Law in North America That Bans the Sale of Animal-Tested Cosmetics and Ingredients

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Globally, growing public pressure to end animal testing for cosmetics is pushing industry away from animal tests and towards greater use of nonanimal *in vitro* and *in silico* methods. The US Food and Drug Administration does not require specific tests for cosmetics, leaving companies responsible for ensuring their products are safe. However, in 2018, the state of California joined the European Union and other regions in banning the sale of cosmetic products or ingredients that have been tested on animals. Starting January 1, 2020, no cosmetic may be sold within the state if it, or its ingredients, were tested on animals after January 1, 2020. Enumerated exemptions allow for testing conducted in response to a requirement by a state, federal or foreign regulatory body under certain conditions. However, the law requires that companies selling cosmetics or ingredients tested on animals under one of the exemptions must also use nonanimal methods to substantiate the safety of the product. Because the law applies to cosmetic companies as well as any third party suppliers or contractors, many ingredient manufacturers will be required to use *in vitro* or *in silico* methods, even if they are also conducting an *in vivo* study for the same endpoint to support the ingredient in another sector. Training and outreach efforts to ensure ingredient suppliers are ready to comply with the law are needed. Indeed, there are numerous nonanimal methods available for assessing the potential hazards of cosmetics and their ingredients. This presentation will outline the law and describe approaches available for use to meet legislative requirements.

PL 0335 Manganese-*Mycobacterium tuberculosis* Interactions: Central and Peripheral Effects

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The role of the immune system, namely neuroinflammation, in the mechanisms of manganese (Mn) neurotoxicity is well established. Recent evidence indicates that in certain occupations, such as mining, there is a distinct possibility of overexposure to Mn and bacterial burden with the highly infectious and persistent bacterial pathogen *Mycobacterium tuberculosis* (Mtb). Yet, the nature of such interaction has not been studied experimentally. This study was designed to assess neurological and peripheral effects of occupationally modeled Mn exposure interfaced with Mtb infection. Male C57BL/6 mice, some carrying a transgene for microglia identification, were exposed to Mn (0.5 mg/kg; bilateral intranasal instillation) 5 days per week for 5 weeks. Three weeks into the Mn treatment, the mice were inoculated with Mtb (H37Rv Dsred) using a microsyringe intratracheally. One day, 1 week, and 3 weeks after termination of the Mn treatment (2, 3, and 5 weeks post Mtb inoculation), mice were sacrificed and various indices of Mtb pathology and/or Mn neurotoxicity were evaluated. From the data analyzed so far, Mn treatment resulted in significantly increased liver granulomas and pericellular vacuolation (PCV) compared to mice only challenged with Mtb 1 day after the last Mn dose. In the brain, mice that were exposed to Mn and Mtb had decreased striatal dopamine levels at all three time points, with the effect being more robust early (1 day post Mn), and late (3 weeks post Mn). Thus, Mtb infection in the presence of increased Mn burden leads to both increased bacterial pathogenesis, which appears to be transient, and in a neurological dysfunction, i.e., neurotransmitter imbalance. In the striatum, the dopaminergic toxicity of Mn in Mtb-infected mice is long lasting. *Support: R21ES026383 (NIEHS).*

PL 0340 Downregulation of SDF1/CXCR4 Signaling Mediates Ethanol-Induced Craniofacial Anomalies and Cranial Nerve Defects in Zebrafish Embryos by Disrupting Neural Crest Cell-Placode Interaction

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One of the hallmarks of Fetal Alcohol Spectrum Disorders (FASD) is the defects of craniofacial skeletons and cranial sensory system. During embryonic development, cranial neural crest cells (CNCCs) generate a wide range of cell types including cartilage and bone that make up much of the cranial skeletons. Epibranchial placodes that give rise to sensory neurons that form the

distal ganglia of the Vth, VIth, IXth, and Xth cranial nerves develop in proximity to CNCCs. The interaction between CNCCs and placodes is known to play a major role in the development of cranial skeletons and nerves. Our study aims to investigate whether ethanol exposure can induce craniofacial anomalies and cranial nerve defects by the impairment of CNCC-placode interaction through the disruption of stromal cell-derived factor 1 (SDF1)/C-X-C chemokine receptor type 4 (CXCR4) signaling, a chemokine/receptor axis that mediates chemotaxis and migration. Using twist1a as a marker for CNCCs, *in situ* hybridization of zebrafish embryos at 30 hours post fertilization (hpf) revealed that exposure to 1% (v/v) ethanol resulted in significant inhibition of CNCC migration. As indicated by epibranchial placode marker Sox3, ethanol exposure also inhibited the migration of cranial placodes. Exposure to ethanol also resulted in craniofacial and cranial nerve abnormalities in zebrafish embryos. We also found that the mRNA expression of placode-secreted ligand SDF1 was significantly decreased in ethanol-exposed zebrafish embryos. Knockdown of SDF1 in control zebrafish embryos resulted in the inhibition of the migration of both CNCCs and epibranchial placodes, and abnormalities in craniofacial and cranial nerve development. Knockdown of SDF1 also exacerbated ethanol-induced inhibition of the migration of CNCCs and placodes and dysmorphology. In contrast, overexpression of SDF1 reversed the ethanol-induced inhibition of CNCCs and epibranchial placode migration and diminished ethanol-induced dysmorphology in zebrafish embryos. However, the rescued effects of SDF1 overexpression were abolished by the knockdown of SDF1 receptor CXCR4. These results demonstrate that ethanol exposure can induce craniofacial and cranial nerve defects by disrupting CNCC-placode interaction and inhibiting the migration of CNCCs and placodes, and strongly suggest that the downregulation of SDF1/CXCR4 signaling contributes to ethanol-induced craniofacial and cranial nerve abnormalities. *Supported by NIH grants AA021434, AA020265, and AA024337 (S-Y.C).*

PL 0347 Evaluating Mechanistic Data in Hazard Assessment: Integration of Key Characteristics and Mode of Action

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There is a need to expedite the assimilation, development and application of mechanistic toxicological data as a basis to tailor efficient and predictive testing strategies to meet demanding regulatory mandates to assess more efficiently much larger numbers of chemicals. Advances in technology to measure effects at lower levels of biological organization in higher throughput technologies have potential to contribute in this area. Potential regulatory application of these mechanistic data necessitates harmonization of reporting protocols and their systematic organization and assessment, consistent with developments for documentation internationally and continually increasing legislative requirements for transparency. An organizing construct for the assimilation of mechanistic data based on 10 key characteristics of carcinogens (KCCs) has been proposed and applied in the hazard classification of carcinogens by the International Agency for Research on Cancer (IARC). However, there has been limited attempt to better define and integrate this approach with systematic approaches to weighting and assessment of mechanistic data within and across lines of evidence, including the development of chemical agnostic adverse outcome pathways (AOP) and mode of action (MOA) analysis for specific chemicals. Potential for further development and integration of the construct for KCCs is considered in the context of recent developments in systematic consideration of the weight of evidence for higher tier approaches to assessment of mechanistic data. Recommendations are included to address identified discrepancies in terminology and necessary additional critical assessment to permit potential application in regulatory hazard assessment. These recommendations draw upon increasing international experience in purpose-based assessment of hazard in increasing tiers of discrimination and stages of data identification and assessment of mechanistic data (e.g., assimilation, establishment of lines of evidence and their weighting for integration) documented in a recent review.

PL 0368 Teratogen Assay Using Gene Expression Profiles of Developmental Regulators in Morphogenetic Aggregates of Human Embryonic Stem Cells

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Animal alternatives are highly desired to identify chemical exposures that potentially disturb embryo development in pregnant mothers. The goal of our study is to establish an *in vitro* assay system using human embryonic stem cells

(HESC) to detect teratogenic exposure levels of chemical compounds. We have formulated a novel culture condition that induces 3-dimensional aggregates of HESC to undergo robust growth and morphological changes in 5 days. The culture condition entails the medium containing small molecule modulators of WNT, NODAL, and retinoic acid signaling pathways, which altogether promote differentiation of paraxial mesoderm and neuroectoderm, as suggested by temporal gene expression profiles. These embryological tissues are precursors of the axial skeleton and central nervous system, which are often malformed in various teratogen-induced birth defects. Thus, we hypothesize that teratogenic chemical exposures would significantly alter the unique gene expression profiles of the HESC aggregates. To test the hypothesis, we examined the impact of 12 compounds, whose teratogenic potentials are well-documented through rodent and human studies. Of these, 10 compounds (abacavir, all-trans retinoic acid, caffeine, dabigatran, ethylene glycol, glycolic acid, MEHP, methanol, nilotinib, and ramelteon) were previously shown in rats for specific links between their *in vivo* concentrations (e.g., plasma C_{max} levels) and developmental toxicity (e.g., fetal malformation or demise). The other 2 compounds (thalidomide and valproic acid) are human medications, whose therapeutic plasma concentrations and teratogenic potential have been unequivocally demonstrated. We cultured HESC aggregates in the presence of these compounds at various concentrations for 5 days, and measured the expression levels of genes associated with paraxial mesoderm (*MEOX1*, *FOXC2*, *MESP2*, and *UNCX*) and neuroectoderm (*PAX6*, *SOX2*, *NEUROG2*, and *OLIG3*) by quantitative RT-PCR. The expression levels of these genes were significantly altered in HESC aggregates that were exposed to teratogenic levels, but not to non-teratogenic levels, for most compounds. The types of genes that were altered, and also the nature of alterations (i.e., increased or decreased), were different depending on which chemicals were tested, implicating that each compound exhibits distinct molecular mechanisms of teratogenic actions. These results suggest that our novel culture system of HESC aggregates is a promising assay tool to evaluate teratogenic exposure levels of various chemicals. Further investigations are warranted to reveal applicability and limitation of the assay.

PL 0383 Function of lncRNA NR_030777 in Affecting Cell Proliferation and Apoptosis and Protection of Motor Function by Regulating Zfp326/Cpne5 in Nerve Cell Damage Induced by Paraquat

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Parkinson's disease (PD) is a common age-related degenerative disease of the central nervous system. It is generally believed that PD is caused mainly by environmental and genetic factors. Previous studies have shown that paraquat (PQ) may be one of the environmental factors leading to PD, but its specific mechanism remains to be further elucidated. lncRNAs generally refer to non-coding RNA transcripts greater than 200 nucleotides in length and researches have shown that lncRNA plays irreplaceable roles in neurodegenerative diseases. However, the exact roles and mechanisms of lncRNAs remain unclear. In this study, we identified lncRNAs related to PD caused by PQ and clarified the biological functions and regulatory mechanisms of a specific lncRNA in nerve cell damage induced by PQ. We found changes in lncRNA expression profile of mice substantia nigra (SN) induced by PQ using microarrays, and six of them validated by qRT-PCR and two by FISH. Bioinformatics analysis was applied to predict the functions of lncRNAs and predict the targeted genes. Among the differently expressed lncRNAs, the dysregulation of NR_030777 which is highly homologous with its gene symbol-Zfp326 draw our attention. We observed that the changes of Cpne5 expression through CNC prediction and mRNA microarrays was related to the high expression of NR_030777. So we reasonably supposed that NR_030777/Zfp326/Cpne5 pathway plays a crucial part in nerve cell damage induced by PQ. *In vitro*, RNAi and lentivirus infection were used to knock down and overexpress NR_030777 expression in order to explore the regulatory mechanism and biological functions of NR_030777 in cell damage caused by PQ. We found that NR_030777 regulated the expression and stability of Zfp326 and Cpne5, and thereby regulated cell proliferation and apoptosis induced by PQ. In addition, we further discovered that by scavenging of ROS by N-Acetyl-Cysteine (NAC), NR_030777 expression was suppressed. *In vivo*, we found that NR_030777 overexpression in central nervous system regulated the expression of Zfp326 and Cpne5 in substantia nigra and hippocampus, and reduced the motor function injury induced by PQ in mice. In summary, NR_030777 plays a protective role against neurotoxicity of PQ by regulating the expression of Zfp326/Cpne5. Our study first reveals the novel role of NR_030777 in PQ-induced nerve cell damage and provide a potential method to prevent and treat neurodegenerative diseases caused by common environmental neurotoxins such as PQ.

PL 0405 Comprehensive Analysis for Immunological Characteristics of Patients with Malignant Mesothelioma and Diffuse Pleural Thickening

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To date, our previous studies have demonstrated suppressive effects of asbestos exposure on NK and T cell functions, the part of which was also observed in peripheral blood mononuclear cells (PBMC) in patients with malignant mesothelioma (MM), where increases in expression of Treg marker and concentrations of various inflammatory cytokines were characteristic compared with people positive for pleural plaque, asbestos exposure sign, not having tumors. Exposure to asbestos also causes benign diseases including diffuse pleural thickening (DP), which is not associated with MM. Therefore, we comprehensively examined and compared immunological characteristics of MM and DP patients. Plasma and PBMC were prepared and used to measure concentrations of multiple cytokines, cell surface markers on monocytes, CD4⁺ T (Th), CD8⁺ T (CTL) and CD56⁺ NK cells and mRNA levels in each sorted cell population, by luminex, flow cytometry and realtime RT-PCR respectively. The surface expressions of GITR and CD69 on Th cells and those of CXCR3 and CD69 on CTL were high in MM compared with DP. The mRNA levels of RORC in Th and of c-Rel were high in MM, whereas the mRNAs of GATA-3 in Th and PRF1 in CTL were high in DP. There were no differences in plasma cytokine concentrations between MM and DP. Those 8 parameters showing the significant differences were statistically examined by factor analysis, which resulted in extraction of the two factors. The high factor loading in the first factor was shown by GITR and CD69 on Th, CXCR3 and CD69 on CTL and mRNAs of RORC in Th and c-Rel in NK, whereas mRNAs of GATA-3 and PRF1 in CTL showed that in the second factor. The 2D plot with these two factors clearly distinguished the two groups of MM and DP. Those findings indicate that MM differs in characteristics of anti-tumor immunity from DP, in particular about Treg and CTL, which might be related with development of those diseases following exposure to asbestos.

PL 0407 Aryl Hydrocarbon Receptor (AhR) Signaling Regulates the Inflammatory Potential of Dendritic Epidermal T Cells

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Dendritic epidermal T cells (DETC) are unique resident innate immune cells in the murine epidermis. DETC participate in the maintenance of skin homeostasis and initiation of inflammatory responses by attracting other immune cells. Moreover, DETC exhibit potent cytotoxicity against cancer cells. The AhR is a transcription factor, which is activated by small molecules of planar structure and controls expression of xenobiotic metabolic enzymes. AhR-ligands can be found in polluted air or food, like polycyclic aromatic hydrocarbons, such as dioxin. In the epidermis, the ligand 6-formylindolo(3,2-b)carbazole (FICZ) is generated by conversion of tryptophan under UV-light. The AhR is highly expressed in DETC and has been shown to be necessary for their maintenance in the epidermis after the initial immigration around birth. However, very little is known about the role of the AhR in DETC function, including related transcriptional changes. We therefore (i) analyzed the gene expression profile of DETC from AhR-full-knockout (AhR^{-/-}) shortly after epidermal seeding, (ii) treated DETC-cell lines with AhR-ligands, and (iii) modified the intake of AhR-ligands by diet in wild-type mice to mimic different levels of endogenous AhR-ligands. Our microarray data showed that more than 80 genes were differentially regulated in AhR^{-/-} vs. AhR^{+/+}-DETCs. According to literature those genes are mainly involved in survival, cell-contact and inflammatory response. Interestingly the immune suppressive factor Interleukin-10-Receptor (IL10R) was absent in AhR^{-/-}-DETCs on the gene- and protein-level. Furthermore, AhR^{-/-}-DETC had a rounded morphology, increased cytoplasmic F-actin and decreased intracellular Calcium, which correlated with a decreased expression of the actin-severing gene *scinderin* and the Ca-activated potassium channel *kcna1*. Treatment of DETC-cell lines with the AhR-ligand FICZ as well as adding the AhR-ligand Indol-3-carbinol to the diet increased the expression of IL10R and the reputed immune suppressor CD69 on DETC. This might indicate that an AhR-activation potentially renders DETC more sensitive to IL-10 mediated dampening of function. These data underline the importance of the AhR for the balance of skin homeostasis possibly by dampening immune responses of DETC, which could contribute to prevent outbreaks of skin inflammation. Further experiments with our recently established inducible DETC-specific AhR^{-/-} mice will allow more precise analysis of AhR-dependent function in DETC.

PL 0415 Mitochondrial Respiration and Metal Bioaccumulation in Zebrafish Developmental Stages (*Danio rerio*) Evaluating Three Functionalized Groups of CdTe Quantum Dot Nanoparticles

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Understanding how specific nanoparticles interact with cells and cell systems is essential to predict their toxicity with respect to either clinical or environmental exposure. Zebrafish are well established models for research and can be used to evaluate nanoparticle biocompatibility. Cadmium selenide (CdSe) and Cadmium Tellurium quantum dots (CdTe QDs) represent two of the most commonly utilized QDs for biological applications, with their extremely bright fluorescent properties making them powerful labeling agents for diverse *in vivo* applications. Exposure of CdTe QDs to zebrafish embryos lead to a range of developmental and behavioral disturbances, most likely due to cadmium ions, as little is known about the toxicity of tellurium. Previous studies indicate LC₅₀ values of CdTe QDs to be around 186 nM for 120 h, inducing malformations and delayed hatching. Thus, our study assessed nanomaterial uptake over time as well as basal oxygen consumption rate (OCR), a measure of mitochondrial respiration. Three different functionalized QDs (COOH⁻, NH₄⁺, PEG) were tested on zebrafish developmental stages, from 24 hpf to 96 hpf following principles of the OECD fish embryo test. Inductively Coupled Particle Mass Spectrometry was applied to quantify the metal concentrations within the exposed organisms and XF24 extracellular flux analyzer (Seahorse Bioscience) was used to measure the OCR of zebrafish embryos *in vivo*. Exposure to all functional groups of QDs caused dose dependant metal uptake within the organism. Exposures also resulted in significant changes in the OCR baseline when compared to controls. The results will contribute to the current understanding of the potential ecotoxicological effects of nanoparticles and support the sustainable development of nanotechnology.

PL 0422 CdTe Quantum Dots Increase Microglial Polarization in Mice and Trigger Damage to Hippocampal Neurons

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The CdTe quantum dots (QDs) we used in this study were synthesized by electrochemical method. The average size of the CdTe QDs was 4.7 ± 0.7 nm, and the emission wavelength was at 618 nm, which meant the fluorescence of CdTe QDs was red. CdTe QDs are quasi-one-dimensional fluorescent nanomaterials which can pass through the blood-brain barrier. 12.5 nM/g CdTe QDs were injected in mice through the tail vein. 3h later, according to the quantitative analysis of the fluorescence signal and cadmium from CdTe QDs, QDs accumulated in the brain tissues of mice, and the hippocampus was one of the parts with the highest concentration of CdTe QDs in the brain. The immunofluorescent staining of hippocampi further indicated that the number of NeuN⁺ neurons decreased. CdTe QDs treatment significantly increased the number of CD16⁺/Iba1⁺ M1 microglia cells and CD206⁺/Iba1⁺ M2 microglia cells in hippocampi within 3 hours after the injection. However, the number of M2 microglia cells decreased as time came to 6h and 12h after the injection. Our *in vitro* experiments also confirmed the polarization of microglia cells (BV-2 cell line) induced by CdTe QDs. Not only the shift of CD11b⁺ and CD40⁺ were detected, but also the increase of the inflammatory cytokines such as IFN- α , TNF- α , IL-1 β were confirmed by qRT-PCR and ELISA. Except for the oxidative damage in neurons directly induced by CdTe QDs, we found that the polarization of microglia cells induced by CdTe QDs can also trigger damage to neurons. The culture medium of normal BV-2 cells shown no significant toxicity to the hippocampal neurons (HT-22 cell lines). But after BV-2 cells were pre-treated by 1.25 μ M CdTe QDs for 6h, as BV-2 cells were activated, the culture medium of BV-2 cells significantly decreased the cell viability of the HT-22 cells. The polarization of BV-2 cells could be regulated by TLR2/MyD88/NF- κ B signaling pathway, as the expression of TLR2 and MyD88 increased significantly in CdTe QDs-treated BV-2 cells. The expression of phosphorylated I κ B and p65 from nucleoprotein increased, while I κ B and p65 from cytoplasmic protein decreased significantly. In conclusion, the CdTe QDs we synthesized can polarize microglia cells to M1 microglia cells, the polarization could be regulated by TLR2/MyD88/NF- κ B signaling pathway. The polarization of microglia cells could damage neurons through the inflammatory response. *Funding Information: National Natural Science Funds of China (31671034, 81473003, 21876026, 81673218, 81573186 and 81302461).*

PL 0452 3- to 7-Ring Polycyclic Aromatic Hydrocarbons Are the Main Inducers of the *In Vitro* Prenatal Developmental Toxicity Observed with Some Petroleum Substances

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Prior studies have noted a strong relationship between prenatal developmental toxicity (PDT) induced by some heavy petroleum substances (PS) and the level of specific polycyclic aromatic hydrocarbons (PAHs) they contain. This is corroborated by data from PDT studies with highly refined base oil (HRBO) and gas-to-liquid (GTL) products, which are a similar PS and a non-petroleum based analogue, respectively, that both do not contain PAHs, and for which no developmental toxicity was observed. Hence, it is hypothesized that PDT as observed with some PS is caused by particular constituents present in these products, namely 3- to 7-ring PAHs. To test this hypothesis, the present study evaluated *in vitro* PDT potency of the DMSO-extracts of 17 PS (varying in 3- to 7-ring PAH content, including HRBO that contains no PAHs) and 2 GTL products (also devoid of PAHs), in the embryonic stem cells test (EST). The obtained results show that all DMSO-extracts of PAH-containing PS inhibit ES-D3 cell differentiation in a concentration-dependent manner at non-cytotoxic concentrations, and that this potency is proportional to the amount of 3- to 7-ring PAHs they contain (stronger effects were seen with substances that have higher 3- to 7-ring PAH levels, and this trend was observed both within and between petroleum product categories). Moreover, the DMSO-extracts of all PAH-containing PS also show aryl hydrocarbon (AhR)-mediated activity, as quantified in the AhR CALUX assay. On the contrary and as expected, the DMSO-extracts of HRBO and GTL products, which all contain no PAHs, tested negative in the two assays. Co-exposure of ES-D3 cells with the selected DMSO-extracts of PS and the AhR-antagonist trimethoxyflavone (TMF), successfully counteracted the PS-induced inhibition of ES-D3 cell differentiation into cardiomyocytes in the EST, confirming the role of the AhR in mediating the observed PDT of PS extracts in the EST. Furthermore, a good correlation exists ($R^2=0.95$) between the *in vitro* PDT potencies of the PS and GTL extracts under study and the published rat *in vivo* data on their PDT, implying the suitability of the EST to assess the PDT potency of complex substances like PS. To conclude, our findings corroborate the hypothesis that PS-induced PDT is caused by 3- to 7-ring PAHs present in these substances and that the observed PDT is, at least partially, mediated via the AhR activation. Overall, these data further underpin read across hypotheses in regulatory submissions for petroleum products (e.g., under REACH) and in this way help to significantly minimize the required animal testing for this endpoint.

PL 0472 Cadmium Telluride Quantum Dots Exposure Causes Functional Impairments in Monocyte-Derived Dendritic Cells and Alters CD4⁺ T Cells Differentiation

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Cadmium telluride quantum dots (CdTe QDs) are one of the most utilized nanomaterials in many fields due to their superior optical properties. Beyond enjoying the convenience of QDs applications, more and more researchers are beginning to pay attention to the toxicity of QDs. We have conducted a series of studies on the neurotoxicity and hepatotoxicity of CdTe QDs. However, their immunotoxicity has not yet been elucidated, especially in human primary immune cells. Human peripheral blood monocyte-derived dendritic cells (DC) could intake QDs and non-toxic dose of QDs impaired the differentiation and functions of DC. CdTe QDs exposure could promote transcription factor EB (TFEB) phosphorylation then into nucleus, which reduced the expression of co-stimulating molecules on the surface, lowering their ability to activate T cells and alter CD4⁺ T cells differentiation. CdTe QDs-treated DC drove a Th2-biased polarization, but impaired development of Th1 and Th17 cells. Beside, CdTe QDs-treated DC induced suppressive CD4⁺CD25^{hi}Foxp3⁺ regulatory T cells. Our results suggest that CdTe QDs induced TFEB-mediated autophagy promotes tolerogenic functions in human monocyte-derived DC, therefore, QDs-treated DC could regulate inflammatory reaction, which would be benefit to proinflammatory T cell mediated pathologies. *This work was Supported by National Natural Science Funds of China (No. 21876026; 31671034; 81673218; 81502783; 81573186).*

PL 0481 Nano ZnO Exposure Alters *In Vitro* Lipidomic Profiles of Human Immune Cells

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Zinc Oxide (ZnO) nanoparticles (NPs) are used in several industrial and biomedical applications, including sunscreens and skin care products. Our previous research has shown that ZnO nanoparticles and organic chemical UV-filters used in sunscreens are equally well tolerated by human immune cells *in vitro* (O'Keefe *et al.*, 2016). However, cytotoxic effects occur after NP uptake and intracellular dissolution, following exposure to high concentrations of ZnO NPs. The present study investigated early cellular responses in this process via lipidomic profiling using a metabolomics approach. Human THP-1 monocytes were exposed to ZnO NPs (39±4 nm; identical to OECD Standard Reference Material "NM-112") at 0, 10, 15, 20 and 25 µg/mL for 24 hrs, at 37°C and 5% CO₂, which resulted in a 10, 21, 25 and 32% decrease in cell viability, respectively. A time-course study also examined exposure for 4, 8, 12 and 24 hrs to 10 µg/mL. Lipid metabolites were extracted from exposed cell cultures using monophasic liquid extraction with selected deuterated-labelled internal standards, and analysed using liquid chromatography coupled with triple-quadrupole mass spectrometry (LC-QQ, Agilent). Processed data were analysed using MetaboAnalyst software (version 3). The results showed clear dose- and time-dependent shifts in the profiles of lipid classes, and individual phospholipids and triacylglycerols. Overall, ZnO NP exposure decreased the abundance of phospholipids containing polyunsaturated fatty acids (PUFA) with a relative increase in saturated lipids—including effects on specific phosphatidylcholine (PC), lyso-PC, ceramide and sphingomyelin lipids. Similar changes were seen in individual triacylglycerols and cardiolipins—the latter indicating mitochondrial membrane disruption from oxidative stress. As Zn-dependent perturbations in cellular processes resulted in decreased unsaturated lipid levels, this suggests that reactive oxygen species have reacted with C=C double bonds in PUFAs and caused lipid fragmentation. Co-exposures to ZnO NPs and hydrophilic (Vit. C) or lipophilic (Vit. E) antioxidants were also investigated to further elucidate the mechanisms of ZnO NP interactions with cells that may lead to cytotoxicity. This metabolomics approach to lipid profiling has assisted in identifying potential cellular biomarkers of effect, and also those cellular organelles specifically targeted in early cytotoxic events, following ZnO NP exposure. *Reference: O'Keefe SJ, Feltis BN, Piva TJ, Turney TW, Wright PFA (2016). ZnO nanoparticles and organic chemical UV-filters are equally well tolerated by human immune cells. Nanotoxicology, 10(9):1287-1296.*

PL 0498 Silver Nanoparticles Promote Procoagulant Activity of Red Blood Cells: A Potential Risk of Thrombosis in Susceptible Population

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Silver nanoparticles (AgNP) are widely used in medical practices owing to their distinct antibacterial, antiviral and anticancer activities. However, with increasing use of AgNP, concerns over its potential toxicity are also escalating. Here, we demonstrated the potential thrombotic effect of AgNP which was mediated by the procoagulant activity of red blood cells (RBCs). In freshly isolated human RBCs, AgNP, but not silver microparticles (AgMP), elicited morphological changes, phosphatidylserine (PS) exposure and microvesicles (MV) generation, the key indicators of procoagulant activity in RBCs at concentration ranges (≤ 100 µg/mL) that were free of significant hemolysis. In line with this, AgNP potentiated thrombin generation and adherence of RBCs to endothelial cells, while AgMP did not. Oxidative stress, intracellular calcium increase, and ATP depletion were found to underlie the procoagulant activity of AgNP, which led to altered activity of membrane aminophospholipid translocases. These *in vitro* findings were well reproduced in rat *in vivo*, where intravenously exposure to AgNP promoted venous thrombosis significantly. Of note, RBCs isolated from cancer patients, who inherently convey the risk of thrombogenesis, were more sensitive to the procoagulant effects of AgNP. In addition, AgNP significantly potentiated the procoagulant effects of a chemotherapeutic drug, paclitaxel. Collectively, these results suggest that AgNP may have prothrombotic risks by promoting procoagulant activity of RBCs and caution shall be taken for its use in the population sensitive to thrombosis like cancer patients.

PL 0527 Development of New Risk Assessment Guideline of Food Contact Materials Based on the TTC Concept in Food Safety Commission of Japan

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In Japan, food utensils, containers and packaging (UCP) are regulated under the Food Sanitation Act. In current regulation, the use of only substances on which the government stipulates individual specifications and standards is restricted (Negative List system), which means the substances whose use is not permitted in foreign countries cannot be regulated immediately unless individual specifications and standards are set. In addition to regulations based on the Act, voluntary management based on the list of substances drawn up in accordance with voluntary standards set by trade organizations are being implemented (Positive List system). Although voluntary management have played a certain role in securing safety of UCP, business operators that do not affiliate to any trade organization fall outside the framework of such measures. The EU countries and the United States (US) apply a national regulatory system for the management of UCP that are made mainly of synthetic resins based on the mechanism of essentially prohibiting the use of substances other than those that have been approved for use as the result of a safety assessment (Positive List system). In 2018, Japanese Ministry of Health, Labour and Welfare (MHLW) decided to adopt a new Positive List system for food contact materials (FCM) of synthetic resins in order to impose restriction on substances that may be used in raw materials of food utensils, containers and packaging materials. In such situation, the Food Safety Commission of Japan (FSCJ), responsible agency for the risk assessment of food related materials, consider to develop new risk assessment guideline. In both the EU and the US systems, different levels of toxicity data are required for inclusion in the Positive List according to dietary concentration, which based on the Threshold of Toxicological Concern (TTC). TTC is a concept that refers to the establishment of a generic exposure level for all chemicals below which there would be no appreciable risk to human health, have been used in the risk assessment of chemicals to which humans are exposed at very low levels. Since FCM is not intentionally added to food, the levels of migration are assumed to be limited. Thus, FSCJ applied TTC concept for new risk assessment guideline, in which FCM's classified into four classes according to the dietary concentration calculated from the migration test, and information of toxicity test required for risk assessment for the lower concentration class are reduced. In this presentation, outline and concept of new risk assessment guideline of FCM applying TTC in FSCJ will be introduced.

PL 0564 *In Vitro* Model for the Prediction of Respiratory Sensitization

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Exposure to chemicals in occupational scenarios can lead to the development of respiratory allergies. At present, no *in vitro* methods are available for the prediction of respiratory sensitization potential of chemicals. To mimic the alveolar-capillary barrier we developed a 3D *in vitro* model grown at the air liquid interface including alveolar type II epithelial cells (A549), endothelial cells (EA.hy926), macrophages like cells (PMA differentiated THP-1) and dendritic like cells (DC) (non-differentiated THP-1). This model was used to test chemical sensitizers and protein allergens. This model was originally developed using medium containing Foetal Bovine Serum (FBS) for cell culture and exposure. With the aim to replace completely animal experimentation, the model was also adapted and tested using FBS-free medium. Exposure to the respiratory sensitizers PA and TMA leads to the upregulation of the expression of CD54 on THP-1 cells up to respectively 185% and 204% relatively compared to vehicle controls while no increase was observed after exposure to irritants. The same was observed for expression of TSLPr with an upregulation up to 162% and 151% after PA and TMA exposure. After protein allergens exposures, OX40L expression was regulated up to 150% after HDM exposure and to 160% after Betv1 exposure, while OX40L expression decreases after irritants exposure, down to 85% for MeSa and to 62% for Acr. Gene expression and cytokines release allowed discriminating sensitizers from irritants. In conclusion, the presented *in vitro* model represents a useful *in vitro* tool to predict respiratory sensitization, allowing discrimination between respiratory sensitizers, either chemicals or protein sensitizers, and respiratory irritants.

PL 0585 The Cross-Laboratory Testing and Evaluation of Two Liver Microphysiological Systems

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Drug-induced liver injury (DILI) and a need for a better understanding of human pharmacokinetics have long been cited as important reasons for drug failure in clinical trials or post-marketing. This is in part due to the limitations of existing animal and *in vitro* models to predict drug efficacy and toxicity in humans. To address these limitations, microphysiological systems (MPS) that model the liver are being actively developed; however, the adoption of these and other MPS has been slow due to lack of wide-spread use of these devices outside of the developer laboratories. Here, we conducted the cross-laboratory testing of two previously established 3D, human liver MPS. One is a sequentially layered multi-cell liver (SQL-SAL) model from the University of Pittsburgh, and the other is a multichannel microfluidic liver model from University of California at Berkeley. These models were transferred from the developers to the Texas A&M Tissue Chip Testing Center. Human primary hepatocytes and induced pluripotent stem cell-derived hepatocytes were seeded into these MPS and baseline secretome was evaluated. Next, terfenadine (10 μ M) was applied to monitor metabolic capability of each liver MPS. Finally, these models were challenged with a panel of hepatotoxic compounds (rosiglitazone, pioglitazone, troglitazone, trovafloxacin, tolcapone, LPS, or a combination of trovafloxacin and LPS) or negative controls (vehicle, and caffeine) to measure response. These studies were carried out in parallel with standard 2D well-based cultures as a comparator. Albumin, lactate-dehydrogenase, urea nitrogen, TNF α , viability, and the presence of drug metabolites were monitored over 10 days to evaluate liver functionality, and stress on the tissues within each of these models, as well as demonstrate the effects of different culturing conditions (2D vs 3D), and cell sourcing. *This research was funded by grants from NIH (U24 TR001950 and T32 ES026568).*

PL 0588 Gestational and Lactational Exposure to the Di(2-Ethylhexyl) Phthalate (DEHP) Increases Mammary Gland Weight in Adult Female Rats Potentially by the Peroxisome Proliferator-Activated Receptor (PPAR) Pathway Activation

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Phthalates are plasticizers added to a wide variety of plastic products to increase their flexibility. Because they do not form covalent bonds with plastic, phthalates are gradually released from products, resulting in a chronic exposure for humans. Phthalates can be detected in various human fluids, including blood serum and breast milk. Exposure to di(2-ethylhexyl) phthalate (DEHP), one of the most extensively used phthalates, has been associated with pathologies such as infertility and breast cancer due to its endocrine disruptive (ED) properties. Exposure during pregnancy has also been linked to metabolic syndrome, which is associated with increased placental peroxisome proliferator-activated receptor (PPAR) levels, and particularly PPAR γ which is involved in adipogenesis. It is now recognized that exposure to ED compounds have more severe consequences if the exposure happens during a window of sensitivity, such as during perinatal life, especially for endocrine-sensitive tissues. However, the effects of DEHP on mammary gland development remain poorly understood. The aim of this study is thus to determine the effects of a perinatal exposure to DEHP on mammary gland development. To investigate this issue, female rats were gavaged during pregnancy and lactation with DEHP and the mammary glands of pups were collected and studied at different key stages of development: post-natal day 3 (PND3), PND21 (weaning), PND46 (puberty), and PND90 (adults). While no effects were observed on morphological parameters such as epithelium area and ductal elongation using whole-mount analysis, an increase of the ratio of mammary gland weight to total body weight was measured in adult rats. Microarray analysis for gene expression was followed by an *in silico* analysis for altered gene expression; an upregulation of the PPAR pathway and adipogenesis transcripts was noted. Consequently, qPCR analysis was done, and it showed an increase in PPAR γ and PPAR β/δ transcripts levels; western blot analysis revealed a trend consistent with the qPCR data. Genomic and proteomic analyses are ongoing in order to determine whether these alterations are also observed at PND3, 21 and 46, and to identify mechanisms involved. Together, our results suggest that a gestational-lactational exposure to DEHP affects PPAR pathway and adipogenesis in the mammary gland at

puberty, a mechanism that is potentially linked to increased mammary gland weight, and long-term effects on normal breast development. *Funded by CRS (IP), FQCS (IP), CIHR (BR), and FRQS (IP, EK).*

PL 0593 Prenatal Exposure to Toxic Metals Modulates Global and Specific DNA Methylation and Expression of Repair Genes

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In utero development is a critical window for exposure to contaminants, such as metals, which has been associated with alterations in DNA methylation, as well as with genetic and oxidative damage in newborns. DNA methylation is an essential epigenetic modification during embryonic development, because the methylation patterns dynamically change to establish the specific patterns of tissues, making it a susceptible stage to epigenetic dysregulation, becoming a risk of developing diseases. Modifications in the methylation of promoter regions, particularly of repair genes, change their expression, and consequently alter the mechanisms of DNA repair. Mexico City Metropolitan Area (MCMA) is one of the most industrialized, populated, and polluted in the country. Previous studies have shown the presence of oxidizing pollutants in the air of this region, such as metals, as well as genetic damage in children. Our interest was to investigate the impact of prenatal metal exposure on the global DNA methylation (LINE1) and specific DNA methylation in promoters of repair (OGG1 and PARP1) and antioxidant (Nrf2) genes (evaluated by pyrosequencing), as well as their mRNA levels (by q-PCR) in umbilical cord blood. A cross-sectional study was conducted in women residents of the MCMA who attended to a hospital of the IMSS (Mexican Institute of Social Security) by childbirth. The prenatal exposure to toxic and essential elements was determined by ICP-MS in maternal urine. The DNA methylation of LINE1, OGG1, PARP1, and Nrf2 was significantly associated with the concentrations of potentially toxic (As, Hg, Mn, Mo, and Pb) and essential (Cu, Se, and Zn) elements. We also observed changes in global and specific DNA methylation patterns due to the interaction between potentially toxic and essential elements. Additionally, an increase in the OGG1 methylation at sites CpG2, CpG3, and CpG4 was associated with decreased mRNA levels, likewise, the methylation at CpG5, CpG8, and CpG11 sites was associated with its reduced mRNA expression. In contrast, the CpG7 site of PARP1 was positively associated with its mRNA levels. No association was observed between the methylation of Nrf2 CpG sites and its expression. Our data suggest that changes in DNA methylation of specific promoters is influenced by prenatal exposure to metals, which may alter the expression of repair genes and, therefore, contribute to alterations in the DNA repair capacity of newborns and thereby in the development of future diseases. *Study supported by Conacyt-Mexico (Grants #233710 and #280296).*

PL 0597 Effect of Silver Nanoparticles on Neural Differentiation in Human iPSCs

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Silver nanoparticles (AgNPs) are widely used in consumer products, because of their anti-bacterial and anti-viral activities. However, AgNPs have been reported to cause various types of cytotoxicity, including developmental toxicity and neurotoxicity. The effects of AgNPs on early fetal development has not been well elucidated. In the present study, we examined the effects of AgNPs on neural differentiation in human induced pluripotent stem cells (iPSCs) as a model of human fetal stage. Exposure to AgNPs reduced the expression of several differentiation markers, such as Otx2, in iPSCs. Since neural differentiation requires ATP as a source of energy, we next examined the intracellular ATP content in iPSCs. AgNPs decreased intracellular ATP levels in iPSCs. We further examined mitochondrial dynamics, which is a critical function to keep homeostasis. As a result, AgNPs induced mitochondrial fragmentation and reduced the level of mitochondrial fusion protein mitofusin 1 (Mfn1). Moreover, knockdown of Mfn1 inhibited neural differentiation via Otx2 downregulation in iPSCs. Taken together, these data suggest that AgNPs induce cytotoxicity, including neurodevelopmental toxicity, via Mfn1-mediated mitochondrial dysfunction in iPSCs. Thus, mitochondrial function in iPSCs can be used for assessing the neurotoxicity associated with nanomaterials.

PL 0599 Establishment of a Reference Chemical Database to Evaluate *In Vitro* and *In Silico* Approaches to Assess Respiratory Sensitization to Facilitate Development of IATA

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Currently no single method or strategy is validated for the detection of respiratory sensitizers, despite public health and regulatory needs. An Adverse Outcome Pathway (AOP) for respiratory tract sensitization by low molecular weight organic chemicals has been published and is proceeding through the Organisation for Economic Co-operation and Development review process. The key events of the AOP facilitated identification of several promising *in vitro* and *in silico* test methods and combinations thereof which could form the bases for Integrated Approaches to Testing and Assessment (IATA); however most of these methods have only been assessed with a few well-known respiratory sensitizers (e.g., toluene diisocyanate or trimellitic anhydride). This presentation outlines our approach to build a comprehensive database which would serve as a list of reference chemicals to facilitate the assessment of testing approaches. We aim to cover a range of chemical classes and include "challenging" chemicals, such as respiratory sensitizers thought to elicit effects through dermal exposure and those for which specific-IgE has not been detected in humans. We first conducted a review of established structure-based profilers, recent literature, and human clinical reports, focusing on data verified in humans for translatability to human health outcomes. To allow for a weight of evidence analysis, we are collecting all data but qualifying the data to reflect quality. We are also making use of the Abstract Sifter literature review tool (Baker et al., 2017) to identify additional potential respiratory sensitizers. Briefly, a set of PubMed MeSH terms describing adverse effects (AEs) for 92 known sensitizers was used to query a large database of chemicals and AEs, yielding over 7000 chemicals of potential interest. Work is ongoing to identify query terms which yield the most relevant papers. The resulting chemical list, which will be shared with regulatory agencies and the public, is an important step towards the assessment of potential test methods and the creation of internationally-harmonized integrated testing approaches for the detection of chemical respiratory sensitizers. *Does not necessarily represent US EPA policy.* Baker N, Knudsen T and Williams A. Abstract Sifter: a comprehensive front-end system to PubMed [version 1; referees: 2 approved]. *F1000Research* 2017, 6(Chem Inf Sci):2164 (<https://doi.org/10.12688/f1000research.12865.1>).

PL 0643 Docosaheptaenoic Acid as a Prophylactic Treatment for Chronic Particle-Exposed Balb/c Mice

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There is a rising trend in the use of nanomaterials in various consumer products; increasing the risk of human exposures. Inflammation is a vital contributing factor to pulmonary diseases which can be triggered by exposure to airborne toxicants such as nanomaterials. However, there are no effective treatments for chronic inflammation. One potential treatment shown to have anti-inflammatory effects is the dietary supplement docosaheptaenoic acid (DHA); an omega-3 polyunsaturated fatty acid found in fish oil. This study investigated DHA as a prophylactic treatment for particle-induced chronic inflammation. Balb/c mice were fed a control diet or a 1% DHA-containing diet for a total of eighteen weeks. At weeks two, three, four, and five the mice were anesthetized and oropharyngeal instilled once a week with dispersion media (50 μ l) or a highly inflammatory multi-walled carbon nanotube (FA21; 50 μ g). Thirteen weeks after the last instillation lung histology, macrophage NLRP3 inflammasome activation (IL-1 β release) and lung lavage fluid (LLF) levels of lactate dehydrogenase (LDH) and protein levels were evaluated. Inflammatory cytokines were evaluated in both LLF and plasma of the mice. DHA's impact on macrophage phenotype was also examined. LLF levels of LDH and protein decreased in mice fed the DHA diet. Mice exposed to FA21 on the DHA diet had decreased pro-inflammatory cytokines in the LLF; including IL-1 β , TNF- α ,

and IFN- γ ; compared to the control diet-fed mice. There was also a decrease of IL-33 and an increase of IL-10 (a marker for M2 macrophage phenotype) in the plasma of these same mice. DHA could potentially be used as a prophylactic treatment since it was demonstrated to have a number of anti-inflammatory effects on Balb/c mice exposed to a common nanomaterial. *Work supported by F31 ES028100, R01 ES023209, R01 ES027353.*

PL 0655 Protective Effect of Resveratrol on the Integrity of Alveolar and Intestinal Epithelial Barrier in Staphylococcal Enterotoxin B-Induced Acute Lung Injury

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Acute lung injury (ALI) is a state of inflammation that breaks down the lung endothelial and epithelial cell barriers. Transepithelial migration of inflammatory cells into alveolar spaces and escape of normal alveolar microbiota into surrounding interstitial tissue causes crucial dysfunction in pulmonary system. Also, intestinal epithelial cell barrier plays a critical role in controlling homeostasis of internal environment. SEB, a superantigen and a toxin secreted by *Staphylococcus aureus*, is well-known for causing food poisoning and ALI. In the current study, we investigated the role of resveratrol (RES) in regulating the expression and functions of tight junction proteins (TJP) in epithelial cell responses following exposure to this superantigen. To this end, C3H mice were given resveratrol orally twice prior to intranasal challenge with SEB which causes ALI and is lethal. 16S rRNA results showed that there were microbes transported in the blood in addition to the lung and colonic tissues. For this purpose, we used a reporter *E.coli*-GFP labeled bacterium to monitor and examine the viability of this bacterium in case of leak from lung into blood and other body compartments during the peak of ALI. The flow cytometry results showed significant reduction in copy numbers of this reporter in the blood in RES-treated mice in comparison to vehicle-treated mice. However, bacteriological examination for viability by culture of the blood showed all were negative for all experimental groups. Mouse transcriptome array showed increase in TJP gene expression in colonic and alveolar epithelial cells of RES-treated mice. Some of these genes were validated with qPCR. We found that there was an increase in the expression of zonula occludens (Zo-1), occludin, E-cadherin and claudin genes following RES treatment. Commensals and probiotics are also known to decrease intestinal barrier dysfunction caused by inflammatory cytokines. Therefore, mass spectrometry (MS) analysis showed increase in the metabolome in colons of RES-treated group that help in strengthening of intestinal barrier such as L-tyrosine, L-tryptophan and L-cysteine metabolites. In conclusion, RES treatment increased directly or indirectly the rigidity of cell boundaries resulting in amelioration of ALI-dependent complications. *Supported by NIH P01AT003961, P20GM103641, R01AI129788 and R01AI123947.*

PL 0684 The Sex-Specific Effects of Arsenic on Immune Function, Infectious Disease Risk, and Vaccine Outcomes

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It is thought that environmental exposures contribute to the persistent global burden of (vaccine-preventable) infectious diseases. Inorganic arsenic (iAs), a potent carcinogen and immunotoxin, poses a risk to millions of people worldwide through contaminated drinking water and food. iAs increases the risk of gastrointestinal and respiratory tract infectious diseases, such as tuberculosis (TB) and influenza, often in a sex-dependent manner. Moreover, iAs is associated with reduced vaccine immune responses against varicella zoster and Bacillus Calmette-Guerin (BCG). However, the sex-specific molecular mechanisms explaining how arsenic increases infectious disease risk and impairs vaccine protection are unknown. We hypothesize that iAs causes a sex-specific immune imbalance leading to increased infectious disease risk and reduced vaccine immunogenicity. We focused on the effects of iAs on macrophages, innate immune cells known to influence TB pathogenesis. We observed dose- and sex-specific differences in nitric oxide (NO) and cytokine production by activated bone marrow-derived macrophages *in vitro* treated with iAs, in the form of sodium (meta)arsenite, either during differentiation or after maturation. We found similar changes in activated lung interstitial macrophages from mice exposed to iAs *in utero*. These results indicate that iAs alters innate immunity in a sex-specific manner and we are currently investigating how these alterations

influence TB risk. Using an influenza A virus (IAV) mouse model, we studied the effects of iAs exposure on the immune responses to natural IAV infections and vaccinations. We observed exacerbated disease severity, proinflammatory cytokine responses and mortality in iAs-exposed females compared to males upon primary IAV infection. Vaccinated females, but not males, also displayed decreased anti-IAV IgG antibody titers and neutralization capacity in following IAV challenge. We are currently investigating the mechanisms driving these immunomodulatory effects of iAs on antiviral immune responses and vaccine immunogenicity. Overall, our data clarifies how arsenic alters host immune responses, thereby influencing infectious disease risk as well as vaccine outcomes in exposed populations. *R00ES024808 (FS) and T32ES07141 (KR, SA, EI).*

PL 0685 THC Treatment Improved Neuronal Functions by Decreasing HIV gp120-Mediated Neurotoxicity

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HIV-associated neurocognitive disorders (HAND) including motor dysfunction, cognitive impairment and progressive neurodegeneration leading to morbidity are due to mainly infiltration of monocytes and increase in pro-inflammatory cytokines in the CNS. HIV proteins such as gp120 shed by infected microglia and macrophages are involved in triggering inflammation and neuronal damage associated with HAND. Tetrahydrocannabinol (THC) is one of the active ingredients found in *Cannabis sativa* that has psychotropic effects and is used to treat anorexia, nausea as well as an analgesic. In the current study, we investigated the therapeutic potential of THC on neurotoxic effects of immune cells. To this end, 7-month-old GFAP-gp120 transgenic (Tg) mice were treated intraperitoneally with vehicle or THC. THC treatment decreased total number of peripheral white blood cells including lymphocytes and monocytes. Furthermore, the anti-inflammatory myeloid-derived suppressor cells (MDSCs) were found in the brain tissue and peritoneal cavity following THC treatment. Additionally, T regulatory cells were increased in the brains of THC treated mice. Behavioral effects were also examined in vehicle- and THC-treated gp120 Tg mice. Thus, nociceptive responses were examined to heat stimuli by the hot plate test. We found that the latency period to the first sign of paw licking in vehicle-treated group was significantly shorter than in THC-treated gp120 Tg mice. Furthermore, the responses to mechanical stimuli were also examined by manual Von Frey test. The vehicle-treated Tg mice demonstrated lower threshold for paw withdrawal, licking or shaking of the paw in the von Frey test when compared to THC-treated group. Together, our data suggest that THC attenuates gp120 induction of neuroinflammation and neuronal damage by induction of anti-inflammatory response. *Supported by NIH grants P01AT003961, P20GM103641, R01AI129788, and R01AI123947 to PN and MN, and MoHESR fellowship for AKM.*

PL 0699 The Research of Health Risk Factors in the Field of Occupational Health and Environmental Toxicology in Laboratory Workplaces

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According to many health hazards in laboratory workplaces, the initial research was aimed to study the health risk factors of laboratory workplace to establish the policy recommendation and the development of the information of occupational health and safety and environmental toxicology of laboratory workplace to manage health impacts which led to prevent and control of diseases of workers and people in community. The methodology was proceeded through out the Thai fiscal year of 2009 (October 2008 to September 2009) to study of health hazards, occupational health and safety, and environmental toxicology of five kinds of the specific sampling laboratory workplaces located in 10 provinces in Thailand. The five kinds of laboratory were governmental high school laboratory, governmental graduate school laboratory, governmental hospital laboratory, non-governmental factorial laboratory, and governmental official laboratory. The walk through survey, questionnaire, and interviewing the one manager, one head of production line and 28 workers of production lines were proceeded. The obtained data were analyzed and interpreted by the descriptive statistic. The percentage of results were presented. The results showed 80 percent of specific sampling laboratory workplaces had no any occupational health process, waste management, safety measures and legislation to manage the laboratory workplace. At the present time the results of the research

leading to establish the guideline of the occupational health and safety and environmental toxicological management and the recommendation of health examination by risk should be processed for prevention and control of diseases and danger from laboratory. However, the policy recommendation and the development of the occupational health and safety and environmental toxicology of laboratory workplace to manage health impacts concerning with prevention and control of diseases of workers and people in community should be further concerned.

PL 0700 Effect of MLL Modified H3K4me3 on Aluminum Induced Cognitive Impairment—Both Population and Animal Epigenetic Studies

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Epigenetic modifications play critical roles in cognitive function. Brain-derived neurotrophic factor (BDNF) is involved in synaptic plasticity and may be modified by tri-methyl histone H3 lysine residues 4 points (H3K4me3), which may be modified by mixed-lineage leukemia protein (MLL), a zinc finger-rich enzyme, thus affecting cognition. This study aims to explore mechanism of this epigenetic modification. 1. 235 male Al-exposed workers were recruited. An occupational epidemiological investigation questionnaire and cognitive tests were performed. The contents of H3K4me3 in lymphocyte and BDNF in plasma were determined by enzyme-linked immunosorbent assay. 2. 24 healthy SD male rats were randomly divided into four groups by weight. The rats drank water containing different doses of aluminum chloride (AlCl₃) (0, 2, 12, and 72mg/kg Al³⁺) for 120d. The neurobehavior of animals was tested, and expression of H3K4me2 and MLL was detected with western blot. With the increasing of blood aluminum level, the cognitive function of Al-exposed workers decreased, the expression levels of H3K4me3 decreased, and BDNF decreased. Multiple correlation analysis showed that blood aluminum concentration was negatively correlated to H3K4me3, BDNF, and cognitive function, respectively. With the Al dose increasing, the neurobehavior of animals decreased, the expression of MLL and H3K4me3 decreased too. Aluminum inhibits MLL by replacing zinc, then the activity of MLL decreases, the methylation of H3K4 increases, the expression of H3K4me3 decreases, then BDNF decreases. *References: [1] Anna Cascante, Susanne Klum, Moumita Biswas, Beatriz Antolin-Fontes, Fanie Barnabé-Heider and Ola Hermanson (2014): Gene-specific methylation control of H3K9 and H3K36 on neurotrophic BDNF versus astroglial GFAP genes by KDM4A/C regulates neural stem cell differentiation [J] Mol Biol, (426):3467–3477. [2] Black, J.C., Van Rechem, and J.R. Whetstone (2012). Histone lysine methylation dynamics: establishment, regulation, and biological impact [J] Molecular Cell, 48(4): 491-507. [3] Qiu J, Shi G, Jia Y, Li J, Wu M, Li J, Dong S and Wong J (2010). The X-linked mental retardation gene PHF8 is a histone demethylase involved in neuronal differentiation. Cell Res, 20(8): 908-18. This work is supported by NSFC 81430078, 81372968.*

P101 - 0073 In Vitro Buccal Membrane Absorption (IVBMA) Model for Tobacco Constituent Testing: Membrane Integrity Assays

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Assays which provide clear information regarding the influence of tobacco harmful and potentially harmful constituents (HPHCs) on buccal permeability and absorption via the buccal pathway are sparse in published, peer-review literature. Therefore, an *in vitro* buccal membrane absorption (IVBMA) model was developed by adapting *in vitro* dermal absorption system methodologies using porcine buccal mucosa as a tissue surrogate for human buccal mucosa given similarities in morphology and permeability. Membrane integrity assays were compared to use for validating test tissues. Porcine buccal mucosa was isolated from connective tissue and cut with a dermatome to a thickness of 400-500 μm . Buccal mucosa was mounted in flow-through diffusion cells and saliva permeability coefficients (Kp) were measured using tritiated artificial saliva, pH 7.7. The average baseline Kp value for porcine buccal mucosa using tritiated saliva was $3.15 \times 10^{-2} \text{ cm/h}$ ($n = 4$). Buccal Kp values were compared to porcine and human dermal Kp values. The average baseline Kp value for porcine and human skin using tritiated saliva was $3.7 \times 10^{-3} \text{ cm/h}$ ($n = 3$) and $2.7 \times 10^{-3} \text{ cm/h}$ ($n=3$), respectively. Saliva Kp values were compared to transepidermal water loss (TEWL) and transepithelial electrical resistance (TEER) measurements to determine baseline criteria for the IVBMA and dermal tissues. Buccal mucosa mounted on transwell plates exhibited average normalized TEER levels of $86.60 \Omega\text{cm}^2$, compared to $78.35 \Omega\text{cm}^2$ for porcine skin and $78.58 \Omega\text{cm}^2$ for human skin. TEWL values for the buccal mucosa averaged $85.16 \text{ g/m}^2/\text{h}$, compared to 40.9 and $6.14 \text{ g/m}^2/\text{h}$ for porcine and human skin, respectively. Saliva Kp values were about 10-times higher in buccal mucosa compared to skin (porcine and human). TEWL values were about 2-times higher in buccal mucosa compared to porcine skin and 10-times higher than human skin. TEER values did not seem to correlate with changes in Kp values across tissues. Therefore, TEWL may be a better measure of membrane integrity than TEER for this model and tissues. Supported in part by the Research Participation Program at the US FDA, administered by the Oak Ridge Institute for Science and Education.

P102 - 0162 The Interchangeability of In Vitro Key Event-Based Skin Sensitization Assays—Impact on Defined Approaches

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There are a growing number of *in chemico/in vitro* assays that measure skin sensitization, driven by a global move away from animal testing. These assays are linked to particular key events in the skin sensitization adverse outcome pathway. Currently there are OECD test guidelines covering the Direct Peptide Reactivity Assay (DPRA), which measures the molecular initiating event (covalent binding to proteins), the KeratinoSens and LuSens assays, which measure key event 2 (keratinocyte activation), and the h-CLAT, U-SENS, and IL-8 Luc assays, which measure key event 3 (dendritic cell activation). Each assay has an intrinsic reproducibility, which are reported to range between 75% and 100%. Where multiple assays cover the same key event, there is also the question of how interchangeable the different tests are. It is important to gauge the magnitude of the interchangeability, as it will impact the uncertainty of both the individual assay predictions as well as any predictions resulting from defined approaches that are based on these test results. This research aimed to collect publicly available *in chemico/in vitro* skin sensitization data for chemicals tested in the 6 assays that currently feature in OECD guidelines, and to analyze the interchangeability between different assays covering the same key event. To this end data were collected for 394 unique chemicals variously tested in the DPRA, KeratinoSens, LuSens, h-CLAT, U-SENS, and IL-8 Luc assays. The interchangeability between assays covering key event 2 was calculated to be 85%, and the interchangeability between assays covering key event 3 was found to range between 74% and 92%. In a worst-case scenario, these figures could lead to hazard classifications differing for approximately one-third of compounds evaluated in a number of published defined approaches. Assay interchangeability will have an impact on key event-based hazard predictions, and any uncertainty will be propagated when assay results are combined within a defined approach. This analysis could be useful when characterizing the uncertainty remaining in skin sensitization predictions using nonanimal approaches, which is an important part of the process to gaining acceptance in these emerging methods.

P103 - 0219 Transcriptional Profiling of Cytochrome P450 Genes in Adult Liver and in Whole Embryo Fry of Zebrafish in Some Developmental Stages

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Increasing use of zebrafish in biomedical, toxicological, and developmental research requires explicit knowledge of cytochrome P450 (CYP), which plays the central role in oxidative biotransformation of xenobiotics and many regulatory molecules. Here we established a comprehensive picture of CYP gene expression in adult zebrafish liver and whole embryo fry of zebrafish (4–144 hours post fertilization [hpf]) using qPCR techniques for 14 CYPs, including CYP1A, 1B1, 1C1, 1C2, 1D1, 2A2, 2K18, 2N13, 2R1, 2Y3, 3A65, 3C1, 3C2/3, and 3C4. In both sexes of adult zebrafish liver, CYP1A, 2A2, 3C1, and 2Y3 are major CYP genes. Significant sex difference in the transcript levels of some CYPs (mostly CYP1s) was identified in the adult liver. Fourteen CYPs showed various changes in their expression pattern during development. Most CYP transcripts abruptly increased after the occurrence of the liver around 96 hpf; however, some of CYP1s (1B1, 1C1 and 2, 1D1) and CYP2R1 reached maximum level before 72 hpf, suggesting extrahepatic expressions. Surprisingly, transcript levels of most CYPs except CYP1A, 2A2, 2K18, 2R1, 2Y3, and 3C1 in whole sac fry were rather higher or comparable to those of adult liver. The present study identified several “orphan” CYP genes as dominant isozymes at transcript levels, implying crucial roles of these CYP genes in development, physiology, and drug metabolism in zebrafish. The current results establish a foundation for studies with zebrafish in drug discovery and toxicology.

P104 - 0253 Evaluation of Skin Barrier Protection Effect of *Lactobacillus rhamnosus* GG Using 3D Reconstructed Skin Model

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The main function of the skin is to protect the body from the environment. Skin is the physical and chemical barrier against allergens, irritants, and microbes. If the skin barrier is disrupted, it may exacerbate the symptoms of dermatologic patients. *Lactobacillus rhamnosus* GG (LGG, ATCC 53103) is one of the most widely used probiotic strains. Various benefits of *Lactobacillus rhamnosus* GG have been reported especially in gastro-intestinal diseases and immune system. However, the effect of *Lactobacillus rhamnosus* GG in the skin barrier has not been studied yet. In this study, we aimed to evaluate the effect of *Lactobacillus rhamnosus* GG on the skin barrier using 3D reconstructed human epidermis model. The skin barrier protection effects were determined in Keraskin model using WST-1 assay and MTT assay. *Lactobacillus rhamnosus* GG was applied to both normal skin model and sodium lauryl sulfate (SLS)-induced perturbed skin model. The tissue viability of both normal skin model and disturbed skin model is increased in *Lactobacillus rhamnosus* GG applied tissues. After cell viability assay, Keraskin tissues were fixed with 4% paraformaldehyde and processed for immunohistochemistry staining. The results showed that *Lactobacillus rhamnosus* GG have protective effects on the skin barrier.

P105 - 0274 Using *C. elegans* for Predictive Oral Toxicity Assessments

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New toxicological tools that better predict human response with reduced time and expense ratios will allow increased evaluation of individual compounds of concern as well as increased toxicity testing of mixtures. The 2016 update to the Toxic Substances Control Act mandates that US federal agencies develop leading edge predictive toxicology methods that can replace the use of vertebrate models. The US Food and Drug Administration's Predictive Toxicology Roadmap supports the integration of emerging methods and new technologies into regulatory safety and risk assessments. Many pathways involved in organismal development, neuronal function, and cellular metabolism are conserved from worms to people. The digestive tract of the *Caenorhabditis elegans* nematode has several features that are analogous to the mammalian digestive system, making this simple model organism a potential candidate for predictive oral toxicity testing. *C. elegans* studies have demonstrated con-

cordance for developmental toxicity or altered motor activity when exposed to mammalian developmental toxins or neurotoxins. We have designed a novel worm Development and Activity Test (wDAT) that maps the timing of *C. elegans* developmental milestone acquisition as well as stage-specific activity levels. The wDAT was able to detect both developmental delay and hyperactivity for arsenic, lead, and mercury, developmental neurotoxins that have been associated with hyperactivity in children. Binary mixtures of arsenic, lead, and mercury produced at most additive effects on developmental delay and/or hyperactivity; no synergistic effects were detected. The wDAT can be completed by a single technician in 4 days using a relatively inexpensive activity tracker, features that would make it a cost-effective addition to integrated approaches to toxicity testing. A planned 20-compound, blinded qualification study will help determine how the wDAT might provide "fit-for-use" data to support developmental neurotoxicity testing strategies. We are also developing an egg versus sperm reproductive toxicity assay, as well as higher-throughput methods for the detection of pathway of toxicity perturbations. Initial results indicate that for *C. elegans*, as in mammals, arsenic is a male reproductive toxin and that oxidative stress is a key mode of action for arsenic toxicity.

PS P106 - 0353 Cytotoxicity Differences of Troglitazone on HepG2 and CD133 Liver Cancer Stem Cells

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The objective was isolation and identification CD133 liver cancer stem cells in HepG2 cells, to investigate the cytotoxicity of troglitazone on HepG2 and its liver stem cells. The CD133-positive and negative population in the HepG2 cell line were sorted by flow cytometry; the cells of CD133 sphere formation, colony formation assay and Transwell invasion migration assay, BALB/c nude mice *in vivo* tumor formation are used to identify proliferative capacity; liver cancer stem cells drug resistance was evaluated by MTT; the toxicity of Tro to CD133 liver cancer stem cells was detected by MTT assay, Automatic biochemical analyzer was used to detect the contents of AST, LDH, TP, ALB and BUN changes in cell supernatants after administration. The effect of Tro on the total activity of CYP and the level of ROS was examined by fluorescence method to compare the cytotoxicity differences. CD133 expression in liver cancer stem cells were found to be $(0.72 \pm 0.05)\%$ in HepG2 and 98.7% in CD133⁺ cells; CD133, c-Myc and Oct4 protein in CD133⁺ cells were higher than those in parental cells. Sphere formation, colony formation assay and Transwell invasion migration assay of CD133⁺ cells ($P < 0.05$, $P < 0.01$), compared with HepG2 group, the ability of CD133⁺ cell tumor formation was significantly increased ($P < 0.01$). At the same time, CD133⁺ cells were mostly in G0/G1 phase, G2/M phase was not blocked, and the drug resistance of sorafenib was higher ($P < 0.01$). Tro treatment 12 h, 24 h, 48 h, the IC₅₀ of liver cancer stem cells was $(150.52 \pm 1.25) \mu\text{mol/L}$, $(99.08 \pm 1.90) \mu\text{mol/L}$, $(43.96 \pm 0.71) \mu\text{mol/L}$ and $(14.81 \pm 1.30) \mu\text{mol/L}$ respectively, which was significantly lower than that of HepG2 ($P < 0.01$), with a significant dose-effect relationship. TP, LDH, ALB and BUN in liver cancer stem cells treated with $80 \mu\text{mol/L}$ Tro for 48 h increased significantly, and the total activity of CYP ($P < 0.05$) and ROS ($P < 0.05$) were significantly inhibited. Successful screening and identification of highly proliferative CD133⁺ HepG2 tumor stem cells are more sensitive than HepG2 cells in showing liver cytotoxicity, and show significant cytotoxicity difference, which provides a new idea for the study of CD133⁺ HepG2 cells specific toxicity.

PS P107 - 0367 Novel Deep Learning Methodology to Enable Drug-Induced Abnormality Detection Using Cultured Neurons on Microelectrode Arrays

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Drug-induced seizures are a major cause of clinical failure and drug withdrawal from the market. Despite this common safety issue, preclinical tools to predict seizure liability are not widely validated. A reliable non-clinical, *in vitro* model to predict seizure liability of drug candidates would enable earlier identification of liable compounds and support improved drug development. This study was designed to validate the use of cultured neurons on microelectrode arrays (MEAs) for seizure liability prediction and to develop more efficient analytical methods. We treated cultures with compounds with defined seizure liability profiles and used the neuronal activity profiles to identify activity signatures associating with specific liability profiles with the goal of using those known

profiles to identify other unknown compound liabilities. Here we show how our novel deep learning algorithm can extract numerical data from neuronal activity profiles on MEAs and enable effective seizure liability prediction. Primary cortical neurons were prepared from fetal Wistar rats, 18 days post-coitum, and then seeded on 24-well MED-Q2430M plates for extracellular recording using MED64-Presto. 5 concentrations of 12 compounds (pentylenetetrazole, picrotoxin, 4-aminopyridine, linopyridine, amoxapine, strychnine, pilocarpine, amoxicillin, chlorpromazine, enoxacin, phenytoin and acetaminophen) were added to the cells ($n = 6$) to see concentration-dependent phenotypic changes on spontaneous electrical activity in neural networks at 19 days *in vitro*. We developed a deep neural network algorithm entrained to drug untreated data that can predict the future window (several milliseconds) from the past window (several hundred milliseconds). After the training, the algorithm tested to see if it could accurately predict the liability profile of the compound. We compared the data from our manual analysis with the deep learning algorithm (quantified by mean squared error (MSE)) to determine the accuracy of the algorithm to detect the compound activity profile. For the 12 compounds tested, the algorithm correctly reported the liability profile of the compound. All of the MSE time-course patterns were consistent with synchronized burst time-course patterns from spike train data analysed manually. Our deep learning methodology can detect drug-induced abnormalities in synchronized bursting of neuronal networks recorded cultured on MEAs.

PS P108 - 0369 Tris(1,3-Dichloro-2-Propyl) Phosphate Exposure during Early Development Alters the Normal Trajectory of Zebrafish Embryogenesis

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Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) is a high-production volume organophosphate flame retardant used in residential and office furniture, automobile seats and other commonly-used products. Due to leaching from end-use products into indoor dust and subsequent detection in neonatal and postnatal microenvironments, concerns remain about its potential impacts on development. Using zebrafish as a model, we showed that initiation of TDCIPP exposure during early development induced severe gastrulation defects at 6 h post fertilization (hpf), represented by a delay or arrest in epiboly; these phenotypes were the most severe when exposures were initiated within the cleavage/early-blastula stages (0.75-3 hpf). Later in development (~24 hpf), TDCIPP-exposed embryos were dorsalized, mimicking the effects of dorso-morphin (DMP), a bone morphogenetic protein (BMP) pathway antagonist. Pre-treatment with 4'-hydroxychalcone—a BMP agonist—partially blocked these effects, suggesting that TDCIPP-induced abnormalities are driven by BMP signaling inhibition. However, mRNA-sequencing and *in situ* localization of p-SMAD1/5/9 (a BMP-specific readout) revealed that, unlike DMP, there were minimal impacts of TDCIPP on BMP signaling. In addition, TDCIPP exposure resulted in decreased levels of transcripts regulating mesoderm differentiation (tbx16, tbx6, tbx6l, msgn1) and disruption of TBX16 (a mesodermal marker) localization *in situ*, suggesting that TDCIPP directly impacts mesodermal integrity. Therefore, we evaluated mesoderm-derived embryonic structures and found that TDCIPP-exposed embryos displayed (1) notochord and somite malformations; (2) decreased red blood cell abundance; and (3) abnormal cardiac development—phenotypic data that were also associated with alterations in transcripts related to heart morphogenesis and hematopoiesis. Overall, our results suggest that initiation of TDCIPP exposure within the first 3 h of development lead to downstream impacts on epiboly, germ layer formation, and organogenesis. Therefore, our ongoing studies are focused on identifying potential mechanisms underlying TDCIPP-induced epiboly defects. Based on our most recent data, we have identified two nuclear receptor ligands—ciglitazone (a peroxisome proliferator-activated receptor γ , or PPAR γ , agonist) and 17 β -estradiol (an estrogen receptor α , or ER α , agonist)—that partially block TDCIPP-induced epiboly defects. Therefore, we are actively investigating the potential role and interplay of nuclear receptors (PPAR γ and ER α), epigenetic reprogramming, and/or embryonic lipidome in mediating TDCIPP-induced epiboly defects.

PS P109 - 0373 A β -Galactosidase-Expressing *E. coli* Culture as an Alternative Test to Identify Skin Sensitizers and Nonsensitizers

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Although the Organisation for Economic Co-operation and Development has adopted several *in vitro* methods with reasonable predictive capacity, alternative methods to identify skin sensitizers and nonsensitizers with sufficient reliability and simplicity are still required for more efficient and economic prediction. The present study was to design an *in vitro* system with the use of a β -galactosidase-expressing *E. coli* culture for simpler but sufficiently accurate classification of skin sensitizers and nonsensitizers. A *LacZ* gene-containing *E. coli* strain that is capable of producing β -galactosidase enzyme was induced by isopropyl β -D-1-thiogalactopyranoside with concomitant treatment with test chemicals. After 6-hr incubation, cells were lysed and β -galactosidase enzyme activity was monitored colorimetrically by using O-nitrophenyl-D-galactopyranoside as a substrate. Following optimization of several experimental conditions, 22 skin sensitizers and 11 nonsensitizers were examined to assess predictive capacity of this method. The results indicated that predictivity was as follows: 90.9% sensitivity, 81.8% specificity, and 87.9% accuracy, when 17.3% of control activity was used as the cut-off value to separate sensitizers from nonsensitizers. The results suggested that the current bacterial system expressing β -galactosidase may serve as a useful alternative test for classifying skin sensitizers and nonsensitizers, without the utilization of animals or mammalian cell cultures. *Supported by the grant from National Research Foundation, Korea (NRF-2017RIDIA3B04033313).*

PS P110 - 0381 A Simple *In Chemico* Method for Testing Skin-Sensitizing Potential of Chemicals Using Small Endogenous Molecules

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Among many of the validated methods for testing skin sensitization, direct peptide reactivity assay (DPRA) employs no cells or animals. Although no immune cells are involved in this assay, it reliably predicts the skin sensitization potential of a chemical *in chemico*. Herein, a new test method was developed using endogenous small-molecular-weight compounds, cysteamine and glutathione, rather than synthetic peptides, to identify skin sensitizers and non-sensitizers with an accuracy as high as DPRA. The percent depletion of cysteamine and glutathione by test chemicals was measured by an HPLC equipped with a PDA detector. To detect small-size molecules, such as cysteamine and glutathione, a derivatization by 4-(4-dimethylaminophenylazo) benzenesulfonyl chloride (DABS-Cl) was employed prior to the HPLC analysis. Following optimization of the test method, a cut-off criterion of 7.14% depletion was applied to differentiate skin sensitizers from non-sensitizers in combination of the ratio of 1:25 for cysteamine:test chemical with 1:50 for glutathione:test chemical for the best predictivity among various single or combination conditions. Although overlapping HPLC peaks could not be fully resolved for some test chemicals, high levels of sensitivity (100.0%), specificity (81.8%), and accuracy (93.3%) were obtained for 30 chemicals tested, which were comparable or better than those achieved with DPRA. *Supported by the grant from National Research Foundation, Korea (NRF-2017RIDIA3B04033313).*

PS P111 - 0382 PM_{2.5} Induced Adverse Effect on Life Span of *Caenorhabditis elegans*

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Exposure to atmospheric fine particulate matter (PM_{2.5}) could cause adverse effects on health. In this study, water-soluble components of outdoor PM_{2.5} samples collected in the north of China in winter were used for the study of acute and chronic toxic effects (including life span) of *Caenorhabditis elegans*. On this basis, longevity-related genes specific mutants and real-time PCR, RNA-sequencing technology were utilized to explore the mechanism. Three groups were set up including blank control group (ddH₂O), PM_{2.5} aqueous solution group A (air mass index AQI $\leq$ 100) and group B (100 < AQI $\leq$ 200). The final concentrations of PM_{2.5} in group A and B were 94 and 119 μ g / mL, respectively. After treatment with PM_{2.5} for 24h, acute lethal toxicity to *C. elegans* was not

found, whereas pharyngeal pump movement was reduced by 30%, body length and body width were reduced by 8% and 6% respectively in group B. In the chronic toxicity study (5 & 10 days exposure), the average survival time were shortened by 26% (group A) and 31% (group B) in the heat stress state. The whole life cycle treatment of PM_{2.5} showed that the average life span of *C. elegans* was shortened by 11% (group A) and 13% (group B), suggesting that PM_{2.5} might affect lifespan by reducing *C. elegans* thermal tolerance. The mechanism investigation showed that 5 day treatment of PM_{2.5} could down-regulate the expression of *daf-16* and *hsp-16.2* in nematode by PCR, PM_{2.5} treatment shortened average life span by about 1/10 in wild type *C. elegans* group and about 1/3 in DAF-2 mutant group. Moreover, left shift of the survival curve was observed in DAF-16 mutant group. These suggested that the effect of PM_{2.5} on *C. elegans* longevity may be mediated by insulin signaling pathway. In addition, RNA-sequencing test screened out 31 differential expression genes in 5-day PM_{2.5} exposure, and the enrichment of 24 genes in 29 GO terms were statistically significant, indicating that above effect of PM_{2.5} could related to lipid metabolism and transport, as well as intestinal ROS production of nematodes. *Supported by NSFC in China #81273108 & #81641184, The capital health research and development of special Project #2011-1013-03.*

PS P112 - 0409 Effects of Cyanobacterial Bloom Metabolites on NSC Survival and Differentiation *In Vitro*

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Cyanobacterial blooms are occurring more frequently with climate change and eutrophication of waters. Besides a large variety of bioactive secondary metabolites produced by cyanobacteria, there is an increasing evidence of the ubiquitous production of retinoid compounds that have already been shown to cause teratogenicity in aquatic animals but are indispensable for vertebrate development. Another highly abundant class of compounds are microcystins, cyclic heptapeptides that are suggested to be involved in many of the bloom-linked health effects and are detected in brain tissue. Finally, the neurotoxic anatoxin-a, also known as very fast death factor, is responsible for early neurological symptoms like respiratory paralysis. Humans can be exposed to these compounds either during recreational water activities or when drinking water is derived from cyanobacteria infested freshwater bodies. Either way, exposure is most likely to occur to a mixture of bioactive compounds co-occurring in the cyanobacterial bloom. To investigate neurotoxic effects and altered neural differentiation in a human-health relevant model, we used *in vitro* culture of human pluripotent stem cell-derived neural stem cells (NSCs). Acute toxicity was assessed upon 4-day exposure of NSCs to single bioactive cyanobacterial compounds (all-trans retinoic acid - atRA, microcystins, anatoxin-a) and mixtures defined based on their environmental co-occurrence, from which IC50 and IC10 values were derived. Prioritized compounds/mixtures were further tested in a 21 day-long differentiation assay when NSCs are induced to form neurons and glia. Endpoints evaluated were neurite outgrowth, neuron:glia-ratio, and the expression of differentiation-specific markers, including β III-tubulin, MAP2, NESTIN and others. Our results reveal altered neurodifferentiation patterns of NSCs upon exposure to the studied compounds in the nanomolar range; the most pronounced effects were observed with atRA—the most potent representative of the retinoid group. Furthermore, the endpoints examined in this study address key events in adverse outcome pathways (AOPs) on developmental neurotoxicity and may contribute to the development and validation of novel *in vitro* developmental neurotoxicity models for regulatory purposes. Our observations are in line with previous *in vitro* results on the teratogenicity of retinoids from cyanobacterial blooms on the early development of *X. laevis* (African clawed frog) and *D. rerio* (zebrafish), strengthening the need for further investigation of cyanobacterial bloom teratogenicity for environmental and human health risk assessment.

PS P113 - 0432 Effects of Electrical Stimulation on Human iPSC-CMs Responses to Cardiac Ion Channel Blockers

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Human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) have been widely used in assessing cardiac safety profiles of new drug candidates, particularly for drug-induced proarrhythmia. However, as iPSC-CMs are firing spontaneously, the variable beating rates among wells and replicate assay plates can be a confounding factor that interferes with data interpretation. In

this study, we compared the response of human iPSC-CMs, specifically iCell², to the challenges of classic cardiac ion channel blockers including lidocaine, dofetilide, nifedipine, JNJ303, ivabradine, and 10 CiPA compounds under spontaneous beating and electrical stimulation (E-pacing, 1-2 Hz) conditions, utilizing the Axion Maestro E-Stim plates. We found that compared with the responses of spontaneous beating iCell² cardiomyocytes, (1) E-pacing shortened rate-corrected field potential duration (FPDc) in a stimulation rate-dependent manner; (2) E-pacing reduced well-to-well and plate-to-plate variabilities; (3) E-pacing attenuated repolarization effects of I_{Kc} channel blockers and revealed reverse use dependence of dofetilide; (4) E-pacing eliminated effects of multiple compounds on spike amplitude including I_{Na} blocker lidocaine; (5) E-pacing rate-dependently attenuated the sensitivity of I_{Ks} channel blocker JNJ303-induced prolongation of FPDc; (6) Effects of E-pacing on iCell² responses to I_{CaL} channel blocker nifedipine were limited; (7) Under spontaneous beating condition, I_r blocker ivabradine dose-dependently prolonged FPD, but there were no prolongations of FPDc after rate correction. Whereas under E-pacing (1Hz), ivabradine dose-dependently prolonged FPD and FPDc, although the effects were less than 10%. In conclusion, analyzing the responses of human iPSC-CMs in both spontaneous beating and E-pacing conditions may help better assess the effects of test compounds on cardiac electrophysiology.

PS P114 - 0445 mRNA-Sequencing Identifies Liver as a Potential Target Organ for Triphenyl Phosphate in Embryonic Zebrafish

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Triphenyl phosphate (TPHP) is a commonly used organophosphate flame retardant and plasticizer in the United States. Over the past decade, there has been a marked increase in the use of TPHP due to the phase-out of certain brominated flame retardants. Using zebrafish as a model, previous studies have shown that TPHP exposure from 24 to 72 hours post fertilization (hpf) results in severe cardiac looping defects by 72 hpf—a phenotype that is dependent on exposure during pharyngula (24-48 hpf) and mitigated by pre-treatment with non-toxic concentrations of a pan-retinoic acid receptor (RAR) agonist (fenretinide). Therefore, the objectives of this study were to (1) rely on mRNA-sequencing to identify pathways before and after cardiac looping (30 and 48 hpf, respectively) that may be impacted following exposure to 10 μ M TPHP from 24-48 hpf and (2) determine whether pre-treatment with 2 μ M fenretinide from 24-30 hpf mitigates cardiotoxicity-related pathways within embryos exposed to TPHP from 30-48 hpf. Based on mRNA-sequencing, TPHP exposure from 24 to 30 hpf and 24 to 48 hpf significantly affected the abundance of 305 and 274 transcripts, respectively, relative to vehicle (0.1% DMSO) controls. In addition to minor effects on cardiotoxicity- and nephrotoxicity-related pathways, Ingenuity Pathway Analysis (IPA) of significantly affected transcripts from 30- and 48-hpf embryos revealed that hepatotoxicity-related pathways were strongly affected following exposure to TPHP alone. Moreover, while pre-treatment with fenretinide mitigated TPHP-induced cardiac looping defects at 72 hpf, IPA revealed that fenretinide was unable to block TPHP-induced effects on hepatotoxicity-related pathways at 30 and 48 hpf, suggesting that, unlike the heart, TPHP-induced hepatotoxicity may be RAR-independent. Overall, our mRNA-sequencing-based data suggest that, in addition to the heart, the embryonic liver may be highly susceptible to TPHP exposure during early development. Therefore, our ongoing studies in zebrafish embryos are focused on (1) confirming that TPHP exposure leads to phenotypic indicators of hepatic injury (based on Oil Red O staining) and (2) utilizing CRISPR/Cas9-mediated genome editing and morpholino knockdown to determine if TPHP-induced hepatotoxicity is dependent on peroxisome proliferator-activated receptor (PPAR) activation, as TPHP is a potent agonist of human PPAR γ .

PS P115 - 0503 Inhibitory Effect of Acremonidin E on Melanogenesis Using B16F10 Melanoma Cells and Pigmented 3D Human Skin Model

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Epidermal or dermal pigmentation is a skin disorder produced from excessively accumulated melanins from hyperactivated melanocytes. Various chemicals have been reported as a therapeutic agent for hyperpigmentary disorders that manifests as melasma, post-inflammatory hyperpigmentation, melanoderma, solar lentigo, or freckles. Here, the inhibitory effect of Acremonidin E investigated on melanogenesis in the murine melanoma cell line, B16F10, and human melanoma cell line, MNT-1, as well as a pigmented 3D-human skin model.

Acremonidin E treatment decreased melanin contents in a dose-dependent manner in α -melanocyte stimulating hormone (α -MSH)-stimulated B16F10 cells. Quantitative real time PCR (qRT-PCR) demonstrated that treatment with Acremonidin E down-regulated several melanogenic genes, including tyrosinase, tyrosinase-related protein 1 (TRP-1), and tyrosinase-related protein 2 (TRP-2) while their enzymatic activities were unaffected. The anti-melanogenic effects of Acremonidin E were further demonstrated in a pigmented 3D human epidermal skin model, Melanoderm, and manifested as whitening and regression of melanocyte activation in the tissue.

PS P116 - 0508 Employment of Cytological Assessment of Skin Irritancy for 3D Reconstructed Human Epidermis Model

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Reconstructed human epidermis model uses tissue viability to determine skin irritancy of test chemicals. Generally, colorimetric MTT assay is used but it accompanies issues of color interference from test chemicals. To avoid it, HPLC separation of formazan dye, or histologic analysis has been tried as a new or cross-check assessment method, but they have respective limitation such as cost or limited and subjective representation of irritancy. Here, cytology is being examined as an alternative assessment for skin irritancy. Skin irritation of six chemicals (diethyl phthalate, allyl phenoxy acetate, 1-bromohexane, di-n-propyldisulphide, hexyl salicylate, 5% potassium hydroxide) was assessed on a reconstructed human epidermis. The epidermis viability was determined in Keraskin model using MTT assay. An irritant is predicted if the mean relative tissue viability of three individual tissues exposed to the test substances is reduced below 50% of the mean viability of the negative controls. 1-Bromohexane, di-n-propyldisulphide and 5% potassium hydroxide were categorized as irritants, and diethyl phthalate, allyl phenoxy acetate and hexyl salicylate were categorized as non-irritants. When the Keraskin tissue remaining after MTT formazan extraction was processed and stained with hematoxylin and eosin, the MTT result could be further confirmed. Cells were separated from Keraskin tissues without MTT assay were separated cells from the tissue and proceeded for cytology. The results were compared with MTT assay and histological analysis. The tissues treat with 1-bromohexane, di-n-propyldisulphide and 5% potassium hydroxide showed decreased density of stratum corneum and vacuolization occurred in some parts of basal layer in histology. Furthermore, they not only had few cytopinized cells, but also had a very irregular and abnormal cell shape. In the case of non-irritants except hexyl salicylate, the distinction between nucleus and cytoplasm was clear. Our study showed the possibility of cytology as an alternative assessment for skin irritancy.

PS P117 - 0519 Altered Lipid Profiles in the Skin and Liver of db/db Mice

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To investigate the skin disorders of diabetes mellitus (DM), we investigated the lipid profile of the skin epidermis of db/db mice, a type II DM model, and compared with those in the liver and kidney. We compared the histology and lipid profiles of the skin of db/db mice and db/m. To characterize the lipid alteration, we quantitated 49 lipids (34 ceramides, 14 free fatty acids (FFAs) and cholesterol) in the skin epidermis, liver and kidney using UPLC-MS/MS. Expression of genes involved in the lipid synthesis was also evaluated. With the full establishment of hyperglycemia at the age of 20 weeks, remarkable lipid enrichment was noted in the skin of db/db especially at epidermis and subcutaneous fat bed. UPLC-MS/MS quantitation revealed prominent increases of the ceramides and FFAs (> 3 fold) with medium-chains (< C26) in the skin epidermis as well as liver whereas those with very long chains were not affected. In the kidney, significant but only slight increases (< 3 fold) were observed for 16NS, 18NS, 20NS, 26NDS, C26FA, and C22:1FA. Consistently, LXR α/β and PPAR γ , nuclear receptors promoting lipid synthesis, and lipid synthesis enzymes like elongases 1, 4 and 6, and fatty acid synthase and stearyl-CoA desaturase were highly expressed in the skin and the liver of db/db. Collectively, our study demonstrated a global alteration in local and systemic lipid profiles of DM, which may be related to skin disorders.

P118 - 0528 Establishment of a GFP Reporter THP-1 Cell Line under the Control of Endogenous Interleukin-8 Promoter

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Interleukin (IL)-8 is reported to be a useful biomarker for alternative testing methods for skin sensitization, and IL-8 luciferase reporter assays have been developed. Here, we developed a new reporter assay, namely IL-8 green fluorescent protein (GFP) assay, with monomeric GFP (mGFP) knock-in cell line employing CRISPR/Cas9 genome editing. This assay is convenient for monitoring of IL-8 levels by the use of mGFP as a reporter gene. In addition, this assay is designed to accurately reflect IL-8 expression responding to skin sensitizer exposure by inserting the mGFP allele at just upstream from the stop codon of IL-8 locus. We first connected mGFP to self-cleaving peptide (P2A) coding sequence and thereby mGFP was co-expressed with IL-8 under the control of endogenous IL-8 promoter. For homologous recombination, a donor vector containing P2A-mGFP, sgRNA and Cas9 proteins were introduced into a human monocyte-derived THP-1 cell line by electroporation. After limiting dilution cloning, PCR analysis showed that several cell clones harbor P2A-mGFP allele in IL-8 locus as designed. In addition, sequence analysis determined that one of the cell clones had no mutation in P2A-mGFP and its surrounding sequences. To investigate whether the expression of mGFP is induced by skin sensitizer in mGFP knock-in cells, we observed mGFP fluorescence in the cells treated skin sensitizer 2,4-dinitrochlorobenzene (DNCB). Fluorescent microscopy analysis showed that GFP fluorescent intensity was increased by DNCB treatment. This result suggests that mGFP knock-in cells expresses mGFP in response to DNCB. To confirm that DNCB induce the expression of mGFP in knock-in cells, mGFP fluorescent was determined by flow cytometry. The mean fluorescent intensity in DNCB-treated cells was significantly increased, and a 4-fold increase in 12.5 μ M DNCB-treated cells was observed as compared with those in control cells. These results suggest that this stable THP-1 cell line expresses mGFP in response to skin sensitizer under the control of endogenous IL-8 promoter. In this study, we succeeded to develop IL-8 GFP assay. This new testing method provides not only time-course measurement without substrates or antibodies, but also accurate measurement reflecting endogenous IL-8 expression.

P119 - 0538 Predictive Capacity of Combined Methods for Tiered Approach on Evaluation of Eye Irritation

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A number of *in vitro* test methods have been suggested to replace *in vivo* Draize rabbit eye test, however, stand-alone *in vitro* test method is still unable to fully replace the *in vivo* test. At present, development of the strategy to evaluate eye irritation has been arisen, e.g., IATA strategy or tiered approach. Therefore, this study aimed to set up tiered approach combined with various methods for alternative eye irritation test for convenient use in Korea. This study selected 5 alternative test methods such as Bovine Corneal Opacity and Permeability (BCOP, OECD TG 437), Short Time Exposure (STE, OECD TG 491), Hen's Egg Test Chorioallantoic Membrane (HET-CAM), and Reconstructed human Cornea-like Epithelium test method (RhCE, OECD TG 492) based on OECD Guidelines on IATA (GD 263). We evaluated predictive capacity for 4 drafts of Top-Down approach for 81 chemicals. And we tested some chemicals which have no results or inconsistent results with *in vivo* UN GHS from investigated published papers. Strategy 1 is composed of STE as the first stage and RhCE as the last stage due to its expensive cost. Strategy 2 is designed "BCOP" as the first stage. Strategy 3 is designed "BCOP or STE" as the first stage according to solubility of test chemicals. And Stage 4 is designed "STE and BCOP" or "HET-CAM and BCOP" as the first step according to solubility of test chemicals. Strategy 1 shows the highest accuracy and specificity. Strategy 2 and 3 shows the highest sensitivity. Stage 4 shows the lowest accuracy and sensitivity. We also compared RhCE using EpiocularTM (OECD TG492) and MCTT (New suggested draft TG). It shows few difference between two models. We proposed to choose among draft strategies depend on conditions by considering advantages, limitations, and predictive capacity of each draft strategy.

P120 - 0629 Multigenerational Epigenetic Effect of Deoxynivalenol in *Caenorhabditis elegans*

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Deoxynivalenol (DON) is one of the most widely distributed trichothecene mycotoxins commonly found in cereal grains and its ubiquitous contamination has become a big concern in the field of public health and food safety due to its potent acute and chronic toxicities to animals and humans. We previously demonstrated that DON produced significant toxic effects on growth, brood size and feeding behavior following multigenerational exposure to *Caenorhabditis (C.) elegans* model. Because it has been well known that DON is not a genotoxic agent, we postulate that DON exposure may induce changes in the epigenome that can be used as potential molecular signatures of DON-induced toxicity. In this study, the total RNA in the worm groups treated with 200 μ g/mL DON and control vehicle at different generation time (P0, F1, F2 and F3) were prepared, and the RNA sequencing was performed on Illumina NextSeq 500. The differentially expressed miRNAs were evaluated between the two treatment groups at different generation times. As compared to the control group, totals of 161 miRNAs (83 up- and 79 down-regulated), 177 miRNAs (110 up- and 67 down-regulated), 217 miRNAs (143 up- and 74 down-regulated), and 225 miRNAs (105 up- and 120 down-regulated) were differentially expressed following DON exposure at P0, F1, F2, and F3 generations, respectively. Further functional enrichment and bioinformatics analyses showed that the major alterations of these differentially expressed miRNAs were related to the protein synthesis, transcription, sex determination, balance of redox status, activity of kinases, and biotransformation. These epigenetic mechanisms appear to underlie the multigenerational transmission of DON-induced toxicity, and the signature of epigenetic alterations may be potential biomarkers for DON-affected molecular pathways.

P121 - 0632 Documenting International Acute Systemic Toxicity Testing Requirements to Facilitate Increased Acceptance of Nonanimal Approaches

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The acute systemic toxicity tests—toxicity oral, dermal, and inhalation LD50 tests and variants—are the most common type of toxicity tests conducted worldwide despite ambiguous scientific relevance. In order to ensure that replacement test methods and strategies are accepted, it is important to understand the testing and information requirements of a variety of agencies covering a number of product sectors. While basic testing methods do not vary widely among countries, specific regional regulations, use contexts driving the conduct of these tests, and the degree of acceptance of alternative approaches are diverse. Understanding international requirements will inform scientific development and necessary regulatory policy changes to facilitate the reduction and replacement of animals in safety testing. Here, we present results from an international survey conducted with Organisation for Economic Co-operation and Development (OECD) member and partner countries to identify specific test methods required and accepted for acute systemic single-dose studies within various product sectors (e.g., pesticides, industrial chemicals, pharmaceuticals, consumer products, and cosmetics). The survey also assessed acceptance of alternative approaches such as read-across, (Q)SAR, *in vitro* methods, and the potential for waiving tests or a combination of approaches. Results include not only frequently collected information by regulators but reported uses of information used for regulatory decision-making, which could serve as an information source to facilitate the development of alternative approaches. The presentation will provide recommendations to improve the implementation of alternative approaches within domestic regulations and identify areas for greater international harmonization.

P122 - 0642 Aberrant Activation of Peroxisome Proliferator-Activated Receptor γ (PPAR γ) Disrupts Dorsoventral Patterning during Early Zebrafish Embryogenesis

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Peroxisome proliferator-activated receptor γ (PPAR γ) is a ligand-activated transcription factor that regulates lipid/glucose metabolism and is critical for adipocyte differentiation. While the role of PPAR γ in adipogenesis and diabetes has been extensively studied, little is known about PPAR γ function during early embryonic development. Using zebrafish as a model, recent data from our lab suggest that non-toxic concentrations of PPAR γ ligands may protect against certain environmental chemicals that disrupt gastrulation and dorsoventral patterning within the first 24 h of development. Within zebrafish, maternally-loaded PPAR γ transcripts are only present within the first 6 h post-fertilization (hpf), and transcription of zygotic PPAR γ doesn't commence until ~48 hpf. Therefore, since maternal PPAR γ transcripts are present during early-gastrula, the objective of this study was to investigate the potential role of PPAR γ in regulating gastrulation and dorsoventral patterning during the first 24 h of zebrafish development. To accomplish this objective, we relied on (1) ciglitazone as a potent, selective thiazolidinedione-based ligand to aberrantly activate PPAR γ and (2) a splice-blocking, PPAR γ -specific morpholino (MO) to determine whether functional PPAR γ is required for normal gastrulation and dorsoventral patterning. Our preliminary data show that initiation of ciglitazone exposure by 4 hpf results in concentration-dependent effects on dorsoventral patterning, leading to strongly ventralized embryos by 24 hpf. Ciglitazone-induced ventralization occurred in the absence of epiboly defects during gastrulation, suggesting that ciglitazone-induced ventralization was not due to delayed cell migration during epiboly. Moreover, our preliminary knockdown data show that loss of functional PPAR γ does not affect gastrulation nor dorsoventral patterning. Therefore, we are relying on (1) PPAR γ knockdown to determine whether ciglitazone-induced ventralization is PPAR γ -dependent; (2) molecular docking to estimate ciglitazone-specific binding energies for human versus zebrafish PPAR γ ; and (3) whole-mount immunohistochemistry and amplicon sequencing to identify whether ciglitazone disrupts bone morphogenetic protein signaling—a process critical for ventral cell fate specification.

P123 - 0654 Toxic Effects of Six Essential Oils on the Biological Model *Caenorhabditis elegans*

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Essential oils are a mixture of volatile components produced on the secondary metabolism of plants, whose composition involves a portion of hydrocarbons of the terpene type, together with other compounds almost always oxygenated (alcohols, esters, ethers, aldehydes and phenolic compounds) which are what give oils the aroma that characterizes them. These have been used over time in applications of alternative and/or traditional medicine. However, there is little information about the toxicity of plants used in alternative and/or traditional medicine. In this investigation, the toxicity of the essential oils extracted from peppermint, lime, bergamot, ylang ylang, tea tree and Chinese herb was evaluated against the biological model *Caenorhabditis elegans* taking as toxicity endpoints lethality and locomotion. Lethality was evaluated by measuring the percentage of survival animals after extract exposure. About ten nematodes in larval age L4 were exposed for 24 h to different solutions of each essential oil (0.025, 0.25, 2.5, 25 and 250 mg/mL). Locomotion behavior was assessed by measuring body bend frequency after 24 h of exposure to different concentrations of liquid extracts. Individual nematodes were transferred to a plate with K agar and scored for the number of body bends in an interval of 20 s. A body bend was counted as a change in the direction of the posterior bulb of the pharynx along the Y axis, assuming that the nematode was traveling along the X axis. A total of 30 nematodes were examined per treatment. The essential oils were analyzed with respect to their composition by instrumental technique of Gas Chromatography coupled to Mass Spectrometry (GC/MS). The most abundant components in the six essential oils were: terpenen-4-ol, D-Limonene, Linalool, Terpineol, L-Menthol, L-Mentone, and Carvone. Lethality and locomotion inhibition of the nematodes were concentration dependent in all essential oils, verifying the potential toxicity of these substances. Peppermint oil at 250 mg/mL was the most toxic solution, and the least toxic oil was that of the tea tree. The analysis of variance indicates that there is a statistically significant relationship between the concentration and toxicity: with percentage of dead nematodes (p value = 0.0002) and with the loss of mobility

(p value = 0.0031). In addition, some components found in the essential oils, such as terpenen-4-ol, are considered neurotoxic, which explains the inhibition in the basic movements of the nematode.

P124 - 0658 Modernizing Biocompatibility Testing: Replacing Animal Tests through the United States Food and Drug Administration (US FDA) Center for Devices and Radiological Health (CDRH) Medical Devices Development Tools (MDDT) Program

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In 2018, the US FDA announced in its Predictive Toxicology Roadmap that the agency is upgrading its toxicology toolbox. In part, this upgrade emphasizes the agency's interest in bringing safer medical products to market by replacing the use of outdated animal-based test methods with new methods that reflect recent breakthroughs in the toxicological sciences. To achieve this goal, US FDA/CDRH has introduced the MDDT Program to establish a clear pathway for the medical device industry to cooperate with the agency when submitting new test methods for use in regulatory risk assessments. We describe the process our organization has taken to develop two MDDT Program proposals as a template for companies and researchers interested in validating non-animal test methods for use in medical device regulatory submissions. In collaboration with the Institute for *In Vitro* Sciences and the personal lubricant industry, the US FDA/CDRH accepted our MDDT Program proposal to assess an *in vitro* testing approach based on human reconstructed tissue model(s) that may replace the rabbit vaginal irritation (RVI) test, a routinely recommended biocompatibility test for personal lubricants. The validation program, developed with input from US FDA/CDRH, focuses on personal lubricants with diverse chemical and physical properties and is underway. Paired *in vivo-in vitro* vaginal irritation data generated using commercially available human reconstructed vaginal tissue models will be analyzed against existing *in vivo* RVI data to develop a prediction model for use in product safety assessment. Additionally, we co-organized a workshop with the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) to explore industry interest in developing the monocyte activation test (MAT) as a replacement for the rabbit pyrogen test and the bacterial endotoxin test, both routinely required by US FDA/CDRH for pyrogenicity assessment for many types of medical devices. Workshop participants—including medical device manufacturers, MAT experts, and regulators—agreed on the value of preparing an MAT MDDT Program proposal and resolving questions raised during the workshop, which is currently underway. This approach to organizing collaborative partnerships between industry, regulators and other organizations that support non-animal test methods is an ideal solution for implementing modern predictive toxicology tools that can support regulatory decision-making while minimizing uncertainty about the acceptability of these new methods in regulatory submissions.

P125 - 0664 Elucidating Gene-by-Environment Interactions Associated with Differential Susceptibility to Chemical Exposure: Abamectin as a Case Study

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The severity of adverse health effects from chemical and drug exposures differ between individuals within and across populations. We developed and sequenced a highly genetically diverse Tropical 5D wild type (T5D) and reported that it contains 10.3M single nucleotide polymorphisms (SNPs), much higher than traditional inbred strains including AB (4.3M), TU (3.6M), TL (6.2M) and WIK (8.5M). We aimed to test the hypothesis that inherent population genetic variability and well-controlled laboratory exposures could help to identify genetic loci responsible for differential susceptibility to specific chemicals. We previously completed early life stage high-throughput screening (HTS) of 1,060 chemicals in T5D zebrafish. We leveraged this data to identify chemicals that produce concentration response profiles suggestive of susceptibility variation in the T5D population. We identified only 19 potential chemicals from the list of 1060 that produced variation in population response. Abamectin, an agricultural insecticide, reliably produced the most variable response between individuals so we selected Abamectin for proof of concept Gene-by-Environment Interactions studies. We generated full genome-wide sequencing data (at 5X

coverage) for 276 individual zebrafish displaying susceptible (Affected, N=138) or resistant (Unaffected, N=138) phenotypes following identical chemical exposure to 0.6μM Abamectin. We completed association analysis for each SNP across the genome to assess allele counts correlating to Affected vs. Unaffected individuals. From the data, 3 SNPs exceeded the genome-wide significance threshold. The top hit (smallest p-value) was a novel G/T variant in the distal promoter region of transcription factor *sox7*. Additionally, deep sequencing uncovered a nearby 15bp deletion that correlated with this variant. In an initial follow up validation study, *sox7* expression is significantly lower in Affected vs. Resistant individuals exposed to 0.6μM Abamectin. To our knowledge, this is the first genome-wide association study (GWAS) using a large sample of individually sequenced zebrafish drawn from a diverse population. The results demonstrate that GxE associated with naturally occurring population genetic variation and may play a significant role in mediating individual response to chemical exposure.

PS P126 - 0675 Identification and Characterization of Long Non-coding RNAs in *Caenorhabditis elegans* Exposed to Mixture of Aflatoxin B₁ and Fumonisin B₁

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Aflatoxin B₁ (AFB₁) and Fumonisin B₁ (FB₁) are the most common food contaminants whose co-existence was found in foodstuffs all over the world, particularly in high liver cancer risk areas. AFB₁ is a potent mutagen and Group 1 human carcinogen, as classified by the International Agency for Research on Cancer (IARC), that causes hepatocellular carcinoma (HCC). FB₁ is a class 2B carcinogen (IARC) with strong tumor promotion activity in animal models. Studies in animal models have shown that the co-exposure to AFB₁ and FB₁ elicits considerably stronger toxic and carcinogenic effect than when exposed to either of the toxins alone. *Caenorhabditis elegans* (*C. elegans*) has been a valuable model organism for toxicological studies, with its simplicity, completely mapped genome, as well as several homologies to mammalian systems. We have found that combined exposure AFB₁ and FB₁ elicited stronger toxic response in terms growth inhibition, brood size reduction, lifespan reduction, as well as DNA lesion in wildtype N2 strain, than either mycotoxins alone. In the present study, the total RNA of N2 *C. elegans* treated with EC10 levels of AFB₁, FB₁, and 1:1 combination of the two were extracted and sequenced using Illumina HiSeq 4000, and the differentially expressed long non-coding RNA (lncRNA) was evaluated. Compared to DMSO-treated control group, EC10 level of AFB₁ resulted in 419 differentially expressed lncRNA (163 down- and 256 up-regulated), EC10 level of FB₁ resulted in 425 differentially expressed lncRNA (159 down- and 266 up-regulated), and combined EC10 levels resulted in 439 differentially expressed lncRNA (171 down- and 268 up-regulated). The combined exposure showed 487 differentially expressed lncRNA (225 down- and 262 up-regulated) when compared to FB₁-only exposure, and 535 differentially expressed lncRNA (291 down- and 244 up-regulated) when compared to AFB₁-only exposure. Given the potential role of altered lncRNA in the dysregulation of DNA damage response mechanisms, the specific genes associated with these lncRNA warrants further examination.

PS P127 - 0683 Statistical Analysis of Me-Too Validation Study on Alternative Test Method for Eye Irritation, MCTT HCE

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This statistical analysis of the validation study of an alternative test method named MCTT HCE eye irritation test (EIT) intends to verify the reproducibility and predictive capacity of the new *in vitro* EIT. Following the OECD performance standards (PS), the validation study should be performed via three repeated experiments by three independent laboratories for 30 reference chemicals. The within-laboratory reproducibility (WLR) and the between-laboratory reproducibility (BLR) were evaluated on the concordance of eye irritancy prediction with 95% Wilson's Confidence Intervals (CIs). In addition, the analysis of the Intra-Class Correlation coefficient (ICC) and Bland-Altman plot were applied to confirm the reproducibility and the relevance with reference to EpiOcular EIT. The predictive capacity is calculated in 3 approaches, (1) Mis-prediction over total prediction, (2) Individual lab prediction, and (3) Majority of run-sequences prediction. The predictive capacity indicators (sensitivity, specificity, and accuracy) were presented with 95% Wilson's CIs. Supplemental tests were conducted for a total of 144 reference chemicals to evaluate the predictive

capacity, and the optimal cutoff values were examined with receptor operating curve analysis. In conclusion, WLR and BLR met the criteria set forth in the performance standards, and the ICC and Bland-Altman plot suggested acceptable reproducibility and relevance. The predictive capacity of 30 reference chemicals evaluated by the method of mis-predicted divided by total predicted resulted in a sensitivity which barely failed to meet the criteria of the PS, however, the range of 95% CI was minimal. The bootstrap resampling method showed adequate confidence intervals and predictive capacity. The predictive capacity assessed for 144 reference chemicals exhibited good performance which sufficiently met the criteria stated in the PS.

PS P128 - 0722 Pioneering Better Science to Advance the 3Rs in Acute Toxicity Testing

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Toxicity testing is undertaken to assess the potential impact of chemicals on human health, wildlife populations and ecosystems. The UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) has dedicated work programmes to promote the 3Rs across human toxicology and ecotoxicology. These programmes are supported by working groups consisting of experts from academia, government agencies (including regulators), contract research organisations, and industry (international agrochemicals, consumer goods, and petrochemicals companies). Acute toxicity testing uses large numbers of animals, and involves severe suffering. This poster will highlight cross-sector initiatives led by the NC3Rs to apply the 3Rs to acute inhalation, oral and fish toxicity testing. NC3Rs-led efforts have resulted in adoption of the Fixed Concentration Procedure (FCP) by the Organisation for Economic Co-operation and Development (OECD) as Test Guideline (TG) 433. FCP is a refined method for acute inhalation toxicity studies compared to the other TGs (403 and 436), using fewer animals and avoiding death as an endpoint. FCP instead uses 'evident toxicity' as the endpoint, which is signs of toxicity that predict severe toxicity or death would occur in most animals at the next highest concentration of chemical. Much of the work to support adoption of TG 433 involved analysis of historic acute inhalation toxicity reports to identify clinical signs that are particularly indicative of evident toxicity. The Fixed Dose Procedure (TG 420) which was adopted in 2001, is the equivalent acute oral toxicity method however uptake of this method has been limited, possibly because this TG lacks guidance around the identification of evident toxicity that has now been provided for TG 433. Similar analysis of historic acute oral toxicity study data is currently underway to support TG 420. The acute fish toxicity test (TG 203) has been a cornerstone of ecotoxicology testing for more than 30 years. As death is the endpoint of this TG, there is the potential for significant suffering over the 96 hours of the test. Whilst *in vivo* fish acute toxicity tests are a core requirement under many global chemicals regulations, in some sectors and regions alternative approaches are accepted. The NC3Rs Ecotoxicology Working Group has identified some key areas where it may be possible to harmonize testing requirements both regionally and across sectors. Through these and other activities, the NC3Rs have sought to influence regulatory guidelines and company practices, to apply the 3Rs to acute toxicity testing whilst maintaining protection of human health and the environment.

PS P129 - 0753 A Simple Spectrophotometric Test to Identify Skin Sensitizers and Nonsensitizers *In Chemico*

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For the key event No. 1 in skin sensitization, a low molecular weight hapten should react with endogenous proteins for the initiation of immune reaction. Because most of skin sensitizers are able to interact with amino or sulfhydryl groups in certain proteins, the Direct Peptide Reactivity Assay (DPRA) has been developed as a simple alternative method to identify skin sensitizers and nonsensitizers. Because artificially synthesized peptides and HPLC should be employed in performing DPRA, sometimes the method would not be easily performed. In the present study, a simple spectrophotometrical method to identify skin sensitizers *in chemico* was developed by using cysteamine, a simple and low molecular weight endogenous substance, that contains both functional groups. To quantitate remained cysteamine following the reaction with skin sensitizers, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) was selected for the derivatization. For the optimization of experimental conditions, 2,4-dinitrochlorobenzene (DNCB) and lactic acid were selected as skin sensitizer and nonsensitizer, respectively. The conditions optimized included: incubation

time of cysteamine with test substances, molar ratios of cysteamine to test substances, stability of derivatized products of test substances with optimal concentration and incubation time of DTNB, and spectrophotometrical characteristics of the derivatized product. The optimized conditions, showing that cysteamine was depleted only by the incubation with DNCB, but not by lactic acid, could also be successful to correctly determine several skin sensitizers and nonsensitizers. Because the present method employs a simple endogenous cysteamine as an acceptor for sensitizers and/or a spectrophotometric detection system, it would be a useful test tool for determining skin sensitization potential of small molecules. *Supported by the grant from National Research Foundation, Korea (NRF-2017RIDIA3B04033313).*

PS P130 - 0808 Evaluation of Photo-Ocular Irritancy of Phloxine B on SIRC Cells and 3D Reconstructed Human Cornea Model

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Photo-irritation is an acute toxic response from chemicals applied to the body topically or systemically, which is either elicited or increased after subsequent exposure to light. Many researchers have investigated phototoxic potential of chemicals on skin cells or skin models. However, solar light affect most sun-exposed parts of our bodies, including eyes, but few studies have addressed the adverse effects of photo-irritants on eyes. Phloxine B is one of the tar-color additives permitted for use in both drugs and cosmetics in the United States, and also in foods. Phloxine B is widely used in color cosmetics such as lipsticks and eye shadows. Although it is known to induce severe eye irritation (UN GHS Cat 1), there is no limitation on using this dye around eye area in Korea. Furthermore, photo-irritancy of phloxine B on eyes has not been studied yet. In this study, we investigated the photo-irritancy of Phloxine B under UVA irradiation using *in vitro* assays. Photo-cytotoxicity on Statens Serum Institut Rabbit Corneal (SIRC) cells was evaluated with MTT assay. To see if oxidative stress is induced by phloxine B with UVA irradiation, CM-H2DCFDA fluorescence staining and GSH/GSSG colorimetric assay were conducted. In addition, mode of cell death was identified by flow cytometric and fluorescent image analysis. To complement some limitation of cell assays, 3D reconstructed human cornea model (MCTT HCETM) which exhibits *in vivo*-like morphological and growth characteristics was used to evaluate the photo-cytotoxic effect of phloxine B on human corneal tissue. Based on our results from these assays, toxicity of phloxine B was increased after subsequent UVA irradiation compared to non-irradiated groups in various aspects. Therefore, this study suggests the risk of using phloxine B in cosmetics, particularly eye make-up products under sun-light irradiation.

PS P131 - 0953 Network Analysis of MCF-7 and Human Breast Cancer Tissues

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Problems with cell-line heterogeneity and artifacts can have serious consequences for the translatability of *in vitro* scientific studies to *in vivo* systems. This project focuses on the Michigan Cancer Foundation-7 (MCF-7) cells, a human breast adenocarcinoma cell line that is commonly used for *in vitro* cancer research, with over 33,000 publications in PubMed. Previously, our center has showed that even MCF-7 cells obtained from the same cell batch at the same cell bank can display cellular and phenotypic heterogeneity, which affected reproducibility of experiments using this cell line. As a follow-up study, we compare a large mRNA microarray data set of MCF-7 to a large mRNASeq breast cancer tissue data set. The MCF-7 dataset includes 351 samples obtained from Gene Expression Omnibus, in which cells were treated with a dose-response curve of xenoestrogens and estradiol; the human breast cancer tissue dataset came from the Cancer Genome Atlas, including 1098 breast tissue samples from individuals with breast invasive carcinoma. We used Weighted Gene Correlation Network Analysis (WGCNA)—a method that takes advantages of correlation amongst genes and graph theory—to explore similarities and differences in key transcription factors, signaling mechanisms and pathways in MCF-7 cell line and human breast cancer tissues. Since cancer cell lines are commonly used in both basic and translational research to understand biological mechanisms, drug effects, and Pathways of Toxicity, our comparison of the regulatory networks of MCF-7 and breast cancer tissues can help clarify both the usefulness and the limits of *in vitro* models.

PS P132 - 0052 In Silico Prediction of Severe Cutaneous Adverse Drug Reactions Using the Japanese Adverse Drug Event Report Database

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Idiosyncratic adverse drug reactions (iADR) are unexpected responses and one of the major reasons for the withdrawing from the drug market. Stevens-Johnson syndrome and toxic epidermal necrolysis (SJS/TEN) are typically caused by drugs and are a type of iADR. Because iADR have high species specificity and low occurrence frequency, it is difficult to predict iADR from animal experiments and clinical trials. On the other hand, spontaneous reporting systems are useful for the detection of severe adverse reaction, which is not found in clinical trials. Consequently, they are an important resource to identify potential relevance between drugs and their severe adverse reactions. The Japanese Adverse Drug Event Report database (JADER), which is constructed by the Pharmaceutical and Medical Devices Agency (PMDA), a Japanese regulatory authority, has accumulated spontaneous adverse drug effects reports for more than a dozen years in Japan. Therefore, in this study, we tried to develop the predictive classification model of Severe Cutaneous Adverse Reactions (SCAR), such as SJS/TEN, by machine learning algorithm using the drugs structure information based on JADER. SCAR was defined using the Standardised Medical Dictionary for Regulatory Activities (MedDRA) Queries (SMQ). We collected and curated SCAR-positive and SCAR-negative drugs from JADER using reporting odds ratio (ROR), which is the signal detection method and the number of reports. Chemical structure information on extracted drugs was obtained from Kyoto Encyclopedia of Genes and Genomes (KEGG) and PubChem, and then molecular descriptors were calculated using Dragon 7. Then, we built SCAR classification models and evaluated their performance using independent test dataset. In addition, in order to make more reliable prediction, applicability domain was defined. As a result, we achieved classification model of SCAR with an area under the curve (AUC) of approximately 0.8 by the deep learning method using only the structure information of drugs. Furthermore, we incorporated the influence of concomitant drugs to our prediction model, because the influence of concomitant drugs should be considered for each suspected drug for using spontaneous reporting systems. In this study, we found a method to utilize the database of spontaneous reporting systems for the SCAR prediction by the machine learning method. This method has the potential to predict SCAR in humans and improve screening efficiency in the early stage of new drugs development.

PS P133 - 0064 Withdrawn

PS P134 - 0103 Congener-Specific Half-Lives of Polychlorinated Biphenyls: A Systematic Review of the Literature

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Most toxicokinetic models for polychlorinated biphenyls (PCBs) have been developed to address the behavior of more persistent congeners. However, humans are environmentally exposed to PCBs as complex mixtures including both labile and persistent congeners, all of which may contribute to the development of toxicological outcomes. More comprehensive models that incorporate the full range of kinetic parameters represented in environmental PCB mixtures would be useful for estimating tissue levels of PCBs in exposed humans and animals. Not only would this information be useful for route-to-route and interspecies extrapolation of toxicological effect levels, it could also lead to a better understanding of the relative contributions of individual PCB congeners in the development of various health effects. A first step in the development of such a comprehensive toxicokinetic model for PCB mixtures is to identify biologically relevant parameters for each congener. One key parameter is half-life of elimination; here, we describe a systematic approach used (1) to identify half-life values reported for individual PCB congeners, and (2) to select from the available values those most appropriate for use in kinetic modeling. Studies evaluating the elimination (i.e., clearance) of individual PCB congeners in humans or other mammals were identified through a literature search of three scientific databases (PubMed, Web of Science, and Toxline). Studies reporting whole-body or tissue-specific half-lives were identified by title/abstract and full-text screening of the literature search results. Half-life values were tabu-

lated along with experimental design information important for evaluating the quality of the study and the resulting confidence in its half-life estimation. From the information presented in these tables, we identified half-life values for use in modeling efforts as well as data gaps and future research needs. The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US Environmental Protection Agency.

PS P135 - 0178 *In Silico* Models for the Predicting of the Repeated-Dose Toxicity Based on HESS Database

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In silico prediction is considered to be a useful method in toxicological research. A high-quality toxicity test database and appropriate prediction method are necessary for the appropriate *in silico* toxicity evaluation. Hazard Evaluation Support System Integrated Platform (HESS) by the National Institute of Technology and Evaluation, which is an open toxicological database, contains reliable information on repeated-dose toxicity (RDT). In this study, using HESS database and machine learning methods, we tried to examine the toxicity endpoints that could be predicted high performance by *in silico* model. Focusing on the renal toxicity that is often observed in RDT studies, we investigated renal findings reported in HESS. Various toxicological findings related to the renal toxicity were reported in HESS and the number of data we could collect depended on type of toxicological findings. We built 3 kinds of prediction models: model 1 based on the increase of the kidney weight (159 positive substances, 132 negative substances); model 2 based on the increase of the kidney weight and the renal histopathological findings (105 positive substances, 118 negative substances); and model 3 based on the increase of the level of serum creatinine (Cre) and the blood urea nitrogen (BUN) (23 positive substances, 131 negative substances). All data were collected on male rats 28-days RDT studies. We developed prediction models by the support vector machine (SVM) and the random forest (RF) using descriptors calculated by dragon 7. The performance of each model was evaluated with prediction accuracy (ACC) and matthews correlation coefficient (MCC) using 5-fold cross-validation. Our best performance was model 2, which achieved MCC of above 0.57 and ACC of above 0.78. These results suggest that the increase of the kidney weight and the renal histopathological findings as endpoints may be useful for the appropriate *in silico* prediction of renal toxicity.

PS P136 - 0188 Responses to the US FDA Standard for Exchange of Nonclinical Data (SEND) and Establishment of Global SEND Alliance (G-SEND)

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The Standard for Exchange of Nonclinical Data (SEND), adopted by the US Food and Drug Administration, is a set of regulations for digitalization and standardization of nonclinical study data. Related organizations have begun implementing processes similar to SEND. The Global Editorial and Steering Committee (GESC), which operates the business of the International Harmonization of Nomenclature and Diagnostic Criteria (INHAND), has cooperated in preparing the Controlled Terminology (CT) related to toxicology and pathology for SEND. SEND provides electronic data standards created by the Clinical Data Interchange Standards Consortium (CDISC). CDISC has collaborated in the implementation of SEND. Furthermore, the Pharmaceutical Users Software Exchange (PhUSE) has conducted various activities to promote realistic and effective methods to establish SEND together with the US FDA. As we reported in 2015, there is a significant divergence in the efficiency of disseminating SEND data between pharmaceutical manufacturers and CROs globally. To solve this problem, the Global SEND Alliance (G-SEND) was established in August 2018 as a model to jointly disseminate SEND data between CROs. This paper reports the first method for organizationally and jointly disseminating SEND data between CROs using G-SEND.

PS P137 - 0548 Cross Talks among PPARα, SREBP, and ER Signaling Pathways in the Side Effect of Valproic Acid

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Valproic acid (VPA) is a widely prescribed anti-epileptic drug, and its side effects include hepatotoxicity such as steatosis. Although under intense investigation, its molecular basis is not fully understood. This study aims to foster better understanding of the molecular mechanism of the hepatotoxicity by VPA administration. As a part of the Percellome Project [1], male mice (C57BL/6, 12 weeks old) were orally administered with VPA (0, 50, 150, 500 mg/kg, vehicle: methyl cellulose 0.5%) and their organs (brain, heart, lung, liver, kidney) were sampled 2, 4, 8, or 24 hr post-administration for Affymetrix microarray analysis. The expression intensities were normalized by calculating their copy numbers per cell ("Percellome" method [2]), and the genes that were differentially expressed in a time- and dose-dependent manner were extracted. We performed pathway enrichment analysis by Garuda platform [3] including Reactome [4–5] and NaviCell [6–7]. Our results suggested that VPA affected on the expression patterns of PPARα (Peroxisome Proliferator-Activated Receptor α)-, SREBP (Sterol Regulatory Element-Binding Protein)- and ER (Estrogen Receptor)- target genes in liver. To elucidate the linkage of these signaling pathways, a Boolean network among PPARα, SREBP-1c and ER signaling pathways was constructed. The simulation supported our hypothesis that VPA is involved in hepatotoxicity in a sex-dependent manner by decreasing PPARα/SREBP-1c activation ratio via reduction of ER ligand. This project has been supported by the Health Sciences Research Grants H27-Kagaku-Shitei-001 from the Ministry of Health, Labour and Welfare, Japan. *References:* [1] Kanno, J. *Biomarkers in Toxicology, Academic Press* (2014): 1019-1032. [2] Kanno, J., et al. *BMC Genomics* 7.1 (2006): 64. [3] Ghosh, S., et al. *Nature Reviews Genetics* 12.12 (2011): 821-832. [4] Milacic, M., et al. *Cancers (Basel)* 4.4 (2012): 1180-211. [5] Fabregat, A., et al. *Nucl Acid Res* 44.D1 (2016): D481-7. [6] Kuperstein, I., *BMC Syst Biol* 7 (2013): 100. [7] Bonnet, E., et al. *Nucl Acid Res* 43 (2015): W560-W565.

PS P138 - 0553 Environmental Chemicals and Alterations in Telomere Maintenance as a Factor in Human Diseases: Toxicogenomic Data Mining

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Telomere dysfunction is one of the mechanisms underlying genomic instability, considered an important factor in many diseases. There is an increase in evidence that telomere maintenance (shortening, lengthening, replication and repair of the telomeric DNA sequences) may be affected by environmental chemicals, leading to a number of chronic diseases. The aim of this study was to explore the connection between the alterations of telomere maintenance by environmental chemicals and human diseases using the toxicogenomics data mining approach. The Comparative Toxicogenomics Database (CTD; <http://ctd.mdibl.org>) Set Analyzer tool listed 44 chemicals that affected telomere maintenance. Among them, arsenic interacted with the highest number of genes (29), resulting in decreased telomere maintenance, which might be connected with arsenic's carcinogenicity and immunodeficiency. Particulate Matter (sum of all solid and liquid particles suspended in air) resulted in decreased telomere maintenance, interacting with 18 genes, while 3,4,5,3',4'-pentachlorobiphenyl affected telomere maintenance by interacting with 10 genes. The "Diseases" data-tab on CTD's web page listed human pathologies connected with the alteration of telomere maintenance or its descendants, providing 2,656 results. Twelve chemicals/chemical groups were connected with neoplasms, mainly affecting the two genes, Serine/Threonine Kinase gene (ATM), and Telomerase Reverse Transcriptase gene (TERT) - 3,4,5,3',4'-pentachlorobiphenyl, arsenic, arsenicals, arsenic trioxide, DDT, diazinon, dioxins, mitomycin, toxaphene, tretinoin, trifluralin and zinc. Nine chemicals/chemical groups were connected with asthma, mainly affecting the two genes, Poly(ADP-Ribose) Polymerase 1 gene (PARP1) and RAD50 Double Strand Break Repair Protein gene (RAD50): 2,4-Dichlorophenoxyacetic acid, arsenic trioxide, DDT, diazinon, particulate matter, permethrin, polychlorinated biphenyls, tretinoin, zinc. Among the other diseases were male infertility, diabetes mellitus, cardiovascular diseases, hypothyroidism, Parkinson disease, etc. These findings provide a basis for fur-

ther *in vitro* and *in vivo* investigation in order to better understanding the connection between environmental chemicals, telomere dysfunction and human diseases.

PS P139 - 0579 In Search of Important TiO₂ NPs Characteristics That Determine Exposure and Toxicity

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Assessing the risks of TiO₂ nanomaterials (TiO₂-NMs) requires a systematic approach, including the comparison of effects across many NMs with identified different characters/descriptors. Hence, there is an urgent need to provide toxicological datasets containing comprehensive toxicity, exposure and materials information relating to a diverse spectrum of NPs characters. In the present study, we assessed the toxic physiological and transcriptomic effects (pathways) of a custom design Fe-doped TiO₂ NPs library, for which we have experimentally measured, modelled and theoretical descriptors. We have use these data to study the particle character related toxicity, using various modeling approaches to get further information on key factors governing exposure and toxicity. The experiment were performed using the invertebrate *Enchytraeus crypticus* (Oligochaeta). The Fe-TiO₂ NPs library contained 15 TiO₂ NMs doped with varying percentage Fe. Effects were assessed under UV and non-UV (fluorescent) light, at organism (phenotype) and gene (transcriptomic) level, in order to be anchored and to derive the mechanisms of toxicity. The results were for example: At organism level, TiO₂ NPs toxicity decreased when combined with UV. At molecular level, common mechanisms include impairment in energy metabolism and cell redox homeostasis. Neuron development was also affected across TiO₂ NPs. Unique mechanisms were identified, and size dependent effects were also apparent, i.e., smaller particles interact with embryos while bigger NPs caused reproductive effects via different mechanisms.

PS P140 - 0606 NTP High-Level Summary Data Collections in the Chemical Effects in Biological Systems (CEBS) Database

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The Chemical Effects in Biological Systems (CEBS) database houses data from the National Toxicology Program (NTP) testing program including bioassay and reproductive toxicology, plus toxicology data from other sources. Summary and raw data are available on a study basis from the CEBS homepage (<https://manticore.niehs.nih.gov/cebssearch/>). NTP Data Collections, a CEBS Guided Search, was developed as a tool to allow large or diverse data sets to be integrated into and searchable in CEBS. The tool provides users with the ability to search the dataset using various criteria and return a list of matched studies and test articles. New datasets have been added to the Guided Search to permit the user to integrate NTP conclusions across studies. The first, NTP Treatment Related Findings Collection, includes conclusions for NTP chronic bioassays, Tox21 high-throughput toxicity screening assays, and genetic toxicology studies (<https://manticore.niehs.nih.gov/datasets/search/trf/>). The data include levels of evidence of carcinogenic activity in rats, mice and hamsters, genetic toxicology assessments in Ames, micronucleus, and comet assays, as well as consensus calls for estrogen receptor activity in Tox21 assays. Users can look up assay conclusions for specific test articles or search for test articles with conclusions of interest. The results are integrated with CEBS Test Article data pages that display all assay data and conclusions for the test article selected. To support the high-level conclusions in the NTP Treatment Related Findings Collection, data collections containing more detailed data used to generate the summary data are being developed. For example, the Ames data collection (<https://manticore.niehs.nih.gov/datasets/search/ames>) provides individual trial calls for each chemical, bacterial strain, and microsomal activation status. This collection can be searched for assay parameters. For example, all positive results in the TA100 strain without S9 activation, which returns a list of 276 test articles that are linked to the study data on which the call is based, and to CEBS data pages providing other NTP testing results for those studies. This feature will be expanded with additional datasets containing integrated results for other assays in CEBS.

PS P141 - 0611 Development of Hepatotoxicity Prediction Model Using *In Vitro* Assay Data of Themolecular Key Events

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For applying *in silico* approach to predict toxicity of chemical substances, structural similarity alone is not sufficient and integrating mechanistic information is necessary. In this study, we developed screening-level hepatotoxicity prediction model utilizing test data on *in vitro* assays, which measure key events at molecular levels possibly linking to hepatotoxicity. Firstly, hepatotoxic chemicals were retrieved from HESS (Hazard Evaluation Support System) and TGP (Toxicogenomics Project) repeated dose toxicity databases (n = 176). Secondly, *in vitro* assay data in which hepatotoxic chemicals were tested were gathered by PubChem search. The *in vitro* assay data in which test result of hepatotoxic chemical is "Active" was chosen. Protein targets likely leading to hepatotoxicity were identified using Ingenuity Pathway Analysis (IPA) to further select *in vitro* assay data related to hepatotoxicity. Two predictive models were built based on test data of the selected *in vitro* assays. Model (A) returns "Hepatotoxic" if query chemical is tested and the test result is "Active" in any of chosen *in vitro* assays. Model (B) returns "Hepatotoxic" if an analogue of query chemical (similarity degree 0.9 or more) is tested and the test result is "Active" in any of chosen *in vitro* assays. As a result, 47 *in vitro* assays were selected for building the prediction models, most of which are related to perturbation of hepatic nuclear receptor signaling, CYP inhibition, induction of oxidative stress response and inflammation reaction. External validation of the two models was performed with repeated dose toxicity test data of 130 hepatotoxic and 72 non-hepatotoxic chemicals from ToxRefDB. Sensitivity and specificity were 0.67 and 0.74 for model (A), and 0.72 and 0.72 for model (B), respectively. It was found that our model is able to detect the hepatotoxic chemicals underlying the toxic mechanisms which are not covered by the existing knowledge base model. On the other hand, false negatives in our model were found to be involved in the mechanisms requiring metabolic activation. Since our hepatotoxicity prediction model is based on biological activity of molecular key events leading to the toxicity endpoint, scientific justification would be more acceptable as Adverse Outcome Pathway (AOP) information will become more available. Further, improvement of the predictive performance and applicability is expected as *in vitro* assay data in relation to the molecular key events will become more available.

PS P142 - 0635 Connecting Mechanistic Annotations of Expert Rule-Based Bacterial Mutagenicity Alerts with Statistically Mined Alerts for Assisting ICH M7 Expert Review

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Under the current ICH M7 guidance, both statistical and rule-based QSAR/SAR methodologies must be used to satisfy regulatory requirements for assessing DNA reactive potential of drug impurities. However, a common conception is that the expert alerts are predictive and provide clear mechanistic explanations because they are developed under the careful supervision of human experts. At the same time, it is assumed that statistical methodologies, while being equal or better predictive, suffer from producing a substantial number of unexplainable alerts, i.e., alerts that lack clear mechanistic explanation and pose difficulties for the expert reviewers. Therefore, we developed an algorithm to establish relationships between a set of highly predictive 617 statistical alerts and 180 expert rule-based mutagenicity alerts. These alerts were taken from commercial QSAR software. The algorithm utilizes sets of common compounds containing the alerts to derive probabilistic associations between the statistical and expert rule-based alerts. We found that 92% of the statistical alerts have their rule-based counterparts and can share the same explanation for the mechanism of action. Majority of the well-known Ashby-Tennant alerts also directly overlap with the statistical alerts. The remaining 8% of statistical alerts were analyzed to identify if they represent a relevant toxic functionality, unidentified alerts by the experts or simply statistical artifacts. The results are presented here along with several examples. We concluded that modern statistical mutagenicity models operate with much more reliable alerts which can be supported by mechanistic explanations in addition to being highly predictive.

P143 - 0738 Are the Available Aquatic Toxicity Prediction Models Suitable for Dyes?

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The Max Weaver Library from North Carolina State University is a repository of around 98,000 dyes. The potential uses for these dyes are textiles, paper, packaging, cosmetics, hair coloring, among others. However, little is known about the toxicological properties of these dyes. Prediction models could help to fill this gap and predict their aquatic toxicity. The purpose of this work was to determine the aquatic toxicity of a small subset of the library, using the *Daphnia* acute toxicity assay and verify if the available QSAR tools provide reliable results. First, molecular confirmation of each dye was performed on a high-resolution Quadrupole Time-Of-Flight (QTOF) mass spectrometer via exact mass and tandem mass spectrometry. Fifteen dyes were tested at the limit of solubility and for the toxic ones their EC50 were determined. We employed *Daphnia similis* at 48 hours exposure with photoperiod of 8h light/16 darkness. Ten dyes showed acute toxic effects with EC50 that ranged from 0.35 to 2.95 mg/L. Results were confronted with two prediction models, the ACO-SVM for *Daphnia* and the multi-species predictions tool Epi-Suite. Both models were not able to adequately predict the acute toxicity, probably because the dyes were structurally too diverse from the training set of the two models. Therefore, the next steps will be to select more dyes from the library, to complement the data set of the ACO-SVM model with representative dyes and to develop a new prediction tool to be applicable to the dye library. *Acknowledgements: Fundação de Amparo a Pesquisa do Estado de São Paulo FAPESP grant # 2017/19599-0.*

P144 - 0748 Withdrawn

P145 - 0768 Setting the Basis for the Mutagenicity Assessment of New Dyes—Application to the Max Weaver Dye Library

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The Max Weaver library at North Carolina State University is a dye repository of around 98,000 substances. While the potential uses for these dyes continues to involve textiles, paper, packaging, cosmetics, hair coloring, and a host of other products, researchers are also taking advantage of modern molecular modeling methods to prioritize dyes to be tested in anti-cancer and antibacterial assays. However, little is reported about the toxicological properties of these dyes. Thus, the purpose of this work was to determine the mutagenicity of a representative subset of the library using the Salmonella microsome mutagenicity assay. Purity was determined and molecular confirmation of each dye was performed using high resolution ESI mass spectrometry. Due to the limited quantity of dyes in the library, a miniaturized plate assay (MPA) was used. The dyes were dissolved in dimethyl sulfoxide at the limit of solubility and tested in dose. Fifteen samples were tested using a comprehensive set of strains (TA1538, TA97a, TA98, TA100, TA102, YG1041 and YG7108) with and without metabolic activation (S9). Six of the dyes were mutagenic. The most sensitive strain was YG1041, followed by TA97a > TA98 > TA100 > 1538 > TA102. YG7108 did not detect any mutagenic response. The results are being compared with the predictions made by the expert knowledge-based and statistical-based *in silico* prediction models, Derek Nexus and Sarah Nexus respectively and possible discrepancies will be discussed. Less than 12.5 mg of each dye was needed for the completion of the tests and the MPA was adequate for the required analysis. *Acknowledgements: Fundação de Amparo a Pesquisa do Estado de São Paulo FAPESP grant # 2017/19599-0.*

P146 - 0772 Assessing Model Selection Criteria during the Application of Benchmark Dose Method to Quantal Response Data: Japanese Perspectives

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Benchmark dose (BMD) method has been increasingly used for risk assessment practice, including the determination of the point of departure for toxicological assessment of chemical substances. Besides, model selection criteria have been highly variable by existing guidelines of different organizations. Here we implemented a simulation-based investigation to identify the optimal model selection criteria as applied to quantal response data. Using standard distributions that consist of one or two parameters, the BMD method was employed to analyze three different empirical datasets, each for a single chemical substance, and we first identified the best fit model using the Akaike Information Criterion (AIC). We regard the identified best model for each chemical substance as the unbiased true model with a known lower bound of the benchmark response level at 10% (i.e., unbiased BMDL10). Subsequently, we randomly generated 1,000 sets of data from the best model. For each dataset, we optimized all standard distributions again and compared if a proper BMDL10 estimate, which should be (i) lower than unbiased BMD10 and (ii) ideally close to the unbiased BMDL10, would be recovered by a specified model selection criterion, including model averaging method. Datasets with frequent testing at doses either with high or low response rates alone as well as datasets with doses involving both high and low response rates were examined. For the data with high response rates alone and that with both high and low response rates, model averaging of three best-fit models yielded the most appropriate estimate of BMDL10. The use of AIC was slightly superior to the model averaging method for the data with low response rates alone. The use of the lowest BMDL10 was not optimal even after removal of bad fit models by goodness-of-fit test (p-value) or using the rejection rules of thumb as BMD10/BMDL10 > 10. Among the examined datasets, the use of model averaging and AIC were theoretically supported. The use of the lowest BMDL10 was too conservative, especially for the datasets with only small sample size. Except for "near linear" dose-response curves, the model averaging method may act as an essential technique to implement toxicological risk assessment using the BMD method.

P147 - 0873 A Method for Health Guidance Values Extrapolation across Exposure Durations

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Gaps in the database of Health Guidance Values (HGVs) constitute a major hurdle in public health chemical risk assessments. Methods of computational toxicology and statistics are commonly viewed as an adjunct to laboratory toxicology that can help fill the gaps. In the present work, statistical relationships between oral HGVs for different exposure durations and associated point-of-departures (PoDs) were examined. The endpoints specific to acute, intermediate, and chronic exposure durations were extracted from the ATSDR minimal risk level (MRL), USEPA reference dose (RfD), and USEPA Office of Pesticide Programs (OPP) databases; these sources represent a unique data collection concerning adverse health effects due to low-dose chemical exposures. The strength of association between the acute, intermediate, and chronic oral endpoints was investigated using correlation and regression analyses. The Pearson's correlation coefficients (r) for acute-intermediate (A-I), acute-chronic (A-C), and intermediate-chronic (I-C) log₁₀(HGV) pairs in molar units were 0.95, 0.95, and 0.98, respectively. However, it is commonly believed that all HGVs and their associated PoDs are subjected to large unknown variability. Because of that, and because in imputations these data can be used interchangeably either as a dependent or independent variable, an orthogonal regression analysis was applied. The derived relationships suggested the possibility of cross-extrapolation between HGVs of different exposure durations within the limits of statistical uncertainty. The estimated cross-duration uncertainty factors (CDUFs) for the extrapolation between HGVs for A-I, A-C, and I-C durations were estimated as 5.5, 9.5, and 3, respectively. The analyses at the PoDs level resulted in similar conclusions. Because of the strength of observed correlation, the statistical uncertainty is expected to decrease, as the number of HGVs available for regression analyses increases in the future. The proposed CDUFs are consistent with the earlier findings concerning the extrapolation of endpoints across different exposure durations. *Disclosure: The findings and conclusions in this presentation have not been formally disseminated by the CDC/ATSDR and should not be construed to represent any agency determination or policy.*

PS P148 - 0946 Use of *In Vitro* and Physiologically Based Kinetic (PBK) Models to Support Human Risk Assessment

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Toxicity testing a keystone for human risk assessment. However, most of the toxicity data come from the *in vivo* models and often of these data showing a large inter-study variation. While the extrapolation of toxicity data from *in vivo* models to humans is imperfect due to differences in anatomy and physiology, these traditional models are commonly used because they represent whole organism biology that is not replicated by *in vitro* models. To bridge the gap, a novel method has been developed based on the extrapolation of *in vitro* concentration-response curves obtained with cell line models to *in vivo* dose-response curves using physiologically based kinetic (PBK) modeling-based reverse dosimetry. This model simulates the relationship between the external exposure levels and the internal concentrations of a chemical over time. The *in vivo* dose effect levels and the points of departure (PODs) for risk assessment including benchmark dose (BMD) or lower confidence limit of the BMD (BMDL) can be defined based on the *in vitro* effect levels that are assumed to represent the tissue or blood concentration in which may cause toxicity. All of these were obtained from the integrated *in vitro*-PBK modeling approach. The combined *in vitro*-PBK modeling approach provides PODs for risk assessment with a similar level of uncertainty as observed in the experimental animal studies, supporting the future possibilities of this approach in human risk assessment.

PS P149 - 0094 Fish Sex Ratio for Endocrine Disruptor Identification, Classification, Testing, and Risk Assessment

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In the EU, identification of endocrine disruptors (EDs) is needed for industrial chemicals, pesticides, and biocides. Chronic fish toxicity tests play a critical role in the identification of EDs because they can show adversity and the underlying mode of action (MOA). Fish sex ratio (SR) is an endpoint included in several test guidelines (TG) that can indicate both MOA and adversity. Due to different test protocols and methods used in non-TG studies in the literature, interpretation of SR results becomes challenging. This study intends to evaluate literature SR data and to explore the possibility of using such data in the regulatory context. We compiled 102 studies on SR of zebrafish, medaka, and fathead minnow for 84 chemicals with different MOAs. The preliminary results showed (1) SR was determined by a method different from the TG method in 50% literature studies, leading to variation of SR results; (2) SR can be reversible for estrogenic but not for androgenic and steroidogenic chemicals after a period of depuration in clean water; (3) SR is responsive not only to chemicals with EAS (estrogen, androgen, steroidogenesis) activity but also to those acting on, e.g., thyroid and progesterone pathways; (4) SR is also influenced by fish strain, however the majority of literature studies did not report the strain; (5) a great variation in culture temperature was observed in literature studies, which is known to also influence SR results; and (6) comparison of SR in different life stages and in different fish species was studied. The results showed that SR was influenced not only by chemical exposure but also by nonchemical factors (e.g., fish strain, temperature, stress), leading to difficulty in data interpretation for the ED identification. How to use the literature SR data in the ED identification, classification, and risk assessment is discussed. Based on the overall data analysis, we will propose how to use such data in an environmental testing strategy, which is important for minimizing vertebrate testing and costs.

PS P150 - 0218 Investigating the Interaction between Drug-Induced Oxidative Stress and an Environmental Oestrogen Using Transgenic Zebrafish Models: Potential of Mixture Effects

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Levels of pharmaceutically active compounds in the environment are set to increase due to the growth and aging of the human population. Human drug targets are often highly conserved across animal phyla, and thus, wildlife exposed to these compounds can be vulnerable to their biological effects. Furthermore, wildlife species are exposed to drugs and other pollutants in the environment

as complex mixtures. Studies into the interactive effects of oestrogenic mixtures have shown additive effects, but research into the combined effects of compounds with different modes of toxicity is lacking. Acetaminophen (APAP) is a pharmaceutical of concern in the environment due to its high use across the world and its ability to act as a hepatotoxin at high levels in humans. The metabolism of APAP depletes glutathione stores, resulting in the buildup of reactive oxygen species, which may then induce oxidative damage. There is widespread concern also associated with the contraceptive oestrogen ethinyl estradiol (EE2), which has been proven to induce feminized responses in wild populations. Oestrogens, however, can have some protective role against oxidative stress (OS) by upregulating antioxidant enzymes via intracellular signaling pathways, albeit to date this has only been demonstrated in an isolated liver mitochondrial fraction. Transgenic zebrafish are an increasingly popular system for assessing the biological effects of drugs and other environmental pollutants, but their use for chemical mixtures studies is scant. Using a recently developed novel transgenic zebrafish model for detecting OS mediated via the electrophile response element (Tg-EpRE:mCherry), we show that APAP induces OS in a dose-dependent manner in the liver, pronephric proximal tubule (PPT), and pronephric duct with effects at exposure concentrations as low as 1.25mM (n=12). Daily response profiles for individual embryo-larvae (n=12 animals exposed over 4 days) showed a dramatic increase in OS response to APAP between 72 and 96 hpf in all 3 target organs, with the PPT showing more than a 300% increase in fluorescence intensity during this time period under 1.25mM APAP, compared with just 17% increase between 48 and 72 hpf. As expected, even for high-level exposure of Tg-EpRE:mCherry embryo-larvae, EE2 alone did not induce an OS response. We are now investigating whether EE2 is able to rescue the oxidative effects of APAP across the targeted body tissues. This presentation will thus demonstrate an application of the new EpRE:mCherry transgenic zebrafish for studies into mixture effects of drugs (here APAP and EE2, and also other oxidative drugs) for enhancing our understanding on drug interactions.

PS P151 - 0235 Zearalenone Inhibits the Chemotaxis of T Cells by Influencing Cell Adhesion and Migration

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Zearalenone (ZEA) is a phenolic resorcylic acid lactone mycotoxin produced by several *Fusarium* species that grow on crops in temperate and warmer climate zones. ZEA is particularly toxic to the female reproductive system. Nevertheless, the mechanism of ZEA toxicity to the immune system is not fully understood. Chemotaxis is an important component of wound healing, development, immunity, and metastasis. In the chemotaxis effect, the directional migration of T cells is the necessary condition for the occurrence and completion of immune response. The purpose of this study was to reveal the effects of ZEA on the chemotaxis of T cells and the adhesion and migration proteins involved in the process. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were used to respectively observe the ultrastructural changes inside the cell and cell surface. Transwell was used to study the effect of ZEA on T cell chemotaxis mediated by CCL19 or CCL21. 3D laser confocal technique was used to capture the morphology of perforating cells, and flow cytometry was used to count the number of cells and detect the proportion of T cell subtypes after penetrating membrane. Western Blot was used to detect the expression of cell migration and adhesion associated proteins. In addition, the expression of chemokine receptors on T cells was detected by flow cytometry. Finally, the level of chemokine RANTES, MIP-1 α secreted by T cells was detected by Cytometric Beads Array. ZEA caused ultrastructural damage on the surface of T cells and inside the cells. It also inhibited the chemotaxis of T cells mediated by CCL19 or CCL21 and disturbed the balance of T cell subtypes. The activity of T cell adhesion and migration molecules were also inhibited by ZEA. In addition, the expression of chemokine receptor on T cells was downregulated by ZEA. Finally, ZEA could reduce the secretion of chemokine RANTES, MIP-1 α secreted by T cells. ZEA reduced the chemotactic effect of T cells mediated by chemokines, which may be related to the inhibition of T cell motility. This process was accompanied by a decrease in the activity of adhesion and migration molecules. *Financial support: This study was supported by the National Key Research and Development Program of China (No. 2016YFD0501208) and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).*

P152 - 0238 The Environmental Health Science Aspects of the Reenvisioning of the (US) National Library of Medicine's Toxicology and Environmental Health Resources

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The (US) National Library of Medicine (NLM) is a part of the National Institutes of Health (NIH). The NLM in early 2018 began an effort to reenvision its toxicology and environmental health resources. This is the first collective reenvisioning of these resources, covering several decades of existence for some resources. The goal is to optimize NLM's electronic resources in the areas of toxicology and environmental health by assessing and reenvisioning them to ensure that they continue to meet the changing needs of a diverse set of users. The overall coverage of NLM's resources includes chemicals and drugs, diseases and the environment, environmental health, exposure science, occupational safety and health, poisoning, emergency response, risk assessment and regulations, and toxicology. Examples of global users of NLM's toxicology and environmental health resources include academic researchers, government agencies, industry, not-for-profit organizations, and healthcare providers. A workshop of experts representing a wide range of these types of users was held in mid-2018. The workshop provided in-depth insights into the information and services provided by NLM and what is foreseen for the future. It also identified current information sources from NLM and elsewhere. In addition, it helped to identify critical gaps in the resources NLM offers to improve the function, content, and presentation of desired information resources in the future. Further, it provided in-depth insights to thoughtfully inform the development of a new generation of NLM resources. This presentation will focus on the environmental health science-related aspects of this NLM reenvisioning effort. For example, since mid-2018, new content from NLM includes the addition of a section in ToxTutor covering environmental health, environmental toxicology, and One Health.

P153 - 0315 Physiological and Behavioral Effects of Exposure to the Antidepressant Amitriptyline on Zebrafish Early Life Stages

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Antidepressant drugs are one of the most commonly prescribed pharmaceutical classes in the treatment of psychiatric conditions, such as anxiety and depression. Of these, amitriptyline is a tricyclic antidepressant that has been detected widely in the aquatic environment, albeit at relatively low concentrations. Amitriptyline acts upon the serotonergic and adrenergic systems that are well-conserved amongst diverse animal phyla. Non-target organisms in the aquatic environment therefore are at risk of exposure effects. Here, we present the chronic exposure effects of amitriptyline on zebrafish physiology and behavior. Zebrafish were subjected to aqueous exposures of amitriptyline from 0-28 days post fertilization (dpf) to nominal exposure concentrations of 0.003, 0.03, 0.3, 3, 30, and 300 µg/L, capturing environmentally relevant and human therapeutic concentrations. Recovery from the exposure effects was then assessed during a 14-day depuration period in fish to 42 dpf. Measured concentrations of amitriptyline (via liquid chromatography mass spectrometry) were >80% of the nominals throughout. Mortality and hatching were recorded daily, and morphological and behavioral measurements were made at both the end of the exposure and depuration period. Thigmotaxis, a behavioral index of anxiety that assesses preference for the periphery of an assessment arena, alongside levels of fish movement more generally, were quantified using a videotracking system. This assay included a light phase followed by a sudden change in illumination to complete darkness, incorporated to induce anxiety-like behaviors. There were no effects of the drug exposures on mortality or growth (weight and length); however, embryos hatched earlier at 72 and 96 hpf in the 300 µg/L treatment compared with the controls. Thigmotaxis behavior was not significantly affected at either timepoint. Other features of fish movement, however, were affected in the 30 and 300 µg/L amitriptyline treatments, but only during the dark phase, suggesting that an anxiety-provoking stimulus was required to induce behavioral effects. Interestingly, behavioral assessments of fish after the depuration period indicated recovery from these drug-induced phenotypes. We will also report our investigations into the effects of chronic amitriptyline exposure on neurotransmitter composition in the zebrafish brain and the use of a transgenic zebrafish model with a genetically-encoded pan-neuronal Ca²⁺ indicator, in combination with light sheet microscopy, to identify the effects of amitriptyline on neuronal activity networks in the zebrafish CNS.

P154 - 0346 Cytotoxicity of Surface Water from Akala-Olu Swamp, Ahoada-West, Rivers State, Nigeria

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Surface water from Akala-olu swamp receiving effluent from Agip flow station in Akala-olu community of Ahoada-West Local Government Area (L.G.A), Rivers State, Nigeria, was collected and analyzed using Allium cepa test. The physico-chemical properties of the water sample were determined in accordance with standard methods and were below permissible limit. Five different concentrations (1%, 5%, 10%, 25%, 50% v/v) of the water was prepared. Twelve Onion bulbs were exposed to each concentration for 72 h to determine inhibition to root growth and chromosomal changes. At 48 h, poorly grown roots were collected and fixed for microscopic examination. There was increasing inhibition of root length with increasing concentration of effluent water. An EC50 of 26.30% was obtained from the growth curve. Morphological defects such as twisted and curved roots were observed. Cytological analysis of the root tips after fixing for 24 h, showed reduction in the mitotic index of the roots with increase in concentration. Chromosomal aberration such as C-mitosis, bridges and precocious chromosomes were induced in all the root cells treated with the surface-water. There were evidence of cytotoxicity and chromosomal changes observed which could be a threat to public health of Akala-olu settlement and water ecosystems. Thus the use of physical and chemical properties as the only screening methodology of wastewater is not enough.

P155 - 0356 Neurotoxicity of Aluminum Oxide Nanoparticles and the Mechanism in Dopamine Neurons Injury Involving p53-Related Pathways

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Studies have suggested that the central nervous system is a potential target of Nano-Al₂O₃. However, the response of striatum and dopaminergic neurons to Nano-Al₂O₃ remain ill-defined except for a companion of *in vivo* or *in vitro* study. In this study, we investigated the distribution and toxic damage of Nano-Al₂O₃ in the crucial functional sub-brain regions of rats after intranasal instillation. The mechanism in dopamine neurons injury was also estimated *in vitro*. *In vivo* results showed that Nano-Al₂O₃ delivered via intranasal instillation translocated into the brain and were specifically deposited in the olfactory bulb, hippocampus and striatum. Treatment with Nano-Al₂O₃ also caused ultrastructural changes, oxidative damage and inflammatory responses crucial functional sub-brain regions (olfactory bulb, hippocampus, striatum, cerebral cortex and cerebellum), suggesting that oxidative stress and inflammation may be important for inducing the neurotoxicity of Nano-Al₂O₃. In addition, the histopathological study revealed that intranasal exposure to Nano-Al₂O₃ produced inflammatory responses and cell degeneration in hippocampus, striatum and cerebral cortex. Furthermore, *in vitro* studies were conducted to evaluate the damage induced by Nano-Al₂O₃ in the dopaminergic neuron PC12 cell line. Exposure to Nano-Al₂O₃ affected PC12 cell morphology, decreased cell viability, increased levels of lactate dehydrogenase and oxidative stress activity, impaired mitochondrial function, disturbed the cell cycle and induced apoptosis. These effects were accompanied by a decreased expression of cyclin D1, bcl-2, Mdm2 and phospho-Rb and by an increased of p53, p21, Bax and Rb. These results demonstrate that Nano-Al₂O₃ possibly have a negative impact on the hippocampus, striatum and dopaminergic neuron as well as a potential promoting factors for neurodegenerative diseases. The oxidative stress, mitochondrial dysfunctions and cell cycle arrest might play an important role in the process of dopaminergic neurotoxicity.

P156 - 0404 Investigating Glucocorticoid Drug Effects on Embryo-Larval Development in Zebrafish (Danio rerio)

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Synthetic glucocorticoids are used for their anti-inflammatory properties to treat a wide variety of human health problems including rheumatoid arthritis, asthma and multiple sclerosis. Recent advances in detection methods have resulted in the identification of glucocorticoids in wastewater treatment plants

and rivers (in the low pmol/L concentration range), likely deriving from human excretion. Glucocorticoid drugs are biologically potent compounds and due to the high conservation of drug targets across vertebrate taxa, it is possible that even these low environmental concentrations may pose a risk to exposed fish. These drugs bind to glucocorticoid receptors in cells which are ubiquitously expressed throughout the body, however there is limited knowledge on the mechanisms or the effects of these synthetic steroids in fish. Using zebrafish, *Danio rerio*, as an experimental model, embryo-larvae were exposed to four highly prescribed synthetic glucocorticoids; prednisolone, beclomethasone dipropionate (BDP), fluticasone propionate (FP) and budesonide, and effects quantified on morphological, and more specifically skeletal, development from fertilisation up to 96h post fertilization. Assessments were carried out for each test compound at exposure concentrations ranging between those measured in the aquatic environment, up to 10x the lowest reported therapeutic dose in humans. Effects on the developing skeletal structures were assessed using Alizarin red and Alcian blue staining. For most of the test compounds there were higher incidences of developmental abnormalities and earlier hatching times compared with controls, but these effects were seen at the highest exposure concentrations tested only. Examples of the effects seen include 30% of embryos with yolk sac and pericardial oedemas in the treatment with 19.2 nmol/L (BDP) (n=20) compared with none of the embryos showing this phenotype in the solvent control (n=24). Skeletal staining with Alizarin red and Alcian blue revealed that exposure to FP and BDP resulted in alterations in jaw development, with reductions in intracranial distance and lower jaw length. Specifically, the average lower jaw length in the embryo-larvae treated with 19.2 nmol/L BDP (n=16) was reduced by 15% compared to the solvent control (n=24). Thus, we show that the tested synthetic glucocorticoids can affect development in zebrafish embryo-larvae, for acute exposures to high (not at environmentally relevant) concentrations. We are now investigating whether chronic, low-level exposures to these glucocorticoid drugs have any health implications for fish.

P157 - 0427 Transcriptomic Analysis Identifies Cellular and Molecular Events Involved in Male-to-Female Sex Reversal in *Xenopus laevis*

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Exposure to environmental estrogens during early life reportedly causes reproductive defects and even male-to-female reversal, especially in amphibians and fish. We previously identified that the environmental estrogens caused sex reversal through the estrogen receptor (ER) nuclear pathway. To reveal the molecular events in male-to-female gonads of *X. laevis*, we undertook a large-scale transcriptomic approach by RNA-seq and compared the gene expression profiles in the gonad-mesonephros complex at stages 48, 50 and 52 between E2 treated and untreated genetic males, when male-to-female sex reversal has taken place. Then, we discovered and demonstrated the biological pathways participated in the gonadal feminization, including cell proliferation, cell adhesion and migration, extracellular matrix and complement cascades. Furthermore, we screened the transcriptome to find out the ER target genes by the ERE Finder software, and paid special attention to the ER target genes that play roles in the above cellular events. These data demonstrated that cellular activities participate in the male-to-female sex reversal. In conclusion, we created a novel entry point into the molecular and cellular mechanisms underlying male-to-female sex reversal in amphibians and disorders of sexual development in other species.

P158 - 0447 Health Is More Than Skin Deep: The Functional Relationship between the Skin Microbiome, Chemical Exposures, and Amphibian Health

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Recent studies have identified that natural communities of microbes inhabiting amphibian skin, the skin microbiome, can defend against infection by the pandemic fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), a major driver of recent amphibian extinctions. Given that amphibian species are subjected to multiple stressors in natural environments, we sought to understand the effects of chemical exposures on both the host amphibian, the leopard frog tadpole (*Rana pipiens*), and its skin microbiome to gain insights into tadpole health, skin

microbiome composition, and susceptibility to *Bd* infection. Multiple amphibian species present on US military ranges require active management for range sustainment, thus our study investigated exposures in the larvae (tadpoles), the most sensitive stage of amphibian development, with dose-series exposures to one legacy munition, 2,4,6-trinitrotoluene (TNT), and three new insensitive munitions (IMs): nitroguanidine (NQ), methyl-nitroguanidine (MeNQ), and the IM formulation, IMX-101. The NQ and MeNQ exposures had low toxicity in acute (4d) and chronic (28d) exposures where no mortality was observed for either chemical, even at the highest chronic exposure concentrations of 2,509 and 3,447 mg/L, respectively. IMX-101 elicited mortality with LC50s of 68.1 and 12.5 mg/L in the acute and chronic exposures while the legacy munition, TNT, was by far the most toxic eliciting LC50s of 3.0 and 0.013 mg/L, respectively. None of the chemicals significantly affected tadpole development or developmental rate. Although NQ and MeNQ did not cause mortality, both decreased the alpha-diversity of the tadpole skin microbiome relative to controls, even in the acute exposures where NQ caused significant deviations from controls at 965 mg/L and MeNQ at 5,285 mg/L. IMX-101 significantly decreased alpha-diversity for all acute exposure concentrations (≥ 10.5 mg/L), as did the TNT exposures (≥ 1.0 mg/L). RNAseq assays have been completed for all acute chemical exposures in tadpoles with chronic exposures in queue. Differential transcript expression and functional enrichment analyses have begun with the purpose of providing mechanistic insights into metabolic and cell signaling effects for each chemical exposure. Further, mutual information and frequent item set analyses will be conducted to identify correlations among tadpole and skin microbiome responses to identify potential interactions among host and symbionts. Finally, combinatory exposures of the munitions and *Bd* exposures in factorial treatment arrangements will be conducted this winter to determine if munitions ultimately affect *Bd* infection rates.

P159 - 0483 Evaluation of the Capacity of Adsorption of the Cigarette Butts to Reduce the Toxicity Produced by Hydrocarbons Present in Waters

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Cigarette butts are waste found anywhere, as they are thrown on the streets without any inconvenience. On the other hand, the contamination of water sources by hydrocarbons is a problem that afflicts all living beings, since it generates great damage to the environment. The objective of this research was to evaluate the adsorption capacity of hydrocarbons in water using cigarette butts. Cigarette butts were collected on the beaches of Punta Arena (PAC), Playa Blanca (PBC) and in the urban perimeter of Cartagena—Colombia (PFC), to which total hydrocarbon tests were carried out by means of Oxygen chemical demand analysis (OCD) from the leachate obtained by Soxhlet extraction using ethanol as a solvent and Fourier transform infrared spectroscopy (FTIR). Then, the nematode *Caenorhabditis elegans* (*C. elegans*) was exposed for 24 h to the extracts of cigarette butts in order to establish the toxicity that concerns the cigarette filters, using as a bioassay the lethality of the worm in concentrated solution and diluted 50%. Adsorption experiments were then carried out exposing three sizes of cigarette butts collected in the urban perimeter of Cartagena (whole butts, butts divided into two and four parts) to a heptane-water solution at different concentrations (10, 100 and 1000 ppm) for a time of 2.5 h in continuous agitation and then characterized by means of gravimetric analysis. *C. elegans* was used as bioindicator before and after adsorption in order to establish as soon as the concentration of heptane in the samples studied previously decreased. It was obtained that the CPA and CPB extracts had a lower amount of oxygen to oxidize the organic matter. In the lethality tests, it was obtained that CPB had greater lethality of the nematode, 98% for the concentrated solution and 92.12% for the diluted one. A high adsorption of cigarette butts was achieved in the 1000 ppm solutions with whole cigarette butts, showing a 97% removal of heptane. A lower mortality was achieved in the solutions of 10 ppm and in those of 100 and 1000 ppm there was a low percentage of mortality in solutions exposed to new cigarette butts and whole butts respectively. Thus, it has been demonstrated that cigarette butts can adsorb contaminants present in water and that their adsorption capacity depends on their concentrations. In addition, the two beaches of Cartagena studied have a high rate of contamination, indicating the health of this tourist sector.

PS P160 - 0540 Seasonal Variation in Heavy Metal Contamination in Soil Samples and Selected Water Holes of Old Oyo National Park, Southwest, Nigeria

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Heavy metal contamination is a concern worldwide as it influences the functionality and integrity of ecosystems. The presence of heavy metals in the ecosystem above permissible limits is not desirable as they may have toxic effects and pose severe threats to wildlife species perpetuation. The study was designed to assess the levels of heavy metals in soil samples and selected water holes (rivers) of Old Oyo National Park (OONP). Three (Marguba, Oyo-Ile and Tede) out of the five ranges of OONP were purposively selected based on the availability of perennial water holes. Surface soils [(n=36) (depth: 0-15 cm)] along chosen topographical catena were randomly sampled while water samples (n=24) were collected by grab sampling for four consecutive seasons (two wet and two dry) and analyzed for heavy metals; cadmium (Cd), copper (Cu), lead (Pb), chromium (Cr), zinc (Zn), nickel (Ni), iron (Fe), manganese (Mn) using Atomic Absorption Spectrophotometer after acid digestion of the samples. Data collected were subjected to descriptive (mean, standard deviation) and inferential (ANOVA, T-test) statistics, and compared with WHO permissible limit. Statistical significance was set at $\alpha 0.05$. The result showed that there were significant seasonal variations in the concentrations of heavy metals in the water holes [Zn (t=5.01), Ni (t=5.04)] and soil [Cu (t=7.42), Zn (t=6.28)] samples. The water holes and soils sampled were more contaminated with heavy metals during the wet season than the dry season. Heavy metals in sampled soils [Cd (2.12 ± 1.07 mg/kg) for both seasons] and water holes [Cd (0.08 ± 0.04 mg/l), Pb (0.21 ± 0.03 mg/l), Cr (0.29 ± 0.39 mg/l), Ni (0.11 ± 0.05 mg/l), Fe (10.63 ± 1.97 mg/l) for both seasons], were above WHO permissible limits and mostly attributable to anthropogenic activities by the surrounding communities. The highest mean levels of heavy metals were observed in Oyo-Ile (Cu, Zn, Cd, Fe) and Marguba (Cr, Pb, Ni, Mn) ranges of the park. Cadmium toxicity was observed in the sampled soils while the heavy metals (Cd, Pb, Cr, Ni, Fe) were above permissible limits which indicate that the sampled water holes (rivers) of Old Oyo National Park may not be potable and/or safe for drinking. The presence of heavy metal contaminations and their seasonal variations within Old Oyo National Park has implications on wildlife health within the park. These findings will help the management of Old Oyo National Park in designing a contamination abatement strategy/remediation measures to control heavy metals' biomagnification along the food chain within the park.

PS P161 - 0547 Differential Proteomic Profile, Morphological, and Tissue Alterations in Zebrafish Embryos Exposed to Water Accommodated Fraction (WAF) and Chemically Enhanced WAF (CEWAF) of Crude Oil and Dispersant

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Chemical dispersants such as COREXIT are commonly used as an immediate response to control oil spills. They help to break down the crude oil into small droplets increasing the presence of hydrocarbons compounds in the water column, which can have detrimental consequences to native biota. Dioctyl sulfosuccinate sodium is one of the main components of commercial dispersant COREXIT, and the information about its toxic effect on aquatic organisms is still scarce. The water accommodated fraction (WAF) of crude oil is a complex mixture of water-soluble hydrocarbons that can be chemically enhanced with the addition of a dispersant, being denominated chemical water accommodated fraction (CEWAF). The use of animal models in laboratory settings, like *Danio rerio*, can help to screen the effect of oil and dispersant at molecular, tissue and morphological level. Our aim was to evaluate the effect of WAF and CEWAF of a light crude oil to zebrafish embryos. A static bioassay of acute exposure to WAF and CEWAF with their respective controls was performed during 4 days without water exchange, and the samples were collected at the end of the experiment. A proteomic analysis was performed using mass spectrometry and the results were validated by RT-qPCR and Western Blot analyses. In parallel, morphological alterations were evaluated by scanning electron microscopy and tissue damage was assessed by histological analyses. The quantification of the hydrocarbons compounds in water from both treatments was done by gas chromatography-mass spectrometry. Proteomic analyses revealed that changes in proteins translation at 96 hours of exposure were significantly different in both treatments. We found alterations in the pathways of cell differentiation, embryo development, anatomical structure development, immune

system process, cell death, chromosome organization, circulatory system process, cell motility, lipid metabolic process, nervous system process, sexual maturation, and locomotion. In addition, proteins related to DNA metabolic process associated with developmental maturation were present only in the CEWAF treatment. Morphological damages and deformities such as scoliosis were found in embryos exposed to both treatments, with up to 60% of samples presenting scoliosis type S. In resume, the proteomic profile revealed a sort of proteins that were differentially up and down-regulated associated either to WAF and CEWAF treatments. The study also showed the activation of several key metabolic routes in the development and early development of the zebrafish as a result of the exposure to oil and oil:dispersant mixture.

PS P162 - 0608 2,4-Dichlorophenoxyacetic Acid Containing Herbicide Impairs Essential Visually Guided Behaviors of Larval Fish

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Aquatic herbicides are used worldwide to eradicate nuisance and invasive plants despite limited knowledge of their toxicity toward non-target organisms. 2,4-Dichlorophenoxyacetic acid (2,4-D) is a common active ingredient in commercial herbicide formulations, which causes plant cell death by mimicking auxin, a plant specific hormone. Application practices of 2,4-D commercial herbicides typically coincide with yearly freshwater fish spawning periods, which exposes fish to xenobiotics at their vulnerable larval stages. The full impacts of 2,4-D on larval fish remains poorly understood, and hence, whether it may alter larval survival, larval behavior, fish populations, and ecosystem dynamics. In the present study, we exposed embryonic and larval zebrafish (*Danio rerio*) to the active ingredient 2,4-D or a 2,4-D containing commercial herbicide DMA4®/VM and evaluated morphology, survival, behavior, and nervous system function. At 2,4-D concentrations producing no overt morphological defects during embryonic or early larval stages, we observed reduced survival throughout a 21-day larval assay. Notably, prey capture, an essential behavior essential, was reduced in larval zebrafish and yellow perch (*Perca flavescens*) exposed to 2,4-D. When 2,4-D exposure was restricted to the embryonic period coinciding with visual circuit development, exposed zebrafish larvae showed both reduced prey capture and reduced neural activity in the brain's visual receptive center. Together, our results suggest that 2,4-D alters the development and function of neural circuits underlying vision of larval fish, and thereby impairs essential visually guided behaviors.

PS P163 - 0672 Effect of Atrazine Exposure on Testicular Toxicity in Quails

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Atrazine (ATR) is known as a herbicide used for broadleaf and grassy weed controlled on crops including corn and lowbush blueberries and has been monitored extensively in surface water, groundwater and most of the earth's soil including north and south poles. There is sufficient evidence in experimental animals for the reproduction toxicity of ATR but the study on the mechanism of reproductive toxicity in birds is rare and is focused on the characterized or correlated data of the ATR toxic performance. The study aimed to establish bird models of reproductive dysfunction exposed to different dosages of ATR and highlight the disturb mechanism of ATR-induced environmental reproductive toxicity. Furthermore, we attempted to explore the major metabolic pattern of ATR through CYP450s. 18-day-old male European quail (*Coturnix C. coturnix*) were randomly allocated to four groups and oral administrated with different dosages of ATR (50 mg/kg, 250 mg/kg and 500 mg/kg) for 45 days, respectively. Histological and ultrastructural changes, body weight gain, testis coefficient, oxidative stress biomarkers, serum sex hormones, biochemical indexes levels and the total CYP450 content and CYP450 enzymes activities were determined. The expression of genes changes both on the nuclear receptor and gonad axis were detected by qRT-PCR array. Finally, the changes protein levels of the CYPs and the gonad axis were also tested. ATR treatments caused the testes coefficient decrease and the weight loss was not significant except the highest-dosage group. ATR decontrolled the correct biochemical function of the birds and the redox balance in testis was disordered. The histological structure and ultrastructural changes of testicles were observed. ATR arrested development of testis and the downregulated concentrations of LH, PRL and T were observed in treatment groups and the gene expressions of LH and PRL in pituitary increased significantly. CYP450s content of quails exposure in ATR were unbalanced compared to the control. And the key proteins for testosterone synthesis

were downregulated. Exposure to ATR leads to systemic toxicity of quails and testicles is an important target organ where it prevents sexual maturation of male quail and cause oxidative damage. ATR exposure can activate the expression of nuclear receptors and regulated the expression levels of CYPs and exert the detoxification effect. *Support: National Natural Science Foundation of China (No. 31572586), Excellent Youth Foundation of Heilongjiang Province of China (No. JC2017005), and China Agriculture Research System (No. CARS-35).*

PS P164 - 0762 The Thyroid-Disrupting Effects of Bisphenol S via Thyroid Receptor β Revealed by *In Vitro*, *In Vivo*, and *In Silico* Study

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Numerous studies on human exposure risk of bisphenol A (BPA) promotes increase global usage of the replacement bisphenol S (4-hydroxyphenyl sulfone, BPS) and its analogues (BPSs)¹, resulted in frequent detection in environment media^{2,3}. BPSs may have similar human exposure risks in organisms⁴, and existing data for endocrine effects assessment are still not sufficient. Herein, our recombinant two-hybrid yeast assay reveals that BPSs at environmentally relevant concentrations induce potential thyroid hormone receptor (TR) β disruption. The brominated bisphenols tetrabromobisphenol S (TBBPS) and tetrabromobisphenol A (TBBPA) have more potent anti-TR β activity than BPS and BPA⁵. The mRNA expression level of TR β in zebrafish embryos is altered significantly after the exposure to BPSs for 72 h⁵. BPSs with different moieties cause relative repositioning of helix 12 of human TR β ligand binding domain (LBD), as revealed by 100 ns molecular dynamics simulations⁵. The fluorescence quenching and red shift of TR β LBD also indicate distinct conformational changes of TR β LBD upon binding of BPSs. Our work suggests BPS and TBBPS are involved in TR β disruption and may be not safe BPA alternatives. *References: [1] Chen, D., et al. Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity-a review. Environ. Sci. Technol. 2016, 50(11), 5438-5453. [2] Qu, G., et al. Recent advances in the analysis of TBBPA/TBBPS, TBBPA/TBBPS derivatives and their transformation products. TrAC Trends Anal. Chem. 2016, 83, 14-24. [3] Jin, H., et al. Occurrence and partitioning of bisphenol analogues in adults' blood from China. Environ. Sci. Technol. 2018, 52(2), 812-820. [4] Rochester, J., et al. Bisphenol S and F: A systematic review and comparison of the hormonal activity of bisphenol A substitutes. Environ. Health Persp. 2015, 123(7), 643-650. [5] Lu, L., et al. Thyroid disruption by bisphenol S analogues via thyroid hormone receptor β : in vitro, in vivo, and molecular dynamics simulation study. Environ. Sci. Technol. 2018, 51(11), 6617-6625.*

PS P165 - 0784 Bioconcentration and Exposure Assessment of Polyaromatic Hydrocarbons in *Tilapia guineensis* and *Clarias gariepinus* in Rivers and Dams in Lagos, Nigeria

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In Nigeria, data are sparse for the levels of bioconcentrated polyaromatic hydrocarbons (PAHs), their toxicities as well as human health risk potentials of PAHs due to consumption of local fishes. This study was conducted to evaluate the levels of aromatic hydrocarbons in *Tilapia guineensis* and *Clarias gariepinus* in some natural communal river, as well as in commercial habitats in districts of Lagos State, Nigeria, and to compare with established limit values. Benzo(a)pyrene was the adopted pollution indicator in this study. Human health risk assessment (HHRA) was also conducted based on consumption of these hydrocarbons consumed by humans. Mean PAH values of 12.80 ± 0.61 and 3.70 ± 0.40 $\mu\text{g/kg}$ were recorded for *T. guineensis*; while those for *C. gariepinus* were 13.68 ± 1.14 and 9.39 ± 1.26 , respectively, for natural and commercial locations of Afa River. Conversely, measured values were 24.84 ± 1.48 and 8.64 ± 0.68 $\mu\text{g/kg}$ for *T. guineensis*; and values of 31.37 ± 3.64 and 15.32 ± 0.25 $\mu\text{g/kg}$, respectively, for natural and commercial fresh fish samples from Majidun River. The hazard index was calculated to be 1.18 for the HHRA. In conclusion, there was presence of PAHs due to activity of benzo(a)pyrene in the local Nigerian fish to sub-reference limit levels; however, there could be health related risks associated with long term consumption of PAHs in these fish species consumed by humans in the chosen locations in Lagos, Nigeria.

PS P166 - 0820 Ozonation Alters Zebrafish (*Danio rerio*) Embryotoxicity of Pharmaceuticals Found in Sewage Effluents

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Pharmaceutical residues can reach the aquatic environment via sewage effluents and may cause adverse effects on fish populations. Conventional sewage treatment plants (STP) are not equipped to efficiently remove pharmaceuticals. Ozonation is an advanced treatment method to improve sewage treatment. Ozonation can, however, create potentially toxic ozonation by-products (OBP) which may have deleterious effects on fish. However, little is known of the OBPs and their embryotoxicity in fish. In order to explore this novel field of potential ecologically relevant compounds, an ozonation reactor was used to ozonate pharmaceuticals regularly detected in STP effluents; e.g. carbamazepine and diclofenac. Ozone removal efficiencies of the parent compounds in water solutions, as well as the formation of putative pharmaceutical ozonation by-products (OBPs), were analyzed using liquid chromatography coupled to tandem-mass spectrometry (LC-MS/MS). Two doses of ozone were used in the study, 1-min contact time (0.053 mg/L peak ozone concentration) and 10-min contact time (0.15 mg/L peak ozone concentration). Embryotoxicity screenings were based on the OECD guideline test no. 236 Zebrafish (*Danio rerio*) Embryo Acute Toxicity Test (ZFET). Carbamazepine and diclofenac were largely removed by the high ozone dose ($90 \pm 11\%$ and $97 \pm 3.8\%$, respectively). Targeted chemical analysis showed the formation of two putative carbamazepine OBPs (carbamazepine-10,11-epoxide and 10,11-dihydrocarbamazepine). The ZFET test elucidated that diclofenac embryotoxicity was eliminated while carbamazepine embryotoxicity increased significantly. The lowest observed effect concentration (LOEC) of carbamazepine was reduced from the medium concentration (50 mg/L) to the lowest concentration to (25 mg/L). In order to investigate whether the increasing toxicity was the result of rapidly degenerating OBPs, carbamazepine (25 mg/L) was ozonated and stored at either 22 °C or 8 °C for 2 weeks and tested repeatedly using the ZFET test. The post-ozonation embryotoxicity remained over the whole period at both temperatures. We have thus shown that ozonation alters the toxicity of selected pharmaceuticals. Ozonation increased the toxicity of carbamazepine. Diclofenac toxicity was, on the other hand, eliminated by ozonation. Work on further identifying carbamazepine OBP products, and their toxicity profiles, is ongoing.

PS P167 - 0006 Impact of Nitrates and Nitrites in Drinking Water in a Rural Community in Transylvania, Romania

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Due to pollution from agriculture, industrialization, mining and urban development, water quality continues to be an ongoing concern in Romania. Specifically, in rural communities where there is a sole dependence on unregulated water sources, there is a concern regarding nitrate/nitrite contamination that is as high as 50–500 mg/L in some regions. These types of exposures have been associated primarily with methemoglobinemia, but more research is needed to identify other possible health effects. In light of this, the goals of this study were to (1) investigate and compare the nitrate/nitrite contamination levels in the private water sources in a rural locality between Romanian and Romma communities and (2) compare the association of contaminants in the water to living conditions and water behavioral practices. Fifteen Romanian and Romma families were recruited utilizing convenience sampling. These populations were identified in Buza, a rural locality in Cluj, Romania. Both of these communities solely depend on water from private and unregulated underground sources. Water was sampled from each participant's primary water source and then tested for ammonium, chemical oxygen demand, nitrate, nitrite, hardness, pH, and conductivity levels. In addition, a questionnaire was developed that captured demographics, water consumption behavior, other behavioral practices, and health profiles. Furthermore, a bacteriological assessment was conducted to quantify *Escherichia coli*, *Enterococci*, and total coliform levels found in the water. These communities had mean nitrate and nitrite concentrations of 71.38 mg/L and 0.44 mg/L, respectively. These nitrate and nitrite levels exceeded both the European Union (EU) and US Environmental Protection Agency (US EPA) maximum allowable limits, with some levels as high as 200 mg/L. Ammonium had a mean of 1.29 mg/L in relation to the

0.5mg/L EU maximum concentration limit. There were inverse associations between the chemical oxygen demand and total coliform levels and the chemical oxygen demand and the amount of *E. coli* (p value 0.05; correlation = -0.999) present. The closer the water source was to a pollution source, the higher the nitrate/nitrite contamination (p value > 0.05). The Romma population showed a trend of higher nitrate/nitrite contamination in their water sources in comparison with the Romanians. This preliminary data support published reports that nitrate/nitrite concentrations in rural unregulated water sources are significantly higher than those in urban communities. Future research should focus on the exposure assessment in these communities to create a sustainable intervention that reduces exposure.

PS P168 - 0067 Venomous Snake Bites in Morocco: Results of a Nationwide Study

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Snake bites are a neglected public health problem in many tropical and sub-tropical regions of the world. The aim of this study is to describe the epidemiological characteristics of snake bites in Morocco. This is a retrospective study of snake bite cases, reported to the Moroccan Poison Control Center over a 15-year period from 1999 to 2013. During the period 1999–2013, 2,053 cases of snake bites were occurred in Morocco, 85 of them fatal. Fifty-eight percent of the cases were males with a male-female ratio of 1.38. The average age of the patients was 26.48 ± 17.25 years. Most victims were adults (55.36%) and children aged 1–14 years (26.20%). According to the results, about 75% of snake bite accidents happened in rural areas. Most incidents occurred during the summer and spring months (42.95% and 35.95%, respectively), when snakes were more active. More than half of bites occurred in public places (56.97%) and about one-third at home (33.56%). The most commonly observed clinical signs were nausea, vomiting, local swelling, localized pain, and tachycardia. Snake bite envenoming is an important public health problem in many regions of the world, especially in rural areas. Concerted action is needed to ensure adequate supplies of effective antivenom to develop systems that deliver high-quality health care.

PS P169 - 0069 Acute Chloroquine Poisoning in Mali: Results of a Nationwide Study

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Chloroquine is widely prescribed for the prevention and treatment of malaria in developing countries, where young children and pregnant women are the most affected groups. The present study aims to describe the main epidemiological characteristics of acute poisoning with chloroquine in Mali. This is a descriptive retrospective study of acute chloroquine poisoning cases, recorded between 2000 and 2010, in the medical records and the consultation registers at 15 hospitals in Mali. There were 225 cases of chloroquine poisoning, aged 8 months to 66 years, in Mali, which was 21% of all drug poisoning cases reported during the study period. Of these, 86.7% were females and 4.1% were children under the age of 15 years. The average age of the patients was 20.9 ± 7.4 years. Most victims were adolescents and young adults aged 15–24 years (75.3%). According to the results, chloroquine was used as a means of suicide attempts in 52.2% of cases and for self-induced abortion in 45.5% of cases. The poisoning symptoms were varied, depending on the dose taken and the delay before treatment. The average length of stay in the hospital was 11.5 hours, with a range of 1 hour to 5 days. Among all diagnosed cases, 18 (8%) died. For other cases, the outcome was favorable with or without sequelae. Chloroquine is the most common cause of drug poisoning admission to hospitals in Mali. The real number of victims is probably underestimated because of undiagnosed and unreported cases.

PS P170 - 0070 Drug Poisoning: An Exploratory Study

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Drug poisoning continues to be the most common cause of acute poisoning worldwide. The purpose of this study is to describe the epidemiological characteristics of drug poisoning in the region of Tanger-Tétouan-Al Hoceïma in North West Morocco. This is a descriptive retrospective analysis of drug poisoning cases, reported to the Moroccan Poison Control Center and recorded in the registers at 11 hospitals in the region studied between 2014 and 2016. The data were cleaned up. Several cases had been identified more than once. These duplicates were eliminated. A total of 1,649 cases of drug poisoning were recorded between 2014 and 2016, which was 29.4% of all poisoning cases reported during the period of study. Nearly three-quarters of the cases (73.1%) were females with a female-male ratio of 2.7, and 21.4% were children under the age of 6 years. The average age of the patients was 20.5 ± 13.9 years. Most victims were teenagers and young adults aged 15–24 years (40.2%). About 99% of all exposures occurred in the home. According to the results, drugs were used as a means of suicide in 24.5% of cases. The symptoms of poisoning vary depending on the drug (or drugs) taken, the dose, and the delay before treatment. Among the 1,449 cases for whom the outcome is known, 7 of them died. For other cases, the outcome was favorable with or without sequelae. Drug poisoning remains a major public health problem. The number of victims is probably underestimated, because many cases are likely to be undiagnosed or unreported.

PS P171 - 0112 A Retrospective Survey of Marine Envenomations and Poisonings as Managed Telephonically by a Poisons Center in South Africa, Over a 20-Year Period

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The long South African coastline has an abundance of marine life, with an enormous diversity and complexity of venoms and poisons in marine animals. The potential for hazardous exposure to marine life in South Africa is thus high. To our knowledge, this is the first epidemiological review regarding marine toxicity that has ever been conducted in sub-Saharan Africa. The aim of this review was to investigate marine toxicology data as managed telephonically by the Tygerberg Poisons Information Centre. Marine toxicology cases were retrospectively analyzed for a 20-year period (January 1, 1995, to December 31, 2014). Data were extracted from archived consultation forms. Descriptive statistics are presented and post hoc analyses compared age, sex, province, and caller's background with severity and type of toxicology. The Tygerberg PIC processed 84,558 telephonic consultations during the 20-year study period, of which 311 (0.4%) related to marine toxicology exposures (median per year 15, interquartile range 9 to 20). The 311 calls involved 392 marine toxicology patient-related cases. Most calls involved adults ($n = 317$, 81%) and males ($n = 214$, 55%) and presented with no or minor symptoms ($n = 242$, 62%). Poisoning from ingestion ($n = 239$; 61%) was more commonly encountered than marine envenomation ($n = 153$; 39%), with paralytic shellfish poisoning ($n = 118$; 30%) and scombroid poisoning ($n = 93$; 24%) occurring often. Most of the recorded envenomation was caused by acanthotoxic fish (80/153, 52%), particularly stingrays (36/80, 45%) and sea barbells (30/80, 38%). The coelenterates were responsible for 48 cases (31%) of marine envenomation, which were mainly caused by bluebottle stings (33/48, 69%). Most patients ($n = 242$, 62%) presented with no or minor symptoms, while moderate to severe toxicity occurred in 129 patients (33%). Four deaths (1%) were recorded at the time of the call. Health care professionals were more likely to consult for severe cases (odds ratio (OR) 3.3; 95% confidence interval (CI) 1.9–5.9) and poisoning-related cases (OR 1.8; 95%CI 1.1–2.9). The relative burden of marine toxicology cases reported to Tygerberg PIC (in relation to the total number of calls managed) was low and mostly related to adults. Health care professionals consulted significantly more in serious cases than the general public, and envenomation was in general less serious than poisoning. The ability to identify and manage marine-related toxicology cases is important for providers of acute care, and future poison center studies should focus on robust data collection, including follow-up calls and patient-centered outcome data.

P172 - 0120 Impact of Timing of Arsenic Exposure on DNA Methylation and Arsenic-Associated Skin Carcinogenesis

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As a nonmutagenic human carcinogen, arsenic carcinogenic activity is likely the result of epigenetic changes, particularly alterations in DNA methylation. Increasing evidence has indicated that prenatal and early postnatal exposure, the timing of arsenic exposure, potentially plays a critical role on DNA methylation regulation and the subsequent risks for carcinogenesis later in life. We conducted a family-based cross-sectional study including three generations of an arsenic-exposed population in the Hetao Plain of Inner Mongolia, China, where the timing of arsenic exposure in each generation living in the same household is distinctive: adulthood, *in utero*, and during early childhood, as well as germ lines exposure for grandparents, parents, and grandchildren, respectively. We compared global and genome-wide DNA methylation profiles of 18 arsenic-exposed families and nine control families, as well as 18 arsenical skin lesions/cancer patients. Arsenic exposure left detectable DNA methylation changes even though exposure occurred decades ago, particularly in patients with arsenic-associated diseases. We showed that exposure timing did affect arsenic's effects on DNA methylation, while we were also able to identify a large number of common differentially methylated DNA loci (DML) and regions across generations. Our further analysis suggested that these DML could be used to separate arsenical skin lesions/cancer patients from arsenic-exposed but healthy individuals as well as from nonexposed individuals. Moreover, our analysis suggested that at least for some aberrant DML caused by arsenic exposure, may show a potential inheritable effect affecting not only those directly exposed but also later generations. Arsenic caused aberrant DNA methylations remain stable and detectable decades later after exposure remediation. For those DML and/or DMRs that are both common across generations and manifested in patients, they could be developed as universal biomarkers for identifying exposure individuals at risk for arsenic-induced skin lesions and cancer.

P173 - 0130 A Retrospective Review of Potassium Permanganate Exposures Reported to a Poisons Control Center in South Africa

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Potassium permanganate (KMnO₄) is a powerful oxidizing, highly corrosive, water-soluble chemical, with both medical and nonmedical uses. KMnO₄ is readily available as an over-the-counter medication for use as an antiseptic or dermal anti-infective. In South Africa, KMnO₄ is used widely as a home remedy, and as a constituent in some traditional medicines, for varying complaints such as disinfecting wounds or treating aches, pains, and swelling. Of concern is that children unintentionally ingest the crystals or liquid. Due to its alkaline corrosive effect, KMnO₄ can cause liquefaction necrosis, resulting in gastrointestinal tract oedema, burns, and ulcerations. The aim of this study was to investigate acute KMnO₄ exposures and the severity thereof, as telephonically managed by the Poisons Information Helpline (PIH) of the Western Cape, which services the whole of South Africa. Data were retrospectively analyzed, over a 3-year period (June 1, 2015, to May 31, 2018) by extracting the information from an electronic database. During the 3-year period, 28,561 human-related poisoning calls were received by the PIH. Of these 366 (1.3%) were related to KMnO₄ exposures. The majority of calls were received from two of South Africa's nine provinces, KwaZulu-Natal (n=169; 46.2%) and Gauteng (n= 90; 24.6 %). Most exposures were accidental (n= 288; 78.7%) and occurred in children between 0 and 12 years of age (n=268; 73.2%). The majority of calls were received from health care professionals (n= 359; 98.1%) and the route of exposure was mostly oral (n=358; 97.8%). The majority of patients presented with no or minor symptoms. (n=306; 83.6%). Ninety-two patients (25.1%) presented with vomiting, 64 (17.5%) presented with corrosive injuries of the mouth (e.g., swollen tongue or lip), 41 (11.2%) presented with dysphagia and/or odynophagia, and 35 (9.6%) experienced epigastric discomfort. Eight patients (2.2%) were in respiratory distress, three (0.8%) were unconscious, and one had a seizure. Although most of the enquiries involved patients with no or minor symptoms, the majority of KMnO₄ exposures were reported in children. Almost all the calls were received from health care professionals in a hospital setting, which implies significant medical costs. Moreover, the ready availability of KMnO₄ in South Africa may falsely assure the public that the chemical is nontoxic and that its medicinal use is without risks. Authorities should flag KMnO₄ poisoning as a public health concern in South Africa, with particular emphasis on legislation for childproof packaging to prevent harmful exposures in this vulnerable age group.

P174 - 0140 Heavy Metal Concentrations in Chinese Folk Medicines for Treating Epilepsy in Children in Rural China, and Their Prescription by Village Doctors: A Cross-Sectional Survey

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In areas of rural China inhabited by minority groups, local village doctors often use traditional Chinese folk medicines to treat primary epilepsy in children. However, such folk medicines have been implicated in heavy metal poisoning in children. This study determined the heavy metal concentrations of samples of folk medicines and examined the attitudes of local doctors regarding their use. Inductively coupled plasma-optical emission spectrometry (ICP-OES) was used to identify toxic heavy metals from 33 samples of folk medicines (28 mixtures and 5 monomers) from eight counties in the western of Hunan Province, China, from March 1, 2018, to March 30, 2018. Village doctors in western Hunan Province, China, participated in face-to-face interviews based on a questionnaire focused on knowledge about the heavy metal toxicity of folk medicines. Four toxic heavy metals (cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As)) were identified in the samples. The mean [SD] concentrations of Cd, Pb, Hg, and As were 0.513[0.237], 5.145[2.404], 0.298[0.179], and 0.321[0.106] mg/kg, respectively. Furthermore, Cd was found in 76%, Pb in 68%, Hg in 38%, and As in 24% of the samples. The highest concentration of lead found in a sample was 65.42 mg/kg. The questionnaire was completed by 112 village doctors, of whom 68% considered folk medicine to be safe for treating epilepsy and 77% believed that some heavy metals, especially lead, were efficacious for treating epilepsy. Some folk medicines contain heavy metal concentrations exceeding safe limits. The use of folk medicines by rural village doctors to treat epilepsy might lead to increased heavy metal exposure in children in these rural areas. It is important to focus on the issue regarding using traditional folk medicines with high heavy metal content for treating epilepsy in children may be harmful for their health, and there is a clear need for multifaceted interventions that target village doctors to improve their knowledge of heavy metal poisoning in children.

P175 - 0192 Opioid Prescribing Patterns in Hawaii—2015 through 2017

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Reduction of overuse and abuse of prescription and illicit opioids remains at the apex of US antidrug policy. Between 2010 and 2016, opioid-related overdose deaths more than doubled, from 21,089 in 2010 to 42,249 in 2016. The Hawaii Department of Health wished to characterize opioid prescribing patterns in HI. We obtained opioid prescription data for 2015–2017 by product, by prescriber, by county, by month for the state of HI from IQVIA (www.iqvia.com). Descriptive statistics, graphical displays, smoothing spline fits, and linear and nonlinear regressions were performed using SAS JMP version 12.0.1 (SAS Inc., Cary, NC). We examined change over time (COT) by month via 2nd order (quadratic) regressions and the change, and % change at the end of 2017 (month 36) based on these COT models. Over this 3-year period, 5750 prescribers wrote 1,804,227 prescriptions for 113,232,031 opioid doses. Opioid prescribing in HI was generally declining over this period based on new prescriptions, total prescriptions, quantity of doses, and number of unique patients. The same was true by county. All of these COTs were decreasing at an increasing rate (COTs were concave down). The most consistent COT (largest Rsqr) was the number of unique patients = 4.90E6 - 2412*Year - 541*(Year-2016.5)², Rsqr = 0.819. The 2nd order (quadratic) regression provides a statistical test of this increasing decrease, e.g., number of unique patients had a 2nd order term of 541 with a p-value <0.05. The % change at month 36 was -11.7%/year for number of patients, -13.5% for new prescriptions, -13.3% for total prescriptions, and -12.9% for total # doses. The top 13 (0.23% of) prescribers provided >10%, and the top 1410 (24.5% of) prescribers provided >90% of HI opioid prescriptions. Although the COT was decreasing for 7 of the 10 largest individual prescribers, it was not for the other 3. The COT by prescriber by quartile 1 through 4 of prescriptions likewise showed different patterns with % change at month 36 of -28.1%, -9.5%, -26.1%, and 0%/year. The product received by the most patients was hydrocodone/acetaminophen followed by oxycodone/acetaminophen. The COT for most products was decreasing, but use was increasing for buprenorphine and tapentadol. Although opioid prescribing patterns 2015–2017 in Hawaii and each county are decreasing by every measure, the opioid products and individ-

ual prescribers show considerable variation in overall change over time. These kinds of data and analyses can provide useful direction in the encouragement of opioid prescriber best practices.

P176 - 0198 Alteration of DNA Methylation by Transplacental PCB Exposure in Human

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Polychlorinated biphenyls (PCBs) have been associated with abnormal fetal development, including neurodevelopmental disorders. PCB exposure may cause epigenetic alterations during development, but studies about the association between maternal PCB levels and fetal DNA methylation status are limited. In this study, we have investigated whether maternal PCB exposure changed DNA methylation status in human umbilical cord tissue (a part of fetus) using a 850K methylation array analysis. Samples were obtained from the Chiba study of Mother and Child Health (C-MACH) (Sakurai et al., BMJ open, 2016). Serum concentrations of the 16 main congeners of PCBs were analyzed by gas chromatography (GC) negative ion chemical ionization quadrupole mass spectrometry (NICI-qMS). The DNA methylation profiles of the umbilical cord tissues were determined using the Infinium Methylation EPIC BeadChip. Results of methylation degrees determined by methylation array showed five and one loci that tended to change in methylation degrees of cord tissue from female (cg09878117, cg06154002, cg06289566, cg12838902, cg01083397) and male (cg13368805), respectively. These results indicated that maternal PCB exposure possibly alters the methylated levels at some loci in fetal genes in umbilical cord.

P177 - 0229 Organophosphate Pesticide Exposures and Diabetes among US Adults: Findings from the National Health and Nutrition Examination Survey, 2007–2008

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Organophosphates (OPs) are the most widely used pesticides in agricultural and residential settings worldwide. Many OP residential uses have been phased out due to the implementation of the Food Quality Protection Act (FQPA) of 1996, but some OPs are still used in the US to control insects on food crops, making them the most commonly used pesticides for indoor pest control. Several toxicology studies have demonstrated positive associations between OP exposures and increases in blood glucose and the development of diabetes. Very few studies have evaluated these associations among humans. Our aim was to investigate the associations between OP pesticide urinary metabolites and diabetes in a representative sample of the US adult population. Secondary analyses were conducted on all adult participants in NHANES 2007–2008 (n=5,707) who were assessed for six OP urinary metabolites, including dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), diethylthiophosphate (DETP), dimethyldithiophosphate (DMDTP), and diethyldithiophosphate (DEDTP). We conducted univariate and multivariable logistic regressions to estimate the adjusted odds of lifetime diabetes in relation to OP metabolites above the limit of detection (LOD). Age, gender, income-to-poverty ratio, race/ethnicity, education, body mass index, and waist circumference were included in multivariable models as potential confounders. The mean age was 46.8, with race distributed as 75% non-Hispanic White, 12.5% non-Hispanic Black, 5.3% Mexican American/Hispanic, and 6.6% other races. Seventy two percent of participants were overweight or obese. DMTP was detected ($\geq$ LOD) in 74% of participants, while other OP metabolites were not detected in the majority of participants (DMP: 38.7%; DEP: 31%; DETP: 40%; DMDTP: 21%; and DEDTP<1%). About 8.5% had ever been diagnosed with diabetes and more than 38% had a positive family history of diabetes. Multivariable-adjusted associations between OP metabolites and diabetes were as follow for each OP: DMP (OR:0.98(95%CI:0.67–1.23)); DEP (1.20(0.81–1.93)); DMTP (0.99(0.58–1.98)); DETP (1.12(0.68–1.83)); and DMDTP (0.74(0.47–1.16)). Furthermore, tests of effect modification by age group (<50, $\geq$ 50) and gender showed no clear trends and were not statistically significant. This is the first study of this size among the US adult population investigating the relationship between OPs and diabetes. Given the widespread use of OPs and the growing burden of diabetes, further studies are required to evaluate these associations.

P178 - 0260 Epidemiological Association between Multiple Chemical Sensitivity and Birth by Caesarean Section: A Nationwide Case-Control Study

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Multiple chemical sensitivity (MCS) is characterized by recurrent nonspecific symptoms that are attributed to exposure to trace levels of environmental agents. Although the clinical symptoms of MCS have been described in several studies, the risk factors for this condition remain unclear. Our aim was to clarify the risk factors for MCS and the association between MCS and birth by caesarean section. We conducted a nationwide case-control study of Japanese individuals (aged 20–65 years) with physician-diagnosed MCS (183 cases) and without MCS (345 controls). The study participants were selected from among 150,000 people in a web-based research panel with approximately 1,000,000 registrants. They completed an online survey including questions on their sociodemographic characteristics, birth history (i.e., birth by caesarean section), and other potential risk factors for MCS. Multivariate logistic regression analysis was employed to determine the association between sociodemographic characteristics and the risk of MCS. The proportions of case and control subjects who were born by caesarean section were 39.9% and 7.0%, respectively. The association between birth by caesarean section and MCS was significant even after adjusting for potential confounders (adjusted odds ratio: 6.15; 95% confidence interval: 3.13–12.1). A history of agricultural work, mouth breathing, ≥ 11 vaccinations in the past 10 years, and residing in a new home (<1 year-old) ≥ 3 times were also significantly associated with MCS. Our data indicate an epidemiological link between MCS and birth by caesarean section. Moreover, we show that factors other than chemical exposure may be associated with the development of MCS.

P179 - 0323 Multidisciplinary Approach in the Diagnosis and Treatment of Necrotizing Spider Bites

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Spider bites belonging to the genus *Loxosceles* can have a very serious evolution both locally and systemically. The therapeutic response is entrusted to the multidisciplinary approach that involves general practitioners, toxicologists, emergency doctors, infectivologists, hyperbarists, and plastic surgeons. This approach was applied to 74 cases observed at the Emergency Room of the Great Metropolitan Hospital Niguarda between 1 January 2015 and 30 November 2018, of which 20 needed of hyperbaric oxygen therapy (HBOT). In Italy *Loxosceles rufescens* is present and often takes refuge in homes. Cutaneous loxoscelism is characterized by cutaneous necrosis, sometimes complicated by soft tissue infection or necrotizing fasciitis. The diagnosis is generally based on the recognition of the spider but it must be noted that it is, unfortunately, difficult to capture because its bite is asymptomatic. It is essential to ask the patient when, where, and how the bite may have occurred and which symptoms he complained about (the immediate pain after the bite excludes the *Loxosceles*), to properly evaluate the extent of the edema and the presence of signs of necrosis. After 6-8 hours, in the affected area there is an inflammatory reaction with itching, burning pain, erythema, and hard and painful tumefaction; if no further lesions appear in the next 2-3 days, an evolution towards skin necrosis is unlikely. In the event of an unfavorable evolution, an increase in the size of the erythematous area occurs, which becomes purple, with the appearance of cyanotic macules that evolve towards necrosis and subsequent formation of an eschar in 3-7 days. The histopathology of the lesion is characterized by thrombosis, hemorrhage, inflammatory infiltrate, and colliquative necrosis. In the case of infections, the production of alpha toxin causes myonecrosis, haemolysis, and haemorrhagic alterations. Treatment is initially generic for spider bite with local disinfection, antibiotic, and antihistamine therapy with general pain relief. In case of dermonecrosis, compartment syndrome and necrotizing fasciitis, the HBOT is indicated with compression profiles chosen on the basis of signs, symptoms, and laboratory tests (LRINEC Score: Laboratory Risk Indicator for Necrotizing Fasciitis). The poison control center is the reference center for the diagnosis of loxoscelism and for the pharmacological therapy to be performed. Hyperbaric oxygen treatment promotes the delimitation of the lesion and is determinant in recovery.

PS P180 - 0325 A Systematic Review of the Public Health Burden of E-waste Exposure in Africa

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The recycling of electronic waste, or e-waste, remains a major source of survival for the poor in urban areas in developing countries. Unfortunately, this economic activity is fraught with severe health risk; for instance, during periods of heavy rainfall, much of the e-waste site becomes flooded and run-off waters irrigate farm lands. This study is aimed at cataloguing the extent and size of the public health effect of e-waste exposure in Africa. This systematic search (2011-2018) of four electronic databases (PubMed, Google Scholar, Science Direct, and Scopus) for studies on e-waste in Africa used the following search words: 'e-waste in Africa', 'e-waste in developing nations', 'public health and e-waste', 'environment and e-waste', and 'e-waste and health.' The combined search yielded a total of 6426 articles. After duplicates were removed, 364 articles were left and, following the inclusion and exclusion criteria, 37 articles were used in the present review. Nigeria (Alaba international electronic market and Ikeja computer village all in Lagos) and Ghana (Agbogbloshie, Accra) are the major e-waste dumpsites in Africa. Water, air, soil, dust, fish, and vegetable samples indicate that the e-waste recycling has caused environmental contamination of public health importance. E-waste workers, residents (including mothers) and traders in these major e-waste recycling hubs showed elevated levels of toxic pollutants in various biological samples (blood, urine, breast milk) indicating that not only the e-waste workers are at risk but the general population. Headaches, cough and chest pain, stomach discomfort, miscarriages, abnormal thyroid and reproductive functions, reduction of gonadal hormone, and cancer are common complaints of the e-waste community. Evidence of severely negative human-animal-environmental health impact from informal e-waste recycling is growing in Africa, along with the call for e-waste regulatory framework in sub-Saharan African countries.

PS P181 - 0395 Associate Metabolome/Lipidome of Taiwanese Children with Environmental Exposure of Perfluoroalkyl Substances and Phthalates

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Perfluoroalkyl substances (PFASs) and phthalates are two groups of common artificial chemicals, which can be widely detected in human samples. In this study, we intend to understand effects of environmental exposures of those chemicals on children. Metabolomics and lipidomics were applied to provide the overall pictures of the molecular perturbation in the serum of children caused by those toxicants. 290 Taiwanese children (age 8-10 years) were participated in this study. Numerous PFASs and phthalate metabolites were analyzed in their biofluids. Moreover, changes of endogenous metabolites and lipids in the serum were analyzed by nuclear magnetic resonance (NMR) and ultra-performance liquid chromatography tandem mass-spectrometry (MS). After spectral processing, multivariate statistical analysis and multiple linear regression were used to evaluate the association between each exposure and metabolite/lipid changes. In our NMR-based metabolomic results, different metabolic patterns were discovered in children exposed to different levels of perfluoro-n-undecanoic acid (PFUnDA), perfluoro-n-tridecanoic acid (PFTrDA), mono-isobutyl phthalate (MiBP), and di(2-ethylhexyl) phthalate (ΣDEHP). Numerous serum metabolites, such as VLDL, glutamine, scyllo-inositol, and glycoprotein acetyls were associated with selective PFAS and phthalate exposures, indicating the possible adverse effects on children health. Moreover, the results of MS-based lipidomic approach showed children exposed to different levels of perfluoro-n-octyl sulfonate (PFOS), PFTrDA, perfluoro-n-decanoic acid (PFDA), and mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) had distinct serum lipidomes. Different subclasses of phosphorylcholine-containing lipids were found to be associated with selective PFAS and phthalate exposures. This study demonstrated that metabolomics/lipidomics is a powerful approach to identify metabolic/lipid perturbation caused by background levels of PFASs and phthalates in children serum and to suggest potential health effects and their possible mechanisms of these toxicants.

PS P182 - 0400 The Aftermath of Flood: Diversity of Fungal Toxic Metabolites in House Dust in Croatia

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In the past decade we are witnessing the rising occurrence of climate extremes all around the world. Droughts, hurricanes, storms and floods are life threatening and with multiple consequences including economic loss and health hazards. Changes of temperature and water availability also affects fungi and their metabolic profile. Post-flood mould colonisation of indoors may represent health threat to inhabitants due to inhalation and cutaneous exposure to fungal allergens and toxic metabolites. Vacuumed dust sampling may be more representative in terms of human exposure indoors as they act as a sink for airborne moulds and their secondary metabolites. In spring 2014 heavy rainfall and floods have been experienced in the Eastern Croatia. In this study we aimed to characterize the presence of fungal toxic metabolites in house dust in association with the post-flood water damage. Samples of house dust were collected in winter and summer of 2016 and 2017 in repaired and unrepaired houses in Gunja (flooded village) and Gornji Stupnik (control village). A 30 mg subsample of dust collected from five houses at each location in each sampling period (N= 60) was used for the secondary metabolite analysis. Metabolites were extracted and diluted from dust using acetonitrile/water/acetic acid solution and analysed by HPLC System coupled to a QTrap 5500 equipped with Turbo Ion Spray ESI source in connection with a Gemini C18 column (150 x 4.6 mm i.d., 5 µm particle size). More than hundred different metabolites, namely *Aspergillus*, *Penicillium*, *Alternaria* and *Fusarium* metabolites were detected in dust samples. Among these dominated metabolites produced by *Aspergilli*. Sterigmatocystin (STC) and its precursors were frequently detected in repaired houses in flood affected and control area (from 3.8 ± 1.2 ppb to 73.9 ± 138.4 ppb and 1.8 ± 0 ppb to 20.7 ± 39 ppb, respectively). *Stachybotrys* metabolites stachybotryamide and stachybotrylactam were detected only in one sample from the flood affected area (73.6 and 435.6 ppb, respectively). No specific metabolic profile associated with post-flood water damage was identified. However, exceptionally high concentrations of STC and *Stachybotrys* metabolites at the certain locations in flooded village suggest thorough remediation in order to prevent possible health hazards. *Acknowledgements: This work has been fully supported by Croatian Science Foundation under the project MycotoxA (IP-09-2014-5982).*

PS P183 - 0416 PI3K/AKT Pathway-Mediated Cyclin D1 Expression Participates in Low Dose Arsenic-Induced Transformation in Human Hepatocyte L-02

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Environmental arsenic contamination has been a major public health problem in many parts of the world, because its toxicity and carcinogenicity. Various potential mechanisms of arsenic carcinogenesis have been proposed, but the mechanism is not completely understood. More evidence indicates that the modulating effect of arsenic on the signaling pathways and genes expression related with carcinogenesis may play an important role in the carcinogenesis. In this study, we study whether PI3K/AKT /GSK3β signaling plays a crucial role in arsenic-induced human hepatocyte transformation through induction of cyclin D1. L-02 cells exposed to 0.2µmol/L arsenic for 24 weeks were designated as L-02-As cells. Compared with the control L-02 cells, L-02-As cells displayed a significant increase in cell proliferation with higher colony formation efficiency. Furthermore, the tumorigenicity of L-02-As cells was confirmed in nude mice. These results indicated that repeated exposure of low dose of L-02 cells to arsenic could cause malignant transformation. Western blot assay demonstrated that the transformed L-02-As cells presented a significant increase of cyclin D1 expression and activation of Akt, as compared with those in parental L-02. Further studies showed that Akt activation is required for low dose arsenic-induced transformation of L-02 cells, since inhibition of Akt activity by pharmacological inhibitor (LY294002) attenuated the colony formation in soft agar. Furthermore, our data indicated that cyclin D1 is a downstream molecule involved in PI3K/Akt-mediated cell transformation upon arsenic exposure based on the facts that inhibition of cyclin D1 expression by LY294002, or the knock-down of the cyclin D1 expression by its specific siRNA resulted in impairing of anchorage-independent growth of transformation of L-02 cells. In addition, we

detected that the level of phospho-GSK-3 β (Ser9) increased and the level of phospho-cyclin D1 (Thr286) decreased in the transformed L-02-As cells, which could be impaired by LY294002. These findings suggested that upregulation of cyclin D1 resulted from stabilization due to a lack of phosphorylation mediated by GSK-3 β . In summary, our results demonstrate that the PI3K/Akt/GSK-3 β pathway is involved in arsenic-induced neoplastic transformation of human hepatocyte L-02 through the induction of cyclin D1.

PS P184 - 0557 Impact of Tobacco Use and Environmental Tobacco Smoke Exposure in Perinatal Outcomes in a Portuguese Population

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As tobacco continues to be the leading cause of preventable disease worldwide, research on its effects, particularly among susceptible populations or during susceptible life stages remains imperative. To deepen the knowledge on the risks of tobacco *in utero*, the NEOGENE project is now in course with the aim to assess DNA damage and some specific epigenetic alterations (global DNA methylation and histone modifications) resulting from tobacco use and exposure to environmental tobacco smoke (ETS) and other simultaneous exposures during pregnancy (namely, metals, polycyclic aromatic hydrocarbons, bisphenol, phthalates and brominated polyphenylene). With data obtained in the frame of the NEOGENE project, we characterize here the tobacco use and exposure to environmental tobacco smoke in a Portuguese population and its influence in the main perinatal outcomes (gestational age, birth weight among full-term newborns, and Apgar score). Furthermore, the significance of smoking cessation prior to the third trimester and the potentially additive effects of passive and active maternal smoking in the analyzed outcomes will also be explored. This cross-sectional study enrolled in 593 pregnant women who received prenatal care on Centro Hospitalar de São João, in Porto (Portugal), from May 2017 to August 2018. A standard questionnaire, including sociodemographic and lifestyle variables, clinical history, tobacco consumption and exposure before and during pregnancy was completed for all the participants. The cotinine concentrations were measured by solid-phase competitive ELISA, in maternal urine samples, collected on the day of delivery. Perinatal outcomes were extracted from medical records. The use of reliable information on tobacco use and particularly on ETS exposure, as the one obtained through cotinine validation is essential for an accurate evaluation of the effects of these exposures in perinatal outcomes. By adding information on co-exposures to all this data, new and relevant information will be obtained contributing to the implementation of coherent measures for disease prevention in early stages of life. *This work is supported by FCT and FAPESP (FAPESP/19914/2014); Carla Costa and Joana Madureira are supported by FCT (SFRH/BPD/96196/2013 and SFRH/BPD/115112/2016 grants, respectively).*

PS P185 - 0610 FDALabel Database: Enabling Insights from US FDA Drug Labeling to Advancement of Adverse Drug Reaction Study

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US FDA drug labeling provides diverse drug information including product indications, dosage recommendations, contraindications, drug interactions, target populations, adverse drug reactions (ADRs). FDALabel Database is a web application (<http://www.fda.gov/ScienceResearch/BioinformaticsTools/ucm289739.htm>) that provides a user-friendly interface with customizable searches against the entire text of drug labeling. It provides the full set of approximately 100,000 labeling documents for products including human prescription drugs, biological products, and over-the-counter (OTC) drugs. To demonstrate the FDALabel database's utility, we have conducted an ADR study that applied Medical Dictionary for Regulatory Activities (MedDRA) standard terminologies. ADRs in Boxed Warning (BW), Warnings and Precautions (WP), and Adverse Reaction (AR) sections of drug labeling documents from 1164 New Drug Applications were investigated. The number of MedDRA Preferred Terms (PTs) was 463 for BW, 2023 for WP, and 2961 for AR. We found severe ADRs in BW section were prevalent in MedDRA System Organ Classes such as Nervous system disorders, Psychiatric disorders, and Cardiac disorders. Furthermore, Hierarchical Cluster Analysis (HCA) revealed that drugs within the same therapeutic categories might be associated with similar ADRs. In summary, the

FDALabel database offers the public, researchers and regulatory reviewers an efficient and user-friendly means of accessing and searching a large amount of drug information. It also opens the possibility for novel uses of drug labeling to support ADR studies by, for example, identifying trends in ADRs or by identifying ADRs that are associated with increased risks to public health.

PS P186 - 0838 Scoping Environmental Chemical Exposures Associated with Parkinson's Disease: An Evidence Map

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The National Toxicology Program's (NTP) Office of Health Assessment and Translation (OHAT) conducts fit-for-purpose health hazard evaluations, including evidence maps, scoping reviews, and full systematic reviews, using systematic review methodologies to identify and translate health effects data in scientific literature into useable information to support public health decisions. Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting at least 6 million people globally. Despite the contribution of known genetic factors to PD, the causes of most cases remain unknown. A growing body of evidence suggests that certain environmental exposures may be partly responsible for the rising incidence and progression of PD. To better understand the contribution of environmental chemical exposures to PD, the NTP generated a scoping review and evidence map to systematically identify and categorize scientific publications investigating any environmental chemical exposure and measurement of neuropathological endpoints associated with PD or mechanisms suspected or known to lead to PD in human observational, experimental animal, and *in vitro* studies. Over 95,000 publications of PD-related outcomes were identified using a comprehensive literature search in PubMed. Because of the high number of publications, a combination of manual and semi-automated screening approaches was then used to select studies reporting relevant environmental chemical exposures based on a priori study screening criteria. Study characteristics were extracted and categorized to develop interactive evidence maps of key health effects data (e.g., exposures, evidence streams, measured health endpoints and genes). Of more than 1,800 relevant publications, rotenone (n=791), manganese (n=325), and paraquat (n=319) accounted for the majority of environmental chemical exposures, along with other pesticides and metal-containing compounds including dieldrin and maneb. The most-studied genes included those encoding α -synuclein (SNCA; n=180), tyrosine hydroxylase (TH; n=90), and the dopamine transporter DAT (SL6A3; n=66). Collectively, scoping reviews and interactive evidence maps help protect public health by allowing researchers to: (1) explore study characteristics and selected results; (2) communicate state-of-the-science to researchers, regulators, and the general public; and (3) support evidence-based decisions including data-rich topics for further systematic review or data-poor knowledge gaps for future research.

PS P187 - 0901 PI3K/AKT Pathway-Mediated Cyclin D1 Expression Participates in Low Dose Arsenic-Induced Transformation in Human Hepatocyte L-02

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Environmental arsenic contamination has been a major public health problem in many parts of the world, because its toxicity and carcinogenicity. Various potential mechanisms of arsenic carcinogenesis have been proposed, but the mechanism is not completely understood. More and more evidence indicates that the modulating effect of arsenic on the signaling pathways and genes expression related with carcinogenesis may play a more important role in the carcinogenesis. In this study, we study whether PI3K/AKT/GSK3 β signaling pathway plays a crucial role in arsenic-induced human hepatocyte transformation through induction of cyclin D1. L-02 cells that had been exposed to 0.2 μ mol/L arsenic for 24 weeks were designated as L-02-As cells. Compared with the control L-02 cells, L-02-As cells displayed a significant increase in cell proliferation, had a more colony forming efficiency. Furthermore, the tumorigenicity of L-02-As cells was confirmed in nude mice. These results indicated that repeated exposure of low dose of L-02 cells to arsenic could cause malignant

transformation. Western blot assay demonstrated that the transformed L-02-As cells presented a significant increase of cyclin D1 expression and activation of Akt, as compared with those in parental L-02. Further studies showed that Akt activation is required for low dose arsenic-induced transformation of L-02 cells, since inhibition of Akt activity by pharmacological inhibitor (LY294002) attenuated the colony formation in soft agar. Furthermore, our data indicated that cyclin D1 is a downstream molecule involved in PI3K/Akt-mediated cell transformation upon arsenic exposure based on the facts that inhibition of cyclin D1 expression by LY294002, or the knockdown of the cyclin D1 expression by its specific siRNA resulted in impairing of anchorage-independent growth of transformation of L-02 cells. In addition, we detected that the level of phospho-GSK-3 β (Ser9) increased and the level of phospho-cyclin D1 (Thr286) decreased in the transformed L-02-As cells, which could be impaired by LY294002. These findings suggested that upregulation of cyclin D1 resulted from stabilization due to a lack of phosphorylation mediated by GSK-3 β . In summary, our results demonstrate that the PI3K/Akt/GSK-3 β pathway is involved in arsenic-induced neoplastic transformation of human hepatocyte L-02 through the induction of cyclin D1. *This work was supported by NSFC (81372939;81673109;8183000177).*

P188 - 0059 Effect of Octyl-phenol and Bisphenol A on Cardiomyocytes Differentiation of Mouse Embryonic Stem Cells

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Endocrine-disrupting chemicals (EDCs) have similar structures with steroids hormones, which can interfere with hormone synthesis and normal physiological functions of male and female reproductive organs. Sex steroid hormones influence calcium signaling of the cardiac muscle in early embryo development. Progesterone (P4) has been reported to reduce blood pressure. To confirm the effect of P4, octyl-phenol (OP), and bisphenol A (BPA) on early differentiation of mouse embryonic stem cells (mESCs) into cardiomyocytes, P4, OP, and BPA were treated at two days after attachment and media were replaced every two days. In addition, mifepristone (RU486) is a synthetic steroid that has an affinity for progesterone receptor (Pgr) and was treated for one day starting on day 11. To investigate the calcium signaling, the expression of calcium channel gene and contraction-related genes was analyzed. Beating ratio was decreased in P4, OP, and BPA treatment. The Pgr mRNA level was significantly increased in P4-, OP-, and BPA-treated group. However, the mRNA level of calcium channel gene, Trpv2, was significantly decreased in the P4-, OP-, and BPA-treated group. In addition, expressions of contraction-related genes such as Ryr2, Cam2, and Mlck3 were significantly decreased in the P4-, OP-, and BPA-treated group. Interestingly, treatment of RU486 rescues altered calcium channel gene and contraction-related genes. P4, OP, and BPA treatments resulted in the reduction of intracellular calcium level and the beating frequency. Taken together, these results suggest that OP and BPA may impact the inhibition of cardiomyocytes differentiation of mESCs, results in disruption of cardiomyocytes differentiation of mESCs. *This research was supported by a grant (17182MFD5487) from Ministry of Food and Drug Safety in 2017.*

P189 - 0090 Status of Urinary Metals/Metalloids of School-Age Children in Xuyi, Jiangsu Province, China

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Metal pollution is a widespread and serious problem in the world. In China, especially in industrial and agricultural regions, metal pollution is becoming an increasingly severe environmental problem. Evaluation of exposure in the population has already attracted great attention. Recently, co-exposure to multiple metals has become a major feature for the general population. In this study, an inductively coupled plasma-mass spectrometry (ICP-MS) method was used to evaluate the urinary level of metals and metalloids of 316 school-age children in Xuyi, Jiangsu Province, China. After diluting with 1% nitric acid, the urine samples were analyzed directly by ICP-MS. Levels of 37 metals and metalloids including Be, B, Mg, Al, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Ag, Cd, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Dy, Ho, Er, Tm, Yb, Lu, Tl, Pb, and U were quantified. The detection rates of the 37 metals/metalloids ranged from 17.09% to 100%. Female samples had higher levels than male ones and showed statistical significance ($p < 0.05$), included B (1185.55 versus 1154.56 $\mu\text{g/L}$), Mg (1368.25 versus 763.12 $\mu\text{g/L}$), Co (0.66 versus 0.59 $\mu\text{g/L}$), Zn (407.30 versus 389.52 $\mu\text{g/L}$), Sr (100.84 versus 82.55 $\mu\text{g/L}$), Ba (1.41 versus 1.27 $\mu\text{g/L}$), and Pb (0.69 versus

0.62 $\mu\text{g/L}$). The urinary levels of As (36.37 $\mu\text{g/L}$), Sr (104.19 $\mu\text{g/L}$), Co (0.67 $\mu\text{g/L}$), Cd (0.18 $\mu\text{g/L}$), Pb (0.66 $\mu\text{g/L}$), and U (0.02 $\mu\text{g/L}$) exceeded urinary levels of American children 4.78, 77.80, 0.48, < 0.036 , 0.24, and 0.005 $\mu\text{g/L}$, respectively. The levels of Rb, Sr, and Cs were higher than the general populations in China, while levels of Cd, Cr, Ba, and Pb were lower. These indicated that school-age children in this area had a relative high exposure level of several metals and metalloids (As, Sr, Co, Rb, U). It is very necessary and meaningful to reinforce the monitoring of long-term exposure and evaluation their health effects in the future.

P190 - 0127 Research on the Effects of Heart Injury in Rats Induced by Short Wave Radiation

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This study was to explore the effects of heart injury in rats induced by short wave radiation and to lay the foundation for finding sensitive biological indicators. It would also contribute to provide new ideas for protection. 100 male Wistar rats were exposed to four different average power densities of 0, 5, 10, and 30mW/cm² for 6 min. Rats were sacrificed at 1d, 7d, 14d, and 28d, respectively, after radiation. The contents of Ca²⁺, AST, CK, LDH, and electrocardiogram (ECG) were detected by using an automatic biochemical detector and multiple physiological recorder. Moreover, the changes of heart tissues structural and ultrastructural of rats were observed by the light microscopy and transmission electron microscopy (TEM) at 1d, 7d, 14d, and 28d after short wave radiation. The results of the study are as follows: (1) The content of Ca²⁺ had significant increase, the heart rate lowered, and a small number of myocardial cell nuclei pyknosis, myocardial mitochondria cavitation in rats of 5mW/cm² group at 1d, and recovered at 7d after short wave radiation. (2) the content of Ca²⁺ had significant increase, the heart rate lowered, and the damage of tissue structural appeared at 1d in rats of 10mW/cm² group, and the injuries were the most significant at 7d after short wave radiation, including the myocardial fibers arranged in disorder and the cavitation and swelling of the mitochondria of the myocardial cells. The above changes recovered at 14d after radiation. (3) The contents of Ca²⁺, AST, CK, and LDH had significant increase, the heart rate with significant lower R-H wave amplitude in rats of 30mW/cm² group 1d and 7d after radiation. What's more, the cardiac muscle fibers showed focal lysis and breakage, the mitochondria of the myocardial cells were cavitation, swelled, the significantly increased autophagosomes and the interstitial edema were found at 7d after short wave exposure. All mentioned injuries above did not recover to be normal until 28d after short wave exposure. The conclusions of the study are as follows. (1) The cardiac dysfunctions and histological and ultrastructural damages of rats were caused by 5, 10, and 30mW/cm² short wave radiation. (2) The average power densities of 5, 10, and 30mW/cm² could cause the changes of functions and cardiac structures with clear dose-effect relationship.

P191 - 0293 Knowledge Awareness and Practice Regarding Arsenicosis among the Community Health Care Providers of Bangladesh

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Widespread contamination of arsenic in Bangladesh has been jeopardizing the health of millions of people. Toxicity with arsenic in drinking water has occurred in many countries of the world causing people to be afraid of this hazardous health problem. This cross sectional study was done to assess the level of knowledge awareness and practice regarding arsenicosis among the community health care providers of Bangladesh in four Districts in Chandpur, Nilphamari, Jessore, and Lalmonirhat. 210 community health care providers were interviewed by a structured questionnaire and were selected using purposive sampling. The study period was January 2014 to December 2014. This study suggests that refresher training will help them increase knowledge, awareness, and practice about arsenicosis. Majority (77.6%) community health-care providers' age group was 31-40 years. Most (80.9%) respondents were female and the remaining male. Regarding educational status, the majority of respondents (76.2%) had passed H.S.C level. The study revealed that 69% of respondents had knowledge about arsenic while 31% did not know about arsenic. All (100%) respondents knew that the red mark tube well was the source of arsenic contaminated water in Bangladesh. Regarding knowledge about arsenic contaminated districts, majority (71.4%) respondents had knowledge, while 28.5% did not. The study revealed that the majority (71.4%) respondents had knowledge about signs and symptoms of arsenicosis and 28.5% did not.

Regarding knowledge about red and green mark tube well, 88.1% respondents knew the meaning of red and green tube well while 11.9% respondents did not. In this study most (95.3%) community health care providers attended the arsenicosis patient. 23.8% respondents thought that arsenicosis is a communicable disease and 23.8% said that arsenicosis is a hereditary disease. 71.2% respondents said that arsenicosis is a preventable disease while 28.2% said that it is a not preventable disease. The study showed that all (100%) respondents said that red labeled tube well cannot be used for cooking and drinking and green labeled tube well can be used for this purpose. Most (90%) respondents practiced to advise arsenicosis patients to avoid drinking the red mark tube well water. Regarding practice of medication in pregnant women, 52.3% respondents advised patients not to take Vitamin A, E and C, while 47.7% respondents advised to take these vitamins. Concerning practice of arsenic kit test, all (100%) respondents did not know how to operate arsenic kit test.

PS P192 - 0331 Effect of Low-Dose of Ethanol on Zebrafish Embryogenesis

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Biphasic dose response of ethanol is a well known phenomenon as far as epidemiological studies are concerned. Modulation of zebrafish behavior at high as well as low doses of ethanol treatment has been well studied in literature. To provide a deeper insight into this phenomenon, effects of ethanol have been investigated in *in vivo* zebrafish system, specifically zebrafish embryos. In this study, zebrafish embryos 6 hpf (hours post fertilization) were exposed to five different concentrations of ethanol (0.01%, 0.1%, 1%, 3% and 5% (v/v)) to investigate their hatching ability and morphological alterations during embryogenesis. Additionally, to determine embryonic apoptosis and Reactive Oxygen Species (ROS) generation, Acridine Orange (AO) and DCFDA staining were done respectively. The results of this study indicate that at an ethanol concentration of 0.01% (v/v), the zebrafish embryo exhibited early hatching with no visible alteration in its normal morphology. However, with further increase in concentration, there is decrease in hatching rate with deformity in embryonic morphology. The mortality rate is lower at low dose of ethanol and apoptotic assay suggests that toxicity ensues as the ethanol concentration dose is increased above 1% (v/v). Ethanol exposure also leads to oxidative stress, as it promotes ROS generation with increase in the strength of dose. Thus, the study explores a range of ethanol dose wherein, both toxic and nontoxic window of ethanolic effect has been covered.

PS P193 - 0451 Home-Based and Informal Jewelry Production Increases the Working Families' Exposure to Cadmium

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Biomonitoring of trace elements in biological fluids has a fundamental significance for human health assessment. Their concentrations may vary according to the individual exposure, especially whether considering specific external factors of the environment, as occupational activities. This study aimed to determine the concentration of Potentially Toxic Elements (PTE: As, Cr, Cd, Cu, Mn, Ni, Pb, Zn) in urine samples of a population which are involved with household and informal work with jewelry and fashion jewelry production in Brazil. This study included 52 families, divided into the Exposed group (at least one member of the family worked in the home with assembly or welding of jewelry, n=114) and Control group (no residents working with jewelry, n=78). Urine samples (n=192) of workers, controls and their relatives residing in the same house were collected. PTE concentrations were determined by ICP-MS. The PTE means in total population comply with Brazilian occupational guidelines. However, some samples presented As, Cd, Ni and Zn concentrations higher the values for environmental exposure in US and Canada, and even above the Brazilian occupational limits. A statistically significant difference was found for Cd concentrations between the Exposed and Control group ($p = 0.002$). Furthermore, Cd concentrations were higher in women ($0.19 \mu\text{g/g}$ creatinine; $p = 0.01$), adult workers ($0.25 \mu\text{g/g}$ creatinine; $p = 0.0002$) and smokers ($0.27 \mu\text{g/g}$ creatinine; $p = 0.0001$). The difference between Exposed and Control groups was significant only among non-smokers ($p = 0.0006$), indicating that the informal work represents a significant source of cadmium exposure. We also observed that welders presented higher concentrations of Cd than those who only assembled the parts of jewelry ($p = 0.003$). Considering workers, workers' relatives and controls, there is no difference in the PTE concentrations. All the PTE determined in urine are present in the materials used at some stage of the

jewelry production. The results indicated that informal practices with jewelry potentially exposed family members, including children, to occupational levels of chemical elements with toxic potential. As workers and relatives do not use protective equipment, PTE can be inhaled in the welding fumes or accidentally ingested in the welding powders, especially by children. Considering this exposure scenario, chemicals used in home-based and informal jewelry production may pose a health risk to all family members, not only the workers. *Funded by FAPESP (Grants #2016/11087-8, #2015/21253-0, #2016/07457-4).*

PS P194 - 0462 Brazilian Preschool Children Attending Day Care Centers Located Near Chemicals Hot Spots and in High Vehicle Traffic Density Sites Present High Blood Lead Levels

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In 2013, preschool children attending day care centers (DCC), in São Paulo, Brazil, had blood lead levels (BLL) analyzed. The objective was to investigate whether BLL in preschool children are influenced by the DCC's geographic position in relation to potentially toxic elements (PTE) contaminated sites (hot spots), and by DCC location's vehicle traffic density (exclusive bus lanes/bus terminals). BLL previously analyzed from 2,433 children were used to categorize children exposure as "low" ($<5 \mu\text{g/dL}$, current reference value by CDC); "medium" (between $5 \mu\text{g/dL}$ and $<13.9 \mu\text{g/dL}$), and "high" ($>13.9 \mu\text{g/dL}$, which was the BLL 97.5 percentile found in the cited previous study). PTE hot spots near the DCC were obtained from the contaminated sites list of the environmental agency of the State of São Paulo (CETESB, 2017). The bus terminals and exclusive/preferential bus lanes data were obtained from São Paulo's municipal administration mapping system (Geosampa). Georeferencing software (Q-GIS) was used to map DCC and PTE hot spots near of them, and to calculate DCC and hot spots distance, latitude, and altitude. In addition, we calculated the number of contaminated sites located near DCC by radius distance (0.75; 1; 1.5; and 2 Km). DCC altitude positions were categorized as above/below the nearest PTE contaminated site. The linear distance (in meters) between DCC and the nearest hot spot was studied as a quantitative continuous variable. ANOVA and Kruskal-Wallis tests were used to analyze the data. When children lead exposure were compared by the horizontal distance between DCC and the nearest PTE hot spot, it was found a statistically significant difference between low and medium exposure, and between low and high exposure ($p=0.0004$). Furthermore, children with medium lead exposure were found to be statistically different than children presenting high exposure, considering the altitude position of DCC; in relation to the nearest PTE contaminated site ($p = 0.003$); the distance between DCC and the nearest preferential bus lane ($p=0.005$), and bus terminal ($p=0.002$). Low and high exposure groups showed differences in all distances studied ($p < 0.005$), and at 2 Km radius, all the exposure groups showed to be statistically different ($p < 0.005$). BLL of preschool children attending DCC can be influenced by DCC geographic position, distance and number of PTE hot spots surroundings and vehicles traffic density. These results should be taken into account by the authorities to planning where is secure to build a DCC in order to avoid lead exposure of children. *Funding: FAPESP (#2011/13076-0, #2011/23272-0, #2012/21840-4), and CNPq (#441996/2014-0).*

PS P195 - 0511 Integrated Risk Assessment of Seven Carcinogenic Metals through Oral, Dermal, and Inhalation

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Since some of the heavy metals are known to be carcinogenic to humans, most of the countries operate standards for food contamination. Some countries also had set standards for medicines, cosmetics, hygienic products, workplace, as well as environments based on independent risk management process for each product category. The health risk for consumers, however, needs to be estimated regardless of the product categories or exposure routes. In this report, integrated exposures have been estimated for the seven heavy metals classified as carcinogens by IARC (International Agency for Research on Cancer), including arsenic, cadmium, chromium, cobalt, lead, mercury and nickel. Reported food monitoring data and KNHANES (Korea National Health & Nutrition Examination Survey) 24-hour recall data were used to estimate oral exposure. Monitoring data, where available, and maximum residue standards were used with consumption scenarios to estimate dermal or inhalation exposure from cosmetics, medicines, sanitary products, drinking water, air and

childcare articles. For risk characterization, published toxicological value like BMDL10, oral slope factor, and inhalation unit risk were utilized. The integrated risk assessment result could be used as a basis for the risk management of consumer products as well as environment.

PS P196 - 0638 Tableware Plastic Utensils Supposed to Be Used for Children and Sold in the Brazilian Commerce Present High Lead and Cadmium Concentrations

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Lead (Pb) is known as an omnipresent metal with high neurotoxicity to children, and cadmium (Cd) is a carcinogenic and bioaccumulative contaminant. Both chemicals are included in the United States Environmental Protection Agency (US EPA) Priority Pollutant List, and the ingestion of them through contaminated food or beverages can mean a relevant exposure to those contaminants. Pb is also related to attention deficit, learning disability, IQ decrease, and antisocial and aggressive behavior, while Cd is mainly nephrotoxic. The aim of this study was to determine the concentrations of lead and cadmium present in tableware plastic utensils (cups, mugs, bowls, dishes, and bottles) intended to offer or store food and beverages for children. The concentration of these elements was determined with a field portable X-ray-fluorescence analyzer. The plastic utensils were measured in loco, at popular stores located in the large commercial centers in the city of São Paulo, Brazil. In total, we performed the measurement of 684 plastic pieces being 277 (40.50%) cups, 179 (26.17%) mugs, 103 (15.06%) bowls, 111 (16.23%) plates and 14 (2.05%) bottles. We found 68 tableware utensils with lead and/or cadmium concentration means higher than US legislation (90 ppm for Pb and 75 ppm for Cd). Out of them, 11 (16.18%) were mugs (Pb: 903.09; Cd: 111.00 ppm), 23 (33.82%) were cups (Pb:1596.26; Cd:36.87 ppm), 16 (23.53%) were plates (Pb: 846.63; Cd: 88.13 ppm), 12 (17.65%) were bowls (Pb: 2139.75; Cd:13.17 ppm), and 6 (8.82%) were baby bottles (Pb: 821.17; Cd: 22.00 ppm). The colors associated with the highest concentrations were orange (Pb: 2295.63; Cd: 6.88 ppm), green (Pb: 1405.10; Cd: 63.86 ppm), red (Pb: 1017.13; Cd: 91.09 ppm), and yellow (Pb: 2443.86; Cd was not quantified). Considering the oral via as the main via of exposure to Pb and Cd for children population and that the elements present in the utensils may migrate to the food/beverages, this study showed concerning data and reinforces the urgency to authorities make effort for performing a better regulation and inspection of those consumer products as they may pose a relevant exposure source. *This study was funded by FAPESP (Grants #2014/20945-2, #2016/11087-8, #2017/14392-9), and CNPq (Grant #441996/2014-0).*

PS P197 - 0649 Assessment of Renal Toxicity and Biochemical Effects of Detergent-Processed Cassava on Wistar Rats (*Rattus norvegicus*)

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Detergents are cleaning agent that helps to remove dirt and grease from porous surface (such as fabrics, clothes, non-treated wood) and non-porous surfaces (such as metals, plastics, and treated wood). The use of detergent in food processing has various health effects which include, breathing difficulty, throat swelling, skin burns holes (necrosis) and low blood pressure in humans. Despite the potential health hazard detergent can cause, some local cassava farmers use detergent to ferment cassava root tuber during processing into fufu. The study investigated the potential kidney effects of consuming detergent-processed cassava food. Acute and subchronic toxicity tests were conducted and some kidney function parameter were assayed for possible degeneration. Routine urinalysis showed a significant presence of urobilinogen, bilirubin, blood, protein, glucose and ketone bodies in the urine of rats treated with detergent-processed cassava for 4 weeks. The study concluded that detergent processed cassava-treated food is deleterious to the kidney health of Wistar rats.

PS P198 - 0693 In Vivo Toxicity Assessment of Metal Contaminated Wind Blown Particulate Matter from Abandoned Uranium Mines Surrounding Arizona and New Mexico American Indian Reservations

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Particulate matter (PM) inhalation is known to have adverse effects on the respiratory and cardiovascular systems. PM derived from sediments near an abandoned uranium mine (Claim 28) in Blue Gap Tachee, AZ were found to have high levels of uranium and vanadium and induced greater respiratory toxicity in controlled exposures. Here, we address how much metal-rich PM from the uranium mine site exists in the ambient air in the nearby communities and assessed the toxicity of local ambient PM using *in vivo* inhalation exposure models. C57BL/6 and ApoE^{-/-} (a vascular inflammation model) mice were exposed to concentrated ambient PM in a mobile laboratory located approximately 1 km from the AUM, for 1 or 28 days for 4 hours/day to approximately 80 µg/m³ PM. Lungs and aortas were collected for mRNA markers of inflammatory disease, followed by qPCR to gauge the possible inflammatory responses by using inflammatory-specific probes. Bronchoalveolar lavage was conducted to examine influx of macrophages and neutrophils into the lungs. 1-day exposure to concentrated ambient PM induced increases in the transcription of TNF-alpha, IL6, and TGF-beta levels compared to filtered air (FA) mice. 28-day exposure to PM caused reduced transcription of CXCL1, TNF-alpha, and TGF-beta levels compared to FA mice. Wind stations were also placed above the mobile laboratory and mine site to determine the wind speed direction, which confirmed that the principal wind flow was to the northeast during the daytime, and to the southeast at night. PM toxicity studies are ongoing, but effects are consistent with previous work demonstrated pulmonary inflammation related to PM exposure. The next step will be to assess the metal content of the PM by ICP-MS and to determine the daily wind direction patterns from each site. Future directions would include to selectively antagonize oxidative pathways to further investigate mechanism.

PS P199 - 0708 Organ-Specific Changes in Gene Expression in Microminipigs following a Single Exposure to a Mixture of Perfluoroalkyl Acids (PFAAs)

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It is evident that some PFAAs, a group of globally dispersed pollutants, have long biological half-lives in humans and animals. Previous animal exposure experiments have focused on hepatic, rather than other organs, effects, even though substantial levels of PFAAs accumulate in the kidney, where they exhibit longer biological half-lives than those observed in the liver. Nevertheless, the organ-specific biological effects of PFAAs on the gene-levels have not been elucidated for pigs. Therefore, the present study investigated the transcription-level effects of PFAAs on microminipigs (MMPigs) hepatic and extra-hepatic tissues. We used frozen tissues (liver, kidney, lung, bladder, and brain) from three mature female MMPigs (5.6-8.5 months) treated orally with a single gelatin capsule containing 3 mg kg⁻¹ body weight of a mixture of 10 PFAAs. RNA was extracted from each tissue and genes were analyzed using a real-time qPCR system. The mRNA expression of twelve genes related to either fatty acid metabolism or kidney injury was studied. The mRNA expression of all targeted genes related to fatty acid metabolism was upregulated in the kidney, while only few of these genes were induced in the liver and lung. In general, the expression of these genes was apparently slightly suppressed or unaltered in the brain and bladder. The expression of several kidney injury-associated genes was upregulated in the kidney. Interestingly, the expression of insulin like growth factor binding proteins (IGFBP-genes) was differentially altered in the liver and kidney. Our findings thus identified organ-specific gene expression changes in MMPigs that were associated with various molecular pathways including peroxisome proliferation, lipid metabolism, kidney injury, and apoptosis. The present study provided novel indication that a single exposure to a

mixture of PFAAs can produce changes in MMPig renal gene expression that were observed three weeks post exposure, suggesting that more attention should be paid to the kidney as an important target organ of PFAAs.

PS P200 - 0774 Toxicological Effects of Donepezilo, Eutebrol, and Medazepam on *Caenorhabditis elegans*

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Pharmaceutical products belong to the group of emergent pollutant, and are globally released to the environment in huge amounts, however there are few studies about their effects in the biota. In order to assess the toxicity of different drugs of frequent use, *Caenorhabditis elegans* was exposed to 1.0, 10 and 100 µg/L of donepezilo, eutebrol and medazepam, and to the mixture of them at 1.0 µg/L. The toxicity endpoints evaluated were survival, locomotion and changes in gene expression through the use of transgenic strains with the genes *sod-4* and *gpx-6* associated to the reporter gene *gfp* in order to assess generation of oxidative stress. Survival was not dose-dependent, the drugs studied displayed a general U-shaped behavior, showing endocrine disruptor effect. Donepezil was the most toxic compound at 10 µg/L. Eutebrol did not show significant negative effects on survival at any concentration. *C. elegans* showed no negative effects on survival at the maximum concentration (100 µg/L) of eutebrol and medazepam, and a minimal effect with donepezilo. Locomotion was dose-dependent in donepezilo and eutebrol, however donepezilo inhibited more the basic movements on *C. elegans* than eutebrol, which could be associated to neurological effects. Medazepam showed a U-shaped behavior on locomotion. The expression of *sod-4* presented a U-shape behavior for exposure to all compounds tested. For gene *gpx-6* it was observed that stress expressions were dose-dependent for the drugs medazepam and eutebrol, but exhibited u-shaped behavior for donepezilo. The expression of *gpx-6* increased 1.47-fold and 1.3-fold than the control for 100 µg/L of eutebrol and medazepam, respectively. For its part, donepezil showed the maximum expression of the gene at a concentration of 1.0 µg/L increased 1.94-fold than the control. The mixture of the three compounds exerted toxicity in a synergistic way on *C. elegans* according to response of locomotion. These results verify that toxicity of these three medicaments depends on the concentration, affecting locomotion behavior and increasing the expression of genes related to stress response.

PS P201 - 0853 Are Al, Cd, and Pb Contents in Tofu a Risk?

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Tofu consumption in Europe and USA is increasing. Any new dietary habit needs to be analyzed and evaluated not only from a nutritional point of view but from a toxicological perspective. Tofu is well known for its many benefits derived from its nutritional composition. However, toxic metals (Al, Cd, Pb) in tofu have not been previously toxicologically assessed. The objectives of this research were: (a) to determine the concentration of toxic metals (Al, Pb, Cd) in 130 samples of different types of tofu (standard, flavoured, smoked) by ICP-OES (Inductively Coupled Plasma –Optical Emission Spectrometry), (b) to estimate the toxic metal dietary exposures considering different tofu consumption scenarios and the tolerable intakes set (Al: 1 mg/kg body weight/week; Pb: 0.5 µg/kg bw/day; Cd: 2.5 µg/kg bw/week). Mean values are Al (2.51±1.78 mg/kg wet weight), Pb (0.02±0.01 mg/kg ww), Cd (0.01±0.00 mg/kg ww). Organic flavoured tofu presented a remarkable Al content (6.71±5.65 mg/kg ww), while Pb levels in organic flavoured tofu were 0.04±0.02 mg/kg ww. Statistical differences ($p < 0.05$) were found in the content of Al, Pb, and Cd between the flavoured tofu and the normal and smoked tofu. Considering a consumption of 200 g/day, Al, Pb, and Cd levels in any type of tofu do not pose a health risk for average consumers. Nevertheless, Pb contribution to the tolerable weekly intake (23.4%) from flavoured tofu is relevant. Due to the increase of soil contamination and the consumption of this kind of products, it is necessary to set maximum levels of toxic metals.

PS P202 - 0884 Mitochondrial DNA as Quantitative Tools for Evaluating RT-PCR for Cell Therapy

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Tight safety evaluation is required for developing cell therapy (CT) products. Bio-distribution test is one of the most important aspects of safety evaluation to secure unwanted homing of CT products in patient organs. Various applications, such as imaging modalities, real-time polymerase chain reaction (RT-PCR) analysis and immunohistochemistry, have been adopted for the bio-distribution test. Compared to other applications, RT-PCR has a couple of advantages, determining donor cells without modification of CT products and the lowest detection limit. Therefore, we have evaluated RT-PCR method in this study. Recently, human-specific DNA polymerase chain reaction (PCR) probes have been used for identifying CT products, e.g., intronic Alu repeats. However, measurement of transplanted cells by Alu repeats is confounded by its extensive representation in the genome. Human mtDNA are the DNA located in mitochondria, cellular organelles within eukaryotic cells. In humans, the 16,569 base pairs of mitochondrial DNA encode for only 37 genes for developing genome-specific signatures for additional applications in CT products bio distribution research. Human specific gene in RT-PCR based bio-distribution test, we compared gene level between Alu and mtDNA. In addition, we determined linearity, upper- and lower limit of quantification (ULOQ, LLOQ), limit of detection (LOD), accuracy, precision and specificity of the method based on the KFDA guideline. Human adipose derived-stem cells (hADSC) as a CT product was injected into the tail vein of Athymic NCR-nu/nu mice. After the injection of hADSC, genomic DNA was isolated from main tissues of mice at two time points (1 hour and 7 days). Human specific gene in RT-PCR based bio-distribution test, we compared gene level between Alu and mtDNA. Human adipose derived-stem cells (hADSC) as a CT product was injected into the tail vein of Athymic NCR-nu/nu mice. After the injection of hADSC, genomic DNA was isolated from main tissues of mice at two time points (1 hour and 7 days). As a result, Alu probe is detection of CT value in no template control, however mtDNA probe is not detected. mtDNA gene detected in 7 day lung tissue because mtDNA have low limit of detection value (0.001 ng/µg) compared to Alu (0.05 ng/µL). The results of this study indicate that the mtDNA as well as Alu primer in bio-distribution of CT product. Currently, we are evaluating changes in the absolute amount of mtDNA with changes in cell status. In addition, we are adding the point of development of a predictive model.

PS P203 - 0894 Are Canned Food Containers Safe? Exposure to Nine Bisphenol Analogues in a Duplicate Diet Study

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Bisphenols (BPs) are high-production chemicals used in the manufacture of polycarbonate plastics and epoxy resins. They are contained in a wide range of products, (e.g., food containers, dental fillings, medical devices, toys, thermal paper, and surface coatings of cans), being bisphenol A (BPA) the most largely used. Despite this widespread use, in 2011 the European Commission banned BPA in baby bottles, while in some countries, restrictions have been even harder, as food-contact materials must be free of BPA. Subsequently, industries started replacing BPA by other BPs. These analogues are used under the label "BPA-free." However, since their chemical structure is very similar to that of BPA, they could have similar or even higher endocrine disrupting potential than that of BPA. Hence, taking into account that diet accounts for more than 99% of the exposure to BPs, an accurate exposure assessment is required for the protection of human health. The present study was aimed at performing a duplicate diet study to evaluate the intake and excretion of BPs through the diet. A cohort of 25 people living in Reus (Catalonia, Spain) received a 2-days controlled diet, divided in two groups: (1) a potentially high-BP diet, containing mostly canned food; and (2) control diet, where food is fresh, packed in glass containers or other BP-free materials. On one hand, a portion of the food was homogenised and stored in the laboratory for BPs analysis. On the other hand, blood and urine samples were also collected in the survey. All food and biological samples were analyzed to determine the concentrations of nine BPs analogues (BPA, BPS, BPF, BPB, BPAF, BPZ, BPE, BPAP and BPP). Finally, each participant recorded the amount of food eaten and the total volume of each micturition to know the exact amount of BPs ingested and the amount of BPs excreted. Results will be very valuable to assess the exposure to BPs and evaluate the potential risk of eating canned food.

P204 - 0925 Reproducibility of Collision Cross Section Measurements with Ion Mobility Mass Spectrometry-Mass Spectrometry

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Chemical contamination following environmental disasters profoundly effects the environment and human health. However, due to limitations in the throughput of current analytical technologies, rapidly assessing the nature and extent of chemical contamination is a current research need. To address this need, the Texas A&M University Superfund Research Program (TAMU-SRP) is developing tools for rapid human health risk assessment of exposures after environmental disasters. Specifically, the TAMU-SRP is developing approaches using ion mobility spectrometry-mass spectrometry (IMS-MS) to establish methods with high sensitivity and throughput. Prior to integrating this technology into an analytical workflow for rapid exposure assessment, the reproducibility of collision cross section (CCS) measurements, the unique structural information based on the size and shape of molecules resulting from the IMS-MS assessments, should be established. A combination of over 50 endogenous and exogenous compounds, which have been previously analyzed by Pacific Northwest National Laboratory (PNNL), were used for comparison to the measurements taken at TAMU-SRP. The TAMU-SRP CCS values showed <1% error when matched to the previously collected CCS values, an acceptable error rate that demonstrates reproducibility of the technology across the laboratories. In addition, ion adducts for many of the compounds were detected by TAMU-SRP and their CCS values calculated. A dilution series was also performed to determine linearity and detectability of the compounds. *This research was funded by grants from NIH (P42 ES027704) and the National Academies of Sciences, Engineering, and Medicine's Gulf Research Program (GRP).*

P205 - 0941 Migration Test for Seven Phthalates from Paper Cups

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Phthalate acid esters are widely used as plasticizers used in various industrial applications. In this study, the potential migration of these seven phthalates from paper cups into various food stimulants under different conditions was investigated. The levels of DBP, DEHA, DEHP, and DNOP were in the ranges of 0.07–3.14 mg/kg, 0.16–42.69 mg/kg, 0.45–58.56 mg/kg, and 0.3–2.4 mg/kg, respectively. Meanwhile, BBP, DINP, and DIDP were not detected in most of the tested samples. In the migration test, DEHA was released to 50% ethanol and n-heptane in a time-dependent manner and the maximum migration levels were 65.62 ± 3.61 µg/L and 95.56 ± 19.76 µg/L, respectively. The release of other phthalates was very low or negligible. These results demonstrated that paper cups are not a significant source of phthalate exposure; however, DEHA could be released from paper cups into alcoholic beverages or oily liquid beverages in the human diet.

P206 - 0035 7,10-epoxy Octadeca 7,9-dienoic Acid as a Synergistic Antibacterial Agent against Multidrug-Resistant *Staphylococcus aureus*

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Structural modification of natural lipids by biocatalysis can change their properties or even create novel functionalities. Hydroxy fatty acid, one of oxylipins, can be produced from the microbial bioconversion of natural vegetable oils. Recently 7,10-dihydroxy-8(E)-octadecenoic acid (DOD) was produced with high yield from olive oil containing oleic acid by bacterial strain *Pseudomonas aeruginosa* PR3, and further study confirmed that DOD contained strong antimicrobial activities against broad range of microorganisms. In this study we tried to modify DOD molecules by physical reaction to create new functionality or to enhance the antimicrobial activity of DOD. After the harsh heat treatment, a novel furan fatty acid (EODA) was produced from DOD. We confirmed that EODA presented strong antibacterial activity against multidrug-resistant *Staphylococcus aureus*, and also EODA showed a recuperative effect of the beta-lactam antibiotics activity against methicillin-resistant *Staphylococcus aureus*.

P207 - 0043 4-methylimidazole Induces Skin Aging through Sirtuin 6 Activation

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Skin aging has been reported to be positively related with various chronic diseases. Aging skin occurs from intrinsic factors and extrinsic factors. Additionally, dietary food or food additives may cause skin aging. Caramel color is one of widely used food additives and its major harmful ingredient 4-methylimidazole produced from caramel processing has been reported to exhibit potential toxic or carcinogenic effects to humans. However, the relationship between skin aging and 4-methylimidazole still remains unknown. The purpose of this study is to investigate effects and the potential molecular mechanisms of 4-methylimidazole on skin aging. Skin epidermal keratinocytes (HaCaT) and dermal fibroblasts (Hs68) were used to evaluate effects of 4-methylimidazole on protein expressions of skin aging-related biomarkers. Cells were treated with or without 4-methylimidazole at esculent doses for 72 hours. Moreover, to evaluate effects of continuous exposure to 4-methylimidazole on skin, 4-methylimidazole was delivered to dermal cells every 2 days for 6 passages. 4-methylimidazole significantly induced the activity of senescence-associated β-galactosidase in a dose- and time-dependent manner in Hs68 cells, while there was no significant difference in the activity of senescence-associated β-galactosidase between each group in HaCaT cells. Moreover, 4-methylimidazole also increased activities and protein levels of matrix metalloproteinases 1, 2, and 9 in Hs68 cells, while corresponding collagens (type I collagen and type IV collagen) were gradually decreased. Furthermore, protein expressions of senescence markers and the senescence-related signal (sirtuin 6) were significantly upregulated by the repeated exposure of 4-methylimidazole until 6 passages in Hs68 cells. 4-methylimidazole at esculent doses accelerated activities and protein expressions of skin aging-related biomarkers and signals in Hs68 cells. The present study exhibits the evidence that 4-methylimidazole at esculent doses induces skin aging, which may provide the administrative authorities for amending average daily intake of 4-methylimidazole in the future.

P208 - 0044 4-methylimidazole, a Maillard Reaction Intermediate in Caramel Colors, Promotes Breast Cancer Stemness Characteristics via Activation of RAGE/SIRT6 Axis

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Breast cancer has been exhibited at a higher incidence rate in women and is the second leading cause of cancer death in women. Several risk factors for breast cancer have been proven, such as family history, genetic conditions, breast density, and dietary habits. Caramel color is widely used as a food additive supplemented in foods and beverages as a colorant. 4-methylimidazole (4-MEI), one ingredient of caramel colors, has been reported to have potentially hazardous effects to health, including lung carcinogenicity. However, effects of 4-MEI on breast cancers and its molecular mechanisms remain largely unknown. Therefore, the purpose of this study is to investigate tumorigenic and malignant effects of 4-MEI on breast cancers and the putative molecular mechanisms in human breast cancer MDA-MB-231 cells. MDA-MB-231 cells were used to evaluate tumorigenic and malignant effects of 4-MEI on breast cancers. Cells were treated with 4-MEI at different concentrations and during different exposure periods. The cell proliferation was analyzed by MTT assay. The stemness characteristics of cells were evaluated by sarscosphere formation assay. Protein expressions of stemness-related biomarkers and signals were analyzed by Western blot. 4-MEI sensitively promoted sarscosphere formation in MDA-MB-231 cells with upregulation of ALDH1A1 and CD44 expressions, one of recognized stemness biomarkers, in a dose-dependent manner, although there was no significant difference in cell proliferation between each treated groups compared with control group. Moreover, 4-MEI administered for 24 h upregulated protein expressions of not only cancer stem cell markers CD44 and Nanog but also stemness-related signals, including multidrug resistance-associated protein 1 (MRP-1) in MDA-MB-231 cells. Furthermore, 4-MEI activated protein expressions of receptor of advanced glycation end products (RAGE) and sirtuin 6, while 4-MEI induced expressions of stem cell biomarkers in MDA-MB-231 cells. The present study exhibits evidence that 4-MEI may promote tumor malignancy in triple-negative breast cancer cells (MDA-MB-231).

cells). These results may also provide the basis for health and safety risk assessment of 4-MEI and suggest to the administrative authorities to amend average daily intake of 4-MEI in the future.

PS P209 - 0045 The Esculent Dose of 4-methylimidazole, a Caramel Coloring Ingredient, Promotes Cancer Stemness Characteristics via SIRT6 Activation in Osteosarcoma Cells and Chondrosarcoma Cells

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Caramel coloring is one of common food additives, which can be found in beverages and sauces. 4-methylimidazole (4-MEI), an intermediate of Maillard reaction in caramel colors, has been identified as a possible human carcinogen by the International Agency for Research on Cancer (IARC). Cancer stem cells (CSCs), a rare population of various cancer cells including bone cancer cells, have been discovered to play a key role in tumor development and malignancy. However, effects of 4-MEI on the promotion of CSCs and molecular mechanisms in bone cancers remain unknown. The purpose of this study is to determine the role of 4-MEI at esculent doses in CSCs of human osteosarcoma cell and human chondrosarcoma cells and the putative molecular mechanisms. Human osteosarcoma cells (MG63) and human chondrosarcoma cells (JJ012) were used to investigate effects of 4-MEI on CSCs markers and key-regulated signals. Cells were treated with esculent doses of 4-MEI (<12 μ M) for 24 and 48 hours. The cell proliferation was analyzed by MTT assay. The stemness characteristics of cells was evaluated by sarsophere formation assay. Gene expressions were analyzed by real-time PCR. Protein expressions were analyzed by Western blot. 4-MEI sensitively promoted sarsophere formation in MG63 cells with upregulation of ALDH1A1 and CD133 gene expressions (CSCs biomarker genes). 4-MEI also significantly elevated sarsophere formation in JJ012 cells with upregulation of CSCs gene expressions (ALDH1A1 and CD44). Moreover, 4-MEI upregulated protein expressions of stemness-related signals, including P-glycoprotein (P-gp) and multidrug resistance-associated protein 1 (MRP1) in a dose- and time-dependent manner via activation of sirtuin 6 (SIRT6) protein expression in both MG63 cells and JJ012 cells. 4-MEI at esculent doses may play a critical role in the activation of cancer stem cells, stemness-related signals, and drug resistance of bone cancers via SIRT6 activation. According to this study, people should pay more attention on the daily consumption of 4-MEI and suggest the administrative authorities to amend average daily intake of 4-MEI in the future.

PS P210 - 0135 Toxin Enterosorbents for Inclusion in Food and Water during Emergencies and Natural Disasters

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Following disasters, the mobilization and redistribution of contaminants can increase the risk of exposures to mixtures of hazardous substances and impact the safety of water and food being consumed. In this study, montmorillonite clays have been amended with natural products (i.e., L-carnitine, choline, and chlorophyllin) at 100% cation exchange capacity or processed to simulate broad-acting carbons. The major goal of this study is to use these sorbents in the diet to reduce human and animal exposures to contaminants at the site of disasters. Based on equilibrium isothermal analyses, thermodynamic studies, and mechanistic studies, we have demonstrated significantly increased binding capacities (Q_{max}), affinities (K_d), and enthalpies (Δ H) of sorption for paraquat, glyphosate, and benzo[a]pyrene. From dosimetry analyses, we have extrapolated the daily dose of sorbent needed to meet the regulatory levels for individual hazardous chemicals. This information will also facilitate the determination of dosage requirements for enterosorbent therapy. The hydra bioassay was used as a sensitive *in vivo* indicator of toxicity to confirm the safety and efficacy of sorbents for individual chemicals. Other Superfund chemicals were also shown to be adsorbed with high capacity and affinity, including pentachlorophenol, trichlorophenol, phenol, pyrene, lindane, diazinon, aldicarb, and linuron. The hydra method was used to indicate the toxicity of "Designed" mixtures and "Real" mixtures from contaminated water and sediment from Superfund sites and the ability of sorbents (at low inclusion levels) to protect against these chemical mixtures. We anticipate that a mixture of optimal sorbents developed from this study can be delivered in food, snacks, condi-

ments, flavored water, sachets, or capsules during disasters to reduce human and animal exposure to chemicals and microbes. *Supported by NIEHS SRP P42 ES0277704.*

PS P211 - 0159 Pancreatic Toxicity Induced by Excess Selenoprotein P

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An essential trace element, selenium (Se) has high toxicity, and the optimum range of Se between excess and deficiency is quite narrow. It has been thought that the essential biological role of Se is mediated by Se-containing proteins called as selenoproteins; however, recent evidence has demonstrated the undesired effects of excess selenoproteins. In the present study, we will present the pancreatic toxicity induced by excess selenoprotein P (SeP). SeP (encoded by SELENOP) is a major Se-containing protein in human plasma. SeP is mainly synthesized in the liver and is secreted to blood. SeP functions as a Se transporter to deliver Se to the peripheral tissues and to maintain intracellular selenoproteins. Thus, SeP plays a pivotal role in the metabolism of Se. However, recent evidence indicates that SeP is induced in type 2 diabetes patients and that excess SeP worsens glucose metabolism and advances type 2 diabetes. The mRNA levels of SeP in the liver significantly correlated with blood glucose levels at 120 min after glucose ingestion. SeP protein levels in serum are also positively correlated with fasting plasma glucose, indicating the increase of SeP in type 2 diabetes. To explore the role of increased SeP in the pathophysiology of diabetes, the effects of excess SeP were investigated. Several *in vivo* and *in vitro* experiments indicate that increased SeP worsens glucose metabolism and proceeds diabetes. In this presentation, I would like to talk about the character and function of SeP and its relation to the molecular mechanisms of pancreatic dysfunction induced by excess SeP. I will show the effectiveness of SeP-neutralizing antibody against SeP-induced metabolic disorder, particularly the protective effects of pancreatic β cells. I would like to discuss a molecular mechanism of pancreatic toxicity of excess SeP in type 2 diabetes.

PS P212 - 0195 Shikonin Augments Hyperthermia-Induced Apoptosis through Enhancement of Oxidative Stress

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Hyperthermia (HT) has been employed widely in cancer treatment, and the development of modern devices has made it more efficient and applicable. Shikonin (SHK) is a natural naphthoquinone derivative and has enormously been used in traditional Chinese medicines for decades. Although the anticancer properties of SHK are evident, its sensitizing effects of hyperthermic cancer therapy and the underlying molecular mechanisms are still obscure. In the current study, the effects of combining low doses of SHK with mild HT were investigated in the U937 cell line. The cells were subjected to HT at 44°C for 10 min with or without SHK pretreatment, and parameters reflecting apoptosis, ROS generation, and intracellular calcium elevation were evaluated by using DNA fragmentation, flow cytometry, and western blot analyses. SHK 0.5 μ M significantly augmented HT-induced apoptosis, as evidenced by DNA fragmentation and caspase-3 activation, with concomitant generation of ROS and elevation of intracellular calcium. The combined treatment also synergistically activated proapoptotic proteins and suppressed antiapoptotic proteins. Furthermore, the phosphorylation of JNK and PKC- δ and the dephosphorylation of ERK and AKT were the upstream signaling modifications that may have compounded the induction of apoptosis. The modulatory effects of HT and SHK were blunted with the employment of NAC and JNK-IN-8 validating their major contribution in the induction of cell death. Intracellular calcium was also elevated and responsible for induction of cell death evident by the DNA fragmentation with or without BAPTA-AM. SHK enhances HT-induced apoptosis via a mitochondrial caspase-dependent pathway. The underlying mechanisms involved are increased intracellular ROS generation, elevation of intracellular calcium, and JNK signaling activation.

P213 - 0236 Estimation on Safety of Newly Developed Sugar Substitute, Maltobionic Acid, through Gastrointestinal Dynamics by Prolonged Feeding in Mice

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A newly developed sugar substitute, maltobionic acid (MA), consists of a gluconic acid and glucose linked by an α -1,4 bond and is produced from maltose by enzymatic conversion. Accordingly, when MA is ingested alone, it is expected to pass through the small intestine without undergoing hydrolysis and undergo fermentation on reaching the large intestine. However, we do not yet have a complete picture of the digestion, fermentation, absorption, and metabolism of MA. We investigated gastrointestinal dynamics by prolonged feeding of MA for 19 days in mice and clarified the safety and bioavailability of MA. This study estimated body weight gain, food intake, organ weight, blood biochemical parameters, and remained the amount of MA in feces and in the contents of cecum and colon. This study used MA syrup (95% or higher purity as MA, San-ei Sucochemical Co., Ltd., Aichi Prefecture). Fructo-oligosaccharide (FOS, 98% or higher purity, Meiji Co., Ltd., Tokyo) was used as the positive control in an evaluation of nondigestible sugar. Male C57/BL6 mice were fed AIN93G without cellulose for 1 week during the acclimatization period and assigned to one of the five groups. The control group was fed a modified AIN93G-based diet containing 10% sucrose (cellulose-free feed). The experimental diets contained 2.5, 5.0, 7.5% MA or 5% FOS at the expense of sucrose of the control diet. Mice were fed with the experimental diets for 19 days. Feces were collected 14 days to 16 days. A mild restriction on access to feed was put in place to ensure each mouse ingested the same amount of feed over the same period of time. Body weight gain, food intake, and blood biochemical parameters, e.g. blood glucose, AST, and ALT, were not significantly different among the five groups. MA groups (2.5, 5.0, and 7.5%) were hardly cause osmotic diarrhea during feeding period. Pathological observation of organs was not existed for the all groups. Tissue weight of organs excluding cecum was significantly different among the all groups. Increasing cecum and cecal contents by fed with MA occurred in a dose-related fashion. This phenomenon may be become an active of fermentation by the intestinal microbes in lower gastrointestinal tract. In actually, remained amount of MA in feces, cecum, and colon was detected a tiny amount. In conclusion, MA is safety food ingredient.

P214 - 0243 Elucidation of the Molecular Mechanisms Underlying Proapoptotic Effect of Trans-fatty Acid in Response to DNA Damage

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Trans-fatty acids (TFAs) are unsaturated fatty acids that contain one or more carbon-carbon-double bonds in *trans* configuration, which are mainly produced as byproducts during the food manufacturing processes. Accumulating epidemiological evidence has demonstrated that TFA intake is associated with various diseases, such as metabolic syndrome, neurodegenerative diseases, and cardiovascular diseases. However, there have been few studies regarding the biological effects of TFAs at the cellular or molecular level, and the underlying mechanisms of TFA-related diseases are largely unknown. Here, we show a novel toxic function of TFAs that promotes DNA damage-induced apoptosis through enhancing activation of the mitochondrial c-Jun N-terminal kinase (JNK) signaling. We found that major food-associated TFAs including elaidic acid (EA, 18:1 t9), but not their corresponding *cis* isomers, facilitated apoptosis induced by a DNA-damaging agent, doxorubicin. EA enhanced doxorubicin-induced JNK activation and mitochondrial reactive oxygen species (ROS) generation in a mutually dependent manner, and inhibition of either JNK activation or ROS generation suppressed apoptosis facilitated by EA. The proapoptotic effect of EA was not affected by knocking out either p53, a key transcription factor for DNA damage responses, or apoptosis signal-regulating kinase 1 (ASK1), a previously identified target of TFAs (*J. Biol. Chem.*, 292 (20): 8174-8185, 2017); however, interestingly, the effect was substantially suppressed by knocking down Sab (also known as SH3 domain binding protein 5: SH3BP5), which localizes at mitochondrial outer membrane, acts as an adaptor recruiting activated JNK in response to various stresses including DNA damage, and thereby mediates mutual enhancement of mitochondrial JNK activation and ROS generation. Sab knockdown also reduced EA-mediated increase in JNK activation and ROS generation induced by doxorubicin. Moreover, immunoblot analysis showed that, in the presence of EA, doxorubicin-induced phosphorylation of

JNK was increased in the mitochondrial fraction. These results suggest that TFAs facilitate DNA damage-induced mitochondrial recruitment of activated JNK in a manner dependent on Sab, leading to feedforward amplification of the mitochondrial JNK activation and ROS generation, and ultimately increase in apoptosis. Since the mitochondrial JNK-Sab-ROS axis has been shown to contribute to cell death in a variety of cell types, such as liver cells, neuronal cells, and cardiomyocytes, which is closely related to TFA-related diseases, these findings may provide insight into the pathogenetic mechanisms of TFAs.

P215 - 0272 Research on Toxicological Characterization of Marine Biotoxins in New Zealand

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Consumption of shellfish is an important recreational and cultural pastime in New Zealand. However the presence of harmful algal blooms and uptake of marine biotoxins can pose a significant risk to human health through shellfish consumption. Since 1993, when a large harmful algal bloom temporarily shut down New Zealand's shellfish industry, extensive programmes for monitoring non-commercial and commercial shellfish have been in place. To support these programmes there has been an ongoing process to better characterize the oral toxicities of the different congeners of common and emerging marine biotoxins; first to improve the assessment of risk in relation to concentration in shellfish, and second to replace the mouse bioassay (MBA) with chemical quantification methods. Difference in the acute toxicity of saxitoxin (STX) and gonyautoxin (GTX) congeners have been observed by intraperitoneal (i.p.) injection, gavage, and feeding in mice. The specific activity determined by the MBA was compared to the acute toxicity by i.p. injection and oral administration. The oral toxicity of STX was found to be overestimated by the specific activity indicated by the MBA, however its congener neosaxitoxin (neoSTX) was underestimated by the same assay. Whereas for the GTX congeners, specific activity determined by the MBA were similar to oral toxicities. Following a poisoning event in 2009 of dogs on Auckland beaches with tetrodotoxin (TTX) containing sea slugs, there has been considerable interest in the occurrence of TTX and its congeners in New Zealand shellfish. The toxicity of TTX and mixtures of STX and TTX was determined by i.p. injection, gavage, and feeding in mice and compared to the predicted toxicity of the STX/TTX mixtures. The toxicity of TTX was found to be the same as STX and additivity has been demonstrated. This shows the importance of determination of the overall toxicity of seafood samples rather than treating STX and TTX separately. The results of the toxicology studies undertaken in New Zealand on marine biotoxins has played a role in developing a sustainable strategy for monitoring these toxins in non-commercial and commercial shellfish. This is complemented by anticipating how climate change will influence the risk of marine biotoxins in New Zealand and highlighting future areas of research to ensure that risks to consumers of shellfish can continue to be identified and mitigated.

P216 - 0288 Safety and Efficacy Assessment of Novel Blood Glucose Regulation Supplement: SugarOut

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SugarOut (SO) is a novel food supplement targeting on lowering blood glucose which contains 15% Red yeast rice (RYR) and 7.17% Hedychium Coronarium J. Koenig (HC) extract. The aim of this study was to investigate the efficacy and safety of SugarOut. Ames test, Chromosomal aberration and Micronucleus were performed in genotoxicity study. In the oral toxicity study, groups of male and female SD rats (N=10 in each gender) were orally administered 0, 0.6, 1.2, and 2.0 g/kg/day SO for 90 days and were sacrificed on Day 91. For the efficacy study, Streptozotocin (STZ) 60mg/kg was injected to Wistar rats 30 mins after Nicotinamide 230mg/kg was injected. After diabetes has established, rats (N=8) were orally administered 0, 62, 124, 620mg/kg SO respectively for 2 weeks. There no substance-related effect was found in both genotoxicity study and oral toxicity study. All treatment groups were significantly decreased in the oral glucose tolerance test (OGTT) after treating 2 weeks SO. Hence, the [no-observed-adverse-effect level](#) (NOAEL) of SO was 2.0/kg/day and the effective dosage was 62mg/kg.

P217 - 0296 Potential Health Risk from Heavy Metals via Consumption of Leafy Vegetables in the Vicinity of Warri Refining and Petrochemical Company, Delta State, Nigeria

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This study aimed to investigate the concentrations of lead (Pb), cadmium (Cd), nickel (Ni), and chromium (Cr) in the commonly grown and frequently consumed vegetables in two communities around the Warri Refining and Petrochemical Company, Delta State, Nigeria, and to estimate the potential human health risks via consumption. Leafy vegetable samples comprising of *Telfairia occidentalis* (fluted pumpkin), *Vernonia amygdalina* (bitter leaves), *Ocimum gratissimum* (scent leaves), *Amaranthus hybridus* (green African spinach), and *Amaranthus viridis* (red African spinach) were collected, digested, and analysed using Solar Thermo Elemental Flame Atomic Absorption Spectrophotometer (model: S4=71096). Results from the study were used to estimate the human health risk of these heavy metals. The result showed that the mean concentrations and (range) of total Pb, Cd, Ni, and Cr were 0.059 (0.019-0.178), 0.076 (0.010-0.230), 1.037 (0.411-2.531), and 1.337 (0.383-2.331) mg/kg, respectively. The concentration of Pb, Cd, and Ni were below the permissible limits recommended by WHO/FAO, EU, and China. The concentration of Cr in all the samples exceeded the limits set by EU and China, but was below the limit recommended by FAO/WHO. The human health risk was calculated using toxicological indices such as Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), Hazard Index (HI), and Lifetime Cancer Risk (LCR). From the results, the EDI of Pb, Cd, Ni, and Cr were mostly below the Tolerable Daily Intake of metals, the THQ in the analyzed samples were less than 1, the HI were also less than 1. The LCR of Pb and Cd were within the predicted permissible lifetime risks for carcinogens as stated by US EPA. The result from this study shows that there may be slight heavy metals toxicity.

P218 - 0300 Heavy Metals Contamination and Potential Human Health Risk via Consumption of Vegetables from Selected Communities in ONELGA, Rivers State, Nigeria

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Anthropogenic activities such as excessive oil exploration, automobile emissions with agricultural activities, etc. tend to elevate the concentrations of heavy metals in the soil. These metals are accumulated by plant roots, thereby resulting to heavy metal contamination of such plants. This study evaluated the concentration of heavy metals, namely: Lead (Pb), Cadmium (Cd), Nickel (Ni), Arsenic (As), Iron (Fe), and Copper (Cu) in edible vegetables from selected communities in Ogba/Egbema/Ndoni Local Government Area (ONELGA), Rivers State, Nigeria, and the concentrations of these metals were used to assess the human health risk posed to the consumers of the edible vegetables. Sixteen different vegetable samples comprising *Vernonia amygdalina*, *Talinum triangulare*, *Abelmoschus esculentus*, and *Telfaria occidentalis* were digested and analyzed for heavy metals using Flame Atomic Absorption Spectrophotometer (F-AAS). Results obtained from this study were used to estimate the human health risk of these heavy metals. The results showed that the concentrations of Pb, Cd, Ni, As, Fe, and Cu ranged from 0.168-4.908; 0.364-2.977; 2.780-10.241; 0.157-2.633; 61.589-101.520; and 12.029-46.540 mg/kg, respectively. The concentrations of Pb, Cd, Ni, and As in some of the analyzed vegetable samples exceeded the permissible limit as recommended by WHO/FAO, EC/CODEX, and NAFDAC, respectively. The concentration of Fe and Cu were below the permissible limit as recommended by WHO/FAO. The Estimated Daily Intake (EDI) of Pb and Cd exceeded the permissible tolerable daily intake of metals (TDI) but fall within the upper tolerable daily intake (UTDI) recommended by Institute of medicine, FDA, also Ni, Fe, and Cu fall within the recommended TDI and the UTDI. The EDI values of As exceeded the recommended TDI and the UTDI. The Target Hazard Quotient (THQ) and Hazard Index (HI) values of Pb, Cd, Cu, and Ni were greater than 1. The Life Cancer Risk (LCR) of Pb were within the range of permissible predicted lifetime risks for carcinogens as recommended by US EPA. The result from the present study indicate that the exposed population may be at risk of Pb, Cd, Ni, and Cu toxicity.

P219 - 0308 Nutrition and Toxicity Relationship: Which Is Responsible?

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Food poisoning and role of food in toxicity in children and adults is one of the commonest problems and pediatricians are frequently asked to manage such cases. In fact, there is a great relationship between nutrition and toxicity; nutrition may have great role in toxicity prevention as in lead toxicity. Also nutrition may help in toxicity management—for example, milk as antidote for corrosives and coconut oil as local antidote for aluminum phosphate poisoning, which is a fatal poison. In contrast, nutrients may aggravate toxicity as in case of hepatic enzyme inducers or produce toxicity as in food poisoning, which may be simple as bacterial food poisoning or dangerous as botulism and organophosphate contaminated food. Finally, some vitamins and nutritional supplements may be dangerous in overdose settings. In this brief review, I will illustrate this relationship with more details for different types of food poisoning in children and its management.

P220 - 0319 Development of a Sensitive ELISA and Rapid Immunochromatographic Strip for Detecting Chloramphenicol in Milk

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Antibodies that are specific to Chloramphenicol (CAP) were generated from rabbits that had been immunized with CAP-keyhole limpet hemocyanin (KLH). This work develops a rapid and sensitive competitive direct enzyme-linked immunosorbent assay (cdELISA) and a gold nanoparticle immunochromatographic strip method for detecting CAP in milk samples. In the cdELISA, CTN at a concentration of 0.45 ng/ml causes 50% inhibition (IC_{50}) of the binding of CAP-horseradish peroxidase to the antibodies. The effective on-site detection of CAP is also achieved based on the proposed rapid and sensitive antibody-gold nanoparticle immunochromatographic strip assay. This strip has a detection limit of 2 ng/ml for CAP in milk products. The entire analysis is completed within 15 min. Closely examining 6 milk samples using cdELISA indicates that all 6 samples were CAP-free. The proposed cdELISA and immunochromatographic strip methods are highly sensitive to the rapid screening of CAP in milk samples.

P221 - 0320 Disruption of Brain and Vascular Development by Ochratoxin A in Zebrafish Embryos

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Ochratoxin A (OTA) is a mycotoxin produced by *Penicillium* and *Aspergillus* and is widely found in various foodstuffs, including cereal, coffee, and wine. OTA has been reported to trigger neural tube defect and hydrocephaly in developing rodents, but the molecular mechanisms that are associated with OTA teratogenicity have not been extensively elucidated. In this study, wild type zebrafish embryos at 6 hour post fertilization (hpf) were exposed to various concentrations of OTA and then phenomena of cerebral hemorrhage were found in 72 hpf embryos. The hemorrhagic rate of OTA-exposed embryos was significantly increased in a dose-dependent manner. Additionally, large numbers of extravagated erythrocytes with red fluorescence were observed in the midbrain/hind-brain areas in the double transgenic embryos *Tg(fli1a:EGFP);Tg(gata1:DsRed)* after exposed to 0.5 mM OTA. Images obtained from light-sheet microscopy revealed that the vascular pattern, especially loop-shaped central arteries, was disrupted in OTA-treated embryos; histological analysis also demonstrated a cavity-like pattern in their hindbrain ventricles, implying the possibility of cerebral edema. OTA is known to induce the expression of microRNA-731 (miR-731) in the zebrafish embryos, and OTA-induced blood vessel defects could be reversed by miR-731 inhibitor herein. These results suggest that OTA exposure may have a severe impact on brain and vascular development in developing organisms.

P222 - 0332 Identification of Compound Causing Yellow Bone Discoloration Following α -Glycosyl Isoquercitrin Exposure

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Flavonoids such as quercetin and its glycosides have been shown to possess potential benefits to human health, including reduction of inflammation, pain elimination and cardiovascular protection. Flavonols are available to consumers as dietary supplements, promoted as anti-oxidants; however, incorporation of natural quercetin glycosides into food and beverage products has been limited by poor immiscibility in water. Enzymatic conjugation of multiple glucose moieties to isoquercitrin to produce α -glycosyl isoquercitrin (AGIQ) has been shown to enhance solubility and bioavailability. AGIQ is used in Japan as a food additive and has been granted generally recognized as safe (GRAS) status by the Federal Food and Drug Administration (FDA) in 2007. In a dietary two-year carcinogenicity study evaluating quercetin via dietary exposure, accumulation of yellow-brown granular pigment absorbed to or by the epithelial cells in the gastrointestinal (GI) tract was observed; however, bone discoloration was not mentioned (NTP Technical Report 409, 1992). In a recent 90-day sub-chronic oral toxicity study in rats, systemic yellow discoloration of bones was observed in a dose-dependent manner, without discoloration of the GI tract mucosa (Nyska, et. al., 2016). There were no microscopic changes indicating a lack of toxicological significance, but a follow up study was conducted to potentially identify the metabolite causing yellow bone discoloration. Measurement of quercetin in the urine, and quercetin, quercetin glucuronide, kaempferol, and methyl quercetin metabolites of AGIQ in bone (femur), white and brown fat, and cerebrum of female rats was performed following a 14-day dietary exposure to AGIQ with and without a 14- or 28-day recovery period. Similar to the 90-day study, gross examination of the femur revealed yellow discoloration that increased in intensity with dose. Following the recovery period, yellow discoloration was still present in a dose-related manner at both 14 and 28 days. Quercetin, at levels correlating with AGIQ dose, was measured in the urine of rats following the 14-day exposure period. At 14 or 28 days following cessation of AGIQ exposure, quercetin was still measurable in the urine, albeit at lower concentrations. Quercetin, quercetin glucuronide, kaempferol, and methyl quercetin were present in a dose-dependent manner in the femur following 14-days of dietary exposure; only quercetin and quercetin glucuronide metabolites were present during the recovery period. These data indicate quercetin to be the most likely metabolite of AGIQ causing the yellow discoloration of bone in rats administered dietary AGIQ.

P223 - 0350 Effects of Excessive Ingestion of 1, 5-Anhydroglucitol on Growth and Metabolism in Rats, and the Suppression of Blood Glucose Elevation through Inhibitory Effect on Disaccharidases

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1,5-anhydro-D-glucitol (1,5-AG), which has 60 % of the sweetness of sucrose, is found in low levels in natural plant foods and is produced sparingly in the liver. It is a polyol that is mostly contained in the body and is used as a marker of blood glucose control in patients with diabetes mellitus. The urinary excretion of 1,5-AG is increased by the competitive inhibition of glucose in patients with high urinary glucose, because 1,5-AG is reabsorbed actively by the sodium glucose cotransporter-4 from the renal tubule. The objective of this study is to prove the safety and fate of 1,5-AG which is ingested excessively in rats, because 1,5-AG has been developing as a food ingredient. To investigate the new physiological functions, the suppressive effect for blood glucose elevation through the inhibitory effects on disaccharidases was observed. 1,5-AG (Sunus, Kagoshima, Japan), erythritol (ERT, Mitsui-Busan Food Science, Tokyo, Japan), D-Sorbitol and D-psicose (Matsutani Chemical Industry, Hyogo, Japan), and Fructooligosaccharide (FOS, Meiji, Tokyo, Japan) were used. Wistar young rats (4 wk old, Clea Japan Inc., Tokyo, Japan) were raised with consecutive feeding of diets containing 5% and 10% of 1,5-AG for 31 days. Brush border membrane vesicles (BMV) by Kessler method was used to the assay of inhibitory effect on disaccharidases. The blood concentration and urinary excretion of 1,5-AG following oral administration of 1,5-AG were observed using adult rats and the suppressive effects of 1,5-AG on the elevation of blood glucose level following sucrose administration was also observed using adult rats. The growth and

organ weight of rats were not significantly different among a control group and other 4 groups (n=5 in each group). 1,5-AG did not affect the concentration of blood glucose and triacylglycerol and did not induce the morphological abnormality of organs and tissues without cecal enlargement in FOS group. 1,5-AG distinctly inhibited the activity of disaccharidases such as sucrase and maltase, but barely inhibited trehalase and lactase. 1,5-AG significantly suppressed the elevation of blood glucose and insulin by the ingestion of sucrose. Ingested 1,5-AG was readily absorbed from the small intestine and excreted rapidly into the urine. Accordingly, 1,5-AG ingested does not cause high osmotic diarrhoea and harmful effects. 1,5-AG seems have a very low toxicity and is safe, because a small amount of 1,5-AG is synthesized in the body and is consumed in daily life. These results support the use of 1,5-AG as low energy bulking sweetener in food products.

P224 - 0403 Protective Effect of Honokiol against Oxidative Stress-Induced DNA Damage and Apoptosis of C2C12 Myoblasts

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Honokiol is one of main active components of *Magnolia officinalis* and has been demonstrated to have multiple pharmacological activities against a variety of diseases. Recently, this phenolic compound is known to have antioxidant activity but its mechanism of action is not fully clear. The purpose of the current study was to evaluate the preventive effects of honokiol against oxidative stress-induced DNA damage and apoptosis in C2C12 myoblasts. The result of the present study demonstrated that honokiol inhibited hydrogen peroxide (H_2O_2)-induced DNA damage and mitochondrial dysfunction while reducing reactive oxygen species (ROS) formation. The inhibitory effect of honokiol on H_2O_2 -induced apoptosis was associated with up-regulation of Bcl-2 and down-regulation of Bax, thus reducing the Bax/Bcl-2 ratio that in turn protected the activation of caspase-9 and -3, and inhibition of poly (ADP-ribose) polymerase cleavage, which was associated with the blocking of cytochrome c release to the cytoplasm. Collectively, these results demonstrate that honokiol defends C2C12 myoblasts against H_2O_2 -induced DNA damage and apoptosis, at least in part, by preventing mitochondrial-dependent pathway through scavenging excessive ROS.

P225 - 0418 Effects of Newly Developed Dietary Fiber Materials on Gastrointestinal Morphology and Function by Consecutive Feeding in Rats

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Novel nondigestible saccharides, such as highly cross-linked phosphate starch (HCPS), have been developed focusing to the prevention of the onset or progress of lifestyle-related diseases. The water-insoluble dietary fiber (DF) used as an ingredient in the processed foods instead of wheat flour to diminish the available energy. It has been clarified that the hypertrophy in epithelial cells of cecum in rat was observed on 2-3 days after feeding of nondigestible saccharide. Thereafter, the hyperplasia and the increasing of cecal weight were occurred, and the fermentation by the intestinal microbes increased. On the other hand, the water-insoluble DF may damage the intestinal mucosa and affect the absorption of the nutrients in elderly or under diseases. The final aim of this study is to prove the safety and advantages of water-insoluble dietary fibers to prevent diseases. In this study, we investigated the effect on the morphology in the cecal epithelial cells, the fermentability by intestinal microbes after one week feeding of HCPS, and to clarify the effects on the growth, organs, tissues and blood biomedical parameters by the consecutive feeding of HCPS for 28 days in rats. HCPS is made from tapioca starch by polymerization in the presence of less than 0.5% phosphoric acid. HCPS-N (General Starch Ltd., Bangkok, Thailand) contains 85.7% DF and HCPS-P (Matsutani Chemical Industry Co., Ltd., Hyogo, Japan) contains 84.7% DF by the AOAC 2009.01 method. Wistar male rats (4 w) were raised for one week by feeding of 10% HCPS-N, HCPS-P replaced to cornstarch or control diet and we observed morphology of cecum and hydrogen excretion. And we carried out consecutive 28 days feeding of same diets and we performed the effects on growth, plasma biomedical parameters, fermentation by intestinal microbes. After one week feeding, the wet weight of cecal tissue and contents, hydrogen excretion, production of short chain fatty acids in 10% HCPS-N and -P were significantly much more than

those in control diet ($p < 0.05$). No adverse effect was detected in growth, biochemical parameters and pathological observations after 28 days feeding. In conclusion, HCPS is safety food ingredient as substitute of wheat flour.

PS P226 - 0440 Influence of Heating Temperature on the Toxicity of Olive and Coconut Oil on *Caenorhabditis elegans*

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Olive oil has been studied for its protective role against oxidative stress due to the presence of phenolic compounds. On the other hand, coconut oil has gained popularity due to its potential cardiovascular benefits for its content of medium chain triglycerides that show beneficial properties for health. Both oils are commonly used to fry food. Frying is a complex physicochemical process, in which a high temperature is obtained in order to modify the surface thereof, waterproofing and avoiding the loss of water inside. However during heating, oils can be degraded by oxidation, leading the formation of toxic compounds, such as peroxides, which are relating to oxidative stress process at cell level. In this work, the nematode *Caenorhabditis elegans* Bristol N2 wild-type strain in L4 larval stage was used as toxicological model, and lethality was evaluated by measuring the percentage of survival animals after 24 h-exposure to coconut and olive oil solutions, using unheated and previously heated oils at 100, 200 and 300 °C. In addition, the transgenic strain *sod-4::gfp* in mixed larval stages was used to evaluate changes in the expression of the *sod-4* gene through the measurement of fluorescence by using a multilevel plate reader and band-pass filters at 485 and 525 nm for excitation and emission respectively. In order to identify changes in chemical composition and presence of peroxides due to oxidation during heating, peroxide analysis and Raman spectroscopy was performed. Results showed that the unburnt oils presented no toxicity, while heating to 100 °C increased the toxicity of olive oil (34%) more than coconut oil (18%). Heating to 200 and 300 °C increased the toxicity of coconut oil (23%), while that of olive oil decreased, but there was no significant difference between both oils. The expression of *sod-4* increased with temperature in both oils, indicating oxidative stress as toxicity mechanism and the presence of reactive oxygen species in the heated oils. The changes in toxicity could likely be related to the compounds formed in each heating phase. The presence of peroxides increased with temperature, and was higher in coconut oil than olive oil, probably due to the presence of antioxidant compounds such as polyphenols that avoid oil oxidation. Raman spectra confirmed the presence of peroxides with typical bands between 845 and 885 cm^{-1} . In conclusion, the heating temperature affects the toxicity of olive and coconut oils in the nematode *Caenorhabditis elegans* probably due to the formation of peroxides during oxidation process, and promoting the formation of reactive oxygen species.

PS P227 - 0455 Ethanol Suppresses *In Vitro* Osteoblastogenesis, Chondrogenesis, and Chondrocyte Function via Different Sources of Reactive Oxidative Species

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Alcohol (ethanol, EtOH) use is highly prevalent among adolescents, who concurrently undergo rapid bone formation and longitudinal skeletal growth. EtOH induces reactive oxygen species (ROS) in bone, primarily through NADPH oxidase (NOX) enzymes 2 and 4, and also through decoupling of mitochondrial respiration. Oxidative stress suppresses osteoblastogenesis to reduce bone growth, and suppresses growth plate chondrocyte proliferation, resulting in shorter long bones in mice. Antioxidant co-treatment *in vivo* prevents EtOH-induced effects, and deletion of NOX4 partially protects against EtOH suppression of osteoblast differentiation in *ex vivo* primary cultures. The studies herein were designed to test mechanisms of EtOH-induced suppression of osteoblastogenesis and chondrocyte differentiation involving induction of mitochondrial ROS. Bone marrow mesenchymal stromal cells (BM-MSCs) cultures from 12-week-old male and female C57Bl6/J mice were differentiated in the presence of l-ascorbate phosphate, and with or without 50 mM EtOH. Additionally, a subset of cultures were co-treated with the mitochondrial ROS-targeting superoxide dismutase mimetic, mitoTEMPO (25 nM). After 14 days, cells were stained for alkaline phosphatase as a marker of mature osteoblast colony formation (CFU-OB). EtOH alone significantly suppressed CFU-OB number, but EtOH in combination with mitoTEMPO did not alter the number of CFU-OBs compared to untreated controls, implying a role for mitochondrial ROS in EtOH suppression of osteoblast differentiation. To test EtOH effects on chondrocyte differentiation, primary chondrocytes were isolated from

ribcages of 12-week-old male C57Bl6/J mice and induced to differentiate in the presence of l-ascorbate and beta-glycerol phosphate. At 14 days, cultures were probed for alcian blue-positive staining for hyaluronic acid deposition (cartilage formation). 50mM and 100mM EtOH significantly reduced alcian blue staining (extracted and quantified absorbance at 630 nm) compared to untreated controls. In addition, primary chondrocyte cultures isolated from neonatal (3-6 days old) mouse ribcages showed significant, dose-dependent suppression of aggrecan mRNA with EtOH. Together, these studies provide *in vitro* evidence of EtOH suppression of chondrocyte differentiation and mature chondrocyte function, i.e., cartilage matrix deposition. Interestingly, co-administration of mitoTEMPO did not protect against the effects of EtOH on chondrocyte gene expression, suggesting alternate ROS pathways between osteoblasts and chondrocytes mediating EtOH effects. *Supported in part by F32 AA026480 (J.W.) and R37 AA18282 (M.J.J.R.).*

PS P228 - 0457 Molecular Characterization and Evaluation of Aflatoxin Production Potentials of Fungi Isolated from Animal Feedlots Kept for Food Production

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Fungi in food and feed substrate constitutes a menace to the safety and health of consumers due to the synthesis of secondary metabolites (mycotoxins) in food/feed. Aspergilliosis emanates from the notorious activities of *Aspergillus* spp. in food/feed substrate, however, not all are toxigenic hence, this study investigated the toxin production potentials of isolated fungi using molecular approach. About 58 fungi isolates were cultured and characterized using Internally Transcribed Spacer (ITS) region PCR amplification. Sequenced product were blasted and accession numbers were obtained from gene bank. *Aspergillus flavus* ATCC 259622TM and *Saccharomyces cerevisiae* and DNA free water was used as positive and negative control respectively. Percent occurrence of fungal isolates ranged from 2 – 77.58% been highest in *Aspergillus* genus (77.58%). Percent occurrence of *Aspergillus* strains; *A. ochraceus* (2.2%), *A. oryzae* (13.3%), *A. spp.* (6.6%), *A. niger* (6.6%), *A. terreus* (11.1%), *A. clavatus* (4.4%), *A. tubingensis* (4.4%), *A. amstelodam* (2.2%) and *A. fumigatus* (4.4%). *Aspergillus flavus* (42%) was highest in cattle feed lots kept for food production. Forty-five isolates belonging to *Aspergillus* strains were further studied and assessed for aflatoxin production potential using the conventional and molecular approach. Spores of *Aspergillus* isolates were grown on yeast extract sucrose (YES) agar and on Desiccated Congo Red Coconut (DCRC) agar while the expression of the aflatoxin regulatory genes (*aflR*, *aflJ*, *Nor-1*, *Ver-1*, *Omt-A*) were employed to determine the propensity for aflatoxin production. A total of 86.04% had *Nor-1* gene, *ver-1* (58.14%), *omt-A* (72.09%), *aflR* (88%) and *aflJ* (100%). About 23 (51%) of *Aspergillus* strains were positive for aflatoxin production on YES and DRRC agar and were confirmed on thin layer chromatography. Total aflatoxin (AFTOT) production concentration of selected toxigenic strains ranged from 6.77 – 71453 $\mu\text{g/g}$, AFB1 (3.76 – 70288 $\mu\text{g/g}$), AFB2 (6.77 – 242.50 $\mu\text{g/g}$), AFG1 (1.87 – 745.30 $\mu\text{g/g}$) and AFG2 (1.67 – 768.52 $\mu\text{g/g}$). About 17 (73.91%) of selected toxigenic *Aspergillus* strains produced AFG2, AFG1 (7; 30.43%), AFB2 (17; 73.91%), AFB1 (7; 30.43%) while AFTOT (20:86.96%). Feedlots of animals reared for food are reservoirs of aflatoxigenic strains of fungi which could cause hazards to health and safety of veterinary and humans when animal products are consumed.

PS P229 - 0520 Acrolein Levels following Dietary Exposure and in Stroke Patients

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Acrolein, a α,β -unsaturated aliphatic carbonyl compounds and highly reactive, is formed by heat treatment of carbohydrates, amino acids and especially animal and vegetable fats. In addition, acrolein could also be formed endogenously such as polyamine oxidation. Previous studies have shown that acrolein is associated with various neurological diseases, including ischemic stroke, which is the third leading cause of death and the most common cause of complex disability in Taiwan. Previous studies have suggested that acrolein-conjugated protein (Acr-FDP) and acrolein metabolite, 3-hydroxypropyl mercapturic acid (3-HPMA), might have potential to serve as stroke biomarkers. We hypothesize that dietary exposure of acrolein might contribute to brain dam-

ages during stroke. We therefore conducted human studies to test acrolein exposure following consumption of fried chicken and french fries. Furthermore, the newly diagnosed stroke patients were also recruited following IRB approval. The presence of Acr-FDP in plasma were assayed by slot-blot analysis and 3-HPMA in urine were quantitated by liquid chromatography with tandem mass spectrometry (LC-MS/MS). The results show that urinary 3-HPMA levels increased in healthy subjects started from 2 hrs after the consumption of fried food, and peaked at 12 hrs. And the plasma Acr-FDP levels did not fluctuate following the consumption fast food. On the other hand, the levels of Acr-FDP in the plasma of stroke patients were significantly higher than those of controls, whereas urinary 3-HPMA levels were markedly decreased in stroke patients as compared to controls. The data indicate that dietary source will contribute to the acrolein exposure in healthy subjects. The high Acr-FDP in plasma and lowered urinary 3-HPMA levels suggest glutathione might modulate the acrolein effects in stroke patients. This study suggests that acrolein metabolite might serve as a biomarker in stroke.

PS P230 - 0575 Green Synthesis, Characterization, and Potential Biological Application of Silver Nanoparticles by Using Leaves Extract of *Acmella oleracea*

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Acmella is a genus of plants belonging to Asteraceae family. It is native to the America and has been introduced to Asia, Africa, and Australia. There are about 31 species in the *acmella* genus. Out of 31, *Amella oleracea* is one familiar species which has been widely cultivated for centuries. Spilanthol has been identified as principal active compound present in *A. oleracea* which acts as an analgesic and found to be an effective to promote salivary secretion. These are perennial herbs with branching stems usually reaching 10 to 20 centimeters in length, growing prostrate or erect. It exhibits a wide spectrum of biological activities. The pretty yellow and red cone-shaped flowers and leaves are non-toxic and it is used to enhance the immune system, improve digestion and help nausea. This plant is also known as toothache plant and this is used for the treatment of gum and dental problems. At present, the medicinal plant could be used as an excellent and resourceful green material for the rapid and consistent synthesis of silver nanoparticles. The UV-Vis absorption spectra of silver nanoparticles formed at specific peak at a specific wavelength and color changes are the preliminary confirmation for the synthesis of silver nanoparticles (SNPs). The present procedure is simple, safe, economical, non-toxic and eco-friendly as compared to toxic chemical process. Plant leaves could thus be used as an efficient choice for the cost-intensive conventional methods to synthesize the silver nanoparticles. A silver nanoparticles has many applications like antibacterial, antimicrobial in the field of medicine as well as food and industry. This study opens up a new opportunity for very conveniently synthesizing Ag nanoparticles using natural products which could be useful in various applications. Our findings could be targeted for the promising potential applications including drug formulation and biomedical applications in future. Finally, the green synthesis of silver nanoparticles using plant material was found to be the most ecofriendly and conventional method, in comparison to chemical and physical synthesis methods.

PS P231 - 0615 Food Grade Titanium Dioxide Diet Reduces Hematocrit and Increases Lymphocyte Count and Phagocytic Cells in Mice Serum

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Food grade titanium dioxide is an odorless food additive used to add white color and opacity to food, food supplements and personal care products. In the European population, the maximum exposure scenario is calculated to be up to 10.4 mg/kgbw/day in children under the age of 11 years and 6.8 mg/kg/day for adults by the EFSA. It is classified by the EU as E 171 and allowed in quantum satis which means "that no maximum numerical level is specified and it shall be used in accordance with good manufacturing practice." Titanium dioxide is classified in group 2B as possible carcinogen by inhalation by the International Agency of Research in Cancer, this has raised concern about the effects by the oral route. E 171 can have up to 40% nanoparticles and can increase the number of tumors in a colorectal cancer model and reach the bloodstream with a maximum peak within 6 hours. However, there are no long term studies with E 171 in mice. We aimed to see if E 171 administration in solid diet caused changes in blood parameters and inflammation in colon of C57BL/6 mice com-

paring these effects between young and adult mice. E171 was administrated in the diet for 90 days. First, E171 was supplemented in pellets with at 0.05% to reach a dose of 7 mg/ kgbw/day. Size of the agglomerates of E171 and pellets supplemented with E171 formed in water, gastric (SGF) and intestinal fluid (SIF) were analyzed. To measure blood parameters, we extracted blood after 90 days of exposure and analyzed it with a flow cell cytometer, white cells populations were separated by size and granularity. Pellets in SGF formed bigger agglomerates (500 nm) than SIF and water (200 nm). We found a decrease in hematocrit levels of 20% in both groups (young and adults) while the lymphocyte and phagocytic cell percentage only increased in adult mice. Total lymphocyte count increased in 2% and phagocytic cells increased in 8%. This changes were significant versus control. There were no significant changes in total weight of the mice, weight of the organs and food consumption, also there was no tumor formation after 90 days. In conclusion, E171 could affect blood parameters such as hematocrit and induce a slight increase in immune cells such as lymphocytes and phagocytic cells of adult mice.

PS P232 - 0647 Modulation of Gut Microbiome and Metabolome by Carcinogenic Mycotoxin, Aflatoxin B₁, in F344 Rats

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Gut microbiota and microbial-dependent metabolisms are critical in human and animal health, immunity, nutrition, and physiology. Disturbance of their balances has been linked to pathogenesis of many chronic diseases. In this study, F344 rats were divided into four dosing-groups and were orally treated daily with AFB₁ in DMSO (0, 5, 25 or 75 µg AFB₁ / kg body weight) for 5 weeks, and were sacrificed at 24 h after the last dose. Microbiome DNA was extracted from gut contents collected during the experiments. 16s rRNA was sequenced using the Illumina MiSeq platform. The community biodiversity of the microbiome was analyzed based on sequencing data. Significant differences on microbiome biodiversity were found in AFB₁-treated groups as compared to the control animals. Among control samples, the phylum *Firmicutes* are the major bacteria phylum, followed by the phyla *Bacteroidetes*, *Verrucomicrobiaceae*, *Proteobacteria*, with other phyla being less common. Among AFB₁-dosed samples, the relative abundance of the phylum *Bacteroidetes* increased, whereas *Firmicutes* decreased. The frequency change was dose-dependent. The gut contents were further extracted and analyzed using various high throughput platforms for un-targeted and targeted metabolomic assessments. UHPLC-ESI-TsQ workstation discovered 494 significant responders from a total of 1744 detected features. Among them, the entities of 234 significant responders were found in Human Metabolome Data Base (HMDB) by comparing exact m/z (0.0001), of which 178 showed excellent matching score (Δ ppm $\leq$ 30), and 56 showed acceptable scores ($30 < \Delta$ ppm $<$ 180). Random Forest and ROC models extracted 15 distinctive metabolites from the dataset, which are metabolically associated with the previously reported increase of *Ruminococcaceae* and *Bacteroidaceae*. The changes of nutritional composition in gut microbial metabolome were further analyzed through HPLC-LTQ-Orbitrap MS and GC-El-Q MS with various databases. AFB₁ reduced total content of carbohydrates and amino acids, gut lactic acid bacteria, production of short chain fatty acids and related secondary aliphatic acids. AFB₁ also disrupted the production of amino acid-derived neurotransmitters, e.g., melatonin and 5-HT, the absorption of food-contained long chain fatty acids, as well as bile acid secretion and metabolism. The affected pathways mainly included glucogenesis, glycolysis, metabolism of amino acids and neurotransmitters. These results provide an insight into the impact of AFB₁ on the nutritional composition of gut-microbial metabolome, as well as the affected biochemical pathways.

PS P233 - 0736 Inhibition of Neurotoxicity of the *Spirulina maxima* Extract from Ultrasonic Pretreatment

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Serious concern about the degeneration of human brain due to various oxidative stresses and drugs, etc. have been increased because it could cause to Alzheimer's diseases and Parkinson's diseases, etc. To reduce the toxicity of reactive oxygen species (ROS) in human bodies, especially human brain, natural or synthetic antioxidants have been developed to have the least side effects. However, the safety of these antioxidants have not been fully satisfied yet. Therefore, in this work, marine alga, *Spirulina maxima* was processed to

have high antioxidant activities with less toxicity because it is one of most safe health foods with various effects. The *S. maxima* extract from a nonthermal ultrasonic process proved to have very high concentrations of chlorophylls and C-PC as well as beta-carotene than the extracts from other conventional processes such as hot water or ethanol extraction. Especially, the extract from this unique process contains high amounts of intact forms of chlorophylls and C-PC which are extremely heat labile bioactive substances. The extract showed strong neuroprotective effects of 90% protection against a glutamate-induced HT22 cells in adding 1.0 mg/ml of the extract containing 15-17 mg/g of high amount of total chlorophylls. The extracts also had an ability of enhancing the activities of glutathione reductase and glutathione peroxidase, which can work on improving its neurotoxicity. Interestingly, the addition of the same concentration of chlorophylls showed lower neurotoxicity than those of the extracts, implying that the extract might have a synergistic effects of many various active components in the extract from ultrasonic pretreatment. In both *in vitro* and *in vivo* experiments, the extract showed higher antioxidant activities than the cases of administering the same dosages of chlorophylls and C-PC itself. The extracts was also found to play an important role in reducing the neurotoxicity of both glutamate-induced HT22 hippocampal neuronal cell and scopolamine-induced mice, even similar to donepezil, a positive control. The mechanism of inhibiting neurotoxicity of this extract was confirmed by effectively activating the enzyme activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx), which resulted in protecting the death of neuron cells in the brain by the reduction of ROS production and the Ca^{2+} accumulation. Therefore, the results of this work proved that the extract from natural resources, for this case, *Spirulina maxima* can work as a detoxicant and expand the possibility of developing cheap but effective natural biomaterials of inhibiting the neurotoxicity with less side effects than chemical drugs.

PS P234 - 0785 Histopathological and Toxicological Effects of Long-Term Consumption of Energy Drinks on Some Organs of Wistar Rats

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Energy drinks are popular and widespread; its market and degree of consumption have also expanded exponentially, raising concern about the adverse health effects of these products. This study investigated the anatomical and toxicological effects of consumption of two popular energy drinks (Monster and Bullet) in Nigeria on the kidney, liver, stomach, pancreas, intestine, ovary and testes of Wistar rats. Seventy Wistar rats of both sexes weighing averagely 200 grams were divided into seven groups (A to G) of six rats each for anatomical and biochemical studies while four additional groups of five each were for toxicity study. Group (B-G) were given the energy drinks by gavage at lower (1.5ml), moderate (3.0ml) and higher doses (6.0ml) respectively for 12 weeks while Group A (Control) received feed and water only. Animals were sacrificed after four, eight and twelfth weeks of treatment and blood sample were collected through cardiac puncture for LF [total (TB) and conjugated (CB) bilirubin, total protein (TP), alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphate (ALP), and albumin (ALB)] while kidney function markers [plasma urea, creatine] and plasma electrolytes [sodium, potassium, chloride and bicarbonate] levels were analysed using Landwind LWC 100 plus and ion selective analyser (ISE) in cases and control rats based on standard biochemical principles. In this study, body and organ weight were not significantly affected. Animals administered with the drinks at graded doses for the period of 12 weeks had significant increase in glucose, electrolytes and LF indices ($p < 0.05$). Reversible reduction were observed in chloride in cases when compared to the control. Histopathological examination of the organs revealed severe pathological lesions in liver, kidney, stomach and mild changes in pancreas and ovary with no lesion noticed in testes. However the changes observed were time and dose dependent with Monster administered rats being the most toxic in its action followed by Bullet.

PS P235 - 0799 Ginsenoside Rg3, an Active Ingredient of Ginseng, Can Induce Hemolysis-Dependent Phosphatidylserine Exposure and Thrombosis

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Ginseng and its active gradient, ginsenoside Rg3 (Rg3), are widely used for a variety of health benefits but they are not without toxicity. Previously, it has been reported that Rg3 can cause hemolysis but its health outcome have not

been fully elucidated. Here, we demonstrated that Rg3 can promote the coagulation and thrombosis during the process of hemolysis. In freshly isolated human erythrocytes, Rg3 led to pore formation and fragmentation of the erythrocyte membrane, which is distinct from a classical shape changes caused by lead (II) acetate (Pb), a well-known heavy metal with thrombotic risks. Confocal observation and flow cytometric analysis revealed that erythrocyte debris resulting from the exposure to Rg3, expressed phosphatidylserine (PS), which can promote blood coagulation through providing assembly sites for coagulation complexes. Despite of a distinct pattern from Pb, Rg3 induced procoagulant activation of erythrocytes and promoted thrombosis. These results were further confirmed in rats *in vivo* where Rg3 administration led to PS-bearing erythrocyte debris and increased thrombosis. Collectively, we demonstrated that, during the process of hemolysis, Rg3 can provoke thrombosis through generating PS-bearing erythrocytes debris.

PS P236 - 0830 Daily Intake of Iodine of Adult Residents in the Coastal Fishery Area in Iwate Prefecture, Japan

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Daily intake of trace elements, minerals and environmental toxic elements from the diet is an important factor for health promotion. In this study, iodine intake from typical Japanese styled meals eaten by adult residents in fishery area, Iwate Prefecture, in Japan was assessed. We collected dietary samples from a group of people living in the coast, where the intake of seafood and seaweed are high and where a traditional Japanese diet is still maintained. Daily intake of iodine was evaluated by two methods: (1) iodine intake was calculated, using the values in the Japan Standard Tables of Food Composition (FCT), through the analysis of duplicate samples of complete 24 h food consumption for 19 adult subjects (calculated value); (2) iodine content was also measured by ICP-MS (measured values). The ranges of daily intake of iodine were wide, 15,891 (18 ~ 15,909, minimum and maximum, respectively) µg/day, for calculated value; and 29,542 (90 ~ 29,632) µg/day, for measured value. Both calculated and measured levels in two subjects out of nineteen were higher than 2,200 µg/day, tolerable upper intake level (UL) for iodine. Because the subjects ate large amount of seaweeds that have high iodine contents, 67% of iodine intake were derived from Group 9 (Algae). Japanese use an Alga, Kombu, for the stock of the miso soup and soba soup. Dried Kombu has iodine by 2.4 mg/g; therefore, a 1 g intake immediately raises the iodine level to excess. It is necessary to estimate the appropriate state of both insufficient and excess iodine intake.

PS P237 - 0848 Sugar Substitutes Increase the Lipid Storage in *Caenorhabditis elegans*

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The prevalence of overweight and obesity are increased lately in many developed countries, generating several diseases such as cardiovascular disease and metabolic syndrome. Obesity is defined as an excess of fat mass, which generally results in excess weight. The high intake of sugars plays an important role in obesity. Because of this, the use of sugar substitutes has increased. These are food additives that offer a sweet taste by avoiding the intake of calories from sugar. However, its effectiveness in the weight loss is controversial. The goal of this work was to check the effect of four sugar substitutes: sucrose, erythritol, stevia and sucralose on the storage of lipids using the biological model *Caenorhabditis elegans*, taking into account that this organism have been explored in its insulin signaling, response to dietary glucose and as an obesity model. Lipids are stored as triacylglycerols in droplets in gut granules and in hypodermal cells in *C. elegans*. About 20 nematodes wild strain Bristol N2 were exposed to several concentrations of each sweetener (1, 10, 100 and 1000 ppb) and to the mixture of sucrose and erythritol. Lethality was evaluated after 24 h-exposure counting the number of alive and dead worms. The lipid accumulation was evaluated using Quick red oil O (qORO) for the staining of the lipid deposits. K medium (KCl, NaCl and ultra-filtered water) was used as control and as solvent. Statistical analyzes were performed using Kolmogorov-Smirnov and Bartlett. The differences between the means of the groups were determined with ANOVA and the Dunnett test was used to find significant difference with the control. Lethality was observable in all treatments in comparison to the control, being the exposure to sucrose higher than sugar substitutes. Lethality caused by sucrose had lower at 10 ppb than at 1 ppb, and increased from 100

ppb to 1000 ppb, showing a non-monotonic curve, distinctive of the effect of endocrine disruption. The exposure to all sweeteners caused the formation of lipid deposits, in a concentration dependence tendency. The highest and lowest lipid accumulation was generated by sucrose 1000 ppb and stevia 1 ppb, respectively. The use of sucrose, sugar substitutes and the mixture of sucrose with erythritol increased the deposits of fatty acids in the intestine of the nematode, while the stevia did not cause this effect. This finding is consistent with other studies that have promoted the use of non-caloric sweeteners such as stevia in the diet to prevent the weight gain. In conclusion, stevia exerted the lower toxicity and less accumulation of lipids in *C. elegans* compared to other sweeteners analyzed.

P238 - 0896 Docosahexaenoic Acid (DHA)-Derived Oxylipins Are Decreased in the Heart by Dietary Exposure to 2-monochloro-1,3-propanediol

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Chloropropanols (CP) have been identified as chemical process-induced food contaminants, which occur as by-products of the manufacturing of refined food oils and hydrolyzed vegetable protein. The 3-monochloro-1,2-propanediol (-MCPD) isomer is the most abundant and well-studied of the CP and is a known rodent and 'possible human' (IARC Group 2B) carcinogen, with a regulatory limit set for its occurrence in foods. However, there has been a paucity of research in understanding the hazard of the 2-MCPD isomer, thus forming a regulatory data gap for risk assessment. A 90-day sub-chronic dietary exposure study in F344 rats was conducted, and non-neoplastic histopathological lesions were observed in the heart of 2-MCPD-treated groups, while skeletal muscle was unaffected (Health Canada, unpublished observations). Oxylipins are oxygenated metabolites of polyunsaturated fatty acids that can mediate cellular processes such as apoptosis, inflammation, and cell proliferation; and are emerging as potential biomarkers of toxicity. The main objective of this study was to understand the relationship between dietary 2-MCPD exposure and the oxylipin profile in rat heart and skeletal muscle to determine potential modes of action in these tissues. We conducted oxylipin analyses using HPLC-MS/MS in the hearts and skeletal muscles of male and female rats exposed to control and 2-MCPD (40 mg/kg BW) AIN-93G formulated diets for 90-days (Health Canada). By comparison to the control, 5 of the 6 docosahexaenoic acid (DHA)-derived oxylipins were significantly lower in the 2-MCPD-treated hearts. In contrast, there were no alterations in oxylipin profiles between control and 2-MCPD-treated skeletal muscle. The DHA-derived oxylipins are considered anti-inflammatory, and our results of their lower levels in the 2-MCPD-treated hearts suggests an inability to resolve macrophage-induced inflammation and thus induce cardiotoxicity. This study provides a detailed profiling of oxylipins (in heart and skeletal muscle) and their potential roles as cardiotoxicity biomarkers of 2-MCPD and other chemical exposures.

P239 - 0897 Effects of Ochratoxin A and Aristolochic Acid on the Kidney in Diabetic Animal Model

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Ochratoxin A (OTA) is one of the most important mycotoxins owing to its widespread occurrence in agricultural commodities and is considered a possible human carcinogen with its potent nephrotoxicity. Aristolochic acid (AA) is nephrotoxic and carcinogenic while it is an active component of herbal drugs derived from *Aristolochia* species that have been used for medicinal purposes. As diabetic nephropathy is one of the most common long-term complications of diabetes, a pilot test was conducted to investigate the possible impact of OTA on diabetes. C57BLKS/J (or *db/db*) mice were used to evaluate the effect of OTA (0.5, 1, and 3 mg/kg BW) or AA (1 and 2 mg/kg BW) given alone or in combinations (OTA 0.5 mg/kg bw + AA 1 mg/kg BW) on the progression of kidney damage in development of diabetes or under diabetic conditions. Time-dependent animal study showed that OTA exposure may aggravate diabetes based on the increased glycated hemoglobin (A1C) and ketone levels when OTA was administered for up to 16 weeks. In addition, OTA: (i) decreased weight of major organs particularly the liver, kidney, and testis; (ii) decreased fasting glucose, albumin, alanine transaminase (ALT), and cholesterol levels; and (iii) increased calcium and alkaline phosphatase (ALP). In contrast, AA at the two doses tested (1 and 2 mg/kg BW) showed no significant impact on the

development of diabetes while the levels of A1C and ketone were lower than those of control until 8 weeks. Blood panel also showed short term improvement of diabetic conditions as determined by dose-dependent decrease in fasting glucose until 12 weeks. Low dose of AA (1 mg/kg BW) resulted in increased liver weight and decreased blood urea nitrogen (BUN) during the first 8 weeks indicating acute toxicity while no significant difference was observed in the kidney weight at both doses. In addition, the weights of pancreas and testis were decreased over time while no significant differences in calcium, ALP, ALT, and cholesterol levels. These results suggest that OTA and AA have different mode of action while they can be antagonistic during the initial stage of exposure as observed in organ toxicity and blood chemistry. It should be noted that OTA can complicate diabetic conditions although it is unclear if/how it affects the kidney directly without decreasing insulin sensitivity. Further study is suggested to elucidate the link between OTA exposure and development of diabetes and diabetic nephropathy particularly in the presence of other nephrotoxic substances.

P240 - 0899 The Role of Oxidative Stress in the Ochratoxin A-Mediated Toxicity in Liver and Kidney Cell Lines

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Ochratoxin A (OTA) is a mycotoxin produced by *Penicillium* spp. and *Aspergillus* spp. that commonly occur in food. OTA is a possible human carcinogen and has been shown to be nephrotoxic, hepatotoxic, immunosuppressive, mutagenic, and teratogenic. Though the mechanism of toxicity remains unclear, OTA has shown to induce hepatotoxicity and nephrotoxicity in both *in vitro* and *in vivo* studies via oxidative stress. In this study, human hepatoma cell line (Hep-G2) and renal proximal tubule cell lines from human and porcine (HK-2 and LLC-PK1) were used to investigate the OTA-mediated oxidative stress response. The cytotoxicity tests on these cells showed dose- and time-dependence decrease in cell viability with IC₅₀ of 1 μ M (Hep-G2), 0.382 μ M (HK-2), and 2 μ M (LLC-PK1) at 48 hr. OTA exposure of these cells resulted in a time-dependent elevation of reactive oxygen species (ROS) levels. In the case of Hep-G2 cells, OTA (0.75 μ M) up-regulated the gene expression of catalase (CAT, 3.2-fold), glucose-6-phosphate-dehydrogenase (G6PD, 3.0-fold), glutathione peroxidase 1 (GPX1, 2.6-fold), glutathione reductase (GSR, 2.1-fold), and superoxide dismutase (SOD, 2.5-fold) in a time-dependent manner (6 hr and 72 hr). The mRNA levels of G6PD and GPX1 were down-regulated by 0.67-fold and 0.66-fold, respectively, in LLC-PK1 cells after 72 h exposure to OTA at 1 μ M. In contrast, CAT, GSR and SOD were up-regulated by 2.20-fold, 2.24-fold, and 2.75-fold, respectively, in HK-2 after 72 hr exposure to OTA at 0.125 μ M. From these data, HK-2 cells are more sensitive to OTA-mediated toxicity compared with LLC-PK1. It was also suggested that oxidative stress contributes to the hepatic and nephrotic toxicity of OTA.

P241 - 0958 Effect of Silicon-Rich Water Intake on the Thymocyte Apoptosis and Peritoneal Inflammation of Rats with Chronic Low Levels of Aluminum Ingestion

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Study evaluated effect of silicon-rich water intake on thymocyte apoptosis and functional characteristics of peritoneal macrophages (PMs) of rats that were chronically exposed to dietary aluminum. The 28 study one month-old female Wistar Albino rats were divided into four groups of seven animals. The control rats only received standard tap water (SW group; 6 mg/L of silicon determined by ICP-OES (iCAP 6500 Duo, Thermo Scientific, United Kingdom) or experimental water (EW group; 19 mg/L of silicon) ad libitum. The experimental water was made by adding solution of sodium-silicate (sodium silicate, Si 1.00 g/L, Merck, Germany) in standard tap water. Experimental rats were administered aluminum chloride dissolved in distilled water (1.6 mg/kg body weight in 0.5 mL) by gavage for 90 days. The Al+SW and Al+EW rats were chronically intoxicated with soluble aluminum chloride by gavage, while drinking ad libitum either the standard or the experimental water respectively. The control rats (SW and EW) all underwent sham gavage by 0.5 mL of corresponding water every day. During the course of the study, none of the animals were subjected to procedures that caused pain or discomfort. Proapoptotic activity of thymocytes tissue was measured by Caspase-3 and Caspase-8. Unstimulated and lipopolysaccharide (LPS)-stimulated PMs were assessed in terms of phagocytic activity and cytokine

secretion *in vitro*. The aluminum-exposed rats had higher thymus proapoptotic cytokines than sham-gavaged, unrelated to type of water. Chronic exposition to dietary aluminum significantly increases Caspase-3 (706.9 ± 79 vs. 864.1 ± 99) and Caspase-8 (494.5 ± 67 vs. 703.4 ± 76) levels in rats with experimental water, as well as in groups with standard water (762.5 ± 107 vs. 1038.8 ± 109) and (531.3 ± 82 vs. 664.2 ± 56). The increase of proapoptotic Caspase 3 and Caspase 8 was lower in rats with EW compared to SW. Thus, aluminum increased concentration of proapoptotic caspases was diminished by silicon-rich water. LPS-stimulated PMs from aluminum-intoxicated animals exhibited low phagocytic activity and release of TNF- α , this was significantly improved by silicon-rich water intake. In the presence of silicon-rich water, LPS-stimulated and unstimulated PMs from aluminum-exposed rats produced significantly more IL-10. Chronic ingestion of aluminum, increases thymocyte expression of proapoptotic cytokines, peritoneal inflammation and PM dysfunction. The presence of high levels of the natural aluminum antagonist silicon in the drinking water restored IL-10 and TNF- α PM secretion and diminished thymocyte apoptosis. Thus, silicon intake can decrease the immunotoxicity of aluminum.

P242 - 0014 Presenting in Metals Platform Session (PL03)

P243 - 0050 HER2 and Src Co-regulate and Mediate Proliferation, Migration, and Transformation by Multiple Downstream Signaling Pathways in Arsenite-Treated Human Uroepithelial Cells

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Arsenic is ubiquitous in the environment and is identified as a human carcinogen by the International Agency for Research on Cancer, but the molecular mechanisms remain poorly defined. In the present study, we investigated the role of Src and HER2 in arsenic-induced malignant transformation. Human uroepithelial cells (SV-HUC-1) were treated with different concentrations (1, 2, 4, 8, 10 μ M) of sodium arsenite (NaAsO_2) for 24 h, or continuously treated with 0.5 μ M NaAsO_2 for up to 40 weeks. Western blot was used to detect protein expression. Co-immunoprecipitation was used to investigate interactions between Src and HER2. Cell proliferation and viability were measured with MTT assay. The migratory capacities were evaluated by Transwell assay and wound-healing assay. We used colony formation in soft agar to evaluate the anchorage-independent cell growth. Colony formation assay was used to examine clonogenicity. Acute exposure studies found HER2 expression increased in a time-dependent fashion in response to arsenite. Arsenite induced HER2 at the lowest dose (2 μ M) causing the strongest induction. Src phosphorylation by arsenite was observed with a peak response at 8 μ M. p-Src expression was significantly increased throughout the chronic exposure. Interaction between HER2 and Src was observed. HER2 was involved in arsenic-mediated Src phosphorylation, and arsenite-induced phosphorylation of HER2 was completely abrogated by Src kinase inhibitor saracatinib, suggesting that activation of HER2 by arsenic is dependent on Src. Chronic arsenite exposure also led to an upregulation of proliferation factors (cyclin D1, COX2, PCNA, VEGF, and HIF-1 α). Both short- and long-term exposure to arsenite activated Ras/Raf/MAPK, PI3K/AKT, and JAK2/STAT3 signaling pathways. Importantly, the activation of these signaling pathways and the consequent release of VEGF, cyclin D1, COX2, PCNA, and HIF-1 α were inhibited by HER2 inhibitor lapatinib and siRNA knockdown of HER2. In addition, cells exposed to 0.5 μ M arsenite for 40 weeks achieved hyperproliferation, anchorage independent growth, and enhanced tumorigenicity. Downregulation of HER2 inhibited cell growth, cell migration, and transformation properties of arsenite-treated cells. Furthermore, Src inhibition showed similar results. Combination of lapatinib and saracatinib almost completely suppressed cell viability and migration ability. HER2 and Src co-regulated and mediated proliferation, migration, and transformation by Ras/Raf/MAPK, PI3K/AKT, and JAK2/STAT3 downstream pathways in arsenite-treated SV-HUC-1 cells.

P244 - 0102 Persulfide and Polysulfide Species in Plants to Detoxify Methylmercury

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Methylmercury (MeHg), an environmental electrophile, covalently binds to protein thiols, resulting in functional loss of the modified protein. We found that MeHg also reacts with reactive sulfur species with highly nucleophilicity, such as persulfides and polysulfides, to yield bismethylmercury sulfide [(MeHg) $_2$ S], which has little electrophilicity and less toxicity. The purpose of this study is to find per/polysulfide species to be able to convert MeHg to (MeHg) $_2$ S in edible plants such as *Allium sativum* L. (garlic). We evaluated existence of per/polysulfide species by using (MeHg) $_2$ S assay on the basis of high-performance liquid chromatography (HPLC) and atomic absorption spectrophotometry (AAS). During reaction of garlic juice or *Allium cepa* L. (onion) juice with MeHg, (MeHg) $_2$ S was formed. To address whether the low-molecular compounds could contribute to production of (MeHg) $_2$ S, such low-molecular-weight fraction and high-molecular-weight fractions were separated and tested (MeHg) $_2$ S formation from MeHg. As a result, low-molecular-weight fraction of garlic contained per/polysulfides to form (MeHg) $_2$ S more than the high-molecular-weight fraction and the low-molecular-weight fraction of onion. Hexane extract of garlic has a high efficiency to convert (MeHg) $_2$ S from MeHg compared with the benzene, chloroform, ethyl acetate, methanol, and water extracts. These results indicate that onion and garlic have reactive sulfur species, which are mainly aliphatic hydrocarbons. Our study provides that dietary taken vegetable containing such reactive sulfur species may reduce health risk of environmental electrophiles exposure. This work was supported by a grant-in-aid (#18H05293 to Y.K.) for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and a research funding to Y.A. from Urakami Foundation for Food and Food Culture Promotion.

P245 - 0117 lncRNA Expression Profiling and Its Relationship with DNA Damage in Cr(VI)-Treated 16HBE Cells

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Compounds containing hexavalent chromium [Cr(VI)] were Group I human carcinogens, which are mutagenic and can induce DNA damage. Cr(VI) exposure could cause a lot of changes in mRNA, protein, and microRNA expression as well as DNA methylation. There were still few studies on the role of long non-coding RNA (lncRNA) in the carcinogenic process of Cr(VI). In the current study, lncRNA expression profiling and bioinformatics analysis in 16HBE cells treated by Cr(VI) were performed. The Cell Counting Kit-8 (CCK-8) assay and the comet assay were done to assess the cell viability and DNA damage in Cr(VI)-treated 16HBE cells, respectively. The lncRNA expression profile was performed by Arraystar Microarray V3.0 in 16HBE cells treated with 0.00 and 10.00 μ mol/L Cr(VI). Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR) was applied to verify some significantly altered lncRNAs. Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, and mRNA-lncRNA network analysis were conducted to identify related biological processes, signal pathway, and critical lncRNAs. It was found that Cr(VI) could induce cells viability decline and alter lncRNA expression profile of 16HBE cells. 1868 lncRNAs were significantly up-regulated and 2203 lncRNAs were significantly downregulated, which formed a complex regulation network. With the increase of Cr(VI) concentration, some lncRNAs increased or decreased gradually. The differentially expressed lncRNA profiling induced by Cr(VI) were associated with immune response, cell cycle, DNA damage and repair, and so on. RP11-388M20.9 and AC092620.3 were non-linearly decreasing with the change of the DNA content of comet tails (Tail DNA), tail length (TLL), tail moment (TM), and Olive Tail Moment (OTM), and the fitting results of Tail DNA and TM were statistically significant ($P < 0.05$). It was possible for RP11-388M20.9 to regulate DNA damage by interacting with the target gene after Cr(VI) exposure and was likely to be a potential biomarker of DNA damage in Cr(VI)-treated 16HBE cells.

P246 - 0118 Search for Splicing Factors That Maintain Normal Splicing of Human Arsenic (+3 Oxidation State) Methyltransferase (AS3MT) mRNA

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It is known that inorganic arsenic with high toxicity is mono-methylated and further di-methylated by arsenic methyltransferase (AS3MT) to excrete outside the body in a state of low toxicity. Studies using samples of chronic arsenic poisoning patients have revealed that the proportion of mono-methyl and dimethyl arsenic is related to toxicity and is attributed to AS3MT polymorphism. On the other hand, immature mRNA transcribed from DNA becomes mature mRNA via a splicing function that binds only the exon. Diverse mRNA is produced by intentional alternate splicing, but induction of unintentional alternative splicing promotes the production of incomplete mRNA and protein. Our laboratory reported that HepG2 cells showed that AS3MT mRNA underwent alternative splicing and that its transcription product was deficient in the methylation ability of arsenic. In addition, we found a novel splicing variant of AS3MT mRNA was triggered by exposure to hydrogen peroxide (H_2O_2). In this study, we aimed to explore the factors involved in the alternative splicing of AS3MT mRNA. Database analysis showed that serine/arginine splicing factors 5 (SRSF5) were more likely to bind to some of the 11 exons of AS3MT mRNA, so we prepared HepG2 cells artificially reducing the levels of SRSF by siRNA technique. When AS3MT mRNA was detected by RT-PCR, unfortunately only reduction of SRSF5 could not cause abnormality in the splicing of AS3MT mRNA. Therefore, in order to find the factors involved in the splicing of AS3MT mRNA, we investigated AS3MT mRNA of various cells. Compared with mRNA collected from HepG2, the levels of WT mRNA were lower, and several splicing variants of AS3MT mRNA were detected in acute myeloid leukemia HL-60 and acute promyelocytic leukemia NB4 and chronic myeloid leukemia K562 cells. Furthermore, we compared the levels of SRSF family of HepG2 and these leukemia cells. Almost SRSF families showed the equivalent levels, but SRSF9 showed lower levels in leukemia cells than HepG2 cells. From these results, it was found that SRSF 9 is a putative factor involved in the splicing of AS3MT mRNA. Currently, we are studying whether the alternative splicing of AS3MT mRNA is triggered by downregulation of SRSF9.

P247 - 0136 Induction Mechanism of TNF- α Expression by Methylmercury in Microglial BV-2 Cells

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Methylmercury is an environmental pollutant known as the cause of Minamata disease and severe central nervous system damage. However, little is known about the mechanisms of methylmercury toxicity and cellular protective mechanisms against such toxicity. We recently reported that the expression of TNF- α (tumor necrosis factor- α), an inflammatory cytokine, was specifically induced in the brains of mice, which administered with methylmercury, and may cause neuronal damage. We also found that microglia are mainly involved in the induction of TNF- α expression by methylmercury in the mouse brain. Thus, elucidation of the induction mechanism of TNF- α expression could be a clue to reveal the mechanisms related to methylmercury-induced neurotoxicity. This study aimed to elucidate the induction mechanism of TNF- α expression by methylmercury using the microglial BV-2 cells. We examined the effect of methylmercury on TNF- α expression using the microglial BV-2 cells. Exposure of the cells to methylmercury increased TNF- α mRNA level in a dose- and time-dependent manner, and this increase was abolished by pretreatment of transcription inhibitor. These results suggest that methylmercury induces TNF- α expression in microglia via the activation of some transcription factors. It has been reported that transcription factor NF- κ B and AP-1 are mainly involved in the induction of TNF- α expression in microglia. Both transcription factors are activated through phosphorylation and then migrate into the nucleus. Exposure of the cells to methylmercury resulted in the phosphorylation of p65 and c-Jun, which are subunits of NF- κ B and AP-1. However, transfection of the siRNA for c-Jun did not affect induction of TNF- α expression by methylmercury, while the siRNA for p65 partially suppressed the induction. Furthermore, we found that methylmercury induced TNF- α expression in primary mouse microglia and also increased the phosphorylation of p65. Our results indicate that methylmercury induces TNF- α expression through the activation of the transcription factor NF- κ B in microglia.

P248 - 0138 Elucidation of the Mechanisms Involved in Methylmercury Toxicity Enhancement Showed by the Novel Transcription Factor tmRT1

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Methylmercury is an environmental pollutant that is toxic to the central nervous system; however, the molecular mechanisms underlying its toxicity remain unclear. Our previous studies have suggested that the novel transcription factor tmRT1 is involved in the enhancement of methylmercury toxicity by inducing the expression of various genes in human embryonic kidney 293 (HEK293) cells and in mouse neural stem cells. Most transcription factors regulate the gene expression by binding with either an activator or a suppressor. In this study, we searched for the proteins that can bind to tmRT1 in order to elucidate the mechanisms involved in methylmercury toxicity enhancement indicated by tmRT1. We examined the intracellular distribution of tmRT1 and found that it is mainly located in the nucleus, and the exposure to methylmercury does not have an effect on its distribution. Therefore, we prepared a nuclear fraction from the tmRT1-V5 expressing HEK293 cells, and incubated with recombinant tmRT1 tagged with both GST and His-tag (GST-tmRT1-His) for pull-down assay using Ni affinity beads. The recombinant GST-tmRT1-His was eluted with imidazole, subjected to SDS-PAGE, and stained with CBB. Several proteins, presumably those bound to GST-tmRT1-His, were observed on the gel. Among them, the bands with a relatively larger amount bound to tmRT1 were excised, trypsinized, and analyzed by LC/MS. We identified X-ray repair cross complementing protein 6 (XRCC 6: DNA repair related factor), heat shock protein 1A1 (HSP1A1: chaperone molecule), histone H2BJ, and histone H2BK (core histone) as the tmRT1 binding proteins and further confirmed these by immunoprecipitation. However, there are no reports that indicate the tmRT1 binding proteins identified in this study are involved in the regulation of transcription factor activity so far. It is expected that examination of the role of these tmRT1 binding proteins on tmRT1 activation by methylmercury will shed light on the novel mechanisms involved in the control of tmRT1 activity.

P249 - 0144 Limit Values for Metals: Do We Need Interspecies Extrapolation Factors?

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Risk assessment/limit value setting for metals at the workplace is often based primarily on animal data. Epidemiological data providing information on quantitative exposure-response relationships are rarely available but should be used preferentially if of sufficient quality. If animal data are used as points of departure, a default interspecies extrapolation factor in the range of 2–5 is usually applied by regulators. Animal and human data for zinc oxide, chromium(III) oxide, and copper will be compared. Zinc oxide is an example for which animal and recent human data are available. In an inhalation study exposing rats over five days for six hours daily, increased concentrations of inflammatory mediators in the bronchoalveolar lavage fluid and in the serum were found already at the lowest applied concentration of 0.55 mg/m³; in another study rats exposed to a concentration of inhaled zinc oxide nanoparticles of 2 mg/m³ for four weeks only showed macrophage infiltration in the alveolar space without other inflammatory cell infiltration. In a human study covering exactly this concentration range from 0.5 to 2 mg/m³, a concentration-dependent systemic response after inhalation of nano-sized zinc oxide particles had been noticed. An increase in acute phase proteins and neutrophils in blood and an increase in body temperature were observed, while zinc oxide exposure had no effect on club cell protein, interleukin 6, various clotting factors in blood, fractional exhaled nitric oxide, and all lung function parameters. In a 13-week inhalation study in rats exposed to chromium(III) oxide concentrations of 4.4 mg/m³ and more, trace to mild septal cell hyperplasia and interstitial inflammation have been observed, while a study of 106 employees exposed to chromium(III) oxide levels of 0.8 to 2.9 mg/m³ for several years did not reveal any increase in the prevalence of respiratory illness. In Germany, a limit value for copper has been proposed by the MAK (maximal workplace concentration) commission focusing on animal data. Based on a 28-day rat inhalation study with copper(II) oxide that demonstrated inflammatory effects starting at concentrations around 200 µg/m³, a MAK value of 10 µg/m³ (respirable fraction) has been derived. However, copper has been used at workplaces for centuries at concentrations even in the mg range without observation of clear health effects. The examples given do not indicate that rodents might be more sensitive to health effects following inhalation of metals than humans. Thus, potential consequences for interspecies extrapolation factors will be discussed.

P250 - 0154 Altered Differentiation of Mouse Stem Cells after Chronic Low-Level Arsenic Exposure

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Arsenic is a contaminant found in drinking water and many varieties of rice. Because of its concentrations and prevalence, millions of people are thought to be exposed to arsenic above the current US EPA and WHO drinking water standard of 10ppb. However, the mechanisms by which it impacts development are still not well understood, although epidemiological evidence indicates that exposure to arsenic during the embryonic and fetal period is associated with reduced weight gain, altered muscle function, and neurodevelopmental effects. In the present study, we have chronically exposed P19 mouse embryonic stem (ES) cells to 0.1mM or 7.5ppb arsenic. The goal is to assess whether arsenic can alter their differentiation into adult cell types, including skeletal myocytes and sensory neurons. The ES cells exposed to arsenic for up to 36 weeks have an impaired ability to differentiate appropriately, as seen by reduced expression of transcription factors such as Ascl1, which is needed to convert stem cells into both neuronal and muscle lineages. The arsenic-exposed cells also overexpress smooth muscle actin mRNA by 2–4X than the controls, suggesting that they are forming more of a fibroblast-like cell. These changes appear to occur after 24 weeks of exposure. However, there are no changes in the transcript levels of Hmx1 or Nrf2, suggesting that the mechanism by which this occurs may not involve ROS. Similarly, there are no changes in Nanog mRNA expression, a marker of pluripotency. Thus, it appears that chronic arsenic exposure alters stem cell differentiation, favoring certain cell lineages over others.

P251 - 0157 BIRC Family Gene Expression Changes by Toxic Metal(loid)s in Neuroblastoma, Kidney, and Hepatic Cells

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Toxic heavy metals and metalloids such as cadmium, mercury, and arsenic can induce apoptosis in their target tissues. We already demonstrated that cadmium induces apoptosis by suppressing expression of the apoptosis inhibitor, BIRC3 (Baculoviral inhibition of apoptosis (IAP) protein repeat containing 3), in human proximal tubular cells (HK-2 cells). BIRC3 is a member of the BIRC family, which consists of eight family members in human and seven in mouse. The BIRC family includes BIRC1/NAIP, BIRC2/cIAP1, BIRC3/cIAP2, BIRC4/XIAP, BIRC5/Survivin, BIRC6/Apollon, BIRC7/ML-IAP, and BIRC8/ILP (not in mouse). In this study, we examined whether cadmium (Cd), methylmercury (MeHg), inorganic mercury (Hg²⁺), or arsenic (As) affects the gene expression of BIRC family members in several different cultured cells. Although the mRNA level of BIRC4 was increased by Cd treatment, those of the other BIRC family members, except for BIRC3, were unaffected by Cd treatment in HK-2 cells. MeHg increased the mRNA level of BIRC5 and decreased the mRNA level of BIRC2 in human IMR-32 neuroblastoma cells. In HK-2 cells, on the other hand, MeHg increased the mRNA levels of BIRC2, BIRC5, and BIRC8 and decreased the mRNA levels of BIRC3 and BIRC6. Hg²⁺ increased the mRNA levels of BIRC2 and BIRC5 and decreased the mRNA level of BIRC3 in HK-2 cells. Finally, As increased the mRNA levels of Birc1, Birc3, and Birc7 and decreased the mRNA levels of Birc2, Birc4, Birc5, and Birc6 in mouse normal hepatic AML-12 cells. These results indicate that each metal(loid) toxicant may regulate the BIRC family gene expression in different manners. Therefore, each BIRC family member may play distinct roles in various tissues when exposed to toxic heavy metals or metalloids toxicants. *Lee et al, Sci. Rep., 2017; Lee et al, BPB Reports, 2018.*

P252 - 0160 New Findings on the Genes Involved in Cadmium Toxicity in Human Proximal Tubular Cells

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Cadmium (Cd) is an environmental contaminant that induces toxic effects in various tissues, including the kidney. Proximal tubular cell damage is characterized as Cd-induced renal damage. In our previous study, DNA microarray result showed that Cd treatment not only increased the expressions of 30 genes more than 2-fold, but also decreased those of 21 genes less than a half in HK-2 human proximal tubular cells. In this study, we examined the effects of SLC39A1 coding for Zinc transporter (ZIP1), GPRC5B coding a member of the G-protein-coupled receptor family, HIST1H4C coding the histone H4 pro-

tein, and heat shock protein genes HSPH1 and HSPA8 among the genes from the DNA microarray results on Cd toxicity in HK-2 cells. As a result, the mRNA level of SLC39A1 was significantly decreased by treatment with Cd time dependently. In addition, HK-2 cells transfected with SLC39A1 siRNA were slightly resistant to Cd treatment compared with control cells. It was found that not only did Cd significantly reduce GPRC5B gene expression in HK-2 cells, but also, knockdown of GPRC5B by siRNA transfection strengthened Cd toxicity in HK-2 cells. HIST1H4C knockdown by siRNA transfection enhanced Cd toxicity, and Cd significantly reduced the mRNA level of HIST1H4C. HSPH1 and HSPA8 gene expressions were increased by Cd treatment; also, knockdown of HSPH1 and HSPA8 by siRNA treatment decreased the viability of Cd treated HK-2 cells compared with control siRNA treatment group. These results strongly imply that Cd exerts cytotoxicity through the disruption of the expressions of various genes. *Lee et al., J. Toxicol. Sci., 2013; Lee et al., Fundam Toxicol., Sci., 2014a, 2014b, 2015, 2018.*

P253 - 0175 Potential Adverse Effect of Low Cadmium Intake to Lactation Load against Bone Metabolism and Renal Function of Mother Rat

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Effects of cadmium intake on bone metabolism of mothers during pregnancy and lactation were investigated together with Cd intake and pregnant-lactation. Female Wistar rats (6 weeks old) were given Cd (as CdCl₂) at a dose of 1, 2, and 5 mgCd/kg/day by gastric tube daily for 6 consecutive days a week for 10 weeks. From 3 weeks after oral Cd administration, female rats were paired with male rats at 1:1 ratio for 1 week. Cd was given continuously during the pairing, pregnancy, and lactation. After delivery, mother rats lactated to 8 newborn rats of 4 male and female each. Cd intake in consideration of intestinal absorption was estimated 2, 7, and 14 μg/kg/day respectively in each group of 1, 2, and 5 mgCd/kg/day. Cd is bound by metallothionein (MT) in intestinal tissue, and more Cd was accumulated in kidney rather than in liver. The increased urinary excretion of β₂-microglobulin (β₂-MG) and N-acetyl-D-glucosaminidase (NAG) was found both in the groups of 2 mgCd/kg and of 5 mgCd/kg, in the group of lactation load. But in the group of Cd intake without lactation, this increase of urinary excretion of β₂-MG was not found in both Cd intake groups. Without the renal dysfunction, the increased osteoid volume of femur of mother rats resulted by lactation. On the other hand, the effect of Cd intake together with lactation caused the significant decrease of bone mineral density and of bone bending strength in each group of 2mg/kg and 5mg/kg. Additionally, the increased femoral osteoid volume and of the urinary excretion of calcium also resulted in both experimental groups. Urinary excretion of deoxypyridinoline (DPyr) as the biomarker of bone absorption increased by lactation of all experimental groups compared with that of control group. Although the urinary excretion of DPyr increased, the remarkable increase of osteocalcin of the index of bone formation was not found. The decrease of bone mineral density, bone bending strength, and bone mineral content was found prominently. Although the effect of lactation load to bone metabolism was remarkably than that of Cd intake of mother rats, the additive adverse effect of Cd intake was also found. It was suggested that the effect of Cd intake on maternal bone metabolism should be evaluated in consideration of the degree of pregnancy and lactating load.

P254 - 0189 Cadmium Induces Autophagy and Blocks Autophagosome Fusion with Lysosomes in AML12 Cells

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Cadmium (Cd) is an environmentally common contaminant that induces hepatotoxicity. Autophagy is a self-protective mechanism against various stresses via degrading and recycling macromolecular proteins and damaged organelles in lysosome, which is of vital importance for maintaining cellular homeostasis. Our previous studies showed that Cd exposure could induce autophagy and autophagy plays a protective role in Cd-induced hepatotoxicity. The role of autophagy in Cd-induced hepatotoxicity has to be studied further. In this study, AML12 cells were treated with CdCl₂ (5 μM) for 0, 3, 6, 12, 24 h. In addition, cells were treated with CdCl₂ at 0, 2.5, 5, 10 μM for 12 h respectively. Rapamycin

(RAP) and chloroquine (CQ), Baflomycin A1 (Baf) were used to promote or inhibit autophagy. The levels of ATG5, Beclin-1, LC3, P62, LAMP2, Cathepsin B (CTSB), and Rab7 were detected by Western blotting. LC3 puncta and the colocalization of LC3 with LAMP2 were observed by fluorescence microscopy. And the autophagosomes were detected by transmission electron microscope. Treatment with Lyso-Tracker Red and self-quenched bodipy-conjugated BSA and then the lysosomal acidity and the lysosomal degradation capacity were assessed by immunofluorescence. Finally, the cell index was monitored by RTCA system. The results showed that the levels of autophagy-related proteins ATG5, Beclin-1, LC3II, and P62 were increased. And LC3 puncta and autophagosomes were significantly augmented. Meanwhile, the autophagic flux was blocked, which aggravated Cd-induced hepatotoxicity in AML12 cells. The lysosomal acidity was further acidified induced by Cd, and Cd increased lysosomal degradation capacity. Furthermore, the levels of LAMP2 and CTSB increased, but the colocalization of LC3 with LAMP2 was inhibited. Finally, the levels of Rab7 which the key protein in the fusion of autophagosomes with lysosomes were declined induced by Cd. Our results demonstrate that Cd exposure not only induces autophagy but also inhibits the autophagic flux. Cd exposure inhibits the autophagosome-lysosome fusion, which leads to autophagy blockade, and then aggravate Cd-induced hepatotoxicity. The potential mechanism of Cd-induced autophagy blockade might be related to Rab7 reduction in AML12 cells. The underlying mechanism of Cd-induced autophagy in AML12 cells needs to be studied further. *This work was supported by the National Key Research and Development Program of China [No.2016YFD0501208], the National Natural Science Foundation of China [No. 31702305], the Natural Science Foundation of Jiangsu Province [No. BK20160458], China Postdoctoral Science Foundation [2016M601900].*

PS P255 - 0206 Drinking Water Treatment Using Cucumber Peel Immobilized on Sodium Alginate: Multi-ion Biosorption of Arsenic and Heavy Metals

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The release of heavy metals in the environment by natural and anthropogenic sources is a serious global concern, especially because these are nonbiodegradable toxicants that bioaccumulate over time and are soluble in water. Biosorption of heavy metals from water is a viable alternative to expensive conventional techniques, and low-cost biosorbents are being developed from agricultural solid wastes. In the present work, six fruit and vegetable peels, including apple, banana, cucumber, kiwifruit, orange, and potato, were screened based on their biosorption performance to simultaneously remove toxic ions from aqueous solution. Equilibrium biosorption of cucumber peel immobilized on sodium alginate was found to be 21% arsenic (As V), 91% cadmium (Cd II), 17% chromium (Cr VI), 72% copper (Cu II), 90% mercury (Hg II), 82% lead (Pb II), and 61% nickel (Ni II) with minimum biosorbent dose for 0.1 mg L⁻¹ cocktail solution. It was then selected for treating tap water collected from a village in the Macraes Mine region of East Otago, which is also the largest gold mine of New Zealand. Known concentrations of As V, Cd II, Cr VI, Cu II, Hg II, Pb II, and Ni II were spiked in Macraes drinking water containing other ions to study the simultaneous removal of arsenic and heavy metals over a contact time period ranging from 15 min to 3 days. Results indicate that multi-ion biosorption is possible using cucumber peel "bead" with interference due to the presence of competing ions in solution.

PS P256 - 0283 Arsenite Exerts Cytotoxic Effect through Inhibition of Synthesis of Ribose-5-Phosphate in Human Acute Monocytic Leukemia THP-1 Cells

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Arsenic is a well-known hazardous metalloid that exists widely in the environment. Health hazards caused by arsenic pollution of ground water have been issues of global concern. On the other hand, arsenite, an inorganic arsenic compound, is used to treat patients with acute promyelocytic leukemia. Arsenite is known to induce carcinogenicity and cardiotoxicity. However, the detailed molecular mechanisms responsible for arsenite toxicity remain unclear. To identify novel mechanisms responsible for arsenite toxicity, we have searched for genes conferring cellular sensitivity against arsenite using budding yeast as a model eukaryote, and found that repression of genes involved in pentose phosphate pathway increased sensitivity of yeast cells to arsenite. Pentose phosphate pathway is a metabolic pathway parallel to glycolysis that

generates nicotinamide adenine dinucleotide phosphate (NADPH), pentoses, and ribose-5-phosphate. In the present study, we investigated the relationship between arsenite toxicity and pentose phosphate pathway in human acute monocytic leukemia THP-1 cells. THP-1 cells were treated with arsenite (0-10 μ M) in RPMI1640 containing 10% fetal bovine serum. After treatment, cell viability was measured by a cell counting kit-8, and mRNA and protein levels were measured by real-time RT-PCR and Western blotting, respectively. We examined the effects of arsenite on the expression of genes involved in pentose phosphate pathway and found that arsenite represses expression of transketolase (TKT) and ribose 5-phosphate isomerase A (RPIA), which are enzymes involved in generation of ribose-5-phosphate. Arsenite also decreased the protein levels of TKT and RPIA. Furthermore, supplementation of D-ribose, a precursor of ribose-5-phosphate, to culture medium decreased the toxicity of arsenite in THP-1 cells. These results suggest that arsenite might exert cytotoxicity through inhibition of synthesis of ribose-5-phosphate in pentose phosphate pathway in human THP-1 cells.

PS P257 - 0284 Analysis of Various Renal Injury Biomarkers Using *In Vitro* Evaluation System

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Recently, various urinary biomarkers have been utilized as indicators of proximal tubular dysfunctions of kidney. These biomarker proteins for renal injury are excreted in the urine through the following mechanisms: (1) Low-molecular-weight (LMW) proteins such as β 2-microglobulin (β 2-MG) are excreted into the urine due to the disturbed reabsorption into the proximal tubule epithelial cells (PTECs), or (2) biomarker proteins such as kidney injury molecule-1 (Kim-1) are secreted into the urine following the damage-induced synthesis in PTECs. Chronic exposure to cadmium (Cd) is known to cause proximal tubular injury. However, the detailed mechanisms how Cd affects the reabsorption of LMW proteins or induces the injury-sensitive proteins remain unclear. The lack of *in vitro* assay systems for reabsorption efficiency of LMW proteins in PTECs has hampered the elucidation of the cellular mechanisms of Cd-induced disturbances in reabsorption. Therefore, we developed *in vitro* assay systems for the uptake of proteins by endocytosis using S1 and S2 cells, which are immortalized cell lines derived from the S1 and S2 (convoluted) segments of the proximal tubule of mice. FITC-labeled albumin, β 2-MG, and metallothionein (MT) were used. The uptakes of these fluorescence-labeled proteins by S1 and S2 cells were confirmed by confocal and fluorescence microscopies and the uptake efficiencies were quantitatively evaluated by flow cytometry. Induction of Kim-1 and clusterin were evaluated by the mRNA levels. Exposure of S1 and S2 cells to Cd at non-lethal doses for 1, 3, and 6 days resulted in the decreases in reabsorption efficiencies of β 2-MG and MT, but not albumin. The mRNA levels of Kim-1, but not clusterin, were enhanced by Cd exposure. The protein levels of megalin and cubilin, which are responsible for the endocytosis of LMW proteins at the apical membrane of PTECs, were altered slightly by Cd exposure, but other factors may also be involved in the disturbed reabsorption of β 2-MG and MT. Thus, the assay system we developed for the reabsorption efficiency of LMW proteins and the induction of renal injury-responsive biomarkers may serve as a good tool for *in vitro* evaluation of renal toxicity of metals and chemicals as well as for the elucidation of molecular mechanisms of renal toxicity.

PS P258 - 0304 Sodium Arsenite Inhibits Tissue Plasminogen Activator Release from Cultured Human Vascular Endothelial EA.hy926 Cells

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Arsenic is an environmental pollutant that exists widely around the world. Health impairment due to arsenic pollution of ground water is currently noted among residents worldwide. It is known that chronic exposure to arsenic increases the risk of vascular lesions such as blood circulation disorder and atherosclerosis. Blood coagulation and fibrinolytic system is considered to contribute to the vascular lesions. Fibrinolysis in normally circulating blood strongly depends on the balance between tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) secreted from vascular endothelial cells. We therefore investigated the effect of arsenite on the fibrinolytic system of vascular endothelial cells. Confluent cultures of human vascular en-

endothelial EA.hy926 cells were treated with sodium arsenite (0-20 μ M) in DMEM containing 10% fetal bovine serum for 24 h. After treatment, the cell viability and mRNA levels were measured by the MTT assay and real-time RT-PCR, respectively. The fibrinolytic activity in the conditioned medium was analyzed by fibrin zymography and the t-PA and PAI-1 content in the conditioned medium were determined by ELISA. It was found that sodium arsenite inhibits the fibrinolytic activity in the conditioned medium of EA.hy926 cells without any cell damage. At that time, the secretion and mRNA expression of t-PA were lowered by sodium arsenite; however, those of PAI-1 were not changed. Furthermore, siRNA-mediated knockdown of NF-E2-related factor2 (Nrf2), which was activated by sodium arsenite, diminished the inhibition of the t-PA mRNA expression. These results suggest that sodium arsenite reduces the fibrinolytic activity in the liquid phase of cultured human vascular endothelial cells through selective suppression of t-PA expression depending on the activation of Nrf2.

PS P259 - 0327 Protective Effect of Aqueous Leaf Extract of *Costus afer* (Ker Gawl) on Testicular Injuries Induced by Low Dose Heavy Metal Mixture (Lead, Cadmium, and Mercury) in Wistar Albino Rats

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This study was undertaken to investigate the protective effect of the aqueous leaf extract of *Costus afer* (ker gawl) on Low Dose Heavy Metal Mixture (LDHMM) (PbCl_2 + CdCl_2 + HgNO_3) - induced oxidative damages and histopathological alterations in the testis of wistar albino rats. The animals were divided into six equal groups, i) control, ii) metal mixture (PbCl_2 + CdCl_2 + HgNO_3), iii) combination of metal mixture + *Costus afer* at 750 mg/kg, iv) combination of metal mixture + *Costus afer* at 1500 mg/kg, v) combination of metal mixture + *Costus afer* at 2250 mg/kg, vi) combination of metal mixture + ZnCl_2 . Oxidative stress, induced LDHMM (PbCl_2 = 20mg/kg; CdCl_2 = 1.61 mg/kg; HgNO_3 = 0.40mg/kg through oral gavage, significantly decreased ($p < 0.05$) the activity level of testicular key enzymatic antioxidant biomarkers (superoxide dismutase, SOD; catalase, CAT; and glutathione, GSH), increased ($p < 0.05$) the lipid peroxidation marker (malondialdehyde, MDA), and testicular lead, cadmium and mercury concentrations. Also, LDHMM administration resulted in a significant ($p < 0.05$) increase in the number of sperms with abnormal morphology and plasma prolactin level and a significant reduction in epididymal sperm count, motility, follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone. Furthermore, LDHMM exposure induced histopathological changes to the testis including alterations of the maturing sperm cells in the seminiferous tubules. Notably, oral pretreatment of experimental animals with *Costus afer* (750 mg/kg, 1500 mg/kg and 2250 mg/kg) lowered the extent of the observed heavy metal mixture-mediated toxicity in a dose-dependent ratio, significantly reducing ($p < 0.05$) the resulting lipid peroxidation marker, Pb, Cd and Hg accumulation in testis, histopathological changes of the testis and spermatozoa abnormalities. In parallel, the pretreatment with *Costus afer* also reversed the observed LDHMM-induced inhibition in enzymatic activities of antioxidant biomarkers (SOD, CAT, and GSH). The pretreatment of rats with *Costus afer* significantly recovered the observed LDHMM-mediated increase in the weight of accessory sex organs. Taken together, our findings highlight the role of *Costus afer* as a protective modulator of LDHMM-induced testicular injuries and suggest some therapeutic potential in mammals. Further investigation of therapeutic strategies employing *Costus afer* against heavy metal toxicity in humans is therefore warranted.

PS P260 - 0374 Biological and Toxicological Significance of Selenocyanate as a Selenium Metabolite

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Selenium (Se) is an essential element for humans and animals, and is recognized as a beneficial/bystander element for plants. Because of chemical properties of Se, it can form a carbon-Se bond through the metabolic pathway in animals and plants, and its biological and toxicological effects are very much dependent on its chemical form. The identification of Se-containing metabolites is expected to provide important clues to elucidate the metabolic pathway of Se. As Se is essential but exists in a trace amount, the analyses of selenometabolome require massive amounts of samples, such as urine, tissues/organs, and blood. Indeed, studies of selenometabolome in cultured cells are limited. Hence, we attempted to examine selenometabolome in cultured cells by speciation analysis. When human hepatoma HepG2 cells were exposed to sodium selenite,

an unknown Se metabolite was detected in the cytosolic fraction by HPLC-inductively coupled plasma mass spectrometry (LC-ICP-MS). The unknown Se metabolite was also detected in the mixture of HepG2 homogenate and sodium selenite in the presence of exogenous glutathione (GSH). The unknown Se metabolite was identified as selenocyanate by electrospray ionization mass spectrometry (ESI-MS) and ESI quadrupole time-of-flight mass spectrometry (ESI-Q-TOF-MS). Because exogenous cyanide increased the amount of selenocyanate in the mixture, selenocyanate seemed to be formed by the reaction between selenide, the product of the reduction of selenite, and endogenous cyanide. Rhodanase, an enzyme involved in thiocyanate synthesis, was not required for the formation of selenocyanate from selenide or selenosulfate and endogenous cyanide. We propose to name the effect of endogenous cyanide a reactive cyanogen species (RCS) likened to NO- and HS- of reactive nitrogen and sulfur species, respectively. A precise mechanism underlying the generation of RCS should be clarified in future studies. Selenocyanate was less toxic to HepG2 cells than selenite or cyanide, suggesting that it was formed to reduce the toxicity of selenite. On the other hand, selenocyanate could be assimilated into selenoproteins and selenometabolites in rats in the same manner as selenite. Consequently, selenite was metabolized to selenocyanate to ameliorate its toxicity temporarily, and selenocyanate acted as an intrinsic Se pool in cultured cells exposed to surplus selenite.

PS P261 - 0391 Mercury Reduces Adipocyte Size and Impairs Lipid and Glucose Metabolism in White Adipose Tissue of Rats

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Toxic effects of mercury (Hg) are involved in homeostasis of energy systems such as lipid and glucose metabolism, and white adipose tissue dysfunction is considered as a central mechanism leading to metabolic disorders. The aim of this study was to determine the effects of chronic inorganic Hg exposure at low doses on the lipid and glycemic metabolism. Male Wistar rats were divided into two groups and treated for 60 days with: saline solution, i.m. (Untreated) and mercury chloride, i.m. - 1st dose 4.6 μ g/kg, subsequent doses 0.07 μ g/kg/day - (Mercury). Histological analyses, Hg levels measurement and GRP78, CHOP, PPAR α , PPAR γ , leptin, adiponectin and CD11 mRNA expressions were performed in epididymal white adipose tissue (eWAT). Glucose, triglycerides, total cholesterol and insulin plasma levels were also measured. Hg exposure reduced the absolute and relative eWAT weights, adipocytes size, plasma insulin levels, glucose tolerance, antioxidant defenses and increased plasma glucose and triglyceride levels. In addition, CHOP, GRP78, PPAR α , PPAR γ , leptin and adiponectin mRNA expressions were increased in Hg-treated animals. No differences in Hg concentration were found in eWAT between untreated and Hg groups. These results suggest that the reduction in adipocytes size is related to the impaired antioxidant defenses, endoplasmic reticulum (ER) stress, the disrupted PPARs and adipokines mRNA expression induced by the metal in eWAT. These disturbances possibly induced a decrease in circulating insulin levels, an imbalance between lipolysis and lipogenesis mechanisms in eWAT, with an increase in fatty acids mobilization, a reduction in glucose uptake and an activation of pro-apoptotic pathways, leading to hyperglycemia and hyperlipidemia. Hg is a powerful environmental WAT disruptor that influences signaling events and impairs metabolic activity and hormonal balance of adipocytes. This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico -CNPq (203440/2014-5) and the Spanish government MICINN (AGL2012-32387, AGL2017-89213, and CSIC -Intramural 2015701028).

PS P262 - 0406 Inhibition of Uptake of Iron by Chronic Exposure to Cadmium

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Cadmium (Cd) is a widespread, harmful heavy metal. The chronic exposure to Cd induces iron deficiency. However, molecular mechanism of Cd-induced iron deficiency remains unclear. In this study, we examined the expressions of iron transport-related genes in the duodenum and iron concentrations in the liver and serum. Female 5-week-old C57BL/6J mice were exposed to 300

ppm Cd via diet for 12 to 21 months. Iron concentrations in the tissues were measured by atomic absorption spectrometry after digestion. Expression levels of iron transport-related genes in the duodenum of mice exposed to Cd were measured using real-time RT-PCR. Cd markedly reduced mRNA levels of heme iron transporter *HCP1* (heme carrier protein 1) and reductase enzyme of non-heme iron (Fe^{3+}) *Dcytb* (Duodenal cytochrome b). The suppression of gene expression of *HCP1* and *Dcytb* indicated that Cd down-regulated iron absorption of both heme and non-heme iron via duodenum epithelial cells, because non-heme iron was absorbed by reduction to Fe^{2+} . On the other hand, Cd had no effects on the mRNA levels of efflux transporter of Fe^{2+} , *Ferroportin 1*, and oxidize enzyme of Fe^{2+} , *Hephaestin*, which exist on the basolateral membrane of duodenum epithelial cells. Cd significantly decreased iron concentration in the liver but not in the serum. In addition, UIBC (unsaturated iron binding capacity) and TIBC (total iron binding capacity) were raised by Cd. These results suggest that chronic exposure to Cd may inhibit uptake of iron in the duodenum and suppress accumulation of iron in the liver.

P263 - 0442 Arsenic Exposure, Gut Microbiome and Human Health

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Arsenic is ubiquitous in nature, highly toxic, and is abundant in Southern Asia. Although many studies have focused on areas like Bangladesh and West Bengal, India, disadvantaged regions within Nepal have also suffered from arsenic contamination levels, with wells and other water sources possessing arsenic contamination over the recommended WHO and EPA limit of 10 ppb, some wells reporting levels as high as 500 ppb. This study aims to be one of the first to examine differential arsenic exposure on the gut microbiome structure within two disadvantaged communities in southern Nepal. Fecal samples ($n = 42$) were collected from members of the Mahuawa ($n = 20$) and Ghanashyampur ($n = 22$) communities in southern Nepal. The 16S rRNA gene was amplified from fecal samples using Illumina-tag PCR and subject to high-throughput sequencing to generate the bacterial community structure of each sample. Bioinformatics analysis and multivariate statistics were used. Our results revealed unique assemblages of arsenic volatilizing and pathogenic bacteria positively correlated with increased arsenic concentration in individuals within the two respective communities. Additionally, we observed that commensal gut bacteria negatively correlated with increased arsenic concentration in the two respective communities. Our study has revealed that arsenic poses a broader human health risk than was previously known. It is influential in shaping the gut microbiome through its enrichment of arsenic volatilizing and pathogenic bacteria and subsequent depletion of gut commensals. This aspect of arsenic has the potential to debilitate healthy humans by contributing to disorders like heart and liver cancers and diabetes, and it has already been shown to contribute to serious diseases and disorders, including skin lesions, gangrene and several types of cancers in disadvantaged areas of the world like Nepal.

P264 - 0482 Distribution, Metabolism, and Excretion of Arsenolipids in Mice after Oral Administration

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It is known that marine organisms and seafood contain arsenic-containing lipids (arsenolipids), and they are sources of daily intake of arsenic in humans. However, very little is known about the metabolism and toxicity of arsenolipids in mammals. Thus, we administrated synthesized AsHC332 ($\text{C}_{17}\text{H}_{37}\text{AsO}$), AsHC360 ($\text{C}_{19}\text{H}_{41}\text{AsO}$), and arsenic-containing fatty acid (AsFA) 418 ($\text{C}_{21}\text{H}_{43}\text{AsO}_3$) orally into mice and compared the tissue distribution, metabolism and excretion of these arsenolipids with those of dimethylarsinic acid (DMAV) and trimethylarsine oxide (TMAO⁺). Groups of five mice were administered orally with olive oil alone (control) or arsenic compounds dissolved in olive oil at a dose of 0.5 mg As/kg body weight. Each animal was housed in a metabolic cage and urine and feces samples were collected for 24 h. Whole blood and tissues were collected 24 h after the administration. Arsenic concentration was measured by inductively coupled plasma mass spectrometry (ICP-MS) after acid digestion. The elemental (As) and molecular speciation of metabolites in urine was carried out by high performance liquid chromatography (HPLC)-ICP-MS and HPLC-electrospray ionization mass spectrometry (ESI)-MS/MS, respectively. Approximately 75 to 94% of the dose were excreted into urine and feces in one day in all the treated groups. The TMAO⁺-treated group, most of the dose was excreted in the urine in one day following administration It

was found that the recovery of arsenic in the brain of AsHC332- and AsHC360-treated groups were approximately 2 and 4 times higher than that of DMAV-treated group, respectively. However, there was no significant difference in the arsenic recovery in the brain between AsFA418- and DMAV-treated group. The higher accumulation of arsenic originating from AsHC332 and AsHC360 in brain suggests that AsHC is more easily transported across the blood-brain barrier than AsFA and DMAV. Results of elemental speciation by HPLC-ICP-MS indicated that all arsenolipids were metabolized into DMAV and several unidentified arsenic compounds in urine. Molecular speciation of unidentified urinary AsHC360 metabolites indicated that at least one metabolite showed electrospray mass spectra (scan mode) at m/z 195 $[\text{M}+\text{H}]^+$. Furthermore, a signal at m/z 177 $[\text{M}-\text{H}_2\text{O}]^+$ was detected at collision energy 5 eV, and signals at m/z 105 (possibly $[(\text{CH}_3)_2\text{As}]^+$) and m/z 73 (possibly $[(\text{CH}_3)_2\text{CH}_2\text{COOH}]^+$) for the product ions were detected at collision energy 15 and 25 eV by HPLC-ESI-MS/MS. Taken together, one of the unidentified arsenic compounds in HPLC-ICP-MS analysis was identified as an oxo-dimethylarsenopropanoic acid ($[(\text{CH}_3)_2\text{As}(\text{O})\text{CH}_2\text{CH}_2\text{COOH}]$; Oxo-DMAV).

P265 - 0489 Cadmium Induces Not Only Apoptotic and Necrotic Cell Death but also Necroptotic Cell Death in Human Aortic Endothelial Cells

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The vascular endothelium covers the luminal surface of blood vessels and a key role in a variety of physiological and pathological processes. Cadmium is a toxic heavy metal and exhibits cytotoxicity in a wide variety of tissues. Vascular endothelium is one of the potential targets of cadmium cytotoxicity, however, the aspects of cell death of cadmium-induced vascular damages are not completely clear. In the present study, we examined the effect of cadmium on cell death modalities in human aortic endothelial cells (HAECs). HAECs were cultured in HuMedia EG2 containing 2% fetal bovine serum. Confluent cultures of HAECs were incubated with cadmium chloride (0-200 μM) for 24 h. After treatment, the cell viability was measured by a cell counting kit-8. Apoptotic and necrotic cells were detected by the annexin V-FITC staining kit and flow cytometer. Cadmium at 100 μM and more significantly decreased the cell viability. Apoptotic cells were significantly increased when the cells were treated with cadmium at 50 and 100 μM , however, this increment was not observed at 150 μM and more. On the other hands, cadmium at 50 μM and more significantly increased the necrotic cells in a dose-dependent manner. We next investigated the involvement of the programmed necrosis (necroptosis) in cadmium-induced cell death revealed by phosphorylation of mixed lineage kinase domain-like pseudokinase (MLKL), one of the necroptosis indicators. In the result, phosphorylation of MLKL was increased by cadmium at 50 and 100 μM . In addition, the decreased cell viability of HAECs by cadmium administration was partly recovered by z-VAD-FMK (100 μM), an apoptosis inhibitor, and necrostatin-1 (100 μM), a necroptosis inhibitor, respectively. Furthermore, double knockdown of receptor interacting protein 1 (RIP1) and RIP3, necroptosis-related factors, diminished the cadmium-induced cell death. Taken together, these results suggest that cadmium induces apoptosis and necrosis in HAECs, and necroptosis is also involved in cadmium-induced vascular endothelial cell death.

P266 - 0522 Development of Fluorescent Probe for Hydrogen Selenide, Important Selenium Metabolic Intermediate

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Selenium (Se) has similar chemical properties to sulfur as a typical element, yet possesses chemical properties characteristic of metal. Based on these properties, we performed the Se studies for its toxicity, kinetics, bioimaging, and development of Se containing-functional molecules, named "chemical metal-omics" studies. Because all inorganic and organic nutritional selenium sources have been deduced to be transformed into the common intermediate hydrogen selenide, chemical tools for visualization of the dynamics of selenide in living cells or tissues are required. In order to investigate the fluorescent behavior of xanthene chromophore, one of the major fluorescent compounds, with selenium functional groups, we have developed "SeMFs." SeMFs worked as fluorescent probes for reactive oxygen species (ROS) *in vivo*. In these probes, we used selenol group as a sensor of ROS. By using selenium as a ROS-sensing motif in a fluorescent probe, it enables us to observe reversible fluorescence that relevant to biological ROS signals. We have designed and synthesized fluorescent

selenium compound "SeMFs," based on the intramolecular spiro-cyclization of selenol group. SeMFs are expected to form a spiro ring in reductive condition due to the high nucleophilicity of selenol group, and the fluorescence is quenched. When SeMFs are oxidized by ROS, the spiro ring form is converted to the quinoid form that shows the strong fluorescence. This redox-induced reversible fluorescence response of SeMFs allowed us to visualize the generation of hypochlorite in stimulated neutrophils. Based on the reaction mechanism described above, we have developed another fluorescent probe for bioimaging of selenide. Our selenide probe is a fluorescent bischromophoric dyad that shows a fluorescence resonance energy transfer, and green fluorescence of an acceptor chromophore is quenched by the intramolecular spiro-cyclization with selenide. Based on this mechanism, our novel fluorescent probe showed the decrease of green fluorescence and increase of blue fluorescence by selenide addition, and this fluorescent change did not occur in addition of the other alkylated selenium compounds and relevant sulfide compounds. This fluorescent probe is expected to be useful for monitoring the dynamics of selenide *in vivo*.

P267 - 0589 The Effects of Whole Life, Low-Dose Cadmium Exposure on High Fat Diet-Induced Metabolic Disease and Its Modulation by Zinc

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Metabolic syndrome (MetS), a group of diseases including diabetes, cardiovascular and liver disease, affects <30% of adults worldwide. Obesity is a risk factor for MetS however not all obese individuals develop MetS, suggesting the contribution of other risk factors, such as environmental toxicants. Some studies have investigated the impact of the heavy metal cadmium (Cd), on the development of MetS; however, most use adult exposure paradigms and do not take into account exposure is often lifelong and multigenerational. Therefore, we developed a model to study the effects of whole life exposure to low dose Cd on the development and progression of high fat diet (HFD)-induced MetS. In addition, the project investigates the impact of dietary zinc (Zn) supplementation and deficiency on disease outcome as both obesity and Cd disrupt Zn homeostasis. Adult male and female C57BL/6J mice fed normal diets (ND) are exposed to 0, 0.5 or 5 ppm Cd-containing drinking water for 1 week before breeding. Pregnant dams and offspring (F₁) are continuously exposed to the same Cd concentration. At weaning, offspring are fed ND or HFD. Male mice are further grouped into diets containing normal, supplemented or deficient Zn. Body weights and water consumption are recorded weekly. Prior to sacrifice changes in body fat composition will be assessed using dual-energy x-ray absorptiometry scan technology. Insulin resistance will be characterized via intraperitoneal glucose tolerance tests, and levels of serum insulin, triglycerides and cholesterol will be tested. Animals will be sacrificed 10 or 24 weeks after weaning. Blood and metabolic organs will be harvested and processed for RNA isolation, metals analysis, histology, immunohistochemistry and western blotting. Thus far, exposure to 5 ppm Cd significantly increased the rate of death after birth, decreased body weight and reduced anogenital distance. Four weeks post weaning, Zn supplementation has restored the body weight in mice exposed to 5 ppm Cd back to control levels and Zn deficiency has decreased body weight compared to control. Overall, results from this study will provide insight into the mechanisms by which whole life, low-dose Cd exposure enhances HFD-induced MetS and discern a potential therapeutic role for Zn in MetS.

P268 - 0605 An Alginate-Yeast-Biocomposite System (AY-BY) for the Simultaneous Biosorption of Cadmium, Lead, and Arsenate from Aqueous Solutions

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Cadmium (Cd²⁺), lead (Pb²⁺) and arsenate (AsO₄³⁻), are highly toxic water pollutants. Chronic exposure to contaminated water with low concentrations of these heavy metals may cause different types of cancer. Biosorption is a promising method for heavy metal removal from contaminated drinking water. It consists in the use of innocuous biological sorbents, like biopolymers from

seaweed and baker's yeast. Alginate, a biopolymer extracted from brown algae *Macrocystis pyrifera*, forms stable hydrogels with potential of heavy metal cations removal, like Cd²⁺ and Pb²⁺. Inactivated baker's yeast, *Saccharomyces cerevisiae*, which also has the ability to remove heavy metals cations, can be modified on its cell surface to promote the biosorption of AsO₄³⁻ through the formation of a biocomposite Fe-yeast. The objective of this work was to design a system (AY-BY) for the simultaneous elimination of Cd²⁺, Pb²⁺ and AsO₄³⁻, from aqueous solutions. For Cd²⁺ and Pb²⁺ biosorption, inactivated *S. cerevisiae* was encapsulated in calcium alginate beads (AY) by extrusion in a semi-continuous Büchi Encapsulator B-395 equipment with CaCl₂ as crosslinking agent. For AsO₄³⁻ biosorption, a Fe-yeast biocomposite was synthesized by chemical crosslinking of iron nanoparticles to yeast cell wall. Nanoparticles were synthesized by alkaline precipitation. Crosslinking to the cell wall of inactivated *S. cerevisiae* was performed using polyethylenimine, carbodiimide and *n*-hydroxysuccinimide. To obtain biocomposite alginate beads (BY), the resulted Fe-yeast biocomposite was encapsulated, as described before. All beads obtained were washed in deionized water, characterized by size, morphology, surface charge and texture stability after 30 days of storage at 4 °C. For heavy metals simultaneous biosorption, both types of synthesized beads were packed in a tandem glass column AY-BY system. It consisted in two interconnected columns, first with AY and a second with BY system. A ternary solution (5 µg/mL of each metal, pH 7.0) and 15 min as residence time were applied. Heavy metals residual concentrations were analyzed by voltammetry in a Metrohm 797 VA Computrace equipment. Resulted removal efficiencies were 98.0 ± 0.2%, 84.1 ± 1.4% and 82.5 ± 3.3%, for Cd²⁺, Pb²⁺ and AsO₄³⁻, respectively. AY beads biosorption capacities for Cd²⁺ and Pb²⁺ were 209.8 ± 0.5 µg/g and 180.1 ± 2.9 µg/g, respectively, while BY beads biosorption capacity was of 176.6 ± 7 µg/g for AsO₄³⁻. In conclusion, AY-BY system was effective to remove Cd²⁺, Pb²⁺ and AsO₄³⁻ simultaneously.

P269 - 0662 Manifestation of Endocrine-Disrupting and Other Effects of Metals in Pregnant Women

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Synthetic endocrine-disrupting chemicals are able to interact with endogenous hormone receptors and produce agonistic and antagonistic effects in humans and other mammals. The effects are estrogenic, androgenic, anti-estrogenic and anti-androgenic; and alter the function and expression of key genes. These alterations lead to adverse cardiovascular, reproductive, developmental and neurological effects as seen in humans and other mammalian systems. Although the well-documented EDC include pesticides, industrial chemicals and substances used in trade and commerce; some metals such as Pb, As, Cd and Hg have been identified to show endocrine-disrupting properties and hence classified as EDCs. The purpose of this study was to evaluate the endocrine and other adverse effects from the individual and combined exposure to EDC metals in pregnant women. We have previously shown that exposure to metals such as Pb, Hg, Cd, As and Mn significantly alter hormones related to inflammation, cytokines, chemokines, interleukins, C-reactive proteins, vascular endothelial growth factors and other biomarkers in pregnant women who participated in the MIREX data in Canada. These women were observed to be at an increased risk of delivering a baby that was small for gestational age, preterm and low birth weight; and were at risk to hypertensive effects and gestational diabetes during pregnancy. By virtue of their EDC properties, these metals tend to induce neurobehavioural toxicity and cognitive deficits resulting in reduction in IQ, learning and attention deficits and hyperactivity. Exposure to these types of metals during early development produced protracted lifelong HPA axis dysregulation. Although exact toxicological mechanism of action of these metals individually and in combination is unclear, the available mechanistic models for cognitive dysfunction and adverse birth outcomes and adverse effects during pregnancy are explored. Both endocrine and general and specific toxicity modes are examined and characterized in this study.

P270 - 0676 Cadmium Induced Nephrotoxicity via Modulating the Mitochondrial Unfolded Protein Response and Nrf2 Mediated Antioxidant Defense

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Cadmium (Cd) is a wide spread heavy metal pollutant in the environment which accumulates slowly in the tissues of living organism including birds. This study intends to reveal Cd-induced nephrotoxicity. In this study, male Hyland

white chickens were treated with 0 mg/kg, 35 mg/kg and 70 mg/kg Cd by oral gavage for 90 days. Histopathological and biochemical indices reflecting Cd-induced the kidney injury. Markedly, Cd exposure induced mitochondrial damage by cristae of mitochondria decreased or disappeared, mitochondrial and nuclear malformation. Cd induced toxicities in mitochondrial, including the expression of mitochondrial function-related genes, mitochondrial unfolded protein response (UPRmt) - related factors (SIRT1, PGC-1 α , TFAM, Nrf1, SIRT3 and HTRA2), mitochondrial division fusion protein (OPA1, MFN1, MFN2, Fis1 and MFF). Meanwhile, impacting on oxidative stress indices (MDA, H₂O₂, CAT, POD, T-AOC, SOD, Cu-Zn SOD). Furthermore, Cd activated Nrf2 signal pathway through upregulating Nrf2 downstream target genes. These results revealed Cd exposure induced oxidative stress and mitochondrial dysfunction by activating the Nrf2 antioxidant defense response and interfering UPRmt in chickens that supported Cd induced nephrotoxicity. *Support: National Natural Science Foundation of China (No. 31572586), Excellent Youth Foundation of Heilongjiang Province of China (No. JC2017005), and China Agriculture Research System (No. CARS-35).*

P270 - 0682 Total Antioxidant Capacity, Total Plasma Peroxides, and Oxidative Stress Indices as Markers of Lead and Cadmium Exposure Toxicity in Auto Technicians in Nigeria

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Occupational exposure to lead (Pb) and cadmium (Cd) is a source of metal toxicity among auto technicians (auto mechanics, panel-beaters, battery-chargers, auto painters) who constitute a large percentage of artisans in Nigeria. Although there have been some reports on oxidative stress in persons occupationally exposed to mixed chemicals; however, while few have narrowed down specifically on auto-technicians, stratification of different subgroup exposure is rare. This study evaluated the antioxidant status in auto technicians routinely exposed to lead and cadmium in Ibadan, the largest city in Nigeria and stratified the result into the major subgroups in this vocation. Sixty-five apparently healthy male (aged 18 to 65 years) were selected based on specific inclusion criteria using structured questionnaire. Thirty-four constituted cases consisting of participants routinely working as auto technicians or apprentices (≥ 2 years) while controls were thirty-one non-occupationally exposed male members of staff/students of the University College Hospital, Ibadan, Nigeria. Cases were further grouped into their subgroups as above. Blood was collected from all participants and analyzed for the presence of lead, total anti-oxidant capacity (TAC), total plasma peroxides (TPP) and oxidative stress index was calculated. Urine samples collected from all participants were analyzed for the presence of urinary lead and cadmium using standard laboratory methods. Although values of TAC in cases (22538 $\pm$ 8726.54) was not statistically different from what was obtained for controls (26741.87 $\pm$ 8696.68), TPP and OSI were statistically higher in cases than in controls (183.88 $\pm$ 53.39 and 120.16 $\pm$ 70.54) and (0.93 $\pm$ 0.45 and 0.49 $\pm$ 0.330.49 $\pm$ 0.33) respectively. The blood lead level in cases (10.11 $\pm$ 4.47) was significantly higher than in controls (7.72 $\pm$ 1.22) while elevated urinary lead and cadmium levels were observed in cases (0.65 $\pm$ 0.21), (0.34 $\pm$ 0.11) compared to controls (0.52 $\pm$ 0.19), (0.27 $\pm$ 0.10) for Pb and Cd respectively. Although accumulation of blood lead was highest in auto mechanics, levels of TPP and OSI was highest in panel beaters compared to other auto technicians. Active lipid peroxidation may be a possible biochemical mechanism of various chronic diseases associated with exposure to these toxic metals by these group of artisans.

P271 - 0682 Withdrawn

P272 - 0766 Effects of an Egg White Hydrolysate on the Development of Hypertension and Vascular Dysfunction after Aluminum Exposure in Rats

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Aluminum (Al) adverse effects have been related with increased oxidative stress and inflammation. We evaluated the effects of an egg white hydrolysate (EWH) on blood pressure and vascular effects after Al exposure. For that, three-month-old male *Wistar* rats were divided into four groups, treated orally for 42 days: C. Control -ultrapure water; Al. AlCl₃ - 100 mg/kg bw; EWH. 1 g/kg/day; EWH+Al. Systolic blood pressure (SBP) was measured by plethysmography. Vascular function was studied in aortic and mesenteric resistance arteries in isolated organ bath and concentration-response curves to acetylcholine and sodium nitroprusside. Vasoconstrictor response to phenylephrine (PHE) in presence/absence of endothelium and in presence of NOS inhibitor (L-NAME), potassium channels blocker, NAD(P)H oxidase (NOX) inhibitor (apocynin), superoxide dismutase, non-selective COX inhibitor (indomethacin), selective COX-2 inhibitor (NS 398). We measured: systemic and vascular reactive oxygen species (ROS), lipid peroxidation and antioxidant capacity, situ vascular O₂⁻ production, NOX activity and the quantitative real time PCR for NOX1 and NOX2. Results were expressed as mean and SEM, compared by t-test and ANOVA followed by Bonferroni test (P<0.05). EWH prevented: Increase SBP after Al exposure (C: 117.2 $\pm$ 1.09; Al: 132.4 $\pm$ 5.20*; EWH: 117.6 $\pm$ 3.12#; EWH+Al: 118.4 $\pm$ 1.93# mmHg, n=8 * vs C; #vs Al); endothelial relaxation dysfunction; increased vasoconstrictor response to PHE; restored endothelium vasoconstrictor -modulation, nitric oxide bioavailability and potassium channels involvement; prevented ROS production (NOX) and contractile prostanooids (COX-2); inhibition ROS production, lipid peroxidation and imbalance on antioxidant capacity; prevented vascular O₂⁻ production and the increased NOX 1 mRNA expressions. As a conclusion, EWH seems to be able to counteract the vascular toxic effects of Al, which points to a possible therapeutic effect based on functional foods against a highly widespread environmental contaminant. *Financial support: CNPq (203440/2014-5), CAPES and MINECO (AGL2012-32387; AGL2017-89213; CSIC Intramural 2015701028).*

P273 - 0782 Involvement of Metallothionein-III in Mercury-Induced Chemokine Gene Expression

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Several mercury compounds are considered hazardous, exerting mainly central nervous system (CNS) or renal damage. Methylmercury is a pollutant that causes severe damage and that accumulates in fish by bioaccumulation. Mercury vapor also produces neuronal damages. On the other hand, metallothionein (MT) is a defense factor against harmful metals by binding to metals. MT-III, which is one of the four MT isoforms and present in the brain, has a protective effect against various brain diseases. Recently, it has been reported that methylmercury increases the expression of several chemokine genes specific to the mouse brain. In this study, we investigated the involvement of MT-III in chemokine gene expression in the cerebrum and cerebellum in response to methylmercury and mercury vapor using the MT-III null mice. No difference in mercury concentration was observed between the wild-type mice and MT-III null mice in any brain tissue examined. The expression of Ccl3 in the cerebrum and of Cxcl10 in the cerebellum was increased by methylmercury in the MT-III null mice but not the wild-type mice. The expression of Ccl7 in the cerebellum was increased by mercury vapor in the MT-III null mice but not the wild-type mice. However, the expression of Ccl12 and Cxcl12 was increased in the cerebrum by methylmercury only in the wild-type mice and the expression of Ccl3 in the cerebellum was increased by mercury vapor only in the wild-type mice. These results indicate that MT-III does not affect mercury accumulation in the brain, but that it affects the expression of some chemokine genes in response to mercury compounds. *Lee et al, Toxics, 2018.*

P274 - 0867 Dietary Intake of Trace Elements for a Population Highly Exposed to Canned Food

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The human exposure to heavy metals and trace elements is still a topic of notable concern for the public health. Their toxicity can affect mental development and central nervous system function, alter the blood composition, and disturb the organic function of the human body. Despite heavy metals reach humans through several exposure pathways of, diet has been largely stated as the most important route of exposure. Heavy metals and trace elements can be present in food due to the natural or anthropogenic environmental contamination, or in the case of canned food, by the migration from the packaging material. Metallic food packaging is mostly composed of tinplate (tin coated steel), chromium coated steel, or aluminium, which is mostly coated on the inner side with a resin to protect food of metal contact. However, when the metal is exposed to the food because of coating damage, corrosion is accelerated and some elements, such as tin (Sn), iron (Fe), cadmium (Cd) and lead (Pb) could be released, increasing their levels in the food. The present study was aimed at performing a duplicate diet study to evaluate the intake and excretion of heavy metals and trace elements during a 2-days diet of canned food. Up to 25 people were divided into two groups who consumed: (1) a diet mostly containing canned food; and (2) a control diet, whose food was fresh or packed in glass containers. On one hand, a portion of the food was homogenised and stored in the laboratory for further analysis. On the other hand, blood and urine samples were collected during the survey. Finally, the total amount of food eaten and the total volume of each micturition were recorded to know the exact amount of heavy metals and trace elements ingested and excreted by each participant. All food and biological samples were analyzed to determine the concentrations of a number of trace elements (As, Be, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Sn, Tl, V and Zn) by means of ICP-MS. These results will be very valuable to estimate the exposure to heavy metals and trace elements, as well as evaluating the potential risk of eating canned food instead of fresh or packed in glass containers. Our findings will be also studied concurrently with data regarding the content of other chemical pollutants, such as bisphenol A, in the same food samples.

P275 - 0872 Do Dysregulated Cellular Energetics Play a Role in Hexavalent Chromium-Induced Human Lung Carcinogenesis?

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Hexavalent chromium, Cr(VI), is a known human carcinogen that is a worldwide environmental health concern. It is established that reactive oxygen species, genomic instability, and DNA damage repair deficiency are important contributors to Cr(VI)-induced carcinogenesis mechanism. Some cancer hallmarks remain understudied for the mechanism of Cr(VI)-induced carcinogenesis. Increased *de novo* lipogenesis and the 'Warburg Effect' are important in the mechanism of carcinogenesis and tumorigenesis in multiple types of cancer. We recently reported that Mitochondrial respiratory dysfunction occurs after tumor development and not during the initial Cr(VI)-induced carcinogenesis. Continuing this work we investigated the changes in lipid metabolism and the 'Warburg Effect' in Cr(VI)-transformed human lung cells. We used three human lung cell lines [human lung epithelial cells (BEAS-2B and BEP2D cells) and human lung fibroblasts (WTHBF-6 cells)]. Our results showed an increased lipogenesis (palmitic acid levels) and Cr(VI) and increased expression of lipogenesis proteins [ATP citrate lyase (ACLY), and fatty acid synthase (FASN) expressions]. Using a drug (C75) for inhibition of FASN, we found that C75 treated Cr(VI)-transformed cells had decreased proliferation and loss of colony formation in soft agar. Interestingly, we also found that Cr(VI)-transformed cells had no major changes in their glycolytic function as measured by the Seahorse Analyzer when compared to their passage-matched control cells and there were no observed increases in Cr(VI)-transformed cells' extracellular L-lactate levels. In conclusion, these data show that Cr(VI)-transformation *in vitro* caused increases in lipid metabolism and this increase is important for Cr(VI)-transformed cell cancer properties. In contrast, Cr(VI)-transformed cells did not exhibit increased anaerobic glycolysis ('Warburg Effect'). Therefore, future work is aimed at elucidating the mechanism that is increasing lipogenesis and not increasing anaerobic glycolysis in Cr(VI)-transformed human lung epithelial cells and lung fibroblasts.

P276 - 0959 Slc30a10-Deficient Mice, a Model of Inherited Manganese Excess, Develop Hepcidin Deficiency and Increased Manganese Absorption

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Manganese (Mn) is an essential metal and nutrient yet toxic in excess. There are several populations at risk of Mn toxicity, most notably miners and welders exposed to Mn-rich fumes and particulates. In 2012, the first known inherited disease of Mn excess was reported in patients with mutations in SLC30A10, a Mn efflux transporter highly expressed in the liver, brain, and duodenum and hypothesized to be essential for Mn excretion. Characterized by increased blood Mn levels, dystonia, polycythemia (increased red blood cell counts), and liver cirrhosis, SLC30A10 deficiency is a novel disease that offers a unique opportunity to investigate systemic Mn regulation. Our overall goal is to establish the role of SLC30A10 in mammalian Mn homeostasis by characterizing global and tissue-specific mouse models of SLC30a10 deficiency (KO). Since SLC30a10 is a Mn efflux transporter highly expressed in the liver, it is hypothesized that tissue Mn excess is a result of impaired hepatobiliary Mn excretion. Data from both global and hepatocyte-specific KO mice support this. However, global KO mice also have aberrant Mn absorption, which is likely to exacerbate tissue Mn overload and disease severity. We hypothesize that excess liver Mn acts as a "hypoxia mimetic" to induce HIF signaling and downstream targets such as erythropoietin (Epo) and hepcidin. Epo regulates the production of red blood cells and is aberrantly expressed in the liver of our global KO mice, likely causing polycythemia. Elevated Epo levels have been associated with hepcidin deficiency. Hepcidin is a hormone produced by the liver that inhibits dietary metal absorption. Our global KO mice are hepcidin-deficient and both iron and manganese absorption are increased following gavage. Absorption rates may also be directly influenced by SLC30a10 expression in the gut. Characterization of an intestinal-specific KO mouse reveals minimal tissue Mn excess, however deficiency in both hepatocyte and intestinal SLC30a10 has a synergistic effect in mice, suggesting that both tissues contribute greatly to Mn homeostasis. Understanding these mechanisms of Mn homeostasis is important for developing pharmacological treatment for both inherited and acquired Mn toxicity.

P277 - 0245 Phytoremediation and Mutual Toxicological Effects of Nickel (Ni²⁺) and Cadmium (Cd²⁺) in Coexistence with Zinc Oxide Nanoparticles Using Sorghum (*S. bicolor*) and Alfalfa (*M. sativa*)

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Nickel (Ni) and Cadmium (Cd) are among the most toxic metals to the human beings, due to all their effects in human health at very low concentrations. Their bioaccumulation in agricultural crops has become more plausible along the year, as a result of all the anthropogenic activities where they can be found. Besides, with the current growth and popularization of the nanotechnology industry, thanks to their wide range of uses, the engineered nanoparticles (ENPs) are bioaccumulating easily in water and soils, becoming bioavailable not only for plants and animals, but also for human beings. The coexistence of both, ENPs and heavy metal is a prominent threat whose effects and interactions are of high interest, as well as their remediation methods. The goals of this study is to determine the mutual effects of Cadmium (II) and Nickel (II) in presence of Zinc Oxide Nanoparticles (ZnONPs) on the uptake and accumulation in hydroponically grown Sorghum (*Sorghum bicolor*) and Alfalfa (*Medicago sativa*), and the capacity of both plants to phytoremediate the mixtures. The Alfalfa and Sorghum seedlings were exposed to treatments of 1 ppm and 5 ppm of Cd (II)/ Ni (II) in presence of 50 ppm and 100 ppm for the ZnONPs. Data of physiological and toxicological effects were taken during the contamination time and finally the plants were digested in order to be analyzed along with the treated water through Atomic Adsorption to determine the contents of Cd, Ni, and Zn. In all treatments, chlorosis, necrosis, and root inhibition were found, although Alfalfa was more resistant to cadmium toxicity while sorghum was more resistant to nickel, both cases presenting fewer physiological effects. Significant interactions between ZnONPs and both heavy metals regarding their toxicity and accumulation were found. While for Ni the ZnONPs had a positive impact on its toxicity, alleviating the biomass reduction in both plants, the Cd had its toxicological effects enhanced by the presence of the NPs at 100 ppm, in fact both Cd and NPs had a synergistic toxicity implying its bioaccumulation in both plants. For cadmium phytoremediation, alfalfa was more efficient due

to its high translocation factor, while for nickel both species behaved similarly remaining with constant translocation factors but significant bioaccumulation factors. For ZnONPs-derived Zn, its accumulation was greater in roots than shoots, meaning the low translocation factor for the NPs, but remarkable bioaccumulation factor. Finally, both plant species are promising in the removal of heavy metal from polluted water sources, as well as for nanoparticle removal.

P278 - 0377 Cigarette Smoke Extract Produces Superoxide in Aqueous Solution by Reacting with Bicarbonate

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Cigarette smoke (CS) contains reactive oxygen species (ROS) and other pro-oxidants. In addition, CS is capable of producing ROS in biological media and stimulating cells to generate ROS. This study was performed to characterize CS-induced ROS production in aqueous solutions for *in vitro* study. Cigarette smoke preparations cigarette smoke extract (CSE) and total particulate matter (TPM) were prepared from the mainstream smoke of 3R4F reference cigarettes. CSE and TPM were added to water, Dulbecco's modified Eagle media (DMEM), Hank's balanced salt solution (HBSS), phosphate-buffered saline, and blood plasma, and superoxide generation was measured using water-soluble tetrazolium salt-1 (WST-1) or electron paramagnetic resonance (EPR) spectroscopy. CSE and TPM produced superoxide only in DMEM and HBSS. In the experiments using each component of HBSS, sodium bicarbonate (NaHCO₃) was found to be responsible for CS-induced superoxide generation. Superoxide production by the reaction between NaHCO₃ and CSE or TPM was abolished by pretreating CSE or TPM with peroxidase. Taken together, CSE and TPM can generate superoxide in aqueous solution containing NaHCO₃, and the substrates of peroxidase such as peroxy acids in CS may be involved in the chemical reactions producing superoxide. These results suggest that HCO₃⁻ may be a determinant of ROS-related CS toxicity in biological environments and the experimental conditions such as compositions of solution should be considered in *in vitro* toxicity study of CS.

P279 - 0441 An Integrated Approach to Evaluate the Environmental Impact of Mixtures of Herbicide Formulations Using Zebrafish Embryonic Stages

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The glyphosate-resistant weeds have greatly enhanced the consumption of glyphosate based-formulations in the crops as well as its association with other herbicides to increase the action efficacy compared to glyphosate alone. Imazethapyr, an imidazolinone herbicide, has been associated with glyphosate-based formulations due to its high activity at low application rates and wide spectrum of weed control. Considering that less than 0.1% of herbicides applied to crops reach their specific targets and can move into different environmental compartments, including water resources, we investigated the embryotoxicity and cytotoxicity of individual and joint formulations of Glyphosate Atanor 48 (ATN) and Imazethapyr Plus Nortox (IMZT) using zebrafish model. For that, zebrafish embryo-larval toxicity test was performed according to OECD 236 (ethical approval UFG N° 102/2014) followed by the cytotoxicity assays using Hoechst 33342, Mitotracker red and acridine orange. ATN and IMZT induced zebrafish larvae mortality with LC50-96h of 76.50 mg/L and 71.60 mg/L, respectively. However, both formulation at concentrations much lower than LC50-96h showed differences in DNA content based on the nuclear intensity when compared with their respective controls upon Hoechst exposure. ATN and IMZT also caused swim bladder disorder with EC50-96h of 26.94 mg/L and 53.87 mg/L, respectively. IMZT still had significant buoyancy or balance problems with EC50-96h of 2.7 mg/L. Additionally, a considerable number of apoptotic cells were observed accumulated in the area of head, yolk sac and tail of zebrafish larvae exposure to low concentrations of ATN and IMZT during analysis with acridine orange. Both herbicides reduced the mitochondrial potential (ATN at 50 mg/L and IMZT from 5 mg/L). The ATN + IMZT mixtures did not induce significant embryotoxicity nor cytotoxicity in the zebrafish. In conclusion, an integrated approach to developmental screening and cytotoxicity using the zebrafish model could be used to assess the impact of herbicides on human and environmental health.

P280 - 0550 Toxicogenomics Analysis of Phthalates and Bisphenol A Mixture: Lung Cancer

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Lung cancer is considered one of the most common malignancies and the leading cause of cancer death worldwide. There is an increase in data suggesting the possibility that endocrine-disrupting chemicals (EDCs), such as phthalates and bisphenol A, can contribute to the development of various cancers, including lung cancer. The aim of this study was to explore the relationship between the exposure to the mixture of three widely used chemicals (diethylhexyl phthalate (DEHP), dibutyl phthalate (DBP) and bisphenol A) and lung cancer development, using the toxicogenomics approach. In this investigation, The Comparative Toxicogenomics Database (CTD; <http://ctd.mdibl.org>) was the main data mining tool. While "Cancer" was among the most curated diseases for DEHP, DBP and BPA on CTD, "Lung neoplasms" were among the inferred diseases for all three substances, meaning that the association was established via curated chemical-gene interactions. Set Analyzer CTD tool, used to list genes affected by the investigated chemicals and connected with the development of lung neoplasms, revealed that DEHP, DBP and BPA interacted with 84, 102 and 241 such genes, respectively. MyVenn CTD tool, used to compare these sets of genes, listed 57 genes common to all 3 substances, while GeneMania predictive server (<http://www.genemania.org>) explored the interaction between these 57 common genes, revealing that the majority of them were in co-expression (54.01%) and physical interactions (27.28%). All three substances interacted with Epidermal Growth Factor Receptor gene (EGFR), widely recognized for its importance in cancer, especially non-small cell lung cancer, as well as Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha gene (PIK3CA). Furthermore, all three substances reacted with Cytochrome P450 Family 1 Subfamily B Member 1 gene (CYP1B1), which tumor-specific transcriptional activation has been found in lung cancer. These results provide a basis for further *in vitro* and *in vivo* investigation in order to better understanding the molecular mechanisms behind the DEHP, DBP and BPA mixture contribution to the lung cancer development.

P281 - 0636 Rat Liver and Kidney Post-mitochondrial Dysfunction by Addition of Chronic Mixed-Metal Intoxication and Hepatorenal Wellness Mediated by Phenolic Components from Croton zambiscus Leaves

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Chronic exposure of mixed-metal intoxication has been associated to prolonged oxidative stress and severe hepatorenal damage. This present study demonstrates the hepatoprotective and renoprotective activity of Croton zambiscus (C-ZAMB) leaves, naturally occurring phenolic compounds against chronic mixed-metal (EOMABRSL) induced toxicity. 0.5ml of EOMABRSL via oral route induced chronic hepatotoxicity and nephrotoxicity at both 98 days (non-withdrawal) and 70 days (withdrawal) by abnormal alteration in the levels of endogenous antioxidants. Moreover, EOMABRSL induced hepatorenal damage by increasing the markers of liver toxicity (ALT, AST, ALP, GGT and bilirubin) and kidney failure (creatinine, urea, uric acid, and renal electrolytes-Na⁺ and K⁺). Both non-withdrawal and withdrawal approaches of EOMABRSL-exposed animals exhibited hepatorenal dysfunctions by increasing the activity of eco-51-nucleotidase (51ENT) followed by the decreased in the activity of lactate dehydrogenase (LDH)-index of cellular ATP. These results were further supported by the histopathological examination of nephritic cells, hepatocytes and splenocytes, manifested by hepatocellular necrosis, swelling or degeneration of tubular kidney epithelial cells as well as coalescing splenic periarterial lymphoid sheaths (PALs) and lymphoid haemosiderin. The chronic EOMABRSL intoxication was ameliorated by administration of phenolic antioxidants from C-ZAMB leaves. Therefore, our study supports the view that phenolic C-ZAMB leaves may mediate hepatorenal wellness on chronic exposure to mixed-metal intoxication

P282 - 0666 Application of Generalized Concentration Addition to Predict Mixture Effects of Glucocorticoid Receptor Ligands

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Environmental exposures often occur in complex mixtures and at low concentrations. There is a need for improved prediction models that evaluate the toxic effect of simultaneous human exposures. Generalized concentration addition (GCA) is a method used to estimate the joint effect of receptor agonists that vary in efficacy. GCA models have been successfully applied to mixtures of aryl hydrocarbon receptor (AhR) and peroxisome proliferator-activated receptor gamma (PPAR γ) ligands, each of which can be modeled as a receptor with a single binding site. One requirement of GCA is specification of the mathematical form for the dose response curves. The glucocorticoid receptor (GR) is a homodimer nuclear receptor that is activated in response to stress and by synthetic glucocorticoids. GR has systemic effects on the endocrine, metabolic, cardiovascular, immune, reproductive, and central nervous systems. Here, we evaluated whether GCA could be applied to homodimer nuclear receptors, which have two binding sites, to predict the combined effect of full GR agonists with partial agonists or competitive antagonists. We measured transcriptional activation of GR using a recently developed cell-based bioassay that contains a stably transfected luciferase reporter gene under the control of three glucocorticoid responsive elements. Individual dose response curves for dexamethasone (full agonist), prednisolone (full agonist), medroxyprogesterone 17-acetate (partial agonist), and mifepristone (antagonist) were generated to predict binary mixture effects of GR ligands using GCA. Comparisons between the empirical data and GCA predictions, along with alternative models, will be presented. This research will expand the application of GCA to homodimer nuclear receptors and improve risk assessment of mixtures that represent cumulative human exposures.

P283 - 0678 Sex-Dependent Effect of a Cocktail of 13 Chemicals on Reproductive System in Rats

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Real life exposure to chemicals represents a hot topic for human health as they represent a risk for human health even at levels near regulatory limits. The current study used a rat model to evaluate long term low dose effects of a cocktail of 13 chemicals mixture on organ cytotoxicity in doses much lower than the regulatory limits in an intention to simulate the real life exposure. 4 groups of 10 Sprague Dawley rats (5 males and 5 females) were treated with 0, 0.25x acceptable daily intake (ADI), ADI and 5xADI doses of mixture of methomyl, triadimefon, dimethoate, glyphosate, carbaryl, methyl parathion, aspartame, benzoic acid, calcium disodium ethylene diamine tetra-acetate (EDTA), ethyl-paraben, butylparaben, bisphenol and acacia gum by drinking water. After 18 months of exposure the animals were sacrificed and testis/uterus cytotoxicity was evaluated by touch prep method. This study was conducted in accordance with the EU Commission Directive 2010/63/EU for animal experiments and approved by the Ethical Committee of University of Medicine and Pharmacy Craiova, Romania. After 18 months of exposure to the cocktail of 13 chemicals a dose dependent cytotoxic effect was observed on testicles. In the control group the testicles showed abundant normal cells. For the low dose group the results showed a mild (10%) reduction in cellularity with no degeneration. In medium dose group testicles presented intermediate reduction (>30%) in cellularity and mild (10%) degenerative changes and in high dose group it was observed a severe reduction (>50%) in cellularity and intense degenerative changes. For uterus no significant changes were found in the low, medium and high dose groups. Long term low dose exposure to a cocktail of 13 chemicals can affect male reproductive system in a dose dependent manner.

P284 - 0701 Low Dose Relative Potency Factors for Non-parallel Dose-Response Curves

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The Relative Potency Factor (RPF) method is a standard toxicological technique for examining the combined effect of mixtures. The use of TEFs for dioxin-like compounds is one notable application. An underlying assumption for strict derivation of RPFs is that isoboles are parallel, negatively sloped straight lines for binary combinations (or planes in higher dimensions), meaning that dose-response curves for individual compounds must be parallel when plotted on a log-dose scale with the same maximal effect (efficacy) and shape, differing only in potency. We have examined mixtures of receptor ligands in situations where the RPF assumption is not met due to differences in maximal effect, i.e., mixtures of full and partial ligands. We developed generalized concentration addition (GCA) as a method for predicting the joint effect of such mixtures where neither RPFs nor concentration addition (CA) can make predictions for all effect levels (RPFs are a special case of CA which is a special case of GCA). We have successfully applied GCA to mixtures of AhR ligands, PPAR γ ligands, and most recently androgen receptor ligands. Mixtures of full and partial agonists following GCA have linear isoboles but they need not be parallel or negatively sloped. However, we show here these GCA models do meet the assumption for RPFs at low doses, i.e., the isoboles have the required shape in this region. Remarkably, the calculated RPF then depends not just on a parameter related to potency but also on a parameter related to efficacy. This result has important implications for mixtures toxicology and risk assessment. We are currently verifying these predictions with laboratory experiments.

P285 - 0015 Pulmonary Toxicity of Organomodified Nanoclay along Its Life Cycle: Integrated Exposure and In Vitro/In Vivo Approaches

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Incorporation of organomodified nanoclays (ONC), montmorillonite coated with different quaternary ammonium compounds (QAC), into organic polymers continues to fuel expansion of nanoclay composite technologies. However, little is known about pulmonary health risks along their life cycle. Here, we characterized physicochemical properties and associated toxicities of several ONCs (Cloisite) across their life cycle including as-produced, breakdown products during nanoclay polypropylene composite (NPC) machining, and incineration by-products. Pristine uncoated nanoclay (UC) exposure elicited increased membrane damage, inflammasome activation, and apoptosis in epithelial and macrophage cells, while preincinerated ONCs primarily exhibited a mixed apoptotic/necrotic phenotype, with potency ranking of Clois30B = Clois25A > Clois93A. Preincinerated nanoclays caused a mixed Th1/Th2/Th17 cytokine inflammation response in naïve macrophages, while incinerated nanoclays had minimal effect. Incinerated ONC by-product exposure caused acute toxicity in macrophages only at high doses. Both UC and Clois30B caused increased proliferation, collagen, and reticular fiber synthesis in fibroblasts. *In vivo* exposure in C57BL/6 mice indicated that pre- and post-incinerated Clois30B caused a low-grade, persistent inflammation with profibrotic signaling at day 28 post-exposure, corroborating our *in vitro* model. Lastly, NPCs with different coatings and percent incorporation were sanded with different sand grits and characterized for dust release. NPCs with greater nanoclay dispersion (4% Clois93A) within the matrix with coarse grit sandpaper released the largest amount of respirable particles compared with less dispersed materials (4% Clois25A), virgin polypropylene, and all composites sanded with fine grit sandpaper. In summary, (1) presence of organic coating and incineration status influences potential mode of action of pulmonary responses and (2) percent inclusion, dispersion of nanoclay, and sandpaper grit influenced composite breakdown and particle size/mass distributions. Linking exposure and physicochemical properties to adverse effects in both *in vitro* and *in vivo* models allows for integrated assessment and information to inform and improve prevention-by-design strategies.

P286 - 0075 The Dispersion State of Carbon Nanomaterials Affects Cellular Responses

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The medical applications of carbon nanomaterials (CNMs), which include carbon nanotubes (CNTs) and carbon nanohorns (CNHs), have recently garnered considerable attention. However, evaluating CNM safety, especially that of CNTs, remains difficult and without consensus. One reason for this is that multi-walled CNTs (MWCNTs) possess various shapes and configurations. During our development of sockets for artificial joints, MWCNTs were observed to become tangled during manufacturing. Some reports have addressed this phenomenon, but the tangled MWCNTs (t-MWCNTs) studied were almost always in an agglomerated state. Accordingly, we studied how the dispersion state of t-MWCNTs affected cellular response, such as cytotoxicity, cellular uptake, and immune responses, using two different cell lines, three different sonicators, and two different dispersants (fetal bovine serum and polysorbate 80). Moreover, we compared the cellular uptake and cytotoxicity of fibrous MWCNTs and spherical CNHs of the same degree of dispersion. The particle diameter of dispersed t-MWCNTs could be broadly classified into two types by the sonicator type: less than 200 nm (200t-MWCNTs) and more than 1,000 nm (1000t-MWCNTs). This difference in diameter affected all cellular responses. Although 200t-MWCNTs did not exhibit cytotoxicity regardless of the dispersant, the 1000t-MWCNTs that were internalized into both cell lines showed dispersant-dependent cytotoxicity, which affected the volume of cellular uptake. Immune responses were unrelated to the cellular uptake of MWCNTs, while 200t-MWCNTs also evoked cytokine secretion. Interestingly, well-dispersed CNHs of 170 nm particle size were internalized into macrophage-like cells and showed cytotoxicity. Our results suggested that the receptors related to cytotoxicity and immune responses differed depending on the aggregation state of MWCNTs and surface modification by dispersants and that the receptors recognized by cells varied according to particle shape. In order to apply CNMs in medical applications, it will be important to determine the carcinogenicity and toxicity of each material and identify the biological responses induced by specific shapes, dispersion states, and particle surface modifications.

P287 - 0078 Enhanced SIRT1 Protein via JNK Pathway in Silica Nanoparticle-Induced Pulmonary Damage

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Silica nanoparticles (SiNPs) cause cellular apoptosis in respiratory system with unclear molecular mechanism. It has been reported that silent mating type information regulation 2 homolog 1 (SIRT1), an NAD⁺-dependent deacetylase, mediated several environmental stimuli-associated pulmonary damage. However, whether SIRT1 reposed to SiNPs exposure and its role in SiNPs-triggered pulmonary toxicity remain unknown. We exposed BEAS-2b cells to SiNPs at different doses and measured cell viability and apoptosis. The results showed that SiNPs induced cell viability loss and apoptosis in a dose-dependent manner. Next, we assessed the SIRT1 expression under SiNPs treatment and found that SiNPs reduced SIRT1 protein rather than mRNA levels in a dose- and time-dependent way. Furthermore, overexpression of SIRT1 through transfection with SIRT1-Flag plasmid attenuated SiNPs-induced apoptosis. We also explored the mechanism behind SiNPs-stimulated lower SIRT1 protein expression. Mechanically, SiNPs enhanced SIRT1 protein degradation, which could be inhibited by JNK inhibitor. *In vivo*, SiNPs downregulated SIRT1 protein expression level and induced apoptosis in mice lung tissue. Overall, our study elucidates a new mechanism in SiNPs-induced pulmonary damage, namely that SiNPs accelerate SIRT1 protein degradation via JNK activation, thereby leading to pulmonary cell apoptosis.

P288 - 0164 Comparing 3D-Lung Cell Models in an Aerosol Exposure System with BaSO₄ Nanoparticles

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The growing use of nanoparticles (NP) in many products of our everyday life is accompanied by the need for reliable tools that support toxicological risk assessment (RA). NP could be emitted from products during various phases of their life cycle (e.g., during waste incineration). To understand possible effects of NP, human lung cell cultures are increasingly being used as models in inhalation toxicology. We are validating a novel cell culture exposure system permitting simultaneous exposure of two plates. Besides the use of tumor cell lines and primary cultures, more complex models, like 3D cocultures or mini-organ cultures (MOC), are getting in focus of research. Our aim is to investigate the additional benefit of such models. Viability of A549 cells, cocultures of peripheral lung cells (PLC) and EA.hy926 cells, as well as MOC were determined by Resazurin-assay. In tumor cells oxidative stress was detected by 2',7'-dichlorofluorescein-diacetate, induction of apoptosis and genotoxicity (phosphorylation of H2A.X) were analyzed using the respective kits on a Merck Millipore "Muse" FACS. Test atmospheres with NP aerosols were generated using a PARI LC Sprint Star nebulizer (Pari, Starnberg, Germany) in a cell culture exposure system from TSE-Systems (Bad Homburg, Germany) with newly designed chambers. Aerosols were characterized using a NanoScan SMPS Nanoparticle Sizer Modell 3910 and an optical particle counter Modell 3330, both from TSI (Aachen, Germany). When A549 cells were exposed to BaSO₄NP in suspension viability decreased in a dose-dependent manner. Exposure to 0.25 mg/cm² for 24 or 72 h significantly decreased viability to 54 or 67 %, respectively. ROS level in cell cultures was increased 2.5-fold when cells were incubated with 0.5 mg/cm² for one hour. After 24 h incubation with 0.05 and 0.5 mg/cm² BaSO₄NP there was a significant increase in the percentage of total apoptotic cells. Significant genotoxicity was found at 0.005 and 0.05 mg/cm² after 24 and 72 h of exposure. We could show that MOC remain viable up 74 days in culture. During the whole time, the tissue was characterized immuno-histochemically. Using the setup described above, we could generate stable aerosols of BaSO₄NP and TiO₂NP with median diameters of 49 and 97 nm and mass concs. for PM10 of 10 mg/m³ and 30 mg/m³, respectively. BaSO₄NP exerted toxic effects in A549 when applied under submerged conditions for 24 or 72 h. These findings make them useful model particles to study particle-mediated toxicity of NP in lung cell cultures. We will investigate the transferability of these results to the different models exposed to NP aerosols, as we could show that we have an appropriate system for such experiments.

P289 - 0203 Reproductive Toxicity of Combusted Diesel Additive Containing Nano-ceria: In Vitro Approach

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Cerium dioxide nanoparticles (CeO₂NPs) are widely used in diesel additives. Following their use, CeO₂NPs could be released in the environment as a result of the combustion of diesel fuel. During combustion, the physicochemical properties drastically change (size, shape, kinetics of dissolution, aggregation state), which highlights the need of a better understanding of their toxic potential at each stage of the CeO₂NPs life cycle. The potential toxicity of combusted CeO₂NPs is still poorly investigated, particularly on reproduction. The OECD considers the evaluation of CeO₂NPs potential toxicity as a priority. The aim of the study was to analyze the genotoxic impact of combusted CeO₂NPs on mature male and female rat germ cells and on human sperm exposed *in vitro* to very low concentrations [1 to 1.103 µg.I⁻¹] dissolved in FertiCult medium. Female rat cells were sampled after stimulated ovulation and rat sperm by epididymis dissection. Human frozen sperm from fertile donors were purchased from Germethèque biobank (France). Cells were incubated with combusted CeO₂NPs during 2 hours at 37°C, 5% CO₂, before analysis of DNA damage by alkaline comet assay (ACA) (Olive Tail Moment [OTM] in oocytes and follicle cells, % Tail DNA in sperm). The analysis of chemical (un)stability of combusted CeO₂NPs in the medium using ICP-MS showed that the concentrations of dissolved Ce were below the detection limits (<2.10-4 µg.I⁻¹) whatever the exposure concentration. ACA showed significantly increased DNA damage in

rat oocytes and follicle cells exposed to $1 \mu\text{g}\cdot\text{L}^{-1}$ CeO_2 NPs (mean $\pm$ SEM OTM = 10.26 ± 0.36 and 4.91 ± 0.29 , respectively) compared with unexposed controls (2.21 ± 0.18 and 0.72 ± 0.05 , respectively) and higher concentrations ($p < 0.0001$). In rat sperm, exposure to $1 \mu\text{g}\cdot\text{L}^{-1}$ CeO_2 NPs induced significantly higher DNA damage (mean % Tail DNA $\pm$ SEM = 21.23 ± 0.37) compared with the unexposed control (11.06 ± 0.24) and higher concentrations ($p < 0.0001$). Human sperm exposed to $1 \mu\text{g}\cdot\text{L}^{-1}$ CeO_2 NPs also showed significantly higher damage (mean % Tail DNA $\pm$ SEM = 32.06 ± 0.54) compared with the unexposed control (11.96 ± 0.30) ($p < 0.0001$). Since DNA damage was higher in the lower concentration, we might hypothesize that our results are related to the different aggregation states. At the highest concentrations, CeO_2 NPs could easily form aggregates (reducing the possible interaction with cells), while at low concentrations, aggregation should be reduced (thus allowing a higher interaction with cells).

P290 - 0205 Comparative Mouse Lung Injury by Nickel Nanoparticles with Differential Surface Modification

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Previous studies have demonstrated that exposure to nickel nanoparticles (Nano-Ni) causes oxidative stress and severe, persistent lung inflammation. However, few studies have investigated whether surface modification of Nano-Ni could alter Nano-Ni-induced lung injury, inflammation, and fibrosis *in vivo*. Here, we propose that modification of Nano-Ni surface may change Nano-Ni-induced lung toxicity. Our dose- and time-response studies showed that exposure of mice to Nano-Ni resulted in a dose-response increase in acute lung inflammation and injury reflected by increased neutrophil count, CXCL1/KC level, LDH activity, and concentration of total protein in the BALF. Nano-Ni-induced acute lung inflammation and injury appeared as early as day 1, peaked at day 3, and attenuated over the time. However, their levels were still higher than those of the controls even at day 42 post-exposure. We chose a dose of $50 \mu\text{g}$ per mouse of Nano-Ni, and day 3 post-exposure as short-term and day 42 post-exposure as long-term to compare the effects of Nano-Ni, Nano-Ni-P (partially [O]-passivated), and Nano-Ni-C (carbon-coated) on mouse lungs. At day 3 post-exposure, $50 \mu\text{g}$ per mouse of Nano-Ni caused acute lung inflammation and injury that were reflected by increased cellular and biochemical parameters in the BALF. Histologically, infiltration of large numbers of neutrophils and macrophages in the alveolar space and interstitial tissues were observed in mouse lungs exposed to Nano-Ni. Nano-Ni-P exposure caused similar acute lung inflammation and injury as Nano-Ni. However, exposure to Nano-Ni-C only caused mild acute lung inflammation and injury. At day 42 post-exposure, Nano-Ni caused extensive interstitial fibrosis and chronic inflammation. Lung fibrosis was also found in Nano-Ni-P-exposed lungs, but to a much lesser degree. Only slight or no lung fibrosis were observed in Nano-Ni-C-exposed lungs. Nano-Ni and Nano-Ni-P, but not Nano-Ni-C, caused significantly elevated levels of TBARS in lungs and 8-OHdG in genomic DNA, suggesting Nano-Ni and Nano-Ni-P induce lipid peroxidation and oxidative DNA damage in lungs, while Nano-Ni-C does not. Our results demonstrate that short-term Nano-Ni exposure causes acute lung inflammation and injury, while long-term exposure causes chronic lung inflammation and fibrosis. Surface modification of Nano-Ni alleviates Nano-Ni-induced pulmonary effects; partially passivated Nano-Ni causes similar effects as Nano-Ni, but the chronic inflammation and fibrosis are at a much lesser degree. Carbon coating significantly alleviates Nano-Ni-induced acute and chronic lung inflammation and injury.

P291 - 0208 Amorphous Silica Nanoparticles Would Worsen Their Hepatic Damage through the Acquired Immune System

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In recent years, the usage of nanoparticles has been expanding in various industries because of their innovative functions. For example, amorphous silica nanoparticles (nSPs) are the most widely used in our daily necessities such as foods, cosmetics, and medicine. On the other hand, reduction of particle size could occur the differences in their kinetics compared with conventional materials. Thus, their unexpected biological effects due to their differences have been concerned. In this regard, we previously revealed that mice pretreated

with silver nanoparticles, but not with the silver ions, developed allergic inflammation in response to the silver. The results suggest that nanoparticles have a possibility to induce unpredictable biological effects through the acquired immunity. Some metallic nanoparticles showed hazards through the acquired immunity; however, there is little information whether nonmetallic nanoparticles pose that hazard. Here, we tried to assess the effects of non-metallic nanoparticles on the acquired immunity. BALB/c and C.B-17/SCID mice were ear-intradermally pretreated with PBS or nSPs with 50 nm in diameter (nSP50; Micromod Partikeltechnologie, Germany) once every week for a month, a total of 4 times. One week after last pretreatment, each mouse was injected with nSP50 intravenously to induce the acute toxicity. At 6 hours after injection, we evaluated the effects of nSP50-pretreatment on hepatic damages based on hepatic damage markers, ALT and AST. In the cell depletion experiment, one week after last pretreatment with nSP50, mice were treated with anti-mouse CD8 antibody intraperitoneally to remove cytotoxic T lymphocytes. The plasma levels of ALT and AST in nSP50-pretreated mice were significantly increased compared with those in PBS-pretreated mice. On the other hand, in immunodeficient C.B-17/SCID mice, there were no significant differences in the levels of ALT and AST between the nSP50- and the PBS-pretreated mice. These results suggest that nSP50-inducible hepatic damages would worsen by pretreatment of themselves through the acquired immunity. Furthermore, in the cell depletion experiment, the plasma levels of ALT and AST in mice treated with anti-mouse CD8 antibody were much lower than those in mice treated with isotype control antibody. This indicates that the cell-mediated immunity plays a crucial role in the exacerbation of nSP50-inducible hepatic damage. Now, we try to elucidate the mechanism by focusing on activation of cytotoxic T lymphocytes and gather information for optimal design of nanoparticles to achieve sustainable usage of nanoparticles.

P292 - 0231 Differential Nanoceria Distribution and Effects in Th1- and Th2-Prone Mice

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Nanomaterials are cleared into the mononuclear phagocytic system. Immune status was shown to differentially affect 300 nm PEG hydrogel and 30 nm carboxylate-coated quantum dot nanoparticle clearance in Th1- (C57BL/6) and Th2- (BALB/c) prone mice (Jones et al, JCI 2013). The objective was to assess the effects of immune status on nanoceria organ and cellular uptake, its bioprocessing, and cellular and oxidative stress effects. Female, 7 to 8 week old, C57BL/6 and BALB/c mice were given an i.p. injection of 10 mg/kg citrate-coated nanoceria in iso-osmotic citrate at pH 7.4 (4 nm primary particles, 14 nm hydrodynamic diameter) or vehicle. They were terminated 0.5, 1, 3, 6, or 24 h later and brain, lung, heart, liver, spleen, kidney, and blood plasma collected. Preliminary findings revealed the following: Nanoceria increased cerium, determined by ICP-MS, more in the spleen than the liver, consistent with previous findings, and more in C57BL/6 than BALB/c mice. Light microscopy of toluidine blue stained sections revealed greater vacuolation in the liver of C57BL/6 than BALB/c mice and a nanoceria-induced decrease 3 to 6 h post-dosing in C57BL/6 mice and 1 to 3 h post-dosing in BALB/c mice. Within 24 h HRTEM showed that some nanoceria was bioprocessed in the liver to form nanoneedles. Electron energy loss spectroscopy and energy dispersive X-ray analysis revealed their composition to include cerium and phosphate. They were identical in form, shape, and size to nanoneedles seen in human monocyte-derived macrophages and murine (RAW 264.7) macrophages polarized to M1 and M2 phenotypes. Copious amounts of biomineralized iron nanoparticles within ferritin (Fe-ferritin NPs) surrounded the intact nanoceria and the *in situ* formed cerium phosphate nanoneedles, which were predominantly within organelles, presumably phagolysosomes. The Fe-ferritin NPs, nanoceria, and nanoneedle intra-organelle distribution differed between the Th1- and Th2-prone mice livers. Caspase-1 was elevated in the liver (more in BALB/c than C57BL/6 mice), and protein carbonyls were elevated in the liver of both strains, suggesting inflammasome pathway activation. The results suggest an immune-status influence on nanoceria pharmacokinetics and dynamics. Support: NIH R01GM109195.

P293 - 0234 Remodeling of the Cytoskeleton and Generation of Vacuoles-Like Structures Caused by Tin Dioxide Nanoparticles in Murine Macrophages

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With the advance of nanotechnology, the production of nanostructures around the world has increased. One of the emerging nanomaterials is tin dioxide nanoparticles, which are used in solid-state gas sensors, transparent conductive glasses, lithium batteries, and electrodes because the band-gap of tin dioxide seems to offer more advantages than other semiconductor metal oxides such as titanium dioxide. However, there is a latent risk in the workers who synthesize these nanoparticles because they are exposed by inhalation in the occupational environments; therefore has been taken care in evaluating it is toxicity in the respiratory tract. However, the evidence about tin dioxide toxicity in different cells is poor, while its production every year is increasing. Therefore, the aim was to evaluate the effects of tin dioxide nanoparticles and titanium dioxide nanoparticles in human lung epithelial cells (A549) and murine macrophages (RAW 264.7). The characterization of tin dioxide nanoparticles and titanium dioxide nanoparticles was performed to observe the shape, primary size, hydrodynamic diameter, and zeta potential. On the other hand, human lung epithelial cells and murine macrophages were cultured and exposed to different concentration of nanoparticles and processed for observed ultrastructure, granularity, cell size, cytoskeleton arrangement, murine macrophages activation, and cytokines production. Both nanoparticles were amorphous and less than 100 nm, but in culture medium, tin dioxide nanoparticles and titanium dioxide nanoparticles form agglomerates of ~248 nm and ~427 nm, respectively, with a similar zeta potential (~8 mV). On the other hand, murine macrophages internalized more tin dioxide nanoparticles than human lung epithelial cells; therefore, the more effects adverse were found in RAW 264.7 cells, with an increase of granularity and decrease in cell size. Furthermore, tin dioxide nanoparticles cause the remodulation of actin filaments and microtubules and induced the generation of vacuoles-like structures that cover the entire cytoplasm. For the case of the activation molecules in murine macrophages, tin dioxide nanoparticles induce the overexpression of CD86 and IL-10 production, but the effects were discrete. Apparently, the effects adverse between tin dioxide nanoparticles and titanium dioxide nanoparticles are different in human lung epithelial cells but similar in murine macrophages, where the generation of vacuoles-like structures was evident; therefore, it is necessary to know the consequences of generation of these structures on cellular functions.

P294 - 0263 Nanoparticle Interactions with the Mitochondrial Genome in Primary Human Bronchial Epithelial Cells

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The mitochondrial genome is a small, 16 kbp loop of DNA which encodes proteins vital for electron transport and the maintenance of eukaryotic life. However, its position outside of the nucleus renders it uniquely vulnerable to exogenous insult, and recent research has only begun to illuminate the role of mitochondrial DNA (mtDNA) damage in environmental stress. Nanomaterials, a relatively new class of materials engineered for useful properties derived from their small size, may pose a unique threat to the mitochondrial genome due to their reported ability to penetrate the plasma membrane and enter the cytoplasm. Silver, carbon nanotubes, and cerium dioxide (ceria) are among the most widely used nanomaterials and have the greatest potential for human exposure by inhalation. In this study, we investigated the potential of these materials to impair mitochondrial gene expression and function in human bronchial epithelial cells (BECs). Primary BECs derived from bronchoscopy of human donors were exposed to sub-cytotoxic doses of nano-silver (nAg 1 ppm), multi-walled carbon nanotubes (MWCNT 3 µg/ml), or nano-ceria (CeO₂ 5 µg/ml) for 5 days and assessed changes in expression of all 14 mitochondrial genes, heteroplasmies, insertion/deletions, and mitochondrial copy number. Exposed cells were also measured for metabolic function using Seahorse XF, cytotoxicity,

and mitochondrial abundance. Ultrastructural imaging verified the presence of nanomaterials free-floating in cytoplasm and penetrating the mitochondrial lumen. We found that MWCNT exposure caused an upregulation of mitochondrial genes and a significant decrease in oxygen consumption rate not seen in exposures to nAg or CeO₂. Heteroplasmy and insertion/deletion mutations were not significantly affected by any treatment. However, MWCNTs did induce mitophagy in BECs, as measured by confocal microscopy of mitophagosomes. We conclude that carbon nanotubes may result in mitochondrial dysfunction in exposed BECs via a mechanism unrelated to its reported genotoxicity.

P295 - 0289 Repeated-Dose 28-Day and 90-Day Oral Toxicity of Titanium Dioxide Nanoparticles in Rats

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These days, nanotechnology have become an indispensable techniques which are useful and effective in many areas. As a result, the various nanoparticles are widely used in cosmetic, sunscreen, food packaging, and medicine, etc. However, they can result in safety risks of human health because of their nanotoxicity properties. Therefore, the toxicity test of nanoparticles is positively necessary to recognize how dangerous they are. In this study, we investigated the NOAEL (No Observed Adverse Effect Level) of repeated-dose oral toxicity when the test substance, Titanium dioxide nanoparticles (TiO₂ NPs), was administered to Sprague-Dawley rats for 28 days and 90 days. And then the tested animals were allowed the recovery period during 28 days. The dose levels of groups were set as three levels including 250 (low-dose), 500 (middle-dose), and 1,000 (high-dose) mg/kg/day and they were compared with the vehicle control group. During the treatment period, animals were observed for mortalities, clinical signs (daily clinical signs, and weekly detailed clinical observation), functional observation battery, weekly body weights, food and water consumption, ophthalmological examination, and urinalysis. After termination of treatment, clinical pathology (hematology, blood coagulation time, and serum biochemistry), necropsy (organ weights measurement and gross findings), and histopathological examination were examined. In conclusion, there were no systemic toxicological effects related with the test substance, TiO₂ NPs, in the repeated dose 90-day oral toxicity and 28-day recovery study in Sprague-Dawley rats. Therefore, it is determined that NOAEL of the test substance was found to be 1,000 mg/kg/day and target organs were not detected.

P296 - 0305 Effects of Nanoparticle Core on Cellular Activities under the Controlled Shape, Size, and Surface Chemistries

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Nanotoxicity depends on various properties of nanoparticles. Nanoparticle size, surface chemistry, and shape are known to affect cell activities. However, whether the core material of a nanoparticle also plays a role remains a long-standing question in the field of nano-bio interactions. Here, we studied a combinatorial library of 36 nanoparticles and showed that cytotoxicity depended on the ability of the core material to regulate reactive oxygen species in cells. Three types of core materials (Au, Pt, and Pd) in two sizes (6 and 26 nm), each conjugated with one of six different types of surface ligand were examined. We show that core material affects the hydrophobicity of a nanoparticle and its subsequent uptake by cells. Furthermore, different core material induced varying levels of free radicals and H₂O₂ reduction activities, resulting in different levels of cytotoxicity. Our results show that nanoparticle core material is as important as surface chemistry in nanoparticle-cell interactions, making them a necessary consideration when designing nanomedicines.

P297 - 0307 Effects of Antioxidant and Inhibitor of γ -Glutamylcysteine Synthetase on Acute Toxicity of Silver Nanoparticles Intraperitoneally Administered in BALB/c Mice

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Silver nanoparticles (AgNPs) are, largely due to their antimicrobial properties, one of the most commonly used nanomaterials in consumer products such as cosmetics, clothing, household products, and even in food products and biomedical purposes, including drug delivery and tumor targeting applications. Although reports of human exposure via several routes have been rapidly increasing owing to increases in the manufacture and utilization of AgNPs, few reports exist on the acute toxicity associated with different sizes of AgNPs. Recently, we reported that intraperitoneally administered AgNPs in 10 nm diameter showed acute toxicity in BALB/c mice, e.g., reduced activity and piloerection at 5 hours post-administration, lowered body temperatures at 6 hours post-administration, and death within 24 hours post-administration with histopathological lesions mainly in liver, but the same amount of AgNPs in 60 and 100 nm diameter did not. This study aimed to evaluate the effects of antioxidants on acute toxicity of intraperitoneally administered AgNPs in BALB/c mice. One hour after the administration of N-acetyl-L-cysteine (NAC, 2000 mg/kg bw, i.g.), Vitamin C (200 mg/kg bw, i.g.), Vitamin E (100 mg/kg bw, i.g.) as antioxidant or L-buthionine- (S,R)-sulfoximine (1.6 g/kg bw, i.p.) as an irreversible inhibitor of γ -glutamylcysteine synthetase, 10 nm AgNPs (0.2 mg/mouse) was intraperitoneally administered to 7-week-old female BALB/c mice (5 mice/group) and then sacrificed 6 and 24 hours after AgNPs treatment. In biochemistry, glucose was increased in all AgNPs treated groups, except for NAC + AgNPs group. In histopathology, the lesions observed in AgNPs only group, congestion, vacuolation in the liver, increased cellular component in the liver sinusoid, and apoptosis in white pulp of the spleen were found at 6 hours, while these lesions were eliminated by NAC pre-treatment but not by others. These lesions were remained until 24 hours. Acute toxicity of intraperitoneally administered 10 nm AgNPs was reduced by only NAC of pre-treated antioxidant.

P298 - 0317 The Effects of Exogenous Nanoparticles on Neurological Disorders and Evaluation of Its Induction Mechanism Based on ADMET Analysis

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Recent epidemiological studies demonstrated that agglutination of particulate matter and an increase in the expression of Amyloid β (A β) in human brains living in areas with high concentration of fine particles in the environment. Thus, it is suggested that the association between the exposure of exogenous fine particles and the onset / deterioration of neurological diseases. While concerns of fine particles are being raised on the central nervous system, it is reported that nanoparticles, artificial fine particles being applied in various fields, might directly migrate from the nasal cavity to the brain. However, information about nanoparticles-induced biological effects on central nervous system is hardly understood. Thus, it is urgently needed to evaluate whether these exogenous fine particles are involved in the onset and deterioration of neurological diseases. Here, we tried to elucidate the influence of silver nanoparticles, which are representative artificial fine particles, on A β expression. In order to evaluate the effect of silver nanoparticles with diameter of 10 nm (nAg10) on A β expression, SH-SY5Y, human neuroblast cell line, was treated with nAg10 for 72 hours. After the treatment, the level of A β 40 and A β 42 in the culture supernatant was assessed. As a result, both A β 40 and A β 42 were increased in dose dependent manner in nAg10-treated group compared to control group. In addition, western blotting showed that increase of the expression level of APP, a precursor of A β , in nAg10-treated group. Then, to elucidate its induction mechanism, the association with nAg10-induced elevation of APP and oxidative stress or inflammatory responses was assessed. As a result, antioxidant, N-Acetylcysteine, treatment suppressed the increase of APP expression induced by nAg10. Next, the association with nAg10-induced elevation of APP and the uptake of nAg10 into the cells was assessed. A cellular uptake inhibitor revealed that their pretreatment suppressed the increase of APP expression induced by nAg10. Moreover, when analyzed the amount of transition of silver nanoparticles to mitochondria which is the main production site of oxidative stress, much silver amount was detected as compared with the control group of silver ion. These results suggest that nAg10 increased expression of APP through activation of oxidative stress or inflammatory responses after being taken up into cells. Now,

we are working on elucidating the detailed mechanism by investigating the dysfunction to mitochondria caused by nAg10 and the accompanying oxidative stress production.

P299 - 0321 High Resolution Melt (HRM) in RT-qPCR to Detect the Presence of Gold Nanoparticles (AuNPs) in RNA/DNA Samples

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Studies used to determine cytotoxicity of engineered nanomaterials (ENMs) may be biased by possible interference caused by ENMs, i.e. during the test procedure and analyses. The results reported herein highlight the novel application of high resolution melt (HRM) in RT-qPCR to detect the presence of gold nanoparticles (AuNPs) in RNA/cDNA samples used as starting material. It is possible to detect subtle and hard to distinguish genetic variations by HRM. Briefly, a universal RNA standard was used as an untreated/control sample and was spiked with known amounts of AuNPs. This treatment mimics exposure assessment studies since intracellular residual ENMs may be present in cellular samples, either during or after isolation/purification procedures. These samples were reverse transcribed and 10 reference genes were amplified. The results obtained included amplification plots, dissociation assay profiles, electrophoretic profiles, and HRM difference curves. The clustering feature of the analysis software was used to identify differences caused by the AuNPs. A concentration-dependent alteration in the amplification of the nucleic acid template was observed for 3 of the genes tested. The results were semi-quantitative, where the HRM profiles differed according to the amount of AuNP present. The experiment was repeated to determine if the assay interference was more specific to a particular step within the procedure. It was shown that interference occurred more readily during reverse transcription of RNA than during amplification of DNA. AuNPs, thus, alter the RT-qPCR reaction dynamics, which implies that toxicity studies using this gene expression method must be properly verified for each nanomaterial. The results are being investigated further to develop a diagnostic tool, i.e., where one can screen samples for AuNP interference of the reference gene, whilst at the same time quantifying the gene expression of a target gene. This is an important quality control procedure that should be implemented when using potentially compromised RNA/DNA to quantify the expression of a particular target gene. Lastly, this report describes a method that can be further developed for biological or environmental samples that have been in contact with various ENMs.

P300 - 0361 Development and Evaluation of Analysis System for the Simultaneous Quantification and Physical Property Analyses of Nanoparticles in Biological Samples Using Single Particle Inductively Coupled Plasma-Mass Spectrometry

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Recent progress in nanotechnology has accelerated the development of engineered nanoparticles (ENPs) that are smaller than 100 nm. Due to their beneficial properties such as enhanced tissue permeability compared to other micro or larger sized materials, ENPs are widely used in various products including foods, cosmetics and medicines. The prevalent use of engineered nanoparticles (ENPs) has increased our exposure to these particles. The current available analytical techniques fail to simultaneously quantify and analyze the physical properties of ENPs in biological tissues. Therefore, new methods are required to evaluate the exposure condition to ENPs. Single particle-inductively coupled plasma mass spectrometry (sp-ICP-MS) is an attractive approach that can perform both quantitative and qualitative analyses of ENPs. However, the application of this approach is limited due to the lack of pretreatment methods that effectively recover the ENPs from biological tissues without altering their physical properties. In this study, we evaluated various pretreatment methods and identified the optimal pretreatment conditions for sp-ICP-MS analyses of ENPs in biological tissues using silver nanoparticles (nAg) as a model. We screened five reagents as pretreatment solvents (sodium hydroxide, tetramethylammonium hydride, nitric acid, hydrochloric acid, and proteinase K). Our results showed that treatment with sodium hydroxide is the optimal pretreatment method for detecting nAgs in mouse liver tissues. Moreover, this

pretreatment method could be applied to other organs such as heart, lung, spleen, and kidney. Finally, we evaluated the applicability of the method by analyzing the quantity and physical properties of silver in mouse blood and liver, after intravenous administration of nAg or silver ion (Ag⁺). Our sp-ICP-MS method revealed that nAg administered into the blood was partially ionized and distributed in the particle form (approximately 80%) in the liver and in ionic form (approximately 95%) in the blood. In conclusion, we optimized pretreatment strategies for sp-ICP-MS evaluation of ENPs in biological tissues and demonstrated its applicability by evaluating the changes in physical properties of nAg in the liver and blood. We also showed that the partial change from the particle form to the ionic form of nAg particles could influence their kinetics and distribution when administered to mice. This technique could contribute to risk analysis of ENPs by enabling evaluation of the ENP exposure conditions, elucidating the biological responses to ENPs, and identifying the mechanisms underlying the responses.

PS P301 - 0378 Titanium Dioxide Nanoparticles Disrupted Cytoskeletons and Autophagy in Germ Cell Lines and Disturbed Glucose Homeostasis in Pregnant Rats

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Titanium dioxide nanoparticles (TiO₂ NPs) are widely used or found in varying products such as sunscreen, paint, food, and medicine. In this context, common population could be exposed to them unavoidably. Plenty studies have tested the toxicity of TiO₂ NPs. As important supplement to the safety assessment, data concerning the reproductive toxicity is relatively limited. Here we evaluated the effects of TiO₂ NPs on reproduction both *in vivo* and *in vitro*. Three germ cell lines (GC-2, TM4 and HTR-8/SVneo) were treated with TiO₂ NPs. Apoptosis, autophagy, migration and integrity of microtubules and microfilaments were examined. Results showed that TiO₂ NPs disrupted cytoskeletons and induced cell apoptosis in both GC-2 and TM4 cells via different mechanisms. Migration and autophagy of HTR cells were damaged and expression of miR-96-5p and miR-101-3p were increased by TiO₂ NPs. Rescue experiments indicated that corresponding microRNA inhibitors reversed impairment of migration and autophagy. Since TiO₂ NPs exhibited reproductive toxicity *in vitro*, we further explore their effects in a rodent model. Pregnant Sprague Dawley rats were exposed to TiO₂ NPs by gavage, and the rats' fasting blood glucose levels significantly increased at gestation day (GD) 10 (P<0.05) and GD 17 (P<0.01) after exposure. 16S rRNA sequencing was performed to profile fecal microbiome. Significant changes in gut microbiota was observed in treated rats. PICRUST predicted the bacterial functional activities, and found that the type 2 diabetes mellitus-related gene abundance was changed. Our results collectively suggested that exposure of TiO₂ could be a threat to reproductive health. Future studies are warranted to confirm these findings in human beings.

PS P302 - 0393 Ubiquitin C-Terminal Hydrolase L1 Mitigates Neurotoxicity Induced by Zinc Oxide Particles through Regulation of NF-κB Signaling

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In this study, we aimed to determine the toxic effects of zinc oxide (ZnO) particles with different diameters on DA neurons, the role of ubiquitin C-terminal hydrolase L1 (UCH-L1) for the ZnO particles-induced neurotoxicity and corresponding molecular mechanisms. *In vitro* cell injury model for DA neurons was constructed for analyzing the cytotoxicity of ZnO particles by using SH-SY5Y cell line. Activation of NF-κB signaling pathway was assessed by using flow cytometry and western blotting. Following adenovirus transfection, it was found that the cytotoxicity of ZnO particles was affected by particle size, time and dose of exposure. Meanwhile, the toxicity of ZnO particles with 50 nm or 100 nm diameter was stronger than that of ZnO particles with 1000 nm diameter. Furthermore, ZnO particles exposure resulted in a significant decrease in UCH-L1 expression in SH-SY5Y; while UCH-L1 overexpression led to significantly increased cell viability and sharply decreased ROS level. Accordingly, exposure of ZnO particles with different diameters all activate the NF-κB signaling in SH-SY5Y cells; while UCH-L1 over-expression resulted in increased level of IκBα, an endogenous inhibitor of NF-κB signaling pathway. These results suggested that ZnO particles with different diameters all induced cytotoxicity in DA neurons, which may be related to the free Zn²⁺ in the suspension. Regarding to neuro-

toxic effect of ZnO particles, UCH-L1 protects against and/or alleviates neuronal damages, possibly by regulating deubiquitination of endogenous inhibitor IκBα, which leads to the activation of NF-κB signaling pathway. Therefore, one possible mechanism for ZnO particles-induced neurotoxicity may be mediated by down-regulation of UCH-L1 expression in DA cells.

PS P303 - 0411 Zebrafish, a Multi-Endpoint Approach for Nanoecotoxicology: Does Shape Matter?

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Gold nanoparticles (nAu) are used as drug delivery vectors based on the assumption that they have a low toxicity. The major clinical interest is within the application of novel drug delivery systems. Environmental exposure to nanoparticles can occur in different stages of the lifecycle of the product; from their synthesis, applications, product weathering and their disposal. Zebrafish allow for multi-endpoint assessments where using non-invasive assessments changes in development, embryo activity, growth rates, cardiac output and swimming behaviour within the same organism throughout their larval stages can be measured. As technology has evolved so have the techniques we use to measure phenotypic changes. By using an adverse outcomes pathway approach we are able to use behavior as an early warning sign of contaminant effect. Changes in animal behavior reflect processes occurring at the biochemical and physiological level which manifest to the point of ecological relevance. The principles of the fish embryo acute toxicity test (OECD 236) were employed over a period of 96 h and additional measurements were taken at relevant 24 h time intervals. Video recordings were taken using an Olympus stereomicroscope and analyzed using Noldus DanioScope software. Swimming behavior was assessed at the conclusion of the test at 5 dpf using a Noldus DanioVision chamber which included a startle response test. Spherical and rod shaped gold nanomaterials (14 nm) of the same citrate capping showed magnitude differences in toxic effect; where nAu rods had an EC50 within the µg/L range compared to mg/L ranges within nAu spheres. CytoViva dark field imaging revealed dose dependent surface adhesion of nanogold over time where nanomaterials accumulated around the anterior region, specifically on the surface of the eye and mouth region of larvae. There was a significant effect on larval swimming behavior in both nAu tested. Based on results within this study nanomaterial shape plays a major role in nanomaterial ecotoxicology across a range of endpoints assessed.

PS P304 - 0448 Naked Fe₃O₄ Magnetic Nanoparticles Are Internalized by Rat Brain Microvascular Endothelial Cells by Endocytosis Causing Toxic Effects

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Anthropogenic iron nanoparticles (NPs), like super-paramagnetic iron nanoparticles (SPIONS, mainly in magnetite phase, Fe₃O₄) are released to the environment as a result of the abrasion of combustion cylinders, brake pads and vehicle disc brakes, therefore they can be found in dust samples from high density traffic area. Fe₃O₄ NPs, have been casually found in human brain tissue, but unlike biogenic magnetite found in brain, these magnetite NPs have a mean diameter of 18 nm and an euhedral morphology, and it has been proposed to get directly into this tissue through the olfactory nerve. Their presence in the brain tissue has been associated with neurodegenerative diseases such as Alzheimer's since these particles are strong inducers of oxidative stress and inflammatory responses. In this work we proposed that external magnetite could also get into the central nervous system from the brain microcirculation by crossing the brain-blood-barrier (BBB). A wide spectrum of studies have evaluated the toxicity of engineered and surface modified Fe₃O₄ NPs that are designed to be used for drugs delivery purposes in different experimental models, however there is scarce information about the toxicity of airborne naked magnetite NPs on cells that form the BBB, that could contribute to explain their presence in brain tissue and their role in neurodegeneration. In this work we synthesized, characterized and evaluated the Fe₃O₄ NPs toxicity on an *in vitro* monolayered BBB model using primary rat brain microvascular endothelial cells (rBMECs). The cellular toxicity induction as well as the Fe₃O₄ NPs internalization was evaluated on rBMECs through lactate dehydrogenase (LDH) release, MTS reduction and transmission electron microscopy (TEM), respectively. Results showed that Fe₃O₄ NPs get internalized into the cells through endocy-

toxicity, besides these NPs induced stronger toxic effects on rBMVECs in terms of cellular membrane damage and mitochondrial metabolism than silica-covered magnetite NPs (Fe_3O_4 @SiO₂). These results suggest that naked Fe_3O_4 NPs released to the environment during different human activities could reach the brain through the brain microcirculation and modify the BBB structure and permeability through their toxic effect on the brain microvascular endothelial cells.

P305 - 0504 Biological Responses of Human Lymphatic Endothelial Cells to Carbon Nanomaterials

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Although most biological evaluations of carbon nanomaterials (CNMs) are performed in the lung or abdominal cavity, the physiological reactions to CNMs may differ in other tissues. Recently proposed medical CNM applications include reinforcement materials for artificial joints, drug delivery systems, and bone regeneration. In them, lymphatic vessel endothelial cells may interact with CNMs in the form of wear particles and waste products. Since tattoo ink, which is composed of mainly of carbon black (CB), is known to accumulate in lymphatic nodes, several reports have described that carbon nanotubes (CNTs) follow a similar route after placement in the human body. The present study analyzed the reactions of lymphatic endothelial cells with fibrous multi-walled CNTs (MWCNTs) and spherical carbon nanohorns (CNHs) in terms of several safety parameters. The CNMs tested were MWNT-7 MWCNTs, untreated CNHs, and oxidized CNHs (CNHox), and CB. The test particles were sonicated with 2% bovine serum albumin in a PR-1 sonicator at 140 W for 60 minutes and analyzed for hydrodynamic size using a Zetasizer Nano ZS. Human lymphatic endothelial cells (HLECs) from ScienCell Research Laboratories were exposed to the CNMs at various concentrations for 24 or 48 hours for assessment of cell proliferation, cell uptake, and immune responses. The diameters of MWNT-7, the CNH samples, and CB were approximately 700 nm, 160 nm, and 220 nm, respectively. The proliferation rate of cells exposed to 0.1 mg/ml of MWNT-7 or CNHox was approximately 60% compared with controls at 24 hours. However, while cell proliferation rate with MWNT-7 became decreased to 35% at 48 hours, the rate with CNHox remained at 60% at 48 hours. The proliferation rate of cells exposed to 0.1 mg/ml of untreated CNHs or CB was over 70% at 24 hours. CNHox and CB were internalized into cells, while untreated CNHs were not. Interestingly, although the amount of cellular uptake of CNHox and CB was comparable on phase contrast microscopy, only CNHox evoked strong cell proliferation inhibition. MWNT-7 was not neatly taken into the cells and rather seemed to pierce them. Cells exposed to 10 $\mu\text{g}/\text{ml}$ of MWNT-7 for 48 hours showed increased IL-6 mRNA expression. Fibrous MWNT-7 exhibited cytotoxicity on HLECs in an imperfect cell uptake state as we previously reported for other cell lines. Although CNHox was completely internalized by cells, it showed cytotoxicity. Oxidation-treated CNHs become hydrophilic, which is generally believed to increase biocompatibility. Further research is needed on the mechanism of cytotoxicity by internalized CNHox.

P306 - 0523 Evaluation of Metal Nanoparticles on Skin Sensitization

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Advances in nanotechnology leads to increase of production and usage of nanomaterials (NMs), and the risk of exposure increases in many places of life. The unique physico-chemical properties of NMs, such as small size and high surface area, can lead to various side effects on the human. A number of studies have evaluated potential health effects of NMs, and this study aimed to apply skin sensitization tests to evaluate safety of NMs. *In vivo* skin sensitization tests, OECD test guidelines 442B, was conducted for 4 different metal oxide nanoparticles (NPs) including copper oxide (CuO , 20 nm), cobalt oxide (Co_3O_4 , 80 nm), nickel oxide (NiO , 20 nm), and titanium dioxide (TiO_2 , 15 nm). In the tests of 442 B, N, N-dimethylformamide (DMF) was selected as a dispersion solvent of NPs and mouse serum (3% v/v) was added to improve dispersion stability. Physicochemical properties, such as hydrodynamic size, polydispersity, and zeta potential, were measured for dispersed NPs prior to the tests. Female BALB/C mice (7 weeks old) were chosen and subjected for dispersed NPs treatment following 442B guideline. In the first preliminary tests, we evaluate any clinical signs, either of local irritation or of systemic toxicity, body weight, ear weight and ear swelling. When the results of body weight and ear weight were checked, no response to stimulation was found. However, there was a statisti-

cally significant difference at cobalt, and nickel oxide NPs in the ear swelling evaluation. Statistical differences were observed, but the criteria presented in the guideline were not reached, and as a result of the first preliminary test, 4 types of NPs were confirmed as non-skin sensitizer. Additional tests would be conducted to evaluate skin sensitizing potential. This study would contribute to OECD test guidelines 442B to evaluate NMs properly, if necessary.

P307 - 0542 Short- and Long-Term Administration of Synthetic Amorphous Silica (NM-203) Nanoparticle and Histopathological Effects on Rat Spleen

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Synthetic amorphous silica (SAS) is a nanomaterial consisting of aggregates and agglomerates of primary silicon dioxide (SiO_2) particles in the nanorange. SAS is widely used because of its biocompatibility and ease of functionalization with targeting molecules; indeed, it is utilized as nanocarrier for delivery of drugs, as excipient in drugs and vitamin preparations and cosmetics, without any limitation of particle size. The spleen is the main secondary immune organ, which functions are carried out by white and red pulp different in their architecture, vascular organization, and cellular composition. Both NANOGENOTOX Joint Action and NANOREG projects confirmed spleen as one of the major target organ for SiO_2 nanoparticle bioaccumulation in rats after intravenous (iv) and oral, short and long term exposure. Due to the increasing human exposure to SAS NPs for medical applications, it appears necessary to provide information about the possible long-term effects due to short (iv) and long (oral) administration on the spleen, mimicking the expected human exposure to drugs. The test material was SAS (SiO_2 NM-203, JRC repository) tested in the NANOGENOTOX for toxicokinetic. Characterization and dispersion analysis of NM-203 were performed using NANOGENOTOX protocols. Sprague Dawley rats (both sexes) were treated with 0 (vehicle only –milliQ water) and 20 mg/kg body weight (bw) per day: (1) iv by injection in the tail vein. Rats received a single dose (SD—1 day of treatment) and five repeated dose (RD—5 days of treatment). After a 90-day follow-up period, rats were sacrificed; (2) orally by gavage, 5 days/week for 90 days. 3 hours after the last treatment, rats were sacrificed. At the end of treatment, rats were anaesthetized with a gaseous solution of isoflurane, and sacrificed by CO_2 asphyxiation. The spleen was excised, weighted and fixed in 10% buffered formalin for the histopathological analysis. *In vivo* study, macroscopic observation of spleen of all RD treated rats showed evident splenomegaly, significant differences among groups were found also in absolute weight. The presence of inflammatory infiltrates consisting of both mononuclear cells and granulomas were also observed, significantly higher in the RD group in both sexes. In oral study, no treatment related effects were present concerning spleen weight and histopathological end-points in both sexes. The present data showed that the toxicological effects of NM-203 may be strongly dependent on the route of administration: indeed, NM-203 gastrointestinal absorption (< 1%) is very low and the cumulative internal dose—even after 90 days of oral administration—did not induce any toxicological effects in rat spleen.

P308 - 0543 Improved Aerosol Generation Method and Newly Designed Whole Body Rodent Inhalation Apparatus for the Testing of Nanomaterials in Human-Relevant Exposure Scenario

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Inhalation study is a gold standard for the assessment of respiratory toxicity of the nanomaterials (NM). To generate well-dispersed aerosol, we developed the "Taquann" dispersion method and a "direct injection" whole body inhalation system for the dispersed sample (designated as "Taquann System" J Toxicol Sci. 2013). The Mitsui MWNT-7 was used to evaluate the performance. Taquann method removes the aggregate/agglomerates effectively and enrich the well-dispersed single fibers without changing the length and width distribution. The method is based on two concepts; liquid-phase fine filtration and critical point drying to avoid re-aggregation by surface tension. The bulk sample of MWNT-7 was suspended and dispersed in tert-butyl alcohol (TBA), filtered by 25 micrometer mesh to remove aggregates/agglomerates, snap-frozen by liquid nitrogen, and vacuumed, mimicking the process of critical point drying (a method used for scanning electron microscope sample preparation). Aliquots of dry dispersed MWCNT were loaded in tube-shaped original cartridges, and

the dispersed sample in the cartridges are periodically (every few minutes in most cases) injected to a newly designed inhalation chamber system by the compressed air to maintain a certain range of aerosol concentration. The length distribution of the MWNT recovered from the lungs of the exposed mice was similar to the fibers in original sample and aerosol in the chamber. Now the system is shown to work for Nikkiso MWCNT, three different makes of nano TiO₂, potassium titanate whiskers, and nano-sized agglomerates of double wall CNT. The advantages of the Taquann System include relatively cheap small scale equipment compared to traditional whole body inhalation chamber systems, low running cost, high flexibility for various types of NM samples, low chance of scattering of sample due to closed procedures after making TBA suspension, and low loss rate of sample. To date, we have developed four models of inhalation exposure systems (ver.1.0, 2.0, 2.5 and 3.0). The latest model of the Taquann system is now equipped with computer-controlled automatic cartridge loader/injector that can keep the aerosol concentration for up to 6 hours. The air flow of the chamber is also improved to increase the efficiency of aerosolization. The capacity of the chamber is 25 mice or 12 rats per chamber, and less expensive than previous models. For instance, with respect to MWNT-7, 80% of samples loaded into a cartridge were aerosolized and mass concentration was reached to 6 mg per cubic meters. These improvements made long-term studies possible for testing chronic effects of NMs. Supported by grants from MHLW, Japan.

PS P309 - 0555 MPA-Capped CdTe Quantum Dots Exposure Causes Toxic Effects in BV2 Cells via MyD88-Dependent Toll-Like Receptor Pathway and NLRP3 Inflammasome Activation

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Due to its excellent fluorescence properties, CdTe quantum dots (QDs) is valuable to be used in the basic research of neuroscience and the diagnosis and treatment of diseases related to nervous system. To guarantee the safety of QDs in the nervous system, it is necessary to conduct comprehensive and in-depth neurotoxicity research on CdTe QDs. Our previous studies have found that CdTe QDs can cause inflammation in rat hippocampus, the results of RNA-seq analysis provide several signaling pathways in which CdTe QD can be involved in causing inflammatory effects, but the underlying mechanisms are unclear. This study found that 40nM CdTe QDs exposure increased the expression of cell surface antigens CD11b and CD40 in BV2 cells, confirming the activation of BV2 cells, as the increased expression of CD11b and CD40 always corresponds to the severity of microglia activation, and increase the secretion of pro-inflammatory cytokine IL-1 β . The TLR2 / MyD88 / NF- κ B pathway is the first signal of pro-IL-1 β expression, and NLRP3 inflammasome plays a role in pro-IL-1 β maturation into IL-1 β , all involved in CdTe QD-induced IL-1 β secretion. Therefore, this is highly correlated with activation of the TLR2/MyD88/NF- κ B pathway and ROS-induced NLRP3 inflammatory bodies. These findings provide insight into the potential mechanisms of neuroinflammatory responses to CdTe QDs. *This work was Supported by National Natural Science Funds of China (No. 21876026, 31671034, 81673218, 81573186, 81502783).*

PS P310 - 0556 Effect of Carbon Nanotubes on Atherosclerosis Progression in Animal and Culture Models of Atherosclerosis

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Asbestos and silicon have been shown to activate the NLRP3 inflammasome. Inflammasome activation is triggered by reactive oxygen species, which are generated by a NADPH oxidase. Carbon nanotubes are a valuable industrial product but there is potential for human pulmonary exposure during production and their fibrous shape raises the possibility that they may cause inflammation and oxidative stress. We hypothesized that carbon nanotubes promotes atherosclerosis due to enhancing inflammatory responses triggered by inflammasome activation. The present study investigated the roles of NLRP3 inflammasome on the development of atherosclerosis by double wall carbon nanotubes (DWCNTs) using apolipoprotein E-deficient (ApoE^{-/-}) mice, a model of human atherosclerosis. Male ApoE^{-/-} and age-matched wild type (WT) mice (n = 8 for each group) were treated with short or long DWCNTs (10 or 40 μ g/mouse) once every other week for 8 weeks by pharyngeal aspiration. After treatment, the aortic arch was stained with oil red-O solution to evaluate the extent of atheroma formation. ApoE^{-/-} mice exposed to CNTs at the high dose

showed increased plaque area in the aorta by oil red O staining. RNA was extracted from the thoracic aorta and the expression of inflammasome activation system was analyzed. The expression of NLRP3, which is a central constitutive factor of inflammasome, and the expression of inflammatory factors (VCAM-1, ICAM-1) and cytokine (MCP-1) were significantly increased in ApoE^{-/-} mice treated with long DWCNTs at the high dose. Furthermore, in human umbilical vein endothelial cells (HUVECs) treated with the high dose of DWCNTs the level of cytokine (IL-1 β) was significantly elevated. The results suggested that DWCNTs induced the formation of arteriosclerotic lesion due to enhancing inflammatory responses triggered by inflammasome activation.

PS P311 - 0582 Effects of Pharyngeal Aspiration Exposure to Multi-Walled Carbon Nanotubes on Pulmonary Inflammation in Mice

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Engineered nanomaterials and nanotechnologies are expected to have an impact on society and economy. There are also concerns that these materials may pose environmental and health risks due to their unusual chemical and physical properties. Carbon nanotubes are a valuable industrial product but there is potential for human pulmonary exposure during production and their fibrous shape raises the possibility that they may cause inflammation and oxidative stress. The present study investigated the roles of oxidative stress in multi wall carbon nanotubes (MWCNTs)-induced pulmonary inflammation using nuclear factor erythroid 2-related factor (Nrf2) null mice. Male Nrf2 null mice and age-matched wild type (WT) were divided into three groups of eight each, and exposed once to MWCNTs at 0, 10, 20 μ g/mouse by pharyngeal installation. Bronchoalveolar lavage fluid (BALF) and lungs were collected to evaluate pulmonary inflammation at post-exposure day 7. The mRNA levels of Nrf2-dependent antioxidant enzymes and inflammatory cytokines were measured. MWCNTs increased BALF total protein and total cell counts in WT mice, but not Nrf2 null mice. Although lymphocytes, neutrophils, eosinophils number in BALF were increased in WT mice exposed to MWCNTs, the increases were suppressed in Nrf2 null mice. WT mice showed higher mRNA level of NADPH oxidase 2 (NOX2) and apoptosis-associated speck like protein containing a caspase recruitment domain (ASC) than Nrf2 null mice. The levels of caspase-1 and interleukin-1 β were increased in only WT mice after exposure to MWCNTs. The results suggested that Nrf2-dependent inflammasome activation is involved in pulmonary inflammatory responses by MWCNTs.

PS P312 - 0621 Leveraging the Developmental Zebrafish to Evaluate the Hazard Potential of Metal Oxide Nanomaterials

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Metal oxide nanoparticles (MONPs) measuring between 1 and 100nm are a rapidly expanding technology due to their potential application in medicine, sensing, energy storage, and consumer products. Their potential undesirable effects on human health remain largely unknown. To systematically evaluate the potential for adverse health effects from exposure to nanomaterials, the NIEHS developed the Nanomaterials Health Implications Research Consortium. Metal oxides are a Consortium priority for structure-bioactivity assessments. A suite of 8 MONPs with physicochemical properties characterized by the Harvard School of Public Health-NIEHS Nanosafety Center, were supplied by the Consortium. We exposed zebrafish to individual MONPs (0, 2.32, 5, 10.7, 23.2, or 50 μ g/ml; n=32/conc.) from 6 hours post-fertilization (hpf) to 120 hpf. Morphology and behavioral endpoints were assayed at 24 and 120 hpf. None of the 8 metal oxides were associated with aberrant morphology at 120hpf, but WO₃ (15 nm), ZnO (50 nM), TiO₂ (100 nm), SiO₂ (15 nm) Al₂O₃ (30 nm), and both CeO₂ (10 and 30 nm) were associated with abnormal photomotor behavior. Our next step is to determine if developmental exposure to MONPs from 6 –96 hpf can manifest as behavioral and respiratory fitness deficits in adult zebrafish. Our goal is to establish a systems level approach that anchors these complex phenotypes to underlying transcriptome changes. *The work is supported by NIEHS grant U01 ES027294.*

P313 - 0644 Silicon Dioxide and Titanium Dioxide Nanoparticles Induce Cytoskeleton Disruption and Inhibit Cell Migration

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Production of engineered nanomaterials have increased in the last years and metal oxide nanoparticles (NPs) are one of the most produced worldwide, however there is evidence of toxic effects by inhalation in occupational settings. Beyond the mechanism of toxicity, less has been investigated about cytoskeleton disruption induced after NPs uptake. In this study, we aimed to investigate the effects of two types of metal oxide NPs, amorphous silicon dioxide (SiO₂) and fibers of titanium dioxide (TiO₂) NPs, on cytoskeleton disruption and if those alterations impact migration process. Lung epithelial A549 cells were exposed to SiO₂ and TiO₂ (1, 10 and 50 µg/cm² for 24 h) and labeling with alizarin red was used for uptake analysis by confocal microscopy while cell viability was determined by trypan blue exclusion. Granularity and cell size were determined by flow cytometry, integrity of actin cytoskeleton was evaluated by immunofluorescence and migration process by transwell assay. The results showed that SiO₂ and TiO₂ NPs formed agglomerates of 437 and 659 nm when they are suspended in cell culture media, respectively. Furthermore, the agglomerates of both NPs were internalized by A549 cells and cell viability decreased only with TiO₂ NPs. Granularity and cell size of A549 cells exposed to SiO₂ not showed changes, however, when A549 cells are exposed to TiO₂ NPs at concentrations of 10 and 50 µg/cm² their granularity increased. Furthermore, size of A549 cells exposed to TiO₂ NPs decreased with concentrations of 10 and 50 µg/cm² in comparison with A549 cells unexposed. SiO₂ and TiO₂ NPs disrupted actin cytoskeleton and as a consequence the cells had a migration reduction. In conclusion, SiO₂ and TiO₂ NPs had the ability to form agglomerates around 500 nm and A549 cells can internalize these agglomerates that produce an increase in their granularity and decreased cell size. SiO₂ NPs had no influence on cell viability, however, A549 cells exposure to TiO₂ NPs decreased cell viability in all concentrations analyzed. SiO₂ NPs and TiO₂ NPs induced cytoskeleton disruption and migration process was affected in similar extent and no differences between these 2 different NPs were detected.

P314 - 0646 Relating Nano-metal Oxide Physicochemical Properties to Developmental Toxicity: An Approach to Safer Nanomaterial Design

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As innovation and the global market for nanotechnology accelerates, the presence of engineered nanomaterials (ENMs) in foods, clothing, electronics, and personal care products will increase. The hazard potential of, and human exposure to, the nanomaterials in such products are not well defined. We also lack systematic methods to assess nanomaterial safety. Risk assessors need relevant *in vivo* data to understand the relationship between ENM physicochemical properties and ENM toxicity. Metal oxides are one of the most commonly used nanomaterials in the market and thus a priority for structure-bioactivity assessments. Thirteen metal oxide ENMs, with physicochemical properties characterized by the Harvard School of Public Health-NIEHS Nanosafety Center, were supplied via the Nanotechnology Health Implications Research Consortium. We exposed zebrafish to individual metal oxide ENMs (0, 2.32, 5, 10.7, 23.2, or 50 µg/ml; n=32/conc.) from 6 hours post-fertilization (hpf) to 120 hpf. Morphology and behavioral endpoints were assessed at 24 and 120 hpf. Zinc oxide, aluminum oxide, cerium oxide, tungsten oxide, and iron oxide exposure were associated with abnormal photomotor behavior at 24 and 120 hpf. As an initial foray into the toxic mechanism, we measured expression changes of 25 oxidative stress related genes and anchored the changes to the morphological and behavior effects, and the ENM physicochemical properties. Our next steps will use dosimetry models, developmental transcriptomics and genetics to uncover the molecular pathways functionally responsible for mediating the toxicity of metal oxide ENMs. This systems level approach will provide foundational knowledge for designing safer ENMs.

P315 - 0705 Zinc Oxide and Silver but Not Titanium Dioxide Nanoparticles Induce Innate and Adaptive Immune Response in THP-1 Macrophages

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The market of nanotechnology is expanding fast. Among which, metallic nanoparticles are one of the most widely used nanomaterials (NMs). Despite that there are increasing reports of toxicity exerted by NMs, understanding of the mechanisms of actions is still far from complete. In the current study, THP-1 macrophages were exposed to three metallic nanoparticles (n-TiO₂, n-ZnO, n-Ag) and their bulk-sized/ion counterparts (b-TiO₂, b-ZnO, i-Ag) for 6h and 24h. Genome-wide gene expression changes at low-toxic concentrations (≤ 15% cytotoxicity) were studied by microarray analysis to elucidate molecular effects. And cytotoxic effect of released ions was evaluated by MTT assay. At 6h, n-ZnO, b-ZnO and n-Ag induced various immunity related genes associating to pattern recognition (including toll-like receptor), macrophage maturation, inflammatory response (TNF, IL-1beta), chemotaxis (CXCL8) and leucocyte migration (CXCL2-3, CXCL14). At 24h, especially n-Ag induced the expression of genes related to virus recognition and type I interferon responses. Overall, results suggested that n-TiO₂ had minimal toxicity. N-ZnO had fast ion release (both extracellularly and intracellularly) and fast biological effects as opposed to the prolonged intracellular ion release and biological activity of n-Ag. The fact that n-ZnO shared only 3.1% differentially expressed genes (DEGs) with its bulk-sized counterpart, once again highlighted the role of size in exerting toxicity.

P316 - 0735 Molecular Signature of Asthma-Enhanced Sensitivity to Aerosols of Pristine and Carboxylated CuO Nanoparticles, Identified in 3D Cell Models

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More than 5% of any population suffers from asthma, and there are indications that these individuals are more sensitive to nanoparticle aerosols than the healthy population. Due to a paucity of data on the molecular-level details and mechanisms involved, we leveraged a realistic air-liquid interface model of inhalation exposure, to investigate global transcriptomic responses in reconstituted three-dimensional airway epithelia of healthy and asthmatic subjects exposed to pristine (nCuO) and carboxylated (nCuOOH) copper oxide nanoparticles. A dose-dependent increase in cytotoxicity (highest in asthmatic donor cells) and pro-inflammatory signaling within 24 hours, confirmed the reliability and sensitivity of the system to detect acute inhalation toxicity. Gene expression changes between nanoparticle-exposed versus air-exposed cells were investigated. Hierarchical clustering based on the expression profiles of all differentially expressed genes (DEGs), cell-death-associated DEGs (567 genes) or a subset of 48 highly overlapping DEGs, categorized all samples according to 'exposure severity,' wherein nanoparticle surface chemistry and asthma are incorporated into the dose-response axis. For example, asthmatics exposed to low and medium dose nCuO clustered with healthy donor cells exposed to medium and high dose nCuO, respectively. Of note, a set of genes with high relevance to mucociliary clearance, were observed to distinctly differentiate asthmatic and healthy donor cells. These genes also responded differently to nCuO and nCuOOH nanoparticles. Additionally, because response to transition metal nanoparticle was a highly enriched Gene Ontology term (FDR 8E-13) from the subset of 48 highly overlapping DEGs, these genes may represent biomarkers to a potentially large variety of metal/metal oxide nanoparticles.

PS P317 - 0742 (Un-)Supervised Learning Methods Predict and Characterize the Association between Nanomaterial-Induced Inflammatory Markers and Pathological Outcomes in the Lungs

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The growing use of nanotechnology in a wide range of industrial applications is raising concerns about the potential adverse health effects that may be associated with human exposure to inhaled engineered nanoparticles (NPs). It has been shown that adverse pulmonary outcomes, such as inflammation, sub-pleural fibrosis, and granuloma formation can appear after *in vivo* exposures to NPs, in particular carbon-based NPs (CNPs). Understanding if the mechanisms contributing to the adverse effects are similar or unique when comparing different NPs is important for accurately assessing their risks and for protecting the health and safety of workers involved in manufacturing and use of NPs, as well as for consumers. Identification of a set of protein markers with an attempt to facilitate the grouping of NPs based on the correlations found in both BALF inflammatory proteins and pathological manifestations in the lungs was explored. This study presents a statistical/machine-learning based approach to group together CNPs, in particular carbon black, multi-walled carbon nanotubes (MWCNTs), graphene and their derivatives. To accomplish the goal, a consistent feature that was measured in all samples was prioritized. In this case, a panel of proteins measured in lung bronchoalveolar lavage fluid following exposure was utilized. As a first step, before selecting proteins that could discriminate the responses to different types of CNPs, a hierarchical cluster analysis was performed to group CNPs based on similarities in their pulmonary toxicity profiles. Following this, a latent cluster analysis was performed to see whether similar groupings of various CNPs would emerge based on histopathological alterations in lungs. Following this, we applied a sparse supervised classification algorithm to select protein markers to distinguish between the different groups (e.g., MWCNTs, graphene based materials and carbon black). Specifically, MDC/CCL22 and MIP-3 β /CCL19 were identified as common protein markers associated with both toxicologically distinct groups of CNPs. In addition, the persistent expression of other selected protein markers in BAL fluid from each group suggested their ability to predict toxicity in the lungs, i.e., fibrosis and microgranuloma formation. Identification of specific markers predictive of such biological responses, will not only help us understand the process leading to toxicity but can also be applied as a confirmation or screening tool for grouping NPs into toxicologically relevant groups, thus limiting the amount of testing needed for risk assessment of nanomaterials.

PS P318 - 0771 Oxide Nanoparticles Aggravate Neuroinflammation in a Murine Model of Experimental Autoimmune Encephalomyelitis

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Medicinal preparations of iron oxide nanoparticles (IONPs) have been used as magnetic resonance imaging contrast agents for the diagnosis of hepatic tumors and the assessment of neuroinflammation and blood-brain barrier integrity. However, it remains mostly unclear if exposure to IONPs affects neuroinflammation under disease conditions. The present study aims to investigate the impact of IONPs on autoimmune-mediated neuroinflammation using a murine model of experimental autoimmune encephalomyelitis (EAE) that mimics human multiple sclerosis. Mice were either left untreated or immunized with myelin oligodendrocyte glycoprotein on day 0 followed by two injections of pertussis toxin for EAE induction. The EAE mice were intravenously administered with a single dose of the carboxydextran-coated IONPs (20 mg Fe/kg) and/or saline (as vehicle) on day 18. Symptoms of EAE were daily monitored till sacrifice of mice on day 30. Tissue sections of the brain and spinal cord were prepared for histopathological examinations. Iron deposition, neuron demyelination and inflammatory cell infiltration were examined by Prussian blue, Luxol fast blue, and hematoxylin and eosin staining, respectively. The infiltration of microglial and T cells, and cytokine expression were examined by immunohistochemical staining and/or RT-PCR. The antigen presenting capacity of microglia was examined by co-cultured with ovalbumin-primed splenic T cells. Iron deposition was detected in both the brain and spinal cord of EAE mice 3 days post ferucarbotran treatment. The clinical and pathological scores of EAE, percentage of myelin loss and infiltration of inflammatory cells into the spinal cord were significantly deteriorated in EAE mice treated with ferucarbotran. Furthermore, ferucarbotran treatment increased the number of CD3⁺, Iba-1⁺,

IL-6⁺, Iba-1⁺TNF- α ⁺, CD3⁺IFN- γ ⁺ cells in the spinal cord of EAE mice. A single exposure to ferucarbotran exacerbated neuroinflammation and disease severity of EAE, which might be attributed to the enhanced activation of microglia and T cells. Additionally, the expression of IFN- γ and IL-17a from splenic T cells was increased after co-cultured with IONP-treated microglial cells. These results demonstrated that the pro-inflammatory effect of ferucarbotran on the central nervous system is closely associated with the deterioration of autoimmunity.

PS P319 - 0802 Genetic and Hepatic Damage Induced by TiO₂ (Anatase), ZnO Nanoparticles, and Their Mixture in Mice: A Morphological and Mechanistic Approach

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The discharge of titanium dioxide (TiO₂) and zinc oxide (ZnO) nanoparticles (NPs) into the environment may enhance the likelihood of being exposed to mixtures, which may be detrimental *in vivo*. *In vitro* studies have shown their potentials to induce genetic damage; but there is dearth of information on the ability of their mixtures to induce DNA and systemic damage. This study was aimed at investigating the genetic and hepatic damage induced by the individual NPs and their 1:1 mixture, in addition to elucidating their mechanism of damage. The shape, hydrodynamic diameter (HD), and zeta potential (ζ) of TiO₂ and ZnO NPs were characterized using transmission electron microscopy and dynamic light scattering, respectively. Swiss mice were intraperitoneally administered 9.38 - 150.00 mg/kg bw each of TiO₂, ZnO NPs, and their 1:1 mixture for 10 days to assess genotoxicity through the micronucleus assay. Hepatic damage was evaluated via assessment of relative liver weight, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), oxidative stress parameters [superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and malondialdehyde (MDA)] and histopathology. TiO₂ and ZnO NPs respectively were spherical and rod-like in shape, with a HD (nm) of 1492 and 482.7, and ζ (mV) of +17.2 and +21.4. Both NPs and their mixture induced significant increase ($P < 0.05$) in the micronuclei frequency. The relative liver weight significantly decreased across the concentrations for the group exposed to the 1:1 mixture; with a significant ($P < 0.01$) increase in AST levels, and a significant ($P < 0.01$) decrease in ALT levels across all concentrations. The effects of TiO₂ NPs, ZnO NPs and their mixture on hepatic SOD, CAT, GSH and MDA activities were significantly ($P < 0.001$) altered compared to the control indicating oxidative stress. Multiple foci of single-cell hepatocellular necrosis and dense aggregates of mononuclear inflammatory cells were observed in the liver sections. Data herein showed that oxidative stress is the mechanistic pathway through which TiO₂, ZnO NPs and their mixture induce DNA damage and hepatic injury.

PS P320 - 0803 Withdrawn

PS P321 - 0831 Graphene-Based Nanomaterials Directly Induce Epithelial-Mesenchymal Transition *In Vitro*

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Graphene and its derivatives have exhibited wide potential applications in electronics, structural engineering and medicine. However, over utilization and untreated discharge may cause its distribution into environmental as well as biological chain, which raised the concerns of potential health risk as a potential hazard. Accumulating evidence has demonstrated that graphene derivatives induce lung fibrosis *in vivo*, so overall goal of this study was to explore the molecular mechanisms underlying the pulmonary fibrotic responses of reduced graphene oxide (rGO) and graphene oxide (GO), using *in vitro* assays. Epithelial-mesenchymal transition (EMT) has profound effect on development of pulmonary fibrosis. Herein, we evaluated the EMT effect of rGO and GO samples on A549 and BEAS-2B cells. Firstly, rGO and GO penetrated through the A549 cells membrane into the cytosol by endocytosis observed via transmission electron microscopy (TEM), and were well tolerant by cells. Secondly, rGO and GO promoted the cell migration and invasion capacities at lower doses (below 10 μ g/ml), but significantly inhibited the capacities at 20 μ g/ml. Moreover,

rGO-induced and GO-induced EMT were evidenced by decreased expression of epithelial marker and increased expression of mesenchymal markers. Based on our findings, it is supposed that rGO and GO can effectively induce EMT through altering epithelial-mesenchymal transition markers *in vitro*.

P322 - 0843 Comparative *In Vitro* Toxicity Assessment of Carbon Dots with Positive and Negative Surface Charge

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Carbon dots are a class of carbon-based nanomaterials (NMs) of dimensions below 10 nm exhibiting exceptional fluorescence properties. Photostability and generally low toxicity of carbon dots favor their use in biomedicine, especially as bioimaging agents. Biocompatibility of NMs is known to depend on their physico-chemical properties, with the surface charge being reported as one of the key factors affecting interactions between NMs and cells and potential toxicity. In the present study, we compared two types of carbon dots similar in size (< 10 nm), yet differing in their surface charge: quaternized carbon dots (QCDs) with positive surface charge (+43 mV) synthesized by thermal oxidation of Tris and betaine, and carbon dots with negative surface charge (-29 mV) prepared by solvothermal decomposition of urea and citric acid dissolved in formamide (hereinafter referred to as CDs). Evaluation of toxicity was performed in human lung carcinoma epithelial cells (A549 cell line) and human embryonic lung fibroblasts (HEL 12469 cell line) after 24-hour exposure in concentrations ranging from 1 to 500 µg/mL. Endpoints assessed covered (1) cytotoxicity tested by the WST-1 assay, (2) the cell cycle analysis by flow cytometry, (3) genotoxicity evaluated using the comet assay in variants with and without formamidopyrimidine glycosylase (FPG) to assess DNA strand breaks and oxidized purines, respectively, and (4) cellular internalization confirmed by transmission electron microscopy. While no or only minor effects were observed in cells exposed to CDs even at the highest tested concentrations, QCDs showed toxicity in all the selected assays. A decrease in cell viability started at a concentration of 50 µg/mL, furthermore, changes in the cell cycle, specifically an increase in the percentage of cells in the G0/G1 phase and a decrease of S-phase cells were detected. Sub-cytotoxic concentrations of QCDs (10 and 25 µg/mL) elicited a slight increase in DNA damage (both with and without FPG). Toxic effects of QCDs were detected in both cell lines, however, HEL 12469 cells exhibited higher sensitivity. Observed toxicity of QCDs is presumably related to their positive surface charge. In summary, minimal toxicity of CDs even at high concentrations (up to 500 µg/mL) together with long-term fluorescence, colloidal stability in water and uniformity render them potentially superior to already well-established quantum dots containing toxic heavy metals. Likewise, QCDs in lower non-toxic doses may also be suitable for bioimaging applications. Supported by the MEYS CR (LO1508; LM2015073; CZ.02.1.01/0.0/0.0/16_013/000 1821).

P323 - 0878 Halloysite Nanotube Toxicity Is Predicted by Surface Area

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Halloysite [Al₂Si₂O₅(OH)₄] is an abundant natural clay mineral that can occur as nanotubes of rolled-up aluminum silica sheets (HNTs). HNT size and structure depend on the formation conditions and therefore vary between locations of origin. Diameters are typically <100 nm, with length to diameter ratios up to 200. More than 50 000 metric tons of HNTs are mined annually. Due to the hollow nanostructure that allows for loading and subsequent release of compounds applications are wide, e.g., HNTs may be loaded with antimicrobial agents (natural essential oils) and subsequently incorporated into polymers to form packaging materials that may increase food shelf life. Considering their high production volume and physico-chemical characteristics, where HNTs have the characteristics of poorly soluble high aspect ratio nanomaterials (HARN), knowledge about their toxicity is highly warranted. We assessed the toxicological response to HNTs following airway exposure, an evident occupational exposure route of concern during mining, processing and handling of HNTs. Both a pristine (NaturalNano, NN) and a HNT modified by surface chemi-

cal etching by sulfuric acid (NNetched) were assessed. BET surface area was 4-5 times higher for the NNetched compared to the pristine HNT. First, the potential cytotoxicity of the two HNTs was screened *in vitro* in MutaTMMouse lung epithelial cells. Then adult female C57BL/6J BomTac mice were intratracheally instilled once with 6, 18 or 54 µg HNTs in isoflurane anesthesia and compared to vehicle controls and 162 µg Carbon black Printex 90 (positive inflammation control) on day 1, 3, and 28 after instillation. Lung inflammation was determined by the cellular composition of bronchoalveolar lavage (BAL) fluid, acute phase response by *Saa* mRNA levels (real-time quantitative PCR) in lung and liver tissue, and genotoxicity was analyzed by the alkaline comet assay as DNA strand breaks in BAL cells, lung and liver tissue. None of the HNTs were cytotoxic, affected mouse body weight or induced genotoxicity. At the highest dose level, NNetched increased neutrophil influx and *Saa* mRNA levels in lung at all assessed time points. At day 1 post-exposure, the induced inflammation correlated well with the deposited particle surface area, indicating that the increase in BET surface area due to etching could explain the difference in the inflammatory potential of the studied HNTs. This is consistent with observations of other nanosized particles. EU's Horizon 2020 research and innovation program (grant #720815) supported the Nanopack project. The abstract reflects views of the authors only, the EU Commission is not responsible for any use hereof.

P324 - 0885 Comparative Study of Cytotoxic and Genotoxic Effects of Uncoated and Polyethylene-Glycol-Coated Gold Nanoparticles on Human Kidney (HK-2) Cells

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Gold nanoparticles (AuNPs) have generated a substantial amount of scientific and technological interest due to their ease of synthesis, chemical stability, and unique optical properties. Numerous empirical studies demonstrate their biomedical applications in chemical sensing, biological imaging, drug delivery, and cancer treatment. In considering these applications, biocompatibility and the absence of cytotoxicity of AuNPs are essential. Comparative studies were conducted to investigate whether 25-35 nm poly (ethylene glycol) (PEG) coated and uncoated AuNPs are more or less cytotoxic and genotoxic to human kidney (HK-2) cells. Cytotoxicity, oxidative stress, and genotoxicity were evaluated by the MTT assay, dichlorofluorescein (H2DCF) assay, and single cell gel electrophoresis, respectively. Results showed that uncoated Au particles significantly reduced cell viability and were cytotoxic with an IC₅₀ concentration of 100µM whereas, the PEG coated AuNPs displayed low toxicity even at a high dose of 200µM after 24-hour exposure. Uncoated AuNPs increased reactive oxygen species concentration (ROS), decrease GSH production, and depolarize the mitochondrial membrane potential in a concentration-dependent manner. PEG AuNPs produced no notable increase in ROS or decreased in GSH along with negligible polarization of the mitochondrial membrane potential. PEG AuNPs showed insignificant genotoxic effect of DNA damage represented by 1.07% in comparison to 37.4% exerted by uncoated AuNPs. Overall these findings show that uncoated AuNP appear to be more cytotoxic and more genotoxic than PEG-coated AuNPs. Hence, PEG-coated AuNPs have beneficial clinical implications for application in nanobiotechnology and nanomedicine.

P325 - 0165 Inhibitory Effect of Kahweol on PMA-Induced MMP-9 Expression through Suppressing Akt/JNK1/2/p38 MAPK Signal Transduction and NF-κB-Dependent Transcriptional Activity in Human Fibrosarcoma Cells

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Coffee is one of the popular beverages in the world and contains various bioactive compounds, such as kahweol, cafestol, caffeine, and chlorogenic acid. These compounds play various pharmacological activities for health promotion, including antioxidant, anti-inflammatory, and anticancer effects. Especially, kahweol is a diterpene molecule that is contained in high quanti-

ties in Arabica coffee beans that have been reported the antitumor, anti-inflammatory, and proapoptotic effects in several types of cancer cells. Although kahweol has shown various bioactivities, the inhibitory effect of kahweol on the metastasis and invasion of tumor cells and the underlying mechanisms is still unclear. This study studied the antimetastatic activities of kahweol and revealed the possible molecular mechanisms. Kahweol attenuated PMA-induced cell proliferation, migration, and invasion in a concentration-dependent manner. Kahweol inhibited PMA-enhanced matrix metalloproteinase-9 (MMP-9) expression and activity through suppression of nuclear factor kappa B (NF- κ B). Kahweol suppressed the PMA-induced phosphorylation of Akt, JNK1/2, and p38 MAPK, which are upstream molecules for MMP-9. Taken together, the antimetastatic effect of kahweol might occur through the interfering of Akt/JNK1/2/p38 MAPK and downregulation of NF- κ B, leading to inhibition of MMP-9 expression. Therefore, kahweol, diterpene contained in coffee beans, could be a useful candidate for the discovery of new chemotherapeutic agents that may contribute to inhibiting the metastatic tumor.

P326 - 0166 Stimulatory Effect of Platycodin D on Osteoblast Differentiation via SIRT1/ β -Catenin Pathways

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Platycodi radix, the root of *Platycodon grandiflorum*, has been used as food resource and traditional medicine in the Asian countries. Previous studies reported that Platycodi radix, which contains various nutrients and numerous phytochemicals, has been considered to be healthy food in atopic dermatitis-like skin diseases, airway inflammation, osteoporosis, tumor metastasis, and liver damage. Nevertheless, the protective effects of platycodin D, the active component of Platycodi radix-derived saponin, on their mechanisms of osteoblast differentiation are still unclear. Platycodin D increased the expression and transcriptional activity of osteogenic markers, including ALP, BSP, Col1A1, Runx2, and osterix, subsequently enhancing the bone mineralization in C3H10T1/2 cells. Platycodin D induced β -catenin nuclear accumulation by upregulating GSK3 β phosphorylation. Furthermore, platycodin D enhanced the mineralization process in osteoblast cells via the SIRT1/ β -catenin pathways. These results suggest that platycodin D could be an effective therapeutic compound against osteoporosis because of its regulatory effects during the osteoblast differentiation.

P327 - 0199 IGF-1 Promotes Platelet Recovery in Mice by Thrombopoiesis Facilitation and Radioprotective Effect after Radiation

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Insulin-like growth factor-1 (IGF-1) is a well-recognized cytokine that can promote cell growth and survival, oncogenic transformation, and cancer progression and that is able to protect many cells from apoptosis induced by various toxic factors. However, the influence of IGF-1 on the activity of megakaryocytes (MKs), especially when they are subjected to radiation, is still unclear, although it is known as a hematopoietic cytokine. In our study, we first observed that IGF-1 treatment decreased the apoptosis of the mononuclear cells in bone marrow (BM), and M07e cell, a factor-dependent megakaryoblast cell line, after radiation with a single dose of 5.0 Gy. These results indicate that IGF-1 may have an antiapoptosis effect on MKs in BM against radiation-induced injury. Further research suggested that there was a gradual increase of IGF-1 receptor on MKs within the process that their differentiation and maturation, and IGF-1 thus could promote CD34+ cell expansion, MK differentiation, and proplatelet formation, as well as platelet release. The underlying mechanism analysis revealed that IGF-1 could activate extracellular signal-regulated kinase 1/2 (ERK1/2) and Akt signal pathways in a time-dependent manner. However,

compared to that TPO treatment resulted in a more visible increase of ERK1/2 phosphorylation, IGF-1 stimulation led to a more apparent elevation of Akt phosphorylation. Furthermore, we found that IGF-1 was unable to completely activate Akt signal pathway in MKs if SRC-3 was deficient. These results suggest that the thrombopoiesis facilitated by IGF-1 may be independent of TPO, but requires the assistance of SRC-3. As a result, we observed that there was an elevation of peripheral platelet counts in homozygous TPO receptor mutant mice (C57BL/6J-Mpl^{hob219}/J mice), and a decrease of peripheral platelet number in SRC-3^{-/-} mice post IGF-1 administration. Finally, we investigated that IGF-1 administration had the capacity to promote platelet recovery in C57BL/6 mice suffered from radiation at a dose of 6.0 Gy, and in grafted mice irradiated with 10.0 Gy γ ray. Generally, these results suggest that the promotion of IGF-1 on platelet recovery after radiation not only depends on its capacity on thrombopoiesis facilitation but also relies upon its radioprotective ability on MKs.

P328 - 0225 AHR-Related Microbial Pattern Changes in the Murine Gut Measured by High-Resolution Flow Cytometry

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It is increasingly recognized that the gut microbiome is important for health and disease. It influences the metabolism of its host. Importantly, the microbiome is both involved in the detoxification/degradation and in the toxification of dietary components, xenobiotic chemicals, or drugs. Currently, the microbiome is assessed by deep sequencing methods such as next-generation sequencing. The high cost of these methods is a barrier for screening purposes, as well as for kinetic studies requiring quick and easy methods. We therefore explored a recently described method, which uses flow cytometry to assess the microbial pattern in a given sample. This method discriminates bacteria based on DNA-staining and size/shape. Fresh fecal samples from C57BL/6 mice were collected, fibers removed, and the bacterial suspension fixed with 2% paraformaldehyde. Samples were stained with the DNA-dye DAPI and analyzed on an ARIAll cell sorter (Becton Dickinson, USA). Scatter and fluorescent characteristics of the entire samples were recorded. Based on the freely available R software, we developed a novel and easy approach to evaluate characteristic features of and differences between samples. Briefly, we created a unique gating template, based on all samples used in each experiment. This template was placed over the samples' scatter plots and percentage of particles therein were analyzed by multivariate analysis of variance with Pillai's trace. We assessed effects of sex, age, co-housing, treatment, and different sample storage conditions. This novel method creates a robust and reproducible data output as shown by repeated measurement of samples. In line with data from the literature, we identified sex and co-housing as factors significantly ($p < 0.01$) influencing fecal bacterial pattern. Moreover, we set up an experiment, where we treated mice with 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD dissolved in corn oil; 10 μ g/kg bw; N=22), corn oil (N=20), or left them untreated for up to 7 days. Using our method for monitoring over time, we aim to identify characteristic microbial pattern changes in fecal samples of mice treated with TCDD. Topics we are currently working on are the longitudinal analysis of data from samples measured at several points of time and the handling of the possible dependence between adjacent gates in the template. In conclusion, flow cytometry measurement of bacteria offers a useful and noninvasive method to follow the heterogeneity and dynamic changes in the gut microbiome after experimental interventions.

P329 - 0297 Protective Effect of 12AC3O against High Phosphate-Induced Vascular Calcification

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In recent years, accelerating the spread of processed foods and the Westernization of foods have caused concern about the excessive intake of phosphoric acid in Japan. Phosphate has roles as transporting energy, and constituting cell membranes and bones. Excessive intake of phosphate or chronic kidney disease (CKD) leads to hyperphosphatemia and eventually causes ectopic calcification. Ectopic calcification leads to cerebrovascular diseases such as arteriosclerosis and cerebral infarction, so ectopic calcification caused by hyperphosphatemia is one of the risk factors for these diseases. It has been reported that vascular calcification induced by hyperphosphatemia is caused by increased reactive oxygen species (ROS). Therefore suppression

of ROS was thought to lead to the treatment of vascular calcification. 12AC3O is one of newly synthesized dihydroperoxides (DHPs), which have antioxidant effects by trapping ROS. In this study, we investigated whether 12AC3O inhibits vascular calcification by trapping ROS. Mouse aortic smooth muscle cell line, p53LMAc1 cell, was cultured under high concentration of phosphate. To confirm establishment *in vitro* calcification model, Alizarin red stain and von Kossa stain were used to evaluate calcification. The effect of antioxidants and 12AC3O on calcification under high phosphate loading was examined by the same method. Cell Rox Green reagents was used to carry out the evaluation of oxidative stress. In addition, the effect of 12AC3O on trapping ROS was evaluated using an electron spin magnetic resonance apparatus (ESR). High phosphate loading increased calcium (Ca) deposition in vascular smooth muscle cells. In the treatment of antioxidants, Ca deposition was suppressed, so it is suggested that ROS was involved in high phosphate-induced vascular calcification. 12AC3O also inhibited Ca deposition. Furthermore, 12AC3O showed an effect on trapping superoxide anion (O₂⁻) and hydroxyl radical (·OH). Taken together, this study suggests that ROS is involved in high phosphate-induced calcification, and 12AC3O inhibits vascular calcification through trapping ROS. Suppression of ROS by antioxidants such as 12AC3O is thought to be a therapeutic target in high phosphate-induced calcification.

P330 - 0348 Effect of the Conserved Residues on Reactive Oxygen Species Scavenging in Cytoglobin

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Reactive Oxygen Species (ROS) refers to oxygen-containing free radicals and peroxides that easily form free radicals. *In vivo*, ROS are generated as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis. However, under environmental stress, ROS level can increase dramatically, which may lead to significant damage to cells by causing the oxidation of lipids, proteins, and DNA. ROS not only have direct cytotoxicity, but also have been proved to be related to the pathogenesis of a variety of diseases including various tumors. Therefore, the elucidation of ROS scavenging mechanism can help control oxidative stress and the related diseases. Recent studies have indicated that Cytoglobin (Cygb), the fourth member of the globin family, is supposed to involve in the ROS scavenging and play an important role in cell protection. Cygb was discovered in 2001 as a protein associated with stellate cell activation protein. Afterwards study showed that it is expressed ubiquitously in mammalian tissues and located in cytoplasm. X-ray crystal structure analysis revealed that Cygb has a hexacoordinated heme in which two His residues coordinate with the active center heme axially. Thus, before the exogenous ligand such as oxygen or carbon monoxide binds to Cygb, the distal His residue should dissociate from the heme first, which is expected to be important for the function elucidation of Cygb. In globin protein, it is known that Leu, the B helix's 10th amino acid, in Cygb which called Leu46, is strongly conserved and responsible for affecting the distal His residue's dissociation. So, in this study we have measured various ROS scavenging enzyme activities of Cygb Leu 46 mutant to attempt to elucidate the ROS scavenging function of Cygb. Mutant L46A, in which Leu 46 of Cygb was mutated to smaller Ala, and L46F, in which Leu 46 was mutated to larger Phe, were generated. Compared to WT, distal His residue is easier to dissociate from the heme in L46A and more difficult in L46F. When studying the reactivity with O₂⁻, the result was obtained in proportion to the mobility of the distal His residue. The autoxidation rate, a parameter relating to the O₂⁻ scavenging, also showed the similar result. That is, the O₂⁻ scavenging ability of Cygb is apparently related to the mobility of the distal His residue. Nevertheless, the result of study on the reactivity with ONOO⁻ showed a poor relationship, in which WT was the most active. Therefore, in order to explore the reactivity between Cygb and ONOO⁻, various attempts such as direct reaction and the reaction with the ONOO⁻ decomposition have been investigated and the result will be reported here.

P331 - 0505 Toxicity of Metformin as an Adjunct Chemotherapeutic Agent

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Lung cancer accounts for the highest incidence of cancer mortality worldwide. Receptor tyrosine kinases can play a role in the onset and progression of cancer. This includes the EML4-ALK chromosomal rearrangement, which is involved in 2-7% of lung cancer cases. Crizotinib, the first-line treatment for

ALK+ lung cancer is an effective treatment, however, resistance develops usually after one year. To prevent/overcome this resistance, novel strategies are being explored. Epidemiological studies show a reduction in the risk of cancer with the use of a hypoglycemic agent, metformin. Therefore, work is being undertaken to determine if metformin could be used as an adjunct therapy with crizotinib. We aimed to examine if this combination would produce any toxicity in mice, before beginning efficacy studies. Male balb/c mice (n=6) were orally gavaged with either vehicle (olive oil), metformin (5 mg/kg or 100 mg/kg), crizotinib (5 mg/kg or 25 mg/kg) or a combination (5 mg/kg of crizotinib and metformin or 25 mg/kg crizotinib and 100 mg/kg metformin) once daily for 14 days. Mice were weighed daily and on the 15th day were euthanized. Blood was collected and organs harvested, weighed and frozen. The liver was homogenized and microsomes collected for CYP3A4 analysis. Serum alanine aminotransferase (ALT) and creatinine kits were used to examine liver and renal toxicity. Data was analysed using one-way ANOVAs with Bonferroni post hoc tests. Metformin (5 mg/kg) and crizotinib (5 mg/kg) as single agents showed no significant elevation in serum ALT compared to the control and remained within the normal threshold (77 ± 44 and 46 ± 11 vs. 41 ± 17 U/L). The combination (5 mg/kg metformin and 5 mg/kg crizotinib) also showed no significant increase in ALT (27 ± 5 U/L). Creatinine activity also had no significant differences between metformin (5 mg/kg, 2.05 ± 0.27 mg/dL), crizotinib (5 mg/kg, 1.78 ± 0.16 mg/dL), the combination (1.62 ± 0.22 mg/dL) and the control (1.94 ± 0.27) (p > 0.05). There were no significant differences in body weights between the groups and organ weights (as a percentage of the body weight) remained constant when compared to the control (p > 0.05). Metformin produced no liver or kidney toxicity as a single agent and in combination with crizotinib. This provides justification to move onto efficacy studies examining the effect of metformin alone and in combination in murine xenograft models of lung cancer.

P332 - 0526 Genome-Wide Screening of Formaldehyde Toxicity Using CRISPR-Cas9

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Formaldehyde (FA), the simplest aldehyde widely used in many consumer products and various industries, is a ubiquitous environmental pollutant. FA has been recently classified as a Group I human carcinogen by the International Agency for Research on Cancer. Previously, we reported that FA induced hematotoxicity and chromosomal aneuploidy in exposed workers as well as toxicities in bone marrow and hematopoietic stem cells of experimental animals. Using functional toxicogenomic profiling (loss-of-function screening) in yeast, we identified genes and cellular processes modulating FA cytotoxicity and reported FA-mediated damage to both DNA and RNA. However, specific gene pathways and mechanisms of action of FA in human cells remains unclear. Here, we apply CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats) to test cellular processes modulating FA toxicity in human K562 cells. We carried out a loss-of-function CRISPR screen using the genome-wide Brunello library to identify modulators of FA toxicity. We used sub-toxic doses of FA (40, 100, 150 µM) and assessed the cellular genetic requirements for FA susceptibility and resistance at two time points (day 8 and 20). Using bioinformatic technologies, we identified hundreds of candidate genes that increase sensitivity (e.g., ADH5 and FANCD1 family) or resistance (e.g., ADD2) to FA in K562 cells. Pathway analysis revealed a major role for DNA damage and repair in FA tolerance, as expected. Network analysis revealed additional biological functions and pathways that modulate FA toxicity. In addition, we will generate individual knockouts using CRISPR-Cas9 to validate the top candidates from screen. Our findings suggest that CRISPR-Cas9 genome-wide screening is a cost-effective functional approach for identifying genes and pathways involved in modulating the toxicity of FA and other environmental chemicals.

P333 - 0627 Inhibition of Pyruvate Kinase M2 Reduces Ovarian Cancer Cells Growth and Migration

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The "Warburg effect" is considered a vital hallmark of cancer cells, characterized by an altered metabolism, in which cells rely on aerobic glycolysis. There is evidence that cancer cells proliferation is controlled by expression of PKM2. However, the exact role of PKM2 expression in ovarian cancer cells has not been fully elucidated. The present study was focused on analysis of PKM2 expression

in 97 ovarian cancer cases, mostly of serous histological type. Moreover, relationships between inhibition of PKM2 and ovarian cancer proliferation were examined. The expression of PKM2 was disclosed only in cytoplasm of tumor tissues. In regard to available tissue microarray data, only an increasing trend of PKM2 expression with increasing grade of histological malignancy was found. In ovarian cancer cells, PKM2 inhibitor significantly inhibited the glycolytic rate according to cellular glucose consumption. However, no significant difference in oxygen-consumption rate in SKOV3 cells. Treatment with PKM2 inhibitor suppressed ovarian cancer growth and cell migration. Therefore, we suggest that targeting PKM2 may constitute a promising therapy for patients with ovarian cancer, and clinical trials involving PKM2 inhibitors are warranted.

PS P334 - 0633 Characterization of a Novel Oxygenating Therapeutic

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Ox66 is a novel therapeutic with possible uses in oxygenation of blood and tissues. However, the exact structure and mechanism by which the compound may be able to induce oxygenation is not yet understood. This study aims to understand the chemical structure and formula of Ox66, thereby opening the way to understanding the mechanisms by which oxygen could be released and absorbed following exposure to the drug particles. In order to elucidate chemical structure and activity, various analytical chemical techniques were used, including energy-dispersive X-ray spectroscopy, infrared spectroscopy, mass spectrometry, and X-ray crystallography. All analytical techniques used a nano-sized form of the particle with a particle size of around 600–700 nm. Solubility for purposes of mass spectrometry was achieved using a basic solution of ammonia, water and methanol. The results reveal that the compound is amorphous, is composed mainly of oxygen and aluminum, and contains a larger and more complex structure than simple aluminum compounds such as alumina, aluminum sulfate, or aluminum nitrate. Furthermore, when dissolved in a basic solution containing ammonia, the aluminum ions exhibit a dynamic chemical behavior where various ligands are exchanged off within a liquid. These two properties, complexity and chemical flexibility, point towards two possible hypotheses for the mechanism of oxygenation. The first hypothesis involves the trapping of oxygen within the structure of the drug particles, which may then be released under low partial oxygen pressure conditions. The second involves chemical exchanges between solution molecules and oxygen-containing ligands on the drug particles.

PS P335 - 0677 Knockdown of PKM2 Induces Autophagic Cell Death via Akt/mTOR Pathway in Human Prostate Cancer Cells

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Pyruvate kinase M2 (PKM2) is essential for aerobic glycolysis and is highly expressed in various cancer tissues. Although high PKM2 expression is observed in prostate cancer tissues, its functional role in cancer metabolism is unclear. Here, we investigated the role of PKM2 in regulating autophagy and its associated pathways in prostate cancer cells. PKM2 expression was silenced using various PKM2 small interfering RNAs (siRNAs) and then we measured PKM2-related cellular pathways associated with autophagy. PKM2 siRNA-transfected prostate cancer cells showed significantly reduced viability. Acridine orange staining and immunoblotting analysis showed that PKM2 downregulation markedly increased autophagic cell death. Results of western blotting analysis showed that PKM2 knockdown affected protein kinase B/mechanistic target of rapamycin 1 pathway, which consequently downregulated the expression of glycolytic enzymes lactate dehydrogenase A and glucose transporter 1. To the best of our knowledge, this is the first study to show that PKM2 inhibition alters cancer cell metabolism and induces autophagy. Thus, the present study provides a strategy for the development of PKM2-targeted novel anticancer drugs for the treatment of prostate cancer.

PS P336 - 0717 Establishment of Ionizing Radiation-Induced Human Umbilical Vein Endothelial Cells Aging Model

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The damage of ionizing radiation (IR) is mainly manifested in the breakage of DNA chain and the cell structure destruction. They cause the destruction and imbalance of intracellular homeostasis by the accumulation of DNA damage and the reduction of DNA damage repair efficiency. It is often accompanied by the weakening of cell proliferation and differentiation, which is usually considered to be cell aging. In recent years, the radiation damage caused by ionizing radiation has been concerned in the related fields of cardiovascular system. At present, it is widely believed that endothelial cells are the most sensitive parts of the blood vessel wall components. In this paper, we established a cell aging model by changing the phenotype of cells under different dosage of ionizing radiation. First, the human umbilical vein endothelial cells (HUVEC) were divided into three groups: control (0Gy, untreated), medium dosage irradiation group (4Gy) and high dose irradiation group (8Gy). After irradiation, the cell samples were collected. The expression of p53, p16 and p21 in human umbilical vein endothelial cells were detected with WB, cell proliferation was determined with CCK-8 and cellular senescence was examined with SA- β -gal staining. The results showed that the cell proliferation rate of the irradiated group was significantly less than that of the normal group, and the results of beta galactosidase staining showed that the number of senescent cells in the irradiated groups were more than that of the normal control group. The expression of cell senescence associated protein p21, p16 after irradiation was earlier than that in control group and increased. It suggested that ionizing radiation can induced aging of human umbilical vein endothelial cells.

PS P337 - 0780 Role of Advanced Glycation End-Products in Diabetes-Associated Pancreatic Islet Endothelial Cell Fibrosis

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The intra-islet vascular endothelial cells (ECs) play an important role in β -cell function. An intra-islet endothelium dysfunction has also been implicated to contribute to the progression of diabetes. Fibrotic islet destruction has been suggested to be one of the pathogenic mechanisms of impaired β -cell growth and function during diabetic condition. Advanced glycation end-products (AGEs) are implicated to be associated with pathogenesis of diabetic vascular complications. The experimental evidence suggested that endothelial-to-mesenchymal transition (EndoMT) plays a role in the pathogenesis of fibrotic diseases. The fibrotic effect of AGEs on islet ECs and the role of EndoMT on islet fibrosis remain unclear. Here, we investigated the effects of AGEs on EndoMT and fibrosis in islet ECs. Mouse pancreatic islet endothelial cells (MS1) were cultured and treated with AGEs (10–50 μ g/ml) for 48 h. In some experiments, the streptozotocin-induced diabetic mice or *db/db* diabetic mice were used. The protein expressions were determined by Western blot or immunohistochemistry. We found that the immunoreactivities for AGEs and α -smooth muscle actin (α -SMA) and fibrosis were markedly increased in the diabetic mouse islets as compared with control mice. Non-cytotoxic concentrations of AGEs up-regulated the expressions of receptor for AGEs (RAGE), fibronectin, connective tissue growth factor, α -SMA, vimentin (EndoMT marker), and phospho-Smad2/3, and down-regulated E-cadherin (EndoMT marker) and CD31 expressions in MS1 cells, which could be significantly reversed by RAGE neutralizing antibody or AGEs cross-link breaker alagebrium chloride ($p < 0.05$ vs control or AGEs alone, $n = 5$ /group/experiment). These results demonstrate for the first time that AGEs-RAGE axis is capable of inducing EndoMT and fibrosis in islet ECs. These findings suggest that AGEs-induced EndoMT may be an important mechanism in the pathogenesis of diabetic islet fibrosis.

P338 - 0798 Determination of the Antimicrobial Activity of Nine Brands of Toothpaste on *Streptococcus mutans* Isolated from the Mouth of Students in Veritas University, Abuja, Nigeria

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Dental caries is an infectious microbiological disease of the teeth that ends up in the destruction of dental hard tissues. The use of toothpaste has been used to reduce the bacterial load in the mouth because they contain antimicrobial properties such as triclosan, fluoride, and so on. The aim of this study was to determine the effect of nine brands of toothpaste on *Streptococcus mutans* isolated from the mouth of students in Veritas University. Following isolation of *Streptococcus mutans* from samples collected from the mouths of Veritas students, the antimicrobial activity of different concentrations of the dentifrices was determined by modified agar well diffusion method. In this method, Muller Hinton agar plates were seeded with a loop full of 24 hour cultures of the bacterial isolate. A sterile 8 mm cork-borer was used to cut one central and five wells at equidistance in each of the plates. 0.3 ml of the dentifrice dilutions was introduced into each of the five wells while the same amount of sterile distilled water was introduced into the first well as control. The plates were incubated at 37°C for 24 hours. The antimicrobial activity was evaluated by measuring the diameter of zones of inhibition (in mm). The diameter in which the bacteria were inhibited was indicative of the toothpaste's antibacterial potential on the specific bacteria. The zones of inhibition on the growth of the test strain were defined by the location where visible growth had been inhibited. All the plates were made in triplicates and the experiments repeated twice. The result showed that Mentadent-P was the most effective dentifrice with an inhibition zone of 2.8 mm, Pepsodent had inhibition zone of 2.6 mm, Oral B with 2.3 mm, Close up with 2.2 mm, followed by Colgate with a diameter of 1.9 mm, Special Lucky with a diameter of 1.6 mm, Darbur promise with a diameter of 1.6 mm, MacLean's with a zone of inhibition of 1.5 mm, and MY-MY with the least effective toothpaste with a zone of inhibition of 1.0 mm in diameter. The results also showed that toothpaste containing fluoride as their main antimicrobial agent proved more effective against *Streptococcus mutans* than other toothpaste not containing fluoride as their main antimicrobial agent.

P339 - 0814 Nanofibers as Membrane for Extraction β -Lactam Antibiotics in Urban River Followed by Determination with Capillary Electrophoresis

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A solid phase membrane adsorbent, Nylon 6 nanofibers membrane coated by polypyrrole (PPy-PA6-NFsM), is firstly synthesized and used for extraction of pollutant, which are two β -lactam antibiotics (oxacillin and cloxacillin) in urban river water. The analytes were then detected by high performance capillary electrophoresis with a diode array detector (HPCE-DAD). The experimental conditions were optimized, including the amount use of PPy-PA6-NFsM, pH of the sample solutions, adsorption volume and desorption conditions. Under the optimal extraction and separation conditions, the detection limits is found to be 2.0 ng/mL for both oxacillin and cloxacillin. The linear calibration curves were established in a range of 5.0-250.0 ng/mL. The proposed method was applied to determinations of the two β -lactams in water samples of urban river. The recoveries of these two β -lactams were found to be in a range of 84.2-96.4%, demonstrating that PPy-PA6-NFsM has a high extraction capability for these two antibiotics. The relative standard deviations, ranging from 2.26% to 5.29% for intraday measurements and from 2.38% to 7.02% for inter-day determinations, were derived respectively. The obtained data provide a sensitive analytical method for the detection of toxic substances in water.

P340 - 0888 The Use of Complementary and Alternative Medicine by Cancer Patients in Libya: Beliefs versus Chemotherapy

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There is a widespread use of complementary and alternative medicine (CAM) in Libya which is mainly due to two factors, the first is the firm beliefs that are inherited from one generation to another, believing that CAM plays an important role in cancer treatment. The second reason is economic hardship, because of the war and instability conditions in Libya, the prices of medicines have increased dramatically so the patient looks for a cheaper alternative. In this study, through a questionnaire we look at the beliefs of patients and the most famous herbs that they used to cure cancer from the patients' point of view." The study was conducted in 2018 as a descriptive study with 430 cancer patients attending Oncology Clinic in Benghazi Medical Center (BMC). Benghazi, Libya. 68.3 % of patients believed that CAM was the solution to their treatment from cancer. 31.7% didn't use CAM even though they believed in them but they followed their doctor advice to avoid them during chemotherapy. In conclusion, the use of CAM is widely spread through most of the Libyan society, people have to be more educated about it and possible side effect also more researches and trials are needed to study the popular herbs and their effects and interaction with cancer treatments.

P341 - 0890 Microplastics (MPs): Occurrence and Characterization Using Fourier Transform Infrared Spectroscopic Method Coupled with Attenuated Total Reflectance (FTIR-ATR)

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Lagos Lagoon is one of the most affected by plastic wastes floating on the surface water; nevertheless little or no studies were executed on the occurrence and chemical characterization of microplastics. Data acquired from the field survey between 2016 and 2017; suggests that microplastics particles are widespread in Lagos Lagoon especially in the sampling area. The highest peak occurrence of microplastics (3322.6particles/L) was identified in commodore channel of the Lagos Lagoon. Microplastics litters were classified into five morphological categories (Pellet, fiber, fragment, foam and film) composed mainly of polyethylene (53%), polyvinyl chloride, polystyrenes, polypropylene, polyethylene terephthalate, and polyamide, were present in all plankton tows. Distribution of microplastics within the Lagos lagoon showed that fragments were the most abundant among the microplastics morphology. The mean ratio of 8 to 0.43 was observed between microplastic particles and zooplankton organisms; among which *Acartia nauplius* was the most abundant during the entire field survey. Using the ANOVA analysis on the plastic polymer categories shows significant difference ($P < 0.05$) between all the classifications expect in foam. Duncan multivariates shows the mean significant difference ($P < 0.05$) among the sampling area. Due to magnitude of the plastic disintegration in the coastal environment, microplastics may be ingested by different organisms usually untouched by most of the coastal debris. The occurrence, distribution and magnitudes of the potential impact of microplastics on the aquatic ecosystem and their possible interactions with the aquatic fauna need to be better evaluated.

P342 - 0891 Expression of miRNA Related to Polyhexamethylene Guanidine Phosphate-Induced Epithelial-Mesenchymal Transition (EMT) in A549

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In 2011, a significant number of South Koreans died of pulmonary disease caused by humidifier disinfectants. Many researchers have reported that polyhexamethylene guanidine-phosphate (PHMG-p), which is a major component of the humidifier disinfectant, causes pulmonary fibrosis. Accordingly, there have been many studies on pulmonary fibrosis, which is mainly caused by ep-

ithelial mesenchymal transition (EMT), through a process of losing epithelium and gaining the properties of mesenchymal cell. Moreover, the role of miRNAs in the pathogenesis of disease has been studied extensively in order to investigate the function of miRNAs that regulate the gene expression post-transcriptionally. Nevertheless, the role of miRNAs in EMT is largely unknown. Therefore, we investigated how miR-33b-3p, which decreased significantly in PHMG-p treatment, acts on EMT in order to elucidate the pathogenesis of pulmonary fibrosis. This research is conducted on human epithelial cell (A549 cell) in order to examine the function of miR-33b-3p as an EMT inhibitive factor. Firstly the expression of miR-33b-3p was confirmed by microRNA array and validation was confirmed by real-time PCR. The result that overexpression of miR-33b-3p changes EMT markers induced by PHMG-p treatment was confirmed by real-time PCR and western blot. In addition, cell migration during upregulation of miR-33b-3p was measured by transwell chamber assay and wound-healing assay. The changes in migration-related genes, matrix metalloproteinase (MMP-2) and MMP-9, were also confirmed. After the exposure of PHMG-p for 48 hours, it was confirmed that the treatment with miR-33b-3p mimic (10 μ M) 2.2 μ L attenuated the decreasing levels of E-cadherin, which is an epithelial cell marker, by 2.06-fold and the increasing levels of α -SMA, which is a mesenchymal cell marker, by 0.4-fold compared to the miR-NC transfected cells at the level of protein expression. Furthermore, the overexpression of miR-33b-3p resulted in an inhibition of PHMG-p-induced EMT at the level of mRNA expression. These results demonstrate that miR-33b-3p inhibits PHMG-p-induced EMT through the process of increasing E-cadherin expression and suppressing α -SMA expression. The overexpression of miR-33b-3p resulted in an inhibition of protein expression of MMP-2 and MMP-9 to 0.75-fold and 0.54-fold, respectively. In transwell migration assay, the number of migrated cells was significantly lessened to 0.7-fold in the miR-33b-3p group compared to the miR-NC group. Thus, miR-33b-3p was involved in EMT and migration induced by PHMG-p. A further research is needed to determine exactly which pathway miR-33b-3p inhibits to the PHMG-p induced EMT.

P343 - 0902 4a,5a-Oxidoecdysm-11-en-3-one, a Potential Selective Estrogen Receptor Modulator from *Cyperus rotundus*

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Phytoestrogens are non-steroidal, plant-derived compounds and feature structures resembling estrogen. The classical phytoestrogen groups are flavonoids, lignans, coumestans, and stilbenes, all of which exhibit various estrogenic activities. Estrogenic activity-oriented fractionation and purification of methanol extract from the rhizome of *Cyperus rotundus*, a well-known traditional herbal medicine, led to the isolation of six sesquiterpenes. 4a,5a-Oxidoecdysm-11-en-3-one (**1**) and cyper-11-ene-3,4-dione (**2**) together with four known sesquiterpenes, cyperotundone, caryophyllene α -oxide, α -cyperone, and isocyperol were obtained from the hexane and dichloromethane fractions. Compounds **1** and **2** were newly isolated from natural resources in particular. To identify the possible use of isolated compounds as an alternative to hormone replacement therapy (HRT), estrogenic activity was evaluated by E-screen assay on MCF-7 BUS cells. Among the all isolated compounds from the rhizome of *Cyperus rotundus*, newly isolated from natural resource, **1** exhibited the most potent estrogenic activity. In an estrogen sensitive reporter gene assay, **1** significantly increased transcriptional activities. As a phytoestrogen, **1** was assessed by investigating dual action on ER- α and ER- β in competitive binding assay. It was found that **1** exerted higher binding affinity to ER- β than ER- α and it also showed both estrogenic and antiestrogenic effects depending on the E2 concentration. 4a,5a-Oxidoecdysm-11-en-3-one was identified as a potent phytoestrogen despite not being a member of a well-known phytoestrogen compound family. Our results indicate that newly isolated from *Cyperus rotundus*, 4a,5a-Oxidoecdysm-11-en-3-one (**1**) has biphasic activities on estrogen receptors which could be useful as an alternative HRT.

P344 - 0912 High-Throughput System for Profiling of Epithelial Damage Induced by Fibrotic Agents

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Epithelial cells play an important role in fibrogenesis through several damages including the disruption of the epithelial lining, the alteration in the production of mediator, and the induction of the epithelial-mesenchymal transition. However, it is still unknown which specific genes are related to epithelial dam-

ages caused by fibrotic chemicals. To find the specific gene, we established a screening system of specific gene using high-throughput screening (HTS) and siRNA library associated with apoptosis. Three conditions were determined to set up HTS protocol; type of cell lines used, transfected concentration of siRNA, and treated concentration of fibrosis-induced chemicals (PHMG, bleomycin, and paraquat). Allstars cell death siRNA which induces cell death was transfected to three kinds of epithelial cell lines. Then, WST-1 assay was conducted to evaluate the transfection efficiency, which indicating that beas-2B cells have the highest transfection efficiency. Optimal transfected concentration of siRNA was determined using siGLO Green Fluorescence Transfection Indicator. Fluorescence siRNA was treated from 4 nM to 25 nM and fluorescence was observed by In Cell Analyzer 2200. When 25 nM of siRNA was transfected, fluorescence value was the highest. Using WST-1 assay, LC50 of PHMG, bleomycin, and paraquat were determined 1.5 μ g/mL, 22 μ g/mL, 155 μ M, respectively. In conclusion, we established the protocol that find apoptosis-associated specific gene using the HTS. Understanding the network of epithelial damage related genes is essential to identifying the toxic mechanism of lung fibrosis induced by chemicals. It is needed to screen the key gene via this platform using HTS and siRNA library. *Funding Source: This work was supported by Korea Environmental Industry and Technology Institute (no. 2018002490005). Disclosure Statement: None of the authors have any conflicts of financial interest to declare.*

P345 - 0923 Inhibitory Effects of *Euphorbia tirucalli* lineu (Euphorbiaceae) Diluted Latex on Human and Canine Melanoma Cells

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Euphorbia tirucalli Lineu (Euphorbiaceae) (ET) latex is popularly used to treat cancer. This study aims to evaluate the effects of the diluted ET latex on human and canine melanoma cells. SK-MEL 28 human and CBMY canine melanoma cells were used. CBMY is a canine buccal melanoma cell line, created at SVMAS - USP, and properly characterized by karyotyping and immunohistochemistry. Cells (3x10³ SK-MEL 28 cells/well and 6x10³ CBMY cells/well) were cultured in 96-well plates. After 24, 48 and 72 hours, cells were treated with 50 μ L/well of further dilutions (1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512) of a standard solution containing 1 mg/mL of the ET latex in DMEM. Control group received 50 μ L/well of DMEM. After 24, 48 and 72 hours, cell viability was assessed through MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. There was significant decrease in both human and canine melanoma cell viability at 24, 48 and 72 hours after treatment mainly in higher dilutions of ET latex. The inhibitory effects of ET latex in high dilutions were similar for SK-MEL-28 and for CBMY melanoma cells. We conclude that highly diluted ET latex exerts hormetic effects similarly on canine and melanoma cells at higher dilutions, and further studies are necessary to understand the mechanisms underlying such inhibitory effects.

P346 - 0926 Exosomes Purified from Bronchoalveolar Lavage Fluid of Mice Instilled with Polyhexamethylene Guanidine Phosphate Induce Inflammation in Naive Macrophages

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Polyhexamethylene guanidine-phosphate (PHMG-p), which damages lung epithelial cells and activates macrophages after being inhaled as humidifier disinfectant, has been investigated to induce lung inflammation and fibrosis. Although lungs are known to contain more than 50 kinds of cells and cell-cell communication is important to explain the progress of pathology, there was few research related to the interaction between cells when exposed to PHMG-p. Exosome, one of the extracellular vesicles, contains various substances such as proteins, RNAs, and DNAs and plays an important role in communication between cells. In lungs, exosomes derived from lung epithelial cells and macrophages exist in bronchoalveolar lavage fluid (BALF) and transfer the cargo substances to recipient cells followed by signalings of pathogenesis. Therefore, in this study, we aimed to identify the effect of exosomes in BALF of mice instilled with PHMG-p to the naive macrophage. A week after instilling PHMG-p (1.2 mg/kg) to the mice trachea, BALF was obtained by injecting and aspirating the PBS to the trachea. After removing cells and debris through the differential centrifuge, exosomes were isolated by using ExoQuick. The isolated exosomes were characterized by dynamic light scattering (DLS), transmission electron microscope (TEM) and western blot for exosomes markers, TSG-101 and CD9. The cells in the BALF of mice were counted after Giemsa and Wright

staining. The inflammation response of naive macrophages was confirmed with western blot after treating exosome 10 μ g for 24 hours. An increase of foamy macrophage was observed in mice pulmonary histopathology after 7 days of PHMG-p exposure. Moreover, the number of cells in BALF of PHMG-p-treated mice were significantly more than one of control mice. The average size of exosomes was measured as 201.2 nm with DLS. The size of cup-shaped vesicles ranged from 70 to 160 nm by TEM. The purity of exosomes purified balf was confirmed by above methods. When naive macrophages were treated with exosome phosphorylated p65, subunit of NF- κ B, was increased. Thus, it was elucidated that BALF exosomes from PHMG-p-treated mice could induce inflammation response. For the further research, it is needed to demonstrate which substances in the exosome cause this effect in PHMG-p treatment.

P347 - 0927 The Role of miR-6126 Upregulated by Polyhexamethylene Guanidine Phosphate in Lung Fibrosis via Targeting PCDH9

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Since 2011, a component of humidifier disinfectants, polyhexamethyleneguanidine-phosphate (PHMG-p) has been investigated as a major causative agent of lung fibrosis. When human alveolar epithelial cell, A549 cell was exposed to PHMG-p, it caused epithelial-to-mesenchymal transition (EMT). Here, EMT is the process that epithelial cells transit to mesenchymal cells, which is associated with a lung fibrosis mechanism. In the previous research, it was identified that PHMG-p induced EMT and upregulated miR-6126 in A549 cell. As a further study, we aimed to elucidate relevance of EMT and miR-6126 upregulation which is induced by PHMG-p. With PHMG-p repeated exposure (0.1 mg/kg, 6 times for 4 weeks) to rat, lung histology (*in vivo*) was observed. To identify PHMG toxicity in A549 cell (*in vitro*), 4 μ g/mL of PHMG-p was exposed to A549 cell and induction of EMT was checked by real-time PCR and western blot. In the same condition, upregulation of miR-6126 was quantified in real-time PCR. To demonstrate the effect of miR-6126 to EMT, miR-6126 inhibitor was transfected to A549 cell. It was identified that PHMG-p induced lung fibrotic pathology in rat and EMT like increased α -SMA and decreased E-cadherin in A549 cell. miR-6126 level was upregulated in repeated exposure of PHMG-p to rat and A549 cell. After transfection of A549 cell with miR-6126 inhibitor, 2 μ g/mL of PHMG-p was exposed. We identified that miRNA-6126 increased expression of mesenchymal marker, α -SMA in quantitative real-time PCR. To identify regulation of various signaling pathway by miR-6126, human phosphokinase assay was performed. We exposed 2 μ g/mL of PHMG-p to A549 cell transfected with miR-6126 mimic or inhibitor. As a result, MAPK pathway, EGFR, and Akt pathway protein expression level increased in miRNA-6126 mimic transfection, but in inhibitor, reactivity was opposite. Using miRNA analyzing program, targetscan, we identified that upregulation of miR-6126 significantly decreased protocadherin9 (PCDH9). PCDH9 silencing in A549 cell induced a part of MAPK pathway, EGFR, and AKT pathway upregulation. It was similar with protein expression level of miRNA-6126 upregulated A549 cell. Thus, we decided that miR-6126 upregulation by PHMG-p caused upregulation of EMT and various signaling pathway which could be associated with EMT. Putting together all the data, we suggested that humidifier disinfectant's major component, PHMG-p induces EMT via upregulation of miR-6126 and miR-6126 putatively targets PCDH9.

P348 - 0929 Polyhexamethylene Guanidine Phosphate Induces Endothelial-Mesenchymal Transition in EA.hy926 Cells

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In South Korea, 2011, humidifier disinfectants caused many cases of idiopathic pulmonary fibrosis (IPF), fatal lung disease. As a result of following researches, major component of humidifier disinfectant, Polyhexamethyleneguanidine-phosphate (PHMG-p) was considered to cause IPF via epithelial-to-mesenchymal transition (EMT) inducing mechanism. But, the effects of endothelial-to-mesenchymal transition (EndMT) in lung fibrosis are not defined well now. Because lung alveolar cells are surrounded by capillaries which is composed of endothelial cells, transition of endothelial cells to mesenchymal cells can be one of the toxicity mechanism of PHMG-p in lung fibrosis. We designed mono and tetra-culture model to figure out PHMG-p toxicity in EA.hy926 cells. Mono culture model was directly exposing EA.hy926 cells to PHMG-p in 24 - 72 h with concentration of (0.5 - 4 μ g/mL) to elucidate protein and mRNA EndMT marker expression level using western blot and quantitative real-time PCR, respec-

tively. To study more similar toxicity of PHMG-p with human lung, tetra-culture model mimicking lung environment was used. Tetra culture consisted of apical side, human mast cells (HMC-1), macrophage cells (THP-1), lung epithelial cells (Calu-3) and basolateral side, endothelial cells (EA.hy926) in air-liquid interface. PHMG-p (4 - 16 μ g/mL) was treated to apical side for 24h. After that, PHMG-p toxicity to apical side indirectly influencing on basolateral side was identified as protein marker expression of basolateral side EndMT markers. In mono culture model, PHMG-p (4 μ g/mL) exposure significantly increased mesenchymal markers and decreased endothelial markers in protein expression level for 48 h. mRNA expression level of mesenchymal markers and endothelial markers was significantly responsive for 72 h. In tetra-culture model, mesenchymal markers and other markers like CTGF, PDGF, collagen 1 significantly increased in concentration of 16 μ g/mL. Thus, we identified EndMT expression with treatment of PHMG-p elucidating by altered expression of mesenchymal markers, α -sma, collagen 1 and endothelial markers, CD31, VE-cadherin. In conclusion, PHMG-p toxicity to endothelial cell was demonstrated in regard of EndMT markers expression level of mRNA and protein markers. Hereby, we suggested that PHMG-p can induce EndMT which can cause lung toxicity.

P349 - 0115 Development of Software for Facilitating Quality Control of POPs Detection in Food and Animal Feeds

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Persistent organic pollutants (POPs) are a significant public and environmental health concern due to their toxicity, long-range transportability, persistence, and bioaccumulation in animals. Exposure to these pollutants may cause adverse health effects such as cancers, diabetes, birth defects, and dysfunctional immune and reproductive systems. Consequently, to help protect and promote human health, it is important to monitor POPs in food and the environment. Gas Chromatography (GC) coupled with mass spectrometry (MS) has been widely applied for determining trace concentrations of organics in food and environmental matrices. In the US Food and Drug Administration (US FDA), GC/MS has been used for quantitative determination of POPs in foods and animal feeds. Stringent quality control procedures are needed to ensure reliability and accuracy of POPs detected using GC/MS. However, quality control procedures data processing is very time consuming. To improve efficiency, we developed software to automate and execute the US FDA quality control protocol. The outcome ensures instrument optimization and usability of the data. Additionally, the software provides dynamically updated historical reference data for improving quality control. We tested the software on a diverse set of real samples by comparing quality control measurements determined using the software with those evaluated by expert analysts. The tests confirmed that the software meets US FDA requirements and provides reliable quality control of POPs' GC/MS analytical data. In conclusion, the software improved the efficiency of the analytical process by reducing the need for user intervention and the time for data analysis of POPs in foods and animal feeds.

P350 - 0214 Using Gene-Network Analysis and Cell Model to Investigate the Effect of Dioxin Exposure on Inflammasome Activation and Lymphomagenesis

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Dioxins are a group of persistent organic compounds containing polychlorinated biphenyl structures and have been known able to activate aryl hydrocarbon receptor (AHR) and regulate inflammation in immune responses. NLRP3 inflammasome is a multiprotein signaling complex for controlling the inflammatory response and leading to pyroptosis, which is involved in the pathologic process of cancers. Even epidemiological studies have implicated environmental exposure to dioxins is correlated with non-Hodgkin's lymphoma (NHL). However, it is not clearly known whether exposure to dioxins could initiate inflammasome activation and lead to the genotoxicological effect on lymphomagenesis. First of all, this study used the Cancer Genome Atlas (TCGA) to perform the analysis in differentially expressed genes (DEGs) of diffuse large B-cell lymphoma (DLBCL), the most common type of NHL, and their association with DEGs of human cells exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; the most toxic dioxin). From the gene database analysis, the genes affected by DLBCL were similar to those genes of human cells exposure to TCDD. Additionally, HL-60 cells exposure to TCDD showed that increased

mRNA expression of AhR, TWIST1, CYP1A1, CYP1B1, Caspase-1, Caspase-3, IL-18, GSDMD, and NLRP3 genes, and AhR inhibitors can reduce the increase in the expression of these genes. It suggested that TCDD could enter the nucleus through the binding of AhR and initiate the expression of cytochrome CYP1A1 and CYP1B1 by binding to XRE and the formation of NLRP3 inflammasome, which might activate caspase-3 to promote natural apoptosis, and on the other hand, promote the caspase-1 to cleavage GSDMD and induce the secretion of the inflammatory cytokine IL-18 for pyroptosis. Furthermore, study subjects with higher exposure concentrations of dioxins presented higher gene expression of TWIST1, which has been known involved in epithelial-mesenchymal transition, invasion, and migration in tumor cells. In this study, a bioinformatic analysis was used to analyze the genomic database of DLBCL lymphoma and dioxin exposure for exploring the gene regulatory networks and target genes related to lymphomagenesis potentially underlying dioxin exposure. These candidate target genes for lymphomagenesis were identified to provide a reference for disease prevention and treatment of cancer.

P351 - 0222 Chronic Exposure to Low-Level Organochlorine Pesticides (OCPs) Mixture Causes Type 2 Diabetes-Related Metabolic Dysregulation in Zebrafish Model

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Organochlorine pesticides (OCPs) have been known to increase a risk of metabolic syndrome such as type 2 diabetes (T2D)^{1,2}. Experimental evidences on metabolic effects by chronic exposure to low-level OCPs mixture were limited because most studies focused on individual compounds, high level, and short-term exposure³. To investigate metabolic dysregulation by OCPs mixture (i.e., p,p'-DDT, Chlordane, Heptachlor, beta-HCH [beta-Hexachlorocyclohexane], Hexachlorobenzene [HCB]), female and male zebrafish adults were exposed to 0.05, 0.25, 2.5, and 25 µg/L for three months in automatic flow-through system. After exposure, we measured hematological parameters (i.e., fasting glucose [mg/dL], fasting insulin [mIU/mL], triglyceride [ng/mL], and free fatty acid [ng/mL]), and analyzed mitochondrial complex function, which is associated with impaired metabolism, in zebrafish plasma and liver, respectively. In addition, the alteration on the molecular level was assessed by multi-omics analysis (i.e., transcriptomics, proteomics, and metabolomics) in order to identify specific pathway involved in metabolic dysregulation. In our results, the significant phenotypic effects, including blood indexes (i.e., glucose and insulin level), were not observed at 0.5 to 25 µg/L, but only at 0.05 µg/L, nonmonotonically. These results corresponded with reduced oxidative phosphorylation activity on mitochondrial, in particular, complex III, and a different expression pattern of RNAs, proteins, and metabolites related to metabolic pathway. Collectively, we confirmed the flexibility of zebrafish model for a mechanistic study and demonstrated that long-term exposure of low-level OCPs mixture can induce metabolic adverse effects in a nonmonotonic manner and have the potential to increase the risk of developing metabolic syndrome. *References: [1] Lee D.H., Porta M., Jacobs D.R. Jr, Vanderberg L.N., 2014. Chlorinated persistent organic pollutants, obesity, and type 2 diabetes. Endocr. Rev. 35(4), 557-601. [2] Lowell B.B., Shulman G.I., 2005. Mitochondrial Dysfunction and Type 2 Diabetes. Science. 307(5708), 384-387. [3] Lukowicz C., Ellero-Simatos S., Régnier M., Polizzi A., Lasserre F., Montagner A., Lippi Y., Jamin E.L., Martin J.F., Naylies C., Canlet C., Debrauwer L., Bertrand-Michel J., Al Saati T., Théodorou V., Loiseau N., Mselli-Lakhal L., Guillou H., Gamet-Payastre L., 2018. Metabolic Effects of a Chronic Dietary Exposure to a Low-Dose Pesticide Cocktail in Mice: Sexual Dimorphism and Role of the Constitutive Androstane Receptor. Environ. Health. Perspect., 126(6), 1-18.*

P352 - 0401 Decabromodiphenyl Ether Impairs Glucose Homeostasis in Mice Fed a High-Fat Diet

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Polybrominated diphenyl ethers are additive flame retardants that are widely used in plastics, textiles, adhesives, sealants, coatings and inks. It has become a ubiquitous environmental contaminant and is also present in humans and wildlife. Decabromodiphenyl ether (decaBDE) is a brominated flame retardant used in plastic and textile articles and is found in house dust, sediment, and in the diet, particularly seafood. Although decaBDE has the potential to disrupt the endocrine, nervous and immune systems, the association between

decaBDE and obesity remains unclear. Therefore, we investigated whether oral exposure to decaBDE contributes to initiation and progression of obesity and obesity-related metabolic dysfunction in mice fed a normal diet (ND) or a high-fat diet (HFD). Mice were fed a HFD (62.2 kcal% fat) or a ND (9.0 kcal% fat) from 5 to 20 weeks of age. Mice were exposed to the equivalent of three doses of 0, 0.5 (L-DecaBDE), and 10 (H-DecaBDE) µg/kg/day via drinking water from 5 to 20 weeks of age. Body and tissue weight, biochemical blood tests, and gene expression profiles in liver, skeletal muscle, and adipose tissue were examined. In HFD-fed mice, decaBDE exposure markedly increased both fasting blood glucose levels, which was more prominent in H-DecaBDE-exposed mice. DecaBDE exposure significantly decreased mRNA levels of glucose transporter 4 and thyroid hormone receptor alpha in skeletal muscle and mechanistic target of rapamycin complex 2 in brown adipose tissue compared with vehicle exposure under HFD-feeding. The tendency for hyperglycemia and the remarkable activation of insulin signaling pathway-related genes were observed in ND + DecaBDE mice compared to the ND + Vehicle mice. These results suggest that decaBDE can contribute to enhancement of diet-induced hyperglycemia through disruption of glucose homeostasis.

P353 - 0453 Assessment of Polychlorinated Biphenyls (PCBs) Levels in Surface Water, Sediment, Parasitic Fauna, and Bagrid Catfish, *Chrysichthys nigrodigitatus*, as an Index of Pollution of a Tropical Lagoon

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The unregulated and continuous released of waste laden with persistent bio-accumulative pollutants into aquatic ecosystems has been a major concern due to their genotoxic, carcinogenic, mutagenic and teratogenic potentials. PCB contaminants in fish act as indicators of pollution in aquatic ecosystems and are a potential threat to human health when consumed. The study assessed the risk associated with levels of twelve PCB congeners (PCBs 8, 18, 28, 44, 52, 60, 77, 81, 101, 123, 153, and 156) in water, sediments, parasitic fauna and 150 samples of adult fish *Chrysichthys nigrodigitatus* from Epe Lagoon, Nigeria, following determination with gas chromatography / mass selective detector (GC/MSD). The concentration of total PCBs ranged from 3.2 to 6.0 ppb, 405.5 to 860.7 ppb, 105.0 to 165.4 ppb and 155.4 to 257.7 ppb in surface water, sediment, parasite fauna and fish respectively. The recorded mean contents of PCBs in fish above the World Health Organization limit of 200ppb indicate great health risk to consumers. Parasitological investigations showed that nematodes (*Procamallanus* sp.) and cestodes (*Diphylllobothrium* sp.) with a combined prevalence of 21.33% were the dominant species of parasites while histopathological assessment revealed alterations such as necrosis, villous collapse, hyperplasia, and accumulation of inflammatory debris within the lumen. The present study emphasises the deteriorating water quality of Epe Lagoon and the potential of fish parasitic fauna as bioindicators of PCBs contaminated ecosystems in the area.

P354 - 0463 Comparative Assessment of Per- and Polyfluoroalkyl Substances (PFASs) on Pancreatic Toxicity

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Per- and polyfluoroalkyl substances (PFASs) are used in a variety of industrial applications including production of surface repellant coatings, fire-fighting foams, and fluoropolymer synthesis. Proposals to eliminate the production and use of the legacy PFASs, perfluorooctanoic acid (PFOA), and perfluorooctanesulfonic acid (PFOS), have been put into place due to their environmental persistence, bioaccumulation in the food chain and toxicity in multiple organ systems, including the pancreas. We have previously shown that a 7 day exposure to PFOA in mice triggers ductal hyperplasia, oxidative stress and inflammation in the mouse pancreas. In addition, we have shown that PFOA induces endoplasmic reticulum (ER) stress, triggering the unfolded protein response (UPR) in the mouse pancreatic acinar cell line 266-6 as well as *in vivo* in the mouse pancreas. To address the biopersistence of the legacy compounds, alternative PFASs have been developed. While the toxicity profiles of the legacy compounds have been studied, little is known of the potential toxicities caused by exposure to the alternative products, for which environmental contamination has been documented in the United States, Europe and Asia. In addition, measurable levels of several of these compounds have been documented in wildlife and humans. Here, in comparison to PFOA and PFOS, we evaluated the

effects of three PFAS alternative compounds for their overall cytotoxicity and ability to trigger the UPR in the mouse pancreatic acinar cell line 266-6: ammonium perfluoro(2-methyl-3-oxahexanoate) also known as GenX (CAS# 62037-80-3), perfluoro(2,5-dimethyl-3,6-dioxanonanoic acid) also known as HFPO-TA (CAS# 13252-14-7), and perfluoro(2,5,8-trimethyl-3,6,9-trioxadodecanoic) acid also known as HFPOTeA (CAS# 65294-16-8). In comparison to PFOA and PFOS, GenX and HFPO-TeA exhibited less effects on cell viability while HFPO-TA caused the most cell lethality. This same rank order was observed when stimulation of the UPR was monitored by ATF4 and CHOP mRNA induction. As ER and oxidative stress have been linked to chronic diseases of the pancreas such as diabetes and cancer, our findings suggest that further toxicological evaluation of the replacement PFAS compounds is warranted.

P355 - 0465 Coupling Transcriptomics and Developmental Toxicity in Zebrafish to Classify Polycyclic Aromatic Hydrocarbons (PAHs)

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Polycyclic Aromatic Hydrocarbons (PAHs) are diverse and ubiquitous environmental pollutants that almost always occur as complex mixtures. Several PAHs are known or suspected mutagens, but developmental toxicity data are lacking. It is not yet possible to predict developmental outcomes following PAH mixture exposure. It is also often incorrectly assumed that PAHs have a common mechanism of action. Before effective mixture models can be made, we must understand the mechanisms of action of individual PAHs. We applied a systems biology approach in zebrafish to evaluate, classify, and define mechanisms of PAH toxicity. Using 22 morphological endpoints, 2 behavioral assays, and localization of Cyp1a protein expression, we evaluated the developmental toxicity of 123 PAHs. Based on responses ranging from complete inactivity to acute mortality, we chose 16 representative PAHs and developmentally exposed zebrafish to the concentration that induced 80% phenotypic effect. These animals were used for transcriptomics to identify genes altered as a result of PAH exposure. 6 of the 16 PAHs altered transcription of only a few genes (≤ 10 genes, FDR $p < 0.05$), while the 10 others altered the expression of many more genes. The incidence of developmental toxicity correlated positively with the incidence of altered expression, for example: anthracene did not induce phenotypic responses or Cyp1a expression, and significantly altered the expression of only 1 gene, while dibenzo[a,h]pyrene induced behavioral responses and in situ Cyp1a protein expression in four larval body regions, and significantly altered the expression of 236 genes. Using the Context Likelihood of Relatedness (CLR) program, we inferred a network that links the PAHs based on coordinated responses of genes that are altered as a function of PAH exposure. The PAHs formed two major clusters: Cluster 1 consisted of the less biologically active PAHs, while cluster 2 induced Cyp1a expression in the skin and/or vasculature. Morpholino knockdown of aryl hydrocarbon receptor 2 (AHR2) revealed that cluster 2 PAH bioactivity was dependent on canonical AHR2 signaling. We identified *cyp1c1*, *wfkn1*, *cyp1ba*, *cyp1a*, and *sult6b1* from the top 10 significantly elevated genes from exposure to cluster 2 PAHs, which may be common biomarkers of AHR2 activation. In addition, the variety of uniquely enriched genes associated with cluster 2 likely points to differing bioactivity mechanisms downstream of AHR2 between the PAHs. Our results indicate that a whole animal systems-level approach can be used for PAH classification needed for the eventual ability to predict mixture toxicity.

P356 - 0467 Decolorization of Some Synthetic Dyes Using Partially Purified Peroxidase from Cucumber (Cucumis sativus)

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Synthetic dyes commonly used in textile mills, paper mills, distilleries, paints factories, and tanneries usually find their ways into the environment especially into water bodies. Effluents resulting from ill-disposed industrial waste waters containing these dyes are known to cause nausea, hemorrhage, ulceration of skin and mucous membrane, dermatitis, perforation of nasal septum, severe irritation of respiratory tract and ultimately cancers. The study was aimed at determination of degradation of selected synthetic dyes using peroxidase extracted from cucumber. Peroxidase was extracted, purified and characterized from cucumber (*C. sativus*) purchased from Farin-Gada Farmers' Market, Jos, North Central Nigeria, using standard protocols. The resulting partially purified

enzyme was applied in the degradation via decolorization of the following nine synthetic dyes; Azo Brilliant Black, Azo Citrus Red, Azo Blue 5, Azo Pink, Azo Purple, Azo Trypan Blue, Azo yellow 6, Vat Orange 9 and Vat Green 11. Enzyme concentration and activity, optimum pH and temperature of the enzyme were also determined using standard protocols. The results showed that the protein concentration of the peroxidase was 80mg/ml, 30mg/ml and 40mg/ml for the crude extract, (NH₄)₂SO₄ precipitate and dialysate respectively. Activity of the enzyme was 0.04 IU/ml while optimum pH and pH of the enzyme was found to be 3.5 and 6.0 respectively. The enzyme treatment degraded the dyes with the following results; azo blue 5 (85.4%), azo yellow 6 (73.5%), azo pink (64.6%), azo citrus red (58.03%), vat orange 9 (56.1%), azo purple (54.43%), azo trypan blue (49.7%), azo brilliant black (28.6%) and vat green 11 (15.5%). Industrial waste water effluents could be safely degraded by peroxidase from cucumber and could potentially serve as cheaper and environmentally friendly alternative to chemical remediation of water bodies polluted by some synthetic dyes.

P357 - 0480 The Study of Molecular Mechanism of Dioxin-Induced Neurotoxicity: Targeting the Cholinergic System

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Exposure to and bioaccumulation of dioxins produce a wide variety of toxic and health effects, such as tumor promotion, teratogenicity, endocrine disruption and tissue- and organ-specific toxicities. The study of mechanisms of dioxins' neurotoxicity is a hot area in the past years since significant deficits in cognitive functioning have been reported in humans exposed to dioxins and dioxin-like compounds. The study of mechanisms of dioxins' neurotoxicity is a hot area in the past years since significant deficits in cognitive functioning have been reported in humans exposed to dioxins and dioxin-like compounds. We found novel mechanisms whereby dioxin may produce its biological or toxicological effects by decreasing neuronal AChE activity through a transcriptional down-regulation mechanism via the AhR-dependent signaling pathway. Moreover, muscular AChE expression was also disturbed by dioxin exposure. During myogenic differentiation of C2C12 cells, the mRNA expression of AChE subunit and the enzymatic activity of AChE were significantly suppressed by dioxin exposure in parallel with the disturbances on the myotube formation. However, the addition of AhR antagonist was not able to reverse the suppressive effect of dioxin, suggesting a distinct role of AhR during the myogenic differentiation process. These results further support the notion that dioxin is a novel environmental AChE disruptor which acts on the biosynthesis processes via multiple molecular mechanisms.

P358 - 0502 Halogenated Marine Indoles Compete with High Affinity Ligands for Aryl Hydrocarbon Receptor Binding

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The persistent environmental presence of halogenated aromatic hydrocarbons (HAHs), including polychlorinated dibenzodioxins, polychlorinated dibenzofurans and polychlorinated biphenyls, leaves a variety of species, including humans, at risk of exposure. Consequently, toxicities, such as liver disease, teratogenicity and cancer, are a risk, owing to the interaction of HAHs with a specific transcription factor, the aryl hydrocarbon receptor (AhR). A possible strategy to therefore protect against such exposures could be to introduce a dietary source of non-cytotoxic, competitive AhR ligands, such as indoles, which reduce HAH-AhR binding without inducing toxicity of their own. Three halogenated indoles, 4,7-dibromo-2,3-dichloroindole (23A), 7-bromo-2,3-dichloro-6-iodoindole (23B) and 6,7-dibromo-2,3-dichloroindole (23C), were recently isolated from *Rhodophyllis membranacea* seaweed and tested for such a purpose in both human HepG2 and mouse Hepa1c1c7 liver cell lines. No significant changes in cell viability, as determined by the sulforhodamine B assay, were found following treatment in either cell line with 23A, 23B or 23C (up to 20 μ M), demonstrating the relatively low cytotoxicity of these indoles. To assess their effect on AhR signalling, changes in the activity of cytochrome P4501A1 (CYP1A1), the gene for which is highly regulated by the AhR, were determined via o-deethylation of ethoxyresorufin. Each indole was tested for its individual capacity to activate the AhR, as well as its ability to antagonize two high-affinity AhR agonists, 6-formylindolo[3,2-b]carbazole (FICZ) and β -naphthoflavone. The results showed that 23A (5 μ M) induced a 3.5-fold increase in CYP1A1 activity relative to vehicle control (0.5% DMSO), ($P < 0.05$) after 12-hour exposure in mouse Hepa1c1c7 cells, while 23B and 23C did not alter CYP1A1

activity. Moreover, all three indoles antagonized CYP1A1 activity induced by FICZ and β -naphthoflavone, implying competitive AhR binding. Similar patterns were also observed following 6- and 24-hour treatment. Contrastingly, in human HepG2 cells all three indoles induced CYP1A1 but 23B, not 23A, was found to be the most potent, highlighting the species-specificity of AhR ligand binding. These results suggest that 23A, 23B and 23C are AhR ligands that can outcompete high-affinity AhR agonists and further investigation into their ability to protect against HAH exposure is therefore warranted. However, distinct species differences in AhR ligand preference are also evident and additional exploration is necessary to understand the applicability of these results to a variety of species.

PS P359 - 0533 Decabromodiphenyl Ether Affects Immune Response in Obese Mice and in Adipocyte-Macrophage Coculture System

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Decabromodiphenyl ether (DecaBDE) is an additive brominated flame retardant that has a variety of applications including in plastics, textiles, and adhesives. It has been detected in indoor air or dust, and their health impact is of concern. Recently, we have found that DecaBDE exacerbates hyperglycemia in diet-induced obese mice. In addition, our previous study showed that brominated flame retardants including DecaBDE can stimulate immune cells. In this study, we focused on the inflammation of adipose tissue related to the development of obesity and examined the effects of DecaBDE exposure on immune response *in vivo* and *in vitro*. Male C57BL/6J mice were fed normal (9.0 kcal% fat) or high-fat (62.2 kcal% fat) diet and treated with DecaBDE (0, 0.5, and 10 μ g/kg body weight/day) *ad libitum* in drinking water from 5 to 20 weeks of age. Isolated visceral adipose tissues (mesenteric, renal, and epididymal) were cultured for 2 days and then the production of the inflammatory/anti-inflammatory mediators was measured. Furthermore, chemokine levels in bone marrow fluid and phenotypes of bone marrow cells were examined. *In vitro* study, 3T3-L1 adipocytes were cocultured with RAW 264.7 macrophages in the presence or absence of DecaBDE (0, 1, 5 μ g/ml) for 24 h and then the production of the inflammatory/anti-inflammatory mediators was measured. In high-fat diet-fed mice, DecaBDE exposure tended to increase IL-6 production in epididymal adipose tissue and induced changes in the bone marrow microenvironment (e.g., decreased SDF-1 α levels in bone marrow fluid and CXCR4 expression in bone marrow cells). Furthermore, DecaBDE exposure stimulated macrophages, and enhanced inflammatory response (e.g., CCL2/MCP-1 and TNF- α production) in adipocyte-macrophage coculture system. These results suggest that DecaBDE exposure affects immune response *in vivo* and *in vitro*. The exacerbation of inflammatory response between adipocytes and macrophages may contribute to the development of obesity-related metabolic disorders.

PS P360 - 0590 Low-Dose Chronic Exposure of OCPs Mixture in C57BL/6J Mice Induces the Impairment of Glucose Metabolism

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Organochlorine pesticides (OCPs) are organic compounds and environmental contaminants that are not easily degraded in the ecosystem. There are chemicals to be closely related to various human diseases. In the present study, we used OCPs such as chlordane, heptachlor, p, p'-DDT, beta-HCH, and hexachlorobenzene, which was detected in the human body. We prepared OCPs mixture at a ratio of 1: 1: 1: 1: 1 and conducted single dose toxicity test to set appropriate concentrations before this experiment. The aim of this study was to evaluate whether the OCPs mixture could induce the risk of glucose metabolism by low-dose chronic exposure conditions in C57BL/6J mice. OCPs mixture (0.5 or 2 mg/kg BW) was administered in corn oil by oral gavage for 12 weeks. Then, oral glucose tolerance test (OGTT) was performed and blood glucose level was measured in C57BL/6J mice. Levels of insulin, glucose, FFA, TNF- α in plasma and insulin immunohistochemistry of the islet of Langerhans in pancreas were measured in C57BL/6J mice. OCPs mixture significantly increased fasting blood glucose, glucose level in OGTT, insulin, TNF- α and FFA levels in plasma, homeostatic model assessment-insulin resistant (HOMA-IR), and pancreatic insulin

content in C57BL/6J mice. Based on these findings, we suggest that the low-dose chronic exposure of OCPs mixture may induce the impairment of glucose metabolism without pancreatic dysfunction in C57BL/6J mice.

PS P361 - 0639 Concentrations of Environmental PCBs in Breast Milk and Serum of Lactating North Carolina Women

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PCBs are a family of 209 structurally related congeners and are ubiquitous and persistent in nature. PCBs are lipophilic compounds which bioconcentrate in the food chain and are present in detectable amounts in humans. There is a paucity of data about recent human exposure to PCBs through breast milk, especially in US studies and even fewer studies with multiple measures over time from the same individual. The US EPA Methods Advancement for Milk Analysis (MAMA) Study was designed to develop or adapt methods to measure PCBs, and other environmental chemicals, in milk and serum twice during lactation (at 2-7 weeks and 3-4 months postpartum) in a convenience sampling of 34 North Carolina women. Milk and serum were quantified for thirty-five PCB congeners. The majority of the milk congeners were measured at concentrations above LOD, including PCBs 153, 138, 180, 118, 74, 170, 99, 187, 196, 194, 199, 156, 146, 28, 105, 206, and 183, in descending order of concentration with mean concentration ranges of 19.6 to 0.81 ng/g lipid. Similar trends were measured in serum (mean concentrations ranged from 13.3 to 0.86 ng/g lipid) with minor partitioning differences. There was no depuration between visits. These data suggest that breast feeding North Carolina mothers are exposed to these environmental PCBs and that the PCBs can partition to breast milk. *This abstract is the opinion of the authors and does not represent EPA or NIEHS policy.*

PS P362 - 0669 DEHP Triggers Developmental Abnormality of Ovary and Oviduct in Quails (*Coturnix japonica*) via Disruption of Hypothalamo-pituitary-ovarian Axis Response

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Recent epidemiologic evidence suggests that women have a unique exposure profile to phthalates, which raises concern about the potential health hazards posed by such exposures. Here we review our work and the work of others studying these mechanisms and propose a model for the ovarian action of di-(2-ethylhexyl) phthalate (DEHP). *In vivo*, DEHP (250, 500, 1000 mg/kg BW / day) caused the quail ovarian histopathologic and ultrastructural changes, disrupting sex hormone levels, induced ovarian developmental disorders. DEHP exposure can induce excessive production of lipid peroxides in ovary tissues of quail, inhibit the activities of antioxidant enzymes, and then induce oxidative stress, playing a critical role on ovary damage of quail. DEHP exposure can affect the expression of progesterone receptor and estrogen receptor, interfere with the expression of hypothalamic-pituitary-ovarian axis steroid hormone-related factors and block the synthesis of steroid hormones. *In vitro*, monoethylhexyl phthalate (MEHP) decreases granulosa cell aromatase RNA message and protein levels in a dose-dependent manner. MEHP is unique among the phthalates in its suppression of aromatase and in its ability to activate peroxisome proliferator-activated receptors (PPARs). To test the hypothesis that MEHP suppresses aromatase through PPAR pathways, granulosa cells were cultured with MEHP (200 microM), selective activator, or antagonist of PPAR gamma for 24 h and gene and protein expression were analyzed by real time RT-PCR and western blotting. PPAR gamma activator Rosiglitazone significantly decreased aromatase mRNA like MEHP. The PPAR gamma-selective antagonist GW9662 partially blocked the suppression of aromatase by MEHP, suggesting that MEHP acts through PPAR gamma, but not exclusively. Our model shows that MEHP acts on the granulosa cell by activating the PPARs, which leads to decreased aromatase transcription. Thus, the environmental contaminant DEHP, through its metabolite MEHP, acts through a receptor-mediated signaling pathway to suppress estradiol production in the ovary, interfere with the regulation of hypothalamus-pituitary-ovarian gonadal axis, hinder the synthesis of sex hormones, trigger sex hormone secretion disorders, leading to serious toxicity on the reproductive system with a dose-dependent manner. *Support: National Natural Science Foundation of China (No. 31572586), Excellent Youth Foundation of Heilongjiang Province of China (No. JC2017005), and China Agriculture Research System (No. CARS-35).*

PS P363 - 0797 PFAS Clearance in Cattle

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Farms are often located near sources where PFAS firefighting foams were used (e.g. training grounds of rural fire services, defence bases, buffer areas of oil/gas refineries). However there is limited information available that facilitates management of PFAS affected farms or the animals. The objectives were to gather robust information on serum clearance of PFAS from beef cattle, tissue distribution and tissue partition coefficients, and GIT absorption. A herd of Belted Galloway beef cattle (16 heifers and 3 steers) were exposed to PFAS in water for 2 years. All had access to the same water, constant PFAS concentration, no other water source. All animals bred on property, or brought on at young age. Age known from breeding log. No, or negligible PFAS exposure from soil or grass. No breeding for approximately 20 months prior to assessment (therefore no PFAS loss to placenta, foetus, via lactation or additional intake from placentophagy). All animals at steady state when investigation initiated. All serum and tissue PFAS analysis (full suite) was done by the same commercial laboratory. Animal ethics approval was deemed not necessary by the appropriate authority since the study was motivated as a chemical contamination investigation and for food safety reasons. Plus animals were managed by qualified veterinarians, and all procedures were routine veterinary practice. Nineteen days after transfer to the Department of Agriculture research farm (to ensure PFAS affected gastric contents had passed), all animals were sampled (tail vein) for serum PFAS. Five were chosen for longitudinal repeat blood sampling, 12 euthanized at day 63 for tissue analysis with a subset of 5 also having urine, bile, saliva and GIT content (at 3 sites) sampled. The cattle from the longitudinal study were euthanized at day 215 and tissues analysed for PFAS. From the longitudinal clearance study and serum data at days 19 & 63 serum half-lives were determined. The following was found (n = 16): No delay in depuration. No serum fluctuation early in depuration. Half-life of L-PFOS = mPFOS > PFHpS = di-PFOS > PFHxS. For the measurable carboxylic acids; half-life of PFDA > PFNA. Variability between animals in half-life for L-PFOS and m-PFOS. Although not statistically different mean half-life of mPFOS > L-PFOS. No difference in PFAS half-lives between heifers and steers.

PS P364 - 0919 Applying the Concept of Essential Use to Determine Where and When PFAS Can Be Phased Out

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In 2015, the Madrid Statement established a global call to phase-out the use of per- and polyfluoroalkyl substances (PFAS) where they are deemed not essential or when safer alternatives exist. Efforts to determine when uses of PFAS have an essential function in modern society, and when they do not, has proven difficult. Here, we: (1) present the concept of "essential use" based on a previous approach described in the Montreal Protocol, (2) establish four Categories of Essentiality, (3) present various use cases of PFAS to determine the feasibility of elimination or substitution in each category, and (4) outline some challenges to phasing out PFAS. A phase-out of many uses of PFAS can be prepared because they are not necessary for the betterment of society in terms of health and safety or because functional alternatives are currently available that can be directly substituted into these products or applications. Some specific uses of PFAS would be considered essential because they provide for vital functions and are currently without established alternatives. However, this essentiality should not be considered as forever; rather, innovation is needed to identify novel alternatives. We recommend expanding this concept by applying it in detail to all use cases of PFAS.

PS P365 - 0931 PFAS Tissue Distribution in Cattle

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Deciding when PFAS in beef cattle has declined sufficiently after exposure is stopped, such that the tolerable daily intake by high end meat consumers is not exceeded, requires information on clearance of PFAS from tissues (experimentally problematic for cattle), or from serum (previous poster) and tissue partitioning data. Belted Galloway beef cattle that had accumulated PFAS from water had blood sampled (tail vein) prior to stunning and exsanguination (as per standard abattoir practice) by a veterinarian. Twelve were euthanised 63 days after PFAS exposure had ceased for tissue analysis (muscle, liver, kidney) with a subset of 5 also having urine, bile, saliva and GIT content (at 3 sites) sampled. An additional five cattle used for longitudinal serum sampling (previous poster) were euthanized at day 215 and tissues (muscle, liver, kidney, fat) analysed for PFAS. The data were supplemented by tissue PFAS concentrations (muscle, liver, kidney, fat, saliva, bile and urine) for 6 steers from a separate investigation. In addition to tissue:serum partition coefficients, PFAS profile relative to L-PFOS, and where appropriate for data interpretation absolute PFAS concentrations are reported.

PS P366 - 0932 PFAS Clearance and Tissue Distribution in Sheep

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There is limited information available that facilitates management of PFAS affected farms or the animals. Objectives were to gather robust information on serum clearance of PFAS from sheep and tissue distribution. The study took advantage of the availability of a large flock of sheep that had accumulated PFAS from impacted farm dam water. The flock had been moved to a research farm where PFAS exposure ceased approx. 3 months before the study began. The study was conducted under Australian farm conditions. Animal ethics approval was deemed not necessary by the appropriate authority since the study was motivated as a chemical contamination investigation and for food safety reasons. Animals were managed by qualified veterinarians and all procedures were routine veterinary practice. Blood from 10-15 animals from the following groups was obtained on day 0. Ewes (EL, 4-7 yrs old) that had twin lambed (lambs sampled within 24 hrs of parturition), non-pregnant ewes (ENP, 4-6 yrs), hoggets (H, 1.5 yrs), and rams (R, 4-6 yrs). From each group, individuals (n=5) with the highest serum PFOS concentration were selected for determination of PFAS serum half-life (t_{1/2}). In addition, serum and tissues (muscle, liver and kidney) from 5 newborn lambs, older lambs (3-4 months) from this flock, and various age sheep from a different flock were also analysed for PFAS. All serum and tissue PFAS analysis (full suite) was done by the same commercial laboratory. Findings included: In the clearance study, PFOS and PFHxS were the only 2 PFAS measurable in serum. There was no difference between serum t_{1/2} of branched(b), linear(l) or total(t) PFOS within any animal group but t_{1/2} differed between groups. There was no gender difference in tPFOS t_{1/2} in mature sheep but a suggestion young females (1.5 yo) may clear PFOS quicker than young males. tPFOS t_{1/2} was age dependent in females (ENP, 4-6 yrs vs. H, 1.5yrs) with younger animals having lower t_{1/2}. Lactation increased serum tPFOS clearance although this was not statistically significant. PFAS tissue distribution is proportionally correlated with serum concentrations over a wide tPFOS range (~5 -150 ng/mL). Muscle and kidney partition coefficients are independent of serum concentration up to the maximum tPFOS measured in this study (150 ng/mL). Partitioning into muscle of newborn lambs > older animals. There is no age dependency for tissue partitioning of PFOS into muscle or kidney after animals are 3 - 4 months old, and for liver after age 7 months. Tissue partitioning is different for each PFAS: lPFOS > bPFOS > PFHpS > PFHxS; i.e. decreasing with decreasing chain length (C8 to C6). Partitioning is different in different tissues; liver >> kidney > muscle.

P367 - 0933 PFAS Assimilation from Water by Cattle and Sheep

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The availability of PFAS stock water criteria would greatly assist environmental investigations of farms where PFAS is suspected to be present. Also with management of water and animals on the farm should PFAS be found in stock water. However there is currently a paucity of information available for assimilation of PFAS by stock from water. This study has taken advantage of PFAS data obtained for a farm from a separate investigation done for different purposes. Because the herd of cattle was exposed to only one source of water which was PFAS affected (no other water available), and the water had a constant measured concentration of PFAS for at least 12 months, and there was no other material source of PFAS, and the animals were at a uniform physiological status (no breeding, no lactation), and the animals were at PFAS serum steady state (SS) it is possible to easily determine assimilation factors (AFs) for PFAS from water into serum to achieve steady state in cattle. Assimilation factor $[(\mu\text{g/L serum}) \text{ per } (\mu\text{g/L water})] = \text{Serum SS conc} \div \text{Water conc}$. For cattle, measured data for steady state serum and constant water concentrations were used to calculate assimilation factors. In cattle, the AF for tPFOS = PFHpS > PFHxS, and PFNA ~ PFDA. At an earlier period of the farm investigation sheep and cattle were accessing the same water but PFAS concentrations in water, although relatively constant, were uncertain due to analytical constraints. However since both species were at steady state serum PFAS concentrations, the assimilation factors for sheep can be determined by adjusting those for cattle according to the ratio of sheep to cattle serum concentrations. In sheep, the assimilation factor for tPFOS < PFHxS (other PFAS in sheep tissue were <LOR). PFAS assimilation factors for tPFOS in sheep are approximately 7x lower than in cattle, but for PFHxS only approximately 2x lower. This may be because sheep drink less water per unit body weight than cattle, or they absorb less PFAS from water (unlikely), and/or have more efficient PFAS clearance mechanisms (as suggested by the different half-lives for PFOS in sheep *cf* cattle, see other posters in this series). Variability in PFAS steady state serum concentration, which was normally distributed, reflects the overall biological interactions and variability in individual water intake, PFAS absorption and distribution, and elimination. Therefore an upper estimate of the assimilation factor $[(\text{mean serum} + 2 \text{ SD}) \div (\text{mean water concentration})]$ caters for the combined biological variability between these parameters for 97.5% of animals. The upper 95% confidence limit on the mean is an alternative upper AF estimate; both are conceptually appropriate.

P368 - 0948 An Evaluation of the Utility of Human Cell Models for Characterizing Relative Potency for Dioxin-Like Compounds

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The 2005 WHO human and mammalian Toxic Equivalency Factors (TEFs) for select dioxin-like compounds (DLCs) were informed by a database of relative potency values (REPs) largely based on nonhuman models (*in vivo* and *in vitro*). Since then, there has been a concerted effort to characterize the relative potency of some of these compounds in human cell models, with the goal of identifying potential species-specific differences that might justify developing TEFs based solely on human *in vitro* data. The purpose of the current evaluation is to assess the utility of these human *in vitro* models for developing a TEF for PCB126 (regarded as the most potent DL-PCBs). The relevant research includes 16 published studies in which eight human cell models (five immortalized, three primary) originating from six different tissues were tested. Studies in cells sourced from human liver and breast tissue comprise the greatest portion of this dataset, and the majority of response endpoints were based on CYP1A activity or the induction of genes regulated by AhR. The REPs reported for these human *in vitro* studies span >three orders of magnitude (0.00009–0.16, relative to TCDD). Although tissue-specific differences may contribute to this variation, several features of the models and/or experimental designs used in these studies were identified that likely impact the REP variation observed: cell populations with low/heterogeneous AhR expression (e.g., peripheral blood lymphocytes), primary cells from limited donor populations with unknown DLC-body burden, highly variable response endpoints (e.g., aromatase), inconsistent cell culture methodology, and inadequate methodological and/or outcome reporting. The specific shortcomings of these human *in vitro* studies, coupled with limitations of *in vitro* studies as a whole relative to *in vivo* studies, limit the degree to which these values can be used solely as the basis of the PCB126 TEF. While human *in vitro* methodologies hold much promise for the future of pre-

dictive toxicology, there is a clear need of improved characterization of current human *in vitro* models, as well as development of standardized methodologies for validating the utility of these models and reducing uncertainty in reported outcomes. At this point in time, therefore, the TEF for PCB126 should continue to be based on the full body evidence that includes both mammalian *in vivo* and *in vitro* REPs.

P369 - 0092 A Case of Delayed Organophosphate Poisoning Caused by Fenthion Ingestion

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A 36-year-old Chinese woman who was previously well was brought in by the ambulance for vomiting and drowsiness after drinking a self-made concoction of pesticide, insecticide, and dettol around 500ml. She first called her friend around 2:30 am, threatening suicide, then ingested the mixture around 4 am that day, before arriving at her friend's place. While there, she vomited a few times, which was bilious and pungent, and her friend called the ambulance at 9:30 am. She arrived only at 10:30 am and was immediately decontaminated by thorough washing due to suspected organophosphate poisoning. A scan done post-decontamination confirmed no residual toxic substance. Her vital signs were: T 34.9 deg C, BP 96/50 HR 62 RR 17 SpO2 100% RA. Her initial GCS was E1V3M5, and she had secretions and saliva over the mouth. Both pupils were pinpoint. Heart, lungs, and abdominal examination was normal, and she was moving all 4 limbs equally. An ABG done on RA showed pH 7.335 pCO2 36.3 pO2 100.7 SaO2 97.1%. She desaturated within 24h of admission and was intubated and sent to the ICU. She was then started on IV atropine and Pralidoxime in view of her clinical presentation. An urgent toxicological screen was done, which confirmed the presence of Fenthion, as well as a RBC cholinesterase level, which was reduced at 2644 (range 7700–14600 U/L) on the first day and then decreased to 1336 the following day. We will be looking at delayed onset organophosphate poisoning through Fenthion and management of organophosphate poisoning in this presentation.

P370 - 0143 Developmental Exposure to Glyphosate-Based Herbicide Causes Dysregulation of Dynorphins in the Brain of Adult Rats

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Glyphosate [N-(phosphonomethyl)glycine], the most widely used herbicide worldwide, has been suggested to induce neurotoxicity and behavioral changes in rats following long-term exposure. The aim of the present study was to investigate persistent brain changes in 90-day-old adult male rats following maternal exposure to a glyphosate-based herbicide (GBH) during gestational and neonatal periods. The maternal exposure to 1% GBH in drinking water (corresponding to 0.36% of glyphosate) or drinking water was started at gestational day 5 and continued up to postnatal day 15 (PND15). Matrix-assisted laser desorption ionization (MALDI) imaging mass spectrometry (IMS) was used to evaluate peptide expression in hippocampus (HC) and substantia nigra (SN) directly on brain cryosections from PND90. Histological changes were examined using immunohistochemistry and quantified using CellProfiler. The results indicated that developmental exposure to GBH had a long-term effect on SN, with 188 significant peaks between the groups, while HC showed no significant differences between the groups. Developmental exposure to GBH resulted in a significant ($P < 0.05$) peak intensity reduction of several peptides of the Dynorphin family in SN of PND90: Dynorphin B at m/z 1572 (57%), alpha-neoendorphin at m/z 1229 (50%), and its endogenous metabolite des-tyrosine alpha-neoendorphin at m/z 1066 (50%). The overall quality of the data was evaluated by validation of variability within groups and between group variance of all peaks detected (SN $n=1200$; HC $n=800$). The reduction of Dynorphin was confirmed through immunohistochemistry. The total area of Dynorphin A immunoreactive fibers, as defined as the immunoreactivity not associated with NeuN-positive mature neurons or with NeuN-immunonegative DAPI cell nuclei, was reduced in the SN of PND90. In addition, a small reduction of Dynorphin A-immunoreactivity associated with nonneuronal cells was seen in the hilus of the hippocampus dentate gyrus, whereas no effect was detected in the CA1, CA3, or radiatum. Exposure to GBH during the brain development also induced an increase in the number of nestin-immunopositive neural pro-

genitor cells in the subgranular zone of the dentate gyrus, known as the site of hippocampal neurogenesis, in PND90 rats. In conclusion, the results demonstrate significant long-term effects in SN and HC following developmental GBH exposure, suggesting that GBH may disturb critical neuronal processes during the brain development period. The present findings could be relevant for future regulation of glyphosate, which can have a significant public health and environmental impact.

PS P371 - 0153 Study on Systemic and Reproductive Toxicity of Acetochlor in Male Mice

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Acetochlor is one of the three most abundant herbicides used in China, which is a preemergence herbicide belonging to chloroacetanilides. It is classified as "likely to be carcinogenic to humans" and suspected as an endocrine disruptor by the US Environmental Protection Agency (US EPA) and the European Union Environmental Protection Agency (EU EPA). Therefore, there is increasing concern about the risk of acetochlor to human health and the environment. However, the current research on acetochlor mainly focuses on the assessment of environmental behaviors, whereas toxicity studies mainly concentrate on nonmammals such as zebrafish. So far, only a few reports of its effects on the male reproductive system of animals have been found. In the present study, C57BL/6 male mice and GC-1 spermatogonia (spgs) were used to analyze *in vivo* and *in vitro* toxicities of acetochlor to investigate the relationships among acetochlor-induced oxidative stress, endocrine dysfunction, degenerative diseases of germ cells, and apoptosis. The results show that acetochlor can induce systemic and reproductive toxicity in subacutely exposed mice, such as clear reduction in body weight, liver function damage in mice, oxidative stress damage, and histopathological degeneration in testicular tissue as the concentration of acetochlor increases. In addition, acetochlor might mediate apoptotic pathways that affect the survival of GC-1 spgs; in detail, cell viability decreases, cell cytotoxicity and apoptosis are elevated, and oxidative stress and expression changes of apoptosis-related proteins are observed compared with that for control in GC-1 spgs. In addition, this study also provides a scientific basis for evaluating the toxicological effects of acetochlor as well as its potential damage to mammals.

PS P372 - 0181 Effects of Acephate, Carbendazim, and Mancozeb Isolated or in Mixture on DNA Damages and Hepatic and Renal Histopathology

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Agrochemicals are the most widely used synthetic chemicals in the world, contaminating food, soil, and water. Consequently, the entire population is environmentally exposed to complex mixtures of these toxicants. The present study investigated whether prepubertal exposure of male rats to Acephate, Carbendazim, and Mancozeb, isolated or in combination, causes DNA damages and histopathological alterations in liver and kidney. These agrochemicals were chosen based on Brazilian National Sanitary Surveillance Agency's pesticide residue monitoring program, which reported these three agrochemicals as the most found in food samples cultured in Brazil. Male Wistar rats were distributed into 5 experimental groups: control (n=7) received only the vehicle corn oil; mixture (n=10) received Acephate, Carbendazim, and Mancozeb in combination at 23.6mg/Kg, 50mg/Kg, and 50mg/Kg, respectively; Acephate group (n=9) at 23.6 mg/kg; Carbendazim group (n=8) at 50mg/Kg; Mancozeb group (n=8) at 50mg/Kg. The treatment was given orally by gavage from postnatal day (PND) 23 to 53. Body weight gain and food/water intake throughout treatment period were similar among experimental groups, showing that systemic toxicity was not induced by agrochemicals in these experimental conditions. On PND53, rats were euthanized for organs and fluids collection. There was an increase in relative weight of liver in all treated groups in comparison with control, as well as an increase in fatty degeneration and inflammatory infiltrate in hepatic tissue. In the kidney, it was observed degeneration of proximal and distal tubules in cortical region and hyaline casts and inflammatory infiltrate in renal medulla of the animals treated with agrochemicals in mixture or isolated. Only Carbendazim exerted cytotoxicity on femoral bone marrow cells of pubertal rats. Exposure to agrochemicals in these experimental conditions did not exerted mutagenicity. The obtained results show that the agrochemicals were able to cause damage on hepatic and renal tissues of pubertal animals, without

important effects on DNA integrity. Interestingly, the mixture of agrochemicals had no additive adverse effect on the parameters evaluated. Further studies are being developed to better understand physiological and molecular aspects of the toxicity of the chemicals used in the present study.

PS P373 - 0184 Understanding the Role of Tyrosine in Toxicity Associated with HPPD Inhibitors and Impact on Human Health Risk Assessment

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Herbicides with the pesticidal mode of action of 4-hydroxyphenyl pyruvate dioxygenase (HPPD) inhibition lead to elevated tyrosine levels and are associated with a number of common toxicities in test animal species. Humans have greater ability to catabolize excess tyrosine than some test animal species (humans equivalent to mice and much greater than rats); therefore, in order to choose the most relevant endpoints and test animal species for human health risk assessment, the role of elevated tyrosine in the observed toxicity must be considered. One of the common toxicity findings, ocular opacity, has been experimentally demonstrated to be due to an increase in plasma and ocular tyrosine levels subsequent to HPPD inhibition in mammals. Other toxicities common to HPPD inhibitors include liver and kidney weight increases without histopathology changes; chronic progressive glomerulonephropathy; sciatic nerve demyelination, thyroid proliferation, bilateral hydronephrosis (pelvic dilatation); reduced pup survival and specific ossification changes, or delays. These are associated with elevated tyrosine, but causality is experimentally difficult to prove. In order to establish the role of tyrosine in these toxicity findings, a review of publicly available global regulatory decisions on nine HPPD inhibiting pesticides and one human therapeutic agent, nitisinone, was conducted. Common toxicities observed consistently with HPPD inhibiting molecule pesticides were correlated with tyrosine levels. Further evidence of the role of tyrosine in these toxicities was provided by more frequent occurrence and at lower exposure in male rats. This review provides a weight of evidence to demonstrate the role of tyrosine in specific toxicity findings associated with HPPD inhibitors; coupled with the knowledge of species-specific tyrosine catabolism differences, this information will support consistent and appropriate human health risk assessment of HPPD herbicides.

PS P374 - 0207 In Vivo and In Vitro Effects of a Neonicotinoid Pesticide, Clothianidin, on Mammalian Nervous Function

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Neonicotinoids are widely used pesticides due to their antagonistic effects on the nicotinic acetylcholine receptors (nAChRs) of insects. However, recent studies have revealed that neonicotinoids also exert excitatory effects on rodent neurons mediated by their nAChRs, suggesting that they have unpredictable adverse effects on the nervous systems of mammals. In this study, we assessed the neurobehavioral and neurofunctional effects of a neonicotinoid pesticide, clothianidin (CTD), *in vivo* and *in vitro* by using mature mice and human neuroblastoma cells to provide new insight into risk assessments of neonicotinoids. To investigate the *in vivo* effects of CTD, male C57BL/6N mice were administered CTD (5 or 50 mg/kg) at or below the no-observed-adverse-effect level (NOAEL). Exposure to CTD acutely caused decreases in the total distance traveled and the percentage of open arm entry in the elevated plus maze test, in addition to abnormal vocalizations (4–16 kHz), which are behavioral signs of aversive emotions. We also observed increases in the number of c-fos immunoreactive cells in several brain regions related to stress responses, including the paraventricular thalamic nucleus and the dentate gyrus of the hippocampus. Collectively, these results demonstrated that low-dose CTD induced an exaggerated response to stress in a novel environment in mice. *In vitro* effects of CTD were evaluated using human neuroblastoma SH-SY5Y cells as a model of human neurons. CTD (1–100 μ M) increased the cell number at 24 and 48 hours of culture, and a noncompetitive nAChR antagonist, mecamylamine, inhibited the stimulative effect of CTD. To examine the mechanisms underlying the effect of CTD, we further analyzed intracellular signaling pathways. We found that CTD evoked a transient intracellular calcium flux and the phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) immediately after expo-

sure. In addition, transcriptome analyses revealed that several genes involved in "flux of Ca^{2+} " and "formation of filopodia" were decreased by CTD. These results indicated for the first time that neonicotinoids exert neurofunctional effects on human neuroblastoma cells by disrupting their intracellular signaling. According to epidemiological studies, the detectable amounts of neonicotinoids in human samples are rapidly increasing. Together with our present findings, further studies are needed to assess the risks of neonicotinoids to human health.

PS P375 - 0301 Organophosphate-Induced ADHD-Like Behavior in Adolescent Rats

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In recent years, association between exposure to organophosphate (OP) insecticides during adolescence and attention-deficit/hyperactivity disorder (ADHD) symptoms have been reported in several epidemiological studies. However, no paper suggests plausible mechanism of the relationship. This investigation clarifies whether OP compounds induce ADHD-like behaviors in adolescent rats. This study was carried out in accordance with the Guide for Animal Experimentation of Nagoya City University. After 1-week acclimation and training for elevated plus-maze test, 4-week-old male Wistar rats were treated with single intraperitoneal administration of vehicle (dimethyl sulfoxide) or EOPF, a fluororophosphate analogue (0, 1, 2, 3 mg/kg). At 30 min, 5, 24, and 30 hours after the treatment, rats were placed in the central area of the maze facing an open arm. The locomotion was monitored and recorded with a video camera for 3 min. Behavioral evaluation in the view of entries into open-arm and closed-arm and time spent on open-arm and central area was conducted by a blind examination. Motility was dose-dependently increased in the rats treated with EOPF compared with the vehicle-treated rats at post-30 hours. Time spent on open arm and entries into open-arm were significantly increased at both 2 and 3 mg/kg EOPF and 3 mg/kg EOPF, respectively. Total entries, i.e., exchanging the arms were also significantly increased in 3 mg/kg EOPF-treated rats. No significant difference was observed at 30 min, 5 and 24 hours after EOPF treatment. Thus, OP agent potentially induces ADHD-like behaviors in rats. Further studies should be examined with commercially available OP pesticides.

PS P376 - 0311 Serum and Caecal Metabolomics in Rats Exposed Subchronically to Glyphosate and Roundup

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Glyphosate is the world's most used herbicide ingredient targeting aromatic amino acid biosynthesis through inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) of the shikimate pathway in plants. In addition, as EPSPS and the shikimate pathway also exists in some microorganisms, glyphosate has also been patented as an antibiotic. Nevertheless, there is intense debate on whether the glyphosate interference with aromatic amino acid biosynthesis in microorganisms inhabiting the gastrointestinal tract can be a source of negative health impacts. In order to address this issue, we conducted a sub-chronic toxicity study in female rats to investigate whether glyphosate or its representative EU commercial herbicide formulation MON 52276 (a Roundup glyphosate-based herbicide) has an effect on the host-microbiota interface. General toxicity parameters (body weight, food and water consumption) were measured and pathological analysis of target organs (kidney and liver) was performed. A global serum and caecal metabolomics analysis was performed in groups of 10 animals exposed to three concentrations of glyphosate acid (0.5, 50, 175 mg/kg bw/day) or MON 52276 (0.5, 50, 175 mg/kg bw/day glyphosate equivalent). The results of these ongoing analyses will be presented.

PS P377 - 0312 The Experimental Study of the Effects of a Carboxamide Derivative on the Migration-Water Hazard Indicator

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The study of the effects of a carboxamide derivative on the migration-water hazard indicator (soil-water), which characterizes the processes by which pesticides enter groundwater from the soil, was conducted. The carboxamide group derivative is classified as a highly hazardous compound by its persistence in soil (hazard class 2) and is used as a fungicide on a large spectrum of crops in agriculture. The study was completed in compliance with the requirements for conducting experiments in extreme soil-climatic conditions, enabling the maximum migration of the studied substance from the soil into contact environments. The experiments were carried out on laboratory filtration units under the conditions of free filtration with 1 and 0.2 m columns (the height of the arable layer), 10 cm in diameter, using single imitating soil modelling standard, with sandy soil with the constant granulometric size and physicochemical composition. The substance in the form of an aqueous solution was applied to the upper 20 cm layer of the columns 3 times in the concentrations of: 1N - 0.094 mg/kg, corresponding to the maximum recommended application rate in agriculture (1.125 l/ha); 0.1N - 0.0094 mg/kg; 10N - 0.94 mg/kg. The amount of substance in the filtrates of 100 ml was determined by high-performance liquid chromatography (the lower limit of detection=0.01 mg/l). The dynamics of determination of the residual quantities of the carboxamide derivative in the filtrates showed: (a) 1N - the substance was not detected in the 1 m columns during the entire experiment (45 days), and detected in the 0.2 m columns at the level of the maximum permissible concentration (MPC) in water (0.02 mg/l) or lower (0.0107-0.019 mg/l). (b) 0.1N - the substance was not detected during the experiment (<0.01 mg/l). (c) 10N - the substance was not detected in the 1 m columns 1 to 13 days after the application. During 14-36 days, the concentration of the substance was 0.0015-0.0012 mg/l, respectively. In the following days, the substance was not detected. In 0.2 m columns, the concentration of the substance was higher than the MPC in water and was 0.07 mg/dm³ and 0.06 mg/l from the 15th to the 45th day, respectively. The maximum concentration at the level of 0.476 mg/dm³ was determined on the 20th day of the experiment. Based on the obtained results, the threshold value of 0.94 mg/kg (10N) for the carboxamide derivative was established according to the migration-water hazard indicator, when the migration from the soil to the groundwater is in safe quantity for the human health (≤ the MPC in water).

PS P378 - 0326 The Isolated and Combined Effects of 2,4-D and Methylmercury on the Morphology of Xerogel of Rat Serum

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Studies of the simultaneous effect of the herbicide 2,4-D and methylmercury on the organism, along with the evaluation of the effects of each of the ingredients separately and in combination, are considered timely from the hygiene viewpoint. The experiments were carried out on white rats in the acute dynamic experiment (1, 3, and 7 days) at the LD16 (630 mg/kg b.w. for 2,4-D and 16 mg/kg b.w. for methylmercury). The morphology of the xerogel of serum (MXS) on the first day after the isolated exposure to 2,4-D and methylmercury exhibited the annular symmetry with a pronounced dehydration net (DN), a strongly expressed intensity of dichotomous branching (IDV), a reduction in the number of globular voids (GVs) in the sectors. Combined effects of 2,4-D and methylmercury caused a dysfunction of the MXS expressed by the significant increase in the number of sectors and the reduction in the number of chambers in a sector. IDV in experimental animals was pronounced but differed from the controls. On the third day after the exposure to 2,4-D, MXS of the experimental animals corresponded to the control in terms of qualitative characteristics (symmetry, DN, IDV), the number of GV's was reduced. When exposed to methylmercury, the MXS of rats presented with the annular symmetry, but the IDV was reduced, the number of sectors was increased, the GV's in the chambers were reduced. The combination of the effect of the studied compounds caused significant changes in the MXS characterized by the annular symmetry with the strongly pronounced IDV. The significantly increased number of sectors and the statistically significant decrease in the number of chambers in the sectors were observed. A dysfunction of the structural background of the xerogel was detected 7 days after the exposure to 2,4-D. The number of sectors, GV's and chambers in a sector were reduced significantly, the annular symmetry was disrupted, the DN and the IDV were poorly pronounced. The MXS of animals exposed to methylmercury presented with severe DN, impaired annular symmetry, the number of sectors and chambers in the sector continued to rise, the

number of GV's reached the control level, the IDV was strongly pronounced. Under the combined action of the herbicide methylmercury, the MXS was characterized by a significant increase in the number of the structural elements, the annular symmetry was disrupted. The boundary of the central and the peripheral concentrers was not expressed clearly, IDV showed a hyper-expression. DN was expressed, but in contrast to the control, the dehydration cracks were more pronounced. Thus, it was established that the combined action of studied substances on the MXS of rat has a greater destructive nature.

PS P379 - 0392 Aluminum Phosphide Poisoning: Rare Survival Case

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Aluminum phosphide is a fumigant material commonly used to protect crops from pests. Phosphine gas (PH₃), which is released when the tablet is evaporated, is a highly toxic gas which causes impaired contraction of the heart and severe metabolic acidosis. Aluminum phosphide poisoning accompanied with high mortality rate may reach 35 to 70%. 22-year-old female patient ingested one tablet of Aluminum phosphide, resulted in severe vomiting, severe metabolic acidosis and hypotension. Gastric lavage was done using 5 ampoules of sodium bicarbonate and then activated charcoal was given. 1 liter of saline was given to correct hypotension and sodium bicarbonate was given in a dose 2meq/kg IV to correct metabolic acidosis, then she was admitted to our ICU. Continuous monitoring specially to blood pressure was ordered, and ABG was done for follow up which showed slight improvement. Blood pressure began to drop to 70/40 with slight CVP affection, so noradrenalin was infused in rate 2-3 µg/kg/min, in addition to 500 cc saline every 6 hours. After 12 hours of treatment the blood pressure and metabolic acidosis improved gradually and then the patient discharged after another 12 hours. Aluminum phosphide is highly toxic and the cases developed severe manifestations. However the case responded to supported management and survived; which may be due to that she spelled part of the tablet from her mouth and may be due to role of activated charcoal. Also early supportive care may be beneficial. Aluminum phosphide poisoning is very dangerous with no available antidote so further studies are needed to minimize its mortality.

PS P380 - 0396 Hydrogen Cyanamide Poisoning: Rare, but Serious, Case Study

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Although suicidal poisoning by pesticides is very common, it was the first time to face this type of toxicity in our national toxicology center. 34-years male patient presented to our national toxicology center in 31 Jan. 2015 at 6:30 pm with history of suicidal ingestion of unknown amount of DORMEX (hydrogen cyanamide) since 2 hours. There was history of repeated vomiting and diarrhea. On examination, the patient wasn't conscious with Glasgow Coma Scale GCS 8, blood pressure was 100/60, pulse 110bpm regular, respiratory rate 30/min, oxygen saturation was 86% (97% on T-tube), pupils were bilaterally constricted (looks like pin point). CVP was 8 cm H₂O. Chest examination shows bilateral coarse crackles. The remaining physical examination did not reveal any significant findings. His laboratory parameters on admission were: random blood sugar: 183mg/dl, SGOT: 22U/L, SGPT: 28U/L, serum urea: 42mg/dl, serum creatinine: 1.2mg/dl, HB: 14g%, TLC: 13000, PLT: 268000, PT 13.8, INR: 1.24. ABG shows metabolic acidosis (PH 7.24, PCO₂ 38, HCO₃⁻ 14) serum sodium 136 mmol/l, potassium 4.1mmol/l. Pseudocholine esterase enzyme level was normal. As the patient was drowsy, he was intubated and connected to T-tube which was connected to oxygen source (10L), IV fluids were given to correct hypovolemia. Atropine was given. Gastric lavage was done, activated charcoal (80g) was given via ryle tube. Sodium bicarbonate (25 mEq/h intravenously) was given to correct the metabolic acidosis then ABG was repeated showed slight improvement, so bicarbonate was given accordingly and adjusted by repeated blood gas analysis. At 3 am, the patient was shocked, CVP dropped to 3 cm of H₂O. Dopamine was started at 5 µg/kg/min, and noradrenaline 5 µg/kg/min, then he was stabilized. Suddenly at 8 am patient was arrested. Cardio pulmonary resuscitation was started, adrenaline (1 mg IV) and atropine (1mg IV) were given, followed by intravenous sodium bicarbonate (50 mEq). Adrenaline and atropine were repeated again twice. In spite of all these measures, the patient died. Although insecticide poisoning is frequent in our country, it's the first time to admit a case of hydrogen cyanamide poisoning. The clinical presentation of the patient was similar to organophosphate poisoning, so atropine was given on admission till pseudo-choline esterase level was done. This case may be the first reporting hydrogen cyanamide ingestion with a suicidal intent, leading to persistent metabolic acidosis, refractory shock, and death. So more studies are needed to know the cause of rapid deterioration and how to save life as hydrogen cyanamide has no antidote yet.

PS P381 - 0430 Endpoints as Biomarkers to Assess the Occupational Exposure to Triazoles

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Among the most widely used pesticides are the triazoles class, used as antifungal agents in agriculture, as biocides and in the treatment of fungal infections in humans and animals against rusts, powdery mildews and scabs. Nowadays, spraying of fungicides has become a common practice for controlling plant diseases, but there is concern about residues of these pesticides in food and water, as well as the concern with the worker applying these substances to the crop. The aim of this study was to study of endpoints as biomarkers to assess the occupational exposure to triazoles fungicides. The study was approved by the Research Ethics Committee of the Federal University of Alfenas, Brazil (CAAE: 42673815.6.000.5142) and the volunteers answered a questionnaire to collect clinical data. Hepatic and renal function tests were performed to support risk assessment. In the evaluation of potential biomarkers of exposure to triazole fungicides, a sensitive and accurate vortex-assisted liquid-liquid microextraction-gas chromatography-mass spectrometry method for urinary triazoles was developed and validated, according to national and international standards, in exposed samples (n= 58). In addition, hormonal endpoints (testosterone and androstenedione levels), oxidative stress (malondialdehyde, oxidized proteins, GSH and GSH / GSSG ratio) and plasma levels of bile acids were compared and compared with samples from 30 urban residents. The results showed statistical difference between the groups studied, with increase concentration of bile acids conjugated to glycine, in addition, a significant decrease (p <0.05) in the concentration of androstenedione was observed, suggesting the existence of a deregulatory mechanism of steroidogenesis, prior to the formation of estrogens by aromatase. In conclusion, the endpoints bile acids conjugated to glycine and androstenedione, both in plasma samples, are potential biomarkers to be applied in the biological monitoring of occupational exposure to triazoles fungicides. *Funding: This work was supported by the CAPES and FAPEMIG [grant number APQ-02281-16].*

PS P382 - 0439 Withdrawn

PS P383 - 0475 Development of Data-Derived Extrapolation Factors for Herbicides That Inhibit 4-hydroxyphenyl Pyruvate Dioxygenase (HPPD)

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The exposure level at which no findings or no adverse findings are observed in experimental animal studies is frequently used as the basis for human health risk assessment of environmental chemicals, including agrichemicals. Chemical specific adjustment factors (CSAFs) or, more broadly, data derived extrapolation factors (DDEFs) may be derived to provide a more accurate, and science-based assessment of human health risk compared to default uncertainty factors; however, default uncertainty factors are typically used due to a lack of relevant pharmacokinetic and pharmacodynamic data in animal species and more importantly humans. For mesotrione, an herbicide that inhibits 4-hydroxyphenyl pyruvate dioxygenase (HPPD), pharmacokinetic parameters including half-life, time to maximum concentration, maximum concentration, and area under the plasma concentration versus time curve have been generated in human volunteers allowing for direct pharmacokinetic comparison to data generated in sensitive animal species. HPPD inhibitors lead to elevated levels of the amino acid tyrosine, which is implicated in many of the adverse findings in animal studies. The difference in pharmacodynamics leading to higher elevation of tyrosine in rats compared to mice and humans is understood based on species difference in the activity of the enzyme tyrosine aminotransferase. Tyrosine levels in humans following exposure to mesotrione have previously been measured and can be directly compared to levels in animal species. Collectively, these data serve as a basis for a CSAF derivation for mesotrione. The present research extends the calculation to generate a DDEF for a similar HPPD inhibitor. Pharmacokinetic and pharmacodynamics parameters in rodents, and in animal and human *in vitro* preparations were compared to mesotrione. Based on similarity to mesotrione a DDEF was proposed that provides the basis for more accurate human health risk assessment. In addition to the data on mesotrione, human data are available due to the use of one HPPD

inhibitor, nitisinone, as a human therapeutic. This information, along with compound specific information can be used to develop DDEFs that can be applied to new similar HPPD inhibitors to support more accurate human health risk assessments for this class of herbicides.

PS P384 - 0577 Vitamin E (α -Tocopherol) Improves Mitochondrial Energy Metabolism in Rat Liver following Acute Aluminum Phosphide Exposure

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Aluminum phosphide (AIP) or phostoxin is frequently used as fumigant and pesticide. Phosphine gas (PH₃) which is liberated from AIP lead to multiple-organ dysfunction syndrome (MODS) and mortality. Although the exact mechanism of AIP is unclear, the major mechanism of PH₃ toxicity seems to be related to mitochondrial dysfunction. Our previous clinical research showed that administration of vitamin E (α -tocopherol) along with supportive treatment could be considered in the management of acute AIP poisoning. The aim of the present study was to investigate the molecular and biochemical mechanisms on the protective effects of vitamin E in rat liver following acute AIP exposure to improve the mortality rate. AIP intoxication was carried with AIP (12 mg/kg body weight; LD50) by oral gavage. In treatment groups, vitamin E (50 and 200 mg/kg) was administered intraperitoneally after AIP intoxication. After administration, the animals were monitored until 72 h to examine the effect of vitamin E on AIP-induced death rate. Then, the animals were sacrificed and subjected to isolation of liver tissues for microscopic examination. The evaluation of mitochondrial function (complexes I, II, II/III and IV), production of reactive oxygen species (ROS) and apoptosis induction in liver tissues was analyzed by spectrometric and flow cytometric assays. According to our results, vitamin E at doses of 50 and 200 mg/kg decreased mortality rate and liver toxicity with changes in the histological observations. In addition, Vitamin E significantly improved activity of mitochondrial complexes (II, II/III and IV) in liver tissue. Furthermore, lower apoptosis and ROS production were detected upon vitamin E treatment of poisonous rats. Our results showed that vitamin E by attenuating oxidative stress and mitochondrial dysfunction is supposed to be a promising candidate to manage acute AIP poisoning.

PS P385 - 0620 Effects of Repeated Doses of the Organophosphorus Pesticide Temephos on Sperm Quality and Acetylcholinesterase Activity in Rats

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Organophosphorus pesticides (OP) are widely used in agriculture and in the control of vectors that transmit diseases, such as dengue, Zika, and Chikungunya (i.e. mosquito *Aedes aegypti*). Nowadays, one of the most commonly used OP in health campaigns in endemic areas is temephos (o,o,o,o'-tetramethyl-o'-thio-di-p-phenylene phosphorothionate; TEM) commercially known as Abate. Toxicity of TEM is not clear. WHO classifies TEM in the U category, with a recommended concentration of 1 ppm for drinking water to eliminate the larval phase of the mosquito. According to WHO, the LOAEL of TEM in male rats is 100 mg/kg/day (up to 44 days by gavage) evaluating clinical signs. However, EPA classified TEM in category II, with a LOAEL of 0.9 mg/kg/day in short-term (7 days) and long-term (up to 90 days feeding) studies. Preliminary data from our group suggest that TEM is metabolized by flavin monooxygenases (FMO) as well as esterases, generating toxic metabolites, including tidiophenol sulfone, also known as bisphenol S (BPS), which is identified as an endocrine disruptor. With this in mind and the limited and controversial information of TEM toxicity, we aimed to investigate the effects of repeated doses of TEM (100 mg/kg/7 days by gavage) on the acetylcholinesterase activity (AChE) and sperm quality, sperm integrity (by SCSA) and lipid oxidation (by MDA concentration) in cells collected during the epididymal maturation. Typical cholinergic signs were observed in some animals on day 3 of administration and AChE activity decreased 60% on this day and 70% on day 7. Regarding the effects on spermatzoa, the motility and viability significantly decreased (30 and 10%, respectively). The value of lipid oxidation significantly increase by 10% and no damage was

observed in the sperm DNA. This first approach allows us to suggest that the recommended safe doses of this OP have negative neurotoxic effects as well as on sperm quality, which may contribute to male reproductive toxicity. Current studies are in progress to better know the toxicokinetics of this pesticide that will contribute to know its safe use in endemic mosquito areas.

PS P386 - 0656 A Novel Mitochondrial Dysfunction Mechanism of Atrazine-Induced Nephrotoxicity in Quail (*Coturnix C. coturnix*) via Hindering Cross Talk between Detoxification Response and Nrf2-Mediated Antioxidant Defense

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Atrazine (ATR) is a most used herbicide which is believed as a pivotal determinant of environmental nephrosis, but potential mechanism is still largely unclear. This study intends to reveal a novel nephrotoxicity mechanism of ATR and provide some novel therapeutic targets on the nephrotoxicity mechanism of ATR. Quail were treated with 0, 50, 250 and 500 mg ATR/kg/d by oral gavage for 45 days. Blood samples were analyzed immediately for BUN and Cre content. The kidney tissue was collected and examined for histological and ultrastructural analysis. The activity/content of oxidative stress biomarkers (T-AOC, MDA, H₂O₂, GSH, GSH-PX, SOD and CAT) and expression of mitochondrial function related proteins (Cyt C, PROX3, VDAC-1, SIRT1, SIRT3, NRF1, TAFM, RAF1 and COA6), xenobiotic-sensing nuclear receptors response (AHR, CAR, and PXR) were determined. Nrf2 signaling pathway and detoxification response (phase I functionalization, phase II conjugation and phase III excretion) were detected. Kidney coefficient was decreased, biochemical and morphologic indices reflecting the kidney injury were significantly increased in ATR-exposed quail. ATR exposure upregulated the expression of proapoptotic factors (*Bax*, *Caspase 3* and *FasL*) and downregulated antiapoptotic factor (*Bcl-2*). Notably, cristae of mitochondria decreased, mitochondrial malformation and mitochondrial vacuolar degeneration were observed in ATR-exposed quail. ATR induced the disorder of mitochondrial function related factors expressions and promoted oxidative damage. Furthermore, xenobiotic-sensing nuclear receptors and the transcriptions of xenobiotic-sensing nuclear receptors-related target molecules were influenced in kidney, detoxification response and Nrf2 signaling pathway were blocked by ATR. Mitochondria in quail were the target organelles of ATR induced-nephrotoxicity. ATR induced renal damage via causing mitochondrial dysfunction, influencing mitochondrial function related genes expression, inhibiting detoxification response and modulating Nrf2 signaling pathway. These results suggested that ATR-induced nephrotoxicity in quail was associated with activating xenobiotic-sensing nuclear receptors responses and disrupting crosstalk between detoxification response and Nrf2-mediated antioxidant defense. Support: National Natural Science Foundation of China (No. 31572586), Excellent Youth Foundation of Heilongjiang Province of China (No. JC2017005), and China Agriculture Research System (No. CARS-35).

PS P387 - 0679 Synthesis, Determination, and the Neurotoxic Potential Effect of Temephos Oxidized-Metabolites

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Temephos (Tem) is an organophosphorothiate pesticide used to prevent the growth of mosquitoes responsible for the transmission of dengue, Zika, and Chikungunya viruses. World Health Organization recommended its use as a larvicide to control mosquitoes, midges, blackflies, and other insects at a concentration not exceeding 1 mg/L. The toxicological information about Tem is limited and has been considered innocuous, therefore it was included in the "U" group. However, US EPA classified Tem in the category II. The toxic action of Tem against the target species has been attributed to the inhibition of acetylcholinesterase (AChE) by Tem-oxons in the nervous system including humans. In chlorinated water, Tem is oxidized to temephos-dioxon-sulfoxide (Tem-dox-SO), temephos-dioxon-sulfone (Tem-dox-SO₂), and temephos-sulfoxide (Tem-SO). However, they have not been identified in mammal samples. To date, there is no available liquid chromatographic method for the analysis of these products in biological and environmental samples and the information about data on the dosimetry of Tem and its metabolites to establish a

dose-response relationship and temporal action that support a toxicological relationship is very limited. In this work, the preparation and structural characterization of the oxidation products of Tem is described. The compounds were obtained by the reaction between sodium hypochlorite and potassium periodate. The products were characterized by IR and ^1H , ^{13}C and ^{31}P NMR spectroscopy, and mass spectrometry. A liquid chromatographic method for the analysis of Tem and six metabolites was developed. Tem-dox-SO, Tem-dox-SO₂ and Tem-SO had the capacity to inhibit AChE and butyryl cholinesterase and this effect is depending on the degree of oxidation. This is the first report involving the chemical characterization of two Tem-oxons, allowing for their use as standards. The analytical method developed and validated in this study was successfully applied in water samples and represents an excellent approach for the analysis Tem and its metabolites in biological and environmental samples.

PS P388 - 0767 Stereoselective Copper-Dependent Hydrolysis of Trichloronate by Turkey Serum Albumin

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Trichloronate is a racemic organophosphatate insecticide. It induced delayed neuropathic in hens and humans. The avian are species with greater susceptibility to organophosphorus poisoning due to their low levels of serum paraoxonase-1. However, a copper-dependent hydrolyzing activity of trichloronate was identified in turkey serum. This study characterize of trichloronate enantiomers hydrolysis by turkey serum and commercial turkey serum albumin (TSA) in presence of copper sulphate using chiral chromatography with CHIRALCEL OD column and heptane HPLC as mobile phase. The hydrolysis results of 10 μL of turkey serum incubated with 400 μM of racemic trichloronate (~200 μM of each isomer) and 300 μM of copper at 37 °C, pH 7.4 for 60 minutes showed a significant hydrolysis of both enantiomers ($p < 0.05$). The (+)-trichloronate enantiomer showed a hydrolysis of 32% (residual concentration of 136 μM), while the (–)-trichloronate was hydrolyzed by 65% (70 μM residual). In hydrolysis term, (–)-trichloronate was hydrolyzed significantly higher than its corresponding (+) form. This stereoselective hydrolysis observed in turkey serum was confirmed by the incubation of 200 μg of TSA (amount of this protein in the 10 μL of turkey serum) with 400 μM of racemic trichloronate and 300 μM of copper sulphate at physiological condition of pH and temperature during 60 minutes; hydrolysis values of 90% (180 μM residual) and 72% (144 residual) were obtained for (–)-trichloronate and (+)-trichloronate, respectively. The present study shows the stereoselective hydrolysis of an organophosphatate racemic by an copper-dependent A-esterase activity in turkey serum and identifies to TSA as the cuproprotein responsible of this Cu²⁺-dependent stereoselective hydrolysis of this chiral insecticide.

PS P389 - 0812 Influence of an Intervention Program in the Concentrations of Organochlorinated Pesticides in Adolescents of an Agricultural Community of Mexico

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The Persistent Organic Pollutants (POPs) are a group of chemical compounds of global concern due to the health damage caused by the exposure to them. One of the biggest concerns, is the difficulty to eliminate these compounds from the body due to their physicochemical properties, for that reason, its important to work on strategies that help the body to excrete these substances. The objective was to evaluate the influence of an intervention program in the concentrations of organochlorine pesticides of teenagers living in a community whose main activity is agriculture. This is a longitudinal study, composed of 40 female adolescents between 15 and 17 years old. Two blood samples (pre and post intervention) were collected for the evaluation of 13 organochlorine pesticides in plasma. The intervention was focused on the health of the adolescents in which the main topics were gynecological, nutritional, environmental and psychosocial health issues. This study was approved by the Ethics Committee. We classified these compounds according to the type of molecule. Group 1 aromatics (2-4 'DDE, 4-4' DDE, 2-4 'DDT and 4-4' DDT), group 2 cycloalkanes (HCB, α -BHC, β -BHC and γ -BHC) and group 3 cyclodienes (Aldrin, Heptachlor, Oxidordane, Cis-nonachlor and Trans-nonachlor. In the case of aromatic compounds, in the first sampling the median obtained was significant less than the 2nd sampling (1165.8 ng / g lip vs 259.9 ng / g lip) ($p = 0.000$), in the case of the cycloalkanes statistically significant differences were obtained ($p = 0.000$) between the pre

(1396.9 ng / g lip) and post intervention (225.5 ng / g lip). Finally, in the group of cyclodienic compounds, significant results were obtained with $p = 0.000$ between the medians of the 2 samples (1377.7 ng / g lip vs 1.4 ng / g lip). These results were a great surprise for the research group, since it was never expected to decrease the concentrations of these agrochemicals due to the physicochemical characteristics of these compounds, which makes them lipophilic, so their elimination time of the organism is between 10 to 30 years. By doing an extensive research in the literature, different mechanisms can be proposed by which these significant results have been obtained.

PS P390 - 0911 Assessment of the Influence of Unutilized and Prohibited to Use Pesticides on Health of the Almaty Region Population

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Among the chemical pollutants of the environment, persistent organic pollutants (POPs) are particularly dangerous and adversely affect human health by causing serious diseases. These are 12 chemical substances, 9 of them are especially dangerous and prohibited pesticides. High rates of pollution by pesticides are characteristically for almost all regions of Kazakhstan. Data on the number of warehouses, as well as on the volumes of obsolete pesticides are contradictory. Therefore, the problem of obsolete, unsuitable for use of pesticides is very relevant in Kazakhstan. To determine the effects of long-term pesticidal environmental pollution on the state of public health, we have begun monitoring studies in rural areas of the Almaty region (southeast of Kazakhstan) where the agricultural direction is dominant. The results of monitoring observations have shown that the soil, as well as the natural and drinking water around the former pesticide storage facilities, have polycyclic component pollution, i.e., organochlorine pesticides and heavy metals are contaminated and the most potent pollutants are DDT and its metabolites. The residual amount of the metabolite DDD varied in drinking water from 1.7 to 3.9 mg/dm³, and in natural water from 2.4 to 5.4 mg/dm³. The concentration of pesticides in the soil exceeded the MPC from 60 to 120 times. An analysis of the indicators of the somatic health of the rural population living in close proximity to the locations of obsolete stocks of pesticides revealed that 80-95% of those surveyed have a "low" and "below average" level of health (express evaluation by G.L. Apanasenko). When studying the frequency of chromosomal aberrations, it was found that 23% of the surveyed population has an increased level (2-4%), and 3% a high level (more than 4%) of chromosomal abnormalities in peripheral blood lymphocytes. A comparative analysis of statistical reports for the years 2000-2010 revealed an increase in overall morbidity rates by an average of 50% with the predominance of respiratory diseases. During this period, there was also an increase in the number of congenital anomalies, both in the whole republic, and especially in the Almaty region (by 20%) due to the incidence of the rural population. Preliminary data indicate the existence of a certain risk of developing health disorders among the rural population of the Almaty region. The presented results are part of a comprehensive monitoring research of environmental quality, which will later serve as the basis for developing proposals to reduce environmental pollution by persistent compounds and strengthen public health issues in Kazakhstan. The work supported by STP No.BR05236379.

PS P391 - 0036 Retrospective Analysis of HIV Cure Applications at the US FDA: 2008–2018

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Several landmark moments have occurred in HIV research, one of which was in 1987, with the approval of the first anti-HIV pharmaceutical (AZT). In the following years multiple classes of drugs have been approved, a single pill once a day regimen approved, and pre-exposure prophylaxis (PrEP) is now standard. The future of HIV may now hinge on the case of the "Berlin Patient" who needed a bone marrow transplant and was given cells resistant to HIV. After the procedure, the previously HIV+ patient appeared have cleared the virus and has remained virus-free while off antiviral drugs for several years. This case generated interest in a new field of HIV research (coined "HIV Cure") with goals to discover a pathway for clearing replication competent virus (a sterilizing cure) or enhancing the immune system's ability to control replication without drugs (a functional cure). The US FDA Division of Antiviral Products evaluated IND submission data for a retrospective analysis of HIV Cure applications be-

tween 2008 and 2018. INDs were analyzed for mechanism of action, the date of submission, and the type of institution submitting the IND. The US FDA analysis demonstrated that there was an increase in interest in the HIV Cure field, with up to 25 different INDs by time of publication. The initial IND submissions began in 2008 (around the time of the "Berlin Patient") and rapidly increased around 2013. The increase in 2013 (and subsequent years) may have been due to the extra funding by President Obama for HIV Cure research late in 2013. The INDs under investigation at the US FDA cover various mechanisms, but most of the treatments are designed to deplete the viral reservoir as well as to prevent reinfection of other cells. Unlike many other fields of drug development, the bulk of the research in HIV Cure in the retrospective analysis was in government and academic facilities. The data are an important snapshot of the HIV Cure field over a 10-year period between 2008 and 2018 and a window into the future of HIV treatments, taking into consideration the toxicological profile and safety of these therapeutics.

PS P392 - 0232 Use of Read-Across to Characterize the Health Effects of Substances in the Anthraquinones Group in a Screening Assessment of the Chemicals Management Plan in Canada

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The Chemicals Management Plan is a multiphase, jointly managed initiative between Health Canada and Environment and Climate Change Canada dedicated to protecting human health and the environment from chemical risks. Seven substances known as the Anthraquinones Group are used as colorants in products available to consumers (Solvent Violet 13, Pigment Blue 60, Solvent Violet 59, Solvent Blue 36, Disperse Red 60, Acid Blue 239, and CAS RN 74499-36-8). Given limited health effects information for some substances in the Anthraquinones Group, potential analogues were identified by read-across in Health Canada. As described by the Organisation for Economic Co-operation and Development, this methodology is where endpoint or test results for one chemical is used to predict the same endpoint or test result for another chemical, which is considered to be similar based upon scientific justification. Anthraquinone, or 9, 10-anthracenedione, is a common core structure shared between substances in the Anthraquinones group and their analogues. It was used to inform the human health hazard for some substances in the group. Solvent Blue 36, which is part of the Anthraquinones Group, was also given its structural and/or functional similarities to other substances in the group. The critical health effects of this analogue anthraquinone include kidney tumors, as well as spleen, bone marrow, and liver toxicity. Pigment Blue 60 was not identified as a potential analogue for this endpoint, perhaps due to differences in structural alerts. All substances in the Anthraquinones Group except Pigment Blue 60 were considered to be nongenotoxic carcinogens based on anthraquinone. Margins of exposure between levels of exposure of the general population from use of Solvent Violet 13 in some cosmetics and levels associated with health effects (cancer and noncancer) were considered potentially inadequate to address uncertainties in the health effects and exposure databases, so were proposed to meet the criteria under the Canadian Environmental Protection Act to constitute or may constitute a danger in Canada to human life or health. Margins of exposure were, however, considered adequate for uses of other substances in the Anthraquinones Group (Pigment Blue 60, Solvent Violet 59, Solvent Blue 36, Disperse Red 60, Acid Blue 239, and CAS RN 74499-36-8). The application of a read-across approach was useful to address data needs for the human health screening assessment of the Anthraquinones Group, to conclude upon their potential risk to human health.

PS P393 - 0271 Prospective Application of Human Health Risk Assessment for Rodent Eradication Program on an Inhabited Island

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The human health risk assessment paradigm commonly used for evaluating past environmental releases was applied prospectively regarding potential risks and mitigation measures for a proposed Rodent Eradication Program on a permanently inhabited island—Lord Howe Island (LHI). The second-generation anticoagulant pesticide brodifacoum has been used in successful island eradications of introduced rodents. Proposed use on LHI represents extension

of eradication to an inhabited island with a permanent residential community, resulting in requests from residents for upfront evaluation of potential health impacts. LHI has extensive nature reserves and assessing exposure for these areas along with the residential settlement called for distinct sets of receptors and exposure scenarios. We included upfront engagement with community members to define exposure scenarios of local concern. The exposure assessment step addressed distribution of pesticidal bait pellets throughout the inhabited settlement and nature reserves at differing densities. Child and adult receptor scenarios were included and pathways considered included direct contact with bait pellets, potential transport to drinking water, exposure to degraded pellets in soil, and uptake in produce. For eradications on inhabited islands endpoints related to both long-term and acute toxicity must be addressed. Toxicity assessment included sensitive developmental endpoints related to repeated exposures and potential acute anticoagulant effects from direct ingestion of bait pellets by children. We established Reference Doses and an inhalation Reference Concentration to use for longer term exposures to soil, water, food, and air, and we evaluated case reports of poisoning to define a lowest-effect level for observable anti-coagulant effects from a one-time incident scenario with a child ingesting pesticide bait pellets. To provide protective toxicity values for risk-based decisions, we applied a read-across strategy from the teratogenic potential of warfarin using an adjustment factor applied to a no-effect level for anticoagulant effects, the most sensitive endpoint for brodifacoum. Risk characterization included standard hazard quotients and presenting acute risks in terms of the number of bait pellets that would need to be consumed. This first application of quantitative risk assessment to a rodent eradication program provided information on key pathways and exposures to monitor and control. Demonstrating that input from residents was included in the assessment increased confidence in the comprehensiveness of the assessment.

PS P394 - 0333 Risk Assessment Study on Inhalation Exposure to Benzene among Gasoline Station Workers: Impact on Human Health

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Benzene is human carcinogen and present as a component in gasoline (1% by volume), which can be evaporated into ambient air during the fueling process at gasoline stations. The aim of this study was to assess health risk on inhalation exposure to benzene among gasoline stations workers. The ambient benzene concentration was measured by personal sampling from 150 workers in gasoline stations (118 fueling workers and 11 cashiers) and analyzed by gas chromatography with a flame ionization detector. Additional data was collected via subject interviews using a structural questionnaire. Risk assessment on inhalation exposure was calculated following US EPA (2009) for non-cancer risk and cancer risk of chronic exposure to benzene. The results showed that non-cancer risk from chronic exposure to benzene was found in 51.33% of gasoline station workers (hazard quotient (HQ) ranged from 5.710E-04 to 1.816). The significant differences of HQ between fueling workers and cashiers and among three located zones (urban, suburban, and rural) of gasoline stations were indicated. The cancer risk was between 1.345E-08 and 1.518E-04, that affected 70.67% of those workers for lifetime cancer risk (>1.00E-06). These risk estimations considered at 50% inhalation uptake of inhaled air benzene without respirator use of gasoline workers. Therefore, the health surveillance program is recommended for gasoline station workers by monitoring of ambient benzene concentration and the biomarker of benzene exposure. Moreover, the employers should support the optimum eight working hours/day at gasoline stations and the use of P25 respirator for inhalation protection from benzene exposure.

PS P395 - 0363 Probabilistic Risk Assessment of Perfluorooctanesulfonate (PFOS) by Integrating In Vitro and In Vivo Toxicity with Physiologically Based Pharmacokinetic Models in Multiple Species

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The toxicity of perfluoroalkyl compounds, particularly perfluorooctanesulfonate (PFOS), has been extensively examined in *in vivo* and *in vitro* studies. However, the risk assessment for PFOS is problematic due to the differences in toxicokinetic properties between species and the uncertainty of dosimetry extrapolation from animals to humans. Our group recently developed a Bayesian

population physiologically based pharmacokinetic (PBPK) model for PFOS in multiple species, including mice, rats, monkeys, and humans. The objective of this study was to apply this model to conduct probabilistic risk assessment for PFOS by integrating available toxicity data and by addressing the uncertainty in toxicokinetics across species. In this study, we applied the PBPK model to predict the internal dosimetry (e.g., maximum plasma concentration and area under the concentration curve) in target organs of animals and humans. The internal dose metrics between species were compared to derive the human equivalent dose associated with reported points of departures (PODs) from animal studies. We also compared the predicted dose metrics to the reported potency (i.e., AC50) from *in vitro* assays of US EPA's ToxCast project. Based on the PODs (i.e., no observed adverse effect level [NOAEL] values) in the animal studies, the model-predicted human equivalent dose values of PFOS associated with adverse effects on the liver in monkeys and rats were 0.042 and 0.081 mg/kg, respectively. The analysis of human equivalent doses associated with the reported *in vitro* potency concentrations of PFOS that can disrupt various signaling pathways from EPA's ToxCast study is ongoing. Our preliminary results provide insights into hazard assessment, risk prediction, and reference dose recommendations for PFOS exposure for humans and this study serves as a probabilistic risk assessment framework that could be applied to other perfluoroalkyl compounds if sufficient toxicokinetic data and validated PBPK models are available.

PS P396 - 0417 Cancer Risk Estimation of Smoking Filter Cigarettes and Risk Ranking of Human Carcinogens in Active and Passive Smokers

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Cigarette smoke contains over 70 human carcinogens. Information on the cancer risk of smoking filter cigarettes and the risk priority ranking of carcinogens are valuable for the risk management of public health. The objective was to estimate the cancer risk for active and passive smokers from carcinogen exposure to mainstream and sidestream smoke of filter cigarettes. The levels of 17 human carcinogens, including acetaldehyde, benzo[a]pyrene, 1,3-butadiene and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), present in smoke of filter cigarettes were examined. Carcinogen exposure and risk calculations were based on the scenarios that active or passive smokers are exposed to smoke while smoking one cigarette. Of the carcinogens, 1,3-butadiene was found to produce the highest cancer risk from exposure to both mainstream ($2.8 \times 10^{-5} \sim 2.83 \times 10^{-4}$ /cigarette) and sidestream smoke (7.52×10^{-6} /cigarette), and secondhand smoke ($5.75 \times 10^{-5} \sim 6.02 \times 10^{-5}$ /cigarette). Acetaldehyde was estimated to produce the second highest cancer risk in mainstream ($2.33 \times 10^{-6} \sim 9.76 \times 10^{-5}$ /cigarette) and the fifth highest in sidestream smoke ($7.19 \times 10^{-7} \sim 7.79 \times 10^{-7}$ /cigarette) and the third highest in secondhand smoke ($5.50 \times 10^{-6} \sim 6.96 \times 10^{-6}$ /cigarette). The sum of cancer risks from the exposure to 17 carcinogens generated in one cigarette by mainstream smoke was calculated as $5.82 \times 10^{-5} \sim 6.00 \times 10^{-4}$ /cigarette. The sum of cancer risks from the exposure to 17 human carcinogens generated in one cigarette by sidestream smoke was calculated as $1.01 \times 10^{-5} \sim 1.22 \times 10^{-5}$ /cigarette, approximately 2~5.8 fold lower than in the risk produced by mainstream smoke. The sum of cancer risks from the exposure to 17 human carcinogens generated in one cigarette by secondhand smoke in 20 m³ small room was calculated as $7.65 \times 10^{-5} \sim 9.57 \times 10^{-5}$ /cigarette. Smoking one filter cigarette a day can significantly raise excess lifetime cancer risks in active smokers, and mainstream smoke is more harmful to active smokers than sidestream smoke is. Secondhand smoking by passive smoker can also raise excess lifetime cancer risks.

PS P397 - 0436 Estimation of Cancer Risk Imposed by Arsenic Exposure through Rice Consumption in Chinese Urban Population

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Inorganic arsenic (iAs) is a naturally occurring element widely present in food and the environment, and has been categorized as a human carcinogen by the International Agency for Research on Cancer (IARC). Due to the efficiency of rice in accumulating arsenic from soil and water, its intake is considered as

another major dietary exposure to iAs in addition to drinking water. A recent study showed that, in contrast to an average exposure level of 4.2 µg/day to iAs from drinking water in the US, the Asian and Pacific subpopulations exhibited the highest mean daily exposure of 2.8 µg/day iAs from cooked rice with a 95th percentile of 11.7 µg/day, corresponding to a mean and 95th percentile of rice consumption of 41.6 g/day and 169.3 g/day. However, these numbers may still underestimate the iAs exposure through rice intake in high rice-consumption populations, such as those in East Asian countries. The objective of this project is to estimate the distribution of iAs exposure from rice consumption in Chinese urban population and further quantify the associated cancer risk. We surveyed 1,800 residents in the cities of Beijing, Hangzhou, and Guangzhou (600 participants in each city) to understand the dietary pattern of rice consumption and the types of rice consumed. The survey was conducted through three in-field operations scheduled in winter, spring and summer 2018, and covered both sexes and different ethnic and age groups. Based on the rice type data collected in the survey, rice samples (proportionally composed of different types) were collected from markets in these three cities (140 samples in each city) and measured for iAs concentration. Together with a probabilistic estimation of bioaccessibility of iAs in rice derived from a meta-analysis of eight key studies, the rice consumption data and iAs concentration were integrated for a distributional estimation of daily iAs exposure from rice intake. A number of critical epidemiological studies were identified (consistent with those cited in governmental reports) and used to develop quantitative dose-response relationship between iAs exposure and lung and bladder cancer incidence. The estimates and results derived in dose-response assessment and exposure assessment were integrated for risk characterization. Results show that the Chinese population has a much higher rice intake rate: an average of 120 g/day with a 95th percentile of 300 g/day, corresponding to a median cancer risk about 100 cases per million persons with an upper bound of 250 cases. Findings suggest that adequate regulatory means should be considered to mitigate the risk to better protect human health.

PS P398 - 0460 Risk Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) Levels in Surface Water, Sediment, and Two Edible Fish Species from a Tropical Creek, Nigeria

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The contamination of aquatic ecosystems with Polycyclic Aromatic Hydrocarbon (PAHs) is a major concern due to the potential adverse effects to ecological receptors and human consumers via the food chain. This study assessed the risk associated with the levels of PAHs in surface water, sediment and two edible fish species, *Oreochromis niloticus* and *Chrysichthys nigrodigitatus*, from Agboyi creek, Southwest-Nigeria. Water, sediment and adult fish samples collected during the rainy season were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) following EPA methods. Average levels of PAHs ranged from below detection limits to 1.5 ± 0.27 mg/L, 0.0001 to 210.5 $\pm$ 30.05 mg/kg, 0.0001 to 121 $\pm$ 1.41 mg/kg, 0.0001 to 0.55 $\pm$ 0.00 mg/kg in surface water, sediment, *Oreochromis niloticus* and *Chrysichthys nigrodigitatus* respectively. Generally, the total PAHs burden in water and sediment were predominantly lower molecular weight (LMW) PAHs congeners, indicating petrogenic sources. Of all the total PAHs recorded, the highly carcinogenic Benzo[a] pyrene (0.337 ± 0.00 mg/kg) and Acenaphthene (121 ± 1.41 mg/kg) were also detected in *Oreochromis niloticus*. The recorded mean concentrations exceeded the World Health Organization (WHO) maximum permissible limit of 2 mg/kg in tissues of *Oreochromis niloticus* suggesting potential carcinogenic risk to human consumers. The result of the total toxic equivalent concentrations of carcinogenic PAHs (ΣTEQ_{PAH}) in sediment (44.05 mg/kg-BaP_{eq}) indicates low ecological risk; however, the obtained levels exceeded the international sediment quality guideline values (ISQG). Hence, there is need for continuous monitoring and effective environmental regulation in the area.

PS P399 - 0479 Risk Assessment of Some Heavy Metals in Cow Hides Processed by Different Methods in Nnewi and Environs

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Cow hide is a very common delicacy in Nigeria, and singeing in open fire is the major way of removing hairs from animals' skin. It is thought that the process of singeing could be a source of heavy metal contamination in meat. This study assessed the risk of heavy metals contamination of cow hides processed by various methods in Nnewi and its environs. Thirty six pieces of fresh cow

hides were obtained from the same animal skin at the Awka-Etiti abattoir, and divided into six equal groups as follows: Group I—unsinged (US), Group II—unsinged and boiled for 2 hours in distilled water (USB), Group III—singed with firewood (SF), Group IV—singed with firewood and boiled for 2 hours in distilled water (SFB), Group V—singed with tyre (ST), and Group VI—singed with tyre and boiled for 2 hours in distilled water (STB). After the processing, As, Cd, Cr, Cu, Ni and Pb levels were determined in the hides using the atomic absorption spectrophotometer. The following risk assessment indices were derived by standard procedures: expected daily intake (EDI) of metal, hazard quotient (HQ) and hazard index (HI). Results showed that the metals analyzed were significantly higher ($p < 0.05$) in the ST group than SF and US groups. The heavy metals levels in the samples were significantly lowered ($p < 0.05$) after boiling for 2 hours in water. The EDI of Cd, Cr, Cu and Ni in the samples were lower than the corresponding metal's oral reference dose (RfD), except Pb in the ST group, whose EDI (8.60 $\mu\text{g/kg/day}$) was $>$ RfD (4 $\mu\text{g/kg/day}$), and As in the US (0.40), ST (1.28) and STB (0.72) groups which were $>$ RfD (0.3 $\mu\text{g/kg/day}$). Lead had a HQ of 2.172 in the ST group, while arsenic had 1.333, 4.33 and 2.47 in the US, ST and STB groups respectively. All the samples had a HI $>$ 1, except the USB group (0.808). From this study, singeing of cow hides with tyre or firewood tend to increase the heavy metal contaminants, while boiling reduces the levels of the metals.

P400 - 0485 Exploratory Genetic Association of Type 2 Diabetes Occurrence Using a Pharmacogenetic Panel

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With upward of 400 million people living with some form of the disease, diabetes is an increasing public health concern. Type 2 diabetes mellitus (T2DM), the most prevalent form, is characterized by the acquisition of varying levels of insulin resistance which produces glycemic control issues. In addition to lifestyle and environmental factors, subject genetics are also thought to contribute to the susceptibility of T2DM development. This study sought to explore the association between the single nucleotide polymorphisms (SNPs) included on a pharmacogenetic panel and the presence of T2DM. A total of 63 de-identified subjects were recruited for this study by a third-party IRB-approved entity, thirty-two individuals (50% male) living with T2DM and non-diabetic subjects ($n = 31$, 71% male) serving as healthy controls. Following DNA extraction from whole blood, subject genetic information was analyzed using an iPLEX ADME PGx Pro v1.0 Panel comprised of various SNPs within selected pharmacokinetic-related genes. With the consideration of an additive genetic association model, simple logistic regression along with multiple logistic regression adjusted for available covariates (sex and age) were carried out in PLINK v1.7. Statistical significance was determined at a p -value less than 0.05. Mean subject age was 44.13 ± 11.86 years. Four SNPs (two in the *n-acetyltransferase 2* gene, another in *dihydropyrimidine dehydrogenase*, and one in *ATP binding cassette G2*) showed association with the presence of T2DM. The first was rs1801280 without and with covariate inclusion, ($p = 0.045$) and ($p = 0.031$), respectively. Secondly, rs1799929, without ($p = 0.045$) and with ($p = 0.031$) covariates achieve significant association. Thirdly, rs1801265 displayed significance with covariates ($p = 0.037$). Finally, rs2231142 was significant ($p = 0.033$) without covariates. The remaining SNPs examined did not show significant association regardless of covariate adjustment. In this study, two SNPs were found to be associated with T2DM regardless of adjustment for covariates; while two SNPs showed association depending on inclusion of covariates. Additional studies are required to further explore these findings in terms of confirmation and explanatory mechanisms. *This study was funded in part by a Research and Development Committee Interdisciplinary Grant from East Tennessee State University.*

P401 - 0510 Analysis of Personal Genomic Susceptibility for Atopic Dermatitis Disease by Using Cord Blood DNA in Small-Scale Birth Cohort

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Atopic disease is caused by a complex combination of environmental factors and genetic factors, and studies on influence of exposure to various environmental factors on atopic diseases are continuously reported. However, the exact cause of atopic dermatitis is not yet known. Our study was conducted

to analyze the association of SNPs (Single Nucleotide Polymorphisms) with the development of atopic disease in a small-scale birth cohort. Samples were collected from the Mothers' and Children's Environmental Health (MOCEH) study and 192 cord blood DNA samples were used to identify incidence of atopy due to influence of exposure to environmental factors. Genetic elements were analysed using a asian precision medicine research (APMR) array designed with various SNPs which are more associated with asian populations for personalized medicine. Case-control analysis of atopy disease revealed 253 significant variants ($p < 0.0001$) and SNPs on five genes (CARD11, ZNF365, KIF3A, DMRTA1, and SFMBT1) were variants identified in previous atopic studies. These results are important to confirm the genetic mutation that may lead to the onset of foetal atopy due to maternal exposure to harmful environmental factors. Our results also suggest that a small-scale genome-wide association analysis is beneficial to confirm specific variants as direct factors in the development of atopy.

P402 - 0515 The *In Vitro* Metabolism and Toxic Effects of Emerging Contaminants 2-Chlorothioxanthone Involved in Human CYP1A2 and CYP3A4 Enzymes

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As the additive materials in food packaging or UV curable resins, thioxanthenes (TXs) have been detected in various environmental media.^{1,2} The frequent exposure to TXs might cause adverse effect toward human health.³ Information on the metabolism and toxic effects of TXs is still very limited. Herein, we reported the metabolic susceptibility of 2-Chlorothioxanthone (2-Cl-TX) mediated by human CYP1A2 and CYP3A4 enzymes and *in vitro* investigated its toxic effect on the enzymatic activities, mRNA and protein expression of CYP1A2 and CYP3A4. Metabolites of 2-Cl-TX were identified by quadrupole-time-of-flight (Q-TOF) mass spectrometry and their site of metabolism was *in silico* predicted via calculation of activation energies. The two major metabolites of 2-Cl-TX were formed via the sulfoxidation or hydroxylation reaction.⁴ The enzymatic activities of two CYP enzymes were significantly inhibited by 2-Cl-TX with the IC_{50} of 8.36 and 0.86 μM toward CYP1A2 and CYP3A4, respectively. Further analysis with the real-time quantitative PCR and western blot assay revealed that the mRNA and protein expression level of two enzymes were concentration-dependently up-regulated in HepG2 cells by 2-Cl-TX.⁴ Our study suggests that the metabolic susceptibility and potential metabolic toxicities of chemicals should be seriously considered. *References:* [1] Liu, R.; Lin, Y.; Hu, F.; Liu, R.; Ruan, T.; Jiang, G., *Observation of emerging photoinitiator additives in household environment and sewage sludge in China. Environ. Sci. Technol.* 2016, 50 (1), 97-104. [2] Liu, R.; Mabury, S. A. *First detection of photoinitiators and metabolites in human sera from United States donors. Environ. Sci. Technol.* 2018, 52 (17), 10089-10096. [3] Reitsma, M.; Bovee, T. F.; Peijnenburg, A. A.; Hendriksen, P. J.; Hoogenboom, R. L.; Rijk, J. C., *Endocrine-disrupting effects of thioxanthone photoinitiators. Toxicol. Sci.* 2013, 132 (1), 64-74. [4] Zhan, T., et al. *Metabolic Susceptibility of 2-Chlorothioxanthone and Its Toxic Effects on mRNA and Protein Expression and Activities of Human CYP1A2 and CYP3A4 Enzymes. Environ. Sci. Technol.* 2018, 52, (20), 11904-11912.

P403 - 0549 Repeated-Dose *In Vivo* Oral Toxicity Study to Test Long-Term Effects of the Mycotoxin Enniatin B in Male and Female CD-1 Mice: Focus on Histopathological Data for the NOEL Definition

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Enniatin B (ENN B) is an emerging mycotoxin, secondary product of *Fusarium* fungi, detected in food and feed, in particular in cereal grains, often in combination with other mycotoxins. ENNB showed antibacterial, anthelmintic, antifungal, herbicidal, and insecticidal activities, the main mechanism of action being its ionophoric characteristics, although the exact mechanism remains still unclear. ENNB showed a potent cytotoxic activity in several mammalian cell lines; despite this, European Food Safety Authority stated that acute exposure does not indicate concern for human health. Nevertheless, insufficient data exist to establish a tolerable daily intake and/or an acute reference dose

given the lack of relevant toxicity data following chronic exposure and a risk assessment was not possible. For this purpose, a repeated-dose oral toxicity study has been performed in order to characterize the ENNB potential toxic effects at the whole organism level and upon long-term exposure. Adult male and female CD-1 mice were orally administered with 3 dose levels of ENNB by gavage daily for 42 days with a treatment schedule of 5 treatments/week. LD50 (350 mg/kg b.w.) was used as starting point to calculate the dose levels, namely 18, (=1/20 LD50 considering the long exposure period and in order to avoid overt toxicity), 1.8 and 0.18 mg/kg b.w. per day. Body weight gain and food consumption were recorded three times a week during the treatment period. At the end of treatment, mice were anaesthetized with a gaseous solution of isoflurane, and subsequently were sacrificed by CO₂ asphyxiation. The following organs were excised, weighed and fixed in 10% buffered formalin for the histological and histomorphometrical analysis: liver, spleen, kidneys, duodenum, adrenals, brain, thyroid, lymph nodes, thymus, heart, mammary glands (females only), uterus, epididymis and ovary. Testes were fixed in Bouin's solution. ENNB treatment affected spleen, brain and thyroid in both sexes, whereas thymus, kidneys, adrenals and reproductive system were affected in female mice only, and duodenum in male mice only. Thyroid and adrenals were target of ENNB and specific studies should be considered to verify potential endocrine disrupting mechanism of such mycotoxin. NOAEL for females is 0.18 mg/kg b.w. per day due to histomorphometrical effects on thymus, uterus and spleen. In male mice, the NOAEL is 1.8 mg/kg b.w. per day due to enterocyte vacuolization in duodenum. Overall for these endpoints, taking into account also the severity of the effects, female mice seem more susceptible to ENNB long-term effects.

P404 - 0624 Gaining Insights to Molecular Mechanisms of Combined Air Pollutants Exposures Using PBPK Models and Systems Biology Approaches

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Due to lack of accessibility, transparency and modeling knowledge, publicly available physiologically based pharmacokinetic (PBPK) models are rarely used in site-specific human health assessments. To address this issue, the Agency for Toxic Substances and Disease Registry (ATSDR) has developed a human PBPK model toolkit for multiple environmental priority pollutants using the Berkeley Madonna platform. The current work, extends the ATSDR PBPK toolkit to include code models for additional volatile organic chemicals (VOCs) such as toluene, ethylbenzene, xylenes (TEX) and their combinations. Comparisons were made between simulation profiles and experimental data involving goodness of fit by calculating the percent median absolute performance error and root median square performance error. Results using the recoded and original models, as well as an application of the recoded models to evaluate VOC data are presented. Human gene expression changes in target tissues after short-term and long-term TEX mixtures exposure were assessed. This evaluation was then combined with toxicity enrichment analyses to examine VOC exposure related changes in disease-networks pathways. The combined analysis showed that 236 genes expressed were common between the short-term and long-term exposures. These genes are important for the interconnecting biological pathways potentially stimulated by TEX exposure, likely related to respiratory and immune systems diseases. This kind of work increases the accessibility of PBPK models, encourages their integration into chemical risk assessment, and allows the evolution of advanced methodologies that augment combined chemical dose-response analyses with mechanistic insights. *The findings and conclusions in this presentation have not been formally disseminated by the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry and should not be construed to represent any agency determination or policy.*

P405 - 0694 Risk Assessment for Benz[a]pyrene Carcinogenicity in the Chilean Population

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Benz[a]pyrene (BaP) is a polycyclic aromatic hydrocarbon, ubiquitously found in air, soil, water, food after organic material combustion. This molecule has been classified as human carcinogen (IARC group 1) as it has been linked to digestive, respiratory and dermal cancer. There are no estimations for cancer risks due to BaP in the Chilean population, so here we use an ILCR approach to estimate risk of cancer for the average urban Chilean smoker and for the non-

smoker. We used data from the national health survey, which includes information about type and amount of food consumption and smoking habit. We also searched the literature for data on concentration of BaP in food, water and air. We show that the ILCR for a non smoker is 1.80×10^{-6} (1.8 people in a million) while the ILCR for a smoker is 2.7×10^{-4} (2.7 people in 10,000). Considering that 35% of the people in Chile are smokers, then 1581 people are at risk of developing cancer. We conclude that smoking largely contributes to risk cancer in the average Chilean population and we exclude food as a contributor to the risk.

P406 - 0706 Withdrawn

P407 - 0740 Quantitative Evaluation of Cytochrome P450 3A4 Inhibition and Hepatotoxicity in HepaRG 3D Spheroids

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Predicting drug-drug interactions (DDIs) and hepatotoxicity is an important issue during drug development to avoid unexpected side effects. Cytochrome P450 (CYP) 3A4 is one of major human hepatic phase I enzyme. It is important to test the potential of a drug candidate to induce CYP3A4 expression or inhibit its activity. Spheroid culture protocol using HepaRG cells was assessed and combined with liquid chromatography-tandem mass spectrometry (LC-MS/MS) to assess its ability to predict CYP3A4 inhibition. Midazolam was used to determine the long-term metabolic capacity of CYP3A4. Midazolam was decreased in the 3D HepaRG culture system by ~80% over 7 days. However, 1-hydroxymidazolam was increased by ~40%. Hepatotoxicity was also assessed by determining the cytotoxicity of known hepatotoxicants in HepaRG spheroids, HepG2 cells, and primary human hepatocytes. Significant differences in cytotoxicity were detected in the system using 3D HepaRG spheroids. These results suggest that 3D HepaRG spheroids are a good model for prediction of CYP inhibition and hepatotoxicity in screening of early drug candidates.

P408 - 0783 Concentration-Time Extrapolation of Short-Term Inhalation Exposure Levels for Dimethyl Sulfide

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Dimethyl sulfide (DMS, CAS 75-18-3) is an industrial chemical. It is both an irritant and neurotoxicant that may be life-threatening because of accidental release. The effects of DMS on public health and associated public health response depend on the exposure concentration and duration. However, currently, public health advisory information exists for only a 1 h exposure duration, developed by the American Industrial Hygiene Association (AIHA). In the present work, the AIHA-reviewed data were computationally extrapolated to other common short-term durations. The extrapolation was carried out using the toxic load equation, $C^n \times t = TL$, where C and t are exposure concentration and duration, TL is toxic load, and n is a chemical-specific toxic load exponent derived in the present work using probit meta-analysis. The developed threshold levels were vetted against the AIHA database of clinical and animal health effects induced by DMS. Tier-1 levels were derived based on human exposures that resulted in an easily detectable odor, because DMS is known to have a disagreeable odor that may cause nausea. Tier-2 levels were derived from the lower 95% confidence bounds on a benchmark concentration that caused 10% incidence ($BMCL_{10}$) of coma in rats during a 15 min inhalation exposure to DMS. Tier-3 levels were based on a $BMCL_{95}$ for mortality in rats. Emergency responders and health assessors may consider these computationally derived threshold levels as a supplement to traditional chemical risk assessment procedures in instances where AIHA developed public health advisory levels do not exist.

P409 - 0786 Risk Assessment of Heavy Metals in *Carica papaya* (Pawpaw) from Three Refuse Dump Sites in Rivers State, Nigeria

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The risk associated with three heavy metals (Cr, Cd, and Pb) in *Carica papaya* from three refuse dump sites in Rivers State, Nigeria, was examined. The study was carried out in three locations namely Ikwerre (station1), Oyiabo (station2) and Eleme (station3). The leaf, root, fruit of *Carica papaya* were collected from the three stations, at intervals of six weeks for six months, and transported to the laboratory for analysis. Atomic Absorption Spectrophotometer (AAS) was used to analyse metals concentration (mg/kg). The risk associated with metals concentrations and consumption were evaluated in terms of Estimated Daily Intake (EDI), Target Hazard Quotient (THQ) and Hazardous Index (HI). The metals (Cr, Cd, and Pb) concentrations (mg/kg) were obtained as follows; for the leaf, station1, 4.70 ± 3.59 , 0.54 ± 0.16 , 5.80 ± 1.79 , station2 4.76 ± 4.30 , 0.37 ± 0.19 , 6.62 ± 1.85 , station 3, 4.11 ± 1.45 , 0.44 ± 0.48 , 6.85 ± 1.98 , for the root, station1, 3.72 ± 4.427 , 0.26 ± 0.20 , 6.21 ± 1.59 , station2, 2.09 ± 2.30 , 0.28 ± 0.13 , 3.95 ± 1.04 , station3, 2.40 ± 3.68 , 0.41 ± 0.31 , 4.85 ± 1.31 , and for the fruit, station1, 1.96 ± 0.84 , 0.19 ± 0.09 , 4.62 ± 1.8 , station2, 2.17 ± 0.97 , 0.12 ± 0.07 , 1.62 ± 0.91 , station3, 0.55 ± 0.26 , 0.23 ± 0.11 , and 2.68 ± 1.65 , respectively. EDI values (mg/kg-1bw/day) of metals (Cr, Cd, and Pb) were as follows; for leaf, station1, 7.82×10^{-4} , 8.99×10^{-5} , 9.65×10^{-4} , station2, 7.92×10^{-4} , 6.16×10^{-5} , 1.10×10^{-3} , station3, 6.83×10^{-4} , 7.32×10^{-5} , 1.12×10^{-3} , for the root, station1, 6.19×10^{-4} , 4.33×10^{-5} , 1.03×10^{-3} , station2, 3.78×10^{-4} , 4.65×10^{-5} , 6.57×10^{-4} , station3, 3.99×10^{-4} , 6.82×10^{-5} , 8.07×10^{-4} , and for the fruit, station1, 3.26×10^{-4} , 3.16×10^{-5} , 7.69×10^{-4} , station2, 3.61×10^{-4} , 2.0×10^{-5} , 2.70×10^{-4} , station3, 9.15×10^{-5} , 3.83×10^{-5} , 4.46×10^{-4} , respectively. THQ values for Metals (Cr, Cd, and Pb), were as follows for the leaf, in station1, 0.52, 5.99, 0.69, station2, 0.53, 4.12, 0.79, station 3, 0.46, 4.82, 0.80, for the root station1, 0.41, 2.89, 0.74, station2, 0.25, 3.10, 0.47, station 3, 0.27, 4.55, 0.58, and for the fruit, station1, 0.22, 2.11, 0.55, station2, 0.24, 1.33, 0.19, station3, 0.06, 2.55, and 0.32, respectively. HI for all the metals across the stations were as follows; for the leaf, station1=7.2, station2=5.44, station3=6.08, for the root, station1= 4.04, station2=3.82, station3=5.4, for the fruit, station1=2.88, station2=1.76, and station3=2.93, respectively. This research study showed that metals bioaccumulated in *Carica papaya* were comparatively higher than WHO/FOA (2001/1999) safety limit. Prolong consumption of *Carica papaya* found in the sampled stations may pose health risk. Monitoring, evaluation, and recycling should be inculcated into waste management system.

P410 - 0822 *Aspergillus* Section *Nigri* from Agricultural Products and Cultivated Soil and Their Mycotoxin Production

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Sixty-three isolates of black aspergilli were obtained from 150 agricultural products and soil samples using the direct plating and dilution plate methods. Identification of the fungi was based on morphological characteristic and molecular analysis. Five species of *Aspergillus* section *Nigri* were found comprising *A. niger*, *A. aculeatus*, *A. tubingensis*, *A. acidus* and *A. japonicus*. *Aspergillus niger* was the dominant species followed by *A. aculeatus*, *A. tubingensis*, *A. acidus* and *A. japonicus*. For mycotoxin production, the fungi were cultured on yeast extract sucrose medium and incubated at 28°C for 14 days. They were assessed for mycotoxin by using an Enzyme Linked-Immunosorbent Assay. All isolates could produce ochratoxin A and fumonisin except two isolates each of *A. tubingensis* and *A. niger* were non-toxicogenic. *Aspergillus niger* (KUFC 7206) from brown rice produced the highest amount of ochratoxin A at 131.9 µg/L followed by *A. aculeatus* (KUFC 7860) from peanut at 117.6 µg/L and *A. japonicus* (KUFC 7264) from sugarcane field at 106.9 µg/L. Moreover, *A. niger* (KUFC 7129) from glutinous rice produced the highest fumonisin content at 7.4 mg/L. Other isolates of *A. niger* producing ochratoxin A and fumonisin were found on peanut, brown rice, dried chili, black sesame, garlic and shallot.

P411 - 0866 Nonclinical Development of Cedazuridine, a Novel Cytidine Deaminase Inhibitor for Use in Combination with Decitabine to Enable Oral Administration to Patients with Myelodysplastic Syndromes (MDS)

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Cedazuridine (E7727) is a synthetic nucleoside analog derived from tetrahydro-uridine (THU) and designed as a potent inhibitor of cytidine deaminase (CDA) with improved stability over THU. It is currently being developed in combination with hypomethylating agent decitabine (ASTX727) as an oral option for treatment of MDS and CMML. Concomitant administration of cedazuridine enhances oral bioavailability of decitabine and achieves therapeutic AUC exposures in the clinic at low doses of decitabine (similar to IV dose, in mg) that are well tolerated. Nonclinical development of cedazuridine included full toxicological and DMPK evaluation. Cedazuridine is well tolerated in mice and monkeys (tox species) over 1-cycle of 7 days dosing, (followed by recovery), with NOAEL at 1000 mg/kg and 200 mg/kg, respectively. Subchronic studies of 13-weeks duration with multi-cycles (28 days/cycle with dosing on days 1-7) resulted in NOAEL of 100/300 mg/kg in mice (females/males) and 60 mg/kg in cynomolgus monkeys. The highest non-severely toxic dose (HNSTD) in multi-cycle study was 200 mg/kg/dose in monkeys. Target organs at the NOAEL were lymph nodes in mice and GI mucosa and bone marrow (RBC parameters) in monkeys. These dose levels offer a large safety margin over the clinical dose (and systemic exposures) used for cedazuridine (100 mg fixed dose, in combination with 35 mg decitabine). Cedazuridine in mouse *in vivo* micronucleus study was negative at up to 2000 mg/kg and was negative in *in vitro* Ames and chromosome aberration tests at concentrations that were not cytotoxic. Co-administration with decitabine in mice and monkeys resulted in significant increase in systemic exposures compared with decitabine administered alone. Cedazuridine is metabolically stable in liver microsomes and hepatocytes, does not inhibit major human CYP enzymes, and is not a substrate and/or inhibitor of major human drug transporters. It does not accumulate in tissues and is excreted mainly renally. The main metabolite was its epimer, to which it partially converts in acidic environment in the stomach prior to absorption. In summary, cedazuridine has been well characterized in nonclinical toxicology and DMPK studies and its nonclinical data profile supports late-stage clinical development.

P412 - 0892 Use of *In Vitro* to *In Vivo* Extrapolation (IVIVE) to Support Risk Assessment for Early Life Stages: Life-Stage Physiologically Based Pharmacokinetic (PBPK) Modeling for Pyrethroids

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Under the new toxicity testing paradigm that is largely based on *in vitro* and *in silico* approaches, alternative strategies are needed to address potentially sensitive populations in chemical risk assessment. To address the concern for age-related sensitivity to pyrethroids, life-stage physiologically based pharmacokinetic (PBPK) modeling supported by *in vitro* to *in vivo* extrapolation (IVIVE) was conducted to predict age-dependent changes in target tissue exposure to 8 pyrethroids. The purpose of this early age dosimetry is to calculate a chemical specific adjustment factor (CSAF) to address age-related pharmacokinetic (PK) differences for pyrethroids in humans. We developed a generic human PBPK model for pyrethroids based on our previously published rat model that was developed on *in vivo* rat data. Metabolic constants were determined for human PBPK model input. Intrinsic clearance (Cl_{int}) of each individual cytochrome P450 (CYP) or carboxylesterase enzyme (CES) that are active for a given pyrethroid were measured *in vitro*, which were then biologically scaled to *in vivo* to obtain the age-specific total hepatic Cl_{int} of the pyrethroid together with human metabolism enzyme ontogeny for those enzymes. The results demonstrate that the age-related differences in internal exposure to pyrethroids in the brain are largely determined by the differences in metabolic capacity for

pyrethroids between children and adult. Consistent with the literature, our *in vitro* metabolism data and IVIVE analysis showed that CES1 enzymes are largely responsible for pyrethroids metabolism in humans, contributing to more than 70% of the metabolism of each of these pyrethroids. The most important conclusion from our research is that, given an identical external exposure, the internal (target tissue) concentration is lower in children than in adults in response to the same level of exposure to a pyrethroid. This is due to both chemical-dependent factors such as metabolism and chemical independent (physiologic) factors in children which include: higher liver blood flow as a fraction of total blood flow (cardiac output) and higher liver weight as a fraction of body weight. Our results show that, based on the use of the life-stage PBPK models with 8 pyrethroids, CSAF values at child and adult ages are essentially equal to or less than 1, resulting in a CSAF for age-related PK differences of 1. For risk assessment purposes, this indicates that no additional adjustment factor is necessary to account for age-related PK differences for these pyrethroids.

PS P413 - 0955 Risk Assessment of Lead Contamination Outdoor of Lead Recycling Company

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In relation to several concerns of the population close to a lead foundry and recovery company, located in an industrial area of a mining zone of Chile in Calama, the company decides to assess the risk of lead and other mining related contaminants in 4 quadrants of the city. For this study, we applied the risk assessment criteria of ATSDR in relation to site description, agents of interest and its quantification, environmental routes, human entry ways and population risk. For the description of the area, we considered the components of topography, weather, winds, slopes and geological origin of soils. Regarding the contaminants, we identified other sources of metals to the environment, natural and anthropogenic, legal and illegal. Among the illegal resources, there were lead-acid battery and tires burning in abandoned sites, therefore, we analyzed samples from the surface and profound material. All these samples were assessed by IR-spectroscopy, alongside the quantification of Pb, As and Hg. As baseline, 2 areas were selected. The results show that the 4 areas analyzed, including the baseline samples, were in acceptable environmental levels of Pb according to EPA and international norms for soils in urban areas. For As, one area close to an urban zone had high concentrations, above the Chilean norm and international reference norms, for surface and profound material. This is related to the use of material for stabilize the area, which generates isolated points in urban areas that should be mitigated. For Hg, its levels were below the lowest risk referential level. This study established that the outdoor impact of this foundry doesn't determinate an unacceptable risk, and that there are other environmental sources related to other processes.

PS P414 - 0957 Systematic Update of the Mammalian Relative Potency Estimate (REP) Database for Dioxin-Like Compounds (DLCs)

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Potential exposures and health risks of DLCs are assessed using Toxic Equivalency Factors (TEFs) developed by the World Health Organization (WHO). The TEFs were last reviewed by a WHO Expert Panel in 2005 and the underlying database considered at the time was comprised of 634 REPs derived from *in vivo* and *in vitro* studies. The objective herein was to update the REP database. Using a search strategy employed systematically, >50 studies published between 2005–2018 were identified as eligible for inclusion (based on criteria utilized for the 2005 update). The number of REPs from each study ranged from 1 to 183, representing multiple congeners, models, and endpoints assessed in each study. For each dataset, study information, dose-/concentration-response data, author-calculated REPs, and/or reported benchmark calculations (e.g., EC₅₀s) at the endpoint-model level were extracted using templates in DistillerSR. In contrast to the previous REP database update, graphical data extraction was performed using graph digitization software. The following hierarchy was then used for development of REPs: (1) If concentration-/dose-response data was available, the data was modeled using methods and models similar to those used in Benchmark Dose Software (BMDS) version 3.1, implemented in R to facilitate batch processing, and REPs were computed using fitted models (>200 REPs); (2) If these data were not available, author-reported

REPs were retained (>400 REPs); and (3) If neither of these were available, benchmark ratios were calculated (e.g., REP_{EC50} = EC_{50 ref comp}/EC_{50 congener}) (>80 REPs). REPs were then assigned a weight based on six primary characteristics of study quality (i.e., study type, experimental model, pharmacokinetics, REP derivation method, REP derivation quality, and endpoint relevance). This update has substantially increased the size of the REP database, with over 1200 REP values across the 29 congeners. This transparent and comprehensive approach to update the REP database is anticipated to facilitate determination of TEFs quantitatively using all available data while accounting for quality, relevance, and a consistent approach for determining the TEF for each DLC.

PS P415 - 0960 Environmental Contamination: The Toxic Legacy of a Native American Tribe Continues

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Beginning in the early 1960s through the late 1970s, Ford Motor Co. dumped paint sludge and other toxic metals from its Mahwah plant into the local suburbs, including Ringwood, NJ. As a result, Ringwood became riddled with contaminated soil and water, placing a large environmental health burden on the very people who have lived on this land for generations—the Ramapough Tribal Nation. Upon community request, soil samples were collected from chemically impacted areas of Ringwood by citizen scientists and NYU graduate assistants in order to evaluate soil metal concentrations. Samples were collected by digging 3-5 inches deep into the soil with a plastic trowel. Once dried, samples were crushed and homogenized and digested by adding 2 ml of nitric and 1 ml of hydrochloric acid to 1 g of soil. The soil digest was then diluted with water and filtered using a 0.45mm filter to capture any particulate matter. Using ICP-MS, 36 metals were identified and their levels compared to New Jersey Direct Contact Soil Standards for dermal, ingestion, and inhalation exposure. Vanadium (V), a transition metal readily taken up by plants, was found in several soil samples at levels that exceeded the NJ soil standard of 78 mg V/kg. At high concentrations, ingestion of Vanadium has been correlated with adverse health effects in the human gastrointestinal, cardiovascular and renal systems and its cancer-causing potential is still being debated. Given the elevated V concentrations in the soil samples of this region, residents consuming plants grown in this area or recreating in the area could be at increased risk for adverse health effects. Supported by NIEHS Core Center Pilot Grant.

PS P416 - 0005 Oral Ingestion of a Novel Oxygenating Compound, Ox66™, Is Nontoxic and Results in Increased Oxygenation

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Ox66™ is a novel solid-state oxygenating compound. In order to support the use of Ox66™ as a potential oxygenating supplement to alleviate conditions caused or exacerbated by hypoxia, this study evaluated the safety of Ox66™, its ability to withstand the conditions in the digestive tract, and its potential to increase oxygenation in the mesentery in rats. The toxicity of Ox66™ was evaluated by performing acute (10-day) and chronic (90-day) feeding studies on rats. The stability of the compound in the digestive tract was evaluated via *ex vivo* simulated digestion and subsequent CFDA viability assay on gut epithelial cells. Its capacity for oxygenation in the mesenteric microcirculation was determined by interstitial fluid pressure (P_{isf}) O₂ measurements upon injection into the small intestine of rats. No toxicity was found associated with acute or chronic oral administration of the compound in rats, and the compound was able to withstand the environment of the digestive tract *in vitro*. Based on the animal feeding study, the NOAEL was considered to be 1000 mg/kg/day. This proof of concept study further demonstrates the potential of Ox66™ to function as an oxygenating supplement that might be useful for treating either pathological hypoxic-related conditions or to improve oxygenation levels during or after exercise under healthy conditions.

PS P417 - 0155 A Review for the Best Practices for the Application and Preparation of SEND Datasets

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Since December 18, 2016, Standard Exchange of Nonclinical Data (SEND) datasets have been required for New Drug Applications (NDA), abbreviated new drug applications (ANDA), and certain biologics license applications (BLA), and since December 18, 2017, SEND was required for nonclinical data contained in all Investigational New Drug Applications (INDAs). The preparation of these datasets is an additional step in the process for submission. As always, timing is crucial for submissions with sponsors looking for the most aggressive timelines to reach the market and gain market competitiveness. This article will look at the key steps to optimize delivery of these SEND datasets. This optimization starts with alignment of terminology and the internal scheduling and processing, so that the draft and final SEND datasets can be sent to the client within days of issuance of the definitive reports. The internal processing and scheduling works, too, for legacy studies or studies run by a client at a different CRO. The key factor here is effective and proactive communication with the sponsor to define the format of the data and thereby reduce or minimize the need for any data reentry or manual manipulation of data. This presentation will illustrate the processes for formatting send datasets based on experiences learned to date when preparing SEND datasets for US FDA submission, and looking forward March 2019, some safety pharmacology will be included, with SENDIG3.1.

PS P418 - 0158 Pioneering Better Science to Advance the 3Rs in Pharmaceutical Toxicity Testing

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The UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) is an independent scientific organization committed to advancing the development and application of 3Rs approaches. Toxicology testing for drug safety is an area of vast animal use, with high potential for severe adverse events. We therefore have a wide variety of projects to identify 3Rs opportunities and disseminate the outcomes globally. Our strategy includes the development of new models, tools, and approaches through grant funding, alongside our open-innovation platform CRACK IT to ensure that emerging technologies are incorporated into practice and provide commercial as well as scientific benefit. By engaging with international expert working groups of scientists from industry, academia, and regulatory bodies, we collect, analyze, and share data to support the 3Rs and provide scientific evidence to promote best practice and acceptance of alternative approaches to minimize animal use and suffering. Examples of our projects to address reduction, refinement, and replacement will be presented. Whilst data from animals is still expected for regulatory submission, our projects promote the appropriate use of different species for monoclonal antibody development (in particular to reduce the use of nonhuman primates), minimize the number of animals required by reducing the use of recovery animals, and encourage the application of micro-sampling to reduce the need for satellite animal groups. Promotion of social housing during telemetry recordings and the development of an innovative home cage analysis system (providing 24-7 monitoring of rodent behaviors in a group-housed environment) are refinements to necessary procedures that can reduce stress and improve animal welfare and data quality. Ultimately, replacement of animal models may be realized in the future through the application of adverse outcome pathways, the use of *in silico* modeling, and wider use of human pluripotent stem cell technologies. These methods may also provide valuable mechanistic understanding and higher predictive/translational value through use of human cells/tissues/data. Through these and other activities, we have influenced company practice and regulatory guidelines, highlighting how the 3Rs can be applied without compromising the drug development process, regulatory requirements, or human safety.

PS P419 - 0177 A Review of Procedure-Induced Lesions in Sprague Dawley Rats and Cynomolgus Monkeys When Utilizing Continuous Intravenous Infusion as the Route of Administration

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Procedure-related lesions resulting from continuous infusion can occur in both Sprague Dawley rats and Cynomolgus monkeys. This has potential to confound drug safety evaluation. This review will serve as a background database to help distinguish from test article-related changes and those due to the route of administration. Following four-week intravenous infusion toxicity study of sodium chloride in cannulated rats and monkeys, procedure-related changes were observed in organ weights and macroscopic and microscopic pathology. Group mean absolute and relative spleen weights were increased in male rats. Procedure-related macroscopic changes were noted in injection site (single firm nodule) and spleen (enlarged) in rats. Primary and secondary procedure-related microscopic finding occurred in injection site, adrenal glands, bone marrow, liver, and spleen in rats. Procedure-related macroscopic changes were noted as minimal swelling subcutaneous tissue of the skin in dorsal area of the thorax in monkey. These data together with control data from ongoing studies will help to distinguish test article-related and -unrelated lesions.

PS P420 - 0269 Preclinical Toxicology Testing Data for Alignment with the Regulatory Update of the Eurasian Economic Union

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A recent regulatory update from the Eurasian Economic Union (EEU) is intended to integrate the members of the Union (i.e., Armenia, Belarus, Kazakhstan, Kyrgyzstan, and Russia), and consequently, facilitate free movement of products, particularly medicinal products. This emerging regulation of the EEU emphasizes the requirement of product dossiers with testing data conducted in adherence with international standards and regulations. However, circumstances wherein sufficient safety data is available for a product registered in other regulatory markets, conduct of additional toxicology studies may not be necessary. There is an urgent need for harmonization of this process in the EEU. Although the expectations, according to the current EEU guidelines, are to provide an updated dossier with preclinical testing data conducted under Good Laboratory Practice, there is no clear recommendation on how to adjust the product dossier registered in one of the member states to a centralized EEU format. One of the most important aspects of this "harmonization" process is the expectation of maintaining a comprehensive approach toward provision of data for risk/benefit evaluation using state-of-the-art methods for toxicology testing. Here we discuss two bacterial lysate products that have been marketed earlier in Russia and clinical safety data were available, but nevertheless, toxicology testing was conducted to align with the recent update to the EEU regulations. Both Imudon (oral dispersible tablets) and IRS 19 (nasal spray) products were investigated on 28-day toxicity studies with 14 days of the follow-up period. The final dosage form of Imudon and solution of IRS 19 were administered to Wistar rats at three dose levels by oral gavage and intranasal route respectively. No signs of toxicity were observed during the administration period in all doses tested. The results of the toxicology studies did not reveal any treatment-related adverse effects on behavior, clinical biochemistry, and hematology. Also, no gross necropsy and histopathology changes were detected. These studies on the bacterial lysate products serve as examples to encourage the drafting of a potential strategy for testing and provision of an updated dossier to the EEU for registration of products with sufficient safety data. Insights from these studies indicate that conduct of toxicity testing may not necessarily be initiated as a part of the dossier adjustment strategy at all times. An in-depth risk assessment to address any safety concerns before initiation of further testing on a case-by-case basis may be an approach for consideration of alignment with the EEU dossier update.

P421 - 0435 The Needleless Injection Site Swabbable Connector (NISSC): A Practical and Reliable Tool That Permits Dogs to be Exercised on Continuous Intravenous Infusion Studies

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Allowing dogs assigned to sub-chronic and chronic toxicity studies to get out of their home cage and exercise a few times a week is a standard practice at ITR that provides enrichment, and is well in-line with the recommendations of most Institutional Animal Care Committees. However, historically dogs on continuous infusion studies could not be exercised since they were surgically implanted with a permanent intravenous catheter. ITR has managed to identify a suitable quick release connector which allows the dogs to be disconnected from the infusion system for exercise. We have recently completed a 4-week method development study using the Needleless Injection Site Swabbable Connector (NISSC) in which dogs were surgically catheterized in the usual way but NISSC was used to hook them up to the infusion pumps. During the study, the dogs were disconnected from the pumps for 5x/week and allowed to exercise freely for 20 minutes/day before being reconnected to the pump. There were no abnormal clinical observations, macroscopic signs of infection at the femoral infusion site, or changes in body weights, food consumption and behavior that were considered to be due to the use of the NISSC. There were no incidents during the 20 minute daily exercise and no difficulties were encountered during the disconnection and reconnection of the infusion system prior to and after dog exercise. Therefore, the NISSC was considered to be a safe, viable, low cost and very practical tool in permitting dog exercise on continuous infusion studies.

P422 - 0536 Development of QSAR Models to Predict Potential Nephrotoxic Ingredients in Traditional Chinese Medicines

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Along with the broad use of traditional Chinese medicines (TCMs), incidences of kidney impairment caused by TCMs have attracted considerable interest in investigating the corresponding toxic ingredients. The safety assessment for herbal medicine is challenging since they contain complex mixtures of phytochemicals, most of which have unknown toxicological effects. Quantitative Structure–Activity Relationship (QSAR) modeling is an efficient tool to evaluate the potential toxicity. Several QSAR models have been applied to predict hepatotoxicity caused by TCMs because of the more liver toxicity data and explicit mechanism. QSAR model for evaluating nephrotoxicity induced by TCMs, however, is still in its infancy. The objective was to develop a QSAR model for predicting the potential nephrotoxicity of the ingredients in TCMs. We developed QSAR models for predicting nephrotoxicity based on three types of datasets from 609 compounds, including natural products datasets, drugs datasets and mixed datasets (containing both kinds of data). Each dataset was used for modeling by utilizing artificial neural networks (ANN) and support vector machines (SVM) algorithm separately. Both the internal (ten-fold cross validation) and external validation (30 ingredients from TCMs as external dataset) were performed on each model. The predictive performance of models was evaluated by accuracy, sensitivity and specificity. Six QSAR models for predicting nephrotoxicity were developed and have produced reliable performance in internal validation. The prediction accuracy ranged from 78.8% to 96.9%. For external validation, 30 ingredients from TCMs were predicted well by natural products models (accuracy: ANN 96.7%, SVM 93.3%). Only one or two compounds were predicted with error. Mixed models (accuracy: ANN 76.7%, SVM 66.7%) showed better performance than drugs models (accuracy: ANN 50%, SVM 53.3%). The QSAR models based on natural products datasets or mixed datasets could improve the predictive power of models over nephrotoxic ingredients in TCMs. Particularly, natural products models could produce more reliable results and were more robust in evaluating the potential nephrotoxicity induced by TCMs. Additionally, it has the application not only on screening nephrotoxic ingredients in TCMs, but it is also helpful at prioritizing the subsequent toxicity testing of natural products. *Financial support: This work was supported by the Special Research Project of Traditional Chinese Medicine (201507004-1).*

P423 - 0569 Toxicological Safety Assessment of Novel 5,6-Dihydropyridazine-1(4H)-Carbohydrazide: An Antitubercular Agent

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A novel series of 5,6-dihydropyridazine-1(4H)-carbohydrazides and its analogs was synthesized and characterized spectroscopically. All the compounds were characterized and screened for *in vitro* anti-tuberculosis (anti-TB) activity against *Mycobacterium tuberculosis* H37Rv strains by using resazurin assay utilizing microtiter-plate method. These compounds also showed good antitubercular. Thus, the high level of activity shown by the compounds (7a, 7e) suggests that these compounds could serve as leads for development of novel synthetic compounds with enhanced anti-TB activity. After that, a toxicological safety assessment was conducted on 5,6-dihydropyridazine-1(4H)-carbohydrazides, to predict safety with oral consumption by rats. Two genotoxicity studies were conducted and no evidence of mutagenicity or genotoxicity was observed in the presence or absence of a rat liver S9 metabolic activation system at concentrations up to 5,000 µg of compound/plate in a chromosomal aberration assay. Studies conducted in Wistar rats included a 14-day acute oral toxicity study, and a 90-day repeated oral toxicity study. A 6-month repeated oral toxicity study was conducted in rats. In the 14-day study, the NOAEL was determined to be 5 g/kg bw. While a few statistically significant ($p < 0.05$) findings were observed in the 90-day Wistar rat study, it was considered to be a sound basis for conducting a 6-month study. In the 6-month rat study, the no observed effect level was concluded as 1,000 mg/kg bw/d, the highest dose group tested. Finally, in a developmental toxicity study in rats no fetal abnormalities related to administration of the test article were observed.

P424 - 0609 Prediction of Seizure Liability in Cultured Human iPSC-Derived Neurons Using MEA System

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Human iPSC-derived neurons are expected to be applied to toxicity evaluations in nonclinical studies. Microelectrode array (MEA), measurement system of the electrophysiological activity, are suitable to evaluate the seizure liability of drugs. Human iPSC-derived cortical neurons and astrocytes were cultured on 24-wells MEA plate for extracellular recording using MED64 Presto. Twelve compounds (pentylentetrazole, picrotoxin, 4-aminopyridine, linopyridine, amoxapine, strychnine, pilocarpine, amoxicillin, chlorpromazine, enoxacin, phenytoin, and acetaminophen) and dimethyl sulfoxide were tested at 5 concentrations for each compound ($n > 6$). In this study, we aimed to develop an analytical method enabling the detection of toxicity of convulsants and the prediction of mechanism of action (MoA) using deep learning. We firstly had artificial intelligence (AI) learned the data of convulsants and the data of non-convulsants. Next this AI predicted the toxicity of the data that not used for learning. The toxicity probability of unlearned sample data was 90% or more, and the toxicity probability of the unlearned convulsants was also 80% or more. In addition, the negative probability of non-convulsants and the prediction probability of MoA was more than 80%. These results indicated that this AI analysis method is useful for predicting the convulsion toxicity and the MoA of new drugs using hiPSC-derived neurons.

P425 - 0815 A 10-Week Telemetered Electroencephalographic and Cardiovascular Comparison between Two Sodium Channel Blockers in Sprague-Dawley Rats

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Vixotrigine, a state- and use-dependent Nav1.7 channel blocker that also inhibits other voltage-gated sodium channels, and carbamazepine (CBZ), a marketed non-selective sodium channel blocker that also inhibits on Nav1.7, were evaluated in telemetered male and female Sprague-Dawley rats for 10 weeks. Vixotrigine was dosed at 100 or 300 mg/kg, CBZ was dosed at 100, 200 or 400→300 mg/kg in males and 50, 100, 200→150 mg/kg in females. Data on cardiovascular (electrocardiograph [ECG], blood pressure), electroencephalographic (EEG), and functional observation battery (FOB) parameters were collected and analyzed. In-life parameters including mortality, clinical signs, body weight, food consumption, and body temperature were also evaluated. The study was conducted to demonstrate non-inferiority of the safety profile of vixotrigine to CBZ. To our knowledge, this is one of the longest and most extensive comparative cardiovascular or EEG telemetry studies performed, with the duration based on the limit of telemetry battery life. Study animals that died were evaluated histologically to assess cause of death. There were no vixotrigine-related deaths observed in rats; deaths in vixotrigine groups were associated with experimental procedure or a spontaneous condition. Compound-related death, in addition to demise due to experimental procedure or spontaneous condition, was observed with high dose CBZ. Clinical sign profiles, including reduced activity at higher dose levels, and decreased body weight and food consumption were similar for both compounds. In some cases, extra supportive care was required for individual animals due to decreased body temperature, which may be associated with mechanism. No FOB-related findings aside from decreased group mean body temperature, were observed for either compound, and no EEG-related signals were observed with vixotrigine. An increase in blood pressure was observed with both vixotrigine and CBZ at higher doses. Heart rate was decreased with CBZ, but vixotrigine did not alter heart rate. There were slight increases in QRS, QT, and QTc in males at a high dose of vixotrigine. There were increases in PR, QT, and QTc in males and females at all CBZ doses, and increased QRS in males at all doses. Safety margins for vixotrigine and CBZ against human clinical exposure determined at the no observed adverse effect levels (NOAELs) for each of the compounds in the study were equivalent or slightly greater for vixotrigine versus CBZ. Overall, the vixotrigine safety profile in Sprague-Dawley rats was similar to or better than that of CBZ.

P426 - 0824 Safety Evaluation of Drugs Using PDX (Patient-Derived Xenograft) and Solid Cancer-Derived Organoid Culture Systems

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Organoid is a three-dimensional culture method that enables long-term culture of cells of various differentiation stages including stem cells. We have developed an alternative model for carcinogenicity testing that uses histopathological neoplastic lesions as an endpoint after subcutaneous inoculation of nude mice with organoids exposed to chemicals *in vitro* and have shown its usefulness. With the aim of further developing the *in vitro* test method, we established and characterized PDX (patient-derived xenograft) and organoids by using colorectal cancer surgical specimens of 45 people provided by the National Cancer Research Center Central Hospital. We confirmed that the PDX and organoids transplanted to NOG mouse, whose T cells, B cells and complement activity are deficient, have histological characteristics of human colorectal cancer. Although PDX and organoids are considered to be useful in preclinical tests and personalized medicine, evaluations comparing the characteristics of these two model systems have not been conducted in detail yet. Therefore, in this study, we are promoting acquisition of multi-layered omics information such as NCC oncopanel, DNA microarray analysis, and drug reactivity results using the PDX and organoids as materials. By comparing the results of NCC oncopanel between the surgical specimens, PDX and organoids, most of the orig-

inal tumor mutations were maintained in both PDX and organoids, but only some were detected in the original tumor. This is presumed to be related to the heterogeneity of the original tumor heterogeneity. In addition, while gene expression patterns of PDX and organoids were comparable with the original tumor, the expression of interstitial / immune genes were not reproduced. PDX containing stroma is more similar to the original tumor, but organoids specialized for the epithelial system are useful because of its ease of establishment and culture, and its ability to simultaneously compare multiple test conditions. It has the advantage of being easy and simplifying insights into the mechanism of action. In addition, organoids derived from normal epithelium, which were impossible in other culture systems, have also been established, and can also be used for evaluation of carcinogenicity. These results suggested that considering of the advantages of each resource is required, when we apply patient-derived models for non-clinical studies.

P427 - 0863 Acute and Subacute Oral Toxicity Study of KCFC0051 in Rats

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KCFC0051 is named transformed chemically from the Trifluoperazine. Trifluoperazine is well-known antipsychotic agent used to treat schizophrenia, has been studied as strong anticancer. The present study aims to evaluate the safety of KCFC0051 for the development of anticancer drug. We performed acute and subacute oral administration (2 weeks repeated) in SD rats. The KCFC0051 was administered to male and female SD rats at oral doses of 290, 350, 420, 500 mg/kg (acute study) and 0, 40, 80 and 160 mg/kg (subacute study). Experimental animals were monitored for the mortality, body weight changes, food intake, clinical signs and gross findings during observation or administration period. Subacute toxicity study was also accompanied by hematological and biochemical analyses. As a result, approximate LD50 of acute toxicity study is 516.1 mg/kg (male), 318.1 mg/kg (female), respectively. And our data suggested that under 40 mg/kg/day of KCFC0051 is maximum dose for > 2 weeks subacute study.

P428 - 0887 Assessment of a 28-Day Oral Exposure to the Dietary Supplements Nattokinase and Lumbrokinase Alone or in Combination with Aspirin in Sprague-Dawley Rats

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Nattokinase (produced by *Bacillus subtilis* var. *natto*) and lumbrokinase (produced by earthworms) are fibrinolytic enzymes promoted as health supplements for improving cardiovascular and circulation health in humans. The possibility exists that these fibrinolytic enzymes may increase the risk of bleeding, especially when taken together with other clot-modifying compounds. We report the findings of a 28-day study in which male and female Sprague-Dawley rats were dosed daily by oral gavage with nattokinase or lumbrokinase (1000 mg/kg body weight (bw)/day), individually or in combination with aspirin (10 or 100 mg/kg bw/day). No treatment-related changes were observed in body weight, grip strength or motor coordination behavior assessments, bleeding time, or clinical pathology. Both doses of aspirin inhibited the arachidonic acid-induced platelet aggregation. This effect was observed also when aspirin was co-administered with nattokinase or lumbrokinase, except in the low aspirin plus lumbrokinase combination group. The reduced effect of the low aspirin dose in the presence of lumbrokinase was probably due to the hydrolysis of aspirin by lumbrokinase esterases. Statistically significant effects were observed also in males treated with the high aspirin for a few parameters measured by thromboelastography; the magnitude of the effects was more pronounced in the high aspirin plus nattokinase combination group. At the histopathology level, the only treatment-related observations were an increase in the incidence of mucosal erosion and regenerative epithelial cell hyperplasia in the glandular stomach in the high aspirin groups, individually or in combination with lumbrokinase or nattokinase. This work was sponsored under an interagency agreement between the FDA/NCTR and the NIEHS/NTP (FDA IAG # 224-12-0003/NIEHS IAG # AES12013).

PS P429 - 0905 A Collaborative Effort to Establish Nonanimal Cosmetic Safety Assessment Globally by 2023

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In recent years there has been significant progress globally in advancing the science to underpin nonanimal cosmetic safety assessment. This has facilitated legislative change within some countries; however, to achieve global cosmetic safety assessment without the use of animal testing, there is more to do. A unique collaboration has just been formed between nonprofit groups, industry partners and other interested groups and coordinated by Humane Society International that intends to help shape future cosmetics legislation and invest in community-based learning that will ensure the ongoing ability to meet regulations which require nonanimal safety approaches and enable a world of animal testing-free cosmetics safety assessment within the next five years. The collaboration focuses on delivering three primary objectives: (1) Global harmonization of nonanimal cosmetic safety assessment legislation in at least 50 key beauty markets by 2023. Priority regions are ASEAN, Australia, Brazil, Canada, Chile, Mexico, South Africa, Sri Lanka and the United States. (2) Sharing information on evolving decision-making approaches without new animal testing. These risk assessment processes are necessarily exposure-led, product and use-specific and iterative. The expertise of multiple stakeholders and relevant case studies will be developed to provide clarity on how to make risk-based cosmetic safety decisions through the integration of scientific evidence from multiple sources. (3) Investment in community-based learning: communication between large companies with advanced capacity, smaller companies with less capacity, regulators, contract research laboratories, regulatory compliance consultants and others will be initiated to develop effective tools and curricula for communicating risk based on problem formulation. Problem formulation includes regulatory context, product and use type, as well as considerations of user populations. Capacity building is necessary within both the regulated and regulatory cosmetic communities for the long-term acceptance and implementation of these new approaches. Since this is a global effort, this will require translation and modification of the curricula to meet the needs of different countries. The collaboration is now inviting interested parties to join in developing detailed plans for capacity building and education, and ensuring globally harmonized legislation.

PS P430 - 0949 JSOH Evaluation of 1-Bromopropane Related to Setting an OEL

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The Japan Society for Occupational Health (JSOH) is currently evaluating the toxicity of the volatile cleaning solvent 1-bromopropane (1-BP) to determine a recommended Occupational Exposure Limit (OEL). OELs are set in a conservative fashion to optimize worker safety. The lowest level at which a relevant toxicity is measured is usually established as the quantitative starting point prior to incorporation of additional safety factors. Determination of the appropriate OEL for 1-BP is a challenging task as neurotoxicity, carcinogenicity, and developmental toxicity represent potential toxicities. The largest human neurotoxicity study evaluated 1-BP workers occupationally exposed in China. This study is confounded due to improper application of 128 Hertz manual tuning forks for determination of peripheral toxicity by measuring distal latency (DL). In the United States, DL for research purposes is measured using an automated vibration stimulator, e.g., the CASE IV system. The National Toxicology Program (NTP) 2-year inhalation study on 1-BP suffers from interpretability issues due to problems in extrapolating bronchioloalveolar lung tumors in mice to pulmonary adenocarcinomas in humans. An analysis of the entire NTP database previously published by our group suggests that intestinal adenomas in female rats are the tumors in the 2-year NTP 1-BP inhalation study most appropriate for inclusion in risk assessment. The lowest observed adverse exposure limit (LOAEL) for reproductive damage from 1-BP was determined from three inbred strains of mice. These inbred mouse strains are extremely sensitive to 1-BP due to enhanced metabolism and production of cytotoxic metabolites not seen in rats or other strains of mice. It is problematic to extrapolate these results to humans. The highest quality set of data regarding the risk of inhalation to humans is represented by the development of intestinal adenomas at 500 ppm following 2-year exposure in female rats. These data are the most appropriate starting point for calculation of the OEL for 1-bromopropane.

P101 - 0046 Trace Element Concentrations in Atmospheric (PM₁₀) Particles from La Habana, Cuba

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This study presents one of the first datasets on heavy metals in airborne PM₁₀ from the city of Havana, Cuba, and is compared with levels in another Caribbean city (San Juan, Puerto Rico). Airborne particulate matter (PM₁₀) samples were collected at two sites in Havana for a period of one year and heavy metals determined using ICP-MS. These data are being compared with urban PM₁₀ particulate matter from Puerto Rico, another Caribbean Island. Here we report the airborne concentrations of seven heavy metals (Cu, Zn, Pb, Hg, Co, Cd, and Ni). We found that the highest concentrations were obtained for Cu, Zn, and Pb in Cuba when compared with Puerto Rico. These exceeded the National Ambient Air Quality Standards of the US EPA. On the other hand, concentrations of Ni and V were a lot higher in Puerto Rico air when compared with that in Cuba.

P102 - 0053 Effect of the Exposure to Ambient Particulate Matter on Striatal Dopamine Transmission and Motor Activity

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Exposure to fine and ultrafine particles (FP and UFP) induces oxidative stress and inflammation in the striatum, a neuronal nucleus critically involved in the control of motor activity. We previously showed that acute exposure to FP reduced the density of striatal dopamine D₂ receptors (D₂Rs), whereas the subchronic exposure did not, but decreased agonist-induced G protein activation as evaluated by [³⁵S]-GTPγS binding to membranes. Alterations in dopamine levels, D₂R density, and signaling have been associated with neurological disorders such as depression, autism, and Parkinson's disease. In this study we explored whether the *in vivo* exposure to FP and UFP alters dopaminergic transmission at the cellular level, and its effect on motor activity. Male adult Wistar rats were exposed for 3 days (acute exposure) or 32 days (subchronic exposure) to FP, UFP, or filtered air (5 h per day) by using a particle concentrator. Fine and UFP exposure concentrations were 344 and 135 µg/m³ and 116 and 75 µg/m³ for the acute and subchronic exposure, respectively. The uptake and release of [³H]-dopamine were assessed in striatal synaptosomes, and D₂R affinity for dopamine by the displacement of [³H]-spiperone binding in striatal membranes. Motor activity in the open field test (dark phase) was measured at the beginning and the end of every exposure. The acute exposure to FP or UFP increased [³H]-dopamine release evoked by depolarization to 26 and 42% over control values, respectively, and reduced spontaneous motor activity by ~20%, with no effect on [³H]-dopamine uptake or D₂R affinity. As previously reported, acute exposure to FP or UFP reduced D₂R density by 28.6 and 36.7%, respectively (control, 308 fmol/mg of protein). In addition, herein we show that subchronic exposure to FP and UFP decreased [³H]-dopamine uptake by 18% and 25%, respectively, and reduced spontaneous motor activity by ~15%, with no effect on [³H]-dopamine release or D₂R affinity. These results indicate that both acute and subchronic exposures to particulate matter alter striatal dopaminergic transmission and reduce locomotion in rats, the effects being more notorious for UFP. *Financial support: Conacyt CB 167778 and 220448 to ADVR and JAAM.*

P103 - 0097 Serum Clara Cell Protein as a Pulmonary Damage Biomarker for Fine Particulate Matter Exposure in Chinese Population

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Particulate matter is the key component of air pollutants, mainly produced by emissions of coal-fired plants and road traffic. Exposure to fine particulate matter (PM_{2.5}) pollution is associated with increased morbidity and mortality

for respiratory diseases. However, few population-based studies have been conducted to assess the alterations of circulating pulmonary proteins due to long-term PM_{2.5} exposure. We designed a two-stage study. At the first stage, we enrolled 558 coke oven emission exposed workers (COE cohort) with a wide range of PM_{2.5} exposure levels as the PM_{2.5} exposed group and 210 controls in China. Pulmonary injury was measured by lung function and serum Clara cell protein (CC16), surfactant protein A (SP-A), and surfactant protein D (SP-D). Linear regression models were used to assess the associations between PM_{2.5}, pulmonary injury markers, and lung function. At the second stage, significant initial findings were validated by an independent diesel engine exhaust (DEE) cohort with 50 DEE exposed workers and 50 controls. Serum CC16 decreased in a dose-response manner in association with both external and internal PM_{2.5} exposures in two cohorts. In the first stage, serum CC16 levels decreased with increasing duration of occupational PM_{2.5} exposure history. An IQR (122.0 µg/m³) increase in PM_{2.5} was associated with a 5.76% decrease in serum CC16, whereas an IQR (1.06 µmol/mol creatinine) increase in urinary 1-OHP concentration was associated with a 5.36% decrease in serum CC16 in the COE cohort. In the validation stage, the concentration of serum CC16 in PM_{2.5} exposed group was 22.42% lower than that of the control, and an IQR (1.24 µmol/mol creatinine) increase in urinary 1-OHP concentration was associated with a 12.24% decrease in serum CC16 in DEE cohort. We conducted a two-stage study with different PM_{2.5} exposure levels originated from emissions of coal combustion and vehicle exhaust to evaluate the dose-response relationship. We found that serum CC16 may serve as a sensitive marker for pulmonary damage associated with chronic PM_{2.5} exposure.

P104 - 0141 Estimates of the Global Burden of Meteorological Factors and Air Pollution on Hand, Foot, and Mouth Disease (HFMD) Infectious among Children

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Hand, foot, and mouth disease (HFMD) is a common illness caused by a variety of enteroviruses, such as enterovirus 71 or Coxsackie, and usually affects children. Meteorological factors and air pollution exacerbate HFMD among young populations around the world and may also contribute to new-outbreak HFMD. We aimed to estimate the number of HFMD cases and new-onset HFMD outbreaks globally attributable to temperature, humidity, fine particulate matter (PM₁₀), ozone, and nitrogen dioxide (NO₂) concentrations. We used epidemiological health impact functions combined with data describing population, baseline HFMD incidence and prevalence, and pollutant concentrations. We constructed a new dataset of national and regional HFMD infectious rates among young people with HFMD under 18 years of age using published survey data. We estimated that 5–15 million and 3–5 million annual HFMD infectious globally could be attributable to temperature and ozone, respectively, representing 6–21% and 3–11% of the annual number of global infectious, respectively. The range reflects the application of central risk estimates from different epidemiological meta-analyses. Anthropogenic emissions were responsible for 42% and 81% of ozone and PM₁₀ impacts, respectively. Remaining impacts were attributable to naturally occurring precursors or emissions. The largest impacts were estimated in China and India. These findings estimate the magnitude of the global HFMD burden that could be avoided by establishing climate early change alarm system or take cooperation worldwide to take large-scale action to reduce air pollution.

P105 - 0239 AhR-Mediated Regulation of SOCS3-STAT3 Axis Is Involved in Downregulation of B[a]P-Induced Retinal Lesion

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Most recently, cigarette smoking has been associated with such chronic ocular diseases including retinopathy and age related macular degeneration (AMD). Several studies have indicated that smokers with more severe inflammatory response is a major risk for retinal lesion. However, the underlying mechanism is not well established. Aryl hydrocarbon receptor (AhR) is a ligand-activated receptor that metabolite several chemical in cigarette such as Benzo [a] pyrene (B[a]P). In this study, we investigate the inflammatory effect of AhR expression level with B[a]P exposure in retinal pigment epithelium (RPE). We found that B[a]P may affect the AhR-regulated suppressor of cytokine signaling

3 (SOCS3) feedback axis and cause retinal lesion *in vivo*. The results showed that B[a]P (0.1~10 μ M) decrease both SOCS3 protein and mRNA expression in dose dependent manner which were determined by western blotting and RT-PCR, respectively. Furthermore, knock out of AhR by CRISPR and AhR inhibitor CH223191 treatment both significantly decrease SOCS3 expression in RPE cells. Signal transducer and activator of transcription 3 (STAT3)-inducible element (SIE) reporter assay showed that STAT3 activity increased in AhR knockout cells with B[a]P treatment, but not wild type RPE. Importantly, our study showed that upregulation of STAT3 activation by B[a]P treatment associate with AhR expression level in RPE cells. Last, we found that the interaction between STAT3 and SOCS3 in AhR^{-/-} RPE with higher increase than wild type RPE cells within B[a]P treatment. For further examining the downstream of STAT3 activation, the ELISA assay was conducted and showed the upregulation of IL6 in AhR^{-/-} RPE cells. Taken together, knock out of AhR attenuate B[a]P-induced SOCS3 expression, which may dysregulate the homeostasis of SOCS3/STAT3 axis and accompany with the increase of the pSTAT3 activation. Moreover, we also found the pathological changed of retina in AhR^{-/-} and long-term B[a]P-exposure (90 days) mice. This is the first finding for elucidating the role of AhR-regulated SOCS3 and STAT3 on retina lesion. Our results reveal that the low level of AhR associate with cigarette smoking or other polycyclic aromatic hydrocarbons (PAHs) exposure may lead a cause of retina inflammation, which provide a novel concept and contribute to developing the innovative prevention in ocular diseases treatment.

P106 - 0268 Cytotoxicity of Ultrafine Diesel Exhaust Particles in Endothelial and Microglial Cell Monocultures and Mixed Coculture

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Diesel exhaust particles (DEPs) are a recognized risk factor for several health conditions including neurodegenerative diseases. However, information regarding the toxicity of low doses of ultrafine DEPs in the brain is limited. While *in vitro* studies of cerebral capillary endothelial cell (CEC) monocultures provide insight into the cellular mechanisms by which particulate matter (PM) cause toxicity, they do not account for mitigating or aggravating effects of cell-cell interactions on PM toxicity. The goals of this study were to evaluate the cytotoxicity of low doses of ultrafine (UF) DEPs and to investigate if cell-cell interactions mitigate or aggravate effects of UF DEP exposure. Rat brain microvascular endothelial cells (BMVECs) were used as a simple blood-brain-barrier (BBB) model, and rat microglia as model immune cells of the brain. To determine cytotoxicity of DEP and investigate the effect of cell-cell interactions, this study used LDH and MTT assays on three different *in vitro* cell culture systems: endothelial and microglial monocultures, and a mixed coculture. The cells were exposed to 2 ng/ml, 1 μ g/ml, and 20 μ g/ml UF DEPs in 96-well plates for 24 hours before assaying. Results indicated that low doses of UF DEPs significantly decreased cell viability (i.e., increased LDH leakage and decreased cell metabolic activity) in a dose-dependent manner in the monocultures, but not in the mixed coculture. Additionally, cell-cell interactions in the coculture appear to attenuate the cytotoxic response when compared to the microglial monoculture, but exacerbate the response when compared to the endothelial monoculture. This study demonstrated that exposure to low doses of UF DEPs can disrupt the function of BMVECs and microglia, and that cell-cell interactions can influence the degree of cytotoxic effects from exposure to environmental toxicants. Future studies involve repeating this experiment using a complete *in vitro* neurovascular unit model to further investigate the effect of cell-cell interactions on cytotoxicity of UF DEPs.

P107 - 0380 Ambient Fine Particulate Matter Induced Pulmonary Vascular Lesions in Mice and Its Cellular Mechanisms

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Epidemiological studies have reported the association between exposure to ambient particulate matter (PM) and pulmonary vascular diseases. However, the causal relationship remains unclear due to complex compositions of PM. We collected the fine fraction of PM from the ambient air in industrial region of Taiwan and investigated its health effect and mechanisms. Oropharyngeal aspiration of industrial fine PM resulted in medial thickening and then progressed to intimal hyperplasia in pulmonary small arteries adjacent to the bronchioles in mice within 8 weeks. Smooth muscle α -actin staining revealed that pulmonary arterial smooth muscle cells (PASMCs) were increased in the remodeled vessels. Since dysfunction of medial PASMCs contributes to wall thickening and

intimal lesion formation, we investigated the effects of PM on cell functions *in vitro*. Treatment of mouse primary PASMCs with industrial fine PM increased inflammatory cytokine productions and VSMCs migration and viability, accompanied with decreased PASMCs marker expressions. These results suggest that PASMCs transitioned from a differentiated to synthetic phenotype. These findings provide important new insights regarding the pathophysiological mechanisms for PM-induced pulmonary vascular diseases.

P108 - 0419 The Hematological Effects of Airborne Fine Particles via the Interaction of the Kallikrein-Kinin, Complement, and Coagulation Systems

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Particulate matter (PM) pollution is now a severe suspending environmental problem, and the public concerns about its potential hazards to cardiovascular system are continuously rising. Due to the inevitable contact of airborne PM with the plasma, the potential interaction between PM and plasma components would be highly vital to elucidate haze-induced hematological effects. In this study, the potential effect of PM in hematological homeostasis through triggering the crosstalk of some critical enzyme cascade systems in plasma, including kallikrein-kinin system (KKS), complement, and coagulation systems, was investigated. Using the *ex vivo*, *in vitro* and *in vivo* models, fine particles collected by either quartz fiber filters (QFFs) or polypropylene filters (PPFs) were found to activate the key zymogens of KKS, including Hageman factor XII (FXII), plasma prekallikrein (PPK), and high-molecular-weight kininogen (HK) in a dose-dependent and time-course manner. This process was initiated by the binding of fine particles with FXII, the initial zymogen in the KKS, and its subsequent auto-activation. Additionally, the complement and coagulation cascade activation were promoted upon fine particles exposure. More specifically, these reactions were triggered by the activation of the KKS, which was demonstrated by the inhibitor experiments of aprotinin and corn trypsin inhibitor (CTI). The data provided solid evidences on hematological effects of airborne PM through inducing the activation of the KKS, complement and coagulation systems, which would be valuable in the risk assessment on air pollution-related cardiovascular diseases.

P109 - 0429 Evaluation of Long-Term Exposure to Air Pollution in the Development of Systemic Lupus Erythematosus in NZBWF1 Mice

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Systemic lupus erythematosus (SLE) is an autoimmune disease with manifestations in various organs. Nephritis is one of the most common and serious manifestations. Etiology is still not known, however environmental factors seem to interact with others triggering, aggravating or predisposing to this disease. Air pollution has been systematically associated with systemic inflammation and autoimmunity. Thus, we hypothesized that air pollution may have influence in SLE development. To test this, we analyzed the effects of exposure to fine particulate matter (PM_{2.5}) on mice genetically predisposed to SLE (NZBWF1 mice). Results from this study will allow us to obtain more information about SLE etiology and may contribute to reinforce the need of reduction in air pollutants levels. Female NZBWF1 mice were divided in two groups (n=5): SLE-FA (Filtered Air) and SLE-NFA (Non-Filtered Air). SLE-NFA group were daily exposed to PM_{2.5} using an Ambient Particle Concentrator from day 90 to day 210 (4 months) to a daily dose of 600 μ g/m³. We assessed weight and protein urine content monthly. After day 210 animals were euthanized, and the following parameters were analyzed: body weight, weight of organs and the dosage of protein, creatinine and albumin in urine. Future analyses will include quantification of autoantibodies, cytokines, oxidative stress and kidney histopathology. SLE-NFA group presented higher body weight than SLE-FA in the 1st month of exposure (p=0,003). SLE-NFA animals presented increased percentage weights of kidney (p=0,029) and thymus (p=0,017), suggesting increased local and systemic activation of inflammation. In urine, SLE-NFA presented higher Protein-Creatinine Ratio value when compared to SLE-FA in the 2nd month of exposure (p=0,043). Other urine analyses showed a tendency of proteinuria and albuminuria increase in animals exposed to PM_{2.5} with the progression of exposure, which suggests declines in renal function. NZBWF1 is a well-established model for SLE. Our preliminary data indicate that independent of the exposure, both groups would develop the disease. However, exposure to PM_{2.5} favors a

worse prognostic, possibly due to systemic inflammation induced by air pollutants. NZBWF1 female mice had some SLE manifestations more pronounced in group exposed to air pollution. Although the differences were small, preliminary results are corroborating with the findings from the literature. These data suggest that air pollution can contribute to the aggravation of SLE. *Ethics Committee Approval: CEUA-FMUSP: 095/17.*

PS P110 - 0446 Health Effects and Climate Change Caused by Fossil Fuels Compared with Other Energy Sources

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The production and burning of fossil fuels causes both disease and climate change. The purpose of this presentation is to show that efforts to decrease fossil fuel health effects in occupational and environmental exposures will also make significant impacts on climate change. Health effects from the production and burning of fossil fuels are immediate and quantifiable, whereas the effects of climate change are in the future and often difficult to comprehend and quantify. In a particularly deadly episode in London in 1952 caused by the burning of coal during a weather inversion, an estimated 4,000 people died from respiratory causes. Recent air pollution episodes in India and China dramatically demonstrate similar acute respiratory effects. The World Health Organization estimated that about one-quarter of worldwide deaths from major lung and cardiovascular diseases could be attributed to ambient air pollution. Coal and diesel combustion produce airborne particulates, and chronic exposures result in lung cancer and other respiratory diseases. Climate change is also caused by the production of carbon dioxide by combustion of these fuels. The US Environmental Protection Agency estimated the economic costs of health effects from US fossil fuel power plants at one-half trillion dollars per year. Furthermore, all other use of fossil fuels, and especially auto exhaust, causes harmful air pollution, especially the production of ozone. Natural gas production also leads to ozone due to the release of volatile organic compounds and truck exhaust. The leakage of methane in this process is a major cause of climate change. Historically, coal mining has been associated with coal-miner's lung, and today's hydraulic fracturing using sand can cause silicosis. Nuclear energy production and renewables such as solar and wind power do not contribute to climate change and their effects on health are much less pronounced than those of fossil fuels. This remains true, even after taking into account the health consequences of the Chernobyl disaster and the relatively small amount of environmental damage caused by the manufacture of components for wind and solar energy production. In contrast to these other sources of energy, both climate change and toxic air pollution have the same major cause: fossil fuels. It is important to realize that the immediate, quantifiable health effects that can be identified by toxicologists can provide important guidance for policies that can also prevent climate change.

PS P111 - 0496 Comparative Study on Genotoxic Activity of Different Components of Fine Particulate Matter (PM_{2.5}) Using *In Vitro* Bioassays

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This study evaluates the genotoxicity activity of PM_{2.5} samples collected at Pudong (PD) and Huinan (HN) of Shanghai with urban and suburban characteristic respectively. The PM_{2.5} samples were collected on quartz filters in summer and winter on a monthly basis during the year 2016. Later, the filters were divided into three parts for different treatment, each part was extracted by ultra-pure water and/or dichloromethane, n-hexane to obtain total particulates (TP), organic component (OC) and water soluble fractions (WS). The above extracts were tested for genotoxicity using bacterial genotoxicity test with five *Salmonella typhimurium* strains (TA98, TA100, TA97a, TA1535 and WP2), SOS Chromotest assays with *E. coli* PQ37 and chromosome aberration test with Chinese hamster lung cell line (CHL), respectively. In this study, only direct genotoxicities were observed in all *in vitro* bioassays for both extracts. The genotoxicity of OC can be neglected compared to TP and WS, especially for relatively low exposure dose ($\leq 10\text{m}^3$ actual atmospheric volume). Therefore, the genotoxicity of OC may increased with exposure time and dose. The genotoxicity of TP and WS were in the similar level. In SOS Chromotest assays, the IF (Induction Factor) of TP and WS in winter were identified to be 3.58-4.76 and 3.00-4.05 for PD and HN respectively, in summer were 2.06-3.31 and 2.01-3.07 for PD and HN respectively. The highest mutation rate of *Salmonella ty-*

phimurium strains exposed to TP and WS were $31.43 \pm 3.69\%$ and $27.09 \pm 5.85\%$ for PD and HN in winter, $20.84 \pm 5.19\%$ and $14.07 \pm 7.15\%$ in summer. The mutagenic activity of TP and WS to five different strains was: TA97a> TA100> TA98> TA1535> WP2. The highest aberration rate of CHL cell line exposed to TP and WS were $9.83 \pm 1.89\%$ and $7.17 \pm 2.08\%$ for PD and HN respectively in winter, in summer were $5.67 \pm 1.04\%$ and $2.50 \pm 0.08\%$. Exposure to OC induced only a slight effect in winter. PM_{2.5} in two locations identified significant seasonal differences in genotoxicity levels, the genotoxicity in winter is significantly high. Therefore, the genotoxicity in two seasons could not be neglected. There are no significant locational differences, showed that the pollution in suburb is becoming serious. The genotoxicity of TP and WS were similar, indicating the main genotoxic substances of PM_{2.5} were water soluble and their genotoxic activity may be decreased by metabolism. Further study will be focus on the components of water soluble fractions of PM_{2.5}.

PS P112 - 0497 Role of Calcium-Related Signaling Pathway in Endothelial Cell Permeability Induced by PM2.5

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Epidemiological and experimental data have shown that there is a correlation between PM2.5 exposure and cardiovascular system damage, and PM2.5 leads to inflammatory response in the cardiovascular system. Endothelial cells are the first line of defense in the cardiovascular system, and their damage is related to cardiovascular disease. The role of PM2.5 on vascular endothelial cell function remains to be elucidated. The objective was to investigate the molecular mechanism of PM2.5-induced endothelial permeability. Three doses of 2.5, 5 and $10\mu\text{g}/\text{cm}^2$ PM2.5 were set to the subsequent assays. Dextran-4 fluorescence assay was used for the detection of endothelial cell permeability. The intracellular calcium concentration of EA.hy926 cells was determined by Fluo-3/AM method. After 24 hours of exposure, total protein was extracted and the expressions of cPLA₂, p-cPLA₂, ZO-1 and occludin were detected by western blotting. At the same time, cytoplasm and cell membrane proteins were extracted for the detection of PKC α protein expression. Then, the control group, $10\mu\text{g}/\text{cm}^2$ PM2.5 group with or without Ca²⁺ inhibitor of 2-APB and BAPTA-AM, PKC inhibitor Gö6976, as well as cPLA₂ inhibitor mepacrine were set for the detection of cPLA₂, p-cPLA₂, ZO-1 and occludin protein, together with the expression of PKC α protein in the membrane and cytoplasm as well. After PM2.5 exposure, the concentration of intracellular calcium significantly increased with the increased endothelial cell permeability. PKC was activated and translocated to the cell membrane. Dextran-4 fluorescence assay showed that in the PM2.5 can result in cell permeability within 4 hours. Moreover, the expression of tight junction proteins ZO-1 and occludin was significantly decreased, suggesting that the endothelial tight junction was damaged. After inhibiting intracellular calcium concentration, PKC inhibition, or the decreased level of cPLA₂ phosphorylation, ZO-1 and occludin can be partially rescued compared with $10\mu\text{g}/\text{cm}^2$ PM2.5 group, suggesting Ca²⁺/PKC/cPLA₂ signaling pathway has implications in endothelial permeability induced by PM2.5. PM2.5 damages tight junction of vascular endothelial cells via Ca²⁺/PKC/cPLA₂ signaling pathway. *Acknowledgements: National Natural Science Foundation of China (No. 21876026; 31671034; 81673218; 81502783; 81573186).*

PS P113 - 0524 Sulfur Dioxide Monitoring Associated with the 2018 Kilauea Lower East Rift Zone Eruption

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In 2018, the County of Hawaii (Big Island—population 200,381) experienced months of sulfur dioxide (SO₂) volcanic air pollution from Kilauea Volcano's Lower East Rift Zone (LERZ) eruption. SO₂ as high as 50-100 thousand metric tons on peak days and volcanic ash were the primary pollutants released from vents at Kilauea's summit and the LERZ. Hawaii Department of Health (HDOH) established short-term advisory levels: HDOH Red "unhealthy" (1.01-3 ppm) and HDOH Purple "very unhealthy" (>3 ppm). ACGIH's occupational short-term exposure level (STEL) is 0.25 ppm over 15 minutes. US EPA's AEGL program designates AEGL-2 (disabling) for an 8-hour exposure to SO₂ at 0.75 ppm. OSHA's permissible exposure level (PEL) is 5 ppm Time Weighted Average (TWA) over an 8-hour workday (OSHA PEL). Although these guidelines may not be intended for non-occupational environments, they are the best available standards. Our aim was to evaluate the number of times and extent to

which these SO₂ thresholds were exceeded during the 2018 eruption. US EPA provided a wireless real-time SO₂ system from 14 sensors to a central repository approximately every second. We calculated the maximum and TWA for intervals of 15 min, 1 h and 8 h by sensor and excursions above SO₂ thresholds of interest using SAS JMP 12.0.1 (SAS, Cary, NC, US). We examined 4,019,847 SO₂ levels from the 14 sensors in at-risk communities. The highest 15-minute TWA was 20 ppm (sensor saturation) from the monitor in Lower Leilani Estates, the neighborhood containing Fissure 8 that was evacuated and subsequently overcome by lava. Outside this area, HDOH Red was exceeded at 9 different locations including Kalapana Seaview (2.48% of the time). ACGIH STEL was exceeded at multiple locations including Kaueleau Kamailli Rd (11.43% of the time). The maximum 8-hour TWA outside Leilani Estates was 1.99 ppm at Kalapana Seaview. The 8-hour TWAs outside of Leilani Estates exceeded AEGL-2 at 3 locations including Kalapana Seaview 2% of the time, and Kaueleau Kamailli Rd 3.7% of the time. None of the 8-hour TWAs (excepting Lower Leilani Estates) exceeded the OSHA PEL. All regulatory thresholds were continuously exceeded in Lower Leilani Estates during the operational period except for HDOH Purple and OSHA PEL, which were both exceeded more than 60% of the time. These findings clearly support evacuation. In other locations, short-term SO₂ levels periodically exceeded the HDOH Red and HDOH Purple and the ACGIH STEL. 8-hour AEGL-2s were exceeded but OSHA PEL limits were not reached. Continuing research is recommended on SO₂ and other volcanic pollutants in preparing for Kilauea's next eruption and other similar events.

P114 - 0552 Transcriptomic Alterations Induced by Air Pollution-Derived PM_{2.5} Reflect the Shift from Healthy to COPD-Diseased Human Bronchial Epithelium

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The chronic exposure to air pollution-derived fine particulate matter (PM_{2.5}) is suspected of exacerbating and even causing chronic inflammatory lung diseases. However, the knowledge of the underlying mechanisms is still incomplete. The objectives were to assess transcriptomic alterations in human bronchial epithelial cells exposed to PM_{2.5}, and better understand how this exposure may lead to a shift from healthy to COPD phenotype. Normal human bronchial epithelial (NHBE) or COPD cells were differentiated at the air-liquid interface and repeatedly exposed to PM_{2.5} for 72h. Their transcriptomes were analyzed using human pangenomic microarrays. Basal transcriptomes of both control cell phenotypes were first compared and 1280 transcripts were significantly deregulated in COPD vs NHBE cells. The transcription of 1168 genes was significantly altered by PM_{2.5} exposure in NHBE cells. Interestingly, among these deregulated mRNA, a large number (679) were in common with those found in shift from NHBE to COPD phenotype. Indeed, within this set of genes, we observed increased expression of genes involved in inflammation (e.g. TGF- β , IL-17, IL-33, CXCL1, CXCL3, CXCL6, CXCL8, CXCL14, CCL20), as well as genes associated with extracellular matrix remodeling (e.g. EGF, FGF1, KRT4, MMP9, MMP 13, TIMP-1). By contrast, only 107 transcripts were modulated by PM_{2.5} in COPD cells, evidencing an exacerbation of COPD. These alterations were validated by RT-qPCR and at functional protein level. Overall these results showed that the transcriptomic alterations induced by PM_{2.5} exposure deregulated key pathways involved in COPD pathogenesis.

P115 - 0573 Time Course of Olfactory Epithelium Damages after Cessation of Inhalation Exposure to 2-Ethyl-1-Hexanol in Mice

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In recent years, 2-ethyl-1-hexanol (2E1H), which is emitted from adhesives and carpeting materials installed on concrete floors, has been postulated to cause "sick building syndrome." Patients' symptoms are associated with an increased sense of smell. Our previous study indicated that inhalable 2E1H is toxic to olfactory epithelium (OE) of the nasal cavity. Exposure for 1 week showed inflammation and degeneration, with a reduction in the number of olfactory cells and in epithelial thickness. Regeneration occurred at 1 month, but lymphocytic infiltration and expanded Bowman's glands in the nasal mucosa were observed at 3 months (Miyake et al., 2016). This study aimed to characterize the histopathological changes of the OE after cessation of 2E1H inhalation for

4 weeks in mice. This study was carried out in accordance with the Guide for Animal Experimentation of Nagoya City University. After 1-week acclimation, male 11-week-old ICR mice were exposed to 0 (fresh air) or 70 ppm 2E1H for 8 hours per day, 5 days per week for 4 weeks using a whole-body inhalation exposure chamber system. Exposure concentration was monitored by charcoal tube sampling and quantified by gas chromatography-mass spectrometry. All mice were maintained under a 12-h light/dark cycle at 23–24°C under relatively constant humidity. After 1-month exposure, the exposure was stopped and the mice were placed outside the chambers. At Day 1, Day 3, Day 7, Day 14, or Day 28 after the last exposure, mice were deeply anesthetized and then sacrificed by transcardially perfusion with 4% paraformaldehyde phosphate buffer. After overnight fixation with 4% paraformaldehyde for decalcification, the noses were trimmed, dehydrated, and embedded in paraffin, and transverse serial sections of 4- μ m thickness of the nasal cavities. Sections were stained with hematoxylin and eosin or immunostained and examined using an optical microscope. Histopathological evaluation revealed that no damage was observed in OE at Day 1. Between Day 7 and Day 14, OE showed degenerations such as reduced thickness of mucous layer, dispersed olfactory cells. Expanded Bowman's glands in the OE were also seen at Day 7 and Day 14. Infiltrated neutrophils tended to increase. These changes vanished at Day 28 after the cessation of exposure. The current study showed time lag in OE changes after cessation of 2E1H inhalation exposure for a month. Accordingly, 2E1H inhalation induced degeneration and subsequent regeneration of the OE and the time course of OE damage and recovery is mismatched with the cessation time point.

P116 - 0607 Naphthalene-Increased CYP2F1 Expression Is Mediated via Activation of NF- κ B Signaling Pathway in Retina Pigment Epithelium Cell

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Naphthalene (NAP) is the most widespread environmental contaminant of polycyclic Aromatic Hydrocarbons (PAHs), which was generated from fuel combustion, transportation, and cigarette smoking. In recent years, studies have indicated that PAHs may associate with the increase risk of eye diseases such as dry eyes, ocular tissue inflammation, even affecting blood flow rate of retina. Moreover, cataract and retina injury have been reported following acute and chronic occupational exposure to NAP, the underlying mechanism is still unclear. Due to NAP is the largest proportion of PAH exposure both in daily life and workplace, elucidating the effect of changing retinal physiology is needed. This study focused on NAP-induced pro-inflammatory factor on affecting CYP2F1 expression, the axis may involve in retinal lesion within NAP exposure. In this study, the retinal pigment epithelium (RPE) cells with dose (0.25, 0.5, 1, 3, 10 mM)- and time-dependent treatment of NAP and its metabolite 1,4-naphthoquinone (1,4-NQ) were analyzed by MTT assay, western blotting, and real-time PCR. Our results showed that 1,4-NQ decreased RPE cell viability within 10 mM, but not NAP. Both NAP and 1,4-NQ up-regulated the phosphorylation of p65 which lead to increase nuclear translocation and NF- κ B activation in RPE cells. NAP also increased such pro-inflammatory factor ICAM, iNOS, and COX2 dose dependently. Except to the down-regulation of ICAM expression, the similar effect was found by 1,4-NQ-treated RPE cells. Furthermore, we confirmed that NAP up-regulated CYP2F1 but unaffected both CYP1A1 and CYP1B1 on mRNA and protein expression; in contrast to NAP, 1,4-NQ has no change on CYP2F1 expression. Interestingly, the NF- κ B activation inhibitor, BAY11-7082 not only inhibited NF- κ B, but decrease both SP1 and CYP2F1 protein expression, and the activity of SP1. Taken together, we revealed that low level of NAP exposure can up-regulate CYP2F1 via activation of NF- κ B/SP1 axis, which may contribute to NAP-induced retina lesion. As further studies are needed, this study proposes a possible regulation of NAP-induced inflammatory effect during earlier stages for preventing ocular degeneration.

P117 - 0622 Dynamic Ozone-Induced Gene Expression Changes in Lung, Liver, and Adrenals during Early Time Point and Adaptation: The Influence of Long-Term Glucocorticoid Pretreatment

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Acute ozone (O₃) induced lung injury and inflammation are attenuated following repeated exposure (adaptation/habituation). Ozone-induced pulmonary and systemic changes are mediated through reversible activation of adrenal release of the stress hormones, corticosterone and epinephrine. The diminution of stress hormone levels or the blockade of their receptors reduces O₃-induced pulmonary and extra-pulmonary effects. We hypothesized that O₃-induced transcriptional changes in lung adrenals and liver are attenuated during adaptation and that 1-month glucocorticoid treatment will diminish ozone adaptation at D+4. Male Wistar Kyoto rats (12-week old) were i.p. injected daily with vehicle (VEH, saline) or a therapeutically relevant dexamethasone sulfate dose (DEX, 0.01 mg/kg/day) for 1 month prior to and during daily exposure to air or O₃ (0.8ppm) 4 hours/day for 2 or 4 days (D+2 or D+4) to reflect acute exposure and adaptation phase respectively. Generally, expression of glucocorticoid responsive gene (*Tsc22d3*) was up-regulated while glucocorticoid receptor (*Nr3c1*) gene was down-regulated after O₃ exposure in lung, liver and adrenals at D+2 but not at D+4. O₃ increased lung *Il-6* expression at D+2 but not at D+4. Lung RNA sequencing by TempO-Seq S1500+ platform in D+4 animals showed that O₃ exposure changed the expression of only 105 (157 ↓48) genes in VEH-treated and 77 (125 ↓52) genes in DEX-treated rats. *Comt* gene involved in epinephrine metabolism was down-regulated after O₃ exposure and DEX-treatment in the liver. O₃ increased hepatic *Ppargc1* expression involved in fatty acid storage and glucose metabolism only at D+2, and DEX exacerbated this effect. Genes involved in corticosterone biosynthesis (*Hsd3b1*, *Cyp11b1*) were up-regulated after O₃ exposure and DEX treatment in the adrenals at D+2. Moreover, expression of *Pnmt* which is involved in epinephrine synthesis in adrenals was also up-regulated after O₃ exposure at D+2 and D+4. These results suggest that O₃-induced multiorgan effects on steroidal and adrenergic pathways also show adaptation upon repeated exposure. Long-term glucocorticoid pretreatment had little influence on ozone adaptation. Does not reflect US EPA policy.

P118 - 0661 Mitochondrial Oxidative Stress and Energy Metabolism Disruption Are Involved in COF-Induced Hepatotoxicity

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Cooking oil fumes (COFs) are some of the most common indoor air pollutants. To gain insight into how to control COF-induced diseases, the oxidative damage and mitochondrial dysfunction caused by COFs in a human normal liver cell line (HL7702) were explored in this study. An Methyl thiazolyl tetrazolium (MTT) assay was used to detect the HL7702 cell survival rate after treatment with different doses of COFs. The levels of reactive oxygen species (ROS) and ATP/ADP/AMP in cells were detected by laser confocal microscopy and high-performance liquid chromatography (HPLC), respectively. The activity of mitochondrial succinate dehydrogenase (SDH) was measured with a microplate reader. The openness of the mitochondrial membrane permeability transition pore (mPTP) and the mitochondrial trans-membrane potential ($\Delta\psi_m$) were quantitatively measured with a fluorescence spectrophotometer. The activity of the mitochondrial respiratory chain complex (MRCC I-V) was determined by UV spectrophotometry. The measurements were analysed based on their Spearman correlations. (1) There was a dose-response relationship between cell survival inhibition and COFs. The half inhibition concentration (IC₅₀) of COFs in HL7702 cells was 318.10 µg/ml. ROS levels were positively correlated with COF doses in a linear manner ($r=0.978$, $P<0.01$). (2) The levels of ATP, ADP, AMP and TAN were markedly decreased after COF treatment. The ATP/ADP ratio increased in a dose-dependent manner with COF concentrations between 0 and 40 µg/ml, and the EC value of the 80 µg/ml COF group was significantly higher than that of the other groups ($P<0.05$). (3) COFs inhibited the activity of mitochondrial SDH and MRCC I-V. COFs showed a negative dose-response relationship with $\Delta\psi_m$ ($P<0.05$), but COF concentration was positively correlated with the openness of the mPTP. COFs inhibited the activity of HL7702 cells, which was indicated by an accumulation of ROS, an inhibition of adenylation syn-

thesis, a decrease in mitochondrial enzyme activity, the opening of the mPTP and a reduction in MRCC I-V activity. The mitochondrial dysfunction induced by COFs was associated with ROS accumulation and adenylation synthesis inhibition.

P119 - 0758 Hazard Identification, Exposure Assessment and In Vitro Toxicity Assessment of Dust Emissions from South African Gold Mine Tailings Sites

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Decades of mining in South Africa have produced waste material, which was deposited as tailings storage facilities (TSFs) across the South African landscape. These TSFs have contributed to air pollution due to the lack of proper rehabilitation measures needed to mitigate erosion. Indeed, human populations residing in close proximity to these TSFs have raised concern about high dust levels and the onset of respiratory-related symptoms likely caused by tailings emissions. Currently, South Africa does not have an efficient routine air monitoring system in residential areas of surrounding communities to determine the extent of air pollution. Moreover, it is not as yet known whether these tailings dusts are toxic and could be the cause of such ill effects. In addition, the physicochemical properties that are likely to govern their toxicity have not as yet been identified. The aims of the present study were to: (1) determine the toxicity of the dust emitted from five TSFs in South Africa (*Hazard Identification*); (2) assess the levels of PM₁₀ and PM₄ dust as well as crystalline silica levels in environmental and personal filter samples in communities surrounding these TSFs (*Exposure Assessment*); and (3) conduct *Risk Characterization* where potential risk was calculated for these surrounding communities to likely develop cancer and non-cancer adverse health effects from their lifetime exposure to inhalation of respirable crystalline silica (RCS). The results from *Hazard Identification* show that all tailings dusts exhibited *in vitro* toxicity in two human-derived cell lines studied and was likely governed either by their elemental composition (i.e., high iron content) or a combination of other physicochemical properties. The results from *Exposure Assessment* showed that the ambient PM₁₀ levels in surrounding populations exceeded by several fold compared to the current South African limit of 75 µg/m³. Furthermore, the RCS levels collected from the personal PM₄ filters from surrounding communities exceeded the current international interim RCS limit of 3 µg/m³. Finally, *Risk Characterization* has revealed the possibility of surrounding communities to likely develop cancer and non-cancer adverse health effects resulting from life-time exposure to the RCS levels recorded during this study. These results could therefore provide mechanistic evidence to support future epidemiological studies attempting to link tailings dust exposure to adverse health effects. In addition, this study could serve as a starting point for future ambient and personal sampling campaigns and suggests that a RCS interim exposure limit, particularly for South Africa, be established.

P120 - 0769 Prenatal Urinary Polycyclic Aromatic Hydrocarbon Metabolites, Global DNA Hydroxymethylation, and Neurodevelopment at 2–3 Years of Age

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Prenatal exposure to polycyclic aromatic hydrocarbons (PAH) is a potential risk factor for neurodevelopmental outcomes of offspring. Epigenetic processes play a key role in orchestrating transcriptional regulation during the development of the human nervous system. The current study aimed to investigate whether prenatal PAH exposure was associated with adverse neurodevelopmental outcomes at 2–3 years of age and altered global DNA hydroxymethylation (5-hydroxymethylcytosine, 5-hmC) modification levels in cord blood and to examine potential mediating roles of global DNA 5-hmC. We enrolled 400 mother-newborns pairs and collected their biological samples after written informed consent. In the follow-up, 221 mother-child pairs with 2&3-year-old children were included in this study. We used the Gesell Developmental Scales (GDS) for neurodevelopmental assessments at 2–3 years of age. The global DNA 5-hmC levels were measured by ELASE kit. Eleven PAH metabolites in maternal urinary samples were measured. Multiple linear regressions were used to analyze the associations of maternal urinary PAH metabolites with child neurodevelopment score and global DNA 5-hmC levels, and restricted cubic spline models were further used to examine the shapes of dose-response relation-

ships. A mediation analysis was also conducted. We found both a significant negative association and a trend between 1-OHPyr levels and neurodevelopment scores in general linear models (p for trend < 0.05). These associations were linear and significant when 1-OHPyr was modeled as continuous variables in restricted cubic spline models. Each one unit increase in log-transformed urinary levels of 1-OHPyr was associated with 4.7 decreases for motor score, 5.8 decreases for adaptive score, 6.0 decreases for language score, and 4.6 decreases for social score, respectively. A significant positive relationship was observed between 1-OHPyr ($\beta=0.09$, $p=0.047$) and global DNA 5-hmC levels. However, mediation analysis did not find global DNA 5-hmC to be a direct mediator between PAH and GDS scores. Prenatal exposure to specific PAH was inverse associated with neurodevelopment scores in children aged 2–3 years. Global DNA 5-hmC levels were positive associated with 1-OHPyr in maternal urinary samples. However, the adverse effects of prenatal exposure to PAH on GDS scores did not appear to be directly mediated by altered global DNA 5-hmC levels.

P121 - 0811 Organic Particulate Matter 10 (PM_{10}) in Transported African Dust at an Urban and Rural Town in Puerto Rico: *In Vitro* Implications to the Etiological and Geographical Assessment of Asthma

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Asthma is a relatively common clinical problem, and increased disease severity occurs in the Caribbean islands, including Puerto Rico, during Saharan desert mineral dust storm episodes. The expression of phase I and phase II detoxification genes and the function of immunological surveillance mechanisms may be decreased or absent during severe inflammatory responses to environmental pollutants. The sites at which particulate matter (PM) was collected are described. A sampling procedure to separate different extracts was designed. Soxhlet organic extract identification and organic extraction of $PM \leq 10 \mu m$ in diameter (PM_{10}) were performed using weather maps and chemistry techniques. Afterwards, cell culture, cell exposure, cytotoxicity assays, sampling by the cultivation method, and statistical analysis were performed. Immortalized bronchoalveolar cells (BEAS-2B) experienced increased necrosis at PM_{10} concentrations greater than $25 \mu g/ml$. Glutathione, an agent produced intracellularly to prevent cell membrane damage, was used as an independent positive control and was found to have the capacity to cause necrosis at $8 \mu M$. Environmental pollutants including PM_{10} , Saharan desert mineral dust storm matter, evidenced through aerial photography, and urban organic matter, evidenced by satellite photography, were found to increase necrosis in BEAS-2B cells.

P122 - 0875 Military Working Dogs: Unlocking the Mystery of Deployment-Related Exposures and Health

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With thousands of troops having performed tours of duty in Iraq and Afghanistan since 9/11, concerns have been raised about the relationship between potential exposures to theater-related hazards and adverse health outcomes. Military working dogs (MWDs) were deployed to these same regions and were exposed to the same hazards, and their health records were maintained much like those of military personnel. Post-Deployment Health Services (PDHS) is charged with providing scientific subject matter expertise for VA for policy development in this area. PDHS is partnering with US Army Public Health Center (APHC), which has created a database that can be utilized to examine the health of MWDs with varied military service, to learn more about the possible health implications of deployment to these locations. The database, which will be made available to the public for research, is a consolidated source of MWD records that includes demographic (e.g., breed, sex, permanent duty location), deployment (e.g., location, dates) and health-related information (e.g., medical diagnoses, necropsy findings). As a proof-of-concept, PDHS has begun examining these data to identify the occurrence of organ-level adverse health outcomes. Future work will compare the health of dogs that deployed to the regions of the recent conflicts to those that did not deploy and/or that

deployed to areas with different potential exposure profiles. This database may be used to study MWDs for sentinel health information on military personnel in recent and future deployments.

P123 - 0893 Levels of Trace Elements, PCDD/Fs, and PCBs in the Surroundings of a Municipal Solid Waste Incinerator: Assessing Health Risks

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Since 2008, the environmental occurrence of trace elements, polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs) is periodically assessed in the surroundings of a municipal solid waste incinerator (MSWI) located in Mataró (Catalonia, Spain). The current study presents updated information on the environmental concentrations of those pollutants in the last stage of the environmental surveillance program, conducted between 2015 and 2017. Regarding soils, the levels of trace elements were below the respective maximum limits according to the Catalan and Spanish regulations for urban soils. Furthermore, PCDD/F concentrations in both studies (0.23 and 0.13 ng WHO-TEQ/kg in 2015 and 2017, respectively) were lower than those found in the baseline (2008) survey (0.13 ng WHO-TEQ/kg). Furthermore, PCB in soils showed a notable temporal decrease when comparing the results of the campaign conducted in 2017 with those found in previous surveys. In air, the concentrations of most of trace elements and PCDD/Fs were similar to, or even lower than, those usually reported in industrial and urban areas of Catalonia. In turn, As and V, as well as PCBs, showed a statistically significant decrease in the period 2015–2017. Notwithstanding, and despite the air concentrations of PCBs in 2017 were similar to those reported in other industrial and urban areas of Catalonia, a slight increase was noted since 2008. In any case, this increase was exclusive for PCBs in air, and not for the rest of pollutants and matrices here used as monitors. Altogether, it confirms the low environmental impact of the MSWI over the surrounding zone. Globally, the environmental exposure to trace elements, PCDD/Fs and PCBs of the population living in the surroundings of the MSWI located in Mataró was estimated to be notably low, being far lower than the dietary exposure to these pollutants.

P124 - 0915 Traffic Road Dust-Derived Particulate Matter Aggravates Allergic Inflammation

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The objective was to evaluate the effect of traffic-derived particulate matter (PM) on the allergic inflammation of ocular surface in a murine model. Seven-to eight-week-old BALB/c mice were sensitized with OVA and aluminum hydroxide via intraperitoneal injection. Two weeks later, mice were challenged by OVA eyedrops for 12 days with traffic road dust-derived PM_{2.5} challenges or vehicle. We evaluated clinical signs, the infiltration of inflammatory cells into conjunctiva, serum levels of OVA-specific IgE production, and Th2 cytokines secretion *in vitro* T cells assay through flow cytometry or ELISA. AC development and conjunctival infiltration of eosinophils (CD45+ Siglec-F+; $p = 0.018$ vs. vehicle) and mast cells (CD45+ c-kit+; $p = 0.022$ vs. vehicle) were significantly increased with topical application of PM_{2.5}, or saline vehicle. In addition, PM_{2.5} led to increased production of OVA-specific IgE in serum ($p < 0.001$ vs. vehicle) and Th2 cytokines *in vitro* T cell assays, such as IL-4 ($p = 0.032$ vs. vehicle), IL-17A, and IL-13 ($p = 0.016$ vs. vehicle), compared to vehicle group. Interestingly, PM_{2.5} led to increase the population of APCs in draining LNs ($p = 0.028$ vs. isotype control). Exposure with PM_{2.5} was associated with a significant increase in the conjunctival expression of IL-1 β , IL-6, IL-17, and TNF compared to the vehicle control. Our results suggest that traffic derived PM_{2.5} exaggerated allergic conjunctivitis, indicated by increased inflammatory cells infiltration into conjunctiva and Th2 responses in draining LNs with increased population of APCs. Therefore, our results demonstrated new insight into the hazardous potential of traffic derived PM_{2.5} on allergic diseases including asthma or atopic dermatitis. *Acknowledgement: This work was funded by the Korea Ministry of Environment as Environmental Health Action Program-2016001360005.*

PS P125 - 0916 Methylation Profiles in Newborns from Diverse Air Polluted Localities in the Czech Republic

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Long-term exposure to air pollutants in industrial localities has adverse health impact and negatively affects genome and epigenome. The effects are particularly deleterious for development and morbidity of children. However, underlying molecular alterations during neonatal development remain to be further investigated. In our study, we studied global and gene-specific DNA methylation in newborns from different districts of the Czech Republic. Two districts (Most and Karvina) were industrial, characterized by black and brown coal mining, respectively. The control district of Ceske Budejovice (CB), was located in the clean air area. We assessed global DNA methylation levels using ELISA 5-mC DNA kit in a total of 100 newborns. In a subset of 60 samples, newborns from Most and CB, we performed microarray analysis of 866895 possible methylated sites using Methylation EPIC Beadchips (Illumina). As both genders were analyzed, methylated sites for sex chromosomes was removed. Data were filtered for non-CpG sites, normalized by the SWAN method and batch corrected. Exploratory analysis was processed using multidimensional scaling and principal component analysis. Differential methylation levels were estimated using limma R package. Associated tests were performed on trait pairs based on questionnaires data of mothers. Global methylation level significantly correlated with apgar score ($p < 0.01$) and with ZIP code ($p < 0.01$). Air pollution levels, estimated as PM_{2.5} concentration in the study localities at the time of delivery, were as follows: Karvina, $55.35 \pm 11.50 \mu\text{g}/\text{m}^3$; CB, $16.9 \pm 10.44 \mu\text{g}/\text{m}^3$; Most, $18.8 \pm 18.94 \mu\text{g}/\text{m}^3$. The concentrations differed significantly only when Karvina and CB were compared. Global DNA methylation levels were significantly different ($p < 0.01$). Results showed the following ratios between methylated CpG sites: Karvina $33.9\% \pm 9.7\%$, CB $38.1\% \pm 12.3\%$ and Most $29.8\% \pm 10.4\%$. The samples on principal components were clustered according to the sampling locality (Most and CB). Also, hierarchical clustering of samples based on all methylation values showed the most variable loci those in promotes sites and CpG islands. For tiling methylated regions, we found 3801 sites, 1498 gene sites, 2106 promoter sites and 1065 CpG islands. Our findings demonstrate that residence of mothers in specific localities before and during pregnancy can be associated with DNA methylation changes in their newborns and a significant hypomethylation in subjects from an industrial locality. *The study was supported by The Strategy AV21 - Qualitas and The Ministry of Education, Youth and Sports of the Czech Republic (HAIE, CZ.02.1.01/0.0/0.0/16_019/0000798).*

PS P126 - 0917 Outdoor Physical Exercise and Health Implications

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Although exercise improves overall health, regular outdoor physical exercise can lead to heightened exposures to a variety of ambient air pollutants, namely those resulted from traffic emissions. As such, the health benefits of exercise may be abrogated to some degree by the harmful actions of inhaled pollutants. The aim of this systematic review was to investigate the effects of traffic-related air pollutants (TRAP) exposure, specifically particulate matter and nitrogen dioxide (NO₂), during outdoor exercise on cardio-respiratory health effects. Systematic database searches of PubMed, Web of Science, Scopus and Medline were performed by two researchers to identify peer-reviewed studies from 2000 to 2018. Combinations of keywords related to cardio-respiratory health effects, physical exercise and ambient air pollution were used. Thirteen studies were included, originating predominantly from European countries but also the American. They suggested that exercising in an environment with high TRAP exposure increases markers of respiratory and systemic inflammation, as well as, impairs the vascular function and increases artery pressure, when compared with an environment with low TRAP exposure. In addition, the smaller particles appear to have the most severe health consequences compared with the larger coarse particles and NO₂. This study also provides evidence that specific groups of population appear to have enhanced susceptibility to adverse effects from particulate matter exposure while exercising. There is a need for more studies focused on the relationship between air pollution, physical exercise and health, as large societal benefits can be obtained from healthy environments that can promote outdoor physical exercise. *Authors acknowledge the funding provided by Fundação para a Ciência e a Tecnologia (FCT) through the scholarship SFRH/BPD/115112/2016 (Joana Madureira), FUMDES Fund for Support*

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PS P127 - 0082 Ether-Phosphatidylcholine Characterized by Consolidated Plasma and Liver Lipidomics Is a Predictive Biomarker for Valproic Acid-Induced Hepatic Steatosis

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Valproic acid (VPA) is known to induce hepatic steatosis due to mitochondrial toxicity in rodents and humans. In the present study, we administered VPA to SD rats for 3 or 14 days at 250 and 500 mg/kg and then performed lipidomics analysis to reveal VPA-induced alteration of the hepatic lipid profile and its association with the plasma lipid profile. VPA induced hepatic steatosis at the high dose level without any degenerative changes in the liver on day 4 (after 3 days dosing) and at the low dose level on day 15 (after 14 days dosing). We compared the plasma and hepatic lipid profiles obtained on day 4 between the VPA-treated and control rats using a multivariate analysis to determine differences between the two groups. In total, 36 species of plasma lipids and 24 species of hepatic lipids were identified as altered in the VPA-treated group. Of these lipid species, ether-phosphatidylcholines (ePCs), including PC(16:0e/22:4) and PC(16:0e/22:6), were decreased in both the plasma and liver from the low dose level on day 4; however, neither an increase in hepatic triglyceride level nor histopathological hepatic steatosis was observed at either dose level on day 4. Hepatic mRNA levels of glycerone-phosphate O-acyltransferase (Gnpat), which is a key enzyme for biosynthesis of ePC, was also decreased by treatment with VPA along with the decrease in ePCs. Since the decrease in ePCs are considered to reflect the enhancement of glycolysis as the consequence of mitochondrial dysfunction (inhibition of beta-oxidation) caused by VPA, a decrease in ePCs could be a common phenomenon in the condition of mitochondrial dysfunction. In conclusion, the changes in ePCs (PC[16:0e/22:4] and PC[16:0e/22:6]) have potential utility as predictive biomarkers for hepatic steatosis induced by mitochondrial dysfunction.

PS P128 - 0083 Evaluation of Exosomes as Toxic Biomarkers

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Besides blood cells, RNA contained in exosomes known as secretory granules exist in the blood. Exosomes are lipid bilayer vesicles of several tens to a hundred nanometers and are secreted into the body fluids from cells. The RNA contained therein contains cell-specific RNAs. Also, cancer-specific exosomes as a biomarker of more than 90% of diagnostic accuracy for early cancers are indicated. The aim of our research project is to develop a highly sensitive "next generation type" toxicity test for chemical substances and medicine using exosomal RNA in blood as a biomarker. In this study, we compared the protocols for the isolation of exosomal RNA from mouse blood, including blood collection methods, serum isolation methods, and exosome purification methods, to standardize the isolation of exosome RNA from mouse blood. Quantity of exosomes was determined by Nanosight, a special optical microscopy adapted to quantify small particles, and western blot using anti-CD9 antibody. The results show that serum isolation methods using blood coagulation accelerator and separating agent reduced the efficiency of collection of exosomes, while different exosome purification methods, including ultracentrifugation method, polymer precipitation method, and affinity columns methods, resulted in different exosomal RNA profiles. Therefore, it is necessary to standardize exosome isolation methods to obtain uniform exosomes from mouse blood. Exosomal RNAs were further evaluated by deep sequencing. We succeeded in isolating more than 50 novel small RNAs, which could be used as novel highly sensitive biomarkers for "next generation type" toxicity test. These results will accelerate a rapid evaluation of chemical substances in "Act on the Evaluation of Chemical Substances and Regulation of Their Manufacture" and medicine in Nonclinical Safety Evaluation.

PS P129 - 0201 Comparison of Modeled Internal Exposures to Di(2-ethylhexyl)phthalate from Foods and Cosmetics with Urinary Metabolite Concentrations—Results from the EuroMix Biomonitoring Study

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A human biomonitoring study was performed to study exposure of chemicals present in foods and personal care products (PCPs). For two 24-hour study periods separated by 2–3 weeks, adult volunteers (44 males and 100 females) in Norway kept detailed diaries on food consumption (type/brand, weight, time, and packaging material) and the usage of PCPs (type/brand of product, time, and number of applications). In parallel, 24 hours urine samples were collected. Monte-Carlo simulation was carried out by Oracle Crystal Ball. This software calculates exposure based on the propagation variable of variability and uncertainty given by each parameter in probability function, until a certain number of iterations. Individual external exposure to di(2-ethylhexyl) phthalate (DEHP) from foods, aggregating dietary with nondietary exposure from PCPs was calculated. External exposure estimates were used for internal dosimetry simulation using physiologically based pharmacokinetic (PBPK) models, and 24 hours cumulative urinary concentrations were compared with biomonitoring (BM) data of the urinary metabolites of DEHP. Preliminary results show that diet is the major contributor to DEHP exposure for both males and females, with an external exposure approximately 10 times higher than for the exposure from personal care products (PCPs). The main contributors to the mean dietary DEHP exposure are milk and dairy products, with a percentage contribution of 69.3% and 62.8% of the total exposure for males and females, respectively. The following major food groups contributing to DEHP exposure were grains and grain-based products > fruits and vegetables > meat > fish. There is a significant difference between PCP use for males and females. However, for both males and females, deodorants contribute most to the exposure of DEHP from PCPs, with a 79% and a 53% contribution, respectively. For females, a relatively high contribution was also observed from body lotion (33%) and from perfume (9%). The same PCPs contributed to DEHP exposure in males, but to a much lower degree. Primary validation of these exposure data using forward dosimetry using the PBPK model are giving good convergence with 24 hours cumulative urinary concentration of simulated and measured BM data.

PS P130 - 0202 Exposure to a Mixture of Phthalates as Measured in 24-Hour Urine and Associations with Food Intake and Use of Personal Care Products—Results from the EuroMix Biomonitoring Study

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A human biomonitoring study was performed to study exposure of chemicals present in foods and personal care products (PCPs). For two 24-hour study periods separated by 2–3 weeks, adult volunteers (44 males and 100 females) in Norway kept detailed diaries on food consumption (type/brand, weight, time, and packaging material) and the usage of PCPs (type/brand of product, time, and number of applications). The participants also registered the number of thermal papers (TP) handled. In parallel, 24-hours urine samples were collected. Thirteen phthalate metabolites from seven different phthalates and two metabolites of diisononyl ester (DINCH) were measured in urine from the first study day. Multivariate linear regression (MLR) between estimated urinary concentrations of the phthalates and DINCH (adjusted for molecular weight and log transformed), and food and PCP categories were performed. Males had higher concentrations of all phthalate metabolites in urine than females, but it was only significant for the mono-2-carboxymethyl hexyl phthalate (MMCHP) ($p < 0.05$). The highest mean urinary concentrations found was for monoethyl phthalate (MEP) and mono-n-butyl phthalate (MnBP) of 56.76 and 14.00 ng/ml in males and 27.75 and 11.04 ng/ml in females, respectively. All metabolites determined were present above the limit of detection for nearly all participants, demonstrating widespread exposure. There were no significant differences in food consumption between males and females, although the energy intake was higher in males. The MLR analyses showed positive significant associations that increased with consumption between DPHP and sweets ($\beta = 1.66$, $p < 0.01$) and drinks ($\beta = 1.54$, $p < 0.05$), DiNP and grain ($\beta = 1.43$, $p < 0.05$), and DEHP and butter and oil ($\beta = 1.22$, $p < 0.05$). The associations between urinary phthalates and the cosmetic categories were stronger than for the food categories, and there were differences between males and females. The strongest positive associations found for males and PCPs were di-iso-nonyl phthalate (DiNP) and

hand soap ($\beta = 2.47$, $p = 0.03$) and di(2-propyl heptyl) phthalate (DPHP) and perfume ($\beta = 2.16$, $p < 0.01$). The strongest positive associations for females were between antiwrinkle cream and diethyl phthalate (DEP) ($\beta = 2.43$, $p < 0.01$) and DINCH ($\beta = 1.82$, $p < 0.02$), and perfume and DEP ($\beta = 1.73$, $p < 0.01$). This indicates that PCPs may contribute considerably to the exposure of phthalates.

PS P131 - 0241 P21 Might Be a Potential Early Toxicity Biomarker of DNA Damage and Hematopoietic Depression by Benzene Exposure

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Benzene is a typical hematopoietic toxic substance. Metabolic activation of benzene could cause oxidative stress and DNA damage. Long-term exposure to benzene may cause significant hematopoietic toxicity and lead to hematopoietic dysfunction. P21 gene is the main member of cell cycle G1 / S checkpoint. It is closely related to cell cycle arrest, proliferation inhibition, and senescence induced by DNA damage. In our current study, we found that the transcription level of p21 gene was significantly increased in mice bone marrow cells when exposed to benzene with significant dose and time effect. The p21 expression level significantly increased at 5 days with 150mg/kg group, which was changed earlier than the decrease of white blood cells count. Also, the levels of P21 in mice exposed to 150mg/kg benzene were 6.82 times and 13.55 times higher than those in the control group at 5 days and 30 days, respectively. The Peripheral blood and bone marrow examination showed mice of benzene poisoning at the end of exposure to benzene. Also, the DNA damage of bone marrow cells showed a significantly increase with dose-effect by comet assay. The result showed transcription level of P21 increased significantly at the early stage of benzene exposure, and dramatically increased in mice benzene poisoning model. Furthermore, the level of P21 expression in the peripheral blood of 119 workers exposed to benzene and 95 control workers was detected. We found that the expression of P21 was significantly higher in the exposed workers when adjusted by the gender, age, smoking, and alcohol use, which were 2.33 folds as high as that in the control group. However, there was no significant difference in white blood cell count between benzene exposure group and control group. Therefore, P21 might be a sensitive marker of early toxic effects of benzene exposure. For further study, we hope to identify the exact internal exposure of benzene in workers to find out the dose-effect relationship between P21 expression level and benzene exposure. Also, the role of P21 in benzene toxicity would be clarified. This work was supported by the National Natural Science Foundation of China (Grants no. 81872645, 81730087).

PS P132 - 0282 Molecular Mechanism of Low Expression of miR-486-5p in Lung Squamous Cell Carcinoma

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Increasing evidence has demonstrated that miR-486-5p is an important gene of cancer. In the present study, the aim was to explore miR-486-5p expression and its role in lung squamous cell carcinoma (LUSC). The expression of miR-486-5p was analyzed in LUSC by Meta analysis of the Cancer Genome Atlas (TCGA) database, the Gene Expression Omnibus (GEO) database, and RT-qPCR results. Ten prediction platforms were used to predict the target genes of miR-486-5p. Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Protein-protein interaction (PPI) networks were used to explore the molecular mechanism. Next, lentiviral transfection was used to construct a miR-486-5p overexpressing cell model. Furthermore, CCK8, flow cytometry and Transwell assays were used to detect the effects of miR-486-5p overexpression on the biological function of LUSC. Western Blot (WB) technique was used to explore the possible regulatory mechanism of miR-486-5p. At last, tumor-bearing nude mice was built to study the function of miR-486-5p *in vivo*. The expression of miR-486-5p was significantly low-expressed with high diagnostic and prognostic value in LUSC by analyzed the TCGA database, GEO microarrays, RT-qPCR results, and a comprehensive meta-analysis of them (SMD = 2.25, 95% CI: 3.47~1.03, $P = 0.0003$). According the results of function analysis and PPI network, the PI3K-AKT pathway may be an important signal pathway for miR-486-5p to influence the development of LUSC. CCK8 assay showed that the cell proliferation ability of miR-486-5p overexpression group was significantly lower than that of negative control group at 36h and 72h ($P < 0.05$). The flow

cytometry results showed the apoptosis of miR-486-5p overexpressing cells increased significantly ($P < 0.05$). Transwell assays had no significance. The WB results showed that the expressions of AKT, p-GSK-3 β , PI3K, p-4ebp, mTOR, and p-mTOR were significantly decreased, while the expressions of ERK, GSK-3 β , and caspase-3 protein were significantly increased in the miR-486-5p overexpression group. The tumor bearing nude mice assay results showed that the expressions of AKT, PI3K, mTOR, and p-mTOR were significantly decreased, while the expressions of caspase-3 were significantly increased, which was consistent with cell model results. MiR-486-5p may be a LUSC anti-oncogene, playing an important role by regulating PI3K-AKT-mTOR-caspase-3 pathway, may serve as a biomarker in LUSC.

PS P133 - 0292 8-Hydroxyguanine as an Oxidative Stress Biomarker in Saliva

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Oxidative stress is well known to be a possible cause of many diseases, such as cancer. It is quite useful to know the oxidative status in the body, for the prevention of diseases and aging brought on by oxidative stress. In addition, psychological stress also increases oxidative stress. To prevent diseases potentiated by oxidative stress, a method for the appropriate assessment of the oxidative stress status is needed. Currently, urinary 8-hydroxydeoxyguanosine (8-OHdG) is widely measured as an oxidative stress biomarker. There is a specific advantage if saliva can be used as the sample to measure the oxidative stress biomarker, because saliva is much easier to collect than urine. However, the accurate measurement of 8-OHdG in saliva is impractical, because the quantity of 8-OHdG in saliva is quite low. In this study, we investigated the measurement of 8-hydroxyguanine (8-OHGua, the base moiety of 8-OHdG) in saliva, as an oxidative stress marker. The 8-OHGua levels in saliva were analyzed by a column switching HPLC system equipped with an electrochemical detector (HPLC-ECD). Statistical analyses were performed using the Pharmaco Basic software (Scientist Press Co., Tokyo, Japan). The study was approved by the Ethics Committee of Medical Research, University of Occupational and Environmental Health, Japan. The quantification limit was estimated to be below 0.2 ng/mL of saliva. The average level of 8-OHGua in saliva was 3.80 ng/mL in the ordinary subjects (non-smokers). The salivary 8-OHGua levels of smokers were higher than those of non-smokers. In addition, the average 8-OHGua levels in saliva were 3.3 pg/mL in the same non-smoking subjects. Salivary 8-OHGua may be a useful noninvasive and promising oxidative stress biomarker.

PS P134 - 0338 Biomarkers of Acute Toxicity by Carbon Monoxide in a Cohort Study of Patients in Argentina

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Carbon monoxide poisoning (CO) is preventable and avoidable. In our country, about 200 people die annually from CO poisoning. This work includes the study of patients with acute CO poisoning, their evolution and the search for new prognostic biomarkers. The most frequent source of exposures were boiler (80%) and water heater (20%). The main clinical manifestations were headache (28%), nausea (28%) and confusion (14%). About 70% of patients had blood carboxyhemoglobin levels (COHb) below 10%. Data were collected from the clinical records, including the Folstein minimal status exam (MMSE) and the Addenbrooke's Cognitive Examination Revised (ACE-R) test, of patients who entered to the J. A. Fernández Hospital throughout 2016 and 2017. Peripheral blood samples were extracted from these patients to evaluate genetic damage, by the comet assay and expressed as Tail DNA%, before (preT) and after (postT) receiving the established treatment. Some of these patients (67%) received normobaric oxygen (NBO) therapy and the remaining patients (33%) received hyperbaric oxygen (HBO) therapy. Data are expressed as mean $\pm$ SEM and statistical analyzes were Student's test or two way ANOVA with Bonferroni post-test. No significant differences were found between both groups (NBO: preT 10 $\pm$ 3, postT 6 $\pm$ 1; HBO: preT 3.8 $\pm$ 0.1, postT 6 $\pm$ 1). We found two prediction models which had lineal regression between: i) COHb with gender, age, headache and syncope (R^2 0.75, Raj 0.24); and ii) TailDNA% with gender, age, COHb (R^2 0.67; Raj 0.4). The results obtained in the cognitive tests ($n = 24$) were the following: ACE-R: mean = 83 $\pm$ 8, average total score = 82.7; MMSE: average =

26.7. The most cognitive domains were memory, verbal fluences and language. In conclusion: i) the most important prevention measures should be focused on home; ii) these tests seems to be appropriate to detect early cognitive disorders although it is could be to enlarge each domain to improve the evaluation; iii) the CO-induced damage was not reversed by any of the applied treatments. It is necessary to include more patients in the protocol. *This protocol was approved CODEI N° 201317 Disp.: 2013/1298.*

PS P135 - 0351 The Potential Mechanisms of Cytotoxicity Induced by Halobenzoquinone Water Disinfection Byproducts

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Drinking water disinfection effectively kills pathogens and prevents water-borne diseases, but it also generates a large number of water disinfection by-products (DBPs). Epidemiological studies have consistently linked long-term consumption of chlorinated water with an increased risk of developing bladder cancer; however, the responsible DBP or DBPs have not been identified. Halobenzoquinones (HBQs) are an emerging class of DBPs that are predicted to be potent bladder carcinogens. However, little is known about the toxicological effects of HBQs. Our study investigated the cytotoxicity and potential mechanism of toxicity induced by HBQs. The cytotoxicity of HBQs was demonstrated in several cell lines: T24 human bladder carcinoma cells, CHO-K1 Chinese hamster ovary cells, and SV-HUC-1 human uroepithelial cells. At μ M levels HBQs induced significantly higher toxicity than the regulated DBPs. HBQs generated significant intracellular reactive oxygen species (ROS), weakened the glutathione (GSH) antioxidant system, and caused severe oxidative DNA and protein damage (i.e., increased cellular level of 8-hydroxydeoxyguanosine (8-OHdG) and protein carbonyls). Additionally, toxicogenomic analysis showed that HBQs activate the nuclear factor E2-related factor 2 (Nrf2) antioxidant pathway; the major transcripts differentially up-regulated at both early and late exposure times were HMOX1, followed by NQO1, PTGS2 and TXNRD1. These results support oxidative stress as a key mechanism of HBQ-induced cytotoxicity. Structure-toxicity relationship analysis demonstrated that formation of ROS, cytotoxicity, and genotoxicity were strongly affected by the structure of HBQs (i.e., position, type, and number of substitutions of halogens on the benzoquinone ring). Halogen substitution groups, isomeric structure, and electron distribution were shown to affect HBQ toxicity. The cytotoxicity of di-halogenated HBQs followed the trend: iodo- > bromo- > chloro-HBQs. Additionally, 2,5-HBQs induced greater toxicity than their corresponding 2,6-HBQ isomers. Furthermore, HBQ toxicity was influenced by two major structural descriptors: the lowest unoccupied molecular orbital energy (E_{LUMO}) and dipole moment. These results provide useful insight into the mechanisms of toxic effects of HBQs in *in vitro* systems.

PS P136 - 0371 Subclinical Nephrotoxicity Associated with Chelation Therapy in Young Thalassemia Patients: The Role of Urinary Kidney Injury Molecule

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We conducted this study to investigate renal involvement in young thalassemia patients, using routine kidney indices and novel urinary human kidney injury molecule-1 (KIM-1) in relation to chelation therapy (deferasirox, deferoxamine and deferroproline). We conducted a cross-sectional study on 66 patients with Beta-thalassemia attending the Pediatric hematology clinic, Zagazig University. Their ages ranges from 7 to 22 years. Routine kidney indices and novel urinary human kidney injury molecule-1 (KIM-1) were measured. Estimated glomerular filtration rate (eGFR) was calculated. The median(IQR) level of serum creatinine was significantly higher in patients on deferasirox therapy than patients on deferoxamine and deferroproline therapy [0.85(0.63-0.99), 0.50(0.34-0.58) and 0.44(0.36-0.45) mg/dL, respectively, $P < 0.001$]. The median(IQR) level of eGFR was significantly lower in patients on deferasirox therapy than patients on deferoxamine and deferroproline therapy [63.3(56.5-92.1), 117.3(91.9-162.0) and 136.7(109.4-157.6)] (ml/min/1.73m²), respectively, $P < 0.001$. The mean (SD) level of urinary (KIM-1) was significantly higher in patients on deferasirox therapy than patients on deferoxamine and deferroproline therapy [0.070(0.019), 0.041(0.017) and 0.042(0.015) respectively, $P < 0.001$]. These data suggest that urinary (KIM-1) is an early predictive biomarker for decline in eGFR in patients with Beta-thalassemia on deferasirox therapy.

P137 - 0394 The Effects of Indirect Toxicity to Vascular Endothelial Dysfunction by Respiratory Exposure to Single-Walled Carbon Nanotubes

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The single-walled carbon nanotubes (SWCNTs) is one of the most extensively applied nanomaterials (NMs) in nanotechnology. It has a broad application prospects in electronics, composite materials, tissue engineering and decorative coatings, etc. Epidemiological studies have demonstrated that nanometre particles in polluted air can increase the risk of cardiovascular disease, which is dangerous to mankind. Respiratory tract exposure is one of the main avenues for NMs to enter the human body. However, little is known regarding indirect toxic effects on the cardiovascular system for respiratory tract exposure to SWCNTs. On the basis of the previous studies, cell tests *in vitro* were carried out to investigate the indirect toxic effects and the possible mechanism to vascular endothelial dysfunction by respiratory exposure to SWCNTs. In the present study, the rat alveolar macrophage cell line (RAM) and primary rat vascular endothelial cells (RAECs) were cultured, respectively. Also, cell models of inflammatory cytokines-stimulated and indirect exposure were established, and the SWCNTs directly exposure to RAECs as a comparison. The results showed the morphological changes of RAM, the decreased cell activity, the higher levels of ROS in RAM, and the continuous secrets of IL-1 α , IL-6 and TNF- α by RAM after stimulated by SWCNTs. There were changes for coagulation and fibrinolysis factors secreted by VECs after stimulated by TNF- α . Similarly, after treated by the indirect exposure, the levels of coagulation and fibrinolysis factors secreted by VECs also changed. After indirect exposure model treatment, the lower expression of t-PA protein, the higher expression of JNK and phosphorylated and non phosphorylated p38MAPK protein in RAECs were detected, which compared with normal control groups. All these results indicated some key points as follows: SWCNTs can induce the release of pro-inflammatory factors by RAM, which has a mediated effect on vascular endothelial function. In comparison to the direct exposure, the induced effects on endothelial dysfunction after indirect exposure to SWCNTs may be more significant. Among them, the inflammatory factors play a key role to induce the endothelial dysfunction. This study illustrates one possible way of indirectly effects of SWCNTs to vascular endothelial dysfunction and the possible mechanism as well as the role of inflammatory cytokines.

P138 - 0424 Effect of Estrogen on Tumor Progression in Gastric Cancer Cells-Derived Xenograft Models

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The issue of sex difference has been studied in the field of cancer research. Estrogen, a sex hormone, is one of the factors leading to sex difference. Estrogen is well known to play an important role in development, proliferation and biological function of reproductive system. Thus, estrogen has been associated with tumor progression in estrogen receptor-positive breast cancer derived xenograft model. On the other hand, the action of estrogen is unclear in other cancer cells. In this study, we investigated the effect of estrogen on tumor proliferation in gastric cancer xenograft models. Gastric cancer MKN-1, SNU-638, SNU-16 and SNU-484 cells were used for xenograft models. Among them, SNU-638 and SNU-16-derived tumors significantly showed growth difference in sex-cross xenograft models. Differentially expressed genes (DEG) from their tumors were analyzed by total RNAseq. Furthermore, we minimized the effect of endogenous estrogen through ovariectomy and treated estrogen pellet in human female gastric cancer SNU-16 cell-derived xenograft model. SNU-16 cell-derived tumor growth was suppressed in ovariectomized group more than in control group. The result is coincident with the tumor growth difference between male-derived xenograft model and female-derived xenograft model. Interestingly, estrogen pellet transplantation promoted the tumor growth in ovariectomized group. DEG from estrogen-treated tumors in the total RNAseq of ovariectomized model. In this model, JAK2 was related to the tumor progression by estrogen. Our results suggested that estrogen could enhance tumor growth in female-derived gastric cancer.

P139 - 0428 AdductomicsPT: A New Mass Spectrometry-Based Adductomics Pipeline for Toxicology Studies

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Identification of protein covalent modifications (covalent adducts) is a challenging task mainly due to the lack of data processing approaches for Adductomics studies. Despite the huge technological advances in mass spectrometry (MS) instrumentation and bioinformatics tools for proteomics studies, enabling the identification of several thousands of proteins in a single injection analysis, these methodologies have very limited success on the identification of low abundant covalent protein adducts. Herein we present a novel strategy to identify protein covalent modifications inspired in metabolomics workflows that consists on LC-MS data pre-processing using the open source software MZmine followed by statistical analysis. The usefulness of this strategy was evaluated using experimental LC-MS data of histones isolated from HepG2 and THLE2 cells exposed to the chemical carcinogen glycidamide. Our approach was able to identify more adducts than the commonly used methodology in adductomics studies, which rely on producing comprehensive MS/MS data, using data dependent analysis (DDA) acquisition mode, and then use protein database search engines (e.g., Mascot, MaxQuant) for the identification of adducted peptides. This new untargeted Adductomics workflow consists on a first full scan analysis of samples followed by our metabolomics-inspired data processing strategy that will yield a list of *m/z* values, corresponding to potential adducts. This peak list will be subsequently used for targeted MS/MS analysis of potential adducted-peptides. The selection step is expected to result in higher quality MS/MS spectra of low level adducted-peptides, when compared with DDA and data independent analysis (DIA) approaches, thereby enhancing the chances of identifying low abundant adducted peptides in biological samples. This will exponentially increase the number and accuracy of findings for all fields of Adductomics application, encompassing epigenetic and toxicological studies.

P140 - 0494 ASB15 and RBM45, the Novel Target Genes of HIF-1 α Involved in Benzene Hematopoietic Toxicity

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ASB15, one of the members of the suppressor of cytokine signaling (SOCS) family of proteins, participated in the ubiquitination and subsequent protein degradation in the cell cycle and signal transduction pathways. RBM45 encodes a member of the RNA recognition motif (RRM)-type RNA-binding family of proteins which could regulate the stability of RNA and variable splicing. In our previous study, the HIF-1 α , a transcriptional factor was found significantly degraded in mouse bone marrow cells when exposed to benzene. In this study, we identified the ASB15 and RBM45 gene were novel target gene of Hif-1 α . The mRNA and protein level of ASB15 and RBM45 were decreased with Hif-1 α degraded in bone marrow cells in C57BL/6 mice with treatment of 150 mg/kg benzene by subcutaneous injection. Meanwhile, the mRNA and protein level of ASB15 and RBM45 were upregulated in HIF-1 α overexpression mice. The Chip qPCR detection revealed the Hif-1 α protein could bind to ASB15 and RBM45 gene and regulated its transcription. Then the over expressed of ASB15 and RBM45 cell of k562 were established. Overexpression of ASB15 and RBM45 could increase the proliferation rate of k562 cells. In addition, when the cells was exposed to 10 μ mol/L 1,4-benzoquinone, the final metabolite of benzene, the overexpression of these two genes showed protective effects for the toxicity with 1,4-benzoquinone treatment. The case-control study with 91 benzene exposed workers and 103 unexposed controls were also set up to explore whether the expression level of ASB15 and RBM45 gene were varied in benzene occupational workers. The mRNA expression levels of ASB15 and RBM45 in workers exposed to benzene were 43.5% and 50.0% of the control group, respectively. They were significantly depressed by exposure to benzene when adjusted by the gender, age, smoking and alcohol use. It suggested that ASB15 and RBM45 gene were new HIF-1 α target genes which involved in the benzene hematopoietic toxicity and might be an early toxic effect biomarker for benzene occupational exposure. This work was supported by the National Natural Science Foundation of China (Grants no. 81730087, 81573189).

PS P141 - 0530 The Concept of "Signal Toxicity" for the Mechanistic Analysis of So-Called Low Dose Effect and Delayed Effect after Perinatal Exposure

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The "Our Stolen Future" by Theo Colbone, Dianne Dumanoski and John Peterson Myers (1996) had coined the concept of "Endocrine Disrupting Chemicals" with its mechanistic plausibility to all living organisms. The paradigm shift from the classical toxicology was its so-called "low dose effect", "non-monotonic dose response" and "delayed effect after perinatal exposure." Here, we would like to introduce the concept of "signal toxicity [1]" that would mechanistically explain the paradigm mentioned above. Symbolically, the following contrast could be pointed out between the classical toxicology and signal toxicology. In case of "classical toxicology," it could be outlined that a toxic substance reaches the target molecule such as enzymes, lipid membranes, DNA, and other components and induces malfunction. In contrast, in case of signal toxicity, a chemical bind to a receptor. After that, the chemical itself is not important. The exogenous signal from the receptor initiates a cascade of molecular events that leads to various changes to induce adverse effects. The target would be not only endocrine/reproductive, but also immune, and neuronal systems. If the signal is used for development, there would be a critical period that the disturbance of such signals may leave irreversible changes to the organism. Examples from our Percellome Project [2-3] will be presented to explain the concept of signal toxicity. We performed a series of perinatal exposure studies of neuroactive chemicals at a non-cytotoxic dose that induces aberrant signaling in the developing mouse brain and monitored the abnormal cognitive function in the adult hood [4-5]. In the Percellome Project, we found that a classical herbicide pentachlorophenol orally administered to mice at non-cytotoxic dose triggers interferon signal in the liver [6], indicating a signal toxicity mechanism in acute response. In comparing aryl hydrocarbon receptor (AhR) knock out mouse and wild type mice, we also found another aspect of signal toxicity which can be expressed as "Sensor Effector Mismatch." This concept will be briefly introduced with Percellome transcriptomic data. Supported by the Health Sciences Research Grants from the Ministry of Health, Labour and Welfare, Japan. References: [1] Kanno, J., et al. *J. Tox. Sci.* 2016, 41(Special):SP105-9. [2] Kanno, J., et al. *BMC Genomics*. 2006, 7: 64. [3] Kanno, J. *Biomarkers in Toxicology*, Academic Press 2014: 1019-32. [4] Tanemura, K., et al. *J. Tox. Sci.* 2009, 34 Suppl 2:SP279-86. [5] Furukawa, Y., et al. *Front Neurosci.* 2016; 10:339. [6] Kanno J., et al. *J. Tox. Sci.* 2013, 38:643-54.

PS P142 - 0600 Physiological Biomarkers of Low-Level VX Exposure in the New Zealand White Rabbit

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The advent and advancement of wearable physiological monitoring devices has made data typically available only through a visit to a medical clinician continuously available to end users. As wearable technologies mature, the reliability of measurements will only improve the diagnostic capability of such devices. With this in mind, the present study set out to determine if meaningful changes in routinely monitored physiological signals could yield biomarkers of exposure to chemical warfare agents at exposure levels that do not produce overt signs of intoxication. Sixteen New Zealand white rabbits were implanted with a telemetry device capable of monitoring the electrocardiogram, blood pressure, body temperature, and animal activity. Groups of four rabbits were exposed to VX aerosols resulting in red blood cell acetylcholinesterase (RBCAChE) inhibition ranging between 10% and 90%. Physiological data were recorded for 5 days prior to, including, and 5 days following exposure. Statistical comparison of post-exposure to pre-exposure values resulted in several notable changes. In animals with RBCAChE inhibition ranging between 40% and 60%, mean arterial blood pressure transiently increased to 94.23mmHg during exposure and then was reduced from a pre-exposure mean of 81.56mmHg to 76.10mmHg for the duration of the five day post-exposure period. Heart rates were generally lower (below 95% confidence limits of the baseline mean) for 48 hours following exposure with perturbation or complete abolishment of circadian rhythm in exposures resulting in RBCAChE inhibition greater than 30%. Additionally, disturbances in the circadian rhythm of animal activity and body temperature were observed. Thermoregulatory disturbances were evident for the duration of the 5 day post-exposure period (baseline range: 39.05 - 39.33C;

post-exposure range: 38.83 - 39.52C). Additional analysis are currently ongoing, however current findings indicate that it may be possible to identify exposure to chemical warfare agents through perturbations in normal physiology prior to onset of traditional overt signs of exposure such as miosis, respiratory distress and tremors.

PS P143 - 0603 aProximate Proximal Tubule Model as a Predictive Model of Aminoglycoside Nephrotoxicity Using Clinically Relevant Biomarkers of Toxicity

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Around 50% of preclinical *in vivo* toxicity screens fail to predict subsequent human toxicity, leading to significant attrition of drug molecules during drug development. Renal toxicity accounts for about 20% of the unexpected toxicity at first in man. Understanding nephrotoxicity has been hampered by the lack of a good renal model. Here we demonstrate the utility of aProximate human proximal tubule cell (hPTC) monolayers as an *in vitro* tool to investigate nephrotoxicity by demonstrating the effects of a well characterized panel of aminoglycosides upon the release of the clinically relevant biomarkers NGAL, KIM-1 and clusterin by the cells in response to aminoglycoside challenge. PTCs were isolated from fresh kidney cortex using a collagenase digest and Percoll density gradient protocol. NHP and canine PTCs grown on Transwells formed confluent monolayers and produced average TEER value of more than 90 $\Omega \cdot \text{cm}^2$ after day 7 in culture. To test the utility of NHP monolayers as predictive models of aminoglycoside nephrotoxicity, cells were challenged with a range of aminoglycoside analogues (tobramycin, gentamicin, neomycin, amikacin and streptomycin) in the concentration range 0-300 μM for 72 hours. At 72 hours the apical supernatant was collected and analysed for the biomarkers; KIM-1, NGAL and clusterin using an Mesoscale Discovery (MSD) platform. In addition Transepithelial resistance (TEER) was recorded as a measure of monolayer integrity, cell viability was determined by intracellular ATP levels and cell death was assessed using LDH release as a measure of cell death. In response to neomycin, tobramycin or gentamicin challenge, there was a significant concentration dependent rise in KIM-1, NGAL and clusterin release (e.g., clusterin production was 5.3-fold, 5.1-fold, 4.9-fold above control levels). And a significant fall in cell ATP-content, LDH release and TEER. In contrast, exposure of the cells to amikacin resulted in a more modest 1.5-fold increase in biomarker and cell viability endpoints. 72 hour exposure to streptomycin had no significant impact on any of the nephrotoxicity endpoints measure. These results report the successful application of primary human proximal tubule model as predictive *in vitro* PTC models of drug transport and drug toxicity.

PS P144 - 0712 2-Alkenal Modification of Hemoglobin: Identification of a Novel Hemoglobin-Specific Alkanoic Acid-Histidine Adduct

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a,b-Unsaturated aldehydes generated during lipid peroxidation, such as 2-alkenals, give rise to protein degeneration in a variety of pathological states. 2-Alkenals are highly reactive toward nucleophilic amino acid residues, such as histidine and lysine, to form Schiff base adducts or Michael addition adducts. In this study, upon the reaction of hemoglobin with 2-octenal, we unexpectedly detected a product corresponding to reduced form of 2-octenal-histidine Michael adduct plus 14 mass unit. Based on the LC-ESI-MS/MS analysis of synthetic adduct candidates, the adduct was identified to be *N*-(1-carboxyheptan-2-yl)-histidine (CHH), a novel alkanoic acid-type histidine adduct. The alkanoic acid-histidine adducts were detected in the 2-alkenal-treated hemoglobin and myoglobin but not in the 2-alkenal-treated cytochrome c and transferrin. The addition of heme in the reaction mixture, containing non-heme protein and 2-alkenals, resulted in the formation of the alkanoic acid-histidine adducts, suggesting that a heme iron may play a role in the oxidation of covalently modify proteins. Moreover, using stable isotope dilution method, we showed evidence for the endogenous formation of CHH in red blood cells exposed to hydrogen peroxide. Thus, this study establishes a novel mechanism for covalent modification of proteins by 2-alkenals, in which heme iron is involved in the formation of alkanoic acid-histidine adducts.

PS P145 - 0755 Antioxidant Enzymes and BDNF as Biomarkers of Oxidative Stress–Induced Neurodegeneration Such as Amnesia

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Previously, some phenolic compounds, which exerted cytoprotective action through expression of antioxidant enzymes as well as brain derived neurotrophic factor (BDNF) in glutamate-exposed HT-22 mouse hippocampal neuronal cells, were found to prevent against amnesia induced by scopolamine. Similarly, N-palmitoyl hydroxytryptamine, which exerted neuroprotective action through expression of antioxidant enzymes as well as BDNF, was also observed to prevent against scopolamine-induced amnesia. Further, N-palmitoyl hydroxytryptamine not only enhanced expression of GCLC, HO-1 or NQO-1 through activation of NRF signaling, but also restored phosphorylation of TrkB, CREB as well as expression of BDNF, consistent with activation of TrkB/CREB/BDNF pathway. Likewise, 3,3'-diindolylmethane (DIM), which showed a similar cytoprotective action, enhanced expression of GCLC, HO-1 or NQO-1 through activation of NRF signaling, restored phosphorylation of TrkB, CREB as well as expression of BDNF. Moreover, 3,3'-diindolylmethane which suppressed the formation of apoptotic bodies induced by glutamate, dose-dependently recovered expression of Bcl-2, anti-apoptotic factor, while reducing expression of Bax, calpain, cytochrome C or cleaved caspase-3 as well as AIF, pro-apoptotic factors. Taken together, the neuroprotective compounds which activated NRF signaling as well as TrkB/CREB/BDNF signaling substantially restored the level of antioxidants such as GCLC, HO-1 or NQO-1 as well as the level of BDNF which were markedly reduced in HT-22 cells exposed to oxidative stress. In conclusion, the level of antioxidant enzymes such as GCLC, HO-1 or NQO-1 as well as the quantity of neurotrophic factor such as BDNF in brain cells or tissues are supposed to be biomarkers of some neurodegenerative diseases such as amnesia. Also, the level of GCLC, HO-1 or NQO-1 as well as BDNF in brain cells or tissues may be utilized as biomarkers in screening neuroprotective compounds which may prevent against oxidative stress–induced neurodegeneration.

PS P146 - 0776 The Effect of Betel Quid Chewing on Smoking-Induced Oxidative Stress

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Oxidative stress has been related to many diseases. Urinary 8-Hydroxy-2'-deoxyguanosine (8-OHdG) reflects systemic oxidative stress *in vivo*. The International Agency for Research on Cancer (IARC) has classified cigarette and betel nut as Group 1. Betel quid chewing and cigarette smoking are most important risk factors for oral squamous cell carcinoma in Taiwan. O6-methylguanine (O6-MeG) and N7-methylguanine (N7-MeG) are the DNA adducts from 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). In Taiwan, almost all betel quid chewers are also cigarette smokers, and whether polyphenol rich betel will have any modulation effects on cigarette smoking-generated oxidative stress has not been studied. The aim of this study is to test the interactive potential of betel quid on cigarette smoke as evidenced by the urinary 8-OHdG levels. The study population consists of 206 healthy subjects, and includes 77 cigarette smokers, 6 betel quid chewers, 101 cigarette smoking and betel quid chewers, and 22 without such habits. Urinary 8-OHdG levels were quantitated using liquid chromatography tandem mass spectrometry (LC-MS/MS). Buccal cell O6-MeG and N7-MeG levels were evidenced by slot blot analysis. The concentration of urinary of 8-OHdG are 0.71 ± 0.33 , 1.87 ± 1.97 , 0.61 ± 0.30 and 1.43 ± 2.91 ng/mg Creatinine in normal, smoking, betel and smoking plus betel group, respectively. The ratio of buccal of O6-MeG and methylene blue are 0.3 ± 0.25 , 0.35 ± 0.3 , 0.16 ± 0.16 and 0.31 ± 0.33 in normal, smoking, betel and smoking plus betel group, respectively. The ratio of buccal of N7-MeG and methylene blue are 0.22 ± 0.18 , 0.44 ± 0.34 , 0.48 ± 0.53 and 0.37 ± 0.41 in normal, smoking, betel and smoking plus betel group, respectively. The smoking group generates more 8-OHdG, O6-MeG and N7-MeG as compared with smoking plus betel group, and yet to reach a statistically significant level. Betel chewer have the highest creatinine urinary level ($p < 0.001$) as compared with other groups, and this finding that betel quid chewers tend to have compromised kidney function. In conclusion, these results suggest that chewing betel quid might modulate the cigarette smoke-generated oxidative stress *in vivo*.

PS P147 - 0850 A Toxicology MOOC (Massive Online Open Course) for Different Learning Needs

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A Toxicology MOOC has been created as a result of the Erasmus + project called TOX-OER (Learning Toxicology through Open Educational Resources; <https://toxoeer.com>). The generation of educational social value was the main objective. To this end, a scientific and pedagogical consortium was created with experts in Toxicology, Chemistry, New Technologies and Pedagogy. It was formed by 7 international institutions: Universities of Salamanca (Spain, as coordinators), Porto (Portugal), Bologna (Italy), Charles (Czech Republic), Applied Sciences of Southeast Finland (Finland), Transylvania of Brasov (Romania) and a Research Institute (Bulgaria). This MOOC have 7 different modules, consisting of a total of 22 topics. Each topic contains videos, complementary texts, readings, activities, self-evaluations and final exam. To cover the different needs of learning each topic begins with a basic level that progressively increases. All the material is available in the language of the 7 partners and in English. To know the degree of satisfaction with the MOOC, dissemination programs have been established in different scenarios. Satisfaction surveys have been carried out for the participants. The results have been the following: Professionals from different fields (researchers, veterinarians, health workers, security forces). Presentation campaigns have been carried out in several countries. The professionals showed interest in the materials with higher scientific level. Higher education: TOX-OER materials have been used in Degree and Master programs of consortium universities. The surveys results showed a high satisfaction with the teaching materials, and a real help for the acquisition of knowledge. In addition, dissemination campaigns have been carried out in European universities, so that other professors can take advantage of this material. It must be mentioned the creation of a master's degree from the University of Salamanca (online), with TOXOER materials (<https://toxoeer.com/toxicology-diploma/>). Secondary education: Promotional campaigns with high school teachers showed the usefulness of the most basic part of the subjects for high school students. General population: to access the general public, part of the MOOC was presented as two courses within the international MiriadaX platform. These courses had a high number of students, and even one of them won a prize called by the MiriadaX platform. In conclusion, the TOX-OER toxicology MOOC covers different contents from its most basic aspects to more scientific, so it can meet different learning needs. The project has had a great impact on the education / learning of Toxicology.

PS P148 - 0856 Identification of Protein Markers Predictive of Adverse Effects Due to Chemical Mixture Exposures

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Recent technological advances are being incorporated into toxicology in an overall effort to reduce reliance on traditional animal testing models. A call for development and application of next-generation methods for predictive toxicology was initiated by the US National Academy of Science (NAS) over a decade ago. Key to these recommendations is the integration of signaling pathway data to improve assessment of toxicological risk for environmental chemical exposures. The overall challenge with this approach is identifying measurable/observable nodes or "key events" that are essential to the biological progression toward an adverse health outcome in the myriad of transcriptional, post-transcriptional, post-translational cellular events that occur with chemical exposure. To demonstrate the utility of identifying protein-based key events for complex environmental chemical mixtures, we utilized an *in vitro* model (BEAS-2B human airway epithelial cells) that were exposed to different concentrations of Ni²⁺, Cd²⁺, and Cr⁶⁺ alone and in defined mixtures, an *in vivo* rat model exposed to multiple air pollutants of particulate matter and ozone, and an *in vivo* mouse model co-exposed to environmental chemical of vinyl chloride and high-fat diet. Expression and phosphorylation status of a variety of common signaling pathway proteins and cytokines for immune, cell death, and acute phase responses were measured in these experimental systems, together with cytotoxicity and other tissue apical endpoints representative of cellular adverse outcomes. Least Absolute Shrinkage and Selection Operator (LASSO) multiple regression was used to identify a subset of protein markers that constituted a putative toxicity pathway capable of predicting toxicity or apical endpoints. The identified protein markers of predictive toxicity for these chemical mixtures included phospho-70S6K, phospho-p53, cleaved CASP3, phospho-MAPK8, IL-10, and Hif-1α. Chemical toxicity including mixture tox-

icity was also successfully predicted in a variety of exposed cells and tissues using the identified protein markers predictive of apical endpoint. As this approach does not depend on *a priori* knowledge of either the specific pathways involved or the specific agents inducing the pathway alterations, it may be generally useful for identifying sets of protein markers predictive of adverse effects for a variety of mixtures, including complex environmental mixtures of unknown composition. Utilization of the generalizable and protein-based biomarkers for apical key events linked to adverse human health may assist in hazard identification and prioritization.

PS P149 - 0868 A New Biomarker Capable to Predict Nephrotoxicity Caused by Contrast Media in Cardiological Patients

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Contrast media (CM) administration causes a pathology known as contrast induced nephropathy (CIN), defined as an increase in serum creatinine of at least 25% or 0.5 mg/dl within 3 days after CM administration in absence of an alternative aetiology. The incidence varies from 2% in healthy patients to up to 50% in patients with kidney disease. Besides, it is the third cause of hospital acquired kidney damage and increases the morbimortality. Recently, new biomarkers for the diagnosis of acute kidney injury (AKI) have emerged. These include neutrophil gelatinase-associated lipocalin (NGAL), Kidney injury molecule 1 (KIM-1) and albuminuria. Besides, preclinical studies conducted in our laboratory have identified a urinary marker, ganglioside M2 activator protein (GM2AP) capable of predicting the risk of developing AKI after administration of various potentially nephrotoxic drugs. The aim of this work was to evaluate the usefulness of these biomarkers to predict CIN in cardiological patients treated with CM. A retrospective clinical study was developed in patients from the Cardiology Service of the University Hospital of Salamanca (Spain) who were administered CM for the performance of diagnostic or surgical procedures. A total of 154 patient were included in the study from whom blood and urine samples were taken before CM administration and the days after until the medical discharge. In addition, data about the volume and type con CM used, their anthropometric parameters, drug consumption, other pathologies and renal risk factors was collected. With the data obtained from the blood analysis, patients were distributed in two groups, based on the creatinine increase: (i) Control; those who did not develop CIN according to the criteria mentioned above and (ii) Cases; those who did develop CIN. In the urine sample previous to the CM administration, NGAL, KIM-1 and albuminuria were determined by ELISA, and GM2AP was determined by Western Blot. Of the total number of patients, 123 were assigned to the control group and 31 to the Cases group, representing an incidence of 20.1 %. In both groups, the gender and age distribution were similar as well as the proportion of patients with arterial hypertension, diabetes mellitus, dyslipidaemia and tobacco consumption. From the urinary biomarkers studied, neither NGAL nor KIM-1 showed significant differences among groups. However, albuminuria and GM2AP did were significantly higher in the cases group. This study shows that both albuminuria and GM2AP could be potential biomarkers to predict the occurrence of CIN. In clinical practice it could be useful for detecting and handling patients at risk in a pre-emptive and personalized manner.

PS P150 - 0869 Correlation between Tobacco Use and Biomarkers of Early Kidney Damage

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Smoking is a chronic addictive disease responsible of the death of more than 7 million people every year. The main pathologies that causes are cardiovascular and respiratory disorders and cancer. Although the direct relationship between smoking and kidney damage is not exactly known, there are numerous compounds in tobacco that are capable of triggering mechanisms that can contribute to injury the kidney and cause a nephropathy (i.e. oxidative stress, hypoxia, vasoconstriction and stimulation of nicotinic receptors). The aims of this work were: (1) to elucidate whether tobacco consumption promotes the excretion of any of the most well-known urinary biomarkers of early kidney damage [proteinuria, kidney damage molecule 1 (KIM-1), N-acetyl-beta-D-glucosaminidase

(NAG) and neutrophil gelatinase-associated lipocalin (NGAL)] and (2) to evaluate the existence of a correlation between these biomarkers and the degree of exposure to tobacco, measured in terms of urinary cotinine (a metabolite of nicotine). Urine samples were taken from volunteers from the Primary Care consultation of a Health Center (Cáceres, Spain). These patients were divided into two groups: (i) smokers (n = 56) and (ii) non-smokers (n = 147). A database was created with their main anthropometric parameters, as well as their levels of plasma creatinine, the marker most used in the clinical setting to evaluate renal function. For each patient, urinary levels of total proteins, NAG, NGAL, KIM-1 and cotinine were quantified. The normality of the data was evaluated with a Kolmogorov-Smirnov test and subsequently each marker of renal damage was correlated with cotinine. Finally, the Spearman correlation coefficient was calculated. There were no significant differences between the number of men and women belonging to each group, nor in the main anthropometric variables considered. However, in the case of age, significant differences were observed between both groups, being lower in smokers. No significant differences were found in relation to their plasma creatinine levels. On the contrary, significantly higher urinary concentrations of KIM-1 (p < 0.001), proteinuria and NGAL (p < 0.01) were detected in the smokers group. In addition, these biomarkers showed a significant direct correlation with urinary levels of cotinine in smokers and non-smokers (p < 0.001 for KIM-1 and proteinuria, and p < 0.05 for NGAL). In conclusion, there seems to be an obvious relationship between smoking and the development of early kidney damage. *Financial support: Instituto de Salud Carlos III co-funded by FEDER (PI17/01979).*

PS P151 - 0874 Toxicity of CMIT/MIT, a Humidifier Disinfectant on Eye-Dryness and Metabolomic Profile Using ¹H-NMR Metabolomics in Rats

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5-Chloro-2-methyl-3(2H)-isothiazolone/2-methyl-3(2H)-isothiazolone (CMIT/MIT) was used as a humidifier disinfectant in Korea. Many people used humidifier to improve eye-dryness symptom. Current study was performed to elucidate effect of CMIT/MIT on eye-dryness in rat model using metabolomics. CMIT/MIT was instilled to eyeball of rat showing eye-dryness induced by scopolamine and dry condition. Mucosal damage and changes of immune index after application of CMIT/MIT were determined. Also, we evaluated changes of plasma and urine metabolites by proton nuclear magnetic resonance (¹H-NMR) analysis. In order to induce eye-dryness, scopolamine (3 mg/kg for 7 days) was subcutaneously injected. After induction of eye-dryness, CMIT/MIT (0.001%, 0.01%) were instilled in the eyeball for 5 days. And CMIT/MIT (0.001%, 0.01%) was instilled in the eyeball of normal rat for 12 days. The amount of tears and tear film breakup time (TBUT) levels showed a tendency to decrease both in eye-dryness-induced group and CMIT/MIT treat group compared with the control. Corneal damage also showed a similar tendency. The levels of IL-1β and TNF-α in corneal immunohistochemistry were significantly higher in CMIT/MIT (0.01%) treated group than control. IL-6 levels showed a tendency to increase in CMIT/MIT treated group compared with the control. In conjunctival immunohistochemistry, IL-1β tended to increase and IL-6 increased significantly at high concentrations. Metabolomics study showed that principal components analysis (PCA) and orthogonal projections to latent structures-discriminant analysis (OPLS-DA) score plots showed separated clusterings by each treatment group based on NMR spectrums. Metabolites (VIP > 1.0) showing differences among groups were 8 and 13 metabolites in plasma and urine, respectively. Among them, glucose, 3-hydroxybutyrate and pyruvate were significantly different between control and CMI/MI treated group in plasma. In urine sample, 2-oxoglutarate, 3-hydroxybutyrate, acetone, creatine, methanol, formate, guanidoacetate, ethanolamine and creatine phosphate were significantly different between control and CMI/MI treated group. These results could contribute to understand the metabolic regulation and can be useful for potential biomarkers which is related with eye-dryness and CMIT/MIT.

PS P152 - 0900 An In Vitro Analysis of PPARα and CYP4A as Potential Biomarkers for Perfluoroalkyl Acid Exposure in Cetacean Kidney Cells

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High concentrations of PFAAs have been documented in marine mammal tissues around the world, and there is an increasing concern for potential negative consequences on marine mammal health because of the severe consequences of exposure in other mammals. Two proteins have been suggested as potential biomarkers for PFAA exposure, peroxisome proliferator-activated receptor alpha (PPARα) and cytochrome P450 4A (CYP4A). PPARα is a transcription factor, which induces transcription of CYP4A, and PFAAs have been documented to activate the PPARα in other mammals including mice, humans, and Baikal seals. Additionally, there is a correlation in cetaceans stranded in Hawaii between high concentrations of PFAAs and PPARα mRNA and CYP4A protein expression. We measured mRNA and protein expression of PPARα and CYP4A in *Tursiops truncatus* kidney cells exposed *in vitro* to three PFAAs, PFOA, PFDA, and PFNA. Quantitative PCR was used to measure mRNA expression. The relative expression of each protein was quantified using Western Blot. PPARα mRNA expression was significantly upregulated at higher concentrations of PFOA (64 - 256 mM) and PFNA (128 mM), but was also significantly down-regulated at the highest concentration of PFNA tested (256 mM). CYP4A mRNA expression was significantly upregulated at higher concentrations of PFOA (128 mM) and PFDA (256 mM). There was a significant positive correlation between PPARα and CYP4A mRNA expression for cells treated with PFOA, PFNA and PFDA. At the highest dose (225 uM), all three chemicals induced greater expression of PPARα than the vehicle control, but this increase in expression was not statistically significant ($p < 0.05$). There was a statistically significant difference between the protein expression in cells exposed to the two lower concentrations of PFDA (1 uM and 25 uM) and the highest concentration (225 uM) ($p = 0.0288$). All three chemicals elicited a dose dependent response in expression of CYP4A; however, the change in expression was not significantly different between treatment groups and from the vehicle control ($p < 0.05$). Although we did see a dose-response effect, our results suggest that these two proteins are not ideal biomarkers for PFAA exposure in cetaceans due to the high sensitivity of the pathway in the narrow range of doses tested. However, this does not lessen the concern for how these chemicals are disrupting this pathway in cetaceans, as higher doses induce expression similar to or greater than known positive controls. This is the first *in vitro* study to investigate the potential of PPARα and CYP4A as biomarkers of PFAAs exposure in cetacean cells.

PS P153 - 0920 Betel Quid Containing Safrole Enhances Metabolic Activation of Tobacco-Specific 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)

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Cigarette smoking (CS) and betel quid (BQ) chewing are two known risk factors and have synergistic potential for the development of oral squamous cell carcinoma (OSCC) in Taiwan. Most mutagens/carcinogens are metabolically activated by cytochrome P450 (CYP450) to exert their mutagenicity or carcinogenicity. Previous studies have shown that metabolic activation of tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) by CYP2A6 activity determines NNK-induced carcinogenesis. In addition, safrole affects cytochrome P450 activities in rodents. However, the effect of safrole in BQ on metabolism of tobacco-specific NNK and its carcinogenicity remains elusive. This study demonstrates that safrole (1 mg/kg/d) induced CYP2A6 activity, reduced urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) levels, increased NNK-induced DNA damages including N7-methylguanine, 8-OH-deoxyguanosine and DNA strand break in a Syrian golden hamster model. Furthermore, altered NNK metabolism and increased NNK-induced DNA damages were also observed in healthy subjects with CS and BQ histories compared to healthy subjects with CS histories. In conclusion, BQ containing

safrole induced tobacco-specific NNK metabolic activation resulting in higher NNK-induced genotoxicity. This study provides valuable insight into synergistic mechanisms of CS and BQ-induced OSCC.

PS P154 - 0947 Urine Metabolomics of a Standardised *Andrographis paniculata* Aqueous Extract in a Rat Model of Neuroinflammation

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In this study, the effect of pre-treatment with standardized *Andrographis paniculata* aqueous extracts (APAE) on rats urine metabolomic changes following Lipopolysaccharide (LPS)-induced neuroinflammation was evaluated based on proton-nuclear magnetic resonance (¹H-NMR) spectroscopy. Both principal component analysis and orthogonal Partial Least Square-Discriminant Analysis (OPLS-DA) revealed that LPS (1 mg/kg) induces metabolic alterations. LPS group showed increased urinary levels of oxoglutarate, creatinine, citrate, allantoin, lactate and hippurate. However, rats pre-treatment with APAE for 7 days prior to LPS administration prevented the upregulation of these molecules similar to the control group to confer neuroprotection. Our findings imply that metabolomic procedures based on NMR could be applicable to elucidate neuroinflammation via the biomarkers exploration. In addition, we proposed a mechanistic metabolic pathway for APAE suppression of neuroinflammation.

PS P155 - 0017 Epigenetic Mechanism of Hexavalent Chromium-Induced Cell Transformation, Cancer Stem Cell-Like Property, and Tumorigenesis

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Hexavalent chromium [Cr(VI)] is one of the most common environmental and occupational pollutants. Although Cr(VI) is a well-recognized Group I carcinogen causing lung cancer in humans, the mechanism of Cr(VI) carcinogenesis has not been clearly defined. It is generally considered that Cr(VI) works as a genotoxic carcinogen due to the fact that Cr(VI) undergoes a series of metabolic reductions inside cells to generate various reactive Cr metabolites and reactive oxygen species (ROS), producing a variety of genotoxic effects. On the other hand, studies showed that Cr(VI) exposure also causes nongenotoxic effects such as epigenetic changes, as evidenced by increased DNA methylation and dysregulated histone posttranslational modifications in Cr(VI)-exposed cells and Cr(VI) exposure-caused human lung cancer tissues. However, it remains to be determined whether Cr(VI)-caused epigenetic changes play an important role in Cr(VI) carcinogenesis. In this study, we found that chronic Cr(VI) exposure caused epigenetic dysregulations, as evidenced by the increased levels of histone H3 repressive methylation marks (H3K9me2 and H3K27me3) and the related histone-lysing methyltransferases (HMTases) in Cr(VI)-transformed cells and Cr(VI) exposure-caused human lung cancer tissues. Using pharmacological inhibitors to inactivate HMTases or shRNA knockdown of HMTases significantly decreased histone H3 repressive methylation marks and malignant phenotypes of Cr(VI)-transformed cells. Moreover, knockdown of HMTases in immortalized human bronchial epithelial cells significantly reduced chronic low dose of Cr(VI) exposure-induced cancer stem cell (CSC)-like property and cell transformation. Further mechanistic studies revealed that increased HMTase expression by chronic Cr(VI) exposure caused downregulation of the expression of microRNA-494 (miR-494). Knockdown the expression of miR-494 in Cr(VI)-transformed cells significantly decreased their transformed phenotypes. Furthermore, stably overexpressing miR-494 in immortalized human bronchial epithelial cells significantly reduced chronic Cr(VI) exposure-induced CSC-like property, cell malignant transformation, and tumorigenesis. Together, our findings suggest that chronic Cr(VI) exposure-caused epigenetic dysregulations contribute causally and significantly to Cr(VI)-induced CSC-like property, cell transformation, and tumorigenesis.

P156 - 0019 Arsenic and Benzo(a)pyrene Co-exposure Synergizes in Inducing Cancer Stem Cell-Like Property, Cell Malignant Transformation, and Lung Tumorigenesis in Mice

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Both arsenic and BaP are well-recognized human carcinogens causing lung cancer, and arsenic and BaP co-exposure is very common in humans. However, the combined effect and the underlying mechanism of arsenic and BaP co-exposure are poorly understood. Using cell culture and mouse models, we found that arsenic and BaP co-exposure acts synergistically in inducing cell transformation, as evidenced by forming more than an additive number of soft agar clones compared with arsenic or BaP exposure alone in BEAS-2B cells for 30 weeks. We also determined the combined effect of arsenic and BaP co-exposure in inducing cancer stem cell (CSC)-like property by using a well-established assay known as suspension culture sphere formation assay. It was found that cells transformed by arsenic or BaP exposure alone are capable of forming small numbers of spheres. However, cells transformed by arsenic and BaP co-exposure form more than an additive number of spheres compared with cells transformed by arsenic or BaP exposure alone, indicating that arsenic and BaP co-exposure also acts synergistically in inducing CSC-like property. Since CSCs or CSC-like cells are considered as tumor initiating cells, we first compared the tumorigenic effect of BEAS-2B cells transformed by arsenic, BaP alone, or arsenic and BaP co-exposure by subcutaneous inoculation of 0.25×10^6 cells into the right flank of 6-week-old nu/nu nude mice. It was found that 0 of 5 mice injected with BEAS-2B-Control cells, 0 of 5 mice injected with arsenic exposure alone-transformed cells, and 2 of 5 mice injected with BaP exposure alone-transformed cells develop tumors; however, all 5 mice injected with arsenic and BaP co-exposure transformed cells develop tumors. These results indicate that cells transformed by arsenic and BaP co-exposure have significantly stronger tumor-initiating capability than cells transformed by arsenic or BaP exposure alone. To further investigate whether arsenic and BaP co-exposure acts synergistically in inducing mouse lung tumors, male A/J mice were exposed to vehicle control, arsenic, BaP, or arsenic plus BaP for 34 weeks. No lung tumors were found in control and arsenic exposure alone mice. All mice with BaP exposure developed lung tumors. However, mice exposed to arsenic plus BaP developed significantly more and bigger lung tumors compared with mice exposed BaP alone. Together, these results demonstrate a synergism of arsenic and BaP co-exposure in inducing lung tumorigenesis. Further mechanistic studies revealed that arsenic and BaP co-exposure synergizes in causing epigenetics dysregulation.

P157 - 0042 Nε-carboxymethyl-lysine, a Major Member of Advanced Glycation End Products, Promotes Tumor Malignancy of Breast Cancers via Activation of RAGE/SIRT6 Axis *In Vitro* and *In Vivo*

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Recent population studies provide clues that poor outcomes in breast cancer (BC) patients are observed across diabetes mellitus. Advanced glycation end products (AGEs) may be as a causative factor of diabetic complications, and AGEs as a risk factor attributed to BC progression, malignancy, and intervention are poorly understood. Therefore, the aim of the present study is to investigate the role of Nε-carboxymethyl-lysine (CML), a major member of AGEs, in tumor malignancy of BC and the putative mechanism in *in vitro* and *in vivo* models. In an *in vitro* study, MDA-MB-231 human BC cells were treated with CML at various doses for 48 h. In an *in vivo* study, tumor formation was designed in the xenograft NOD/SCID mouse with streptozotocin (STZ)-induced diabetes model. The cell proliferation was analyzed by MTT assay. The stemness characteristics of cells were evaluated by sarsosphere formation assay. Protein expressions of culture cells and tissues from animals and patients were analyzed by Western blot and immunohistochemical (IHC) staining. CML significantly promoted cell proliferation and also induced sarsosphere formation in MDA-MB-231 cells with upregulation of ALDH1A1 and Nanog gene expressions, biomarkers of cancer stem cells. CML also induced protein expressions of stemness-related signals (ALDH1A1, CD44, and p-glycoprotein) through activation of RAGE/SIRT6 signaling pathway. Moreover, tumor tissues from STZ-induced diabetic NOD/SCID mice with xenotransplantation of MDA-MB-231 cells and BC patients with diabetes also exhibited activated protein expressions of AGEs, CML, ALDH1A1, CD44, RAGE, and SIRT6 compared with nondiabetic mice and pa-

tients with BC. CML induced tumor malignancy by upregulation of stemness characteristics-related signals via activating RAGE/SIRT6 signaling pathway, and hopes to provide possible remedy targets and effective adjuvant therapies of breast cancer under diabetic condition in the future.

P158 - 0119 Seed Oils of *Centranthus ruber* and *Valeriana officinalis* Contain Conjugated Linolenic Acids and Have a Cytotoxic Effect on Cancer Cells

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Polyunsaturated fatty acids, which have a conjugated double bond system, are expected to have anticancer effects. Natural seed oils of some plants (tung, karela, etc.) contain conjugated linolenic acids (CLNs), which are isomers of α-linolenic acid with a conjugated trienoic structure. CLN has an antitumor effect on human tumor cells. Discovering novel plants rich in CLN is important for cancer prevention. In this study, we focused on herb seed oils. Although the therapeutic effects of volatile components of herbs have been actively studied, there is little information about the other components. The purpose of this study is to discover novel herb seed oils containing CLN. The seeds of various herbs were homogenized and the total lipids were extracted from the homogenates according to the Bligh & Dyer procedure. UV spectra were measured in each extracted lipid to examine the presence of CLN. The extracted lipids that showed the characteristic spectrum for CLN were methylated with trimethylsilyldiazomethane and sodium methoxide/methanol and then analyzed by gas chromatography (GC) to determine the fatty acid composition. The structures of the unknown fatty acids were further analyzed using gas chromatography-mass spectrometry (GC-MS). The extracted lipids were fractionated to neutral lipids, glycolipids, and phospholipids by silica column chromatography. The fatty acid compositions in the obtained fractions were measured using gas chromatography. In addition, to examine cytotoxic effect of seed oils that contain CLNs, the survival rate of HeLa cells exposed to the seed oils was measured by using WST-8 method. The lipids extracted from *Centranthus ruber* and *Valeriana officinalis* seeds indicated the characteristic spectrum pattern of CLN. By the GC and GC-MS analysis, it is revealed that the lipid fraction extracted from *C. ruber* seeds contained CLNs such as α-eleostearic acid (α-ESA; 9c, 11t, 13t-18:3), β-eleostearic acid (β-ESA, 9t, 11t, 13t-18:3), and catalpic acid (9t, 11t, 13c-18:3). *V. officinalis* seeds contained α-ESA and β-ESA. These CLNs seemed to exist as a triacylglycerol, because most of them were detected in the neutral lipid fraction. HeLa cells showed significantly decreased survival when exposed to seed oils from *C. ruber* and *V. officinalis*. Besides, the survival was recovered by adding antioxidant substance. Therefore, it was revealed that the seed oils of *C. ruber* and *V. officinalis* contain CLNs and have cytotoxic effect to cancer cells via lipid peroxidation.

P159 - 0156 Inhibitory Effect of Sesamin on CYP1A1 and CYP1B1 Expression in Breast Cancer Cells via Prevention of Aryl Hydrocarbon Receptor

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Induction of cytochrome P450 (CYP) 1A1 and CYP1B1 by environmental xenobiotic chemicals or endogenous ligands through the activation of the aryl hydrocarbon receptor (AhR) has been implicated in a variety of cellular processes related to cancer, such as transformation and tumorigenesis. Sesamin, a lipid soluble lignan from sesame (*Sesamum indicum*), displays anticancer activities through an unknown mechanism. This study investigated the effects of the sesamin on expression of CYP1A1 and CYP1B1 in breast cancer cells. Sesamin downregulated the expression of CYP1A1 and CYP1B1 in MCF-7 and MDA-MB-231 breast cancer cells follow concentration- and time-dependent manner at the transcription and protein level. Besides, sesamin also inhibits the expression of CYP1A1 and CYP1B1 in MCF-7 breast cancer cells in TCDD-induced condition. Finally, sesamin blocks XRE luciferase activity and AhR nuclear translocation. These findings demonstrate that sesamin reduces CYP1A1 and CYP1B1 expression in breast cancer cells by downregulating AhR signaling. Sesamin would be able to act as a potential chemo-preventive agent against CYP1A1 and CYP1B1-mediated carcinogenesis and development of cancer.

P160 - 0196 Adaptive Response of Cadmium with Decreasing Concentrations to Apoptosis in Human Myelomonocytic Lymphoma U937 Cells and Its Molecular Mechanism

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Cadmium (Cd) is a known potential environmental hazard and has been categorized as a human carcinogen by the International Agency for Research on Cancer. The adaptive response of low concentrations of Cd to apoptosis and its underlying molecular mechanisms remain to be clarified. Therefore, we aimed to uncover the signaling alterations related to apoptosis with low concentrations of Cd in human myelomonocytic lymphoma U937 cells. Cell viability assay was performed by CCK-8 viability assay, while intracellular reactive oxygen species (ROS) and mitochondrial membrane potential (MMP) were detected using flow cytometry. Moreover, protein expressions were analyzed by western blotting. U937 cells were treated with 50 μ M cadmium chloride (CdCl_2) for 12 h, and significant induction of apoptosis, generation of ROS, activation of JNK, suppression of Mcl-1, and increases in Bim, Noxa, and tBid have occurred. Contrarily, 1 μ M CdCl_2 pretreatment altered the sensitivity of the cells to apoptosis with marked decrease in intracellular ROS and suppression of JNK. Pretreatment with low-dose Cd inhibited apoptosis, whereas high-dose had opposite effects. The underlying mechanism involved was inhibition of intracellular ROS, JNK suppression, and modulating the balance between the expression of Mcl-1 with its binding partners such as Bim, Noxa, and tBid. Cd-adapted cells developed resistance to high-dose Cd-induced apoptosis by altering the balance in apoptotic proteins, which reveal an important mechanism involved in the carcinogenesis following prolonged exposure to low doses of Cd.

P161 - 0210 Nrf2 Accumulation and PI3K / Akt Activation upon Exposure to Arsenate Bring about Autophagy Deficiency through Novel Signal Transduction Pathway in Human Keratinocyte Transformation

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Transcription nuclear factor erythroid 2-related factor (Nrf2), acting as a major regulator in cellular defense mechanisms against oxidative stress, is involved in the multiple physiological processes including autophagy. The association between Nrf2 accumulation and cancer progression has attracted much more attention recently. However, the precise mechanism of carcinogenesis remains largely unknown. The previous studies have suggested that persistent activation of Nrf2 induced by chronic exposure to arsenite is correlated to cell proliferation and cell death in the malignant transformation. In the present study, a human keratinocyte (HaCaT) cells transformation model was used to investigate the dynamic effects on Nrf2 and autophagy induced by arsenite chronic exposure as well as the internal mechanism. Herein, we showed the overexpression of Nrf2 and the activation of autophagy in arsenite-transformed HaCaT cells and suggested that excessive accumulation of Nrf2 could induce autophagy deficiency. Then a reduction of activity in autophagy initial phase and an inhibition of autophagosome in degradation were observed in HaCaT cells after chronic exposure to arsenite. Moreover, we indicated that chronic exposure to arsenite could activate PI3K-Akt pathway leading to the overexpression of Nrf2, which in turn activated of Bcl2-Bcln1 signaling and upregulated mTOR expression to inhibit autophagy in HaCaT cells. Those experimental results demonstrated that accumulation of Nrf2 could induce autophagy deficiency via Bcl2/Bcln1 and mTOR-mediated pathways by arsenite in human keratinocyte transformation.

P162 - 0215 CYP1B1 Induces XIAP Stability and Activity through Phosphorylation of PKC- ϵ in Human Breast Cancer Cells

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Cytochrome P450 1B1 (CYP1B1) is a key enzyme that catalyzes metabolism of 17 β -estradiol (E2) into catechol estrogens, such as 2-OH-E₂ and 4-OH-E₂. High level expression of CYP1B1 is frequently occurred in a variety of cancer cells and CYP1B1 may play an important role in human carcinogenesis. However, the detailed mechanisms of CYP1B1 action are still not clear. Here, we showed that CYP1B1 significantly affects expression of X-linked Inhibitor of apoptosis protein (XIAP), which is a key member of intrinsic inhibitor of apoptosis proteins. DMBA, a CYP1B1 inducer, increased XIAP level as well as CYP1B1, while TMS, an inhibitor of CYP1B1, downregulated XIAP protein level in MCF-7 cells. When cells were overexpressed with CYP1B1, induction of XIAP protein level was significant. But XIAP mRNA level was not affected by CYP1B1 expression. The estrogen metabolite 4-OH-E₂ generated by CYP1B1 also increased XIAP protein level, whereas 2-OH-E₂ did not show a significant effect. We found that 4-OH-E₂ prevents embelin-mediated caspase-3 activation. In addition, 4-OH-E₂ prevented XIAP degradation through the phosphorylation of XIAP at S87 site. Downregulation of p-PKC- ϵ (S729) level, which may increase phosphorylation of XIAP (S87) by PKC- ϵ siRNA was recovered by 4-OH-E₂ in MCF-7, MDA-MB-231, and HeLa cells. In summary, our study demonstrates that upregulation of PKC- ϵ and XIAP phosphorylation by CYP1B1 may inhibit apoptosis through regulating XIAP stability and activity in cancer cells.

P163 - 0264 Improving Formalin-Fixed Paraffin-Embedded (FFPE) Clinical Samples for Variant Calling and Mutant Detection

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Damage to nucleic acids during formalin fixation leads to latent sequencing artifacts and limits the use of archival FFPE tissue samples for genomic analyses. Previously, we showed that organocatalyst use during RNA isolation protocols improved FFPE RNA-sequencing data. The goal of the current study is to evaluate whether (a) organocatalyst treatment improves FFPE DNA-seq data, (b) RNA-seq variants can be used to filter out formalin-induced sequence artifacts in DNA, and (c) time in formalin alters these FFPE DNA-seq analyses. Here, we isolated RNA and DNA +/- organocatalyst from paired FFPE and frozen human renal and ovarian carcinoma specimens from the National Cancer Institute's Biospecimen Preanalytical Variables program (BPV). Sample collection/processing was prospectively designed in BPV to evaluate common pre-analytical variables. Paired tissue samples were either frozen and embedded in OCT or formalin-fixed for 12 or 72 hours (h) prior to paraffin embedding. Tumor types were microscopically confirmed by EPA's pathologist from adjacent tissue sections. Total RNA-seq library preparation required 4 to 6, 10- μ M thick, FFPE curls and at least 6+ for FFPE DNA where yields were 1.6- to 16.4-fold lower than FFPE RNA yields. Furthermore, 72 h formalin-fixed samples had ~1.8/4.5-fold less RNA/DNA, respectively compared to the 12 h formalin-fixed samples (4.7 $\pm$ 2.6/1.1 $\pm$ 0.9 μ g). Total RNA and DNA-exome sequencing were performed by Q2 solutions (Durham, NC). Use of the organocatalyst improved FFPE RNA yields, but not DNA. This suggests that incubation conditions ideal for reversing formaldehyde damage in FFPE RNA may not be the same for FFPE DNA. Preliminary analysis of the frozen versus FFPE DNA samples indicates that formalin-fixation results in significantly fewer SNPs being identified even with equivalent numbers of input reads. In general, the concordant SNPs identified in paired frozen and formalin-fixed DNA samples agreed on both the genotype and the homo- versus heterozygosity of calls. We are further investigating disagreement between the calls. With the growing importance of large-scale genomic analyses, this work will have important applications in mechanistic toxicology and precision medicine. *This abstract does not reflect EPA policy.*

P164 - 0355 Zearalenone Promotes Colon Cancer Cell Proliferation via GPER

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Colon cancer (CRC) is one of the leading cause of cancer related deaths worldwide and the second leading causes of death in Hong Kong. Over the past 5 year, the survival rate of CRC patient has doubled, however, more than half of the survivor only survived less than 5 years after the diagnosis. The statistics highlight the need for understanding agent that might be involved in the advancement in carcinogenesis. It is widely believed that environmental factor contributes an important role in colon cancer development. There has been a growing interest on the involvement of xenoestrogen. Zearalenone (ZEA) is a non-steroidal estrogenic mycotoxin produced by *Fusarium* fungi. It can competitively bind to estrogenic receptor (ER), due to its structural similarity to 17- β -estradiol. ZEA can be widely found in our diet and animal feeds. It enters human body mainly through oral intake, and has been reported as being linked to tumorigenesis in many hormone-sensitive cancer. However, the association between ZEA intake with colon cancer has received less attention. In this study we investigated the effects of ZEA on colon cancer cells and its underlying molecular mechanisms. Our results demonstrated that the effect of ZEA may contribute to the development of colon cancer by upregulating colon cancer related genes. We found in cell viability and proliferation assay that ZEA increases CRC proliferation in a dose-dependent manner by promoting the G1-to-S phase transition. The growth promoting effect was further demonstrated by the increased level of the proliferation-related cyclin D1 gene and protein in CRC cells. We investigated the involvement of hippo and MAPK/ERK1/2 pathways through the activation of GPER using qPCR and western blotting. Our preliminary result suggests it activates G-protein coupled estrogen receptors which trigger the MAPK/ERK1/2 pathway and decreasing phosphorylation of YAP1. Taken together, our results demonstrated how ZEA are likely to be one of the contributing factor of promoting colon tumorigenesis and subsequently worsen the outcome of colon cancer patients.

P165 - 0454 The Role of NF- κ B and AhR in Pb-Mediated Immune Toxic Effects on A549 Lung Cancer Cells

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Lung cancer is the second most widespread cancer and the first most common cause of cancer related death in the world. Air pollution increased the risk to 45% and responsible for 36% of deaths from lung cancer. Lead (Pb) is recognized as a first pollutant of the top six toxic air pollutants threatening human health and the second hazardous substance according to Agency for Toxic Substances and Disease Registry 2017. Pb exposure is associated with impairment of lung immune system and high incidences of lung cancer. Nuclear factor kappa B (NF- κ B) and aryl hydrocarbon receptor (AhR) signaling pathways are known to be expressed and play an important role in lung immune responses. The exact mechanism of Pb-induced pulmonary immunotoxicity not yet fully explored and there are very limited data regarding the impact of NF- κ B and AhR in Pb-modulated pulmonary immunotoxicity. Therefore, this study was established to investigate the mechanisms of Pb induced-immunotoxicity and the role of NF- κ B and AhR modulation in an *in vitro* human A549 lung cancer cell line. The treatment of human A549 lung cancer cell line with Pb (0, 25, 50, 100, 150 and 300 μ M) decrease the cell proliferation at high concentrations used by <70%. Therefore, Pb concentration 100 μ M was utilized in all subsequent experiments. The percentage of apoptotic cells and oxidative stress and inflammatory markers production were determined using flowcytometry. The potential effects of Pb on NF- κ B and AHR pathways in the lung cells at the mRNA and protein levels was determined using Real-Time PCR and Western blot analysis, respectively. Mechanistically, the direct role of NF- κ B and AHR was examined by using small interfering RNA (siRNA) technology to knock down the gene. The results show that treatment of human A549 lung cancer cells with Pb 100 μ M for 24 hours results in significant increased in the apoptotic cells and oxidative stress markers production. The mRNA levels of CYP1A1, iNOS, IL-10, TNF- α , TGF- α , and P-53 were significantly increased, whereas IL-6, IL-8, GST- α 1, CAT, EphX, and Bcl-2 were significantly decreased. The expression of NF- κ B and AHR were significantly increased at mRNA and protein levels. AhR and NF- κ B expression silencing by siRNA rescued the human A549 lung cancer cells from oxidative stress and apoptosis in response to Pb, suggesting that the induction of AhR and NF- κ B expression has a direct role in mediating Pb-induced immune toxic effects.

P166 - 0501 Pterostilbene Triggers Endoplasmic Reticulum Stress-Mediated Autophagy and Apoptosis in Human Hepatocellular Carcinoma Cells

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Hepatocellular carcinoma (HCC) is the one of the most common cancer worldwide and the leading cause of cancer-related death. Due to the poor response rate and severe side effect of current treatment, new effective therapeutic strategies are urgently needed. Pterostilbene (PT), a natural analog of resveratrol, is known to have diverse pharmacologic activities including antioxidant, anti-inflammation and anti-proliferation. However, the molecular mechanism underlying PT as an anti-HCC agent is not yet fully understood. Here we show that PT inhibited HCC cell proliferation without the induction of apoptosis. However, PT simultaneously induced autophagy and ER stress in HCC cells. Mechanistic investigations suggested that PT modulated the ER stress related autophagy through p-eIF2 α /ATF4/LC3 pathways. Enhancement in phosphorylation of eIF2 α leads to increased cytotoxicity due to increased induction of autophagy and autophagy induced apoptosis. *In vivo* xenograft analysis revealed that PT significantly reduced tumor growth in mice with SK-Hep-1 tumor xenograft. Taken together, our results provide new insights into the pivotal role of PT in ER stress induced autophagy and eIF2 α in autophagy induced apoptosis in HCC cells.

P167 - 0703 Bioactive Fractions of *Rhus trilobata* Induce Apoptosis on Colon Cancer Cells (CaCo-2) by Activation of Caspase-9

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Colorectal cancer has the highest incidence and mortality rates worldwide. Chemotherapies currently used have severe side effects, which has led to the search for new active compounds focusing attention on medicinal plants, considering them as a viable source of molecules with therapeutic potential. Several studies have shown that active components of medicinal plants can inhibit mechanisms of proliferation and induce cell death, contributing to inhibition of progression of carcinogenesis. The extract of *Rhus trilobata* (Rt), a plant that grows in Chihuahua state, can inhibit the proliferation of both CaCo-2 and SKOV-3 ovarian cancer cells, decreasing the number of viable cells by an apoptotic-dependent pathway. The aim of this work was to demonstrate the antineoplastic effect of bioactive fractions (BioF) of Rt on CaCo-2 cells and determine the apoptosis pathway responsible for cell death. Aqueous extract (AE) was carried out and fractionated by solid phase extraction on a BAKERBOND C-18 column working in reverse phase. Cell viability and IC₅₀ for BioF were calculated on CaCo-2 cells using the MTT assay. The antineoplastic potential was determined using the kit ApoTox-Glo to measure viability, cytotoxicity and, apoptosis. To determinate the caspase 8 and 9 activity the kits Caspase-Glo -8 and -9 were used according to the manufacturer's instructions. MTT assay showed that Rt BioF decreases cell viability at concentrations <15 μ g/mL. IC₅₀ of A2 was lower than fractions A3 and A4, with values of 4.72, 9.97 and 8.33 μ g/mL, respectively; those values below 10 μ g/mL confirmed the inhibitory effect of Rt on the proliferation of CaCo-2 cells. These results suggest that the compounds present in BioF can be considered as active compounds according to the National Cancer Institute of the USA. Antineoplastic analysis showed that BioF decreases cell proliferation by induction of apoptosis, increasing over 100% the number of apoptotic cells in the assay by A2 effect against no treatment control. BioF promoted the activity of caspase-9, which is related to the intrinsic pathway of apoptosis. For A2 and A3, caspase-9 activity was dependent on concentration, whereas A4 showed the same response to all concentrations tested. None of BioF promoted caspase-8 activation, which indicates that cell death is not due to the activation of the extrinsic pathway. These results support that Rt fractions decrease the number of cancer cells due to its proapoptotic ability mediated by intrinsic pathway. More studies are necessary to understand the mechanism of action of active components of Rt, which may be related to the modulation of proteins involved in intracellular signaling pathways.

P168 - 0718 lncRNA MALAT1 Mediates PM2.5 Organic Extract Induced-Cells Epithelial-Mesenchymal Transition via miR-204/ZEB1 Pathway in Lung Bronchial Epithelial Cells

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Extensive cohort studies have explored the hazards of PM2.5 to human respiratory healthy, however, the specific molecular mechanisms of PM2.5 carcinogenesis are poorly studied. Long non-coding RNA (lncRNA) is involved in various physio-pathological processes. In the present study, we investigated the effect of PM2.5 on epithelial-mesenchymal transition (EMT) in lung bronchial epithelial cells and the underlying mechanism mediated by lncRNAs. Organic extracts of PM2.5 from Shanghai were adopted to treat human bronchial epithelial cell lines (HBE and BEAS-2B), and we found that PM2.5 organic extract induced cells transformation and EMT. And increasing levels of metastasis associated lung adenocarcinoma transcript 1 (MALAT1), which mediated by NF- κ B, were involved in the induced epithelial-mesenchymal transition (EMT) process. Similar response was observed between both cell lines. In addition, lncRNA MALAT1 interacted with miR-204, and relieved the inhibitory effect of its target gene ZEB1, thereby contributing to EMT and malignant transformation. In sum, these findings indicate that NF- κ B transcriptionally regulated lncRNA MALAT1 to promote EMT by binding with miR-204 and releasing ZEB1. This study offers further understanding of the regulatory network of PM2.5 induced EMT and the potential health risks associated with PM2.5.

P169 - 0729 Modes of Action Underlying Development of Renal Tumors in Rats following Chronic Exposure to Tert Butyl Alcohol

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In the National Toxicology Program's (NTP) 2-year carcinogenicity bioassay of tert butyl alcohol (TBA), after standard sectioning of the kidneys there was a modest but non-statistical increase in renal tubule cell tumors (RTCT) in male rats exposed to TBA administered in drinking water. Recently the EPA issued a draft IRIS assessment of TBA based mainly on the NTP 2-year study, concluding that TBA exerted toxic effects for the rat kidney, citing transitional cell hyperplasia and suppurative inflammation as manifestations of that toxicity. The document also posited that there could be an undefined mode of action for the RTCTs. Standard kidney sections of all rats in the NTP 13-week and 2-year carcinogenicity bioassays (including representative tumor-bearing step sections) were histopathologically re-examined to determine the mode(s) of action underlying the development of RTCTs. The findings are listed as follows: (1) The renal tumors and preneoplastic hyperplasias included several amphophilic-vacuolar tubule proliferations, which are known to be spontaneous growths and not caused by chemical induction. (2) The re-evaluation emphasized alpha-2u-globulin nephropathy (a2u-gN) and exacerbated chronic progressive nephropathy (CPN) as the only pathologic processes operative in the TBA-exposed male rat kidneys. (3) All of the key sequential changes characterizing a2u-gN were present, including hyaline droplets of angular shape, increased cell proliferation in cortical tubules (measured by counting cortical tubule mitoses), granular casts at the junction of inner and outer stripes of outer medulla, and modest linear mineralization in renal papillary tubules. (4) Vesicular change of the renal papilla lining (transitional cell hyperplasia in the draft IRIS document), and sporadic tubulitis (suppurative inflammation), were components of advanced, exacerbated CPN. (5) Exacerbated CPN was also present in female rat kidneys, but not at a level sufficient for encouraging tumor formation or renal papilla changes. (6) It was concluded that the modes of action underlying renal tumor development in male rats after exposure to TBA involved a combination of a2u-gN and CPN exacerbation with no evidence to support involvement of a third mode of action.

P170 - 0793 Targeting ER Stress to Overcome Acquired Resistance to EGFR-TKIs in Non-small Lung Cancer

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Epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI) is a representative chemotherapy for non-small cell lung cancer (NSCLC), however, it will be soon confronted with acquired resistance to EGFR-TKIs. In this study, we established gefitinib or erlotinib-resistant NSCLC cell lines and tried to elucidate therapeutic strategy for overcoming acquired resistance to EGFR-TKIs utilizing bortezomib (BTZ), a proteasome inhibitor, focusing on induction of ER stress-mediated anti-tumor effects. Gefitinib- or erlotinib-resistant lung cancer cell lines (HCC827-GR, H1993-GR, H1993-ER and H1975) showed rapid cell growth, activation of mTOR and increased protein translation rate than their parental cell lines (HCC827 and H1993). More potent anti-proliferation effects and the enhanced expression of apoptosis related proteins (cleaved caspase-3 and cleaved PARP) were found in BTZ-treated EGFR-TKI resistant clones compared to their parental clones. Moreover, the resistant cell lines showed marked activation of canonical unfolded protein response (UPR) signaling pathways (ATF6, IRE1 and PERK) in response to BTZ, which subsequently resulted in the enhanced expression of UPR downstream regulators such as ATF4, CHOP, GRP78 and sXBP1. In addition, mRNA expression of ER stress-inducible downstream target genes (ASNS, ERDJ4 and p58IPK) was significantly increased only in EGFR-TKI resistant cell lines. Xenograft assay using BALB/C athymic nude mice engrafted with H1993-GR cells exhibited that intraperitoneal injection of BTZ significantly suppressed tumor growth. These results could provide a novel therapeutic approach to overcome EGFR-TKIs resistant NSCLC. This work was supported by the National Research Foundation (NRF) grant funded by Korean government (MSIP; Ministry of Science, ICT & Future Planning) (No. 2018R1A2B2003590).

P171 - 0801 Inhibition of Cell Migration and Metastasis of Prostate Cancer Cells by GV1001: Role of GnRHR/Gas/cAMP Pathway in Androgen Receptor/YAP1 Axis

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Gonadotropin-releasing hormone receptor (GnRHR) transmits its signal via two major G α -proteins, primarily G α_q and G α_i . However, the precise mechanism underlying the functions of Gas signal in prostate cancer cells is still unclear. We have previously identified that GV1001, a fragment of the human telomerase reverse transcriptase, functions as a biased GnRHR ligand to selectively stimulate the Gas/cAMP pathway. Here, we tried to reveal the potential mechanisms of which GV1001-stimulated Gas-cAMP signaling pathway reduce the migration and metastasis of prostate cancer (PCa) cells. GV1001 inhibited expression of epithelial-mesenchymal transition (EMT)-related genes and spheroid formation on ultra-low attachment plate. GV1001 suppressed *in vivo* spleen-liver metastasis of androgen receptor (AR)-positive LNCaP cells as well as cell migration *in vitro*. Inactivation or knockdown of AR promoted cell migration of LNCaP cells. GV1001 enhanced the phosphorylation of AR and transcription activity of androgen response element reporter gene through cAMP/protein kinase A pathway in LNCaP cells. Moreover, GV1001 increased phosphorylation of YAP1 and its ubiquitination, and subsequently decreased the levels of AR-YAP1 binding in the promoter region of CTGF. In contrast, both protein and mRNA levels of NKX3.1 known for tumor suppressor gene and AR-coregulator, were upregulated by GV1001 in LNCaP cells. YAP1 knockout using CRISPR/Cas9 significantly suppressed the migration ability of LNCaP cells, and GV1001 did not affect the cell migration of YAP1-deficient LNCaP cells. On the contrary, cell migration was more potentiated in LNCaP cells overexpressing YAP5SA, a constitutive active form of YAP1, which was not changed by GV1001 treatment. Overall, this study reveals an essential role of AR-YAP1 in the regulation of PCa cell migration, and provide an evidence that GV1001 could be a novel GnRHR ligand to inhibit metastasis of PCa via Gas/cAMP pathway. This work was supported by the National Research Foundation (NRF) grant funded by Korean government (MSIP; Ministry of Science, ICT & Future Planning) (No. 2018R1A2B2003590).

P172 - 0857 Definition of Biological Thresholds for the Identification and Classification of Tumorigenic Chemicals in the Rat Liver

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Chemicals induce liver cancer in rodents largely through well-characterized adverse outcome pathways (AOPs). In this study we defined biological thresholds for molecular initiating events (MIEs), downstream key events (KEs), and associated events (AEs) in liver cancer AOPs in short-term assays. We used data in the rat TG-GATES (TG) and DrugMatrix (DM) studies to measure MIEs (gene expression biomarkers for genotoxicity, cytotoxicity, AhR, CAR, ER, and PPAR α), associated KEs (cell hypertrophy/proliferation by liver to body weights (LV/BW)), and AEs (clinical chemistry measurements) across 77 and 86 chemicals, respectively that could be linked to doses with previously established effects on rat liver tumor induction. Thresholds were calculated by determination of maximum and minimum values for exposures that do not lead to liver cancer. For all of the biomarkers and 12 biomarker genes (2 for each biomarker), clear threshold values could be identified that were relatively consistent across training and test sets and between TG and DM datasets. Thresholds for LV/BW and some clinical chemistry endpoints could also be clearly identified. Thresholds for the biomarkers or the 12 genes were derived from a training set of 38 TG chemicals and could be used to identify rat liver tumorigens in a 37 chemical TG test set with 91% accuracy. Thresholds derived from the entire TG study were not predictive of liver tumorigens in the DM study (67-74% for the biomarkers and 62-65% for the genes). In contrast, thresholds derived from the entire DM study were very predictive in the TG study (85-95% for the biomarkers and 89-94% for the biomarker genes) and were most accurate when applied to test sets of 7d treatments for the biomarkers (94%). A training set of clinical chemistry and liver to body weight thresholds derived from 38 TG chemicals was found to be very predictive in a test set of 37 TG chemicals (91%) and was most predictive in the test set of 7d treatments (98%). The biological thresholds defined in this study could be used to predict tumorigenic potential in short-term studies as well as to determine the mode of action that would underlie the tumors. The information derived using this approach could be potentially used to argue against the need for a 2-year rat bioassay when the mode of action is deemed to be irrelevant to humans. *This abstract does not represent EPA policy.*

P173 - 0951 Goniiothalamine Induces Cytotoxicity and Reduces the Migration and Invasion of Human Glioma Cells

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Gliomas are the most common type of primary brain tumor that is very difficult to treat due to the highly invasive and proliferative nature of the cancer. In this study, we investigated the cytotoxic effects of goniiothalamine (GTN) on human glioma cell lines. Cytotoxicity assessment using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay showed that GTN was cytotoxic towards the gliomas. The cytotoxic effect was more potent toward LN-18 cells (Grade 4) as compared to 1321 N1 (Grade 2) and SW 1783 (Grade 3) with IC₅₀ of 21 μ M, 46 μ M and 66 μ M respectively. However, the anti-migration and anti-invasion effects induced by GTN were more potent toward 1321 N1 followed by SW 1783 and LN-18 cells as assessed by Boyden chamber Transwell migration assay kit and *in vitro* scratch assay. The GTN-induced anti-migration and anti-invasion effects is somewhat related to the decrease expression of aquaporin-1 (AQP-1) and matrix metalloproteinase-9 (MMP-9) in 1321 N1 and LN-18 cells. The flow cytometric measurement of reactive oxygen species (ROS) using Mitosox staining demonstrated an increase of ROS level in GTN-treated glioma cells. Interestingly, there was an increase expression of Finkel-Biskis-Reilly murine sarcoma virus (FBR-MuSV)-associated ubiquitously expressed gene (FAU) and NAD(P)H: Quinone Oxidoreductase 1 (NQO1) according to the grade of gliomas as measured based on protein expression and enzyme activity using immunoblotting and 2,6-dichlorophenolindophenol (DCPIP) assay, respectively. The upregulation of FAU and NQO1 suggests that these proteins play a role in the aggressiveness of the glioblastoma multiforme (GBM). Downregulation of FAU by siRNA in LN-18 cells increased the migration activity and reduction in the cells' sensitivity toward GTN-induced cytotoxicity. Notably, an upregulation of AQP-1 and NQO1 expression was observed in FAU-silence cells, which contributed to migration activity and cell resistance. Therefore, this study suggests the role of FAU and NQO1 in GTN-induced cytotoxicity as the novel target for the treatment of GBM.

P174 - 0055 Qualification of *In Vitro* Cardiomyocyte Cell Models for Prediction of Drug-Induced Toxicity Studies

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Detrimental effects upon the cardiac system are a major cause of attrition during drug development, withdrawal of marketed medicines, and development of heart failure in survivors of chronic therapies. Drug-induced cardiotoxicity is a consequence of effects upon a range of molecular pathways involved with either structural or functional attributes of the heart. Current *in vitro* methodologies for assessment of drug-induced cardiotoxicity involve either suboptimal screens involving noncardiac cell lines engineered to express individual cardiac ion channels, or primary animal cardiomyocytes and cardiac tissue with their limited utility for *in vitro* culture and clinical translation. The emergence of innovative technologies combined with the use of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM), with the ability to synchronously beat (contract) *in vitro*, opened up opportunities for improved identification of cardiotoxicity. However, the costly and complex maintenance techniques pose limitations in terms of widespread use. A possible solution to these limitations is the utilization of a panel of cardiac cell lines, with defined attributes for clinical translation, scope for molecular manipulation, and the beneficial capability for continuous and expandable *in vitro* growth. However, the advantages and limitations for these cell models for prediction and detection of the various perturbations of structural and functional cardiotoxicity are as yet unclear. The objective of this study was to characterize and profile the capabilities for toxicity prediction of different cardiac cell lines, specifically the immortalized human ventricular cardiomyocyte cell line—AC10, the immortalized murine atrial cell line—HL1, and the rat embryonic cardiomyoblast cell line—H9c2, relative to primary cardiomyocytes and ultimately hiPSC-CM. This study evaluated these cell systems using impedance based real-time analyses (xCELLigence RTCA) to address the ability of these cardiac cell models to detect both structural and functional drug-induced cardiotoxicity. Data are presented of the evaluation of these cell models against a panel of drugs with known cardiotoxic effects, identifying their advantages and limitations for screening of drug-induced cardiotoxicity.

P175 - 0079 Atherogenic Index of Plasma (AIP) and Its Association with Novel Predictive Circulating Molecular Biomarkers for Cardiovascular Illnesses in Mexican Women Exposed to PAHs

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Cardiovascular diseases (CVD) are the leading cause of morbidity and mortality worldwide. Nevertheless, according to the World Health Organization (WHO), a precise and quick identification of high-risk individuals is imperative to combat those illnesses. Additionally, developing countries need affordable alternatives to effectively prognosticate cardiovascular events. Therefore, the aim of this study was to evaluate well-established clinical predictive biomarkers in Mexican women exposed to polycyclic aromatic hydrocarbons (PAHs) to identify affordable and useful tools to predict cardiovascular events. A cross-sectional study was performed including 340 healthy women. Anthropometric (weight, height, waist circumference, among others) and clinical (systolic and diastolic blood pressure) measurements were acquired from all enrolled individuals. Also, a blood sample of each participant women was obtained to complete biochemical analyses (glucose, triglycerides, total cholesterol, LDL cholesterol, and HDL cholesterol), and serum asymmetric dimethylarginine (ADMA), and adipocyte-fatty acid binding protein (FABP4) determinations. With anthropometric, clinical, and biochemical determinations, atherogenic indices (Framingham risk score (FRS), Castelli's risk index (CRI), and atherogenic index of plasma (AIP)) were estimated. As exposure biomarker of PAHs, 1-hydroxypyrene (1-OHP) was quantified using a high-performance liquid chromatography (HPLC) technique (1.23 $\pm$ 1.4 μ mol/mol creatinine). A mean value of 6.5 $\pm$ 7.2 was calculated for Framingham risk score, 3.7 $\pm$ 1.3 for Castelli's risk index, and 0.12 $\pm$ 0.22 for the atherogenic index of plasma (AIP). Circulating mean ADMA and FABP4 levels found in assessed women were 0.68 $\pm$ 0.34 μ mol/l and 20.3 $\pm$ 16.6 ng/ml, respectively. Furthermore, strong positive associations ($p < 0.05$) between AIP and 1-OHP, serum ADMA, and FABP4 concentrations were detected after adjustment by traditional CVD risk factors. In conclusion, data found in this study suggest that AIP can be used as an affordable prognostic biomarker of cardiovascular events.

P176 - 0098 Nrf2/Keap1 Oxidative Stress-Responsive System Inhibits Neointimal Hyperplasia Response to Vascular Injury by Regulating Vascular Smooth Muscle Cell Migration and Apoptosis

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Abnormal increases in vascular smooth muscle cells (VSMCs) in the intimal region after vascular injury is a key event in developing neointimal hyperplasia followed by vascular occlusive diseases. To maintain vascular function, proliferation, apoptosis, and migration of VSMCs is tightly controlled during vascular remodeling. Reactive oxygen species (ROS) are important signaling mediators in VSMCs, whereas excess ROS-induced oxidative stress contributes to the development and progression of vascular diseases, such as atherosclerosis. Activation of the nuclear factor erythroid 2-related factor 2 (Nrf2)/Kelch-like ECH-associated protein 1 (Keap1) system is pivotal in cellular defense against oxidative stress by transcriptional upregulation of antioxidant proteins. This study aimed to elucidate the role of Nrf2/Keap1 system in increases in VSMCs and neointimal formation after vascular injury. In a vascular injury model, Nrf2 protein was highly expressed in VSMCs on the luminal side of the internal elastic lamina during the early stages (at 7–14 days) of neointimal expansion. In the VSMC proliferative phase (at 14 days), apoptotic (TUNEL-positive) cells were detected in both the developing neointimal and medial layers, which were the two layers composed of VSMCs expressing high Nrf2. In cultured VSMCs, platelet-derived growth factor (PDGF), a key growth factor to accelerate VSMC growth and vascular remodeling, promoted nuclear translocation of Nrf2, followed by the induction of target genes, including NAD(P)H:quinone oxidoreductase-1 (271%), heme oxygenase-1 (366%), and thioredoxin-1 (154%). Nrf2 depletion by small interfering RNA enhanced PDGF-promoted ROS production (150%) and persistently phosphorylated downstream extracellular signal-regulated kinase-1/2. Nrf2 depletion enhanced VSMC migration in response to PDGF (183%) and wound scratch (142%), but not proliferation. An increased expression of nuclear Nrf2 by Keap1 depletion induced features of apoptosis such as positive TUNEL staining and annexin V binding, which was inhibited by simultaneous Nrf2 depletion. *In vivo*, Nrf2-deficient mice showed fewer TUNEL-positive apoptotic cells in the neointimal and medial areas (60%) at 14 days and enhanced neointimal hyperplasia (I/M ratio: 159%) at 28 days after vascular injury. These results suggest that the Nrf2/Keap1 system regulates VSMC movement and apoptosis during vascular remodeling after injury. Our findings provide insight into the Nrf2/Keap1 system and recommend it as a potential therapeutic target to reduce the effects of postangioplasty restenosis and atherosclerosis, which are associated with oxidative stress.

P177 - 0125 Cardioprotective Effect of Gemigliptin on Cardiac Ischemia/Reperfusion and Spontaneously Hypertensive Rat Hearts

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Diabetes is associated with an increased risk of cardiovascular complications. Dipeptidyl peptidase-4 (DPP-IV) inhibitors are a class of medicine that lowered clinically to reduce high blood glucose levels widely used in clinics as an and for their anticancer agent. DPP-IV inhibitors have cardio-protective and cardioprotective effects in addition to their glucose-lowering effect in experimental studies. However, the effect of DPP-IV inhibitors Gemigliptin on the DPP-IV inhibitor gemigliptin on ischemia/reperfusion (I/R)-induced myocardial injury and hypertension is not explored. The present unknown. In this study aimed to assess, we assessed the effects and mechanisms of Gemigliptin on the rat model of myocardial I/R injury and spontaneously hypertensive rats. Gemigliptin spontaneous hypertension. Gemigliptin (20 and 100 mg/kg/d) or vehicle was administered intragastrically administered to Sprague-Dawley rats for 4 weeks before induction of I/R operation. Gemigliptin pretreatment conferred cardio-protective effects against. Gemigliptin exerted a cardioprotective effect on I/R injury by improving hemodynamic function and limiting infarction reducing infarct size. Moreover, receiving Gemigliptin administration of gemigliptin (0.03% and 0.15%) powder mixed to their food for 4 weeks administration reversed hypertrophy and improved diastolic function in spontaneously hypertensive rats. Taken together our study has identified We report here a novel function of DPP-IV inhibitory drug Gemigliptin prevention effect of the DPP-IV inhibitor gemigliptin on I/R injury and hypertensive hypertension.

P178 - 0133 Effect of Acrolein on Epigenetic Modifications of Rat's Vascular Smooth Muscle Cells

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Epigenetics is the study of changes in gene expression without modification of the underlying DNA sequence. Environmental pollutants are among the factors that can exacerbate disease conditions through epigenetic modifications. Acetylation and methylation of histone lysine are the most common type of modifications, with acetylation contributing to gene expression and methylation resulting in gene inactivation. Acrolein, an environmental pollutant and a major component of cigarette smoke, has been implicated in cardiovascular toxicity and several neurological disorders such as Alzheimer's disease. N-acetyl cysteine (NAC) has been reported to protect against acrolein-induced lipid peroxidation through stimulation of the production of glutathione. The goal of the present study was to investigate epigenetic modifications in rat's vascular smooth muscle cells (VSMCs) exposed to acrolein and evaluate the efficacy of NAC in prevention of these modification. Rat VSMCs were treated with 3 µg/ml of acrolein for 6 hours in the presence or absence of 0.2 mM NAC. At the end of the treatment, MTS assay was used to check cells' viability. To evaluate Free Radical Generation, level of 8-Isoprostane production was measured. Western blot analysis was used to determine changes in H3K9 tri-methylation and acetylation. Histone modification multiplex assay was used to evaluate additional Histone H3 modifications. Additionally, immunofluorescence staining was used to analyze cellular localization of H3K9 acetylation and methylation. Acrolein increased the level of 8-isoprostane by 52% compared with control group. Addition of NAC significantly decreased 8-isoprostane level (57%). Acrolein significantly modified different variants of histone H3 and addition of NAC significantly prevented these modifications. Acrolein significantly increased the expression of methyl K9 (266%) and acetyl K9 (66%) compared with control group. Addition of NAC significantly decreased the methyl K9 expression by 76% and acetyl K9 expression by 60%. Immunofluorescence staining further confirmed changes in acetylation and tri-methylation in acrolein-treated VSMCs with or without NAC. Based on our data we are concluding that epigenetic modification of H3 Histones may be involved in acrolein-induced toxicity, and the reversal of these modifications by NAC confirms the protective effects of NAC.

P179 - 0134 Exposure to Volatile Organic Compounds Depletes Circulating Angiogenic Cells

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The Global Burden of Disease study estimates that 4.2 million deaths are attributable to ambient air pollution, with an additional 2.8 million deaths due to household air pollution. Over 90% of pollutant mass consists of gases or vaporous compounds, such as volatile organic compounds (VOCs). VOCs are abundant in both outdoor and indoor air. Major sources of exposure include incomplete combustion of fossil fuels, automobile exhaust, tobacco smoke, paints, wood preservatives, cleansers, and disinfectants. High levels of VOCs are also present at various Superfund and Hazardous Waste Sites. Emerging data suggest that VOCs are associated with many adverse health outcomes; however, little is known about the effect of VOCs on cardiovascular disease (CVD). We hypothesized that VOC exposure prolongs endothelial injury by depleting circulating angiogenic cells (CACs). CACs have been found to be good markers of vascular function and are associated with numerous cardiovascular risk factors, as well as predictive of both cardiovascular events and mortality. Our cross-sectional study consisted of 236 nonsmokers with low to high CVD risk. VOC metabolites were measured in the urine by liquid chromatography-mass spectrometry, and 15 different subtypes of circulating CACs in peripheral blood were characterized and quantified by flow cytometry. A cumulative VOC exposure score was established to assess the effect of overall VOC burden on CACs. Associations between CACs and individual VOC metabolites as well as a cumulative VOC exposure score were examined using generalized linear models. Multipollutant models showed a negative association with urinary metabolites of acrylonitrile, acrolein, and xylene with CAC-4 cells (45+/31+/34+/133+), which was independent of other VOC metabolite levels. Additionally, single pollutant adjusted models showed negative associations with CAC-4 and 1,3-butadiene metabolite (-28% per IQR, 95% CI: -43%, -7.5%), and crotonaldehyde metabolite (-33% per IQR, 95% CI: -54%, -4%). Similar re-

sults were observed for CAC-14 cells (45+/34+/133+). Cumulative VOC exposure score showed a strong negative association with both CAC-4 and CAC-14, with a 51%, and 49% absolute change (per IQR), respectively, suggesting that total VOC exposure has a cumulative effect on CAC levels. Demographics such as sex and race, and preexisting health conditions including diabetes and hypertension, significantly modified xylene-associated CAC depletion. These findings suggest that VOC exposure could deplete CACs and thereby compromise endothelial repair.

PS P180 - 0262 Healthful Nutrition as a Modifier of Environmental Pollutant-Induced Inflammatory Diseases: Implications in Atherosclerosis

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Atherosclerosis and other CVDs are defined as inflammatory diseases, which suggests that multifactorial interactions linked to exacerbated disease pathology include pro-inflammatory chemical and non-chemical stressors. Many environmental pollutants or chemical stressors with pro-oxidant and pro-inflammatory properties include persistent organic pollutants, such as polychlorinated biphenyls (PCBs), that have an affinity for the aryl hydrocarbon receptor (AhR). AhR ligands lead to induction of P450s and free radical formation, followed by inflammation. For example, dioxin-like PCBs can increase endothelial cell dysfunction and activation (e.g., increased adhesion molecule expression and cytokine production). Recent data suggest that genetic and lifestyle factors are independently associated with susceptibility to CVD. Thus, potential biological interactions between chemical and non-chemical stressors and buffers will determine disease outcome. Our data suggest that nutrition, or the type of diet we eat, can modulate environmental insults. For example, fats/oils high in omega-6 fatty acids can act as pro-oxidative and pro-inflammatory stressors. In contrast, foods rich in omega-3 fatty acids and plant-derived polyphenols can act as non-chemical buffers. Many environmental pollutants and pro-atherosclerotic nutrients/diets can activate NF- κ B signaling leading to increased oxidative stress and inflammation, and many anti-inflammatory nutrients can decrease inflammation by activating Nrf2 signaling. Our data, including metabolomic profiling, suggest that the pathology of dioxin-like PCB-mediated inflammatory diseases is complex and may involve disturbances in the gut microbiota, liver, and vascular tissues. In particular, we linked PCB exposure with increased plasma levels of trimethylamine N-oxide (TMAO), a diet-derived metabolite formed through cross-talk between gut microbiota and hepatic oxidation and associated with risk of atherosclerosis. Of special interest are approaches of prevention/intervention to lower disease outcome linked to complex interactions of chemical stressors to modify CVD disease outcome. *Supported in part by NIEHS/NIH grant P42ES007380.*

PS P181 - 0410 NADPH Oxidase 1 Mediates Angiotensin II-Induced Calcium Signaling by Acting on Non-selective Cation Channels in Rat Vascular Smooth Muscle Cells

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Angiotensin II (Ang II) serves as the primary effector in renin-angiotensin system and increases vascular tone and blood pressure by inducing vascular contraction. The crucial event in vasoconstriction is the elevation of calcium (Ca^{2+}) in vascular smooth muscle cells (VSMC). In addition, Ang II is known to activate NADPH oxidase (NOX) and thereby produces reactive oxygen species (ROS) in VSMC. A growing number of evidences are suggesting the potential crosstalk between Ca^{2+} and ROS. Here, Ca^{2+} regulation by ROS was investigated in vascular smooth muscle Ang II signaling. Ang II-induced ROS production was largely mediated by NOX1 and NOX4 in rat VSMC. Such ROS production was biphasic; immediate, tiny generation followed by tardy, large production. ROS formed in first phase was superoxide derived from NOX1, which precedes Ca^{2+} increase. NOX1-mediated, rapid superoxide production was mediated by phosphoinositide 3-kinase (PI3K)-dependent signaling pathway but not by protein kinase C (PKC)-involved signal. Ang II-induced Ca^{2+} elevation was inhibited by NOX inhibitors and knockdown (KD) of NOX1 but not by NOX4 KD. Ang II-caused Ca^{2+} release from the intracellular store was not affected by NOX inhibitors or KD of NOX1, indicating NOX1 is required for extracellular Ca^{2+} influx. Indeed, NOX inhibitors attenuated non-selective cation current (I_{NSC}), but not L-type voltage-gated calcium current. In accordance with cell study, aorta isolated from NOX1-null mice exhibited impaired contractile response to Ang II, and

the rapid pressor response to Ang II was attenuated in NOX1-null mice. In conclusion, NOX1-derived superoxide mediates the vasoconstrictive effect of Ang II by positively regulating Ca^{2+} signal in VSMC. ROS and Ca^{2+} signals are not discrete events but work cooperatively for the full functions of Ang II.

PS P182 - 0412 A Mixture of Chloromethylisothiazolinone and Methylisothiazolinone Disturbs Vasomotor Function by Dysregulating Ca^{2+} and Zn^{2+} in Vascular Smooth Muscle Cells

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Chloromethylisothiazolinone (CMIT) and methylisothiazolinone (MIT) are active ingredients in a preservative Kathon CG which contains ca. 1.5% CMIT/MIT at a ratio of 3:1. They have ever misused for humidifier disinfectant products, which caused serious health problems in Korean population. Here, the effects of CMIT/MIT (Kathon CG) on vasomotor function and vascular smooth muscle cells were investigated to identify cardiovascular toxicity observed in epidemiological studies. In myograph experiments, CMIT/MIT did not alter basal tension of rat thoracic aorta up to 2.5 mg/mL and the pretreatment of 0.1–0.5 mg/mL CMIT/MIT did not affect acetylcholine-induced relaxation. However, 0.5–2.5 mg/mL CMIT/MIT attenuated contraction induced by phenylephrine or 5-hydroxytryptamine (5-HT), and 5–25 mg/mL of CMIT/MIT caused vasoconstriction directly. In primary cultured aortic smooth muscle cells (ASMC), CMIT/MIT suppressed 5-HT-evoked Ca^{2+} transient at 5–25 ng/mL, whereas it elevated intracellular Zn^{2+} and induced cellular contraction at 100–1,000 ng/mL. CMIT/MIT exhibited cytotoxicity at 0.5–2.5 mg/mL, and such cytotoxicity was attenuated by concomitant treatment of cell permeable zinc chelator TPEN. Taken together, CMIT/MIT causes dysregulation of Ca^{2+} and Zn^{2+} , and disturbs vasomotor function depending on exposure strength, which may ultimately contribute to the vascular toxicity.

PS P183 - 0438 Nicotinamide Adenine Dinucleotide Phosphate Oxidase (NOX) Upregulation by PM2.5 Alters Nitric Oxide Level through ADMA Increase in Human Umbilical Vein Endothelial Cell

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Particulate matter (PM), the main cause of invisible haze of air pollution, is an evident public health concern. Detrimental cardiovascular effects of air pollution have been concluded to impair heart function, exacerbate cardiopulmonary disease and contribute to cardiovascular mortality and morbidity increase by the American Heart Association. Accumulating literature, to date, involving potential mechanisms are mutually consistent but are relatively incomplete. Among them, oxidative stress takes up a large part. In the present study, PM2.5 stimulation was shown to increase reactive oxidative stress (ROS) generation, including increased superoxide anion in human umbilical vein endothelial cells (Ea.hy926). Moreover, PM2.5 treatment significantly altered nitric oxide (NO) production level with increased asymmetric dimethylarginine (ADMA) production, which were reversed by glutathione. ADMA, the endogenous inhibitor of NO synthases, has been reported related to markers of ROS, it is unclear that how ROS increase the ADMA levels during PM2.5 induced cardiovascular detrimental effects. Nicotinamide adenine dinucleotide phosphate oxidase (NOX) is an oxidase that can generate hydrogen peroxide to catalyze peroxidative reactions. Hereby we describe the role of NOX on endothelial nitric oxide synthase (eNOS) expression and activity in Ea.hy926. Here, we found that PM2.5 treatment induced upregulation of gene and protein expression level of NOX. Decreased NO production, eNOS expression and phosphorylated ser1177 eNOS expression level, together with increased the ADMA level were found under different PM2.5 treatment concentrations, which were attenuated by glutathione. Furthermore, the NOX inhibitor attenuated PM2.5-mediated eNOS uncoupling and the decrease of phosphorylated ser1177 eNOS expression. It also significantly changed ADMA level together with NO production. Therefore, the present study was designed to test the hypothesis that NOX is a source of ROS in Ea.hy926, enhances ADMA production, and subsequently induces endothelial dysfunction under conditions of PM2.5 exposure. *Acknowledgement: National Natural Science Foundation of China (No. 21876026; 31671034; 81673218; 81502783; 81573186).*

P184 - 0587 Ameliorative Effect of Gallic Acid against Sodium Fluoride-Induced Hypertension and Hepato-renal Complications in Wistar Rats

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Sodium fluoride (NaF) is used globally as an important element in the prevention of tooth decay, and it has proven its effectiveness in dental caries prevention when it is at low level in oral hygiene materials. However, detrimental effects associated with prolonged exposure to NaF include gastrointestinal disturbances, deranged cerebrovascular and cardio-renal integrity, initiation of anomalies in the chromosomes resulting in genetic damage, muscular wasting and mineralization of myocardium. Gallic acid (GA) derivatives occur naturally in plants and it has been reported to possess antioxidant properties against various disease conditions. Here, the ameliorative effect of GA on sodium fluoride (NaF) induced-hypertension and hepatotoxicity was studied. Four groups of seven rats each were used in this study. Group A received distilled water (control), group B received NaF (300 ppm), groups C and D received NaF + GA (60 mg/kg) and NaF + GA (120 mg/kg), respectively, through oral gavage, for 7 days. All data were expressed as mean $\pm$ standard deviation (SD). All results were analyzed using student's t-test and one-way ANOVA followed by Tukey's post-hoc test using Graph prism 5 and p-values < 0.05 considered to be statistically significant. The results showed that NaF alone significantly increased systolic, diastolic and mean arterial blood pressure. Administration of NaF also significantly raised both renal and hepatic hydrogen peroxide, malondialdehyde, protein carbonyl, serum myeloperoxidase and significantly decreased reduced glutathione, glutathione peroxidase, superoxide dismutase and glutathione-S-transferase when compared with the control and those co-administered with GA. Therefore, concomitant administration of Gallic acid with NaF reduced high blood pressure, markers of oxidative stress and improved antioxidant defence system. It also ameliorated structural changes in renal and hepatic tissues. Our findings thus suggest that Gallic acid is a potential drug candidate in the treatment of sodium fluoride induced hypertension and hepato-renal toxicity.

P185 - 0619 Gender Differences in Cardiac Remodeling Induced by a High-Fat Diet and Whole-Life, Low-Dose Cadmium Exposure

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Childhood obesity, which is prevalent in developed countries, is a metabolic risk factor for atherosclerotic vascular diseases, ventricular hypertrophy and heart failure. Cadmium (Cd) is a ubiquitous environmental toxic metal. The deleterious effects of increased serum Cd levels on the cardiovascular system are also well documented. However, the combined effects of whole-life, low-dose Cd exposure and high-fat diet (HFD) on cardiac remodeling remain elusive. Additionally, gender differences in this response have not been explored. The objectives of this study were to determine the effects of combined Cd and HFD exposure on cardiac remodeling and gender-specific differences in the response. C57BL/6J mice were exposed to the low-dose (L-Cd, 0.5 ppm) or high-dose (H-Cd, 5 ppm) Cd via drinking water from conception to sacrifice. After weaning, mice were randomized into two groups receiving HFD (42% kcals from fat) and low-fat diet (LFD, 13% kcals from fat), respectively. At 10 weeks, mice were sacrificed and hearts harvested for metal analysis, histopathology, protein (Western blot) and mRNA (RT-qPCR) measurements. Data were analyzed by ANOVA. H-Cd significantly increased Cd accumulation in heart of parental mice and their offspring. In the offspring, the Cd content in male and female heart was comparable, and HFD showed no added effects on Cd content. H-Cd increased cardiac metallothionein protein levels only in female mice, which was not affected by HFD. Histological analysis revealed that H-Cd combined with HFD induced cardiac hypertrophy and fibrosis in only female mice. This was further supported by high expression of ANP and COL1A1 protein, along with COL1A1, COL1A2 and COL3A1 mRNAs. Consistently, profibrotic markers; PAI-1, CTGF and FN; were also elevated in HFD/H-Cd group. Levels of the oxidative stress marker 3-NT significantly increased in the hearts of HFD-fed female mice, while Cd exposure showed no additional effects. Of all the antioxidant markers examined, CAT levels increased in HFD fed mice, independent of gender and Cd exposure. HFD combined with whole-life low-dose Cd exposure induces cardiac hypertrophy and fibrosis in female, but not in male mice. This response was independent of oxidative stress.

P186 - 0625 Dioxin-Like Pollutants Accelerate Atherosclerosis and Disrupt Gut Microbiota Homeostasis

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Exposure to dioxins can contribute to cardiovascular disease (CVD) risk through multiple mechanisms including the induction of chronic inflammation. Epidemiological studies have shown that leaner individuals may be more susceptible to the detrimental effects of lipophilic toxicants because they lack large fat depots that can accumulate and sequester these pollutants. This phenomenon complicates mechanistic studies of pollutant-accelerated atherosclerosis in experimental animal models where high-fat feeding usually leads to weight gain. Here, we investigated whether a model dioxin-like pollutant, PCB 126, could increase inflammation, modulate the gut microbiota, and accelerate atherosclerosis in Low density lipoprotein (Ldl) receptor deficient mice fed a low-fat atherogenic diet. We fed male Ldlr^{-/-} mice the Clinton/Cybulsky diet (10% kcal fat, 0.15% cholesterol) and exposed to PCB 126 on weeks 2 and 4 (2 doses of 1 μ mol/kg or vehicle gavage). To characterize this model, mice were humanely sacrificed up to 12 weeks post PCB gavage and effects of PCB 126 on markers of systemic inflammation, hepatic steatosis, modulation of gut microbiota, and atherosclerotic lesion size were examined. Mice exposed to PCB 126 exhibited significantly increased plasma inflammatory cytokine levels, increased accumulation of hepatic fatty acids, reduced cecum bacteria alpha diversity, significantly increased Firmicutes to Bacteroidetes bacteria ratio, and accelerated atherosclerotic lesion formation in the aortic root. Exposure to dioxin-like PCB 126 increases inflammation and accelerates atherosclerosis in mice. This low-fat atherogenic diet may provide a useful tool to study the mechanisms linking exposure to lipophilic pollutants to increased risk of CVD.

P187 - 0692 Role of Spliced XBP1 in Palmitic Acid-Induced Endothelial Lipotoxicity

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Lipotoxicity causes multiple organs damages, among them the injury of vascular endothelium is the critical factor to induce metabolic syndrome including cardiovascular disease and diabetes mellitus. With high prevalence and mortality, these diseases raised a significant public health concern. In previous works of literature, endoplasmic reticulum stress (ER stress) and unfolded protein responses (UPRs) are both connected to lipid metabolism and homeostasis. It is still uncertain how UPRs regulate lipotoxicity-induced cell damage under pathological pressure. The role of XBP1s, an adaptive UPR executive transcription factor, is also unknown. Therefore, this study aimed to investigate the potential regulatory mechanisms of XBP1s in endothelial function under the exposure of palmitic acid (PA), a typical pathological FFA. Our preliminary results showed that exposure of PA induced XBP1s expression at both mRNA and protein levels in human umbilical vein endothelial cells (HUVECs). Besides, as expected, PA revealed anti-angiogenic property as demonstrated with wound healing and tube formation assays. Treated cells with 4 μ 8C, an IRE1 RNase inhibitor which selectively inactivates XBP1 splicing, or lentivirus-shXBP1 remarkably slow cell migration and tube formation as well as the expression of eNOS protein. Taken together, we demonstrated that XBP1s exerts a protective role against lipotoxicity. Another pharmaceutical target for treatment or prevention may probably be inspired.

P188 - 0695 The Effect of PBDEs Exposure on Early Vascular Development in Zebrafish

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Polybrominated diphenyl ethers (PBDEs), the ubiquitous environmental pollutants, are known to exert toxic effects on development, such as neurodevelopment. Vascular system which supplies oxygen and nutrients, maintains homeostasis and protects from toxic agents, is crucial for tissue development, such as brain development. However, the adverse effects of PBDEs on vasculature and underlying mechanisms are largely unknown. In this study, we investigated the effects of BDE-47 and BDE-99 on vascular growth and function using zebrafish model. Zebrafish embryos were continuously exposed to waterborne BDE-47 or BDE-99 ranging from 0.05 to 0.6 μ M starting from 2 hours post fertilization (hpf). Fluorescent images of vasculatures in kdrl:eGFP

zebrafish were acquired using confocal microscope. Quantitative RT-PCR was applied to assess the mRNA levels. Vascular barrier function was evaluated by intracardiac injection of 10 kD dextran and the measurement of its leakage from blood vessels. By using a transgenic zebrafish line, we found that BDE-47 exposure did not lead to significant changes in general toxicity, while caused a dose-dependent impairment in early vascular development. The expression of angiogenesis-related genes, including VEGF and Angiopoietin signaling, was affected by BDE-47 exposure. In BDE-99 exposure experiments, we found that BDE-99 exposure did not cause significant change in general toxicity, and the modest changes were observed in vascular development and gene expression related to vascular growth. Strikingly, exposure to BDE-99 dramatically suppressed the expression of genes responsible for vascular barrier function and led to vascular leakage as compared with control group. These results suggest that exposure to low concentrations of PBDEs inhibits vascular development and impairs vascular barrier formation. Vascular barrier function is more sensitive to PBDEs exposure. Developing vasculature in zebrafish may serve as a powerful tool for the assessment of early exposure to PBDEs.

PS P189 - 0726 α -Bisabolol Attenuates NLRP3 Inflammasome Activation and Enhances Autophagic Flux in Isoproterenol-Induced Myocardial Infarction in Rats

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Inflammation plays a vital role in the pathology of myocardial infarction (MI). The activation of the NOD-like receptor (NLR) family, pyrin-domain containing 3 (NLRP3) inflammasome has now been proven to have a close link with inflammation during MI. The protective effects of α -bisabolol against inflammation associated with the NLRP3 inflammasome activation were evaluated in isoproterenol (ISO)-induced myocardial infarcted rats. Male albino Wistar rats were pre- and co-treated with an intraperitoneal injection of α -bisabolol (25 mg/kg body weight) daily for 10 days along with the subcutaneous injection of ISO (85 mg/kg body weight) at an interval of 24 h for two days (9th and 10th day). ISO-induced MI showed elevated activities/levels of serum cardiac marker enzymes and altered oxidative stress markers in the total heart and lysosomal fractions. Cardiac NLRP3 inflammasome was activated via TLR4-NF κ B/MAPK signaling and lysosomal dysfunction showed elevated myocardial interleukin-1 β (IL-1 β) and other proinflammatory cytokine concentrations in ISO-induced MI. Furthermore, the accumulation of inactive autophagic vacuoles has been witnessed even though the machinery of autophagic flux were seen to be upregulated in ISO-induced myocardium of rats. These abnormalities were abrogated by the treatment with α -bisabolol and interestingly its impact augments autophagic flux as evidenced by molecular and ultrastructural findings. Our study has clearly revealed that α -bisabolol abrogates inflammation by inhibiting NLRP3 inflammasome activation driven by oxidative stress-mediated TLR4-NF κ B/MAPK signaling and lysosomal dysfunction in ISO-induced rats. This could be due to its free radical scavenging activity and its potential on augmenting autophagic flux.

PS P190 - 0828 Effects of Arsenite and Fluoride Exposure on Cardiac Injury and Expression of Autophagy Related Genes in Rats

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Arsenic (As) and fluoride (F) are ubiquitous environmental pollutants in nature and associated with human cardiovascular diseases. However, the combined cardiotoxicity of these pollutants and their underlying mechanisms are still unclear. The aim of the present study was to investigate the toxic effects of subchronic exposure to high doses of As and/or F on cardiotoxic injury and how autophagy is involved in cardiotoxic processes induced by As and/or F exposure. The offspring rats were randomly divided into four groups: NaAsO₂ (50 mg/L), NaF (100 mg/L), NaF (100 mg/L) and NaAsO₂ (50 mg/L) in drinking water, or clean water, respectively, from gestation to puberty. The offspring rats were exposed to three months, the cardiac function indicators were tested; the histopathology of rat heart tissue was observed by light microscopy and electron microscopy; the activity of myocardial enzymes were detected in serum; the oxidative stress related indicators and ATPase enzyme were detected in tissues; the distribution and expression of autophagosomes in cardiac tissues were observed and detected by electron microscopy, qRT-PCR and Western-blot. The result revealed that As and/or F exposure altered the

parameters of cardiac function indicators and damaged the histopathology in rat heart tissue. Moreover, As and/or F exposure increased the activity of LDH in serum, and inhibited the activity of myocardial antioxidant enzymes and ATPase, and accumulated the levels of myocardial lipid peroxidation product MDA. Furthermore, As and/or F exposure increased the number of autophagosomes in cardiomyocytes and significantly increased the mRNA and protein expression of autophagy-related genes. These results suggest that sub-chronic exposure to high-dose As and/or F induced cardiotoxicity, changed cardiac function and cardiac pathology and ultrastructure, affected the level of myocardial injury markers. Autophagy is involved in the process of cardiac toxicity induced by As and/or F exposure.

PS P191 - 0956 Multiplex Proteomics Approaches to the Identification of Potential Predictive Biomarkers of Doxorubicin-Induced Clinical Cardiotoxicity

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Treatment of cancer patients with doxorubicin (DOX) can cause cumulative dose-dependent cardiotoxicity, yet currently there are no clinically validated biomarkers for the prediction of cardiotoxicity caused by DOX treatment. In this study, 70 breast cancer patients were enrolled under an IRB-approved protocol and treated with a combination of DOX and cyclophosphamide. Blood samples were collected prior to treatment (T0), and after the first (T1) and second (T2) cycles of DOX treatment. Cardiac function was assessed by a multi-gated acquisition (MUGA) scan before the start of DOX treatment and at completion of four cycles of chemotherapy. The SOMAscan proteomic platform was used to profile 1,305 proteins in each plasma sample and statistical analyses compared protein levels at the three time points in two groups of patients: the cardiotoxicity group (N=14, those patients with a reduction of >10% in left ventricular ejection fraction after the completion of treatment) and the normal function group (N=56, i.e., normal LVEF after the completion of treatment). Fourteen proteins showed different abundance levels in plasma at T0 in the cardiotoxicity group as compared to the normal group. In addition, six proteins at T1 and one protein at T2 were decreased in the cardiotoxicity group. Kaplan-Meier analysis, log-rank test, and Cox regression analysis indicated that plasma levels of these proteins were associated with increased rate and risk of cardiotoxicity. Among these proteins, lower baseline levels of myeloblastin without multiple analysis adjustment (HR=9.94, 95% CI 1.24-79.57, p=0.03) were found in the cardiotoxicity group. Multiplex immunoassays using the Olink technology not only confirmed the differential levels of myeloblastin, but also found that the protein delta homolog 1 (DLK-1) had higher baseline levels in the cardiotoxicity group, and the levels were associated with increased rate and risk of cardiotoxicity as revealed from the statistical analyses. These putative biomarkers may be useful for the prediction of DOX-induced cardiotoxicity, however further validation is needed using a larger cohort of patients.

PS P192 - 0310 Acute Accidental Poisoning in Children: The Situation in Cairo

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Childhood poisoning continues to challenge the diagnostic and treatment skills of the pediatrician. Generally, childhood poisoning can be attributed to suboptimal parental supervision and accessibility of products with poisoning potential. Also children are curious and explore their world with all their senses, including taste. As a result, the home and its surroundings can be a dangerous place when poisonous substances are inadvertently ingested. Acute poisoning carries high morbidity and mortality for children, especially in low economic countries. The aim of the study was to get an idea about pediatric acute exposure to poisons in Cairo, manner of exposure, type of poisons taken, and morbidity and mortality as a result of this exposure. Data were collected from the national institution of clinical and environmental toxicology, Cairo University. Patients below 14 years were selected over 6 months from January to June 2018, and their data as age, sex, poison type, manner of exposure whether accidental or intentional, and outcome were collected. Simple statistical analysis was done for the collected data. Around 802 children below 14 years seek medical treatment the national institution of clinical and environmental toxicology, Cairo University from January till June 2018; 48 (47%) were females

and 54 (53%) were males. About 24% were below 2 years, 51% were from 2 to 3 years old, while 19% were from above 3 to 5 years, and only 6% were above 5 years. All cases were accidental poisoning, except one case (13 years) that was intentional abuse of petroleum product. 49% accidentally ingested house used drugs as analgesics, contraceptive pills, etc. 43% ingested household products, with corrosives the commonest of them, while only 6% ingested rodenticides and one child intentionally inhaled large amount of petroleum product. All cases were managed according to treatment protocol and activated charcoal was given, more than 70% were discharged shortly or after short observational stay less than 6 hours. Only 30% or less were admitted and discharged after improvement with exception of one case (intentional abuser of petroleum product), who was in deep coma and needed more supportive care on mechanical ventilation and unfortunately died. Pediatric exposure to poisonous materials is very common in Cairo, especially in children under 5 years. Most of cases were accidental exposure and needed short observational stay.

PS P193 - 0339 The Nonclinical Innovation and Patient Safety Initiative (NIPSI): Supporting Human-Based Nonclinical Approaches through Global Advances in Regulation, Policy, Science, Education, and Training

Elizabeth Baker. Physicians Committee for Responsible Medicine, Washington, DC, The United States of America.

The integration of modern and state-of-the-art nonclinical approaches for assessing drug safety, efficacy and disease modeling are expected to improve drug development. Recent advances, such as the United States Food and Drug Administration's (FDA) Predictive Toxicology Roadmap and the Interagency Coordinating Committee on the Validation of Alternative Methods' (ICCVAM) Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States, represent a fundamental shift in how drugs will be developed and regulated. Regulators now state the need to move away from animal testing towards new approach methodologies that can be expected to be more predictive for humans, and have mapped their plans for doing so. The Nonclinical Innovation and Patient Safety Initiative (NIPSI) formed to foster stakeholder collaboration—among federal agencies, the private sector, and patient, health and research organizations—that supports innovative human-based science and addresses the factors that impede the uptake of modern, predictive approaches. This presentation describes recommendations for advancing the uptake of human-relevant nonclinical approaches published by NIPSI along with project updates. One project aims to change US Food and Drug Administration (FDA) and International Council on the Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) regulations from requiring “animal” data to “nonclinical,” which encompasses animal *in vivo* and human and animal-based *in vitro* and *in silico* approaches. Another project involves lobbying the US Congress to increase funding allocated for human-based science. The presentation will also include results from a recent review of NDAs for approved drugs that found no inclusion of “tissue chips” or “microphysiological systems” in US FDA submissions for approved drugs. Results will be updated for the meeting.

PS P194 - 0443 Cryotherapy as a Coadjuvant in Fabotherapy in Patients with Bites of Type Crotalos Serpents

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Cryotherapy is attributed benefits on inflammation, muscle spasm and metabolic demand, however its usefulness in the treatment against snake bite is unknown. The objective was to determine the usefulness of Cryotherapy as a coadjuvant to treatment with fabotherapy in patients with Crotalus snake bites in Mexico. Patients from the Clinical Toxicology service of the Hospital Juárez de México were included for an Ophidic accident with a Grade II poisoning according to the Christopher-Rodning classification, in the period from January 2015 to July 2017. One group received faboterapia (anti-vipmin) plus cryotherapy jointly and another group only fabotherapy. Forty patients were included, of which 87.5% were male (n = 35). 55% of the subjects were residents of Mexico City with a basic level of education (34% primary and 30% secondary). 77.5% presented the upper extremity as the site of injury, followed by the lower extremities (22.5%) and 42.5% presented previous manipulation of the lesion. No differences were found in the decrease of edema and pain between the groups. The group that received cryotherapy as an adjuvant to fabotherapy was found to have a decrease in days of hospital stay (p < 0.0001), as well as a lower number of fabotherapies used (p < 0.021). The use of cryotherapy in an

adequate way as a coadjuvant to specific fabotherapy improves the days of hospital stay and reduces the use of less fabotherapeutic dose. Operational definition: Cryotherapy is a modality of superficial thermotherapy that consists in the local or systemic application of cold for therapeutic purposes and in a controlled manner.

PS P195 - 0464 Cannabis and Driving Impairment: Is There a THC Equivalent to 0.08 BAC?

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Some form of cannabis, either medical and/or recreational, has been legalized in 30 US states, Canada, and parts of Mexico, as well as other locations globally, leaving law enforcement in these states and countries to address the challenges of establishing standards for driving under the influence of cannabis. Unlike alcohol, scientific data do not support the use of breath or blood level testing to identify individuals that are impaired. Part of the challenge is multiple products (marijuana flowers; cannabis-infused edibles; marijuana concentrates), and multiple exposure routes including oral, inhalation, and dermal. Furthermore, concentrations of the principal active psychoactive components, THC and CBD, can be variable depending on the plant source, or product formulation. Thus, pharmacokinetic considerations that can dictate exposure-response relationships for impairment are complex, and are often product-specific. Analysis of available studies on the relationship between blood levels of THC or CBD and endpoints related to impairment of driving skills due to alcohol (i.e., horizontal gaze nystagmus, balance and coordination), as well as other endpoints linked to impairment (e.g., reaction times, cognitive function, etc.), do not identify predictable exposure-response relationships. Consequently, a comparison of laws and standards across states in the US show that there is no single standard that has been adopted. This poster will provide an overview of the science and provide suggestions for areas of research that might provide reliable data for setting scientifically based standards for cannabis impairment.

PS P196 - 0476 Betel Nut Chewing Induced Acute Pulmonary Edema

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Betel nut chewing is still a great problem of public health in some countries or areas of Asia. In addition to the potential contribution of carcinogenesis, few cases of acute toxicity on cardiovascular system had been reported. Here, we report a rare case of sudden onset of pulmonary edema associated with betel nut chewing. A 44 y/o male with negative medical history suffered from chest tightness, short of breath and cold sweating while drinking and chewing betel nut with his friends. He was in comatous condition and respiratory failure and intubated immediately at a local hospital. Atropine 2mg were prescribed due to bradycardia. After initial assessment, he was transferred to our hospital. In our hospital, physical examination revealed that blood pressure was 196/127 mmHg, regular heart rate 86/min, deep coma, diffused rales and rhonchi with bronchorrhea, dilated pupils and warm skin. Pulmonary edema was noted on chest X-ray. He was admitted to intensive care unit for nitroglycerin therapy. He woke up in the second day with near normal CXR picture. He was hospitalized for 10 days due to complication of aspiration pneumonia. Alkaloids, such as arecoline or arecaidine in betel nut might play the key role in this event. Cytokine condition had been noted to associate with asthma developing in betel nut chewing. More investigations are needed to reveal the exact mechanisms of betel nut chewing in the future.

PS P197 - 0517 Sex Differences of 5-Fluorouracil-Induced Adverse Drug Reactions in Colorectal Cancer

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Sex-related incidence and outcomes were reported in various cancers, including colorectal cancer. 5-fluorouracil (5-FU) is widely used as an essential chemotherapeutic agent for colorectal cancer. However, sex-based differences in 5-FU toxicity have yet to be reported in human cancer cell lines and xenograft mouse models to date. Here, we investigated, for the first time, sex-based dif-

ferences in 5-FU toxicity using human colon cancer cell lines, xenograft mouse models, and Korean patients' data. Female-derived colon cancer cell lines exhibited greater 5-FU-induced cytotoxicity than male-derived colon cancer cell lines. We established two xenograft mouse models: one with a male-derived human colon cancer cell line injected into male mice (a male-xenograft model) and another involving a female-derived human colon cancer cell line injected into female mice (a female xenograft model). Treatment with 5-FU inhibited tumor growth and led to hematological toxicity in a female xenograft model more potently than in a male xenograft model. We analyzed the data obtained from Korean patients with colorectal cancer to examine sex differences in adverse drug reactions caused by 5-FU. Korean female patients with colorectal cancer who received 5-FU chemotherapy experienced more frequent adverse drug reactions including alopecia and leukopenia than male patients. Taken together, we demonstrated that female may be associated with increased risk of toxicity to 5-FU treatment in colorectal cancer based on *in vitro* and *in vivo* investigations and clinical data analysis. Our study suggests sex as an important clinical factor, which predicts induction of toxicity related to 5-FU treatment.

P198 - 0626 Utility of Purified Swine Hemoglobin in the Therapeutic Approach of Cutaneous Injury by Loxoscelism: Pilot Study of Controlled Clinical Trial

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Poisonous arthropod accidents in Mexico are a major public health problem with a report of up to 3000 thousand cases per year. The loxoscelism is an accidental poisoning caused by the bite of the spider of the genus *Loxosceles* sp. in which there is a diverse spectrum of clinical manifestations ranging from local involvement with deep and extensive skin necrosis, to a systemic picture with multi-organ dysfunction. The objective was to evaluate the therapeutic response obtained with the use of purified porcine hemoglobin in the approach of skin lesions in the patient with loxoscelism. A pilot study of a controlled clinical trial was conducted. In the period of 24 months (December 2015–December 2017) in the Clinical Toxicology service of the Juárez Hospital of Mexico where all patients with the clinical diagnosis of cutaneous loxoscelism. Two groups were established: in the first group, 10 patients in whom highly purified porcine hemoglobin was used as a therapeutic and in the second group a colloidal patch was used. 20 patients were included. 60% corresponded to the female gender. 30% had a comorbidity prior to loxoscelism of importance for the recovery of the wound, more than 95% of the population went to hospital care after 48 hours, 50% of the patients came from Mexico City. The approximate average of the attention time was 110.40 hours. The anatomical location that predominated was in the lower extremities in 75% of the cases. It was observed that 60% of the poisonings occurred in places outside the home. In 50% of the cases there was manipulation of the lesions before hospitalization. In 50% of the patients, immunomodulatory treatment was started from the beginning, 20% with initial surgical treatment and less than 20% with dual therapy. 85% of the patients required a surgical treatment program once the wound had been delimited. Only 35% had wound infection during the treatment. Finally, a statistical significance was shown in the percentage of wound closure in patients with application of purified hemoglobin, presenting a $P < 0.0001$ in the third consultation and remaining until the sixth consultation and discharge of the patient compared to patients in which I apply a coloidal patch. The use of highly purified porcine hemoglobin in patients with cutaneous lesions due to loxoscelism can be considered as a therapeutic option attached to the support management since it showed a progressive decrease in the percentage of extension of the cutaneous lesions in a shorter time compared to other granulation treatments.

P199 - 0641 Understanding the Types and Sources of Online Information Used by US Poison Center Staff

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United States (US) Poison Center (PC) staff manage calls from consumers, healthcare professionals, and others using information from a variety of in-house and online resources. These include published proprietary databases, information from household product manufacturers, US National Library of Medicine (NLM) databases, and in-house authored protocols and guidelines. However, information about which databases are most used by PCs is limited.

Our information gathering included interactions with PC staff supplemented by online searches. The focus was on US PCs but could be extended to non-US PC staff. We found that all 55 US PCs are required to use the POISINDEX (POISINDEX System (electronic version)) from IBM Watson Health so call product and exposure data can be uniformly uploaded in near real-time to the National Poison Data System (NPDS). NPDS is maintained by the American Association of Poison Control Centers. Another resource used by PCs is the ToxEd Clinical Toxicology Poisoning and Drug Overdose Reference, a component of the Gold Standard ToxEd database from Elsevier/Gold Standard. This is an electronic clinical toxicology reference with point-of-care and product information. UpToDate from Wolters Kluwer Health, a point-of-care evidence-based medical resource online software system is also used. Further, PC staff use information (safety data sheets, product labeling, and web site content) from a variety of household product drug (OTC and prescription), and pesticide manufacturers. PCs access online databases from CDC (diseases and toxins), US FDA (drugs), various botanical databases, and US EPA (pesticides). In addition, NLM's TOXNET suite of databases are used. These include the PubMed and TOXLINE literature databases, Hazardous Substances Data Bank (HSDB), LactMed drugs and lactation database, and the Household Products Database. NLM's ToxTutor, a self-paced tutorial covering key principles of toxicology and related topics is an additional resource. Analyzing available on-line toxicology information supports NLM's recent re-envisioning efforts to maintain the relevancy and value of its toxicology and other information resources, and is aligned with the direction of NLM's 2017–2027 Strategic Plan. This includes possible development of a "one stop shop" (or "database of databases") portal to NLM and non-NLM resources, including those useful to PCs and others.

P200 - 0663 The Latest (2019) Content and Plans for the Chemical Hazards Emergency Medical Management (CHEMM) Resource

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The goals of the Chemical Hazards Emergency Medical Management (CHEMM) resource are to (a) enable first responders, first receivers, other healthcare providers, and planners to plan for, respond to, recover from, and mitigate the effects of mass-casualty incidents involving chemicals, and (b) provide a comprehensive, user-friendly resource that is available online, can be downloaded, and is part of the content in the (US) National Library of Medicine's (NLM's) Wireless Information System for Emergency Responders (WISER) iOS and Android apps. CHEMM is produced by the US Department of Health and Human Services, Office of the Assistant Secretary for Preparedness and Response in cooperation with the US National Institutes of Health's National Library of Medicine and many medical, emergency response, toxicology, and other subject matter experts. The first version of CHEMM was released in 2011 after development began in mid-2008, and its content has attracted US and international interest. The most recent content for CHEMM was included in 2019. Examples of the newer content are (a) addition of the second edition of the Primary Response Incident Scene Management (PRISM) reports providing authoritative, evidence-based guidance on mass casualty disrobe and decontamination during a chemical incident, and (b) development of decision-aiding tool based on PRISM called the Algorithm Suggesting Proportionate Incident Response Engagement (PRISM) that can be used for known and unknown chemicals. Other newer content included (a) an expansion of the toxidromes in the CHEMM Intelligent Syndromes Tool (CHEMM-IST) from the original four to seven, (b) a section entitled "In the First Minutes," (c) enhanced content in CHEMM's Information for pre-hospital management and for emergency department/hospital management. Other new content, plans for enhancement of CHEMM, and examples of how CHEMM and CHEMM-IST can be used for training and in response to an incident will be noted.

P201 - 0670 Serum Albumin Binding of Synthetic Cannabinoids and Other Drugs and Dyes Is Detectable by Agarose Gel Electrophoresis and Spectroscopic Techniques

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Albumin is a highly soluble and abundant plasma protein with at least 17 binding sites for many structurally diverse compounds. Albumin binding of drugs and other compounds can affect their plasma concentrations, distribution in

the body and bioavailability and this may impact their toxicity. Synthetic cannabinoids are drugs of abuse that bind and activate cannabinoid receptor integral membrane proteins in the nervous system and peripheral tissues. These cannabinoids include over 200 different compounds in different structural classes. Synthetic cannabinoids abuse occurs worldwide and has been associated with serious side effects. There is limited knowledge on factors influencing their toxicity. There are no previous studies on the interactions of synthetic cannabinoids with serum albumin. It is hypothesized that synthetic cannabinoids will bind to albumin because they contain multiple aromatic rings and are of similar size to molecules reported to bind albumin and because of the versatile nature of albumin binding. This hypothesis was tested by the novel use of agarose gel electrophoresis and by uv-visible and fluorescence spectroscopy to detect changes produced by binding of the synthetic cannabinoids Bay 59-4731 and JWH-015 to albumin. As controls, different dyes drugs and other compounds were assessed for albumin binding. It was found that complexes of both human and bovine albumins with Bay 59-4731 and JWH-015 could be detected by virtue of the fluorescence of these compounds under ultraviolet light after electrophoresis. Similar complexes were observed for fluorescent and/or colored substances such as Nile red, eosin, Bromophenol blue, coumarin, Methyl red and the drug timolol. The colors of eosin, bromophenol blue and Nile red changed in the presence of albumin indicating an influence of albumin on the spectral properties of small compounds. Ultraviolet-visible and fluorescence spectroscopy supported the occurrence of the binding of compounds to albumin. In conclusion, the binding of synthetic cannabinoids to serum albumin was observed for the first time. It was also shown that agarose gel electrophoresis can be used in a novel fashion to detect the interaction of certain colored or fluorescent compounds with albumin.

PS P202 - 0781 The Incidence, Associated Factors, and Clinical Impact of Hyperamylasemia in Self-Poisoning Patients

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This study was conducted to investigate the incidence, associated factors, and clinical impact of hyperamylasemia in self-poisoning patients. This study was based on a toxicology case registry of patients treated from 2009 to 2013 at a tertiary care university hospital. We retrospectively investigated the demographics, clinical variables, laboratory variables, and intoxicants. Hyperamylasemia was defined as an elevation in serum amylase level to above the upper normal limit within 24 hours after admission. We analyzed the predisposing factors and clinical outcomes of patients in the hyperamylasemia group. Hyperamylasemia was identified in 49 (13.3%) of the 369 patients. Using multivariate logistic regression, the odds ratios for HA were 3.384 (95% confidence interval, 1.142-8.013, $p=0.014$), 3.261 (95% confidence interval, 1.163-9.143, $p=0.025$), and 0.351 (95% confidence interval, 0.154-0.802, $p=0.013$) for pesticides, multi-drug use, and sedatives, respectively. In the hyperamylasemia group, the peak amylase levels during 72 hours were correlated with the peak lipase levels ($r=0.469$, $p=0.002$) and peak aspartate aminotransferase levels ($r=0.352$, $p=0.013$). Finally, none of these patients had confirmed acute pancreatitis. Hyperamylasemia occurred rarely in these self-poisoning patients, and pesticide and multi-drug use were independent predictors of hyperamylasemia. Peak amylase levels were correlated with the peak lipase and aspartate aminotransferase levels.

PS P203 - 0804 Mechanism of miR-27a-5p Regulating AA-Induced Apoptosis Induced by Oxidative Stress

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Acrylamide (AA) is a neurotoxic and potentially carcinogenic contaminant, usually generated during food processing. Studies indicated that AA-induced oxidative stress is closely related to the diverse toxicity of AA. However, the details of toxic mechanism of AA are still obscure. MicroRNAs (miRNAs) is a kind of endogenous, non-coding, small RNAs, the expressions of which could be modulated by exogenous compounds. Our former research suggested miR-27a-5p increased induced by AA in most tissues of SD rat. In this study, ccdc88c was identified as target gene of miR-27a-5p by dual luciferase reporter system. In liver of AA-induced SD rats, miR-27a-5p inhibited the expression of CCDC88C in protein level, and also changed several oxidative stress related protein level, like JNK and Akt. Moreover, in normal rat liver cells (IAR20), over-expressed

miR-27a-5p induced ROS accumulation and apoptosis via suppressing ccdc88c. Therefore, we suggest miR-27a-5p-CCDC88C-JNK axis may play a key role in the regulation of AA induced ROS accumulation and apoptosis.

PS P204 - 0816 Safety Assessment of a New CBI Class of DNA Alkylators

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CyclopropaBenzIndolones (CBIs) are a potent class of DNA alkylating agents, making them attractive as anti-cancer agents. To increase the localization of these potent drugs to tumor sites and to reduce potential side effects associated with systemic administration, CBI alkylators were conjugated, via a non-cleavable linker, to antibodies that target various antigens on tumors for indications with high unmet need. A streamlined toxicity testing paradigm was utilized to allow for parallel evaluation of toxicities and therapeutic index (TI) of multiple drugs and drug-linkers with minimal number of animals used (3Rs principle). Rats were used as a screening species for tolerability assessment of CBI-ADCs and candidates that demonstrated a satisfactory acute toxicity profile and indication of adequate TI progress into toxicity testing in cynomolgus monkeys. This abstract focuses on the preclinical toxicity profiles identified for CBI-ADCs in rats and monkeys. In rats, the primary target organs of CBI-ADCs administered 1-2 weekly doses (QW x 1-2) included bone marrow, thymus, and reproductive organs. In cynomolgus monkeys, the primary target organs of CBI-ADCs include bone marrow, thymus, lymph node, spleen, kidney, and female reproductive organs in repeat-dose studies (Q3W x 4 doses). An additional single-dose study in monkeys with an extended drug-free recovery period was conducted to further characterize PK and reversibility of toxicity findings. Interestingly and unexpectedly, an irreversible kidney lesion of interstitial fibrosis, previously not observed in the repeat-dose monkey studies, was observed in this study. In both dosing scenarios, these pathologic changes were not associated with any significant alterations in clinical chemistry indicative of renal dysfunction. While the myelosuppression observed with the CBI DNA alkylators are consistent with those observed an analogue (adzelesin) already in the clinic, we believe that the renal toxicity observed in our studies is a novel finding. These findings were present in multiple CBI drug/drug-linker combinations, regardless of the target antigen used. Due to the current lack of understanding of the mechanisms underlying this irreversible kidney lesion, the lack of clinical monitorability and manageability associated with renal fibrosis, and the failure of candidates to achieve a meaningful TI, the CBI class of ADCs were discontinued from further clinical development.

PS P205 - 0876 Determinants of In Vitro Cross Talk between cAMP and Sirtuins

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Mitochondria are dynamic organelles with a central role in energy metabolism, as well as calcium homeostasis, signaling and overall cell viability. Consequently, mitochondrial dysfunction in human pathologies such as cancer, inflammation, age related, and metabolic diseases make mitochondrial signaling systems attractive candidates for treatment of these diseases. Sirtuins, are lysine deacylases that play a central role in mitochondrial function, metabolic regulation, and aging. Indeed, efficient mitochondria and adaptive changes in oxidative metabolism were shown to be coordinated by signaling pathways, activated by physiological stimuli, controlling an intense cross talk between nucleus and mitochondrial genomes. In this study we used different cell lines, SIRT3^{-/-} mouse embryonic fibroblasts (MEFs), SIRT1^{-/-} MEFs, as well as wild-type MEFs as a control cells. Cells were incubated with novel sirtuin activators and a sAC inhibitor and mitochondrial function was evaluated. As a result, we observed an improvement of mitochondrial function in SIRT3^{-/-} MEFs and SIRT1^{-/-} MEFs by observing a dose- and time-dependent increase in mitochondrial membrane potential. Moreover, no changes were observed for reactive oxidative species formation, but we observed an increase in nuclear-encoded mitochondrial genes. From the molecular to the physiological level, we are starting to understand the cross talk between two major signaling systems in two cell compartments. Pharmacological or alternative therapies offer promising approaches to target and manipulate these systems leading to the maintenance of mitochondrial content and function, and ultimately prevention of cellular dysfunction. *Acknowledgements: This*

work was financed by the European Regional Development Fund (ERDF), through the Centro 2020 Regional Operational Programme: project CENTRO-01-0145-FEDER-000012-HealthyAging2020, the Portugal 2020 - Operational Programme for Competitiveness and Internationalisation, and the Portuguese national funds via FCT - Fundação para a Ciência e a Tecnologia, I.P.: project POCI-01-0145-FEDER-016770 (PTDC/BIM-MEC/6911/2014), as well as by UID/NEU/04539/2013 (CNC.IBILI Consortium strategic project). JAA is recipient of a scholarship from FCT (PD/BD/114173/2016).

PS P206 - 0952 Zinc Encephalopathy in Adult Related to Recent Tattoo (Clinical Case Report)

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Clinical case of a young man, previously healthy, professional member of a health institution, who presents symptoms of headache, commitment of the general state, feverish for 5 days, and during his shift at the emergency unit, presents loss of consciousness and seizures. In his epidemiologic record, there is no direct contact with infectious patients outside his working area, and there is the antecedent of getting a large tattoo in his right arm 1 day before the initial symptoms. He's admitted in the medical centre as encephalopathy in study, viral or chemical compounds as the origin. The treatment starts with benzodiazepines as seizure control, and toxicological studies are requested. The results show absence of abuse drugs, and presence of paracetamol in high therapeutic range (46.2 µg/mL), lead (0.8 µg/dL), è-ALA in urine (0.31 mg/dL), cadmium (blood: 0.46 µg/L; urine: 0.16 µg/L) and zinc (blood: 719.4 µg/dL; urine: 1,827 µg/24h). High levels of zinc are detected in blood and urine, in values exceeding 10 times for blood and 4 times for urine. Due to the initial finding of discretely high levels of paracetamol, and considering the suspicion of heavy metals, the patient received N-Acetylcysteine for 5 days in doses of 9 grams/day (in 3 portions), complemented with anticonvulsants and general support measures. On the 5th day, he is discharged without sequels and with normal physical examination. Zinc levels in blood and urine are controlled at 45 days post discharge and were in normal range.

PS P207 - 0149 Tributyltin Decreases the Expression of Nuclear Respiratory Factor-1 via Epigenetic Changes

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Organotin compounds, such as tributyltin chloride (TBT), have been widely used as antifouling paint for marine vessels. Due to an ability to disrupt endocrine potency, the use of TBT in antifouling paint has been restricted in 1980s. However, TBT has continued to be detected in marine sediments even now. We previously reported that exposure to TBT inhibits DNA binding activity and expression of nuclear respiratory factor-1 (NRF-1), leading to neurotoxicity. NRF-1 is a transcription factor that regulates genes related to mitochondrial respiration. In this paper, we examined TBT-induced hypermethylation of DNA and investigated whether TBT decreases NRF-1 expression via DNA hypermethylation in SH-SY5Y human neuroblastoma cells. Exposure to 300 nM TBT for 72 h decreases NRF-1 expression in SH-SY5Y cells. Under the conditions, TBT caused hypermethylation of the NRF-1 promoter region and increased the amount of methyl-CpG-binding protein 2 (MeCP2) bound to the NRF-1 promoter. TBT altered the expression of three kinds of DNA methyltransferases and DNA demethylation enzymes. In this way, epigenetic change plays a pivotal role in the regulation of NRF-1 expression. Taken together, TBT induced epigenetic changes such as hypermethylation, leading to increased recruitment of MeCP2 to the NRF-1 promoter and decreased NRF-1 expression. This research provides new evidence of the epigenetic action caused by organotin.

PS P208 - 0169 Effect of Tetrabromobisphenol A on MMP-9 Expression through Enhancing of NF-κB/AP-1 and Akt/MAPKs-Dependent Pathway in Breast Cancer Cells

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Tetrabromobisphenol A (TBBPA) is a widely used brominated flame retardant that acts as a cytotoxic, neurotoxic, and immunotoxicant, causing inflammation and tumors. Matrix metalloproteinase-9 (MMP-9) plays a key role in angiogenesis, tumor invasion, metastasis, and inflammatory tissue destruction. However, the mechanism of TBBPA-induced MMP-9 expression in human breast cancer cells is not clear. In human breast cancer MCF-7 cells, treatment with TBBPA significantly induced the expression and promoter activity of MMP-9. Transient transfection with MMP-9 mutation promoter constructs verified that NF-κB and AP-1 response elements are responsible for the effects of TBBPA. Furthermore, TBBPA-induced MMP-9 expression was mediated by NF-κB and AP-1 transcription activation as a result of the phosphorylation of the Akt and MAPKs signaling pathways. Moreover, TBBPA-induced Akt/MAPK pathways and MMP-9 expression were attenuated by specific NADPH oxidase inhibitor and ROS scavenger. These results suggest that TBBPA can induce cancer cell metastasis by releasing MMP-9 via ROS-dependent Akt and MAPKs pathways in MCF-7 cells.

PS P209 - 0217 Effects of Bis (2-Ethylhexyl) Phthalate, Endocrine-Disrupting Chemicals, and Alternative Chemicals on Estrogen and Androgen Receptors

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Plastic products are closely entwined with modern life. Some plasticizers used in making plastics, such as phthalates, are reported to be endocrine-disrupting chemicals. Plasticizers can be released into the environment, and health risks related to plasticizer exposure have been reported. In addition, due to plastic waste that flows into the ocean, microplastics have been found in marine products, including nonbiological seawater products such as sea salt. Plastics can affect the body via a variety of pathways, and therefore, safer alternative chemicals are needed. Three such chemicals to replace bis(2-ethylhexyl)phthalate (DEHP), a typical plasticizer, have been evaluated. The endocrine-disrupting activities of each chemical, including estrogenic or antiestrogenic activity, androgenic or antiandrogenic activity, steroidogenesis, and estrogenic properties via a short-term screening test using the uterotrophic assay were assessed in accordance with the Organisation for Economic Co-operation and Development guidelines for chemical testing. Our results showed that DEHP, acetyl tributyl citrate (ATBC), triethyl 2-acetyl citrate (ATEC), and trihexyl O-acetyl citrate (ATHC) possess no estrogenic activity, whereas DEHP, ATBC, and ATHC demonstrate antiestrogenic activity and ATBC antiandrogenic activity. DEHP and ATHC exhibited a disruption in steroidogenesis activities. Additional tests are necessary, but our results suggest that ATEC is a good candidate plasticizer providing a suitable alternative to DEHP.

PS P210 - 0601 Bisphenol A-Induced Expression of Peroxisome Proliferator-Activated Receptors in Testis and Its Implication

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Environmental and dietary exposure to bisphenol A (BPA) and its toxicological consequences are extensively reported. BPA has multiple cellular targets. One of the mechanisms of action of BPA involves interaction and activation of nuclear receptors (NRs) including peroxisome proliferator-activated receptors (PPARs). PPARs regulate genes involved in adipogenesis, and metabolism of glucose, lipid and cholesterol. Study of tissue and dose specific PPAR expression may decipher the toxicity outcome of BPA exposure in different tissues. We studied expression of three forms of PPARs in mouse testes exposed to BPA for 14 days. mRNA and protein expression of all three forms of PPAR increased non-monotonously and specific effects were observed. However, histopathological study showed a dose-dependent pattern of pathological changes in testis demonstrating a monotonous effect. These findings imply that other PPAR-independent mechanisms may play a role in BPA-induced pathological

changes. There is overwhelming evidence showing vulnerability of male reproductive system to BPA. The present study warrants exploration of role of BPA-induced PPARs and their non-monotonous expression on male infertility.

PS P211 - 0614 Effect of Parental Obesity on the Transgenerational Reprotoxicity of Bisphenol A in F1 Generation Rats

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There is a global concern about adverse health effects of endocrine disrupting chemicals (EDCs) which are ubiquitously distributed in our food chain. Among EDCs bisphenol A (BPA) has pre-eminent place. It has obesogenic and estrogenic properties. Due to its omnipresence, the populations of all age groups are unavoidably exposed to BPA, though at low level. Transgenerational exposure and effect scenario for BPA have also been recognized. On the other hand, obesity has been a scourge in all communities, especially in developed and developing countries. We studied the transgenerational effect of parental obesity with concurrent exposure to low dose BPA (10 ppm for 6 months) and its reproductive consequences in siblings of both the genders of F1 generation. The exposed F0 generation animals were crossed and F1 generation was analyzed 35 days after birth for indications of reproductive toxicity. We observed changes in reproductive hormonal levels, and increase in the C-reactive protein. Besides, there was an increase in serum cholesterol and triglycerides along with higher birth weight and rapid weight gain in those animals. Histopathological findings revealed presence of regressive and inflammatory changes in the ovary and testis. Findings demonstrated an additive effect of parental obesity in transgenerational reproductive toxicity of BPA.

PS P212 - 0657 Screening and Prioritization of Phase 1, 2, and e1K ToxCast Chemicals for Potential Inhibition of the Sodium-Iodide Symporter (NIS)

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The sodium-iodide symporter (NIS) mediates the uptake of iodide into the thyroid, which is the initial step in thyroid hormone synthesis. Inhibition of NIS function by xenobiotic chemicals has been demonstrated to suppress circulating thyroid hormones and perturb related physiological functions. However, few high throughput (HTP) assays were available for screening target sites of thyroid hormone disruption in the US EPA's Endocrine Disruptor Screening Program (EDSP). Previously, a radioactive iodide uptake (RAIU) assay using our newly developed human (h)NIS-EPA-HEK293T cells was validated as a high throughput screen for NIS inhibitors. Following validation and confirmation of this robust, highly reproducible and reliable assay with average Z' scores of over 0.7, approximately 1800 chemicals from the ToxCast ph1v2, ph2, and e1K libraries were screened and evaluated for hNIS inhibition. The blinded chemical samples were initially screened at 1×10^{-4} M with the RAIU assay, and those chemicals that had >20% RAIU inhibition were further tested in parallel RAIU and cell viability assays at six concentrations (1×10^{-4} - 1×10^{-9} M). These assays delivered consistent performance and highly reproducible results based on QC metrics and internal replicate responses. Dose-response evaluation showed that 430 chemicals actively inhibited RAIU, with 129 showing no cytotoxicity at one or more concentrations. Further dose-response analyses using a previously developed cytotoxicity-adjusted potency scoring system (with the reference chemical sodium perchlorate having a standardized score of 200) revealed that only 12 chemicals scored >100. The highest ranked chemicals were subsequently screened in a secondary rat thyroid follicular cell (FRTL5) RAIU assay to confirm their activity and to compare cross-species potency. This information will assist in the prioritization and selection of chemicals for further *in vivo* confirmation. *This abstract does not necessarily reflect EPA policy.*

PS P213 - 0665 Potential Effects of Benzophenones on Sex Differentiation and Their Toxic Effects on Zebrafish Larvae and ZFL Cells

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Ultraviolet (UV) filter chemicals found in sunscreen are common environmental contaminants that represent a growing health concern due to their presence in water, fish, and human systems. Benzo[*a*]resorcinol (BP1, CAS 131-56-6), oxybenzone (BP3, CAS 131-57-7), and dioxybenzone (BP8, CAS 131-53-3) are three such chemicals that have been detected in environmental samples and linked to alterations in estrogen receptor signaling pathway and oxidative stress. Zebrafish is a well-established model to study molecular genetics, toxicology, and trans-generation effects of chemicals. In this study, zebrafish liver cell line (ZFL) and larvae were employed to investigate the potential risks of the benzophenones (BPs), and the molecular mechanism of their toxic effects. 24-hour and 96-hour chemical exposures were conducted on both ZFL (*in vitro*) and embryo-larvae (72 hpf, *in vivo*) to determine half lethal concentrations (LC50s) for each chemical. Gene expression profiles on estrogen receptor (ER) pathway and gender differentiation have been detected by using quantitative real-time PCR (qRT-PCR). Preliminary data showed that BPs strongly activated the expression of estrogen receptors (ER α , ER β 1, GPER), vitellogenin and sex-determining genes (BRCA2, CYP19A, DMRT1, SOX9A) both *in vivo* and *in vitro*. The sex differentiation genes of zebrafish could be caused by a combination of factors: up-regulation of BRCA2 and CYP19A is likely to result in the differentiation of larvae into females, while enhancing the expression of CYP19A, DMRT1 and SOX9A in sexually mature individuals can lead to gonadal malformation or gender transformation. Based on this result, it was speculated that BPs will cause serious interference to endocrine systems. Moreover, the tolerance of larvae to BPs was weaker than ZFL. The BPs that directly entered ZFL can rapidly participated in metabolism and strongly induced the expression of related genes, whereas affected individuals for a sustained period of time and accumulated in larvae. *Research supported by a Direct Grant from Faculty of Science, Chinese University of Hong Kong.*

PS P214 - 0668 Endocrine-Disruption Effects of Three Different Emergent Pollutants on *Caenorhabditis elegans*

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Human production of chemical compounds is growing, greatly benefiting civilizational progress yet generating a great variety of less positive effects on nature. Bisphenol A (BPA) is a chemical compound massively used as a plasticizer in several daily use elements. Propylparaben (PPB) and triclosan (TCS) are chemical compounds that extend the shelf-life and avoid contamination in personal care products. Biological models have been proven an effective evidence of toxicity and its mechanisms. *Caenorhabditis elegans* (*C. elegans*) is a nematode widely used due to its multiple beneficial characteristics for biological models. In this study, three stages are contemplated: an evaluation of toxicity of the three molecules described in the biological model *C. elegans*, considering endpoints such as mortality, growth, fertility and progeny size; a study of fatty acid deposit quantity, and the expression of *C. elegans* genes related to oxidative stress, by reporter genes associated with green protein fluorescence (GFP). The lethality of *C. elegans* exposed to BPA, PPB and TCS was concentration dependent, and the LC50 after 24 h of exposure was 113.5, 261.7 and 43.2 μ M, respectively. At concentrations greater than 0.5 μ M, BPA, PPB and TCS caused lethality, with statistical differences related to the control. At lower concentrations, only TCS (0.05 μ M) was bioactive. Body length increased with BPA, decreased with PPB, and was unaffected by TCS. Body width mostly increased, although the BPA response was bimodal. Body length-width relationship was somewhat augmented, but PPB was the most active, suggesting an association with obesity. The largest brood sizes after BPA and PPB exposure were reached at 5 μ M and 0.5 μ M, respectively, then decreased in response to higher concentrations. In contrast, TCS decreased this aspect following a concentration-dependent trend. The most sensitive genes were *sod-4*, *hsp-4*, *hsp-16.2* and *skn-1*. These genes increased their expression after exposure to all compounds, indicating a toxic response related to the generation of reactive oxygen species (ROS). There was no evidence of concentration dependency; however, low and high concentrations caused overexpression of some genes. BPA, PPB and TCS caused a concentration-related increase in the accumulation of lipids in exposed nematodes when staining with q-ORO, which was consis-

tent with the recorded body width-length relationships. In summary, molecules bisphenol A, propylparaben and triclosan exert toxicity in *C. elegans* and its endocrine-disruption effects are qualitative and quantitatively measurable on the biological model.

PS P215 - 0671 Identification the Target Receptor of Sunscreen Chemicals, Bezophenones and Octocrylene, Using Reporter Gene System in a Human Cell Line of Embryonic Kidney, HEK293

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Ultraviolet (UV) filter chemicals found in sunscreen are common environmental contaminants that represent a growing health concern due to their presence in water, fish, and human systems. Benzoresorcinol (BP1, CAS 131-56-6), oxybenzone (BP3, CAS 131-57-7), dioxybenzone (BP8, CAS 131-53-3), and octocrylene (OC, CAS 6197-30-4) are four such chemicals that have been detected in environmental samples and linked to alterations in estrogen receptor signaling pathway and oxidative stress. Zebrafish is a well-established model to study molecular genetics, toxicology, and trans-generation effects of chemicals. In this study, the genes of ER and AhR were cloned from the zebrafish liver cell line (ZFL) and recombined with the corresponding promoter to construct luciferase reporter systems to determine the biological activities of these UV filters on ZFL at the gene transcription level. Human Embryonic Kidney Cells 293 (HEK293T) is used to provide cellular environment due to its high transfection efficiency than ZFL cells. Preliminary data showed that ER α and GPER are the main receptors for BPs on ER pathway; and AhR1b and AhR2 are the main receptors of BPs on AhR pathway. Depending on the fluorescence intensity, BPs had a significant effect on ER pathway, even exceed its natural ligand estrogen. AhR and ER are probably not the receptors for OC, because the fluorescence signal of the binding of OC to the receptor is weak regardless of the ER or AhR pathway. *Research supported by a Direct Grant from Faculty of Science, Chinese University.*

PS P216 - 0689 High Content Imaging and Machine Learning-Based Single Cell Analysis of Adverse Effects Induced by Bisphenol A and Its Analogs Bisphenol S, Bisphenol AF, and Tetrabromobisphenol A in a Three-Dimensional Testicular Cell Coculture Model

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With the recent advances in automated microscopy and the development of high-performance computational image analysis, high content analysis (HCA) has emerged as a powerful tool for toxicity assessment. However, due to the complexity of the high-dimensional and large-scale data produced by HCA, there is an increased demand for an effective computational strategy to characterize and quantify large-scale image data at a single-cell level, enabling the identification of unique subpopulations. The purpose of this study is to develop an HCA and machine learning-based single cell analysis to examine complex phenotypic changes and molecular mechanisms after exposure to BPA and its analogs in an *in vitro* three dimensional (3D) testicular cell coculture model. Testicular cell cocultures were treated with doses of BPA and its analogs for 24, 48 and 72 hrs, and multi-channel images (49 fields per well) targeting the nuclear, cytoskeleton, DNA damage and cell cycle were then automatically acquired using an Arrayscan VTI HCS reader. Cell segmentation, localization, and measurements of multiple features of a single cell were conducted using the open-source software CellProfiler. The output of over 100 cellular features, including size, shape, intensity, and texture of nuclei, F-actin, and γ -H2AX in a single cell was further used to establish a supervised machine learning pipeline to characterize the phenotypes associated with the testicular toxicity in the CellProfiler Analyst using a Random Forest classification model. We observed the treatments of BPA or its analogs resulted in the loss of spatial arrangement in the 3D structure of cytoskeleton and induction of M phase arrest. Specifically, BPAF significantly induced multinucleated cells, which was associated with an increase of DNA damage response and impaired actin filament structure. BPA and its analogs exerted differential toxicities on multiple endpoints in the coculture model, and BPAF exhibited the most toxicity compared to TBBPA, BPA and BPS. In summary, this finding suggests that the HCA integrated machine

learning algorithm empowers the traditional HCA approach, and provides a deeper insight into the cellular dynamics and heterogeneity in response to BPA and its analogs. *Supported by R44 ES027374 funding.*

PS P217 - 0690 Occurrence of Fibrates and Their Metabolites in Source and Drinking Water in Shanghai and Zhejiang, China

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Fibrates, which are widely used lipidaemic-modulating drugs, are emerging environmental pollutants. However, fibrate concentrations in the environment have not been thoroughly surveyed. Here, we determined concentrations of the most commonly used fibrates and their metabolites in source water and drinking water samples from ten drinking water treatment plants in Shanghai and Zhejiang, China, using solid-phase extraction and liquid chromatography-tandem mass spectrometry. All the target compounds were detected in at least some of the source water samples, at concentrations ranging from 0.04 ng/L (fenofibrate) to 1.53 ng/L (gemfibrozil). All the compounds except fenofibrate were also detected in at least some of the drinking water samples, at recoveries ranging from 35.5% to 91.7%, suggesting that these compounds are poorly removed by typical drinking water treatment processes. In a peroxisome proliferator-activated receptor α agonistic activity assay, the target compounds showed no significant activity at nanogram per litre concentrations; therefore, our results suggest that the fibrate concentrations in drinking water in Shanghai and Zhejiang, China, do not significantly affect human health. However, because of the increasing westernization of the Chinese diet, fibrate use may increase, and thus monitoring fibrate concentrations in aquatic environments and drinking water in China will become increasingly important.

PS P218 - 0752 B Cell Survival Is Regulated by Bisphenol A and Its Substitutes

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B cells are an important contributor to the production of inflammatory cytokines and antibodies, presentation of autoantigens, and interactions with T cells. Bisphenol A (BPA) is widely used to make certain plastics such as water bottles and sports equipment. Epoxy resins containing BPA are also used to line water pipes, as coatings on the inside of many food and beverage cans and in making thermal paper such as that used in sales receipts. BPA is a xenoestrogen, exhibiting estrogen mimicking hormone-like properties, which lead to the development of its substitutes. Here, we investigated whether B cell cytotoxicity is regulated by BPA and its substitutes using WIL2-NS human B lymphoblast cells and mouse spleen cells. When cytotoxicity was measured by MTT assay, cell titer Glo assay and flow cytometry analysis with propidium iodide staining, cytotoxicity by BPA was higher than that by substitutes. ROS production by BPA was also higher than that by substitutes. Cytotoxicity and ROS production were attenuated by the treatment with N-acetylcysteine (NAC), a ROS scavenger. We also investigated the mechanism of action on the regulation of B cell cytotoxicity by BPA and its substitutes. Taken together, these results implicate that B cell cytotoxicity by substitutes should be lower than BPA. Data suggest that substitutes can replace BPA in the field of manufacturing BPA-used products.

PS P219 - 0761 Glucocorticoid Biosynthetic Pathway Analysis Divulges the Susceptibility of Adrenal Gland against EDCs by Novel Approach Uncovering Endocrine-Disruption Mechanism

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The adrenal gland is a less focused endocrine organ for the endocrine-disrupting effect of endocrine-disrupting chemicals (EDCs) leading to the neglected study of glucocorticoid biosynthetic pathway as the target of EDCs. The effects of two extensively used phthalate esters viz. di-ethyl hexyl phthalate (DEHP) and di-butyl phthalate (DBP) on adrenal gland were observed in Wistar rats in the present study to check the susceptibility of the adrenal gland and glucocorticoid biosynthetic pathway against the exposure of these extensively used

plasticizers which are well-known EDCs. Wistar rats were divided into seven groups ($n = 5$) and received the treatment for fourteen days. Group I was the control and received only corn oil which is used as a vehicle. Group II, III and IV received a daily dose of 250 mg/kg-BW, 750 mg/kg-BW and 1500 mg/kg-BW of DEHP respectively while group V, VI and VII received a daily dose of 100 mg/kg-BW, 500 mg/kg-BW and 1000 mg/kg-BW of DBP respectively. The comparative microscopic study of histological slides of endocrine glands, i.e., pituitary, pineal, thyroid, parathyroid, adrenal gland and testes, revealed the susceptibility of adrenal gland towards the DEHP and DBP. Glucocorticoid biosynthetic pathway was analyzed by molecular docking study of DEHP and DBP with the enzyme proteins of the pathway using Maestro Schrodinger 9.4 software showing the potential of DEHP and DBP to inhibit these proteins comparable to the known inhibitors of these enzymes. The expression study of enzymes of this pathway, i.e., StAR, 3 β -HSD, CYP21A1, CYP11B2 and CYP11B2, on exposure to DEHP and DBP by real-time PCR has also assessed the sensitivity of the pathway. However, the 2D-nano-LC-MS-MS data of adrenal gland tissue is yet to come which will display the differential expression of proteins and will clear the picture of the glucocorticoid biosynthetic pathway as a potential target of EDCs and will elucidate the novel approach of the mechanism of endocrine disruption.

PS P220 - 0832 Phthalate-Induced Dysfunction of Blood-Brain Barrier (BBB) Endothelial Cells Mediated by Impairment of Tight Junction Proteins

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Phthalates are plasticizers which are commonly used in consumer products and food containers. Concerns for the adverse health effects of phthalates, including the reproductive toxicity as endocrine disruptors, have been reported. Interestingly, the association between phthalates and cardiovascular diseases such as stroke has been also reported. Here, we investigated the effects of phthalates on brain endothelial cells, which are important components of blood-brain barrier (BBB). We investigated if phthalates affect brain endothelial cell viability/permeability and tried to elucidate the underlying mechanisms. We expanded our study with potential of phthalate in enhancement of ischemic damage in brain endothelial damage using *in vitro* hypoxic condition of oxygen-glucose deprivation (OGD). Three representative types of phthalates, di-ethylhexyl phthalate (DEHP), dibutyl phthalate (DBP) and diisobutyl phthalate (DIBP), increased cytotoxicity and the susceptibility of OGD-induced damage, with different potency in brain endothelial cell line bEnd.3. The functional integrity of BBB, as determined by permeability assay, was impaired by phthalate treatment. Potential mechanisms including degradation of tight junction proteins and impairment of mitochondrial membrane potential were examined. Taken together, we demonstrated that phthalates induce brain endothelial damage and permeability impairment, suggesting that these effects can contribute to phthalate-induced cardiovascular damage or ischemic stroke.

PS P221 - 0833 The Sensitivity of Taxane-Resistant Prostate Cancer Is Improved by Targeting Plk1 and Androgen Receptor

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In spite of the development of targeted and immune therapies for cancer treatment, taxanes are still the first choice of treatment for several solid malignant tumors. However, taxanes have limitations such as chemoresistance. To overcome the paclitaxel-resistance, genetic alterations have been investigated in paclitaxel-resistant cancer. As expected the levels of MDR1 and MRP1 were upregulated in the cancers. In addition, the levels of Plk1 were high in the paclitaxel-resistant cancers. Plk1 is a valuable target for chemotherapy, since recent lots of studies demonstrated the clinical relevance between Plk1 and malignancy. Thus Plk1 inhibitors volasertib, BI 2536, and genistein were treated in the paclitaxel-resistant cancers to increase the sensitivity of paclitaxel-resistant cancer cells. Treatment with Plk1 inhibitors reduced cell viability through downregulation of MDR1, MRP1, and Plk1 in the paclitaxel-resistant cancer. Especially genistein has a tendency to be more effective in the paclitaxel-resistant prostate cancer at the GI_{50} level. Genistein also known as a suppressor of androgen receptor or prostate specific antigen expression, in addition to Plk1 inhibitor. Then, Plk1 inhibitors and androgen receptor antagonist bicalutamide were combined. These combinations were effective in paclitaxel-resistant pros-

tate cancer by inhibiting Plk1 and androgen receptor. From these results, we suggest that targeting Plk1 and androgen receptor may be effective in advanced chemoresistant prostate cancer to overcome the taxane-resistance.

PS P222 - 0834 TP53-Mutated Cancer Cells Are Sensitive to Genistein, a Direct Plk1 Inhibitor

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Due to the mutation of *TP53* gene is one of major causes of carcinogenesis and overexpression of polo-like kinase 1 (Plk1) has been detected in several cancer, Plk1 has been emerged as an advantageous target for anticancer drug development. As an effect of anticancer drug development, genistein is detected as a direct Plk1 inhibitor, determined as FRET and ADP-Glo kinase assays. Kinase profiling showed that genistein is relatively selective to Plk1 compared with other kinases, including receptor tyrosine kinase such as EGFR, InsR, and HER2, which were tested. In addition, genistein induced programmed cell death in cancer cells of diverse tissue origins, but it did not in non-transformed cells. Previous study showed that depletion of Plk1 with shRNA resulted in a greater sensitivity to apoptosis in p53-mutated cancer cells. Thus, we also observed selectivity of genistein in *TP53*-nonfunctional cancer cells using shRNA targeting *TP53*, showing that treatment of genistein was selective against *TP53*-nonfunctional cancer cells. Treatment of genistein induced G2/M arrest and mitotic catastrophe by inhibiting Plk1. Thus genistein would be effective to *TP53*-mutated cancer targeting Plk1.

PS P223 - 0835 7-O-Methylwogonin Induces Mitotic Disturbance by Inhibiting Plk1 Activity in Hep3B Cells

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A mitotic kinase Plk1 has been recognized as a valuable target for anti-cancer drugs development, because of the correlation between overexpression of Plk1 in cancer patients and low survival rates of the patients. To find new Plk1 inhibitors, the compounds isolated from *Scutellaria baicalensis* were screened using Plk1 kinase assay. From the screening, we found that 7-O-methylwogonin and related flavones, including baicalein, dihydrobaicalein, and viscidulin II, directly inhibited Plk1 kinase activity. Dihydrobaicalein showed the highest Plk1 inhibitory activity among these four compounds, but it also exhibited inhibitory activity against Vaccinia-related kinase 2 and Plk2. Viscidulin II and baicalein also exhibited low selectivity to Plk1 because viscidulin II and baicalein inhibited Plk2 and Plk3, respectively. However, 7-O-methylwogonin displayed relatively selective Plk1 inhibitory effects, when *in vitro* kinase assays was performed based on FRET and ADP-Glo kinase assays. Treatment of 7-O-methylwogonin displayed mitotic morphology in Hep3B cells. In addition, immunostaining using specific antibodies for the mitotic markers, MPM 2 and p-histone H3, in Hep3B cells showed that 7-O-methylwogonin treatment increased mitotic cell populations. These phenomenon may be caused by mitotic arrest as a result of Plk1 inhibition. These patterns were similar to those of BI 2536, a specific Plk1 inhibitor. Taken together, we suggested that 7-O-methylwogonin disturbed mitotic progression by targeting Plk1, indicating that it might be a potential anticancer agent.

PS P224 - 0851 Assessment of Androgen Receptor Agonistic/Antagonistic Effects on Bisphenol Analogues by OECD In Vitro Assays

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Organisation for Economic Co-operation and Development (OECD) has provided international standardization test methods to confirm the potential effect of chemicals on human endocrine system. Among the OECD Test Guidelines, *in vitro* stably transfected transcriptional activation assays using chinese hamster ovary cell line, AR-Ecoscreen accepted as OECD TG458 to detect the human androgen receptor (AR) agonist/antagonist. The aim of this study is to assess AR agonistic/antagonistic effects on bisphenol A and its 13

analogues by OECD TG458. In results from AR agonist assays, two analogues (bisphenol AF and bisphenol AP) have been found to determine an AR agonist. In case of AR antagonist assays, seven analogues exhibited the AR antagonistic effects alongside bisphenol A by OECD TG458. These data provide information about AR agonistic/antagonistic effects of bisphenol analogues by OECD *in vitro* assays. Although these results may not correlate directly with risks to humans, it would be offer the basal scientific information about food, drug and cosmetic ingredients with safety management.

PS P225 - 0910 Exposure to Low Doses of Oxybenzone during Perinatal Development Alters Mammary Gland Morphology in Male and Female Mice

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Oxybenzone (benzophenone-3) is a UV filter commonly used in personal care products including sunscreens, textiles and inks, and food and beverage containers, among others. Due to its widespread use, human exposures to oxybenzone are widespread. Oxybenzone is considered an endocrine disrupting chemical due to its antiestrogenic and antiandrogenic properties. We evaluated the effects of oral exposures to oxybenzone on the growth and morphology of the mammary gland in BALB/c mice exposed to 30, 212 or 3000 µg/kg/day *in utero* and during lactation. Developmental exposures to oxybenzone reduced the size and growth of mammary gland in males prior to and during puberty. In exposed females, oxybenzone reduced mammary cell proliferation, decreased the number of cells expressing estrogen receptor α , and altered mammary gland morphology in adulthood. These results suggest that even low doses of oxybenzone can disrupt hormone sensitive organs during critical windows of development.

PS P226 - 0037 Tissue-Specific Global Epigenetic Alterations Induced by Acrylamide in Mice

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Acrylamide has been classified as a "Group 2A carcinogen" (probable human carcinogen) by the IARC. The carcinogenicity of acrylamide is attributed to its well-recognized genotoxicity. In the present study, we investigated the effect of acrylamide exposure on epigenetic alterations in mice. Female B6C3F1 mice received acrylamide in drinking water for 28 days at doses previously used in a 2-year cancer bioassay (0, 0.875, 0.175, 0.35, and 0.7 mM), and the genotoxic and epigenetic effects were investigated in the lung (target) and liver (non-target). Acrylamide exposure resulted in a dose-dependent formation of N7-(2-carbamoyl-2-hydroxyethyl)guanine and N3-(2-carbamoyl-2-hydroxyethyl)adenine in lung and liver DNA at the similar levels. In contrast, the profiles of global epigenetic alterations differed between the two tissues. In the lung, acrylamide exposure resulted in a decrease of histone H4 lysine 20 trimethylation (H4K20me3), a common epigenetic feature of human cancer, while in the liver there was increased acetylation of histone H3 lysine 27 (H3K27), a gene transcription activating mark. Treatment with 0.7 mM acrylamide also resulted in substantial alterations in the DNA methylation and whole transcriptome in the lung and liver; however, there were substantial differences in the trends of DNA methylation and gene expression changes between the two tissues. Analysis of differentially expressed genes showed activation of the gene transcription regulation pathway in liver but not lung. This corresponded to DNA hypomethylation and increased histone H3K27 acetylation in liver in contrast to hypermethylation and transcription silencing in lung. Our results demonstrate that acrylamide induced global epigenetic alterations independent of its genotoxic effects, suggesting that organ specificity in acrylamide-induced tumorigenesis may be a consequence of different patterns of tissue-specific epigenetic alterations.

PS P227 - 0139 Exosome-Packaged miR-1246 Contributes to Bystander DNA Damage by Targeting LIG4

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An increasing number of studies have recently reported that microRNAs packaged in exosomes contribute to multiple biological processes such as cancer progression; however, little is known about their role in the development of radiation-induced bystander effects. Cell proliferation was analyzed by CCK-8 and colony forming assays. To monitor DNA damage, the DNA double-strand break (DSB) biomarkers 53BP1 foci and micronuclei were detected. DSB non-homologous end joining (NHEJ) efficiency, expression of DNA repair genes, and the miR-1246 targeting sequence of the DNA ligase 4 (LIG4) mRNA 3' untranslated region (UTR) were assessed by luciferase reporter vectors. miR-1246 was increased in exosomes secreted from 2 Gy-irradiated BEP2D cells and inhibited the proliferation of nonirradiated cells. An miR-1246 mimic, exosomes from irradiated cells, and radiation-conditioned cell culture medium increased 53BP1 foci and micronuclei in nonirradiated cells, and decreased NHEJ efficiency. miR-1246 downregulated expression by directly targeting the LIG4 3'-UTR. Our findings demonstrate that miR-1246 packaged in exosomes could act as a transfer messenger and contribute to bystander DNA damage by directly repressing the LIG4 gene. Exosomal-derived miR-1246 may be a critical predictor of and player in bystander DNA damage.

PS P228 - 0151 Epigenetic Effects of Low-Level Sodium Arsenite Exposure on Human Liver HepaRG Cells

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Inorganic arsenic is an abundant environmental contaminant and an IARC "Group 1 carcinogen" (known to be carcinogenic to humans). Among different molecular alterations attributed to arsenic's carcinogenicity, epigenetic alterations are of particular interest. We have previously reported that prolonged exposure of human hepatic progenitor and mature HepaRG cells to a low concentration of sodium arsenite elicits different transcriptomic responses. Considering the well-recognized role of epigenetic mechanisms in the control of gene expression in physiological and pathological states, we have investigated the effect of arsenic exposure on the status of DNA methylation and histone modifications. By using comprehensive reduced representation bisulfite next generation sequencing analysis of genome-wide DNA methylation, we identified 4255 and 4585 differentially methylated regions in arsenic-treated progenitor and mature HepaRG cells, respectively; however, there was a minimal overlap between the methylation profiles in these cells. There were also substantial differences in the trends of DNA methylation between arsenic-treated progenitor and mature cells characterized by increased hypomethylation in progenitor cells and increased hypermethylation in mature cells. This corresponded to a difference in the overall response of cells to the treatment. In contrast to changes in DNA methylation, no arsenic-induced histone modification alterations were found. A detailed analysis of genes that exhibited an inverse correlation between their expression and methylation in the promoter or first intron regions identified genes critically associated with different functional responses of progenitor and mature cells to arsenic exposure. These findings emphasize the importance of DNA methylation alterations as a key molecular component in the pathogenesis of arsenic-associated pathologies, including arsenic-induced liver carcinogenesis.

P229 - 0187 The Persistence of 1,3-Butadiene-Induced DNA Damage, Transcriptional Effects, and Chromatin State Alterations in C57BL/6J and CAST/EiJ Mice

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Epigenetic alterations are known to play a role in chemical-induced carcinogenesis; however, it is not well known whether epigenetic effects of chemicals are transient or persist after cessation of exposure. Our previous work demonstrated that exposure to 1,3-butadiene, a known human carcinogen, results in DNA damage and disruption of the chromatin landscape in different mouse tissues. Effects of 1,3-butadiene on DNA damage and epigenetic alterations are both strain- and tissue-specific, but these studies examined the phenotypes immediately after exposure. In this study, we investigated the persistence of 1,3-butadiene-induced DNA damage, as well as its transcriptomic and epigenetic effects. We tested the hypothesis that 1,3-butadiene-induced DNA damage, transcriptomic responses, and epigenetic alterations do not persist after exposure. Male mice of two strains (C57BL/6J and CAST/EiJ) were exposed to clean air or 625ppm of 1,3-butadiene by inhalation (6 hr/day, 5 days/week) for 1 or 2 weeks. Animals were sacrificed at the end of each exposure, or 1 or 2 weeks after the 2-week exposure. We evaluated genotoxicity in the liver, a target tissue of 1,3-butadiene-induced carcinogenesis, by measuring THB-Gua adduct levels. We observed increasing adduct levels in C57BL/6J and CAST/EiJ mice after 1 and 2 weeks of 1,3-butadiene exposure. DNA adduct levels declined after cessation of exposure. For assessment of the changes in chromatin states, we used the assay for transposase accessible chromatin (ATAC) followed by sequencing, using the lung. We found interstrain differences in chromatin states upon exposure to 1,3-butadiene. In addition, total RNA sequencing was done in liver, lung, and bone marrow. After 2 weeks of 1,3-butadiene exposure, CAST/EiJ mice presented more genes that were significantly altered relative to C57BL/6J mice in all three tissues. Transcriptomic effects declined dramatically in the liver and lung of both strains after exposure was stopped; however, changes in gene expression persisted for 2 weeks after 1,3-butadiene exposure in bone marrow of C57BL/6J, but not CAST/EiJ mice. This persistent transcriptional signature included genes involved in carcinogenesis as well as *RGSS*, a tumor antigen of leukemia, which is one of the disease outcomes associated with human exposure to 1,3-butadiene. Our results indicate that the effects of 1,3-butadiene on DNA damage and most of the transcriptomic responses do not persist after the cessation of exposure; however, there may be tissue- and strain-dependent persistent effects.

P230 - 0256 Arsenite-Induced Histone H3 Modification and Its Effects on *EGR1* and *FOS* Expression in HeLa Cells

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It is evident that trivalent arsenicals do not have mutagenicity, but they are human carcinogens. Recently, epigenetic modification has been considered as one of the important causes of arsenical carcinogenicity. Here we examined global histone H3 modification by trivalent inorganic arsenite (iAs(III)) and its contribution to gene expression in HeLa cells. iAs(III) induced histone H3K9 dimethylation (H3K9me2) and trimethylation (H3K9me3), histone H3S10 phosphorylation (H3S10p), histone H3T11 phosphorylation (H3T11p), and histone H3K9S10 trimethyl-phosphorylation (H3K9me3S10p). Among these modifications, H3S10p, H3T11p, and H3K9me3S10p were observed as a punctate signal in interphase cells, which seems to associate with remodeling of the chromatin structure at the specific locus. A chromatin immunoprecipitation assay was performed to examine histone H3 modifications around the *FOS*, *EGR1*, and *IL8* promoters, as previous studies revealed some relation between histone H3 modification and induction of these genes. iAs(III) increased H3S10p and H3K9me3S10p in the *FOS* promoter around the SRE/ELK1 binding site (–400 to –200) and CRE-binding site (–50). In contrast, histone H3 around the *EGR1* promoter of SRE/CRE-binding site (–200 to –50) was modified to H3S10p and H3K9me3S10p by iAs(III). Reporter gene assays with deletion mutants of the *FOS* and *EGR1* promoters revealed that the around SRE/ELK1 site is important for iAs(III)-mediated *FOS* induction, and SRE/CRE site for *EGR1* induction. Collectively, these results demonstrate that iAs(III) induces histone H3 modifications around the transcription factor binding sites of the *FOS* and *EGR1* promoter, and these modifications seem to be important in transcriptional activation of these genes.

P231 - 0280 LncRNA Lnc-RI Regulates Homologous Recombination Repair of DNA Double-Strand Breaks by Stabilizing RAD51 mRNA as a Competitive Endogenous RNA

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DNA double-strand break (DSB) repair is critical for the maintenance of genome stability. The current models of the mechanism of DSB repair are based on studies of DNA repair proteins. Long non-coding RNAs (lncRNAs) have recently emerged as new regulatory molecules, with diverse functions in biological processes. In the present study, we found that expression of the ionizing radiation-inducible lncRNA, lnc-RI, was correlated negatively with micronucleus frequencies in human peripheral blood lymphocytes. Knockdown of lnc-RI significantly increased spontaneous DSBs levels, which was confirmed to be associated with the decreased efficiency of homologous recombination (HR) repair of DSBs. The expression of RAD51, a key recombinase in the HR pathway, decreased sharply in lnc-RI-depressed cells. In a further investigation, we demonstrated that miR-193a3p could bind with both lnc-RI and RAD51 mRNA and depressed the expression of lnc-RI and RAD51 mRNA. lnc-RI acted as a competitive endogenous RNA (ceRNA) to stabilize RAD51 mRNA via competitive binding with miR-193a-3p and release of its inhibition of RAD51 expression. To our knowledge, this is the first study to demonstrate the role of lnc-RI in regulating HR repair of DSBs. The feedback loop established in the current study suggests that lnc-RI is critical for the maintenance of genomic stability.

P232 - 0366 Dynamic Alterations in DNA Methylation Precede Tris(1,3-dichloro-2-propyl)phosphate-Induced Delays in Zebrafish Epiboly

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After rapid erasure of maternal and paternal methylation marks following fertilization, zygotic genome activation, tissue-specific gene expression, and normal somatic development of vertebrate embryos are dependent on steady, *de novo* genome-wide cytosine methylation by DNA methyltransferases during the maternal-to-zygotic transition. Therefore, alterations in *de novo* DNA methylation marks during early embryonic development have the potential to change the trajectory of normal development and, if inherited, persist across multiple generations. We previously showed that tris(1,3-dichloro-2-propyl)phosphate (TDCIPP)—a high-production volume organophosphate flame retardant widely used within the United States—induces genome-wide hypomethylation during cleavage within early zebrafish embryos, an effect that may be associated with TDCIPP-induced delays in epiboly during late-blastula and gastrula. Therefore, using zebrafish as a model, the objectives of this study were to (1) quantify concentration-dependent impacts of TDCIPP on epiboly; (2) determine whether co-exposure with folic acid (FA)—a methyl donor—mitigates TDCIPP-induced impacts; and (3) using ten previously identified TDCIPP-susceptible loci, rely on bisulfite amplicon sequencing (BSAS) to monitor CpG methylation dynamics across multiple TDCIPP concentrations in the presence or absence of FA. Although TDCIPP delayed epiboly up to 3 h by 6 h post-fertilization (hpf) and induced malformations by 24 hpf, FA was unable to mitigate TDCIPP-induced effects at all stages evaluated. Moreover, while no differences in global methylation were detected using a 5-methylcytosine (5-mC) DNA ELISA, BSAS revealed that TDCIPP-induced effects on CpG methylation were dependent on concentration and developmental stage, and that early effects on methylation do not persist despite continuous exposure. Our findings demonstrate that TDCIPP delays zebrafish epiboly, a phenotype that is preceded by complex, dynamic alterations in DNA methylation.

PS P233 - 0456 Gene Expression and Epigenetic Alterations in Human Hepatocytes Treated with Low Doses of Chemical Carcinogens

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The increasing number of man-made chemicals in the environment that may pose a carcinogenic risk emphasizes the need for the development of the reliable time- and cost-effective approaches for carcinogen detection. To address this issue, we have investigated the utility of human hepatocytes for the *in vitro* identification of genotoxic and non-genotoxic carcinogens. Induced pluripotent stem cell (iPSC)-derived human hepatocytes (iCell Hepatocytes 2.0) were treated with the genotoxic carcinogens aflatoxin B₁ (AFB1) and benzo[a]pyrene (B[a]P) and with the non-genotoxic liver carcinogen methapyrilene at non-cytotoxic concentrations for 7 days, and transcriptomic and DNA methylation profiles were examined. 1569 (892 protein-coding and 677 non-coding), 1693 (922 protein-coding and 771 non-coding), and 2061 (1465 protein-coding and 596 non-coding) differentially expressed genes (a Benjamini-Hochberg adjusted $P < 0.05$ and a fold change ≥ 2) were detected in cells treated with AFB1, B[a]P, and methapyrilene, respectively. Exposure of iPSC-derived hepatocytes to test chemicals resulted in profound DNA methylation alterations evidenced by the 4091, 4038, and 4444 differentially methylated regions found in AFB1-, B[a]P-, and methapyrilene-treated cells. A combined gene expression and gene-specific DNA methylation analysis in cells treated with AFB1, B[a]P, and methapyrilene demonstrated that 117, 133, and 172 genes showed an inverse correlation between gene expression and methylation. This indicates that carcinogens, regardless of their mode of action, may act as epigenotoxicants by causing functional disturbances of the cellular epigenome. The results of our study suggest that the evaluation of carcinogen-induced functional epigenetic alterations by using a combined gene expression and cytosine DNA methylation analysis may assist and enhance in the identification of carcinogenic chemicals.

PS P234 - 0546 Correlation between Heavy Metal and 8-OHdG Levels in Turkish Welders

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Welding is one of the key components of numerous manufacturing industries, which poses potential physical and chemical health hazards. These workers expose a number of hazardous compounds which can occur during the welding process such as ultraviolet (UV) radiation, electromagnetic fields, toxic metals and polycyclic aromatic hydrocarbons (PAHs) with the particle size typically within the range of fine to ultrafine particles. The welding fume generated during the welding process, possesses a number of metals, including chromium (Cr), manganese (Mn), nickel (Ni), copper (Cu), arsenic (As), cadmium (Cd) and lead (Pb). The biomarker 8-hydroxy-2-deoxyguanosine (8-OHdG or 8-oxodG) has been established as a commonly measured and sensitive marker of DNA damage due to the hydroxyl radical attack. Results showed that this damage may contribute to mutagenicity and cancer promotion. The aim of this study is the determination of correlation between the heavy metal and 8-OHdG levels in 48 Turkish welders and their controls. According to our results, there were important correlations between Cr, Mn, Cu and Pb and 8-OHdG levels were found.

PS P235 - 0616 Gene-Specific 5'-UTR Methylation versus Promoter Methylation in Leukocytes from Workers Exposed to Different Levels of VOCs

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As a result of exposure to toxic substances, different human and animals cells have displayed either global DNA hypomethylation or gene-specific hypermethylation. Specifically in occupational volatile organic compound (VOC) exposure, we previously found hypermethylation in *TOP2A*, *SOD1* and *TNF-α* promoter area in leukocytes from people exposed to a complex VOC mixture, while participants exposed to a single VOC did not show any differences compared to controls. The goal was to analyze the methylation pattern in the 5'-UTR region, considered for some authors as an "internal/alternative promoter area" in people occupationally exposed to a single VOC compared to VOC mixtures

exposure as well as a reference, non-exposed group. PCR-pyrosequencing was performed in order to analyze methylation status in CpG's located at the 5'-UTR region of the *TOP2A*, *SOD1*, *IL6*, *TNF-α* and *CYP2E1* genes in leukocytes from Mexican workers laboring at four different scenarios with VOC exposure. We analyzed this methylation status in a subgroup of participants who participated in a former study with a larger sample. Labor scenarios were as follows: A leather shoe factory (LS, n=19); gas station attendants (GS, n=19); a plastic shoe factory (PS, n=17); a tannery (T, n=19) and a control group (administrative workers, C, n=19). We also analyzed the promoter methylation status in this groups. LS workers exposure was higher for four VOCs other than BZ while BZ was higher in GS workers. Tannery workers (T) were exposed only to toluene. 5'-UTR region from *IL6* and *TOP2A* genes showed higher mean methylation values one exposed population workers compared to controls; while promoter methylation did not showed changes. Methylation in the 5'-UTR region of the *SOD1* gene did not show differences between groups; while promoter methylation behaved in the same way observed for a larger sample; same case for *TNF-α* methylation. DNA obtained from the reference group showed higher mean methylation values in the 5'-UTR region from the *CYP2E1* gene compared to tannery workers. 5'-UTR region in certain genes is more prone to undergo epigenetic modifications compared to promoter area as a result of VOC exposure. 5'-UTR methylation status must be considered in a near future as an early biomarker of effect in human chemical toxicity.

PS P236 - 0807 Association between Methylation of DNA Damage Response-Related Genes and DNA Damage in Hepatocytes of Rats following Subchronic Exposure to Vinyl Chloride

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This study investigated the association between methylation of DNA damage response-related genes such as *cyclin-dependent kinase inhibitor (CDKN)2A*, *Ras association (RasGDS/AF-6) domain family member (RASSF)1A*, *O6-methylguanine DNA methyltransferase (MGMT)*, *Kirsten rat sarcoma viral oncogene homolog (KRAS)*, and *spleen-associated tyrosine kinase (SYK)* and DNA damage in hepatocytes of rats following subchronic exposure to vinyl chloride (VC). A total of 64 healthy rats were randomly divided into three VC exposure groups (5, 25, and 125 mg/kg) and an untreated negative control group (n = 16 each). VC was administered by intraperitoneal injection every other day for a total of three times a week. Eight randomly selected rats from each group were sacrificed at the end of 6 and 12 weeks, and liver tissue was harvested for the comet assay and for assessment of DNA methylation level and mRNA expression of related genes by PCR. We found that overall methylation levels in the genome of hepatocytes in VC-exposed rats were higher than those in the control group at 6 and 12 weeks ($P < 0.05$), although no differences were observed according to the dose ($P > 0.05$). After 12 weeks of exposure, differences in the methylation of *RASSF1A* and *MGMT* promoter regions were observed between the high-dose group and other groups ($P < 0.05$), whereas no differences were observed for the *KRAS*, *SYK*, and *CDKN2A* promoters ($P > 0.05$). These results suggest that DNA damage and increased genome-wide methylation are biomarkers for VC exposure and that *RASSF1A* and *MGMT* promoter methylation is related to the carcinogenic mechanism of VC.

PS P237 - 0898 The Period of Germline Epigenetic Reprogramming Represents a Critical Window of Chemical Sensitivity

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The germline lineage is fundamental in maintaining genetic and epigenetic information across generations through a series of specialized cellular and genomic programs. Chromatin remodeling, including DNA methylation and histone posttranslational modifications, are associated with pivotal developmental stages including the establishment of totipotency and the differentiation of the mammalian germline from primordial germ cells (PGCs) during development. However, due to accessibility and number issues, little is known of the sensitivity of PGCs in the face of potential environmental influences, such as chemical exposures. We leverage a previously described and well-characterized model of mouse embryonic stem cells (ESCs) engineered with two specific PGC fluorescent markers *Blimp1* (Prdm1) and *Stella* (Dppa3) that efficiently generate Primordial Germ Cell-like Cells (PGCLCs) from epiblast-like cells (EpiLCs). We examined the effect of several known epigenetic disruptors in dose-responses before and over the course of PGCLC differentiation at precisely defined de-

velopmental windows. The EpiLC viability was measured by propidium iodide staining solution and analyzed by flow cytometry. The PGCLCs were stained by primary antibody H3K9/K27me3 (histone 3 lysine 9/27 trimethylation) and secondary antibody Cy3/Cy5, then analyzed by flow cytometry. We found that EpiLCs, the developmental precursors to PGCLCs, and differentiating PGCLCs show widely different sensitivity to epigenetic disruptors. The EpiLC viability was significantly reduced by exposure of Garcinol (10-100uM, $p<0.05$), DZNep (50-100nM, $p<0.001$), Valproic acid (10-1000nM, $p<0.05$), 5-aza-2'-deoxycytidine (1-100nM, $p<0.05$), and SN38 (50-100nM, $p<0.05$). Garcinol 100uM led to the strongest decrease in viability, with 86.5% ($p<0.05$) reduction in total viable cells compared to DMSO control. PGCLCs displaying heightened sensitivity to histone methyl transferase inhibitors. GSK343 500nM led to 52.3% ($p<0.01$), reduction in total generate efficiency and also led to 25.5% ($p<0.01$) and 19.3% ($p<0.01$), decrease in H3K9me3 and H3K27me3 expression compared to DMSO control. Herein indicate that H3K9/K27me3 deficiency leads to impair mammalian PGC differentiation and our chemically induced system could therefore be used for screening of epigenetic modifiers.

PS P238 - 0935 Characteristics of Chemical Compounds Affecting Global DNA Methylation in Multipotent Stem Cells

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Maternal and developmental exposures to environmental chemicals, such as drugs, pesticides, or food additives, have been concerned to induce reproductive or developmental aberrance through the epigenetic regulation of gene expression. Therefore, comprehensive detection of epigenetic abnormality is a key factor to assess the developmental toxicity of numerous environmental chemicals. An assay system using cultured cells has been developing as an alternative approach to the laboratory animal experiment. Especially, the assay system with multipotent stem cells such as ESCs and iPSCs is established as an *in vitro* model for detecting developmental toxicity. In this study, we developed the *in vitro* assay system to evaluate effects of chemicals on the global epigenetic status in iPSCs. The fusion gene consisted of the methyl-CpG-binding domain (MBD), the nuclear localization signal, and the red fluorescent protein mCherry was introduced into iPSCs. By using the engineered iPSCs, we assessed the epigenetic toxicity of various chemicals, measuring the fluorescent expression pattern of the fusion protein as an index. As a result, some chemicals, such as biotin, propyl gallate, or etoposide, increased the mCherry signal intensity. In contrast, the other chemicals such as theophylline or thalidomide, decreased the intensity. To investigate the alteration of DNA methylation status according to the global epigenetic changes induced by these chemicals, DNA methylome analysis was carried out by using robust methylation profiling microarrays with unparalleled coverage of CpG islands, genes, and enhancers. The DNA methylome analysis showed that chemicals with up-regulated MBD fluorescent intensities increased the DNA methylation rate, but the other chemicals which decreased the intensity did not show relationships with down-regulation of DNA methylation rates, suggesting that down-regulation of DNA methylation rates was affected by a variety of DNA methylation patterns. Moreover, the gene ontology analysis of genes, in which DNA methylation level changed by exposure to chemicals, revealed that influenced genes were related to the regulation of transcription or the histone demethylase activity. We also examined relationships between the patterns of global epigenetic changes and other bioactivities of test chemicals using a database in Toxicology Testing in the 21 century. The statistical analyses indicated that the epigenetic toxicity had relationships with the genotoxicity or the HDAC inhibitory activity. These results suggest that our assay system is useful for detecting the effects of chemicals on global DNA methylation.

PS P239 - 0937 Examining the Transgenerational Effects of Environmental Cues in *C. elegans*

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In sexually reproducing organisms, germ cell development is vital for faithful genome and epigenome transmission across generations. Recent studies have shown that germ cell development is affected by different environmen-

tal toxicants, resulting in a decrease in germ cell health and number. Here, we examine and compare the transgenerational impact and mechanisms of two prevalent toxicants, the plastic chemical Bisphenol A and ethanol. Both have well-described impacts on the developing fetus; however, their effects on developing germ cells and subsequent generations are less explored. We analyze the transgenerational effects of both compounds in *Caenorhabditis elegans*. We hypothesize that exposure disrupts the epigenetic machinery in germ cells, causing changes in histone modifications, fertility defects, and germline dysfunction in a transgenerational manner. First, we showed that BPA exposure causes a transgenerational germline chromatin desilencing coupled with a reduction and redistribution of histone H3K9me3 and H3K27me3. We showed that the alteration of repressive histone mark levels is required for the observed transgenerational increase in germline apoptosis and embryonic lethality. Similar to BPA, ethanol exposure at human-relevant doses also causes transgenerational chromatin desilencing and germline dysfunction, although to a lower extent than BPA's. Current work examines whether the same histone demethylases involved in BPA's transgenerational responses also apply to ethanol. This project identified BPA's and ethanol's transgenerational effect on the germline epigenetic machinery and reproductive health. We hope to further understand how it can induce germline dysfunction, carrying important implications for human reproductive health in the context of environmental exposures.

PS P240 - 0954 Epigenetic Mechanisms of Memory of Environmental Chemical Exposure

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While the intricate steps of germ cell development have long been hypothesized to provide unique windows of sensitivity to environmental insults, little is known of the effect of chemical exposure on the epigenome of germ cells and of the mechanisms of inheritance of these effects. Here, we describe the use of several model systems, including *C. elegans* and ES cells-derived mammalian germ cells, to better understand how the complex developmental program unique to germ cells is affected to environmental exposures. The combination of these models allows us to dissect the genetic pathways at the root of germline dysfunction, epigenetic impact and transgenerational effects. We will present our work showing that BPA causes a transgenerational redistribution of repressive histone modifications such as H3K9me3 and H3K27me3 that correlates with reproductive toxicity effects (including germ cell apoptosis and embryonic lethality). Furthermore, we show that a network of H3K9 and H3K27 histone demethylases acts to sustain the germline toxicity response elicited by Bisphenol A across 5 generations. Together, our work highlights the value of leveraging multiple platforms in the fields of genetics, stem cell biology and computational toxicology, to uncover new windows of reproductive sensitivity and mechanisms of transgenerational inheritance.

PS P241 - 0962 Fusaric Acid-Induced Promoter Methylation of DNA Methyltransferases Triggers Global DNA Hypomethylation in Human Hepatocellular Carcinoma (HepG2) Cells

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Fusaric acid (FA), a mycotoxin contaminant of maize, displays toxicity in plants and animals; however, its epigenetic mechanism is unknown. DNA methylation, an epigenetic modification that regulates gene expression, is mediated by DNA methyltransferases (DNMTs; DNMT1, DNMT3A and DNMT3B) and demethylases (MBD2). The expression of DNMTs and demethylases are regulated by promoter methylation, microRNAs (miR-29b) and post-translational modifications (ubiquitination). Alterations in these DNA methylation modifying enzymes affect DNA methylation patterns and offer novel mechanisms of FA toxicity. We determined the effect of FA on global DNA methylation as well as a mechanism of FA-induced changes in DNA methylation by transcriptional (promoter methylation), post-transcriptional (miR-29b) and post-translational (ubiquitination) regulation of DNMTs and MBD2 in the human hepatocellular carcinoma (HepG2) cell line. FA induced global DNA hypomethylation ($p<0.0001$) in HepG2 cells. FA decreased the expression of DNMT1 ($p<0.0001$), DNMT3A ($p<0.0001$) and DNMT3B ($p<0.0001$) by upregulating miR-29b ($p<0.0001$) and inducing promoter hypermethylation of DNMT1 ($p<0.0001$) and DNMT3B ($p<0.0001$), and promoter hypomethylation of DNMT3A ($p<0.0001$). FA decreased the ubiquitination of DNMT1 ($p=0.0753$), DNMT3A ($p=0.0008$) and DNMT3B ($p<0.0001$) by decreasing UHRF1 ($p<0.0001$) and USP7 ($p<0.0001$).

FA also induced *MBD2* promoter hypomethylation ($p < 0.0001$) and increased *MBD2* expression ($p < 0.0001$). Together these results indicate that FA induces global DNA hypomethylation by altering *DNMT* promoter methylation, upregulating miR-29b, and increasing *MBD2* in HepG2 cells.

PS P242 - 0105 Follow-Up of Oxidative Stress and DNA Damage Markers in Young Physicians Occupationally Exposed to Inhalation Anesthetics during the Medical Residency

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Worldwide, millions of operating room professionals are occupationally exposed to inhalation anesthetics, which have potential toxic effects. It is known that there is a link between oxidative stress and DNA damage. Therefore, human biomonitoring can be a useful tool to assess the health of exposed professionals. In addition, it is unknown the consequences of occupational exposure to inhalation anesthetics on young physicians, including the medical residents. Thus, the study was designed to evaluate the possible early events by monitoring the possible toxic effects of short-term exposure to the most widely used inhalation anesthetics in healthy physicians. This study was previously approved by the local IRB, and all the subjects signed the informed consent. This follow-up included 26 young physicians of both sexes who worked in operating rooms and were occupationally exposed to inhalation anesthetics (halogenated isoflurane, sevoflurane, and desflurane, and also the nitrous oxide gas) during the first year of medical residency in anesthesiology or surgery in a Brazilian university hospital. Blood samples were collected at three time points, as follows: before the start of the medical residency program (before exposure-control), and after six months and after twelve months of exposure to monitor oxidative stress (lipid peroxidation marker—malonaldehyde: MDA by High-Performance Liquid Chromatography and protein oxidation marker—protein carbonyls by Enzyme-Linked Immunosorbent Assay) and genotoxicity (DNA breaks by the comet assay). Additionally, all the trace concentrations of the anesthetics were measured in the physicians' breathing zone by a portable infrared analyzer. All the anesthetic concentrations were above the recommended limit established by the National Institute for Occupational Safety and Health (NIOSH, US). The results showed a statistically significant increase in lipid (MDA) and protein (protein carbonyls) oxidation markers at six months of exposure, and the protein carbonyls showed progressive increase until twelve months of exposure. However, no statistically significant changes were found regarding the genotoxicity biomarker. Therefore, the findings highlight that a relative short exposure to high concentrations of inhalation anesthetics leads to detrimental effects in young professionals occupationally exposed. This study contributes to the occupational and environmental fields and emphasizes the need to further minimize this occupational exposure. *Financial Support: Brazilian agencies CNPq and CAPES.*

PS P243 - 0107 In Vitro Genotoxicity of TEMPO and Its Derivatives

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TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl), one of the most commonly used members of the nitroxide family, is a stable nitroxyl radical that has a variety of uses related to its role as radical scavenger and stabilizer. However, under certain conditions, TEMPO can have pro-oxidant activity and may induce adverse effects. We investigated the cytotoxicity and genotoxicity of TEMPO in mammalian cells using the mouse lymphoma assay (MLA) and *in vitro* micronucleus assay. Our results indicated that TEMPO is mutagenic in the MLA and induces micronuclei and hypodiploid nuclei in TK6 cells. TEMPO also induced apoptosis, as demonstrated by increased caspase-3/7 activity, an increased proportion of annexin V stained cells, and decreased expression of anti-apoptotic proteins including Bcl-2, Bcl-xL, and Mcl-1. 4-Methoxy-TEMPO, a TEMPO derivative, also caused time- and concentration-dependent intracellular ROS generation and glutathione depletion. 4-Methoxy-TEMPO induced DNA damage characterized by increased levels of DNA tail intensity and expression of γ -H2AX, p-CHK1, and p-CHK2 proteins. In addition, we quantified the genotoxicity of TEMPO and three of its derivatives (4-hydroxy-TEMPO, 4-oxo-TEMPO, and 4-methoxy-TEMPO). All four nitroxides caused DNA strand breakage. The mode-of-action (MoA) analysis by a multi-endpoint DNA damage pathway assay classified all four nitroxides as clastogens. These results suggest that TEMPO and its three derivatives are cytotoxic and mutagenic in mammalian

cells through a mechanism that involves strand breakage and large alterations to DNA. The potency rankings indicate that the different TEMPO derivatives vary in their mutagenic and DNA-damaging potential.

PS P244 - 0113 Genome-Wide Characterization of Mutations Induced by Genetic Carcinogens Using Next-Generation Sequencing

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Mutations are heritable changes in the nucleotide sequence of DNA that can lead to many adverse effects, such as cancers. Genotoxicity assays have been used to identify chemical mutagenicity and carcinogenicity. Currently recommended mutation assays, such as the Ames test and mouse lymphoma assay, predict mutagenicity of test agents in the genes that allow mutant cells to be positively selected when mutations occur in the genes. These assays only detect mutations related to the genes, but not the whole genome. The mutations induced by the test agents may bias to certain types of mutations due to the target genes' natures. Although the assays have been used for many years, a new mutation assay that can directly measure all types of mutations in the genome has been expected for a long time. Recently developed next-generation sequencing (NGS) technology allows us to detect genome mutations in the cells directly. In our laboratory, we have used the whole genome sequencing method to screen mutagens using *Salmonella typhimurium* TA100 cells, a bacteria system, to detect germ line mutations in *Caenorhabditis elegans*, a worm system, and to evaluate mutational spectra in mouse lymphoma cells, a mammalian system. The results show that NGS technology can sensitively detect mutation induction caused by genetic carcinogens and effectively evaluate the different types of mutations, including base pair substitutions, insertions and deletions (indels), loss of heterozygosity, and chromosome number changes, suggesting that the unparalleled advantages of NGS for evaluating mutagenicity of chemicals can be applied for the next generation of genotoxicity tests.

PS P245 - 0114 Quantitative Comparison of In Vitro Genotoxicity between Metabolically Competent HepaRG Cells and HepG2 Cells Using the High-Throughput High-Content CometChip Assay

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In vitro genotoxicity testing that employs metabolically active human cells may be better suited for evaluating human *in vivo* genotoxicity than current bacterial or nonmetabolically active mammalian cell systems. In the current study, 28 compounds, known to have different genotoxicity and carcinogenicity modes of action (MoAs), were evaluated over a wide range of concentrations for the ability to induce DNA damage in human HepG2 and HepaRG cells. DNA damage dose-responses in both cell lines were quantified using a combination of high-throughput high-content (HTHC) CometChip technology and benchmark dose (BMD) quantitative approaches. Assays of metabolic activity indicated that differentiated HepaRG cells had much higher levels of cytochromes P450 activity than did HepG2 cells. DNA damage was observed for four and two out of five indirect-acting genotoxic carcinogens in HepaRG and HepG2 cells, respectively. Four out of seven direct-acting carcinogens were positive in both cell lines, with two of the three negatives being genotoxic mainly through aneugenicity. The four chemicals positive in both cell lines generated HTHC Comet data in HepaRG and HepG2 cells with comparable BMD values. All the nongenotoxic compounds, including six nongenotoxic carcinogens, were negative in HepaRG cells; five genotoxic noncarcinogens also were negative. Our results indicate that the HTHC CometChip assay detects a greater proportion of genotoxic carcinogens requiring metabolic activation (i.e., indirect carcinogens) when conducted with HepaRG cells than with HepG2 cells. In addition, BMD genotoxicity potency ranking is useful for quantitatively evaluating CometChip assay data in a scientifically rigorous manner.

PS P246 - 0128 Thai Desserts and Snacks Reduce the Formation of Mutagen, Mutagenesis, and Their Antioxidant Activity

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Thai desserts are made from various natural ingredients and colors that are a good source of antioxidants. In this study, we evaluated anti-formation of mutagen and anti-mutagenesis of ten types of Thai desserts and their recipes development using the Ames test (*Salmonella typhimurium* reverse mutation assay). 1-Aminopyrene interacted with sodium nitrite used as a model that produces direct mutagens and their mutagenicity in *Salmonella typhimurium* strains TA98 and TA100 of the Ames test. We found that all of sample extracts, namely, santol compote (Kra Thon Loi Kaew), pumpkin cake (Khanom Fak Thong), black sticky rice pudding with taro (Khao Niew Dam Piak Phuek), crispy flower (Dok Jok), mung bean rice crepe (Tua Paep), steamed Thai dessert made of mixture of flours and syrup (Nam Dok Mai), black coconut sweet pudding (Piak Pun), taro paste yolk (Med Kanoon Sai Phuek), fruit-shaped mung beans (Luk Chup), and crispy gelatin (Wun Krob), were not toxic and mutagenic on both tester strains. For anti-formation of mutagen approach, 3 out of 10 Thai desserts, namely, Khao Niew Dam Piak Phuek, Dok Jok, and Med Kanoon Sai Phuek, had strong activity through TA98. In evaluating the anti-mutagenicity of each sample, we found that Med Kanoon Sai Phuek moderately inhibited the activity on both tester strains while Dok Jok and Tua Paep did only on TA 98. Additionally, each extract from 10 Thai desserts was confirmed antioxidant activities by DPPH scavenging and FRAP reducing power assays. It was noted that some samples (e.g., Nam Dok Mai, Luk Chup, and Wun Krob) that posed no inhibitory effects in the Ames test could be modified to be active in anti-formation of mutagen and anti-mutagenesis by incorporating some natural colors, namely, sappan color. This evidence suggests that we could modify the anti-mutagenicity and antioxidant activities of any samples using natural colors. Our findings indicate that the ingredients of Thai dessert provide some phytochemicals posing their antioxidants that scavenged mutagen and/or could alter bacterial mutagenesis.

PS P247 - 0161 Fate of Micronuclei and Micronucleated Cells

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Micronuclei arise during mitosis and are a sign of genomic instability. Although there is detailed knowledge about the formation of micronuclei, comprehensive studies about the fate of micronuclei and micronucleated cells are lacking. Several possibilities have been proposed: extrusion, reincorporation into the nucleus, degradation, persistence of micronuclei and/or cells, apoptosis, and chromothripsis. A long-term live cell imaging study in Hela-H2B-GFP cells was conducted to reveal which micronucleus fates are most relevant. A variety of genotoxic agents like tert-Butyl hydroperoxide, vinblastine, etoposide, or radiation in different doses have been used to clarify, if different treatments result in different fates for micronuclei or micronucleated cells. Cell death, mitosis, and persistence of the cell as well as the formation of micronuclei were monitored for the duration of several cell cycles. Timing and duration of these events were analyzed. Preliminary results with tert-Butyl hydroperoxide show that most frequent fates for micronuclei are persistence and reincorporation; other fates such as extrusion and degradation seem to occur only rarely, if at all. Results for other genotoxic agents under various experimental conditions will be presented. These findings will contribute to the important question whether genomic instability in the form of micronuclei and micronucleated cells is primarily cause or consequence in the context of carcinogenesis and chromothripsis.

PS P248 - 0180 Comprehensive DNA Analysis for DNA Modifications and Reporter Gene Mutation Assay to Investigate Genotoxicity of Elemicin and Its Mechanisms Using gpt Delta Rats

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Elemicinis, a natural alkoxybenzene compound, is a flavor component of several plant species such as nutmeg and mace. Our previous data demonstrating significant increases in the number and areas of GST-P-positive foci in the livers of rats treated with elemicin at a dose of 400 mg/kg/day for 13 weeks indicated that elemicin was a possible hepatocarcinogen. In the present study, to clarify genotoxicity of elemicin and its mechanisms, we carried out comprehensive DNA adduct analysis by liquid chromatography with tandem mass spectrometry (LC-MS/MS) and reporter gene mutation assay in the livers of *gpt* delta rats treated with elemicin by gavage at doses of 0, 25, 100, or 400 mg/kg/day for 13 weeks. Comprehensive DNA adduct analysis demonstrated the existence of five spots as putative DNA adducts at m/z 434, 458, 474, and 496 in the livers of rat given 400 mg/kg bw. In addition, MS spectrum analyses showed that all spots included the fragment ion peaks showing loss of deoxyribose ([M+H-116]⁺) and m/z207 corresponding to 2-allyl-3,4,5-trimethoxybenzene structure, indicating that those spots originated from elemicin-derived deoxyguanosine (dG), deoxyadenosine (dA), and deoxycytidine adducts. Peaks of all adducts were detected from low-dose group by multiple reaction monitoring (MRM) analysis, and those areas were increased in a dose-dependent manner. Significant increases in *gpt* mutant frequencies in the livers were detected in rats given 100 mg/kg/bw and above. Mutation spectrum analysis of *gpt* mutants showed that mutation frequencies of A:T-T:A and G:C-C:G transversion and G:C-A:T and A:T-G:C transition were significantly increased in rats given 400 mg/kg bw. Characteristic fragment ions observed in MS spectrum analyses strongly suggested that all spots originated from elemicin-modified nucleoside. It is highly probable that these DNA adducts formation leads to increases in *gpt* mutant frequencies. The fact that mutations of A:T site were predominant independently of the amount of modified base each might imply that mutagenic potential of elemicin-derived dA adducts is higher than that of dG adduct. Overall data suggest that elemicin is able to form the specific DNA adducts with mutagenic potentials.

PS P249 - 0191 Arecoline, an Alkaloid of Betel Quid, Inhibits p53-Mediated DDB2 Expression and DNA Repair in Human Head and Neck Cancer Cells

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Arecoline is the major alkaloid in areca nut, which is carcinogenic to human (IARC class I). Areca nut is an essential constitute of betel quid (BQ) and is the fourth most common addictive substance in the world. Epidemiological studies show that BQ-chewing significantly contributes to the occurrence of head and neck cancer (HNC). Arecoline exhibits obvious genotoxic activity, such as induction of DNA damage, chromosome breaks, and micronucleus formation; however, the underlying mechanism is not fully elucidated. Previously, we have reported that arecoline (0.3 mM) inhibits nucleotide excision repair (NER) in a p53-dependent manner. Here we further investigated the effect of arecoline on p53-regulated NER genes, DDB2 and XPC. Gene expression was examined using western blot and quantitative RT-PCR. NER capacity was measured by host cell reactivation (HCR) assay. The effect of arecoline on p53's DNA-binding and transactivation domains was elucidated by luciferase-reporter and chromatin immunoprecipitation (ChIP) assays. A cell model under long-term arecoline exposure was established by daily repetitive treatment of arecoline (6–8 h/day for 60 days). DDB2 expression in BQ-exposed HNC specimens was evaluated using quantitative RT-PCR after approval of Institute Review Board. RNA sequencing data was retrieved from the Cancer Genome Atlas (TCGA) data portal and analyzed using SPSS software. Arecoline (0.3 mM, 24 h) specifically inhibited the expression of DDB2, but not that of XPC in HNC cells. HCR assay showed that ectopic DDB2 expression restored NER activity in arecoline-treated cells, suggesting that DDB2 downregulation was critical for arecoline-mediated NER inhibition. Luciferase-reporter and ChIP assays showed that arecoline inhibited p53-induced DDB2 promoter activity through the DNA-binding but not transactivation domain. Both NER and DDB2 promoter activities declined in the arecoline long-term exposed cells. DDB2 mRNA level was downregulated in

HNC specimens with a history of BQ exposure but not in those without BQ exposure (TCGA cohort). Arecoline inhibits NER capacity through downregulating 53-regulated DDB2 expression, which may contribute to HNC development. Financial support: MOST106-2320-B-037-001-MY3.

P250 - 0324 Toward *In Vitro* Approaches Linking Chemical Exposure to Mutagenesis Risk

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DNA adducts are a source of mutation due to their altered recognition by DNA polymerases during replication and are therefore candidates for early biomarkers to predict mutagenesis risk. However, the causal link between DNA adduct location and type with mutation patterns found in cancer genomes is highly complex and not generally predictive. A limitation to understanding this relationship is the confined availability of *in vitro* models for characterizing both DNA adduct formation and immortalization due to accumulation of specific mutations in key driver genes. The goal is to find a suitable cell model to bridge the gap between DNA damage distribution and formation of subsequent mutations. Mouse embryonic fibroblasts (MEF) are known to immortalize upon carcinogen treatment. Benzo[a]pyrene (BaP), a ubiquitous environmental and tobacco-relevant carcinogen, was chosen as a starting point to validate the model. We observed a decrease in primary MEF viability, with BaP concentration ranging from 0.1–20 µM exposed for two days or six days, including two days of recovery. Surprisingly, no activity was detected for CYP1A1 and CYP1B1 metabolic enzymes, reported to be responsible of BaP transformation into the carcinogenic epoxide BPDE, but BPDE-dG DNA adducts nonetheless formed. These data observations suggest the activity of alternative metabolic pathways. BPDE-dG DNA adducts were quantified using liquid chromatography coupled to tandem mass spectrometry, and there was a dose-dependent increase in BaP-derived adducts. Furthermore, following BaP exposure, we confirmed that chemically treated MEF immortalized faster and more often than the negative control group. These results support the use of the MEF *in vitro* model for relating DNA damage with mutation outcomes as a basis to understand chemical mutagenesis.

P251 - 0334 The Impact of OPRM1 A118G Polymorphism on Addiction in Turkish Population

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Some single nucleotide polymorphisms in particular genes may affect the tendency to alcoholism and opiate addiction, subsequent relapse and their optimum treatment. The aim of our study was to assess the frequency of OPRM1 A118G gene polymorphism in a Turkish population and to investigate whether OPRM1 A118G gene polymorphism was associated with opiate and other substance addiction. In addition, we examined the association between OPRM1 A118G polymorphism and psychiatric patients as a risk factor for addiction. A hundred and three addicted patients to opiate, cocaine, ecstasy, alcohol, Lysergic acid diethylamide (LSD), cannabis and sedative/hypnotic substances were included in the study. Eighty three healthy volunteers with similar demographic features participated in our study as a control group. Addicted patients were examined and assessed by a psychiatrist specialized in substance abuse and all subjects conformed to the criteria for opiate dependency. The control subjects did not have any personal or familial history of alcohol addiction. After the informed consent, ten milliliters of peripheral blood was collected in a sterile EDTA container via venipuncture from each subject to determine OPRM1 A118G gene polymorphism by Polymerase chain reaction-restriction fragment length polymorphism assay. DNA was extracted from the peripheral blood and PCR-RFLP was performed for the amplification of A118G polymorphism. The 193 bp fragments were obtained and digested. The digested fragments were separated by electrophoresis. Consequently, the A118A, A118G and G118G genotypes resulted in 193 bp; 193 and 169 bp; and 169 bp respectively. A statistically significant difference was observed on genotype frequencies between addicted (32.0%) and control group (16.9%). G allele in addicted Group was 16.1% and 8.4 % in Control Group. A118G polymorphism in OPRM1 gene is widely studied for addiction disease, mediating the rewarding effects of addicted substances. We found the difference between groups was significant

p=0.031. According to our results; there was a significant association between OPRM1 A118G gene polymorphism and substance addiction. References: [1] Kapur S, Sharad S, Singha RA. A118G Polymorphism in Mu Opioid Receptor Gene (OPRM1): Association with Opiate Addiction in Subjects of Indian Origin. Journal of Integrative Neuroscience 2007; (6):511–522. [2] Kim SG, Kim CM, Kang DH Association of functional opioid receptor genotypes with alcohol addiction in Koreans. Alcohol Clin Exp Res 2004b; (28):986–990.

P252 - 0423 A Comparative Study of the Genotoxic Effects of the Neonicotinoids Nitenpyram and Imidacloprid in Human Cell Line U937 Utilizing a Novel Statistical Approach to Evaluate Comet Data

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Neonicotinoids are nicotine derivatives used in insecticides and veterinary antiparasitic medicines. They are nicotinic acetylcholine receptor (nAChR) agonists which is considered to be the mechanism of action behind their acute neurotoxicity. Since the neonicotinoids have much greater affinity toward the invertebrate nAChR than the vertebrate counterpart they have been considered safe for use against pests in plants and animals. However, their potential for low dose induced DNA-damaging effects has not been fully explored and it remains unclear if human metabolism results in bioactivation or detoxification. It has previously been shown that 2-chloropyridine, a common substructure of neonicotinoids, may introduce DNA-damage in the presence of rat S9 fractions. The DNA-damage may be triggered by interactions with 2-chloropyridine and one of its N-oxidized metabolites. Moreover, epoxides and quinones are common DNA-damaging metabolites of halobenzene derivatives and may also potentially be formed. However, their potential for low dose induced DNA-damaging effects has not been fully explored and it remains unclear if human metabolism results in bioactivation or detoxification. In this work the DNA-damaging effect in the human cell line U937 of two neonicotinoids Imidacloprid (N-[1-[(6-Chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl]nitr- amide) and Nitenpyram ((E)-N-(6-Chloro-3-pyridylmethyl)- N-ethyl-N'-methyl-2-nitrovinylidenediamine) was investigated at a 5–50 µM concentration range using the alkaline single-cell gel electrophoresis, also known as the comet-assay; a method for measuring single-, double-strand and alkali-labile sites breaks in individual cells. It is well known that comet assay data is difficult to analyze properly because the distribution of the data is highly skewed, often contains large amounts of zero values and does not follow standard parametric distributions even after data transformation. Therefore, in this work, we propose using a proportional odds model tailored to continuous outcomes as a statistical evaluation method of the comet data. The model does not need any transformation of data and can take the hierarchical structure of the data into account thus eliminating the need of data aggregation. Our study did not support previous evidence that the neonicotinoids Imidacloprid or Nitenpyram trigger DNA-damage, nor that they are subjected to bioactivation resulting in DNA-damaging metabolites via the human metabolic S9 system.

P253 - 0500 The Reproductive Toxicity of ZishenYutai Pill on Rats and Offspring in Perinatal Period

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ZishenYutai Pill (ZYP) is one of the most commonly used Chinese medicines in prevention of early pregnancy loss due to threatened and recurrent miscarriage. Although it is widely used, its toxicity during perinatal period has not been well understood. Its main components include Radix Codonopsis, Radix Dipsaci, Polygoni Multiflori, Atractylodes macrocephala, Morinda officinalis, Eucommia Ulmoides, Semen Cuscutae and Radix Rehmanniae Praeparata. In recent years, the research on ZYP has been increased year by year. Previous studies have shown that ZYP combined with phloglucinolysis significantly effective in the treatment of threatened abortion. Pregnant rats (F0) were exposed to 6g/kg, 12g/kg and 24g/kg body weight/d of ZYP by intragastric administration from gestation day 15 (GD₁₅) to through parturition and lactation up to weaning, i.e. post-natal day 21 (PND₂₁). Water and propylthiouracil (PTU, 15mg/kg) were used as the negative control and positive control, respectively. The mating was done between the treatment (ZYP or PTU) group and negative control group when the F1 pups were born 63 days. The reproductive capacity of F0 and F1 generation decreased significantly after PTU exposure ($P < 0.05$);

however, the body weight and reproductive ability of F0, the physical development and feed consumption of F1 as well as the reproductive ability and survival rate of F2 rats were not significantly changed in the ZYP group compared with the negative control group ($P > 0.05$). There was no statistically significant evidence of perinatal toxicity under ZYP exposure.

P254 - 0518 Repeated-Dose Toxicity Study and Explore for Safety Assessment of *Thesium chinense* Particles in Juvenile Rats

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The objective was to explore the non-clinical safety evaluation method of Chinese herbal medicine in weaning rats, and to observe the toxic effects of *Thesium chinense* extraction powder administered for 4 weeks on weaning SD rats. One hundred and twenty juvenile SD rats were randomly divided into 4 groups with 30 of each gender in each group. The rats were orally administered with *Thesium chinense* extract at doses of 0, 0.6, 9.0, and 15.0 g $\cdot$ kg⁻¹ $\cdot$ d⁻¹ qd from PND₂₁ for 4 weeks respectively. Two thirds of the animals were killed immediately after the last administration, and the others were observed for 2 weeks after medicine withdraw. Factors including animal age and the choice of developmental toxicity parameters were explored to address strategies for juvenile toxicity assessment. Parameters were examined including general condition, body weight, food intake, development index, hematological and biochemical index, blood coagulation parameter, urinalysis value, immune system, hormone, organ coefficient, and histopathological examination. The juvenile rats of both sexes in the high dose group had changes in growth and development indexes (male crown-rump length decreases, female vaginal opening time delay), hematology indexes (RET^a reticulocyte count and RET % obvious increase trend), organ weight, the ratio of visceral weight to body weight, the ratio of visceral weight to brain weight (thymus decrease, kidney or liver weigh increase) in both sexes at 15.0 g $\cdot$ kg⁻¹ $\cdot$ d⁻¹ and significant decrease in body weight, blood biochemical (ALT, BUN and TBIL) in male rats. The influence of the tested substances on male juvenile rats was more obvious. The influence can be reversed. Under the test conditions, the no observed adverse effect level (NOAEL) of *Thesium chinense* particles for juvenile rats was 9.0 g $\cdot$ kg⁻¹ (equivalent to 164.9 g crude drugs $\cdot$ kg⁻¹) when administered by gavage for 4 weeks, which is 32.3 times of rat dosage and 194 times of clinical dosage.

P255 - 0827 CRISPR-Cas9 Generated Appb Zebrafish Mutants with Early Developmental Defects and Behavioral Alterations in Adulthood

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The amyloid precursor protein (APP) plays a central role in the pathophysiology of Alzheimer's disease due to its sequential proteolytic cleavages that generate toxic β -amyloid peptide (A β). Although APP is expressed widely in the central nervous system, its physiological function in the development and maintenance of the central nervous system remains elusive. Our previous studies show that the zebrafish APP homologue, Appb is strongly expressed during early development. However, thus far, its role during neuronal development has not been studied thoroughly through knockout strategy. In this study, we characterized zebrafish with a homozygous *appb* mutation for the first time generated with CRISPR/Cas9 technology. We studied the developmental abnormalities by phenotypic analysis and behavioral changes by monitoring the locomotor activity. We report that *appb* mutants were significantly smaller at early developmental stages and adult mutants displayed anxiolytic behavior in novel tank diving test. Embryos displayed disturbed cell adhesion phenotypes at blastula stage which was the result of defects in cellular integrity and weak cell-cell adhesion. Moreover, *appb* mutants that survived beyond 48 hpf developmental stages did not show obvious morphological defects and exhibited no behavior defect at 6 days post fertilization. While investigating the reason, we found the altered expression of other *app* family members; *appa* and *apl2*.

All together, these results indicate that the loss of *appb* results in early aberrations in the development that strongly indicate a requirement of *appb* in cell adhesion during development, however the genetic deficit are likely compensated by the altered expression of other App family members.

P256 - 0877 Withdrawn

P257 - 0879 Withdrawn

P258 - 0906 Evaluation of Black Cohosh Extract for Genotoxicity and Epigenetic Alterations Using the CometChip Assay

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Black cohosh extract (BCE) is marketed to women as an alternative to hormone replacement therapy for alleviating symptoms of menopause. Due to widespread use of BCE and lack of toxicity data, the National Toxicology Program (NTP) is evaluating BCE for toxicological effects in rodents and in humans. While results of the NTP 2-year chronic carcinogenicity study are pending, results of subchronic studies revealed increased erythrocyte micronucleus (MN) frequencies in peripheral blood of female mice and rats. Follow-up tests showed increased MN frequencies in human lymphoblastoid TK6 cells, particularly when cells were cultured in physiological (~120 nM) levels of folate. In the present study, we evaluated DNA damage, epigenetic alterations, and large deletion mutations in human and rodent cell lines. Human HepG2, HeLa-S3 and TK6 cells were cultured in media with folate ~2600 nM (standard) and ~120 nM (physiological) concentrations and exposed to 0-250 μ g/ml BCE. Higher doses of BCE induced significant increases in % tail DNA in TK6 cells cultured in standard folate media following both 4 and 24 h exposures ($P < 0.01$), whereas DNA migration was not increased in HepG2 or HeLa-S3 cells cultured under similar conditions. Physiological folate level in culture media did not enhance the level of DNA damage induced by BCE in TK6 cells. BCE (250 μ g/ml) also induced a significant increase ($P < 0.01$) in Spi- mutant frequencies in the mouse lung fibroblast cell line, GDL-1. Studies using the Epicomet assay to measure alteration in global DNA methylation showed no significant change in BCE-treated human cells and these results were confirmed by Methylated DNA Immunoprecipitation (MeDIP) quantitative PCR analysis of long interspersed nucleotide elements (LINE) 1 analysis. These results indicate that BCE is genotoxic *in vitro* and additional studies to define its potential toxicity to humans are warranted.

P259 - 0922 The Comparative Evaluation of the Genotoxicity of Different Titanium Dioxide Particles Using *In Vitro* Comet Assay

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The consumption and exposure of nanomaterial products is also increasing. Especially, titanium dioxide (TiO₂) is an important inorganic compound which is widely applied in real life, for example, as a white pigment, plastic, paint, rubber and UV filter in cosmetics. However, the potential risk of nanomaterials is not fully understood. It is required to propose the method for the toxicity of them. In this study, we aimed to evaluate and compare the genotoxicity of four nanomaterials in the way of quantified genotoxic method. Nanomaterials were dispersed by sonicator for 20 minutes at 20% amplitude in 2% Bovine Serum Albumin (BSA) whose size was measured using dynamic light scattering (DLS). Cytotoxicity of nanomaterials in human bronchial epithelial cells (BEAS-2B) was measured by Water Soluble Tetrazolium (WST-1) assay. We assessed the genotoxicities of four types of TiO₂ (NM-101, NM-103, NM-105 and sunclear-T30). We are evaluated with the nanomaterial concentrations that 70% of cells were viable. The genotoxicity of nanomaterials was quantified by applying mathematical toxicity calculation methods of EC50, LOEC, CDI and SCDI. In the result, all nanomaterials except sunclear-T30 induced dose-dependently the cytotoxic responses. TiO₂(NM-101) did not show any cytotoxicity until 1000 μ g/ml, LC70

of NM-103 and NM-105 were evaluated, which were approximately 300 µg/ml and 800 µg/ml, respectively. In addition, all TiO₂ caused significant DNA damage. Sunclear-T30 showed intense DNA damage even though the concentration of treatment was much lower than other TiO₂. In quantified genotoxic value, sunclear-T30 induced the strongest genotoxicity in all value.

P260 - 0032 Either IL-4 Administration or Zinc Supplementation Mitigates Aggravated Inflammatory Response Associated with Increased White Blood Cells in Zinc-Deficient Rats

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Nutritional zinc deficiency leads to immune dysfunctions such as inflammatory diseases. We previously elucidated the cause of inflammatory responses related to Th2 lymphocytes and M2 macrophages via the loss of GATA-3 or interleukin (IL)-4. Furthermore, previous data on inflammatory response suggested that WBC and platelet numbers increased with zinc deficiency. Here, we investigated whether the increased immune cells in the blood due to zinc deficiency could be either inhibited by IL-4 administration or recovered by zinc supplementation. Five-week-old male SD rats were fed a standard diet (17 g/day), and two groups were fed a zinc-deficient diet (n = 7 each). Each group was also injected with saline or IL-4 (zinc deficient/IL-4 i.p.). The SD rats were also fed a zinc-deficient diet for 6 weeks but were returned to the standard diet for 4 weeks thereafter (zinc deficient/standard). After the dietary manipulation, the number of WBCs, RBCs, Hb, Hct, MCV, MCH, MCHC, and platelets in the blood was determined using a cytometer. Furthermore, the measurements of CD3, CD8 (cytotoxic T lymphocyte), CD4 (helper T lymphocyte), CD11b, and granulocytes (neutrophils) double positive cells were done using flow cytometry. The number of WBCs and platelets in the blood was significantly higher in the zinc-deficient group than in the other groups. Conversely, the number of RBCs, Hb, Hct, MCV, MCH, and MCHC in the blood was significantly lower in the zinc-deficient group than in the other groups. The population of cytotoxic T lymphocytes in the blood was significantly decreased in the zinc-deficient group compared with that in the other groups. In contrast, the population of helper T lymphocytes and neutrophils was significantly increased in the zinc-deficient group compared with that in the other groups. Thus, we suggest that the inflammatory states resulting from zinc deficiency may be related to the effects of hematocyte and immune cells in blood. The previous study data demonstrated that inflammatory response in the spleen caused by zinc deficiency could be inhibited with either IL-4 administration or zinc supplementation. Therefore, hematocyte and immune cells in the blood caused by inflammatory response of zinc deficiency could be recovered by IL-4 administration or zinc supplementation.

P261 - 0072 Development of Th1-Related Diseases during Omalizumab Treatment: A Case Series of Severe Asthma

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Asthma is a common chronic respiratory disorder characterized by allergic airway inflammation, which is primarily a Th2-weighted process in many patients. Omalizumab is a recombinant humanized monoclonal antibody that selectively binds to free immunoglobulin E (IgE) and inhibits the binding of IgE to high-affinity receptors, which significantly improves disease control in terms of decreasing the rate of exacerbations. However, the potential side effects of omalizumab are unclear in asthma patients. Between July 2009 and July 2018, 269 adult Japanese patients with severe asthma received omalizumab in National Hospital Organization Sagami Hospital (Kanagawa, Japan). Four patients with severe asthma who received omalizumab treatment developed a Th1-related disease: sarcoidosis (n = 2) or polymyalgia rheumatica (PMR; n = 2). Case 1 was of a 56-year-old never-smoker woman with a history of aspirin-exacerbated respiratory disease (AERD). After 3 months of omalizumab treatment, she complained of worsening dyspnea without wheezing. Chest computed tomography indicated ground-glass opacities, interlobular septal thickening, and centrilobular nodules. Video-assisted thoracic surgery of the right middle lobe indicated a non-caseating epithelioid cell granuloma with sarcoidosis based on histopathology. Case 2 was of an 86-year-old never-smoker woman with a history of AERD. After 36 months of omalizumab treatment, indurated a

reddish-purple nodule appeared on her face. Histopathology of the skin nodule showed non-caseating epithelioid cell granuloma with sarcoidosis. Case 3 was of a 51-year-old woman with a history of aspirin-tolerant asthma (ATA) and chronic rhinosinusitis. After 57 months of omalizumab treatment, she described morning stiffness in the shoulders, hip girdle, and neck for over an hour. According to the classification criteria of the European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR), she was diagnosed with PMR. Case 4 was of an 83-year-old man with a history of ATA. After 12 months of omalizumab treatment, he described morning stiffness in the shoulders, hip girdle, and neck for over an hour. According to the classification criteria of the EULAR/ACR, he was diagnosed with PMR. We encountered the development of sarcoidosis or PMR in 4 patients with severe asthma treated with omalizumab. Although omalizumab is an important medication in the treatment of severe asthma, physicians should pay attention to the development of Th1-related diseases in patients treated with omalizumab.

P262 - 0087 Evaluating the Pharmacodynamics and Protective Efficacy of Catalytic Bioscavenger following Subcutaneous Administration in Guinea Pigs

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Previous studies determined the guinea pig to be a viable animal model for evaluating subcutaneous (s.c.) injection as an alternative route of administration of catalytic bioscavengers, enzymes designed to render nerve agents nontoxic in systemic circulation following exposure. Recent studies have focused on evaluating the pharmacodynamics (PD) and protective efficacy (PE) of the lead bioscavenger candidate, organophosphorus hydrolase (OPH) variant, YT, in both unmodified and modified (PEGylated) versions of the enzyme. A comparative study was conducted with YT and three progressively sized PEG-YT variants (934, 935, and 936) following a single bolus s.c. dose (5 mg/kg). Bioavailability increased linearly with molecular size such that the largest sized PEG-YT variant, 936, afforded the highest bioavailability, thus promoting 936 to subsequent PD and PE studies. Dose dependence on bioavailability was also examined via three cohorts of guinea pigs receiving doses (5, 15, 20 mg/kg) of either YT or 936 in which a linear dose response was also found. Guinea pigs receiving either YT or 936 (5mg/kg) via intramuscular (i.m.) or s.c. routes had superimposable PD profiles regardless of route, suggesting an identical absorption pathway for the drugs. Protective efficacy was also evaluated by exposing the animals to 2xLD₅₀ of sarin (GB) 7 days after enzyme administration; only the animals receiving 936 (i.m. and s.c.) survived 24 hours post-exposure. Periodic s.c. dosing strategies were also evaluated with 936 in which three injections were administered either 2 or 24 hours apart for a total dose of 15 mg/kg followed by a protective efficacy assessment 8 days post-administration. Overall these data support the use of s.c. injection as a practical route of administration for catalytic bioscavenger with PEGylation, synergistically enhancing both bioavailability and extending drug half-life in circulation. The views expressed in this abstract are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the US Government. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. This work was supported by the Defense Threat Reduction Agency-Joint Science and Technology Office, Medical S&T Division.

P263 - 0088 Assessing the Skin Irritation and Sensitizing Potential of Concentrates of Water Chlorinated in the Presence of Iodinated X-ray Contrast Media

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Chemical disinfection of water provides significant public health benefits. Disinfectants like chlorine can react with naturally occurring materials in the water to form disinfection by-products. Natural levels of iodine have been reported to be too low in some source waters to account for the levels of iodinated disinfection by-products detected. Iodinated X-ray contrast media (ICM) have been identified as a potential source of iodine. The toxicological impact

of ICM present in source water at the time of disinfection has not been fully investigated. Iopamidol, iohexol, iopromide, and diatrizoate are among the ICM most frequently detected in water. In this study, source water containing one of these four ICM was chlorinated; nonchlorinated ICM-containing water samples served as controls. Reactions were conducted at an ICM concentration of 5 μ M and a chlorine dose of 100 μ M over 72 hr. Water concentrates were prepared by XAD-resin/ethyl acetate extraction and DMSO solvent exchange. We used the MatTek reconstituted human epithelial skin irritation model to evaluate the water concentrates and also assessed the dermal sensitization potential of these concentrates using the LLNA:BrdU ELISA in BALB/c mice. None of the concentrations tested (i.e., 5000X, 2500X, 1250X, 625X, and 312.5X) resulted in a skin irritant response in the MatTek skin irritation model ($n = 3$). Likewise, none of the concentrates produced a skin irritation response in mice: erythema was minimal; the maximum increase in ear thickness was less than 25% ($n = 6$). Importantly, none of the concentrates produced a positive threshold response for skin sensitization at any concentration tested in the LLNA:BrdU ELISA ($n = 6$). We conclude that concentrates of water disinfected in the presence of four different ICM did not cause significant skin irritation or effects consistent with skin sensitization at the concentrations tested. *This abstract does not represent US EPA policy.*

PS P264 - 0194 Mercury and Arsenic Disrupt Inflammatory Responses by Inhibition of Inflammation Activation

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Exposure to heavy metals can cause several diseases associated with the immune system. Although the effects of heavy metals on production of inflammatory cytokines have been previously studied, the role of heavy metals in inflammasome activation remains poorly studied. The inflammasome is an intracellular multiprotein complex that detects intracellular danger signals, resulting in inflammatory responses such as cytokine maturation and pyroptosis. In this study, we elucidated the effects of four heavy metals, including cadmium (Cd), mercury (Hg), arsenic (As), and lead (Pb), on the activation of NLRP3, NLRC4, and AIM2 inflammasomes. In our results, mercury and arsenic inhibited interleukin (IL)-1 β and IL-18 secretion resulting from canonical and noncanonical NLRP3 inflammasome activation in macrophages and attenuated elevation of serum IL-1 β in response to LPS treatment in mice. In the mechanical studies, mercury interrupted production of mitochondrial reactive oxygen species, release of mitochondrial DNA, and activity of recombinant caspase-1, whereas arsenic downregulated expression of promyelocytic leukemia protein. Both mercury and arsenic inhibited Asc pyroptosome formation and gasdermin D cleavage. Thus, we suggest that exposure to mercury and/or arsenic could disrupt inflammasome-mediated inflammatory responses, which might cause unexpected side effects.

PS P265 - 0270 lncRNA MALAT1 Interacts with Nrf2 to Regulate Septic Shock in Mouse: Molecular Mechanism and Potential Therapeutic Application

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The metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a long noncoding RNA which has been found to play an important role in regulating diverse cellular processes and pathogenesis especially tumorigenesis. Our recent studies showed that MALAT1 interacted with Nrf2/ARE as a negative co-regulator and its deletion led to activation of genes in the Nrf2/ARE pathway. Nrf2 is a pleiotropic transcriptional factor that regulates the cytoprotective genes required for resistance to oxidative stress which induces reactive oxygen species and is the common etiological factor for many diseases. Indeed, we found MALAT1 ablation led to the activation of the insulin pathway and the mice become sensitized to the hyperglycemic challenge. In the current study we found MALAT1-Nrf2 interactive axis act as a key regulator for the LPS-induced inflammatory response. Using LPS-induced septic shock and clinically relevant septic shock model induced by cecal ligation and puncture (CLP), we showed MALAT1 null mice were highly resistant to the septic shock with significantly higher survival rate and subsided "cytokine storm." We performed a time-dependent LPS challenge in peritoneal macrophage isolated from MALAT1 null and wildtype mice and found the initial proinflammatory cytokines including TNF α and IL1 β were significantly lower in the supernatant of the cultured peritoneal macrophages, as well as in the serum from the LPS-challenged mice. Consistently, the expression of proinflammatory related

genes were significantly inhibited by MALAT1 ablation. Collectively, our research indicates MALAT1-Nrf2 interactive axis regulates multiple diseases induced by oxidative stress and may be targeted for treatment of these diseases.

PS P266 - 0294 Obovatol Attenuates NLRP3, AIM2, and Caspase-11 Inflammasome Activation

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Obovatol, a biphenolic chemical originated from *Magnolia obovate*, has been utilized as traditional medicine for inflammatory diseases. Inflammasome matures inflammatory cytokines in response to intracellular danger signals and its dysregulation induces inflammatory diseases. The effect of obovatol on inflammasome activation has not been reported although its anti-inflammatory property has been progressively studied. Obovatol were treated macrophages with inflammasome triggers, and the secretions of interleukin (IL)-1 β , IL-18, and caspase-1 were measured as readouts of inflammasome activation. In addition, Asc pyroptosome formation, caspase-1 activity, and mitochondrial reactive oxygen species (ROS) production were analyzed for mechanical studies. Anti-inflammasome properties of obovatol were confirmed in animal model. Obovatol inhibited NLRP3, AIM2, and caspase-11 inflammasomes through inhibition of Asc pyroptosome formation and mitochondrial ROS generation. As well, obovatol disrupted the priming step of inflammasome activation and inhibited the transcription of inflammatory cytokines. In mice, obovatol attenuated serum IL-1 β elevation in response to monosodium urate crystals. Obovatol is suggested as an inhibitor for NLRP3, AIM2, and caspase-11 inflammasomes.

PS P267 - 0316 Red Ginseng Extracts Inhibit Cytokine Expression via TLR4-MyD88-NF-kappaB Signaling

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Ginsenosides of Korean Red Ginseng extracts (RGE) and its saponin components suppress secretion of inflammasome-mediating cytokines, whereas the non-saponin fraction (NS) of RGE oppositely stimulates cytokine secretion. Although direct exposure of NS to macrophages in mice induces cytokine production, oral administration of NS has not been studied in inflammasome-related disease in animal models. Mice were fed RGE or NS for 7 days and then developed peritonitis. Peritoneal cytokines were measured, and peritoneal exudate cells (PECs) were collected to assay expression levels of a set of toll-like receptors (TLRs) and cytokines in response to NS ingestion. In addition, the role of intestinal bacteria in NS-fed mice was assessed. The effect of pre-exposure to NS in bone marrow-derived macrophages (BMDMs) on cytokine production was further confirmed. NS ingestion attenuated secretion of peritoneal cytokines resulting from peritonitis. In addition, the isolated PECs from NS-fed mice presented lower TLR transcription levels than PECs from control diet-fed mice. BMDMs treated with NS showed downregulation of TLR4 mRNA and protein expression, which was mediated by the TLR4-MyD88-NF κ B signal pathway. BMDMs pretreated with NS produced less cytokines in response to TLR4 ligands. NS administration directly inhibits TLR4 expression in inflammatory cells such as macrophages, thereby reducing secretion of cytokines during peritonitis.

PS P268 - 0365 Gefitinib, an Anticancer Drug Inhibiting EGFR, Stimulates IL-1 β Secretion by Activating Drp1 and p38 MAPK

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Gefitinib is a molecular-targeted anticancer drug that suppresses tyrosine kinase activity of epidermal growth factor receptor (EGFR). It has been reported that gefitinib causes inflammation-based side effects, such as pneumonitis. However, the underlying mechanisms remain unknown. Here, we provide evidence that gefitinib activates macrophages and specifically promotes the synthesis of pro-IL-1 β (precursor of IL-1 β) and the release of mature IL-1 β . The IL-1 β release is mediated by the caspase-1-associated multiprotein complexes called inflammasomes, which process pro-IL-1 β into mature IL-1 β . We found that gefitinib-induced IL-1 β release was strongly abrogated by deletion of either of the components of the NLRP3 inflammasome, such as NLRP3, ASC, and

caspase-1, indicating that gefitinib promotes the NLRP3 inflammasome activation. Mechanistically, gefitinib-induced NLRP3 activation is mediated by dynamin-related protein 1 (Drp1), a large GTPase of the dynamin superfamily that has been reported to govern mitochondrial fission induced by viral infection. On the other hand, we also found that gefitinib transcriptionally upregulates pro-IL-1 β by activating p38 MAPK. Collectively, these results suggest that gefitinib facilitates the process of IL-1 β maturation at both the transcriptional and post-translational levels in macrophages. Thus, our results may provide insight into the mechanisms by which gefitinib causes unwanted inflammatory side effects.

PS P269 - 0413 Whole Body Microwave Irradiation and Rat Peritoneal Macrophages

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In the modern world, microwaves have been used for many years. Their potential health hazards have been considered as a new form of environmental pollution (WHO) and in 2011 it was classified as "possibly carcinogenic to humans" (Group 2B) by the IARC. The incomplete knowledge of RF/MW-related cancer risks has initiated the search for biological indicators sensitive enough to measure the "weak biological influence" of RF/MWs. One of the main candidates is the immune system, which is able to react in a measurable way to discrete environmental stimuli. Macrophages like phagocytes play an important role in the body's defence and immune system. It is known that the phagocytic mechanism of macrophages related with abnormal inflammation and cancer immunity will contribute to the explanation of several human diseases. To establish the potentially adverse effects of every day exposure to radiofrequency radiation (RF) on humans we performed a controlled animal study that aimed to investigate the influence of RF radiation on rat peritoneal macrophages. Eighteen adult male rats were divided into two groups of nine, sham-exposed control animals and exposed group with total body irradiation two hours daily during two weeks. A 915 MHz RF field, power density of 2.4 W m⁻² and strength of 30 V m⁻¹ was generated in a Gigahertz Transversal Electromagnetic chamber. The specific absorption rate (SAR) was 0.6 W kg⁻¹. Immediately after the last exposure, the animals were sacrificed, peritoneal cells were collected and their quantity, viability, morphology, migration, and adhesion index were determined. Phagocytic processes were examined by means of phagocytosis of latex beads by adherent and nonadherent cells and their ingestion capacity as well as through the production of intracellular free radicals-superoxide anion which is the first response in the respiratory burst. There were no differences in the number, viability and differential count of collected peritoneal macrophages. The adherence and migration index, phagocytosis capacity, both phagocytic index and phagocytic efficiency of adherent and nonadherent cells and production of intracellular free radicals-superoxide did not differ between the exposed and sham-exposed group. Mann-Whitney U-test showed no statistically significant evidence of an unfavourable effect of the applied RF radiation on peritoneal macrophages and their activity. Based on these results, we can conclude that exposure to 915 MHz electromagnetic radiation does not represent a significant risk factor for rat peritoneal macrophage and immunity.

PS P270 - 0570 Effect of Pesticide Exposure on Immune Parameters in Agricultural Workers: The Twenty Years Experience at the International Centre for Rural Health

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Pesticide exposure has been associated to several effects on the immune system, varying from a slight modulation of immune functions to the development of clinical immune disorders. The International Centre for Rural Health has long been interested in studying the effects of pesticides on the immune system. With this work, we wanted to collect all the data obtained in the last twenty years through the assessment of several serum, cellular, and functional immune parameters. Studies conducted on workers exposed to Mancozeb, propanil and very recently to glyphosate, chosen based on the evidence in the laboratory animals of effects on the immune system, demonstrate the ability of these pesticides to modulate the immune response in exposed agricultural workers compared to controls living in the same geographical area. The alterations are usually observed in absence of any sign of overt disease; in some cases, data suggest the presence of a dose effect relationship. Overall, our results point out a method adequate to reveal immune-modifications in workers occupationally exposed to potential immunotoxic compounds, based

on a whole blood assay and the collection serum parameters (i.e., cytokine profile). The prognostic significance of the observed changes is still unclear. Conclusions on the prognostic significance of the slight changes often observed will be reached only by validating the hypothesis generated by field studies with an epidemiological approach.

PS P271 - 0594 Study of Generic Tebuconazole Hematotoxic Activity after Acute Intoxication on Wistar Hannover Male Rats

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Pesticides are extensively used in agriculture today. Fungicides based on derivatives of triazole are the most widespread all over the world. Tebuconazole (TEB) is one of the most frequently used substance of this group. The aim was to assess the hematotoxic effect of generic TEB, 97 % on peripheral blood (PB), hematopoiesis in bone marrow (BM) and spleen of Wistar Hannover male rats in conditions of acute oral toxicity study. 10 healthy males of Wistar Han rats were equally divided into control (0 mg/kg/bw) and experimental groups. Dose 1700 mg/kg/bw (1/2 LD₅₀) was established experimentally and was administered once orally by gavage to 5 experimental rats. Peripheral blood was studied at 0 and 1, 3, 7 day after exposure (DAE). RBC, HGB, HCT, MCV, MCH, MCHC, WBC and PLT were measured, reticulocytes, hemogram, morphological disturbances of cells were studied. Succinate dehydrogenase (SDH) fermental activity in lymphocytes was evaluated. At 7 DAE total amount of myelocaryocytes, myelogram and splenogram with morphological assessment of hematopoietic cells were studied. Significant RBC, HCT increase, MCHC decrease due to hypoxia on 3 DAE, anemia with HGB decrease on 7 DAE were shown in PB. Established increase of erythroblasts, pronormocytes, basophilic normocytes in BM with a simultaneous decrease in the erythrocyte maturation index and reticulocytopenia in PB indicates inhibition of the late stages of erythropoiesis. Development of erythrosuppression stimulated extramedullary hemopoiesis in the spleen (increase of erythroblasts, pronormocytes). In response to anemia, compensatory neutrophilia in PB (elevated band and segmented neutrophils) and stimulation of neutrophilic granulocytopenia in BM were observed. Lymphocytopenia in PB with subsequent lymphocytosis in BM were compensatory. Activation of monocytopenia was demonstrated. SDH activity was significantly increased. Due to obtained results the generic TEB, 97 % had hematotoxic action on Wistar Hannover male rats blood in conditions of acute oral toxicity study.

PS P272 - 0596 Sex-Specific Influenza Pathology with Concurrent Chronic Arsenic Exposure in Adult C57BL/6 Mice

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Inorganic arsenic (As) is a common drinking water contaminant and immunotoxicant. Roughly 200 million people worldwide are exposed to over 10 ppb As in their drinking water—the World Health Organization's safety standard. There is mounting epidemiological evidence that exposure to arsenic is associated with increased risk of respiratory infection—infections like influenza A virus (IAV), which remain among the top ten causes of death worldwide. While a few previous studies demonstrate effects of As exposure on IAV susceptibility in mice, none to date have assessed the sex- and dose-specific effects of As on the immune system's response and the effects on IAV pathogenesis. The goal of this study is to determine the sex- and dose-associated mechanisms of As exposure on IAV disease susceptibility and pathogenesis in a mouse model. We hypothesize that exposure to As skews the immune response in a sex-specific manner, resulting in increased pathology and mortality in males and females. We exposed seven-week-old C57BL/6 male and female mice to As (sodium meta-arsenite) at 0, 10, 100 or 1000 ppb chronically via ad libitum drinking water. At 5-wk of exposure, mice were intranasally infected with 10¹ 50% tissue culture infective dose (TCID₅₀) of mouse-adapted A/California/4/2009 (H1N1) [ma2009]. Results demonstrate that exposure to As results in significant dose- and sex-dependent decreases in survival over the course of the infection. Additionally, all doses of As in female mice and 100 and 1000 ppb in male mice significantly increases clinical signs of disease pathology and mortality at 12 days post-challenge (DPC). Analysis of bronchoalveolar lavage fluid at 3 DPC reveals that IL-2 secretion is reduced in males and females exposed to As, while

many proinflammatory cytokines and chemokines associated with chemotaxis and colony stimulation of innate immune cells were significantly increased at all doses in females as well as males exposed to 1000 ppb As compared to control. We are currently investigating the source of the exacerbated innate inflammatory response and how As exposure leads to higher mortality in females compared to males. We are assessing lung viral titers at 3, 7 and 9 DPC, histopathology at 9 DPC, and performing flow cytometric immune cell profiling in the lung and mediastinal lymph nodes following infection. Our data support the sex-dependent immunomodulatory effects of As in drinking water on influenza response and pathology. These results will help elucidate the increased respiratory pathology seen in epidemiological studies of individuals exposed to high levels of drinking water As and help indicate points of intervention. *Funding: 5T32HL007534-35.*

P273 - 0659 Investigating the Role of Metallothionein and Gene Expression as Predictors of Immune Response in *Hypsibius dujardini* following Exposure to Cadmium

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This research focuses on understanding the processes leading to immunological response following exposure to heavy metals such as Cadmium, using for analysis tardigrades which are resilient, microscopic invertebrate aquatic organisms that belong to the phylum Tardigradia. Their widespread distribution makes them potential bio-indicators especially when exposed to environmental stressors. Tardigrades possess the ability to arrest their metabolic activity in response to exposure to dryness and enter an ametabolic "tun" state which allows them to tolerate exposure to various chemical and physical extremes. There is variable interspecies survival following exposure to extremes stress with terrestrial tardigrades being able to adapt faster. Various proteins have been identified as playing a role in the survival of these extremophiles. Of interest to this study are Hsp70 (heat shock protein) and damage suppressor protein (Dsup). Hsp70 proteins play a key role in recovery from stress. Dsup confers on the tardigrade the ability to tolerate extreme radiation. As invertebrates, they possess only innate immunity. Typically, when immunity is discussed, it is in response to microbial organisms and the specific responses that the host cellular mechanism mounts up against the attack. However, other non-pathogenic stimuli such as environmental stress can elicit an immune response. Heavy metals in the environment are an example of such stressors. Cadmium is a heavy metal that occurs in nature. It is an environmental toxicant that causes damage to cells via depletion of antioxidants leading to accumulation of reactive oxygen species and has also been shown to cause DNA strand break and inhibition of repair. Because there is insufficient information regarding the immune system of tardigrades, other members of the Ecdysozoa Superphylum such as *Caenorhabditis elegans* and *Drosophila melanogaster*, that have previously been sequenced with genes and pathways involved in immune response, can be used as templates for comparison to the tardigrade *Hypsibius dujardini*. In the absence of a well-defined/specialized immune pathway for tardigrades, this study proposes that the role of metallothionein in signaling immune pathways, DNA repair mechanism induced by damage from environmental stressors, and the presence of Reactive Oxygen species (ROS) can be used to demonstrate the presence of a conserved immune response. *Hypsibius dujardini* will be cultured and preconditioned before exposure to various concentrations of cadmium. DNA will be extracted and sequenced pre and post exposure. yH2AX screening and mRNA amplification will be performed.

P274 - 0674 Role of the Aryl Hydrocarbon Receptor (AhR) in Inflammatory Responses Induced by Dioxin and Persistent Organochlorinated Pesticides

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The exposure to environmental pollutants including persistent organochlorinated pesticides and dioxins is associated with chronic inflammatory conditions leading to significant adverse health effects. Numerous persistent environmental pollutants in our environment bind to the aryl hydrocarbon receptor (AhR), which controls the response of an organism to these compounds. The AhR is involved in the regulation of immune responses and has been described as a critical ligand-binding transcription factor modulating the development, differentiation and function of macrophages, dendritic cells (DC) and T cells. Different ligands of the AhR including persistent organochlorinated pesticides and dioxins have been reported to have immuno-suppressive or pro-inflammatory effects on the development of pathological immune responses and endpoints. The effects of the specific ligand and the downstream activated AhR pathway on immune regulated endpoints *in vivo* and *in vitro* is rather complex and may depend on the presence and participation of the specific cell types and their environment. In the current study we investigated the effects of various ligands of the AhR such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or Dioxin) and organochlorine pesticides including Hexachlorobenzene (HCB) and Dichlorodiphenyltrichloroethane (DDT) which have been shown to interact with the AhR signaling pathway on the expression of cytokines in TLR activated and non-activated DC. Results show, that TCDD, HCB, and DDT induce the expression of CYP1A1 in DC. The expression of interleukin (IL)-1 β and cyclooxygenase 2 (COX-2) was also significantly induced by the treatment with TCDD, HCB, and DDT. However, in TLR activated DC we found antagonistic and agonistic effects of AhR ligands depending on the TLR ligand and the target gene. The TLR-mediated induction of IL-6 for instance was significantly suppressed by TCDD, HCB, and DDT whereas IL-1 β and COX-2 were further induced by AhR ligands in TLR activated DC. The results indicate that the specific TLR ligands may direct the outcome of TCDD, HCB, and DDT and the regulatory or immunogenic response in DC. Elucidating the role of AhR modulating the TLR-regulated inflammatory responses of immune cells is important to determine the possible involvement of AhR in the development of chronic inflammatory diseases induced by dioxins and persistent organochlorinated pesticides.

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P275 - 0733 Allergenic Oxidized Fragrances in Consumer Goods: Understanding the Sensitizing Potential and Related Oxidative Stress in an Epidermal Skin Environment

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Allergic contact dermatitis is the clinical outcome of skin sensitization, one of the most important occupational and environmental health issues, considered also as the most frequent expression of immunotoxicity in humans. Fragrance compounds, ingredients in up to 60% of cosmetic and household products, remain the most commonly detected allergens in consecutive dermatitis patients in Europe. Among them, allylic hydroperoxides ROOHs derived from autooxidation of natural odorant terpenes such as linalool and limonene. To improve the prediction of their toxicity in contact with the skin, upstream investigations are required to understand the molecular mechanisms involved in the allergic-inflammatory reactions induced by these compounds, this way complementing existing non-animal alternative methods for the evaluation of the skin sensitization potential of chemicals. ROOHs are suspected to form the sensitization antigenic triggers through binding to skin proteins via radical-mediated processes. Still, the link between free radicals actually formed in the epidermis and mechanism(s) of action are far from being unraveled. We present in here a new Electron Paramagnetic Resonance combined to spin-trapping methodology to identify free reactive radicals derived from ROOHs in a reconstructed human epidermis 3D model (EpiSkin) in a real life exposure situation, and examination of their properties to activate the immune system, namely dendritic cells in their natural context of keratinocytes and the epidermal skin environment. Formation of C-radicals in the epidermis model was confirmed suggesting a key role in the antigen formation process. The Coculture Activation Test (COCAT) with HaCaT keratinocytes-THP1 dendritic cells showed up regulation of CD86/CD54 in a ROOHs concentration-dependent manner and a link to the *in situ* formation of radicals could be hypothesized. For the first time, free radical formation from allergenic xenobiotics has been proved in the epidermis and can be related to the important oxidative stress promoted by chemical allergens and known to induce skin dendritic cell activation in allergic contact dermatitis disease.

PS P276 - 0734 Formation of Free Radicals from the Skin Sensitizer Ascaridole in a 3D Epidermis Model and Activation of the Innate Immune Response

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Skin contact allergy is a major public health problem and is considered as the most important immunotoxicity reaction in humans. Because of the increase in the prevalence, it is necessary to predict the sensitizing potential of all chemicals before their use in consumer products. Thus, understanding all mechanisms leading to allergic reactions is essential in order to evaluate the molecules at risk. Chemicals form antigenic triggers for skin sensitization and further contact dermatitis by binding to skin proteins through nucleophilic-electrophilic mechanisms but also by radical-mediated processes. Ascaridole (Asc), the oxidative metabolite of α -terpinene, is considered to be one of the components responsible for contact allergy to essential oils derived from *Chenopodium* and *Melaleuca* species. The endoperoxide chemical function can be degraded and form radical intermediates evolving via different mechanisms to form carbon radical intermediates together with electrophilic reactive compounds. Our aim was to understand the role of free radical versus electrophilic intermediates derived from Asc in the sensitization processes by (i) evaluating the reactivity profile towards amino acids, (ii) studying their formation in a reconstructed human epidermis (RHE) 3D model, closer to what may happen *in vivo*, (iii) evaluating the properties to activate the immune system by using the Coculture Activation Test (COCAT) with HaCaT keratinocytes-THP1 dendritic cells and THP-1 cells cocultured with RHE. The use of Electron Paramagnetic Resonance combined to spin-trapping in the EpiSkin 3D RHE model showed for the first time that C-radicals derived from Asc are formed in this epidermal-like skin environment. To our knowledge so far no publication demonstrated directly by spin-trapping the activation of this endoperoxide in the skin. Yet, radicals formed in RHE were not the reactive species towards amino acids but the electrophilic intermediates derived from radical degradation of Asc. The fact that time played a crucial role in dendritic cell activation after exposure to Asc leads us to hypothesize that this could be promoted as a combined effect of formation of electrophilic reactive species together with a depletion of keratinocytes scavenging properties. This said, the importance of danger signals from free radicals formed and also the involvement of radical oxygen species and induced oxidative stress in skin sensitization to Asc need to be considered, because innate immune activation following stress signaling may promote T lymphocyte activation/polarization.

PS P277 - 0743 Effect of Immunosuppressive Drugs on Viral Proliferation

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Immunosuppressive drugs are used to make the body less likely to reject transplanted organs or to treat autoimmune diseases. In this study, five immunosuppressive drugs including two glucocorticoids (dexamethasone and prednisolone), one calcineurin inhibitor (cyclosporin A), one non-steroid anti-inflammatory drug (aspirin), and one antimetabolite (methotrexate) were tested for their effects on viral proliferation using feline foamy virus (FFV). The five drugs had different cytotoxic effects on the Crandell-Ress feline kidney (CRFK) cells, the natural host cell of FFV, depending on their concentration. Dexamethasone pretreated CRFK cells were susceptible to FFV infection, but pretreatment of prednisolone, cyclosporin A, aspirin, and methotrexate showed obvious inhibitory effects on FFV proliferation, by reducing viral production to 29.8-83.8% of that of an untreated control when drug concentration were not toxic to CRFK cells. These results were supported by western blot, which detected viral Gag structural protein in the infected cell lysate. These results produce a correlation between immunosuppressive drugs and susceptibility to viral infections and so could alert people who are especially vulnerable to viral infection to use immunosuppressive drugs for health.

PS P278 - 0789 Ovalicin Alleviated Pruritic and Inflammatory Signaling through Inhibition of IL-31 and ROS in DH82 Cells

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Atopic dermatitis is one of the most common diseases related to the hypersensitivity. It induces pruritus and inflammation by an immunologic aberration through interleukin 31 (IL-31). Steroidal drugs are commonly prescribed for the canine atopic dermatitis, but their usage should be carefully monitored due to side effects. The effort to find alternative anti-pruritic, anti-inflammatory medication has been made for a long time. Ovalicin is extracted from *Cordyceps militaris* and has been used for dandruff, seborrheic dermatitis treatment, and agricultural insect pests. Although the potential of ovalicin to reduce scratching behavior has been studied, the detailed mechanism is not known. In the present study, DH82 cells, a canine macrophage cell line was used to elucidate the effect of ovalicin on the inflammatory signaling. Treatment of LPS-stimulated DH82 cells with ovalicin suppressed the mRNA expression of interleukin 4 (IL-4), interleukin 31 (IL-31) and interferon γ (IFN- γ). The effects were more than those of dexamethasone that was included as a positive control. In addition, increased IL-31RA and OSMR which are dimer of IL-31 receptor were significantly decreased by ovalicin treatment to LPS-stimulated DH82 cells. Furthermore, JAK2 and STAT-3 expression were also significantly suppressed. Ovalicin treatment suppressed the expression of scratching behavior-targeted genes, TRPV1 and histamine R2, through the reduction of intracellular calcium amount. These effects were similarly reproduced by treatment with JAK2 inhibitor. Ovalicin treatment showed an anti-inflammatory effect via suppression of ROS concentration. Inflammatory markers including iNOS, COX2, and NF- κ B were significantly reduced by ovalicin treatment. Our data suggest that inhibition of IL-31 pathway suppression may be the potential mechanism of anti-inflammatory effect of ovalicin.

PS P279 - 0810 Cholera Toxin Can Exert Mucosal Adjuvanticity via ERdj5 in Innate Immune Cells

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Cholera toxin (CT) is one of most strong enteric toxin in human as well as mucosal adjuvants for experimental vaccine development. However, the importance of endoplasmic reticulum (ER) molecules involved in CTA retro-translocation to induce immune responses remained to be investigated. In this study, we investigated the physiological relevance of ERdj5 in immune stimulation by CT. ERdj5 is an ER protein that may associated with reduction the disulfide linkage of CT. ERdj5-knockout (ERdj5 KO) mice had decreased production of antigen-specific IgG in the serum and IgA in the mucosal secretion after intranasal immunization with Ag and CT. Especially, IgG2c isotypes were specifically reduced in the absence of ERdj5. ERdj5 KO dendritic cells (DCs) failed to full activation with decreased expression of costimulatory molecules, such as MHC class II, CD80, and CD 86. In addition, ERdj5 KO DC secretion of pro-inflammatory cytokines, such as IL-1 β , TNF- α , and IL-6, was reduced. The cytokine signatures of several helper T cells were reduced in ERdj5 KO mice following intranasal CT immunization. However, ERdj5 KO DCs enhanced ER stress protein by CT stimulation. These results suggested that ERdj5 contributed as a decisive factor to the immunostimulatory capacity of CT via CTA1 retro-translocation.

PS P280 - 0224 Prompt Elimination of Uremic Toxin Alleviates AKI to CKD Transition

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Acute kidney injury (AKI) is a sudden loss of kidney function and inadequate recovery, including post-AKI fibrosis, will cause chronic kidney disease (CKD). Indoxyl sulfate (IS) is a protein-bound uremic toxin produced by bacterial metabolism of the amino acid tryptophan, which excreted through organic anion transporter on renal tubular epithelial cells into urinary and generally accumulates in patients with CKD. Although the adverse effects of IS on CKD pro-

gression have been well documented, its impacts on post-AKI fibrosis are still unknown. Accordingly, we investigated whether AKI induces an increased level of serum IS and its role in AKI to CKD transition. Also, the therapeutic potency of early elimination of uremic toxins by absorbent Kremsin (AST-120) was tested. In animal study, the two-step unilateral ischemia reperfusion injury with contralateral nephrectomy (hereafter abbreviated as UIRI) was used as a post-AKI fibrosis model. Briefly, C57BL/6 mice were subjected to 30 minutes unilateral ischemia reperfusion injury and contralateral nephrectomy were conducted at day 10, and then sacrificed at day 15. All mice are randomly divided into (1) Sham, (2) UIRI, (3) UIRI + indole, (4) UIRI + indole + AST-120, and (5) UIRI + AST-120. For *in vitro* investigation, HK-2 cells were treated with 1mM IS for 48 h, followed by 12 hours of hypoxia and 24 hours of reperfusion (H/R) to explore the underlying mechanism. In our animal studies, we observed an increased serum IS levels without simultaneous accumulation of small uremic toxin molecules following 10 days of UIRI. Furthermore, AST-120 administration actually reduced deterioration of renal function caused by UIRI combined with high protein intake. We also revealed the possible mechanisms underlying the IS-enhanced proximal tubule damage after AKI. IS exposure potentiated H/R-induced G2/M cell cycle arrest, which also aggravated ER stress-mediated epithelial mesenchymal transition (EMT), as demonstrated by 4-PBA-treated rescue experiments. Our results support the pathological role of uremic toxins in post-ischemic AKI fibrosis, and low protein diets would be recommended to prevent advanced kidney disease progression. Furthermore, elimination of IS by AST-120 is likely to prove useful for the prevention/treatment of post-AKI renal impairment.

PS P281 - 0328 Effect of Moon Seed Vine (*Triclisia gilentii* Staner) on Ethane-1,2-Diol-Induced Urolithiasis and Its Renotoxicity in Wistar Albino Rats

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The prevalence of renal toxicity and nephrolithiasis as a result of exposure to environmental toxicant and drugs is of the increase. Ethane-1,2-diol had been used to model nephrolithiasis in animals as a source of environmental exposure to chemicals and nephrolithiasis model. The present study was undertaken to explore the efficacy of *Triclisia gilentii* a folkloric therapy in the management of renal-related ailment against ethane-1,2-diol induced model of nephrolithiasis and nephrotoxicity in rats. Phytochemical screening, LD₅₀, and *in vitro* anti-nucleation activity of aqueous methanolic leaf extract of *Triclisia gilentii* (TGME) were performed. Nephrolithiasis was induced in male Wistar rats with 1% ethane-1,2-diol in drinking water for 28 days with or without co-administration of (50, 100, and 200 mg/kg b.w. TGME or 5 µg/kg b.w. tamsulosin hydrochloride) throughout the experimental period. Urinalysis was carried out. In addition, levels of nitrogen-containing molecules (creatinine, urea, and uric acid), electrolytes (calcium, potassium, sodium, chloride, and bicarbonate) glucose, total protein, and albumin were accessed in both plasma and urine. Markers of redox status (malondialdehyde (MDA) produced, reduced glutathione (GSH) level, protein carbonyl (PC) concentration, ferric reducing antioxidant power (FRAP), glutathione peroxidase (GPx) activity, superoxide dismutase (SOD) activity), anti-inflammatory activity; myeloperoxidase (MPO) activity, and markers of membrane integrity (complex I activity, glutamine synthetase (GS) activity, Na⁺ - K⁺ ATPase activity, lactate dehydrogenase (LDH) activity), as well as histopathology of the kidney were accessed. Phenols, steroids, saponins, flavonoids, are present in the TGME with a total antioxidant capacity of (30.36 ± 1.90 (mg GAE/g extract), LD50 greater than 5000 mg/kg b.w. and *in vitro* anti-nucleation activity (IC₅₀ = 7.09 mg/ml). Calcium oxalate stone formation as a result of oxalate from ethane-1,2-diol was evident by hypocalcemia, and further electrolyte imbalance, and decreased glomerular filtration rate. Enhanced oxidative milieu in hyperoxaluria was evident by increased MDA, PC and decreased enzymatic and non-enzymatic antioxidants as well as renal membrane enzymes activities. The renal histopathological study further emphasized oxalate induced damage. The abnormal biochemical, redox electrolyte, membrane integrity and histological alterations by ethane-1,2-diol were attenuated by TGME which affirms its usage as nephroprotectant.

PS P282 - 0349 Acute Kidney Injury Associated with Ingestion of Star Fruit

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Star fruit is popular fruit in tropical countries due to both nutritional and medicinal uses, especially in diabetes mellitus due to its hypoglycaemic effects. Star fruit induced nephrotoxicity, manifesting as acute kidney injury (AKI) and neurological symptoms in renal impaired patients is well documented. We report a unique case of star fruit nephrotoxicity with acute renal failure need haemodialysis in a patient with normal renal function. A 78-years-old male with known past medical history of Hypertension, Diabetes mellitus and Hyperlipidemia with no history of diabetic nephropathy presented to the ED with hiccups, nausea and vomiting and generalized weakness. In the last 1 week, he had consumed 3 bottles of concentrated juice which was made by him from 1 kilogram of small sour star fruits. His vital signs and clinical examination were normal. Serum creatinine from baseline normal was now >1300 µMOL/L with urine full report showing trace amounts of protein. His urine culture was normal and bedside ultrasound of the kidneys did not show hydronephrosis. He was diagnosed as Acute kidney Injury secondary to starfruit induced nephropathy. He was admitted to nephrology unit and treated with Haemodialysis. His serum creatinine normalized and he was discharged uneventfully. The content of oxalate varies according to the type of star fruit, being higher in the sour type than in the sweet. Homemade and medicinal preparations of pure sour star fruit juice often have high concentrations of oxalate. Many patients with star fruit toxicity present within hours of ingestion with gastrointestinal symptoms such as vomiting and abdominal pain. These are suspected to be due to direct corrosive effects of dietary oxalates rather than systemic effects. This may be followed by a reduction in urine output due to acute kidney injury. Accumulation of the toxin caramboxin in patients with renal impairment results in the neuropsychiatric manifestations of star fruit toxicity which include hiccoughs, confusion, insomnia and seizures. Our patient had intractable hiccoughs on presentation, a possible clue to the underlying diagnosis. In Asian countries where the star fruit is consumed routinely, it is essential to prevent star fruit nephrotoxicity by educating the public and especially diabetics to avoid consuming star fruit, especially on an empty stomach or in a dehydrated state to avoid acute renal failure.

PS P283 - 0469 Characterization of Early Kidney Damage in a Child Population Exposed to a Mixture of Environmental Pollutants from Apizaco, Tlaxcala, Mexico

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Hospital discharges for kidney diseases tripled in public hospitals in Tlaxcala from 2004 to 2012. Some risk factors for the development of renal issues could be exposure to environmental pollutants. The diagnosis of chronic kidney damage (CKD) is made in the late stages of the disease, so it is necessary to use biomarkers of early kidney damage that allow to evidence of damage in apparently healthy individuals. The objective was to evaluate the relationship between exposure to environmental pollutants and the urinary concentration of biomarkers of early kidney damage in children. The methodology was a cross-sectional study in 109 children (6-15 years old). The anthropometric characteristics were evaluated; fluoride (F) was quantified using an ion selective electrode, the heavy metals were determined by ICP-MS. A general urinalysis was performed, the glomerular filtration rate (eGFR) was calculated using Bedside-Schwartz and the biomarker of renal damage concentration (KIM-1, Clusterin, a-1-microglobulin, Osteopontin, NGAL and Cystatin-C) were determined by Luminex Magpix. Individuals with abnormalities in urine samples (microalbuminuria, blood and leukocytes) were found. The average concentrations of F, potassium (K), vanadium (V), magnesium (Mg), calcium (Ca), copper (Cu), strontium (Sr), arsenic (As), and selenium (Se), exceeded the reference values. The average concentrations of the biomarkers in the population were: a-1-microglobulin 58 ng/mL, clusterin 237.2 ng/mL, cystatin-C 1.74 ng/mL, osteopontin 106 ng/mL, KIM-1 0.60 ng/mL and NGAL 5.3 ng/mL. The decreased eGFR, as well as the presence of urinary abnormalities revealed a CKD prevalence of 4.6%. The concentrations of V, As, Mg, Ca, Sr, Se, F and K above the reference values indicate a worrying exposure of the population. The analysis of

principal components grouped the V, K, As and F, the more toxic elements that were found in the population; their presence in high concentrations suggests the alteration in biodistribution and/or bioavailability of other essential or toxic substances, causing early kidney damage that was revealed by the presence of specific biomarkers in urine. *Funded by Conacyt (grant 239689) and Secretaría de Salud de Tlaxcala.*

P284 - 0474 Cross-Species Assessment of Amphotericin B-Induced Renal Tubular Injury and Related Profile Changes in miRNAs, Genes, and Proteins

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We evaluated drug induced kidney injury (DIKI) biomarker panel changes in mice from C57BL/6J mice, Sprague Dawley rats, Beagle dogs and Cynomolgus monkeys following repeated exposures to Amphotericin B (AmpB) for 4 or 10 days. Amongst the microRNAs examined, miR-31-5p and miR-205-5p were found to be significantly upregulated in the urine following AmpB treatment. In AmpB-treated mice, blood urea nitrogen (BUN) on day 1 (D1); urinary albumin (Ur ALB) on D1 and D3; urinary osteopontin (Ur OPN) on D2; and urinary neutrophil gelatinase-associated lipoprotein (Ur NGAL) and kidney injury molecule 1 (Ur KIM-1) on D5 indicated early detection of AmpB-induced minimal renal medullary injury characterized by multifocal intraluminal granular eosinophilic material located in the loop of Henle. In AmpB treated rats, increases in urinary cystatin C (Ur CYS C), total protein (Ur TP), Ur ALB, and Ur OPN on D4 and increases in urinary clusterin (Ur CLU) on D4, D8 and D11 revealed cortical and medullary tubular alterations, interstitial inflammation, intratubular granular and inflammatory cell casts, acute pelvic inflammation and tubular mineralization. In AmpB treated dogs, increases in Ur ALB on D1 through D4 and increases in Ur CLU and Ur KIM-1 on D1, D2, and D4 appeared as indicators of acute multifocal necrosis, regeneration, dilatation, and mineralization. In AmpB treated monkeys, increases in serum creatinine appeared only on D11, while increases in urinary N acetyl β D glucosaminidase, urinary alpha 1 microglobulin, Ur ALB, and Ur TP appeared as early as D2 indicated early/progressive tubular damage characterized by increased intratubular crystal deposition (interstitial), increased intratubular casts and increased cortical tubular dilatation and basophilia. The miRNA expression pattern changes correlated with altered renal tubular-enriched gene/protein DIKI biomarkers, providing alternative tools when monitoring for noninvasive indicators of nephrotoxicity across species during preclinical toxicity studies.

P285 - 0617 Role of NGAL/24p3 Receptor in the Handling of CdMT-FITC along the Distal Nephron with Decreased Endocytic Capacity

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Cadmium (Cd) is filtered by the glomerulus because of the small molecular mass of most circulating Cd conjugated forms. The proximal tubule (PT) contributes to the reabsorption of Cd, mainly through Cd-metallothionein (MT), because this segment is responsible for reabsorption of components of primary urine. Although megalin:cubilin is a high-capacity receptor complex for endocytotic reabsorption of filtered proteins and has been claimed to mediate receptor endocytosis of CdMT *in vivo* to cause renal Cd accumulation and toxicity, evidence indicates that the affinity of MT to megalin:cubilin is too low to be relevant. Furthermore, some filtered proteins may bypass reabsorption in the PT, either as the consequence of their low affinity to megalin:cubilin. The high-affinity protein receptor of NGAL/Lipocalin-2/24p3 (24p3R) in the distal nephron (DT) could also contribute to protein reabsorption. For this reason, CdMT but also other Cd-conjugates are likely to reach DT when these proteins are not reabsorbed in the PT. The objective was to determine renal handling of CdMT, we evaluated in an *in vivo* model with a decreased reabsorptive capacity of PT induced by gentamicin (GM) exposure, the CdMT-FITC absorption by the 24p3R in the DT. We evaluated the administration of 10 and 40 mg of GM for 7 days via subcutaneous injection in male C57BL/6J mice over renal function.

GM did not modified the glomerular function but the protein levels of renal biomarker Kim-1 increased in a dose-dependent manner after GM treatment. Moreover, as the dose of GM increased, the expression of megalin receptor decreased in PT while the levels of calbindin-D 28k decreased in DT but only for the highest dose of GM. Under physiological conditions, double staining for 24p3R, megalin (PT marker) and desmoplakin (DT marker) revealed that 24p3R is expressed specifically in the apical membrane and cytoplasm of epithelial cells of the DT while no expression was detected in PT segment. After exposure to the highest dose of GM, a decrease in 24p3R expression was observed. *In vivo* evaluation of the reabsorption of CdMT-FITC conjugate showed that exposure to GM decreased the absorption of CdMT-FITC complex in proximal and distal tubule. It is possible that the decrease in the endocytic function of PT and DT cells is due to the decrease in the expression of both Megalin and 24p3R and that both receptors could be involved in the proteinuria observed during early stages of kidney diseases. *Funded by Fonciacyt (grant 267755).*

P286 - 0681 Dendropanax moribifera Attenuates Nephropathy via SIRT1/AMPK Pathways in Streptozotocin-Induced Diabetic Rats

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Dendropanax moribifera (DM) is a traditional folk medicine as used for prevention of various diseases including hypertension, diabetes, or inflammation. The water extract of DM is popular beverage in Korea, and their preventive and therapeutic activities for metabolic disorders have been widely reported. However, the molecular mechanism of DM against diabetic-induced nephropathy has not been clearly studied. Diabetic-induced nephropathy is one of the most common causes of end-stage renal disease (ESRD). The present study was undertaken to evaluate the protective efficacy of water extracts DM against streptozotocin (STZ)-induced diabetic nephropathy in rats. Diabetes was induced by a single intraperitoneal (i.p.) injection of STZ (60 gm/kg). DM (50 mg/kg) or metformin (50 mg/kg) were administered orally in STZ-treated rats for 4 weeks. At the end of the experiment, all rats were fasted overnight and sacrificed; serum was collected for measurement of glucose, meanwhile liver, kidney and pancreatic tissues were dissected. Antioxidant enzymes activities and oxidative stress markers were measured in the kidney tissues. Kidney injury biomarkers were measured in serum or urine of rats to characterize the STZ-induced nephrotoxicity. DM treatment significantly protected body and organ weight changes in STZ-treated groups. Glucose, lactate dehydrogenase (LDH), creatinine, alkaline phosphatase (ALP), total cholesterol, and triglyceride levels were significantly reduced in STZ-treated group followed by DM administration. In addition, serum albumin and total protein levels were exhibited the normal range as compared with STZ-treated group. DM administration significantly reduced urinary excretion of KIM-1, SBP-1, and PKM2 levels, while increasing the superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) levels in diabetic rats. DM also markedly ameliorated the diabetic-induced cytokines production and expression of fibrosis markers in the kidney. Our data suggest that administration of aqueous extract of DM protects the diabetic-induced kidney damages.

P287 - 0754 The Role of the Inducible Calbindin-D28k Protein in Advanced Glycation End Products-Associated Renal Proximal Tubule Fibrosis

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Diabetic nephropathy is a common diabetic complication associated to calcium dyshomeostasis. Previous studies suggested that diabetes-associated advanced glycation end products (AGEs) are associated with the increase of extracellular matrix (ECM) proteins synthesis and renal fibrosis induction. Protein expression of calbindin-D28k, which plays a role in the calcium reabsorption in the renal distal convoluted tubules, has been shown to be increased in diabetic kidney. Here, we found that calbindin-D28k could be induced in the proximal tubules of the *db/db* diabetic mouse kidney. The increase of AGEs and ECM proteins were also observed in the *db/db* diabetic mouse kidney. We further explored the role of calbindin-D28k in AGEs-induced human renal proximal tubule cell (HK2) injury and fibrotic changes. AGEs significantly induced the

expression of calbindin-D28k in HK2 cells, but not in mesangial cells. AGEs also significantly induced the expressions of fibrotic molecules, ECM proteins, epithelial-mesenchymal transition (EMT) markers, and endoplasmic reticulum (ER) stress-related molecules in HK2 cells. Calbindin-D28k-siRNA transfection significantly reduced the cell viability and markedly enhanced the protein expressions of fibrotic factors, EMT markers, ER stress-related molecules, phosphorylated smad 2/3, and phosphorylated p38 in AGEs-treated HK2 cells. Our findings suggest that the inducible calbindin-D28k plays a protective role against AGEs-induced ER stress and EMT induction and cell injury in renal proximal tubule cells.

PS P288 - 0914 Association of Genetic Polymorphisms in Multispecific Transporters with Mercury Nephrotoxicity in Artisanal and Small-Scale Gold Mining

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The kidneys are one of the main target organs for elemental mercury (Hg). However, in a historically gold-mining town in Colombia, we have reported that, despite statistically significant differences in blood and urinary Hg levels, kidney function monitoring parameters were found unaltered in miners compared to a control population. To investigate whether genetic variation in genes that could alter Hg toxicokinetics, single nucleotide polymorphisms (SNPs) derived from genes involved in Hg transport—either into or out of cells—were analyzed in our study population: 160 subjects from mining towns and 121 non-occupationally exposed subjects from non-mining towns. All the SNPs included were genotyped by TaqMan-based allelic discrimination assay: ABCB1 rs1202169, ABCG2 rs1885301, SLC22A6 rs4149170, and SLC22A8 rs4149182. A multivariable linear regression model was used to assess the effect of SNP on Hg levels and on two kidney function biomarkers: estimated glomerular filtration rate (eGFR) and beta-2 microglobulin (B2M) while adjusting for possible confounders. Empirical significance (P values < 0.05) was determined based on 5,000 permutations. Statistical analysis was performed with R software. Alone, none of the SNPs showed any association with blood- or urine-Hg levels. However, there was a significant interaction between the SNPs SLC22A8 rs4149182 and ABCB1 rs1202169 (P = 0.025) on urine-Hg levels; where urine-Hg is higher in individuals with rs4149182-G and rs1202169-T alleles. Regarding kidney function assessment, statistically significant associations were found for an interaction between urine-Hg levels and the epistatic pairs SLC22A6 rs4149170 and ABCB1 rs1202169 with both eGFR and B2M. Thus, when urine-Hg level increases, individuals with rs4149182-A and rs1202169-C alleles showed lower levels of eGFR (β = -84.96, P = 0.040) and high levels of B2M (β = 743.38, P = 0.0009). This correlation was observed only in the control group, but not in miners. The non-risk ABCB1 rs1202169-TT genotype was significantly found more frequently in miners than in controls (19.2% vs 8.5%; P = 0.021), although no significant difference in SLC22A6 rs4149170 genotypic distribution was found between the groups. This analysis indicates that the ABCB1 rs1202169 SNP may modulate the pathogenesis of Hg nephrotoxicity. We propose that the increase expression associated to rs1202169-C allele would favor Hg detoxication—e.g., via glutathione conjugation. However, the high levels of conjugates of Hg in the luminal space of renal tubules could induce mechanisms involved in their reabsorption and, thus, the long-lasting nephrotoxic effects of Hg.

PS P289 - 0063 Connexin Hemichannels and Pannexin Channels as Drug Targets in Liver Toxicity and Disease

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Because of its unique function in the body, it is not surprising that the liver is a frequent target in toxicity and disease. Liver pathology is typically associated with cell death and inflammation. Connexin (Cx) hemichannels and pannexin (Panx) channels have emerged as key players in a plethora of tissues in both processes over the past few years. In this research, it was investigated whether this also holds true for hemichannels consisting of Cx32 and Cx43 as well as Panx1 channels in the liver. Focus was hereby put on acute liver failure, non-alcoholic steatohepatitis (NASH), and liver fibrosis. It was found that inhibition of Cx32 and Cx43 hemichannels by using TAT-Gap24 and TAT-Gap19 peptides, respectively, in acetaminophen-induced acute liver failure counteracts inflam-

mation, while their coadministration leads to diminished liver damage in mice. Likewise, mice with diet-induced NASH treated with TAT-Gap19 or TAT-Gap24 peptides display less inflammation and steatosis. Along the same line, TAT-Gap19 peptide reduces scar formation in carbon tetrachloride-evoked liver fibrosis in mice. Furthermore, inhibition of Panx1 channels by ¹⁰Panx1 peptide or genetic ablation of Panx1 alleviates liver damage, inflammation, and oxidative stress in acetaminophen-intoxicated mice. Similar results are seen upon inducing NASH in whole body Panx1 knockout mice, while these genetically modified animals respond in distinct ways to different experimental triggers of liver fibrosis. Collectively, these results suggest that Cx32 and Cx43 hemichannels as well as Panx1 channels may represent promising pharmacological targets for the treatment of acute and chronic liver toxicity and disease.

PS P290 - 0111 Mechanistic Investigation of Anti-retroviral Related Liver Injury through the Use of Metabolomics and Lipidomics Techniques

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Drug-induced liver injury (DILI) refers to a variety of related disease states that arise from exposure to hepatotoxic pharmaceuticals. Various population studies have identified DILI occurring at rates of 13.9 to 19.1 per 100,000 patients annually. Out of all current anti-retroviral drugs used to treat HIV, 35% have been strongly linked to DILI. It is therefore important to develop a greater understanding of the mechanistic and metabolic perturbations associated with anti-retroviral exposure that lead to DILI. Metabolomics is a promising tool for investigating and developing an understanding of DILI and the mechanisms that could be responsible for specific forms of liver injury. In this *in vitro* study, HepaRG cells (differentiated hepatocytes) were utilized to assess metabolic perturbations induced by treatment with anti-retroviral drugs. MTT assays of abacavir, didanosine, nevirapine, ritonavir, stavudine, and zidovudine were performed to ascertain the dose dependency of hepatotoxicity *in vitro*, followed by metabolomics, lipidomics, and CARS microscopy studies. Briefly, HepaRG cells were drug treated for 48 hours and harvested for metabolite extraction and Liquid Chromatography Mass Spectrometry (LCMS)-based metabolomics and lipidomics analyses. Additionally, CARS (Coherent Anti-stokes Raman Spectroscopy) microscopy was performed to assess the occurrence of lipid accumulation after a 48-hour drug treatment with anti-retroviral compounds. Metabolomics analysis showed changes associated with a decrease in bile acids, including taurocholate, across several anti-retroviral compounds. Furthermore, there were significant changes observed throughout the lipidome with the levels of glycerides and phospholipids showing increases and decreases in lipid abundance respectively. CARS microscopy allowed visualization of the occurrence of lipid accumulation in the HepaRG cells following drug treatment. Drug-induced changes to metabolite levels provided insights into the mechanisms that are associated with specific forms of liver injury. Specific changes in bile acid metabolism may be indicative of cholestasis-based liver injury, and changes to lipid abundance may be indicative of steatosis-based liver injury, which is supported by the visualization of lipid accumulation by CARS microscopy. These data illustrate the role of metabolomics and lipidomics as potential tools for investigating and identifying mechanisms of DILI.

PS P291 - 0116 Population Variability in the Severity of Alcoholic Hepatitis in a Mouse Model

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Alcoholic hepatitis (AH) is a severe acute-on-chronic form of alcoholic liver disease. The etiology and pathogenesis of AH in humans is poorly understood; mortality is high in subjects who develop AH as a complication of chronic alcohol. There are few animal models of advanced forms of AH. Previously, we combined carbon tetrachloride (CCl₄)-induced liver fibrosis with chronic intra-gastric alcohol feeding in C57BL/6J mice. We observed that combined treatment with CCl₄ and alcohol resulted in severe liver injury with histopathology and transcriptomic changes similar to human AH. In this study, we tested the hypothesis that the Collaborative Cross (CC) mouse population can be used to study inter-strain differences in the severity of liver fibrosis and/or AH. Female mice from ten CC strains were treated with CCl₄ (0.2 ml/kg, 2 days/week) for 6 weeks to induce chronic liver fibrosis. Subsequently, ethyl alcohol (EtOH) (25 g/kg/day) was administered intragastrically as a continuous infusion for 4 weeks. We observed inter-strain differences in liver fibrosis in animals treated with CCl₄. Three of ten strains that were treated with a combination of CCl₄ and EtOH demonstrated a marked exacerbation of liver fibrosis, which was evident

by the development of hepatic inflammation, marked steatosis, and pericellular fibrosis. This study shows that in addition to previously demonstrated inter-strain differences in alcohol-induced steatohepatitis, we find that some of the CC strains are sensitive to the development of AH. This animal model shows that individual differences in genetic and other factors may contribute to the severity of alcohol-associated liver disease, especially the development of the severe acute-on-chronic AH.

PS P292 - 0123 The Role OATP2A1 in Hepatoprotection against CCl₄-Induced Liver Injury

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Prostaglandin (PG) E₂ has hepatoprotective effect against several liver injury by enhancing hepatocyte proliferation and regulating expression of cytokines. In general, PGE₂ is synthesized by cyclooxygenase (COX)/PGE synthase (PGES) pathway and inactivated by 15-prostaglandin dehydrogenase (15-PGDH) at cytoplasmic domain. Recently, we reported that organic anion-transporting polypeptide (OATP) 2A1, a known major PGE₂ transporter, plays a role in PGE₂ disposition in several tissues under pathological conditions. However, the role of OATP2A1 in liver injury still remains unclear. In this study, we investigated the role of OATP2A1 in regulation of hepatoprotective effect of PGE₂ in CCl₄-induced liver injury model mice. Liver injury was examined by serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. Hepatic PGE₂ levels were examined using enzyme immunoassay. RNA and protein expression of PGE₂-related gene was evaluated by qPCR and western blotting. Localization of Oatp2a1 and 15-Pgdh in the liver was examined by immunohistochemistry. Hepatic PGE₂ levels increased significantly in CCl₄-induced liver injury model in comparison with control mice. Although hepatic Cox-1, Cox-2, mPges-1, and mPges-2 were not upregulated, 15-Pgdh expression decreased significantly in CCl₄-induced liver injury mice at 24 hr after CCl₄-treatment. Moreover, hepatic Oatp2a1 were upregulated in CCl₄-induced liver injury mice. Immunohistochemical study indicated that Oatp2a1 and 15-Pgdh seem to be partially expressed in sinusoidal endothelial cells in liver. In order to clarify the role of Oatp2a1 in liver injury, suramin, a known potent inhibitor for OATP2A1, was co-treated with CCl₄. Compared with CCl₄ control, the suramin cotreated group exhibited a significant increase of plasma AST and ALT levels at 24 and 48 hr after CCl₄-treatment. These results suggest that OATP2A1 may play a hepatoprotective role by adjusting PGE₂ disposition, and hepatic PGE₂ amount seems to be regulated by downregulation of 15-PGDH in CCl₄-induced liver injury. Further investigations are necessary to clarify the role of OATP2A1 in PGE₂ disposition under liver injury condition.

PS P293 - 0142 Hepatoprotective Effect of Polyphenolic Rich Fractions from *Lauridia tetragona* (L.f.) R.H. Archer on Acetaminophen-Induced Liver Injury

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Lauridia tetragona is routinely used in traditional medicine; however, its hepatoprotective property is yet to be scientifically proven. To this effect, the hepatoprotective activity of the polyphenolic rich fractions (PPRFs) was investigated against acetaminophen (APAP)-injured HepG2 cells. The ability of the PPRF to scavenge free radicals was tested against 2,2-diphenyl-1-picrylhydrazyl (DPPH), and [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS). The ferric ion reducing power (FRAP) was also evaluated as a cell free antioxidant assay. The hepatoprotective activity was then investigated by observing the effect of PPRF against APAP-induced reduction in cell viability of HepG2 cells. Also, the concentrations of alanine aminotransferase (AST), aspartate aminotransferase (ALT) were evaluated. Thereafter, the isolated PPRFs were identified using LCMS. All the six fractions of the PPRFs isolated showed significant antioxidant properties that were evident by the effective scavenging of DPPH and ABTS radicals together with the higher FRAP. The hepatoprotective activity using the IC₅₀ was in the order of PPRF3 > PPRF4 > PPRF6 > PPRF5 > PPRF1 > PPRF2. There was also significant decrease in the levels of AST and ALT against APAP injuries. The LC-MS analysis revealed the presence of a wide variety of polyphenolics such as ellagic acid, ellagitannins, and flavonol derivatives among the dominant constituents. In conclusion, this study demonstrates that the isolated PPRFs have potential hepatoprotective activity that may be attributed to their antioxidant potential.

PS P294 - 0168 Suppressive Effect of Sesamin on Lipogenesis through Activation of CaMKK/AMPK in Hepatocytes

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Nonalcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease worldwide and may even lead to liver cancer or liver failure. Therapeutic options targeting hepatic lipid metabolism are therefore crucial to the management of NAFLD. Sesamin, a lipid soluble lignan from sesame (*Sesamum indicum*) which exerts antioxidant effects and improves lipid metabolism of the fatty liver, but the underlying mechanism is still not clear. This study investigated the effects of sesamin in reducing lipid accumulation in palmitate-treated HepG2 cells. Sesamin suppresses palmitate-induced lipid accumulation and inhibited fatty acid synthase (FASN) expression by modulating sterol regulatory element binding protein (SREBP)-1c activation in HepG2 cells. Moreover, use of the compound C (pharmacological AMPK inhibitor) revealed that AMPK is essential for suppressing SREBP-1c expression in sesamin-treated cells. Finally, sesamin induced the calcium/calmodulin-dependent protein kinase kinase (CaMKK) phosphorylation in cells. These results indicate that sesamin prevents lipid accumulation in HepG2 cells by blocking the expression of SREBP-1c and FASN through CaMKK/AMPK activation, suggesting that sesamin is a novel AMPK activator with a role in the prevention and treatment of obesity.

PS P295 - 0170 Suppressive Effects of Platycodon Acid A on TGF-β1-Induced Hepatic Stellate Cell Activation through Blocking Smad-Dependent and Smad-Independent Signal Transduction Pathways

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Platycodi radix, the root of *Platycodon grandiflorum*, has healthy effects due to its various nutritious compounds and is considered as a functional food in the Asian countries. Aqueous extract and saponin derived from Platycodi radix have been reported to show a variety of suppressive effects, including atopic dermatitis-like skin diseases, airway inflammation, osteoporosis, tumor metastasis, and liver damage. Although Platycodi radix has been studied on various bioactivities, the effect of platyconic acid A (PA), the active component of Platycodi radix-derived saponin, on activation of hepatic stellate cells is still unclear. The present study examined the suppressive effects of PA and revealed the related mechanisms. PA strongly suppressed the TGF-β1-stimulated cell proliferation in rat hepatic stellate cells. Moreover, PA decreased TGF-β1-induced α-SMA and collagen Ia1 mRNA and protein expression in a concentration-dependent manner. PA suppressed TGF-β1-induced SBE4 luciferase activity via blocking of smad2/3 phosphorylation. By contrast, PA abolished TGF-β1-reduced smad7 and PPARγ expression. Also, PA inhibited TGF-β1-enhanced Akt, ERK1/2, JNK1/2, and p38 MAPK phosphorylation in a concentration-dependent manner. These results suggest that PA decreases hepatic stellate cells activation via inhibition of smad-dependent and smad-independent pathways. Consequently, PA may potentially be a useful chemotherapeutic agent that may contribute to improvement to the chronic liver injury.

PS P296 - 0171 Inhibitory Effects of Rutaecarpine on Acetaminophen-Induced Hepatotoxicity through Enhancing of Nrf2-Mediated Antioxidant Enzymes in Mice

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Rutaecarpine, an alkaloid isolated from the unripe fruit of *Evodia rutaecarpa*, has traditionally used for the treatment of headaches, abdominal pain, postpartum hemorrhage, dysentery, and amenorrhea. Although rutaecarpine has shown various functions, the inhibitory effect of rutaecarpine on acetaminophen-induced acute liver injury remains unknown. This study assessed the inhibitory effects of rutaecarpine against APAP-induced hepatotoxicity in mice

model. Pretreatment with rutaecarpine significantly inhibited serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities and hepatic malondialdehyde (MDA) content by induction of acetaminophen. Acetaminophen-induced proinflammatory cytokines was reduced and anti-inflammatory cytokine expression was induced by rutaecarpine treatment. Furthermore, rutaecarpine induced activation of nuclear erythroid 2-related factor 2 (Nrf2)-mediated antioxidant enzymes, such as glutamate-cysteine ligase catalytic (GCLC), heme oxygenase-1 (HO-1), and NAD(P)H quinone oxidoreductase 1 (NQO1). These results suggested that rutaecarpine attenuates APAP-induced liver damage through the activation of Nrf2-mediated antioxidant enzymes. Therefore, rutaecarpine could be a useful candidate for the discovery of new chemotherapeutic agents that may contribute to inhibiting the liver injury by hepatotoxicants.

P297 - 0279 An Inhibitor of the Protein Kinases IKKε and TBK1 Ameliorates Acetaminophen-Induced Oxidative Stress in Mice

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Acetaminophen (APAP) overdose is the main cause of acute liver injury (ALI). Amlexanox is a clinically approved small-molecule therapeutic presently used to treat several forms of acute or chronic diseases such as organ injury, obesity, metabolic disorders, and cancers. This compound acts as an inhibitor of the noncanonical IκB kinases (IKK-ε) and TANK-binding kinase 1 (TBK1). However, the role of IKK-ε and TBK1 on APAP-induced hepatotoxicity remains uncharacterized. Mice were intraperitoneally injected with 300 mg/kg APAP to induce ALI. After 1 hour of APAP treatment, mice were orally administered with amlexanox (50 mg/kg) and were sacrificed 12 hours after APAP administration. To induce *in vitro* hepatotoxicity, primary hepatocytes were treated with 10 mM APAP. Inhibition of IKK-ε and TBK1 by amlexanox treatment attenuated APAP-induced ALI as confirmed by decreased plasma levels of aspartate aminotransferase and alanine aminotransferase. Furthermore, amlexanox significantly decreased hepatocellular apoptosis in injured livers of mice as confirmed by histopathologic observation. Consistently, reduced oxidative stress was confirmed by increased hepatic glutathione concomitant with lower levels of malondialdehyde. Also, increased expression levels of Nrf2 target genes including heme oxygenase 1, NAD(P)H quinone oxidoreductase 1, and glutamate-cysteine ligase were observed in injured livers of mice treated with amlexanox. Mechanistic insights into the mode of action of amlexanox were involved in increased phosphorylation of AMP-activated protein kinase (AMPK) and nuclear translocation of nuclear factor E2-factor related factor (Nrf2). Similar results were observed in *in vitro* results, showing that reduced oxidative stress was observed by treatment of amlexanox as confirmed by increased protein levels of phosphor-AMPK and nuclear Nrf2 and consequently enhanced expression levels of Nrf2 target genes in primary hepatocytes. Furthermore, the protective effects of amlexanox on APAP-induced hepatotoxicity were abolished by compound C, AMPK inhibitor. Taken together, our findings suggest that amlexanox exerts anti-oxidative activities against APAP-mediated hepatotoxicity via AMPK-Nrf2 pathway.

P298 - 0286 Amlexanox Attenuates the Severity of Hepatotoxin-Induced Liver Fibrosis and Biliary Fibrosis in Mice

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Liver fibrosis is the excessive accumulation of extracellular matrix proteins occurring in chronic liver injury and subsequent abnormal wound-healing response. Since it can progress to irreversible and lethal cirrhosis with serious complications in patients with liver disease, it is valuable to understand the pathogenesis of liver fibrosis and its associated inflammation. Although numerous studies have suggested that canonical IκB kinases (IKK) play a key role in the progression of liver fibrosis, the role of non-canonical IKKε and Tank-binding kinase 1 (TBK1) on the development and/or progression of liver fibrosis remains unclear. To demonstrate such issue, repeated injection of carbon tetrachloride (CCl₄, 2:5 v/v in corn oil) was used to induce hepatotoxin-mediated chronic liver injury and biliary fibrosis was induced by 0.1% diethoxycarbonyl-1, 4-dihydrocollidine (DDC) diet feeding for 4 weeks. Mice were orally administered with amlexanox (25, 50, and 100 mg/kg) during the experimental period. Significantly increased levels of IKKε and TBK1 were observed in fibrotic livers or hepatic stellate cells (HSCs) isolated from livers of mice with fibrosis.

Interestingly, treatment of amlexanox significantly inhibited the phosphorylation of IKKε and TBK1 accompanied by a reduction of liver injury as confirmed by decreased plasma levels of aspartate aminotransferase and alanine aminotransferase. Also, decreased fibrotic lesion was confirmed by histopathologic observation in the livers of mice treated with amlexanox. Consistently, treatment of amlexanox significantly decreased the expression levels of inflammatory and fibrogenic-genes in fibrotic livers. Additionally, treatment of amlexanox promoted the fibrosis resolution as well as reduction of fibrosis progression. In accordance with these findings, reduced phosphorylation of IKKε and TBK1 in HSCs was observed by treatment of amlexanox and consequently suppressed HSC activation as confirmed by decreased production of extracellular matrix. Furthermore, amlexanox decreased the protein level of phosphorylated NFκB and inflammatory responses in kupffer cell (KCs). Collectively, we found that inhibition of the IKKε and TBK1 by amlexanox is a promising therapeutic strategy to cure liver fibrosis.

P299 - 0337 Cyp2b-Null Male Mice Are Susceptible to High-Fat-Diet-Induced Obesity Due to Changes in PUFA Metabolism and Response to Hepatic Lipids as Measured by RNAseq

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CYP2B6 metabolizes xenobiotic and endobiotic compounds, including drugs, pesticides, plasticizers, steroids, and polyunsaturated fatty acids (PUFAs). Recent studies using a conditional hepatic cytochrome P450 oxidoreductase knockout mouse revealed steatosis coupled with induced Cyp2b10. Data from our laboratory using RNAi-mediated Cyp2b-knockdown mice indicates age-onset obesity. To better investigate the role of CYP2B in lipid metabolism, a Cyp2b triple knockout mouse lacking Cyp2b9, Cyp2b10, and Cyp2b13 was developed. Wildtype (WT) and Cyp2b-null mice were fed a normal diet (ND) or a 60% high-fat diet (HFD) for 10 weeks. Male but not female Cyp2b-null mice weighed 15% more than WT HFD-fed counterparts, primarily due to increased (55%) white adipose tissue mass. Liver to serum triglyceride ratios increased in Cyp2b-null (ND and HFD) compared to WT mice, indicating a role for Cyp2b in fatty acid metabolism regardless of diet. In addition, serum β-hydroxybutyrate, leptin, and cholesterol were increased in HFD-fed Cyp2b-null male mice compared to HFD-fed WT mice. Interestingly, RNAseq demonstrated that hepatic gene expression in ND-fed Cyp2b-null mice is similar to HFD-fed WT mice, indicating that Cyp2b-null mice are reacting as if they are receiving a HFD even if they are not. Gene ontology and KEGG pathways show perturbations in lipid metabolism pathways, including PUFA metabolism, fatty acid elongation, and glycerophospholipid metabolism. Lipidomic results confirm changes in PUFA metabolism, and random forest analysis also shows that ND-fed Cyp2b-null mice share many important lipid species with HFD-fed WT. Overall our data demonstrates a role for Cyp2b in hepatic fatty acid metabolism and lipid signaling; therefore, the repression or chemical inhibition of CYP2B may exacerbate metabolic disorders and cause obesity.

P300 - 0359 The Toxicological Profiles of 1,3-Dichloro-2-Propanol Determined by a Repeated-Dose 28-Day Oral Toxicity Study in F344 Rats

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1,3-Dichloro-2-propanol (1,3-DCP) is a contaminant found in various foods such as cheese, meat, and fish products. Although 1,3-DCP shows unequivocal carcinogenicity in the liver, kidney, thyroid, and tongue of rats, its toxicity and genotoxicity are poorly understood. Thus, we investigated the mechanisms underlying the carcinogenicity of 1,3-DCP in a medium-term F344 *gpt* delta rat model, which is useful for evaluating the mutagenicity and tumor-promoting activity of chemicals. The toxicological profiles of 1,3-DCP were evaluated in a preliminary dose-finding study using wild-type F344 rats. A total of 20 male 6-week-old F344 rats were divided into four groups and given 0, 25, 50, or 100 mg/kg/day 1,3-DCP in distilled water, as the vehicle, by gavage for 28 days. General toxicity was examined in all rats, and liver tissues from rats in the 0, 25, and 50 mg/kg groups were subjected to immunohistochemical, western blot, and real-time reverse-transcription PCR analyses. All rats in the 100 mg/kg group had died or been sacrificed in moribund condition by day 2. In this group, severe centrilobular necrosis in the liver and slight vacuolation and necrosis in the proximal tubules of the kidney were observed by histopatho-

logical examination. In the 25 and 50 mg/kg groups, whole body weight and food consumption showed no changes, whereas red-blood-cell-related parameters were decreased, and the absolute/relative weights of the liver and kidney were increased. In serum biochemical analyses, the alanine aminotransferase level was increased in the 50 mg/kg group. In the histopathological examination, single cell necrosis of hepatocytes and positivity for cleaved caspase 3 were increased in the central zone of the liver in the 25 and 50 mg/kg groups. The mRNA and protein levels of glutathione S-transferase placental form (GST-P) were increased in the liver in the 25 and 50 mg/kg groups. Immunohistochemistry revealed that centrilobular hepatocytes were positive for GST-P, while there were no obvious preneoplastic GST-P-positive foci. The number of Ki67-positive hepatocytes, which were detected mainly in the central zone, and the mRNA expression of genes responsible for cell cycle acceleration were significantly increased in the 25 and 50 mg/kg groups. Acute liver injury may have been the cause of death in the 100 mg/kg group. Increased cell proliferation in the livers of rats in the 25 and 50 mg/kg groups may be related to the tumor-promoting activity of 1,3-DCP.

PS P301 - 0364 CTR1 Overexpression Enhances Copper Toxicity in Zebrafish Liver Cell Line ZFL

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Copper is essential for all organisms because it serves as cofactor of many enzymes to carry out biological and cellular functions. Therefore, Cu homeostasis is maintained in the body and at an optimized cellular level under homeostatic control, because Cu ions could be toxic with oxidative stress. Inadequate or excessive amount of Cu free ions caused by genetic diseases known as Menkes disease (MD) and Wilson's disease (WD) respectively, leading to the discoveries of copper transporters: ATP7A and ATP7B. Mutations in human ATP7A cause MD, whereas mutations in ATP7B cause WD. ATP7A and ATP7B transfer Cu⁺ to nascent proteins synthesized in the Golgi apparatus and eliminate Cu⁺ from the cell. In human or mammals, ATP7B is highly expressed in liver, kidney and placenta, and ATP7A is expressed in many tissues except liver. There are other major Cu transporters CTR1 on plasma membrane related to cellular Cu uptake, and ATOX1 for intracellular Cu transport. Zebrafish (*Danio rerio*) is a powerful vertebrate model organism for toxicology and developmental biology studies with an almost completed genome sequence showing high homology with human, except that zATP7A is highly expressed in the liver instead of zATP7B and zATP7B mainly expressed in other tissues. To understand how zebrafish handles Cu stress, four Cu transporters were studied in ZFL. Cellular Cu content was determined by Atomic Absorption Spectrometry in ZFL and Cu exposure increased the cellular Cu contents. The expression profile of Cu transporters in ZFL was determined by qPCR. zAtp7a and zAtp7b were slightly induced by 4 h and 12 h Cu exposure and 12 h and 24 h Cd exposure while zCtr1 and zAtox1 stayed the same by Cu and Cd exposure. Zn exposure also changed the expression of Cu transporters. Gene methylation did not alter much the expression of Cu transporter genes. To study the function of Cu transporters, Cu transporters overexpression stable cell lines of ZFL were created. Overexpression of zCTR1 increased Cu toxicity in ZFL but overexpression of zATP7A and zATP7B merely reduced Cu toxicity. Overexpression of zCTR1 will highly increase the cellular Cu content as well. It is suggested that overexpression of zCTR1 increased Cu uptakes in ZFL and causing Cu toxicity. However, Cu contents also increased after Cu exposure in overexpression of ZFL with zATP7A and zATP7B, perhaps overexpressed zATP7A and zATP7B could bind to Cu and reduced the toxicity.

PS P302 - 0461 Early-Life Activation of mTOR Pathway by Environmental Xenobiotics Permanently Reprograms Metabolism of Lipids and Carbohydrates by Liver

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An emerging hypothesis links epidemic of metabolic diseases with chemical exposures during vulnerable windows of *in utero* and early life development. Molecular mechanisms of metabolism reprogramming by environmental xenobiotics remain elusive. In this study we dissect key mechanisms that participate in permanent reprogramming of liver lipid and carbohydrate metabolism by environmental brominated flame retardant 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) in laboratory rodents. Developmental exposures to BDE-47 result in non-alcoholic fatty liver disease (NAFLD) like phenotype, and reduced insulin sensitivity. We demonstrated previously that BDE-47 can activate mTOR signaling in mouse livers and human hepatocellular carcinoma cells and can permanently alter expression of fatty acid translocase *Cd36* in hepatocytes.

Cd36 is a membrane receptor responsible for fatty acids uptake. Based on this evidence we hypothesize that exposure to BDE-47 during specific sensitive windows of liver development may reprogram expression of *Cd36* and liver/blood balance of triglycerides (TG) via mTOR pathway. To test this hypothesis we conducted series of exposure experiments with wildtype mice and mice knockout for Raptor (element of mTOR complex 1) or Rictor (element of mTOR complex 2). Tissue samples were analyses for transcriptomic changes, changes in protein expression and lipid profile in blood and liver. Our experimental data demonstrate that developmental exposures to BDE-47 result in permanent changes in mTOR controlled functions at transcriptional level. Pre- and neonatal exposures to BDE-47 produce very similar long-lasting effects on liver gene expression and blood/liver TG balance, suggesting that hepatocytes maturation is a sensitive window of liver lipid metabolism reprogramming. Further we have found that low (0.2 mg/kg BW) and moderate (1 mg/kg BW) doses of BDE-47 have opposite effects on liver gene expression and TG balance and changes in TG liver/blood balance. Inactivation of mTORC2 abolished BDE-47-induced changes in expression of *Cd36* and dyslipidemia. Inactivation of mTORC1 abolished effect of exposure on expression of several key genes of lipid and carbohydrate metabolism including *Irs1* and *Irs2*—key players in insulin signaling cascade. mTOR pathway can be activated by broad variety of xeno- and endobiotics, suggesting urgent in understanding its role in the developmental programming of metabolism.

PS P303 - 0468 Withdrawn

PS P304 - 0470 How Similar among Different Toxicogenomics Study Designs?

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Toxicogenomics (TGx) is an important tool to gain an enhanced understanding of toxicity at the molecular level. A broad range of TGx study design has been reported, some based on the well-established animal models (e.g., one-day short-term assay or repeated dosing for 28 days) and the other applied *in vitro* systems (e.g., cell lines from rat, humans and cancer). A question is naturally raised: How similar among different TGx study designs? In fact, this question can be asked in many different ways, for example: (1) Is a one-day *in vivo* short-term assay able to replace the 28-day standard and expensive toxicological assay? (2) Are some biological processes more conservative across different preclinical testing systems than others? (3) Do these preclinical testing systems have the similar resolution in differentiating drugs by their therapeutic uses? (4) Is it possible for *in vitro* to *in vivo* extrapolation? And (5) can genomic profiles from a cancel line predict drug-induced liver injury? In this presentation, these questions will be explored using several large genomics datasets including Open Toxicogenomics Project-Genomics Assisted Toxicity Evaluation System (TG-GATES) and L1000.

PS P305 - 0486 The Regulatory Mechanism of Hepatic PGE₂ Disposition in Carbamazepine-Induced Liver Injury

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Carbamazepine (CBZ), a widely used anti-epileptic drug, causes idiosyncratic liver injury. Previous study have suggested that prostaglandin (PG) E₂ treatment ameliorates liver damages in CBZ-induced liver injury model mice. Generally, PGE₂ is synthesized endogenously instead of PGE₁ by cyclooxygenase (COX)/PGE synthase (PGES) pathway and degraded by 15-prostaglandin dehydrogenase (15-PGDH) at cytoplasmic domain. Although PGE₂ has hepatoprotective effect under liver injury condition caused by several toxicants, regulatory mechanism of hepatic PGE₂ disposition in CBZ-induced liver injury has not been cleared yet. In this study, we aimed to clarify the regulatory mechanism of hepatoprotective PGE₂ disposition in liver under CBZ-induced injury condition. CBZ was orally administered to mice at a dose of 400 mg/kg for 4 days and 800 mg/kg on the 5th day and then, plasma AST and ALT levels were determined to confirm extent of liver injury. Hepatic PGE₂ amount was assayed by enzyme immunoassay. Expression of PGE₂-related gene was quantified by qPCR and western blotting. Localization of 15-Pgdh was investigated by immunohistochemistry. Significant increase of plasma ALT and AST levels were observed in 20% of CBZ-treated mice 24 hr after last CBZ administration. Hepatic PGE₂ level increased significantly in CBZ-induced liver injury mice comparing with vehicle-treated mice. Although hepatic expression of Cox-1, Cox-2 and mPges-2 did

not increase, significantly downregulated expression of 15-Pgdh was observed in CBZ-induced liver injury mice. Moreover, expression of mPges-1 was not detected by qPCR. Immunohistochemical study indicated that 15-Pgdh seems to be expressed in non-parenchymal cells, especially in sinusoidal endothelial cells (SECs). Hepatic expression of Oatp2a1, a major PGE₂ transporter expressing in SECs, was also decreased in CBZ-induced liver injury mice. Our results suggest that 15-PGDH-mediated PGE₂ degradation in SECs may be critical factor for increase of hepatic PGE₂ level in CBZ-induced liver injury. Further investigation is necessary to clarify the relationship between alteration of 15-PGDH expression and degree of liver damage in CBZ-induced liver injury.

PS P306 - 0487 Diclofenac Induces Lipid Accumulation in Liver by Inhibiting Chaperone-Mediated Autophagy

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Intracellular proteins are subjected to turn-over through synthesis, degradation and recycling process which is called autophagy. Autophagy allows the orderly degradation and recycling of cellular components. In macroautophagy, cellular components are sequestered in vesicles that form in the cytosol and then fuse with lysosomes and transfer their contents to lysosomes, and degrade the components. However, not all autophagy processes involve vesicles. Proteins can be targeted from the cytosol to the lysosomal membrane and then gain access to the lumen of this organelle by directly crossing its membrane. This process is known as chaperone-mediated autophagy (CMA). It is well reported that lipid droplet (LD)-associated proteins perilipin 2 (PLIN2) is CMA substrates and PLIN2 is degraded by CMA preceded lipolysis. Thus, the inhibition of CMA blocks degradation of PLIN2 and results in LD accumulation in cells. Nonsteroidal anti-inflammatory drugs (NSAIDs) are a class of drugs that provides analgesic and antipyretic effects, and, in higher doses, anti-inflammatory effects by inhibiting cyclooxygenase (COX). Diclofenac, one of the nonsteroidal anti-inflammatory drugs (NSAIDs), induces steatosis in liver. But the mechanism of steatosis by diclofenac is poorly understood. Here, we investigated how diclofenac induces lipid accumulation in liver by CMA inhibition. In this study, diclofenac induces lipid accumulation in HepG2 cells and primary mouse hepatocyte. And, diclofenac significantly increases CMA substrate—GAPDH and PLIN2. Also, diclofenac inhibits interaction between PLIN2 and lysosome-associated membrane protein 2 (LAMP2A), which is the key protein in CMA process. In addition, treatment of CMA activator (AR7) and starvation reduces diclofenac-induced lipid accumulation. Taken together, our data suggest that diclofenac induces lipid accumulation in HepG2 cells by CMA inhibition. These results indicate that the mechanism of steatosis by diclofenac may be the inhibition of CMA and further studies are needed to elucidate the effect of diclofenac on CMA.

PS P307 - 0490 Diclofenac Inhibits Autophagic Flux and Sensitizes Hepatocarcinoma Cells to Sorafenib-Induced Cell Death

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Diclofenac is a nonsteroidal anti-inflammatory drug that provides analgesic and antipyretic effects by inhibiting cyclooxygenase (COX). Autophagy is a self-degradative process that involves the degradation of long-lived proteins and organelles. Recent studies show that inhibition of autophagy may enhance the sensitivity of the anticancer drugs. In this study, we investigated the effect of diclofenac on autophagy and whether diclofenac enhances the anticancer effect of sorafenib in hepatocarcinoma cells. Treatment of HepG2 cells with diclofenac increased the GFP-LC3 puncta in cells transfected with GFP-LC3 as well as the protein level of LC3B, SQSTM1/p62 and NBR1 in HepG2 cells. Diclofenac suppressed the autophagic flux by impairing the lysosomal function, which was demonstrated in HepG2 cells transfected with tandem fluorescence-tagged LC3B. Suppression of autophagy by diclofenac enhanced the chemosensitivity of sorafenib-induced cell death in HepG2 cells. Treatment of the cells with sorafenib in the presence of diclofenac increased the cell death synergistically. In summary, these data show that diclofenac is a novel autophagy inhibitor and could function as a potent anticancer drug in combination therapies to overcome chemoresistance.

PS P308 - 0613 Regorafenib Is Far More Toxic Than Its Pharmacologically Active Metabolites in Hepatocytes and Cardiomyocytes

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The US Food and Drug Administration (US FDA) has approved 47 small molecule kinase inhibitors (KIs) as of November 2018, with 31 of them having hepatotoxicity warnings in the product labels. We examined the hepatocyte toxicity of these approved KIs and identified regorafenib as the most cytotoxic drug. Regorafenib (Brand name Stivarga) is an oral broad-spectrum KI approved for treating colorectal cancer, gastrointestinal stromal tumors and hepatocellular carcinoma. Regorafenib carries a black box warning for hepatotoxicity and a warning for cardiotoxicity in its label. It is metabolized by liver CYP3A4 and UGT1A9 producing two pharmacologically active metabolites M2 and M5, whose steady-state blood concentrations (~8 µM) are similar to regorafenib. We compared the cytotoxicity of regorafenib, M2 and M5 in primary cultured rat, canine and human hepatocytes and found that regorafenib was remarkably more cytotoxic than M2 and M5. In human hepatocytes, regorafenib caused nearly 100% ATP depletion at 2.5-fold C_{max}, while M2 only injured 60% of the cells and M5 was essentially non-toxic. A clear species difference was also observed, with hepatocytes from rats being the most sensitive to toxicity, followed by canine and human. We next examined the cytotoxicity of regorafenib and its metabolites in human induced pluripotent stem cell derived hepatocytes (iPSC-Hepatocytes) and cardiomyocytes (iPSC-CMs). In both cell types, regorafenib showed significantly stronger cytotoxicity than M2, with M5 being non-toxic even at the highest testable concentration (40 µM). The remarkable difference in cytotoxicity is at least partially due to the direct mitochondrial liability, as regorafenib caused complete uncoupling of oxidative phosphorylation and significant cytochrome c release at the concentration equal to C_{max} (8 µM), while M2 and M5 showed similar effects only at much higher concentrations (>16 µM). In human hepatocytes pretreated with CYP3A4 inhibitor ketoconazole, the cytotoxicity of regorafenib was significantly increased. These results provide strong evidence that regorafenib is far more toxic than its pharmacologically active metabolites M2 and M5, indicating the latter might be further explored as safer new drugs than regorafenib.

PS P309 - 0640 Human CYP2B6 is an Anti-obesity Enzyme Involved in Unsaturated Fatty Acid Metabolism

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Multiple factors in addition to overconsumption are believed to lead to obesity and non-alcoholic fatty liver disease (NAFLD) in the United States and elsewhere. Recently, we showed diet-induced obesity develops in Cyp2b9/10/13-null mice that lack the primarily hepatic Cyp2b enzymes due to increased white adipose tissue, coupled with increased liver triglycerides. Therefore, studies were undertaken to determine a putative mechanism by which Cyp2b enzymes, including human CYP2B6, may be anti-obesogenic. Vivid human CYP2B6 recombinant enzyme was used to determine fatty acid inhibitors of CYP2B6. Several unsaturated fatty acids including arachidonic acid, linoleic acid, DHA, and α-linolenic acid were inhibitors with EC₅₀'s below 10mM. LC-MS/MS revealed that CYP2B6 metabolized DHA, arachidonic acid, linoleic acid, and α-linolenic acid. However, there was a 20-fold preference for metabolism of α-linolenic acid to 9-HOTre, a monohydroxylated product. This data indicates that CYP2B6 metabolizes a specific polyunsaturated fatty acid (α-linolenic acid) to a specific metabolite (9-HOTre) with unknown function. Interestingly, incorporation of Nile Red, a triglyceride sensor, is greater in α-linolenic acid treated CYP2B6-transfected HepG2 cells than untransfected HepG2 cells, indicating that metabolism of α-linolenic acid by CYP2B6 perturbs lipid utilization and transport. We recently developed a humanized CYP2B6 mouse on our Cyp2b-null background and provided a high-fat diet to humanized and -null mice. The presence of CYP2B6 significantly reduced metabolic disease as measured by glucose tolerance tests. This study is ongoing. In addition, several pharmaceuticals and environmental chemicals were recently screened using the Vivid CYP2B6 recombinant enzyme; we have found triclosan, diazinon, endosulfan, atrazine, ticlopidine, parathion, chlorpyrifos, jet fuel, nonylphenol, and several other chemicals to be CYP2B6 inhibitors. Further studies are being performed to quantify and determine lipid localization in HepG2 and CYP2B6-HepG2 cells following exposure to inhibitors using Nile Red incorporation coupled with confocal microscopy. It is interesting to speculate that these chemicals have the potential to increase diet-induced obesity or metabolic disease through inhibition of CYP2B6 and the disruption of polyunsaturated fatty acid metabolism. Funding support was provided by NIH R15ES017321.

PS P310 - 0680 Estrogen Deficiency Potentiates Thioacetamide-Induced Hepatic Fibrosis

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Liver fibrosis is characterized by the persistent deposition of extracellular matrix proteins including collagen that occurs in most types of chronic liver diseases. In particular, chronic liver diseases, such as fibrosis or cirrhosis, are more common in men than in women. This gender difference may be related to the effects of sex hormones on the liver. The aim of the present work was to investigate the effects of estrogen on thioacetamide (TAA)-induced fibrosis of the liver or kidney in rats. Fibrosis was induced in male, female, and ovariectomized (OVX) rats by TAA administration. All the groups were treated with TAA (150 mg/kg) for 7 days. At the end of experiment, all the rats were killed to measure serum biochemical parameters for hepatic or renal functions. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and total cholesterol were significantly increased in OVX female and male rats compared with normal rats by TAA treatment. Furthermore, vimentin content, alpha-smooth muscle actin (alpha-SMA) and type I collagen levels significantly increased in the liver of male and OVX female rats induced by TAA administration. The fibrotic response of the female rats to TAA treatment was significantly weaker than that of male liver. Therefore, we suggest that estrogen deficiency in postmenopausal state might be increased the risk of hepatic or renal fibrosis among postmenopausal women.

PS P311 - 0696 Adipose FGFR1 Regulates Binge Alcohol-Induced Adipolipolysis and Fat Accumulation in Liver in Mice

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Normally fat (triglyceride, TG) is stored in adipose tissues, but under pathological conditions such as obesity or alcoholism, fat can also be accumulated in liver and causes steatosis (fatty liver). Usually 65% fat in the liver is from adipose tissues. During the development of fatty liver, fat "flows" from adipose tissues to liver via blood in the form of free fatty acids (FFA). Fibroblast growth factor 21 (FGF21) is mainly produced in liver and liver is a major source of blood FGF21. FGF21 exerts effects through FGF receptor 1 (FR1), which is extensively expressed in multiple organs including liver and adipose tissues. Previously, we showed that liver-specific deletion of FGF21 enhanced alcohol-induced fatty liver. However, liver-specific abrogation of FR1 did not affect alcoholic fatty liver. In this study, we test the hypothesis that liver-derived FGF21 binds to adipose FR1 and initiate a release of FFA, which contributes to alcohol-induced fatty liver. Adipose tissues-specific FR1 knockout (FR1 KO) mice, created by crossing FR1 floxed mice with ap2-Cre mice, and their wild type control (FR1 WT) mice, were treated ethanol at 5 g/kg by gavage. The mice were sacrificed after 3, 6, and 9 h. Blood was collected and serum was isolated for FFA and TG assays. Liver tissues were collected for hepatic TG assays. After binge ethanol treatment, liver TG in FR1 WT mice was increased by 1.6 times at 3 h, and further increased to 2.2 times at 6 h and 2.6 times at 9 h, respectively. In FR1 KO mice, liver TG was also increased at 3 h and further increased at 6 h, but it decreased to almost normal levels at 9 h. Serum FFA in FR1 WT mice was increased by 1.5 times at 3 h and 6 h, and 1.2 times at 9 h; however, in FR1 KO mice, serum FFA remained unchanged at 3 h and 6 h, but it was decreased by 50% at 9 h. Serum TG was unchanged at 3 h, but it was decreased at 6 h and further decreased at 9 h identically in both FR1 WT and FR1 KO mice. These results suggest that adipose FR1 regulates alcoholic fatty liver through regulating adipolipolysis.

PS P312 - 0792 The Influence of Alantolactone on Cholesterol Metabolism in Liver Cells

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Alantolactone (ALA) is a sesquiterpene lactone that is being studied as a novel anticancer agent, especially against liver and breast cancer. It has shown pro-apoptotic and pro-oxidative effects, downregulation of transcription factor NF- κ B and inhibition of STAT3 transcription factor phosphorylation in liver HepG2 cells and breast cancer MDA-MB-231 cells [1,2]. We have performed a target prediction analysis using BATMAN-TCM tool for ALA and garmacrone, two structurally similar compounds. Six common targets were discovered for

both compounds. Among them, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) and aromatase CYP19A1 caught our attention, since HMGCR and CYP19A1 are important for cholesterol and steroid hormone biosynthesis and can influence proliferation, differentiation and survival of both healthy and cancer cells. The effect of ALA was tested on differentiated HepaRG cells, which biological functions, biotransformation of xenobiotics and regulatory pathways are comparable to primary human hepatocytes. To evaluate potential DNA damage caused by ALA to HepaRG cells, COMET assay was performed. Lovastatin (HMGCR inhibitor), letrozole (CYP19A1 inhibitor) and hydrogen peroxide (DNA damage) were used as positive controls. Expression of lipid metabolism-related genes (*Scd*, *Fasn*, *Acacb*, *Gpam*, *Cidec*, *Plin2*, *Plin4*, *HMGCS1*, *SREBP2*) and protein levels of SREBP-2 and LDL-R were measured. To analyze the binding location, binding energy and other criteria of ALA to HMGCR and CYP19A1, the webtool DockingServer was used. From preliminary results, ALA showed higher half-maximal inhibitory concentration (IC_{50} = 60 μ M) in HepaRG cells in comparison to tested cancer cell lines (IC_{50} = 30-40 μ M). ALA 60 μ M caused significant decrease in HMGCR protein expression after 24-h exposure, while it induced HMGCR mRNA after 6-h treatment. Moreover, ALA 30 μ M caused increase in CYP19A1 mRNA level after 6 hours of exposure, which returned to normal after 24 hours. Obtained results can contribute to the understanding of mechanisms by which sesquiterpene lactones influence cholesterol metabolism in liver cells. *The study was supported by the Czech Science Foundation (grant No. 18-09946S). References: [1] Chun, J., et al., Alantolactone selectively suppresses STAT3 activation and exhibits potent anticancer activity in MDA-MB-231 cells. Cancer Letters, 2015. 357(1): p. 393-403. [2] Khan, M., et al., Alantolactone induces apoptosis in HepG2 cells through GSH depletion, inhibition of STAT3 activation, and mitochondrial dysfunction. BioMed research international, 2013. 2013: p. 719858-719858.*

PS P313 - 0800 Docosahexaenoic Acid Inhibits SREBP-1-Mediated Lipogenic Enzyme Expression via G-Protein-Coupled Receptor 40 in Primary Hepatocytes

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Non-alcoholic fatty liver disease is the most copious liver malady and if proper treatment is not applied, it may progress to cirrhosis, the end stage liver disease. Omega-3 fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are clinically proven to lower serum triglyceride (TG) levels. It has been shown that a variety of physiological activities of omega-3 fatty acids are due to their agonistic actions on G-protein coupled receptor 40 (GPR40) and GPR120. Bodipy staining for lipid droplets (LD) accumulation in hepatocytes confirmed that DHA treatment reduced the number of > 10 m2 LD as well as the total area of LD. Moreover, DHA lowered protein or mRNA expression levels of lipogenic enzymes such as fatty acid synthase (FAS), acetyl CoA carboxylase (ACC) and stearoyl-CoA desaturase-1 (SCD1) in primary hepatocytes incubated with liver X receptor (LXR) agonist, T0901317 or high glucose/high insulin. DHA also inhibited protein expression of both nuclear and total sterol response-element binding protein (SREBP)-1, a key lipogenesis transcription factor. We further found that exposure of murine primary hepatocytes to DHA for 12 h increased mRNA levels of GPR40 and GPR120. To assess whether DHA's anti-lipogenic effects are mediated through GPR120 or GPR40, specific agonists (Compound A for GPR120 and AMG-1638 for GPR40) and hepatocytes from GPR120 knock-out mice or GPR40 selective antagonist (GW1100) were used. Compound A did not decrease the protein expression of SREBP-1 and FAS in hepatocytes exposed to T0901317 and high glucose with insulin. Moreover, DHA's down-regulating effects on lipogenesis enzyme expression were sustained in GPR120-null hepatocytes. In contrast, AMG-1638 lowered the protein levels of SREBP-1 and SCD-1 as DHA did. Additionally, GW1100, a GPR40 antagonist, reversed the anti-lipogenic effects of DHA. Collectively, our data demonstrate that DHA downregulates the expression SREBP-1-mediated lipogenic enzymes via GPR40 in primary hepatocytes. *This work was supported by the National Research Foundation(NRF) grant funded by Korean government (MSIP; Ministry of Science, ICT & Future Planning) (No. 2017M3A9C8028794).*

P314 - 0855 A Human Hepatocellular Steatosis Model for *In Vitro* Toxicity Assessment

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Hepatic steatosis is a pathological condition that can alter xenobiotic metabolism and increase susceptibility to environmental toxicants. Here, we used the metabolically competent human hepatocyte HepaRG cell line to model the steatotic condition for *in vitro* toxicity assessment. Cells were exposed to media containing an oleate: palmitate mixture at varying ratios and concentrations. Optimum free fatty acid ratio, concentration, and exposure time were chosen to achieve measurable lipid accumulation with minimal toxicity. The hepatotoxicant pesticide rotenone was used as a case study to assess chemical toxicity in our steatotic model. After 48h chemical exposure, measurement of cell viability by intracellular ATP assay demonstrated increased sensitivity to rotenone corresponding to increased levels of lipid accumulation (IC50 values of 0.29 < 0.35 < 0.36 < 0.41 corresponding to 750, 500, 250, and 0 μ M dosed free fatty acid conditions). Conversely, lipid accumulation did not enhance sensitivity to the hepatotoxicants aflatoxin, troglitazone or tamoxifen. Additionally, qPCR measurement of cytochrome P450 gene expression at the highest free fatty acid concentration showed marked reduction in expression of several Cyp genes including Cyp3a4, the most active P450 enzyme in rotenone metabolism. P450 activity is a major factor in limiting rotenone toxicity as the metabolites of rotenone are less active than the parent compound. These results suggest that our HepaRG steatosis model can be a useful tool for evaluating steatosis as a risk factor in chemical toxicity studies. Future addition of high content analysis (HCA) oxidative stress and mitochondrial dysfunction parameters to the model may enhance its predictive capability in human hepatotoxicity susceptibility screening. *This does not represent the views of the EPA.*

P315 - 0860 Interactions between Epigallocatechin-3-Gallate (EGCG) and Hydroxy Citric Acid Potentiate EGCG Hepatotoxicity

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The rise in popularity of complementary and alternative medicine has seen an increase in concomitant use of herbal medicines with conventional medications as well as the concomitant use of multiple herbal preparations (polyherbacy). Cytochrome P450 (CYP) is a superfamily of enzymes responsible for phase 1 metabolism of various xenobiotics, sometimes leading to the formation of toxic compounds. Interactions between the components of herbal medicines are poorly understood. Herbal supplements that inhibit or induce CYP450 enzymes are a public health concern as these interactions may inhibit the breakdown of toxic parent compounds to less toxic daughter compounds, as well as affecting therapeutic efficacy. A recent case report detailing severe hepatotoxicity requiring a liver transplant after the ingestion of supplements with epigallocatechin-3-gallate (EGCG) and hydroxycitric acid (HCA) suggests a possible role for adverse interactions. EGCG has previously been shown to produce hepatotoxicity. This investigation was undertaken to determine whether HCA could produce hepatotoxicity alone and whether hepatotoxicity might be potentiated by EGCG, in a hepatocyte model cell line HepG2. The toxicity of EGCG and HCA in a model of intestinal epithelium, Caco2 cells was also investigated. EGCG and HCA individually produced concentration-dependent (20 -100 μ M) toxicity in HepG2 and Caco2 cells. Combining threshold toxic concentrations of EGCG with HCA potentiated the toxicity of HCA in an additive manner. Similarly combining threshold toxic concentrations of HCA with EGCG potentiated the toxicity of EGCG in an additive manner. Without required evidence of the pharmacokinetics of many of the ingredients contained in supplements before entering the market, consumers are at risk of possible herb-herb interactions.

P316 - 0861 Herbal Phytochemical Interactions Produce Synergistic Toxicity: Implications for Polyherbacy

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Use of complementary medicine products, including herbal medicines, is increasing in Australia. Though herbal medicines are widely considered safe, severe adverse effects and fatalities have been reported from their use; and use of multiple preparations represents a poorly appreciated source of adverse

reactions. A recent case of fatal hepatotoxicity was believed to be due to pharmacokinetic interactions between the *Psoralea corylifolia* toxic component, psoralen, and CYP3A4 inhibitors astragaloside IV (AST-IV) and atractylenolide I (ATR-I) from *Astragalus propinquus* and *Atractylodes macrocephala*. The toxicity pathways of psoralen are unknown, though may follow similar pathways to the related toxic chemical coumarin, thus CYP3A4 inhibition would result in increased toxicity. With over 50% of prescription medicine metabolised by CYP3A4 enzymes, and many inhibiting these enzymes, investigating the effect of CYP3A4 enzyme modulation on herbal toxicity is important. The aims were to investigate the effect of CYP3A4 modulation on coumarin toxicity in the presence of AST-IV and ATR-I. Coumarin, AST-IV and ATR-I were tested for toxicity in liver (HepG2) and intestinal epithelial (Caco2) cell models untreated and treated with CYP3A4 inducer, rifampicin. Cell viability assessed with MTT colorimetric assays. Significant coumarin toxicity was observed at 400 μ M-1000 μ M in untreated HepG2 cells, with similar toxicity seen in treated cells; 1000 μ M in untreated Caco2 cells, and at 600 μ M and 1000 μ M in treated. AST-IV was nontoxic in untreated HepG2 and Caco2 cells, but toxic at 300 μ M in both treated cell lines. ATR-I was nontoxic in both untreated cells and toxic at 200-300 μ M in treated Caco2 cells. AST-IV (100 μ M) + ATR-I (50-300 μ M) was not toxic in both cell lines. Coumarin (200 μ M) + AST-IV (50-300 μ M) showed synergistic toxicity in untreated Caco2 cells but simple additivity in treated or HepG2 cells. Coumarin (200 μ M) + ATR-I (50-300 μ M) showed synergistic toxicity in untreated Caco2 cells, but not in treated cells; HepG2 cells showed additive toxicity. No significant increase in toxicity over the interaction seen with two phytochemicals was observed in three-component interactions. The results demonstrate that modulation of CYP3A4 enzymes may influence coumarin toxicity and that common herbal phytochemicals have synergistic toxicity. This may be a potential source of adverse effects in polyherbacy.

P317 - 0870 Lichen Secondary Metabolite (Atranorin) Ameliorates Adriamycin-Induced Hepatotoxicity: Targeting the Proinflammatory Cytokine, Oxidative Stress, Apoptosis, and TGF- β 1/ STAT-3 Signaling

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Adriamycin (AOX) is an anthracycline antibiotic that is frequently employed for treating the various form of malignancies. Hepatotoxicity is one of the grave complications of AOX. The objective of this study was to investigate the effect of different doses of atranorin on Adriamycin-induced hepatotoxicity in mice. Sixty male BALB/C mice were taken for the study. These were divided into six groups having the same number of animals. Groups are as follows: control group (CTRL), AOX group (AOX), Atranorin-small dose group (ATR-S), Atranorin-large dose group (ATR-L), AOX + Atranorin - small dose group (AOX+ATR-S), AOX + Atranorin large dose group (AOX+ATR-L). Liver wt/body wt ratio, food intake, serum albumin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and total bilirubin were measured. Tissue antioxidant enzymes, transforming growth factor beta 1 (TGF- β 1), nuclear factor (erythroid-derived 2)-like 2/heme oxygenase-1 (Nrf2/HO-1) content, tumor necrosis factor alpha (TNF- α), interleukin 6 (IL-6) and signal transducer and activator of transcription-3 (STAT-3) was analyzed. A part of liver tissue was used taken for histopathological examination. Atranorin administration to AOX-treated mice induced significant optimization of serum ALT, AST, ALP, total bilirubin, tissue TGF-1 β , TNF- α , IL-6 and liver weight/body weight ratio. This is associated with a significant increase in food intake, serum albumin, tissue Nrf2/HO-1 content, STAT-3, and antioxidant enzymes. Histopathological pictures of different groups compared to AOX group showed significant improvements. This enhancement was significant with AOX + Atranorin large dose as compared to AOX + Atranorin small dose. Atranorin possess antioxidant, anti-inflammatory and hepatoprotective properties. Also, it affects TGF- β 1/STAT-3 signaling pathway and inhibits apoptosis. This presents atranorin as a promising candidate for cancer research, as it ameliorates AOX-induced hepatotoxicity, in a dose-dependent manner. All the methods followed here were conducted according to the guidelines provided by CPCSEA for animal experimentation.

P318 - 0903 Transcriptomic Landscape in Murine Plasma of Hepatocellular Liver Injury for Biomarker Discovery

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Drug-induced liver injury (DILI) is one of the adverse drug reactions that occasionally causes severe damage to patients and often leads the withdrawal of approved drugs. In both clinical and non-clinical assessments, transaminases, bilirubin, and alkaline phosphatase are used for the diagnosis of liver injury. However, their sensitivity and specificity are not enough. Recent studies indicate a potential utility of microRNAs as circulating biomarkers for DILI. However, to the best of our knowledge, no study analyzed effects of liver injury on mRNA and long non-coding RNA (lncRNA) levels in plasma. Given that the expression of hepatic lncRNAs have been dysregulated in several liver diseases, we hypothesized that distinct lncRNAs are secreted into the circulation upon hepatocyte injury. In this study, we determined their landscape in plasma of hepatocellular type of liver injury in mice. Eight-week-old male BALB/c mice were treated with thioacetamide (TAA; 75 mg/kg, *i.p.*) or carbon tetrachloride (CCl₄; 0.5 mL/kg, *i.p.*). Plasma and liver were collected 24 hours after the treatment. Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined. Total RNA was extracted from the pooled plasma. Using the RNAs, the cap analysis for gene expression-sequencing (CAGE-seq) was performed to determine and quantify gene transcription start sites by sequencing the 5' capped ends of mRNAs and non-coding RNAs. Plasma ALT and AST levels were significantly increased in the mice receiving TAA or CCl₄ compared to the mice receiving vehicle. By the histological analysis, severe centrilobular necrosis was noted both in TAA- and CCl₄-treated mice. By the CAGE-seq of plasma RNAs, a total of 11,000 lncRNAs or mRNAs were detected in either vehicle-, TAA- or CCl₄-treated mice, among which 8,958 in vehicle-, 8,837 in TAA-, and 7,411 genes in CCl₄-treated mice were detected. We observed a high correlation coefficient value ($r = 0.76$) in the plasma lncRNA and mRNA levels between TAA- and CCl₄-treated mice whereas those between vehicle- and TAA-treated mice ($r = 0.33$) and vehicle- and CCl₄-treated mice ($r = 0.02$) were not high, suggesting that plasma mRNA and lncRNA levels reflected liver injury. In TAA- and CCl₄-treated mice, 1,617 and 2,277 genes, respectively, exhibited log₂ fold change > 4 compared to vehicle-treated mice and 39% (1,099 genes) were common. We found that plasma mRNA and lncRNA levels were dramatically affected upon liver injury in mice. They could be potential circulating biomarkers for DILI.

P319 - 0904 Carbon Monoxide-Releasing Molecule-3 Protects Oxidative Stress-Induced Cell Death in HepG2 Cells: Role of ERK-Mediated Nrf2 Activation

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Oxidative stress is one of the important factors in the development and progression of most liver diseases including a variety of liver injuries and non-alcoholic steatohepatitis. It has been reported that carbon monoxide (CO) exerts several beneficial effects such as anti-apoptotic, anti-oxidant and anti-inflammatory effects. Among CO-releasing molecules (CORMs) designed to release and deliver CO to cells in a controlled manner, CORM-3 [tricarbonylchloro(glycinato)ruthenium (II)] is water soluble and its therapeutic potential has been extensively studied. However, to our knowledge, this is the first study to investigate the hepatocyte-protective effect of CORM-3 against tert-butylhydroperoxide-induced oxidative injury and the underlying mechanism. The cell viability assay showed that CORM-3 completely prevents apoptosis stimulated by tert-butylhydroperoxide when pretreated for more than 15 hours in HepG2 cells. CORM-3 pretreatment also attenuated ROS generation and restored the decreased glutathione level. CORM-3 stimulated the activation of Nrf2, a key transcriptional factor to regulate diverse antioxidant enzymes and cytoprotective molecules as demonstrated by the enhanced antioxidant response element (ARE)-luciferase activity and nuclear NF-E2-related factor-2 (Nrf2) accumulation. In addition, real-time PCR analysis revealed that mRNA levels of heme oxygenase-1, glutamate-cysteine ligase and NADPH:quinone oxidoreductase 1 increased, which suggests the possible role of Nrf2 and antioxidant system in the cytoprotection by CORM-3. Both ERK and AMPK signaling pathways were activated by CORM-3, but only the blockade of ERK activation by PD98059, a MEK inhibitor significantly attenuated the Nrf2 activation induced by CORM-3. Finally, our data suggest that CORM-3 has the potential for the protection of hepatocyte against oxidative stress possibly through the Nrf2 activation mediated by ERK signaling.

P320 - 0908 Effect of Molybdate on Hepatic Steatosis in Mice Given a Choline-Deficient High-Fat Diet

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Hepatic steatosis can progress to fibrosis through a development of non-alcoholic steatohepatitis (NASH). Anti-fibrotic effects of sodium molybdate (SM) have also been demonstrated in rats with cholestatic liver. In the present study, we evaluated the effects of SM on hepatic steatosis, fibrosis and associated disturbances in mice fed a choline-deficient high-fat diet (CDF). Male C57BL/6 mice at 10 weeks of age were fed a CDF and bottled water containing SM for four weeks. The SM treatment markedly attenuated CDF-induced accumulation of lipids, mainly triglycerides, in the liver. Particularly, the expression of lipolysis-related proteins, such as adipose triglyceride lipase and monoglyceride lipase, was up-regulated by SM in the CDF-fed mouse livers. Lipid catabolic autophagic pathways were activated by SM in the CDF-fed mouse livers, as evidenced by decreased p62 levels. In addition, SM treatment markedly alleviated CDF-induced hepatic fibrosis by down-regulating the expression of pro-fibrogenic genes, such as collagen type I alpha 1 and tumor necrosis factor-α. The CDF-induced oxidative and cellular damage and apoptotic cell death was alleviated by SM in the liver. Taken together, these findings suggest that molybdate may be a new therapeutic strategy against hepatic steatosis and NASH.

P321 - 0961 4-Methylpyrazole Is a New Therapy for Massive Acetaminophen Poisoning in Mice and Human

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Acetaminophen (APAP) overdose is the most common cause of acute liver failure in western countries. APAP is safe at the recommended therapeutic dose of 4 grams/day. However, unintentional (moderate) and intentional (severe) overdose do occur and leads to 500 deaths every year. APAP mediated liver toxicity is due to cytochrome P450 generation of the toxic reactive metabolite, NAPQI (N-acetyl-p-benzoquinone imine), which depletes hepatic glutathione and subsequently induces activation of MAP kinases like c-Jun N terminal kinase (JNK) and mitochondrial oxidant stress. N-acetylcysteine (NAC), a glutathione precursor, is the only current clinical treatment for APAP overdose. However, NAC is not as effective in cases of severe overdose. Interestingly, a recent case report of acute APAP overdose (Zell-Kanteretal,AmJTherap,20:104-106,2013) suggested that 4 Methylpyrazole (4MP), an antidote for toxic alcohol poisoning, may contribute to better clinical outcomes. Thus, we examine the mechanistic basis of 4MP protection in C57BL6J mice receiving 300mg/kg APAP followed by 4MP (50mg/kg) 90 minutes later. Delayed 4MP treatment protected against alanine transaminase elevation 3, 6 and 24h after APAP overdose, without influencing APAP bioactivation. 4MP also blocked mitochondrial dysfunction by preventing continuous JNK activation. The inhibition of JNK activation was not restricted to the APAP model, since 4MP also strongly blocked JNK activation in both phorone/tetrabutylperoxide and Galactosamine/endotoxin models of liver injury. Molecular docking, thermal stability and *in vitro* kinase assays suggest that 4MP directly binds the JNK1/2 active site and prevents kinase activity. A clinical trial in human volunteers performed with our collaborators, indicates that 4MP is also very effective in humans as a P450 inhibitor. In conclusion, delayed treatment with 4MP is very effective against APAP overdose by acting via an alternate mechanism to that of NAC. Thus, clinical treatment with 4MP could complement NAC, especially in cases of severe APAP overdose.

P322 - 0009 The Role of Hepatic Cytochrome P450s in the Cytotoxicity of Dronedaron

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Dronedaron is used to treat patients with cardiac arrhythmias and has been reported to be associated with liver injury. We investigated the role of cytochrome P450s (CYPs)-mediated metabolism in the dronedaron-induced toxicity using our previously established HepG2 cell lines expressing individually 14 human CYPs (1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, 3A5, and 3A7). We demonstrated that CYP3A4, 3A5, and 2D6 were the major enzymes that metabolize dronedaron and that CYP3A7, 2E1, 2C19, 2C18, 1A1,

and 2B6 also metabolize dronedarone, but to a lesser extent. Our data showed that the cytotoxicity of dronedarone was decreased in CYP3A4-, 3A5-, or 2D6-overexpressing cells compared with the control HepG2 cells, indicating that the parent dronedarone has higher potency than the metabolites to induce cytotoxicity in these cells. In contrast, cytotoxicity was increased in CYP1A1-overexpressing cells, demonstrating that CYP1A1 exerts an opposite effect in dronedarone's toxicity, comparing with CYP3A4, 3A5, or 2D6. We also studied the involvement of topoisomerase II in dronedarone-induced toxicity and demonstrated that the overexpression of topoisomerase II caused an increase in cell viability and a decrease in gamma-H2A.X induction, suggesting that suppression of topoisomerase II may be one of the mechanisms involved in dronedarone-induced liver toxicity.

P323 - 0100 Toxicokinetic Characteristics of Fusarenon-X and Its Major Metabolites in Goats

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Fusarenon-X (FX), a type B trichothecene mycotoxin, is mainly produced by the *Fusarium* fungi, which naturally occurs in agricultural commodities. Toxicokinetic profiles of FX and its metabolite have been reported in mongastic animals, but to the best of the authors' knowledge no data exist on its toxicokinetics in ruminants. The main aim of this research was to evaluate the toxicokinetic characteristics of FX and its metabolite, nivalenol (NIV), in goats. The amounts of FX and NIV in post-mitochondrial, microsomal, and cytosolic fractions of diverse tissues of the goat were also investigated. FX was intravenously (iv) or orally (po) administered to goats at dosages of 0.25 and 1 mg/kg bw, respectively. The concentrations of FX and NIV in plasma, feces, and urine were quantified by liquid chromatography tandem-mass spectrometry. The concentrations of FX in plasma were quantified up to 8 h with both routes of administration. A large amount of NIV was quantifiable in plasma, urine, and feces after both administrations. The C_{max} value of FX was 413.39 ± 206.84 ng/ml after po administration. The elimination half-life values were 1.64 ± 0.32 h and 4.69 ± 1.25 h after iv and po administration, respectively. *In vitro* experiments showed that the conversion FX-to-NIV mainly occurs in the liver microsomal fraction. In addition, deepoxynivalenol was detectable in feces and urine samples of goats by high resolution tandem mass spectrometric detection. This is the first study that evaluates the fate and metabolism of FX in ruminant species.

P324 - 0106 Withdrawn

P325 - 0126 Withdrawn

P326 - 0132 Quantitative Metabolism of Senecionine—A Carcinogenic Pyrrolizidine Alkaloid by LC/MS/MS Analysis

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Pyrrolizidine alkaloids (PAs) are toxic and carcinogenic phytochemicals widespread in the world. There are three principal metabolic pathways of PAs: (i) hydrolysis of the ester functional groups to form retronecine; (ii) *N*-oxidation of the necine bases to PA *N*-oxides; and (iii) ring-oxidation followed by dehydration to produce dehydro-PAs, which are highly unstable, never been isolated from biological reactions, and are readily hydrolyzed to 6,7-dihydro-7-hydroxy-1-hydroxymethyl-5*H*-pyrrolizine (DHP). Radioactive [¹⁴C]PAs were used for the quantitative metabolism study, through quantifying the amounts of retronecine, PA *N*-oxide, and DHP to account for these three pathways, and the formation of DHP has been used to account for the metabolic activation pathway. We previously found that besides DHP, 7-glutathione-DHP (7-GS-DHP), 7-cysteine-DHP, 7-*N*-acetylcysteine-DHP (7-NAC-DHP), and 9-CHO-DHP are also DNA-reactive metabolites, capable of binding to cellular DNA leading to the formation of four DHP-DNA adducts designated as DHP-dG-3, DHP-dG-4, DHP-dA-3, and DHP-dA-4. We have determined that DHP-DNA adduct levels correlated with liver tumor potencies for a number of PAs with different struc-

tural features. Accordingly, we have proposed that these DHP-DNA adducts are biomarkers of PA exposure and PA-induced liver tumor initiation. In this study, we conducted the quantitative metabolism of senecionine, a carcinogenic PA, by rat liver microsomes. With all the synthetic standards prepared, we were able to identify and quantify these five reactive pyrrolic metabolites (DHP, 7-GS-DHP, 7-cysteine-DHP, 7-NAC-DHP, and 9-CHO-DHP) as well as senecionine *N*-oxide, and retronecine by LC/MS/MS multiple reaction monitoring (MRM) mode measurements. These results represent the first time using an LC/MS MRM method for quantitative metabolism of a PA and quantify the five DNA reactive metabolites. This provides an excellent methodology for studying metabolic activation pathways of PAs through quantifying multiple DNA-reactive pyrrolic metabolites. *This abstract is not an official US Food and Drug Administration (FDA) guidance or policy statement. No official support or endorsement by the US FDA is intended or should be inferred. The authors declare no competing financial interest.*

P327 - 0172 Buprenorphine and Metabolites Concentrations in Neonate Cord Blood: An Exploratory Pharmacogenetic Analysis

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As suggested by the Substance Abuse and Mental Health Services Administration guidelines on opioid use disorder treatment during pregnancy, both methadone and buprenorphine are safe pharmacotherapy options. Due to increased accessibility and comparatively less severe withdrawal in the newborn, women often receive the latter. Therefore, most cases of neonatal abstinence syndrome (NAS) are caused by buprenorphine. Single nucleotide polymorphisms (SNPs) may influence the expression and/or activity of the cytochrome P450 (CYP) 3A subfamily, which produces an active metabolite (norbuprenorphine), and uridine diphosphate-glucuronosyltransferase 1A1 (UGT1A1), which yields buprenorphine-glucuronide, an inactive metabolite. In this subsection of a previous study concerning NAS prediction, we sought to identify associations between umbilical cord blood drug and metabolite concentrations and select SNPs. In the IRB-approved double-blind nonintervention study, cord blood samples were collected following maternal consent. Inclusion criteria consisted of a gestational age greater than 36 weeks and an absence of congenital anomaly as well as the absence of maternal poly substance use. Following protein precipitation and solid-phase extraction, buprenorphine, norbuprenorphine, and buprenorphine-glucuronide concentrations were assayed via LC-MS. Samples were genotyped using an iPLEX ADME PGx Pro v1.0 Panel. Multiple linear regression adjusted for covariates (gestational age, birth weight, and maternal drug dose) was conducted using an additive genetic association model in PLINK v1.7 with statistical significance set at $p < 0.05$. The cord blood of fourteen newborns, presenting with an average gestational age of 38.89 ± 1.71 weeks and a mean birth weight of 3.00 ± 0.39 kg, was sampled. Additionally, a mean maternal buprenorphine dose of 10.29 ± 6.22 mg was noted. The concentration of buprenorphine (6.43 ± 4.76 ng/mL) was associated with one SNP (rs3745274; $p = 0.003$) in CYP2B6. No significant association was detected between norbuprenorphine cord blood concentration (15.30 ± 7.33 ng/mL) and neonate genotype. However, the concentration of buprenorphine-glucuronide (15.38 ± 8.19 ng/mL) was associated with three SNPs, rs2273697 ($p = 0.049$), rs9282861 ($p = 0.016$), and rs3745274 ($p < 0.001$), in *ATP-binding cassette C2, sulfotransferase 1A1*, and CYP2B6, respectively. No CYP3A subfamily or UGT1A1 SNP associations were found. These exploratory results may suggest the utility of pharmacogenetic exploration in buprenorphine usage during pregnancy. However, further confirmation studies are required.

P328 - 0174 Changes in Hepatic Expression of Cytochrome P450 and Glutathione S-transferase in Mice Exposed to Cigarette Smoke

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The present study aimed to determine the effects of cigarette smoke on the regulation of hepatic cytochrome P450 (CYP) and glutathione *S*-transferase (GST) enzymes in male BALB/c mice exposed to nose-only cigarette smoke for 4 days. There were no significant increases in serum liver injury markers (alanine aminotransferase and aspartate aminotransferase) or oxidative stress (total antioxidant capacity, malondialdehyde, and glutathione disulfide/reduced glu-

tathione) following cigarette smoke exposure, but malondialdehyde was elevated in the bronchoalveolar lavage fluid of smoke-exposed mice. Additionally, the hepatic microsomal protein levels of Cyp1a and Cyp2b, and the activities of ethoxresorufin O-deethylase, pentoxyresorufin O-dephenylase, and chlorzoxazone 6-hydroxylase, were elevated in smoke-exposed mice. Interestingly, the hepatic activities of GST toward 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene, and ethacrynic acid, but not cumene hydroperoxide, were enhanced by cigarette smoke exposure, which was consistent with the increased expression levels of mu- and pi-class GSTs, but not alpha-class GSTs, observed in immunoblot analyses. These findings indicate that the short-term inhalation of cigarette smoke induces drug-metabolizing enzymes such as CYP1A, CYP2B, and mu/pi-class GSTs in the absence of hepatic injury and oxidative stress. Furthermore, smoking may alter hepatic drug metabolism, as well as the disposition and toxicity of xenobiotics, including some therapeutic drugs and cigarette smoke constituents.

PS P329 - 0254 Characterization of Role of Cytochrome P450 in Hepatic Metabolism of Fimasartan

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Fimasartan (FMS), an angiotensin II receptor antagonist, is metabolized to FMS S-oxide, FMS N-glucuronide, oxidative desulfurized FMS (BR-A-557), and hydroxy-n-butyl FMSs. The purpose of this study was to characterize enzymes involved in NADPH-dependent FMS metabolism using recombinant enzymes such as cytochrome P450 (CYP) and flavin-containing monooxygenase (FMO), as well as selective chemical inhibitors. The results showed that CYP, but not FMO, plays a major role in FMS metabolism. CYP2C9, CYP3A4, and CYP3A5 were involved in the formation of FMS S-oxide, which was further metabolized to BR-A-557 by CYP3A4/5. CYP2C9 played an exclusive role in n-butyl hydroxylation. The specificity constant (k_{cat}/K_m) values for S-oxidation by CYP2C9, CYP3A4, and CYP3A5 were 0.21, 0.34, and $0.19 \mu\text{M}^{-1}\cdot\text{min}^{-1}$, respectively. The k_{cat}/K_m values of hydroxylation at the 1-, 2-/3-, and 4-n-butyl group in CYP2C9 were 0.0076, 0.041, and $0.035 \mu\text{M}^{-1}\cdot\text{min}^{-1}$, respectively. The k_{cat} and K_m values provide information for the prediction of FMS metabolism *in vivo*. In addition, simultaneous determination of the FMS metabolites may be used to evaluate CYP2C9 and CYP3A4/5 activity.

PS P330 - 0257 Metabolism of [3H]-BADGE in Rodents: A Spotlight on the Complex *In Vivo* Fate of the Diglycidyl Ether Analogue of Bisphenol A

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Many man-made molecules present in our environment and/or food chain are endocrine disrupting chemicals (EDC). Bisphenol A (BPA) is widely used for the synthesis of polycarbonates and epoxy resins. Strong assumptions that BPA has adverse effects on human health have led Canada and Europe to restrict its use. French Authorities banned BPA's use for the manufacture of baby items as well as food packaging material, since 2015. Nevertheless several analogs, in particular bisphenol A diglycidyl ether (BADGE), are still used for the synthesis of polymers and similar epoxy resins, including those used in food contact packaging. Available bibliography reports human exposure levels around $5.9 \mu\text{g}/\text{person}/\text{day}$. However, BADGE's metabolic fate remains largely unknown and it is currently unclear whether human monitoring should focus on the parent compound or on metabolites arising from the biotransformation and/or the degradation of this reactive molecule. This *in vivo* study aims at exploring BADGE's metabolic balance and identifying the different metabolic pathways involved in its metabolism. It aims to identify candidate biomarkers with the perspective of further human biomonitoring studies, and also seek for metabolites which structure may hint for bioactivation pathways. We investigated the metabolic balance of a low dose of radio-labeled BADGE in male and female Wistar rats. Rats were orally doses with a single dose of $50 \mu\text{g}/\text{kg}$ b.w. of [3H]-BADGE ($1.6 \text{ MBq}/\text{kg}$ b.w.). After 24h in metabolic cages, rats were euthanized and blood, urines, feces, and tissues (including digestive and genital tracts, glands, adipose tissue, organs, skin, and carcasses) were collected for radioactivity quantification. Extractions were performed on digestive contents, feces, and livers. Analytical methods were developed to explore fecal and urinary metabolic profiles, based on high-performance-liquid-chromatography coupled to on-line radioactivity detection (radio-HPLC). BADGE was quickly ab-

sorbed, and mainly excreted in feces. BADGE (the parent compound) was not detected in urinary radio-HPLC profiles, revealing an extensive and relatively fast metabolism. Four main metabolites were detected in male urine, six in female urine, seven in male feces, and five in female feces. Some of these metabolites have been tentatively identified through HPLC-MS/MS approaches. Several metabolites underline the chemical reactivity of this BPA analogue.

PS P331 - 0362 Investigation of Mechanism of Rodenticide Resistance with Closed Colonies of Rodenticide-Resistant Rats from Tokyo

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Anti-blood coagulation rodenticides, such as warfarin, have been used around world. They inhibit vitamin K epoxide reductase (VKOR), necessary for producing several blood clotting factors to cause lethal hemorrhage. However, heavy usage of them has led to the appearance of rodenticide-resistant rats. To eliminate them, second-generation-rodenticide have developed but their high toxicity caused secondary damage to wild species especially raptors. Thus, it is necessary to reveal the mechanisms of resistance to establish effective elimination strategy. There are two major mechanisms of the resistance: (I) mutation of VKOR, (II) enhanced metabolism of rodenticide. However, it is often difficult to observe these factors with wild rats because they have large individual differences and external factors like environmental chemical can affect metabolic ability. To solve this problem, we established two closed colonies of roof rats (*Rattus rattus*). One is warfarin susceptible rats (S-rats), the other is resistant ones (R-rats). They enable us to perform experiments without external factors. (I) Assessment of VKOR activity and affinity to rodenticide. We established *in vitro* test which uses tris(3-hydroxypropyl)phosphine as reductant for VKOR which is more suitable to measure natural VKOR activity compared to dithiothreitol commonly used reductant. R-rats showed significantly lower activity of VKOR but showed higher IC_{50} value to warfarin. (II) Assessment of metabolism. *In situ* liver perfusion of warfarin was performed to examine hepatic metabolism. R-rats had enhanced hepatic warfarin hydroxylation than S-rats. *In vitro* warfarin metabolism assay was performed to investigate cytochrome P450 (CYPs) having role in warfarin hydroxylation. We prepared two fractions from their liver. One is microsome (Ms) containing CYPs, the other is S9 fraction, including CYPs and natural NADPH secretion ability. NADPH was added to Ms, NADP was added to S9. The V_{max} of Ms of R-rats showed modest difference, however, that of S9 of R-rats was significantly higher than that of S-rats. These results indicated the enhanced metabolism shouldn't be due to CYPs but NADPH production ability. (III) We measured NADPH production activity by pentose phosphate pathway; major source of NADPH. R-rats showed enhanced NADPH production. There were not significant differences in amino acid sequence and expression level of glucose 6 phosphate dehydrogenase (G6PD) playing role in NADPH production in pentose phosphate pathway. G6PD are known to have post-transcriptional regulation. These results indicated mechanism of higher NADPH production may lie in post-transcriptional regulation of G6PD.

PS P332 - 0386 Role of Intestinal Microbiota in Metabolism of Voglibose *In Vitro* and *In Vivo*

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Some of trillions of microorganisms that reside in the intestine would have roles in metabolism and detoxification of toxicants and drugs. Voglibose is an anti-diabetic drug that inhibits alpha-glucosidase. Studies proved that none or very less metabolism of voglibose occurred in liver. In the present study, the possible metabolism of voglibose by intestinal microbiota was investigated both *in vitro* by using intestinal contents prepared from saline- and antibiotics-treated mice and *in vivo* by using normal and diabetic mice. Three metabolites of voglibose were identified from the incubation of voglibose with intestinal contents in this study. For animal studies, male ICR mice were treated with streptozotocin ($40 \text{ mg}/\text{kg}$) for 5 days to induce diabetes, followed by the treatment with either antibiotics or saline for 3 days to control the numbers of intestinal microbiota. On the next day of last administration with antibiotics, mice in both models were fasted for 6 hr. Voglibose ($10 \text{ mg}/\text{kg}$) followed by

maltose (50 mg/kg) was administered and the blood glucose level was measured at several time points. Pretreatment with antibiotics would cause lesser metabolism of voglibose and, thereby, lower the blood glucose level more than in control animals. The results suggested that the pharmacological activity of certain drugs would be modulated by the metabolism by intestinal microbiota. *Supported by the grant from National Research Foundation, Korea (NRF-2017RIDIA3B04033313).*

PS P333 - 0506 Glutathione Conjugation of the Reactive Metabolite of Acetaminophen Catalyzed by Human Glutathione S-transferase Isoforms

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Acetaminophen (APAP) is one of the mostly used analgesics and antipyretics. It is considered to be safe drug when used at therapeutic doses. On the other hand, the APAP overdosing is the most frequent cause of acute liver failure in men. At therapeutic doses, APAP is detoxified by three major pathways in the liver. The most of APAP dose is conjugated by glucuronidation and sulfation. Remaining part of APAP is oxidized by cytochrome P450 to a toxic metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI). NAPQI is detoxified by either spontaneous or enzymatic reaction with glutathione (GSH) resulting in a conjugate APAP-SG. However, there is little research to identify GSH S-transferase (GST) isoforms involved in generation of APAP-SG. In the present study, we investigated enzymatic reaction of NAPQI with GSH catalyzed by various human recombinant purified GSTs (13 different isoforms). To optimize incubation conditions for enzymatic reaction of NAPQI with GSH, we examined concentrations of GSH, incubation time, and pH dependency. As a result, lower GSH concentration and pH and longer incubation led to efficient enzymatic generation of APAP-SG. GSH conjugation activity toward NAPQI was measured by LC-MS/MS and quantified using standard of APAP-SG that was synthesized according to published methods. LC-MS/MS analysis revealed that the conjugation activity was detected in hGSTA1-1, hGSTA2-2, hGSTM1-1, hGSTM2-2, and hGSTP1-1. Of those isoforms, hGSTP1-1 had the highest activity. It is generally known that protein expression level of hGSTP1-1 in human normal liver is lower than that of other GST isoforms that have conjugation activity toward NAPQI. Our results suggested that several GST isoforms involved in enzymatic GSH conjugation of NAPQI.

PS P334 - 0572 Withdrawn

PS P335 - 0687 Toward Understanding Metabolism of Synthetic Cannabinoids and Model Compounds by Using *In Vitro* Oxidation by Microsomes and Chemical Oxidant Reagent

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Synthetic cannabinoids (SCs) are designer drugs that bind the CB1 and CB2 cannabinoid receptors on neurons and other cells. Over 200 SCs exist in different chemical groups. Some are classed as Schedule 1 drugs because they cause harm. Many emergency room visits and adverse events are reported for SCs. Detection of metabolites of SCs in biological matrices is key to detecting abuse of these compounds and toxicity studies. An analytical problem is the fact that novel cannabinoids are brought into the market after banning of the prevalent ones and standards and identification of metabolites of novel compounds need to be identified. Phase 1 metabolism of compounds involves cytochrome p450 enzymes which produce oxidative and other changes in their substrates. It is hypothesized (1) that human liver microsomes and cytochrome p450 will produce metabolites of the SCs JWH-015 and Bay 59-3074 that are relevant to *in vivo* metabolism (2) that oxidation of SCs with chemical reagents may produce some of the compounds produced by cytochrome p450 enzymes and enable production of metabolite standards. JWH-015, Bay 59-3074 and the controls eosin and coumarin were incubated with human liver microsomes (HLM) and recombinant cytochrome p450 2D6 (CYP 2D6) and were also subjected to chemical oxidation by sodium hypochlorite. Preliminary experiments showed that the synthetic cannabinoids JWH-015 and Bay 59-3074 and the controls

eosin and coumarin were detectable by thin layer chromatography (TLC) and uv-visible spectroscopy. TLC analysis revealed changes in eosin and coumarin after incubation with HLM. The JWH-015 and Bay 59-3074 were not detected on TLC plates because they were most soluble in organic solvent and large dilutions had to be made to allow incubations with HLM and CYP 2D6. In contrast, uv-vis spectroscopy detected absorbance increases in the JWH-015 and absorbance decreases in Bay 59-3074 after incubation with HLM compared to the controls. The Rf value of JWH-015 but not that of Bay 59-3074 on TLC plates was decreased after incubation with sodium hypochlorite oxidant. Eosin and coumarin incubated with sodium hypochlorite showed differences detected by both TLC and uv-visible spectroscopy relative to controls. In conclusion, future work must solve the problem of the necessity to work with high dilutions of synthetic cannabinoids with HLM and CYP 2D6 to minimize protein denaturation by the organic solvents in which they are soluble. Chemical oxidation and *in vitro* incubation with human liver microsomes may produce metabolites of the synthetic cannabinoids Bay 59-3074 and JWH-015 for chemical characterization and standards for detection of these compounds in biological samples.

PS P336 - 0698 Cytochromes P450 Bioactivate Lamisil (Terbinafine) through Multiple N-dealkylation Pathways

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Lamisil (terbinafine) may cause idiosyncratic liver toxicity through a mechanism involving the reactive metabolite 6,6-dimethyl-2-hepten-4-ynal (TBF-A), yet relevant pathways leading to TBF-A formation remain unknown. We combined computational modeling experimental kinetics to assess contributions of three N-dealkylations yielding ultimately TBF-A and hepatic P450 isozymes responsible for them. A deep learning model predicted a high likelihood for N-demethylation followed by N-dealkylation to TBF-A and marginal contributions from other pathways. For experiments, we used novel labeling methods for metabolites to carry out steady-state kinetic studies with pooled human liver microsomes. Those efforts revealed efficient TBF-A formation and first order decay during metabolic reactions; actual TBF-A levels would then reflect the balance between those processes as well as impact of adduct stabilization with biological molecules like glutathione. Model predictions and experimental studies agreed on the significance of N-demethylation and insignificance of N-denaphthylation in microsomal metabolism, yet the model failed to identify direct TBF-A formation as the most efficient microsomal pathway. These microsomal studies provided insights on the collective capacity of P450 isozymes to catalyze reactions, but not their individual contributions that vary in the population and determine risks. Our models for nine P450 isozymes predicted a common high likelihood for N-dealkylation for two pathways yet minor contributions for direct TBF-A formation. Experimentally, we identified P450 isozymes metabolizing terbinafine and then carried out steady-state studies with recombinant isozymes. Based on kinetics, CYP2C19 dominated all N-dealkylation pathways followed by CYP3A4, whereas other isozymes were more selective and less efficient for pathways. Extrapolation of *in vitro* kinetics to *in vivo* clearance indicated that CYP3A4 was the major contributor (>30%) to all pathways followed by CYP2C19. Moreover, CYP1A2 and 2C9 contributed to direct formation of TBF-A. Other P450s were minor contributors to metabolism. Consequently, variations in CYP3A4 and to a lesser extent CYP1A2, 2C19, and 2C9 would likely impact drug clearance and TBF-A generation in patients impacting toxic risk. Through this combination of approaches, we are the first to identify pathways and P450 isozymes leading to generation of TBF-A for assessing its role in idiosyncratic adverse drug interactions.

PS P337 - 0760 Functional Characterization of Five CYP2A13 Allelic Variations

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CYP2A13 is mainly expressed in the human trachea and lung, whereas CYP2A6 is found in the human liver. Therefore, CYP2A13 enzyme can be considered as the primary enzyme responsible for the metabolic activation of many tobacco-specific carcinogens. Genetic variations can significantly influence the toxicological outcomes derived from tobacco smoking. Here, the *in vitro* functional activities of five CYP2A13 genetic variations (R257C, 133_134insT, R101Q, I331T, and R257C/I331T) in CYP2A13*2, *3, *4, and *10 alleles were analyzed. Mutant clones were constructed and their recombinant enzymes were expressed in

Escherichia coli. CYP2A13 mutants containing R257C, 133_134insT, I331T, and R257C/I331T displayed P450 holoenzyme spectra. The R101Q mutant was not expressed. CYP2A13 enzymes displayed the typical type I binding spectra to coumarin and the calculated binding affinities of R257C, R257C/I331T, and 133_134insT mutants were decreased from approximately 3 to 7 times. In catalytic analyses of purified mutant enzymes for coumarin and nicotine, the R257C and I331T mutants displayed decreased k_{cat} values with catalytic efficiencies reduced up to approximately 20%. The double mutation of R257C/I331T induced increased K_m values and decreased k_{cat} values that resulted in >50% decrease in catalytic efficiencies. For the 133_134insT mutant, the catalytic activities were not saturated but the measured rates at the highest concentrations were much lower than those of the wild type or other mutant enzymes. Functional analysis of these variations in CYP2A13 allelic variants can help understand the consequence of CYP2A13 polymorphism in the bioactivation of many tobacco-derived carcinogens.

PS P338 - 0840 Barcoded DNA Measurements Yield Unexpected Interference with High-Throughput CYP-Mediated Cytotoxicity Assay

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In vitro chemical tests commonly use cell lines that lack chemical metabolism, hindering the use and relevance of cell culture in high-throughput toxicity screening. To address this challenge, we engineered HEK293T cells to over-express cytochrome P450 monooxygenase (CYP) recombinant transgenes, which are commonly involved in human chemical metabolism. Each of the five clones (CYP1A1-, CYP1A2-, CYP2E1-, and CYP3A4-overexpressive and one empty vector control) was mated to a unique DNA barcode, which acts as a surrogate of cell count. Therefore, if chemical toxicity is mediated via one or multiple of these CYPs, it can be detected in mixed clonal culture by probe-based PCR, making it easily adaptable to high-throughput screening. As a proof of concept, we analyzed clonal toxicity to aflatoxin B1 (AFB1) and staurosporine (STS). Using CellTiter-Glo and CellTiter-Blue cytotoxicity assays (Promega), CYP3A4- and 1A1-expressive clones demonstrated greater susceptibility to both AFB1 and STS, supporting their previously documented roles in forming cytotoxic metabolites. Fluorometric DNA quantitation of cell lysates further showed a loss in cellular DNA associated with chemical exposure. Surprisingly, when clone-associated DNA barcodes were measured by droplet digital PCR (ddPCR) and targeted sequencing, we observed significant increases in barcode counts from CYP3A4- and CYP1A1-expressive clones after exposure to both toxic compounds. Similar results were seen when testing a larger panel of chemicals. To address these inconsistencies, a direct lysis method with multiple modifications was developed to circumvent any possible interference due to DNA isolation. These modifications included sonication, restriction enzyme digest, QIAshredder homogenization, and various lysis buffers. Despite these efforts, augmented ddPCR barcode measurements were still observed with chemical-induced cytotoxicity suggesting issues with PCR. Overall, the data suggests a need for an *in situ* method of cell death determination that avoids amplification, such as Fluorescence *in situ* Hybridization. *This abstract does not necessarily reflect US EPA policy.*

PS P339 - 0864 Toxicokinetics of QiLin Pills following Single Oral Administrations in SD Rats

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QiLin Pills (QLP) is one of the most commonly used Chinese medicines and it is used to treat kidney deficiency, deficiency of blood gas, weakness of waist and knee, fatigue, poor complexion, semen thinning, impotence and premature diarrhea, and menstrual irregularity in women. Although it is widely used, its toxicity not been well understood. Albiflorin is one of its main components. So the purpose of this study was to determine the toxicokinetics of QLP following single oral (PO) administrations in SD Rats, and to develop an ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method to determine the concentration of albiflorin in SD Rats. Chromatographic separation was achieved on a Zorbax SB-C₁₈ (4.6mm*150mm, 5μm) column at 40°C with an auto sampler temperature of 4°C after the proteins precipitated with methanol. The mobile phase was 0.1% formic acid (A) and methanol (B), the elution program was as follows: 0min, 70%B; 0-3min, 70%-90%B; 3-5min, 90%B; 5-5.2min, 90%B-70%B. The flow was 0.5 mL/

min. Electrospray ionization (ESI) with a negative-ion and multiple reactions monitoring mode was used. The precursor ion→product ion transitions m/z 525.2→449.2 for albiflorin and m/z 321.0→151.9 for the internal standard were monitored. The calibration curve was linear in the range of 1 to 60 ng/ml with a correlation coefficient of 0.9995, and the limit of detection (LOD) of albiflorin in plasma was 0.1 ng/ml. The accuracy was 87.4%-108.7%, the extracted recovery were 92.6% -104.6%. And the maximal concentration (C_{max}) and the mean apparent half-life ($t_{1/2}$) were (23.46± 4.65) ng/ml and (292.47±52.36) min. The UPLC-MS/MS method was quick and sensitive with a simple protein precipitation, which can be used for toxicokinetics studies of albiflorin in SD Rats.

PS P340 - 0909 Edaravone Free Radical Metabolite Formation and Its Consequences

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Edaravone (Radicava) is a relatively recent drug that is considered to work as an antioxidant *in vivo*. As a criterion of an antioxidant is its obligatory one-electron oxidation, we investigated the free radical species of edaravone itself using neutrophil myeloperoxidase (MPO). We observed that edaravone was a substrate of MPO and that edaravone enhanced MPO halogenating activity in a biphasic manner. Edaravone was itself oxidized to a stable metabolite by MPO, which was detected by UV-Vis spectrophotometry. Further studies using LC/MS revealed a number of MPO-catalyzed metabolites of edaravone, which appeared to be dimers, trimers, and tetramers, originated from the edaravone free radicals. To unequivocally characterize edaravone free radical formation, electron spin resonance (ESR) spin trapping with 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) was used, where a carbon-centred radical metabolite of edaravone was identified. As it was unclear if the carbon atom in question was acidic, NMR studies revealed that there are two ionizable hydrogens, one of which was indeed on the α-carbon, further solidifying the formation of the carbon-centred edaravone radical. Interestingly, the formation of this radical did not appear to potentially oxidize glutathione when compared with phenol (a prototypical glutathione oxidant). To assess if the intracellular formation of the edaravone free radical would be detrimental, HL-60 cells (rich in MPO) were treated H₂O₂ and edaravone. We observed that edaravone was able to reverse the toxicity induced by H₂O₂ that we considered led to the formation of hypohalous acids (e.g., HOCl). These findings suggest that edaravone free radical formation is not necessarily detrimental in a cellular system, although further studies are necessary to elaborate on other possible targets of edaravone free radicals.

PS P341 - 0936 Pharmacokinetics and Metabolism of Acetyl Triethyl Citrate in Rats

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Acetyl triethyl citrate (ATEC) is an aliphatic ester of citric acid that is used as a plasticizer in cosmetics, coating tablets, and food packaging. In this study, the pharmacokinetic properties of ATEC were investigated in rats. ATEC was intravenously or orally administered to rats and its plasma concentrations were determined using liquid chromatography-tandem mass spectrometry. The metabolic stability was evaluated in plasma and liver microsomal fractions and the *in vivo* metabolite profile was investigated in rats. The pharmacokinetic study showed that the volume of distribution and mean residence time of ATEC were higher after oral administration than after intravenous administration, pointing to extensive first-pass metabolism and distribution in tissue. In addition, the plasma concentration profile of the postulated metabolites of ATEC was investigated in plasma, urine, and feces. The resulting data indicated that ATEC was extensively metabolized and excreted mainly as metabolites rather than as the parent form. These data may be useful for understanding the safety and toxicity of ATEC.

P342 - 0022 Damaging Effect on Spatial Cognition Behavior by Bioengineered ZnO Nanoparticles of *Trianthema portulacastrum* Linn. in Wistar Rats

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Over the recent years nanoparticles are considered as rapidly growing modern technology; still, there is unavailability of adequate data for their possible health consequences on human beings. The key objective of the current study was to explore the effect of n-butanol fraction of *Trianthema portulacastrum* Linn. hydroethanolic extract (BFTP) and bioengineered zinc oxide nanoparticles of BFTP (ZnOTP) on the spatial cognitive process through a behavioral model, and evaluation of possible underlying mechanism by assessing the enzymatic antioxidant level along with estimation of acetylcholinesterase (AChE) activity and brain Zn content along with histological changes in the hippocampal region in mice. Strongest *in vitro* antioxidant and AChE inhibitory activity bearing fraction, BFTP, was subjected to determine inhibition kinetics by Lineweaver-Burk and Dixon plots. BFTP was further utilized for synthesis of ZnOTP and characterized by using different analytical methods. Old male swiss albino mice were administered for 21 consecutive days and subsequently analyzed for estimation of spatial memory using the Elevated plus maze (EPM) and Hebb's William maze (HWM). Brain homogenate supernatant was used for enzymatic antioxidant level and AChE inhibitory activity determination. Data exhibited that scopolamine induced extended transfer latency (TL) and time taken to reach reward chamber (TRC) was not significantly reversed by the ZnOTP-administered group, while BFTP-treated animals significantly ameliorated the scopolamine effect by reducing the TL and TRC as compared with scopolamine-treated groups. ZnOTP reduced the activity of various antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), AChE inhibition, and accelerated lipid peroxidation level. Increased brain Zn content was observed in comparison with donepezil and BFTP administered groups. Histological studies further supported the impairing effect of ZnOTP. The study concludes that ZnOTP damages the spatial learning and memory process by elevation in oxidative stress along with decrease in AChE inhibition activity and imbalance in Zn haemostasis in the brain hippocampus.

P343 - 0023 Quercetin Attenuates Metronidazole-Induced Neurotoxicity through Downregulating Nitric Oxide Release and Regulation of NF- κ B Pathway

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Metronidazole (MET), a 5-nitroimidazole derivative, is a widely used agent for the treatment of trichomoniasis, giardiasis, amebiasis, protozoal, and anaerobic infections, and also in the treatment of Crohn's disease. It is a nontoxic drug and has been shown to cross the blood brain barrier very rapidly. Side effects of MET are uncommon and very rare and include mild abdominal pain, headache, nausea, and a persistent metallic taste. Peripheral neuropathy and encephalopathy is an uncommon side effect, with long-term use of this drug in patients with infectious colitis at high cumulative dose. Furthermore, unusual cases have also been reported for development of rapid onset peripheral neuropathy at low cumulative dose. Currently, no information is available about the putative mechanism involved in MET neurotoxicity/neurodegeneration and neuroprotective effects of quercetin in MET-induced neurotoxicity using a rat model. Herein, we investigated the effect of MET on survival/death of neurons and associated mechanism of neuroprotection of quercetin in MET-induced neurotoxicity a rat model. The quercetin restored the functional deficits in MET-induced neurotoxicity rat model as signified by neurobehavioral assay, confirming the protection of neuron degeneration caused by MET. Quercetin also improved the biochemical and antioxidant status in the brain tissue by decreasing the lipid peroxidation and inducing the reduced glutathione release in tissue. Pretreatment with quercetin also ameliorated the neuronal cells degeneration, which could be perceived from histopathological findings. Quercetin also attenuated the MET-induced NF- κ B, Bax, and caspase-3 activation. On the contrary, quercetin increases the decreased expression of Bcl2 due to the administration of MET. MET demarcated a well-defined peripheral inflammation as perceived through the plasma inflammatory markers (i.e., NO, SGOT, and SGPT). The MET (oral) administration causes remarkably neurotoxic effects, precipitates peripheral inflammatory reactions, and the same can/ could be attributed to the fact that MET has the ability to cross the blood brain barrier and damage the neuronal cells. Consequently, quercetin exerts a neuro-

protective effect through modulating the oxidative stress, neuroinflammation, apoptosis, and reversing the neuron degeneration, suggesting its therapeutic role in neurotoxicity by enhancing the regenerative capacity.

P344 - 0084 Behavioral Disorders Caused by Acute Carbon Monoxide Poisoning and Its Relationship with Prognostic Biomarkers

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Carbon monoxide poisoning (CO) is preventable and avoidable. Hundreds of people die by acute intoxication to CO, and many of them suffer the so-called late neurological syndrome (LNS) with irreversible consequences. The aims of the present work were to assess the effects of an acute exposure to CO on the behavior, memory, anxiety, and gait dynamics and to relate these changes to some prognostic biomarkers. To this end, adult rats were exposed to CO at acute doses (350 ppm for 20 seconds) capable of causing deterioration of the sensorium, and different tests were performed seven days post-intoxication. Compared with control animals (C), rats exposed to CO showed changes in the (i) footprint test expressed as cm (C: 6.5±0.9; CO: 9.1±0.7; $P<0.05$) and left overlap (C: 1.1±0.1; CO: 1.8±0.2; $P<0.01$), compatible with ataxia by exhibiting a greater maximum difference in the stride length; (ii) open field test by exhibiting a greater exploratory activity and memory deficit ($P<0.05$); (iii) elevated plus maze test by manifesting a greater degree of anxiety ($P<0.05$); and (iv) inhibitory advance test by displaying a lower latency to enter the dark compartment ($P<0.01$). In order to search for some prognostic biomarkers, we evaluated the genetic damage in different cells using the comet assay. Compared with C, CO-exposed rats exhibited a higher genetic damage, expressed as TailDNA%, in (i) peripheral blood (PB, CO: 14±3; C: 5.1±0.6; $p<0.05$) and bone marrow (BM, CO: 31±3; C: 15±2; $p<0.01$) at 1 h post intoxication; and (ii) BM (CO: 27±2; C: 10±2; $p<0.001$) and brain (B, CO: 36±3; C: 27±3, $p<0.05$) at 7 days post-intoxication. These results suggest that the behavioral evaluation, gait, and detection of genotoxicity may inform early disorders prior to the development of the LNS.

P345 - 0110 Immunohistological Analysis of Peripheral Nerve Injury in Methylmercury-Exposed Rats

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Methylmercury (MeHg) is known as the causal substrate of Minamata disease, which induces central and peripheral nerve injury. MeHg-induced neural degeneration has been investigated mainly by using the brain because the principal pathological phenotype is caused by the impairment of central nervous system (CNS). In contrast, MeHg-induced neural injury in peripheral nervous system (PNS) is not well investigated. To clarify the aspects of neural injury in PNS induced by MeHg, 9-week Wistar male rats were exposed by MeHgCl solution orally for 5 days and not exposed for subsequent 2 days (day 7). This cycle was continued to the next week again (day 14). On day 7 and 14, rats were anesthetized and fixed and their dorsal root ganglion (DRG), motor, and sensory fibers were excised and cryosectioned. Co-staining of axonal marker NF and myelin marker MBP showed axonal degeneration in sensory but not in motor fibers on day 14. The peripheral sensory neurons in DRG were also degenerated, especially middle to large sized neurons on day 14. In fact, DRG has many different subtypes of neurons that sense pain, pressure, itch, etc. so that neurons were classified by different markers such as NF, PLXNC1, TrkA, and TH; however, this classified staining did not show any differences of neural degeneration between each subtype. We also analyzed another cell type using several cell type-specific markers, such as microglia (Iba1), macrophage (CD68), astrocyte (GFAP), fibroblast (Vimentin), epithelial cell (CD31), and Schwann cell (SOX10). Microglia, macrophage, and Schwann cell were significantly increased on day 14 DRG and sensory fibers. These results suggest that MeHg affects peripheral neural degeneration in DRG and axonal degeneration in sensory fiber but not in motor fiber with concerting several cells.

P346 - 0148 Cellular Recovery and Resilience—A New Angle to Look at Toxicity Testing

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To date, most *in vitro* toxicity testing focuses on acute effects of compounds at high concentrations. However, measurement of cytotoxicity cannot explain different susceptibility to exposures and organ selectivity. In addition, humans are mainly exposed to low doses of the chemicals and sometimes early in life, which then can manifest in the form of disease later in life. Therefore, we hypothesized (Smirnova et al. ALTEX 2015) that it is rather the way the cells can cope with the stress and respond to the next exposure and not their immediate response to the toxicant makes them differently susceptible. We used a dopaminergic neurons as a model, because they cannot cope with the same stress as other types of neurons. 3D neuronal cultures overcome the limitations of monolayer cultures in studying chemical withdrawal and recovery. We used a human 3D dopaminergic LUHMES model to determine whether effects of short-term rotenone exposure (100 nM, 24 h) are permanent or reversible (Harris et al, Arch Tox, 2018). A decrease in complex I activity, ATP level, mitochondrial diameter, and neurite outgrowth were observed acutely. After compound removal, complex I activity was still inhibited; however, ATP levels were increased, cells were electrically active, and aggregates restored neurite outgrowth integrity and mitochondrial morphology. We identified significant transcriptomic changes after 24 h that were not present 7 days after wash-out. To study cellular resilience, 3D LUHMES were reexposed to rotenone after recovery period. Preexposed cells maintained higher metabolic activity than controls and presented a different expression pattern of rotenone sensitive genes. In summary, our study shows that dopaminergic neurons can recover from short-term exposure to rotenone and are more resilient to a second hit; however, it is not clear yet whether this resilience is beneficial or detrimental in the long term. *References:* [1] Smirnova L, Harris G, Leist M., & Hartung T. (2015). *Cellular resilience*. *Altex*, 32(4), 247–260. [2] Harris G, Eschment M, Orozco SP, McCaffery JM, MacLennan R, Severin D, Leist M, Kleensang A, Pamies D, Maertens A, Hogberg HT, Freeman D, Kirkwood A, Hartung T, Smirnova L. (2018). *Toxicity, recovery and resilience in a 3D dopaminergic neuronal in vitro model exposed to rotenone*. *Arch Toxicol*. 28;31(15):441–20.

P347 - 0176 Molecular Mechanism of Methylmercury-Induced Neurotoxicity via Endoplasmic Reticulum Stress

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Methylmercury (MeHg), known as an environmental electrophile, evokes neuronal cell death. However, the mechanism is not fully determined yet. Previously, we found that MeHg caused S-mercuration of protein disulfide isomerase (PDI), and this covalent modification resulted in the loss of enzymatic activity. The disruption in PDI function by MeHg induced endoplasmic reticulum (ER) stress via the accumulation of misfolded proteins. Although this stress triggers the unfolded protein response (UPR) to maintain ER homeostasis, it is unclear whether MeHg affects the UPR signaling. Here, we report that MeHg modulates UPR branches to promote cell death. Treatment with MeHg activated protein kinase RNA-like endoplasmic reticulum kinase (PERK) and activating transcriptional factor 6 (ATF6) pathways. On the other hand, inositol-requiring enzyme 1 α (IRE1 α) pathway was attenuated by this challenge. Since MeHg suppressed the endonuclease activity of IRE1 α , we next attempted to determine the target cysteines of MeHg in IRE1 α RNase domain. Site-directed mutagenesis analysis revealed that C931S IRE1 α significantly prevented MeHg-induced inhibition of its RNase activity. These results suggest that C931 in IRE1 α might be a target of MeHg. IRE1 α branch is known to function as an antiapoptotic pathway. Indeed, MeHg-induced cell death remarkably attenuated in C931S IRE1 α expressed cells. Hence, MeHg might accelerate cell death by both inhibition of IRE1 α branch and activation of proapoptotic signaling via the PERK/ATF6 branches. Furthermore, to elucidate whether MeHg evokes ER stress *in vivo*, we utilized ER stress-activated indicator (ERAI) transgenic mice expressing luciferase in the presence of ER stress. Bioluminescence imaging showed that ER stress occurred in the brain by MeHg administration. Taken together, we demonstrated

that MeHg resulted in ER stress both *in vitro* and *in vivo*. Additionally, our study indicates that MeHg interfered antiapoptotic signaling based on the IRE1 α branch via the oxidative modification of IRE1 α in cells.

P348 - 0204 Mitigating Effects of Resveratrol Supplementation on Arsenic Trioxide Induced Neurotoxicity in Mouse Hippocampus

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Arsenic toxicity is a global health concern, as millions of people are exposed to this toxicant via drinking water, and arsenic exposure affects almost every organ system in the body, including the brain. Its chronic exposure induces behavioral and cognitive deficits in both human populations and in rodent models. The beneficial effects of naturally occurring antioxidants have been explored in various neuropsychiatric disorders, metal toxicities, and oxidative damage. The present study investigated the effects of resveratrol (antioxidant) on cognitive functions, oxidative stress parameters, and estrogen receptor profile in mice hippocampus (CA-1) following As₂O₃ exposure. Adult female mice of reproductive maturity (monitored by estrus cycles) were divided into control and experimental groups. As₂O₃ (2 and 4 mg/kg bw) alone or along with resveratrol (40 mg/kg bw) was administered orally to experimental groups for 45 days. Animals were subjected to a battery of behavioral tests. On Day 46, the animals were sacrificed either by perfusion fixation or by euthanasia and the brain tissues, thus obtained, were processed for morphology, estrogen receptor localization, and estimation of oxidative stress endpoints, respectively. Behavioral study suggested enhanced anxiety-related behavior and cognitive deficits in As₂O₃ alone-exposed animals when subjected to open field test, elevated plus maze and morris water maze. However, resveratrol supplementation restored these behaviors in As₂O₃-exposed animals. Oxidative stress parameters (GSH and NO) in hippocampal tissue of As₂O₃ (2mg/kgbw) alone-exposed animals were altered. Hippocampal sections of As₂O₃ alone-treated animals also showed significant changes at the histo-architectural level, which were normalized on simultaneous treatment with resveratrol. Therefore, these observations provide preliminary evidence of mitigating role of resveratrol on functional, biochemical, and structural parameters of the hippocampus in mice subjected to chronic As₂O₃ exposure.

P349 - 0251 Age-Dependent Dopaminergic Dysfunction following Early-Life Exposure to Atrazine in SD Rats

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The herbicide atrazine (ATR) is used worldwide and its contamination in the environment has resulted in documented human exposure. It has also been shown that ATR results in dopaminergic neurotoxicity, however, few studies have investigated the long-term effects of ATR following *in utero* and pubertal exposure. Therefore, we evaluated the effects of ATR exposure in Sprague Dawley rats during gestational and pubertal on the offspring dopaminergic system development. Pregnant and pubertal dams were treated with oral ATR at 0, 25, 50 mg/kg/day. (1) In this study, we examined the hypothesis that ATR could cross the placental barrier and have long-term adverse effects on the synthesis, degradation, and reuptake of DA in the brain. (2) For this purpose, we examine the concentration of levodopa (L-DA), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) in striatum. (3) The mRNA and protein expression of orphan nuclear hormone (Nurr1), tyrosine hydroxylase (TH), vesicular monoamine transporter 2 (VMAT2), dopamine transporter (DAT), monoamine (MAO), and catechol-O-methyl transferase (COMT) in the midbrain were examined by fluorescence PCR and Western blot when the offspring reached six months old or one year old. When measured 6 months post-treatment, the level of DA and expression of Nurr1, VMAT2, DAT, and TH were reduced in the striatum and Substantia nigra, respectively. (4) The juvenile period is particularly vulnerable to environmental agents, but only few studies have investigated the long-term effects of atrazine following exposure during the pubertal development. (5) Clearly, juvenile exposure to ATR results in a significant decrease of Nurr1 expression. In addition, our data demonstrate that in the case of high occupational exposure, ATR may inhibit vesicular uptake and storage of DA. The decreases in DA and TH suggest that ATR is a dopaminergic toxicant and may be a risk factor for PD.

PS P350 - 0258 An Innovative Tool for Detecting Alcohol Withdrawal Neurotoxicity: Focus on Limbic Memory in Rats

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Chronic excessive alcohol consumption is associated with cognitive and emotional deficits, as a result of the profound decrease in dopamine neurotransmission in the limbic brain regions (Melis et al., 2005). These deficits can compromise detoxification efforts, promoting compulsive alcohol consumption and relapse, and persist even with prolonged sobriety. Current *in vivo* evaluation of alcohol neurotoxicity focuses on spatial or neutral declarative memory (Vorhees & Makris, Neurotoxicol Teratol. 2015), disregarding the pivotal role of limbic brain regions in shaping episodic memory and behaviour (Pignatelli et al., Neuron, 2017), and often fails to unveil alcohol-related deficits. Herein we report the identification of an original marker of alcohol-related neurotoxicity, i.e. "limbic memory," and an innovative tool for its assessment, the Emotional-Object Recognition test (EOR, Brancato et al, JNeurosci Methods, 2017). Alcohol dependence was modelled in rats by a 21-day liquid alcohol diet. At 12 h, 48 h, and 14 days of abstinence, the evaluation of withdrawal signs was followed by the assessment of limbic memory by the EOR. The effects of dopamine stimulation by L-DOPA/benserazide (L-DOPA) were investigated at the three time points in order to revert the hypodopaminergic state. Our results show that alcohol-dependent rats displayed a significant impairment in emotional object avoidance and target zone aversion at 12 h of abstinence with respect to alcohol drinking- and control rats ($p < 0.001$). Notably, limbic memory deficit in alcohol withdrawn rats persisted at 48h and 14 days of withdrawal ($p < 0.01$). When administered at 12 h of abstinence L-DOPA was able to decrease behavioral signs of withdrawal ($p < 0.01$) and to rescue limbic memory with respect to vehicle ($p < 0.001$), up to alcohol-naïve control rats' level. Long lasting limbic memory disruption in alcohol-withdrawn rats was not rescued by late stimulation of dopamine transmission. The assessment of limbic memory is an early and sensitive marker for detecting alcohol-induced neurotoxicity, beyond the conventional paradigms used, and allows to screen specific drug strategies able to counteract the unbalance in dopaminergic neurotransmission typical of alcohol addiction.

PS P351 - 0273 Metabolomic Changes Associated with Genetic and Toxicological Perturbation of the Vesicular Monoamine Transporter (VMAT) in *Caenorhabditis elegans*

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Neurodegeneration is a complex phenomenon that has environmental, genetic, and aging risk factors. Altered expression of the vesicular monoamine transporter 2 (VMAT2; SLC18A2) has been associated with Parkinson's disease, observed in both animal and human studies. VMAT2 transports monoamines into neuronal vesicles, protected from cytoplasmic degradation, where they reside until released into the synapse. Apart from monoamines, the toxicant MPP+ (1-methyl-4-phenylpyridinium) is also sequestered by VMAT2, preventing the toxicant from exerting its effect on the mitochondria. MPP+ is a complex I inhibitor and was briefly used as a pesticide and that is also the primary metabolite of the parkinsonism-inducing MPTP. This raised our interest in understanding the effect of its exposure on neuronal health. In our lab and others, altered expression of VMAT2 has been associated with susceptibility to MPP+ and Parkinson's disease, seen in both animal and human studies. To measure holistic changes in biochemical responses from genetic mutations and chemical exposures in *C. elegans*, we have developed a high-resolution metabolomics framework using a Thermo QExactive LC Orbitrap that leverages the sensitivity of high-resolution mass spectrometers and the unparalleled genetic tractability of the nematode model. In studies using both a mutant strain lacking the VMAT2 orthologue *cat-1* and the potent neurotoxicant MPP+, we detected changes in features suggestive of altered monoamine metabolism. Differentially expressed metabolic features were evaluated using metabolic pathway enrichment, which detected altered fatty acid beta-oxidation, which is known to be regulated by the monoamine serotonin in *C. elegans*. In wildtype L4 worms exposed to 1 mM MPP+ for 4 hours, we detected changes in tryptophan metabolism and altered levels of putatively identified 5-hydroxyindole acetate, the primary metabolite of serotonin, suggesting additional changes in serotonin pathways. Wildtype worms in the first larval stage exposed to 2mM MPP+ for 48 hours show an increase in neuronal blebbing (p -value < 0.0001) compared to controls. Using fluorescent markers of neuronal integrity and me-

tabolomics, we can identify genetic and environmental contributors to neurodegeneration and compare metabolomic profiles to elucidate underlying biochemical pathways of neurodegeneration.

PS P352 - 0285 Autophagic Cell Death via Inhibition of Akt/mTOR Signaling Pathway Contributed to Antimony-Induced Neuronal Damage

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Antimony (Sb), as a naturally occurring metal presented in air and drinking water, has been reported to be found in human brain. Furthermore, Tanu et al. has provided experimental evidence that Sb has a toxic effect on neurobehavioral in mice. Although Sb presented potential brain toxicity, no investigators paid their attention to underlying mechanism by which Sb damaged brain. In the present study, we exposed PC12 cells to antimony potassium tartrate hydrate at different dose and duration. We found that Sb induced cell apoptosis in a dose- and time-dependent manner. Mechanically, Sb triggered autophagy as indicated by markedly increased expression of LC3-II and accumulation of GFP-LC3 dots, and upregulated Atg5 as well as Atg7 mRNA levels. Moreover, Sb enhanced autophagic flux and p62 degradation. However, both p62 mRNA and protein was increased under Sb treatment. Subsequent analyses demonstrated that autophagy inhibition protected PC12 cells from apoptosis. Furthermore, Sb treatment decreased p-Akt and p-mTOR expression levels. *In vivo*, mice treated with Sb also showed higher expression of LC3-II and p62 in brain, consistent with *in vitro*. In conclusion, Sb induced autophagic cell death possibly through inhibition of Akt/mTOR pathway. Our study provided the first mechanism of Sb-associated brain toxicity, namely that Sb induces autophagy via inactivation of Akt/mTOR axis, therefore leads to neuronal apoptosis.

PS P353 - 0329 The Attenuation of Glutamate-Induced Oxidative Toxicity and Induction of Antioxidant Genes by Resveratrol in HT22 Murine Hippocampal Neuronal Cells

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Glutamate-induced toxicity in nerve system has been related with reactive oxygen species (ROS) and oxidative stress. It is the one of crucial factors promoting the loss of neurons in neurodegenerative disorders, which anti-neurodegeneration remain unclear. Resveratrol, bioactive compound found in various berries that has been recognized for strong antioxidant health benefits. This study aimed to investigate the effect of resveratrol on toxicity of glutamate-induced oxidative stress in HT22 murine hippocampal neuronal cells. The cells were pre-treated with resveratrol and then exposed to glutamate. The results showed that resveratrol significantly decreased the glutamate-induced cytotoxicity. Resveratrol inhibited the apoptotic cell death in response to glutamate examined by Annexin V-FITC/propidium iodide stains. Moreover, pre-treatment of cells with resveratrol suppressed the intracellular ROS generation, using DCFH-DA assay. Additionally, we confirmed the function of endogenous antioxidant genes in this cell model. Real-time polymerase chain reaction analysis revealed that resveratrol also increased the gene expression of antioxidant genes (superoxide dismutase, catalase and glutathione peroxidase). Thus, resveratrol could modulate neuronal defences in neuroprotection suggesting for further development for treatment of neurodegenerative diseases.

P354 - 0343 ER Stress Inhibitor Salubrinal Attenuates Deltamethrin-Induced Reduction of Hippocampal Neurogenesis in Adult Mice

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Environmental factors, including pesticide exposure, are thought to be significant contributors to the neurodegeneration and cognitive deficits. Emerging evidence indicates that adult neurogenesis in the hippocampus is important for learning and memory. Approximately 1400 newborn neurons are added to adult human hippocampus every day and provide about 2% annual turnover during aging, suggesting that adult neurogenesis contributes significantly to human hippocampal function (Spalding et al., 2013). In our previous study, repeated exposure to deltamethrin (3 mg/kg) caused ER-stress, reduction of hippocampal neurogenesis, and deficits in learning and memory in adult mice. Here, we sought to determine the potential relationship between ER stress and hippocampal neurogenesis following exposure to deltamethrin. Immunohistochemistry of various markers along with bromodeoxyuridine (BrdU) were used to examine neurogenesis. We found that single oral exposure to deltamethrin (3 mg/kg) resulted in reduction of progenitor cell proliferation as BrdU- and Ki67-positive cells were significantly decreased in the dentate gyrus (DG) of the hippocampus. Furthermore, deltamethrin-treated mice exhibited significant reduction in the expression of doublecortin (DCX)-positive cells, suggesting potential impairment of hippocampal neurogenesis. These were accompanied by ER stress as the protein levels of CHOP and GRP78 were significantly increased in the hippocampus following exposure to deltamethrin. To determine whether ER stress is associated with inhibition of neurogenesis, a group of mice were treated with two intraperitoneal (i.p.) injections of 1 mg/kg salubrinal (eIF2 α inhibitor) 24 h and 30 min before the administration of deltamethrin. The data revealed that salubrinal prevented ER stress and attenuated deltamethrin-induced reduction of BrdU-, Ki67-, and DCX-positive cells in the DG of hippocampus. Collectively, these results demonstrate that exposure to deltamethrin leads to ER stress mediated inhibition of adult hippocampal neurogenesis, which may subsequently contribute to deficits in learning and memory in mice. *Supported in part by R01ES027481.*

P355 - 0372 Regulation of Ubiquitin-Proteasome System in ERAD via S-nitrosylation of UBE2D1

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Nitric oxide (NO) functions as signaling molecules and modulates various physiological roles such as regulation of blood pressure and memory formation. A regulatory mechanism through modification by NO at the cysteine thiol of the target proteins has been proposed. Appropriate amounts of NO function as signaling molecules in maintaining cellular homeostasis. On the other hand, excessive amounts of NO contribute to pathogenesis of various diseases including neurodegenerative diseases and cancers. We previously demonstrated that protein disulfide isomerase (PDI) could be a target of NO. PDI catalyzes thiol-disulfide exchange, thus assisting formation of disulfide bonds and rearrangement reaction of the newly synthesized immature protein in ER lumen. S-nitrosylation of PDI (SNO-PDI) resulted in the attenuation of its enzymatic activity. Thus, inactivation of PDI via S-nitrosylation abrogates its chaperone activity and stimulates unfolded protein response (UPR) leading to neuronal cell death. ER-associated degradation (ERAD), a component of UPR, and is activated when accumulation of misfolded proteins in ER lumen and promotes degradation by ubiquitin-proteasome system via substrate transport from the ER to the cytosol. In this study, we attempted to solve the role of NO on the ERAD system induced by the dysfunction of PDI. We initially succeeded to identify ubiquitin-conjugating enzyme E2 D1 (UBE2D1) as a target of NO by the biotin-switch technique and subsequent liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The target cysteine residue for S-nitrosylation was Cys85 which is ubiquitin binding site. Also, we detected the SNO-UBE2D1 formation in cells by treatment with NO donor. Those results indicate that NO results in the dysfunction of UBE2D1 via S-nitrosylation. We therefore performed *in vitro* ubiquitination assay to confirm whether NO affects the enzymatic activity of UBE2D1 in ERAD. The level of ubiquitinated proteins was decreased by exposure to NO. From these findings, it was suggested that SNO-UBE2D1 formation attenuates its E2 activity in ERAD and results in the accumulation of unfolded proteins.

P356 - 0376 Atypical Antipsychotic Olanzapine Induces Oxidative Stress in the Hypothalamus

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Olanzapine, classified as multi-acting receptor targeted antipsychotics (MARTA), is used for treatment of schizophrenia and bipolar disorder. Olanzapine shows lower risk of neurological adverse effects such as extrapyramidal symptoms than typical antipsychotic drugs but appears to have increased risk of hyperglycemia and weight gain. Recent study demonstrated that oxidative stress in hypothalamus causes leptin and insulin resistance and, thereby, induces onset of diabetes. Therefore, we asked in the present study whether olanzapine causes the oxidative stress in the hypothalamus in mice. Male BALB/c mice were treated to olanzapine (5, 10, 25 mg/kg, i.p.), or vehicle (5 mL/kg, i.p.) and hypothalamus was obtained 3-16 h later. In some experiments, mice were repeatedly administered olanzapine for 7 days and hypothalamus was collected 24 h later. The gene expressions of Heme-oxygenase (HO-1) and NAD(P)H quinone oxidoreductase 1 (NQO-1) were determined by real-time PCR analysis. Olanzapine induced HO-1 expression, a typical marker for oxidative stress, in the hypothalamus in a time and a dose dependent manner. A significant increase in HO-1 expression was observed 6 h and returned to the base level 12 h after olanzapine administration. On the other hand, the significant increase was not shown after 12 h of administration of olanzapine, suggesting recovery. On the other hand, HO-1 expression did not change in hippocampus and cortex. These results suggest that olanzapine specifically causes oxidative stress in the hypothalamus in mice. Currently, markers of oxidative stress are examined for tissues after repeated administration.

P357 - 0379 Possible Role of Endogenous S-persulfidation in the Enzymatic Activity of ER Protein

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Increasing evidence indicates that modification of cysteine thiol in target proteins is an important mechanism responsible for the biological functions. Recently, reactive persulfide species (RSS) including cysteine persulfide (CysSSH) have been identified in mammalian cells. CysSSH functions as a potent nucleophile or an antioxidant and could play an important role in the redox signaling. Especially, the chemical reactivity of RSS is believed to be involved in the catalytic activity of particular proteins. In fact, a wide variety of proteins are regulated by the RSS-induced modification (S-sulfhydration, S-persulfidation or S-polysulfidation). Thus, the identification of novel S-persulfidated proteins has become an emerging theme in the analysis of the biological significance of RSS. In this report, we investigated a possibility that some enzymes located in endoplasmic reticulum is a target of RSS. We initially attempted to detect S-persulfidated or S-polysulfidated proteins in cells with a modified biotin switch assay (tag-switch technique). We succeeded to identify some modified proteins in SH-SY5Y cells. Subsequently, we focused to analyze a protein located in ER lumen that demonstrated to be a target of NO. So far, there have been identified four enzymes to produce CysSSH including cystathionine b-synthase (CBS), cystathionine g-lyase (CSE), cysteinyl-tRNA synthetase (CARS), and 3-mercaptopyruvate sulfurtransferase (3MST). These proteins are thought to participate in endogenous CysSSH formation and protein modification. Therefore, we tried to determine the enzymes involved in the S-persulfidated modification in cells. Knockdown analysis by specific siRNA revealed that CBS and CARS, but not 3-MST, contributed to endogenous modification in this protein. On the other hand, the recombinant protein purified from bacteria possessed the enzymatic activity. But the treatment with DTT completely lost the activity and the modification. Interestingly, the enzymatic activity was ameliorated by challenge with RSS donors such as sodium tetrathiolate (Na_2S_4), sodium trisulfide (Na_2S_3), or sodium disulfide (Na_2S_2) in a concentration-dependent manner. These results suggested that the RSS-induced modification regulates the endogenous enzymatic activity. We will discuss the role of modification by RSS in this meeting.

P358 - 0414 Utilization of Highly Sensitive LC-MS/MS to Detect Changes in the Central Nervous System (CNS): Comprehensive Profiling of Neurotransmitters and Proteins in Cerebrospinal Fluid in Rats

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In vivo behavior testing of experimental animals for central nervous system (CNS) toxicity assessment during the development of CNS-targeted drugs, such as functional observation battery (FOB) test, has limitations. Biochemical evaluation methods such as microdialysis or cerebrospinal fluid (CSF) analysis are well established; however, comprehensive analysis is a challenge due to low concentration of each component. The purpose of the present study was to establish a novel use of highly sensitive LC-MS/MS for comprehensive detection of changes in neurotransmitters and proteins in CSF for CNS toxicity evaluation. Kainic acid at 7.5 or 10 mg/kg was administered once intraperitoneally to male SD rats (n=7 or 5, respectively). CSF was collected after clinical observation under anesthesia at 3 hours after dosing of 7.5 mg/kg, or 72 hours after dosing of 10 mg/kg. Control animals (n=5) received saline and CSF was collected similarly. The neurotransmitters including monoamines, amino acids and their metabolites in CSF were derivatized by e.g. dansyl chloride, followed by the quantitation by highly sensitive LC-MS/MS systems. Proteins in CSF were quantified by shotgun proteomics workflow using nanoLC-MS/MS with Tandem Mass Tag labeling. Two of 7 rats at 7.5 mg/kg showed salivation and increased aggression. All 5 rats at 10 mg/kg exhibited salivation, increased aggression, twitching. In the 2 animals at 7.5 mg/kg with increased aggression, apparent increases were observed in neurotransmitters related to dopamine/norepinephrine pathways. The protein analysis revealed overlapping changes not only between the 10 mg/kg group and the 2 rats at 7.5 mg/kg with increased aggression but also the 5 rats at 7.5 mg/kg without any abnormal clinical signs. These included an increase in creatine kinase (brain-type) and changes in the pathways of glycolysis/gluconeogenesis. We succeeded in comprehensive detection of changes in neurotransmitters and proteins associated with CNS toxicities using highly sensitive LC-MS/MS. The method was sensitive enough to detect changes in the CSF samples from animals without any abnormal clinical signs. This method of evaluation can easily be incorporated in preclinical toxicity studies and will help understand the mechanism of toxicity and search for new safety biomarkers for CNS toxicities.

P359 - 0426 Dimethylglycine Alleviates Behavioral Disturbances Induced by Adolescent Toluene Exposure

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Sniffing glue and other inhalants, especially those that include toluene, is prevalent among adolescents. Adolescent toluene exposure results in persistent psychosis-like behaviors including social withdrawal, cognitive impairment, and enhanced head twitch response to 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a hallucinogenic 5HT_{2A} agonist in mice. Altered N-methyl-D-aspartate receptor (NMDAR) function has been postulated as a possible mechanism of toluene-induced behavioral disturbances. The present study examined the restorative effects of dimethylglycine (DMG), a derivative of glycine and acting as an NMDAR glycine binding site partial agonist, on behavioral disturbances after toluene exposure during adolescence. Mice were exposed to toluene (600 mg/kg, i.p.) during postnatal days (PN) 38–40 and toluene (750 mg/kg, i.p.) during PN41–42 and PN45–49. The novel object recognition test (NORT) and three chamber social test were performed to confirm toluene exposure produced recognition memory impairment and social deficits. Subsequently, DMG (30 or 100 mg/kg, i.p.) was given during PN63–76. NORT, social interaction, three chamber social test, and DOI-induced head twitch response were monitored. DMG ameliorated the behavioral deficits in adolescent toluene-exposed mice. These results suggest that DMG may be a promising compound to treat behavioral dysfunction associated adolescent toluene exposure.

P360 - 0491 Psychopharmacological Effects of Pyrolysates of Synthetic Cannabinoids

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Synthetic cannabinoids (SCs) are chemicals designed to mimic tetrahydrocannabinol (THC), a main psychoactive component in cannabis. SCs generally show analgesia, hypothermia and suppressed locomotor activity. People who have smoked SCs show abnormal excitation, but its mechanism of action has been obscure. UR-144 [(1-pentyl-1*H*-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone] and its fluoro derivative XLR-11 [(1-(5-fluoropentyl)-1*H*-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone] are the SCs containing a cyclopropyl ring substituent. The cyclopropyl ring of these compounds are known to undergo thermal rearrangement. The aim of the present study was to evaluate effects of the pyrolytic components derived from UR-144 and XLR-11 on the psychomotor function in mice. Smoke of UR-144 and XLR-11 contained the respective degradants at levels much higher than the parent compounds. The UR-144 degradant displayed approximately four-fold higher agonistic activity for the CB₁ receptor compared with UR-144, as demonstrated by human CB₁ expressed CHO-CB₁ cells. Male Balb/c mice inhaled the XLR-11 smoke showed jumping, as previously described as a "popcorn effect" of THC, at the beginning of exposure, resulting a significant increase in locomotor activity. The stimulated psychomotor function could be resembled with excitation behavior seen in human who smoked SCs. Mice treated i.p. with the degradants of UR-144 and XLR-11 at doses comparable to those smoke inhalation showed similar excitatory behavior to the smoke inhalation of SCs. On the other hand, UR-144 and XLR-11 showed no excitatory action. As similar to hypothermia and catalepsy, the excitatory action of XLR-11 degradant was suppressed by AM-251, a CB₁ receptor antagonist, indicating involvement of CB₁ in this action. Extracellular dopamine and glutamate in the nucleus accumbens shell and the hippocampus did not change during the treatment of XLR-11 degradant, suggesting that these excitatory transmitters are not involved in the action. Collectively, the simulated excitatory actions observed in the exposure of UR-144 and XLR-11 smoke to mice would be attributed to those pyrolytic products. Therefore, smoking certain kinds of SCs may lead to profound and unexpected pharmacological and toxicological effects. In addition, the excitatory action caused by XLR-11 degradant could be mediated by CB₁ receptor, although precise mechanism of action remain to be determined.

P361 - 0499 Prescription Drugs Interrupt Cholesterol Biosynthesis in Primary Cultures of Neurons

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Dyshomeostatic sterol metabolism is the cause and result of many neurological disorders. Yet, targeting neuronal cholesterol (chol) metabolism as a molecular initiating event causing an adverse outcome has been less studied for the following reasons: (1) separating chol from other bioactive sterols presents an analytical challenge, (2) measuring neuronal chol presents an additional challenge since it does not freely cross the blood-brain-barrier, and (3) there is conflicting evidence about the capacity of neurons to make chol raising a question of whether neuronal chol biosynthesis is a relevant issue. To address the former two concerns, *in vitro* rat neuronal cultures can be used to look exclusively at neuronal chol while reliable analysis of sterols can be obtained with established LC/MS methods. One such analytical method involves the derivatization of sterol extracts with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) followed by a less than one-min UPLC/(SRM)-MS analysis. When matched isotopically-labeled standards are used, this PTAD method can quantify the amount of four sterols in the chol biosynthetic pathway: lanosterol (Lan), 7-dehydrocholesterol (DHC), desmosterol (Des) and chol. To test the capacity of neurons to make chol, cultures consisting of more than 95% neurons were grown for 5, 12, and 26 days *in vitro* (DIV) and a reference sterol profile was obtained. In parallel, neurons were also exposed to four prescription drugs identified to impact chol biosynthesis at three concentrations of 1, 10, 100 nM for 48 h. Reference control cells accumulate all sterols analyzed from DIV5 to DIV12, but only chol continues to increase from DIV12 to DIV26 reaching a total chol content of 86±3 nmol/10⁶ cells, or a 5.8-fold increase compared to DIV5 neurons. Neurons exposed for 48 h to simvastatin, a drug used to treat cardiovascular disease, had significantly lower Lan, DHC, Des and chol levels at all ages tested. Lan and Des were elevated when DIV12 neurons were exposed to 100 nM econazole and 10 nM pregnenolone, respectively. Cariprazine, an antipsychotic medication, elevated DHC at all aged cultures tested in a dose-dependent manner. These data demonstrate neurons grown *in vitro* can synthesize chol and that prescribed

pharmaceuticals are able to interrupt chol metabolism showcasing the need to better understand the human health consequences of xenobiotic inhibition of chol biosynthesis. *Work in part supported by NIH-ES-022936, NIH-ES-024133, NIH-HD-064727 and NIH-ES-007028.*

P362 - 0534 Alteration of Social Behavior and Neuroimmune Biomarkers in Fragile X Mental Retardation 1 Knockout and Valproic Acid-Induced Autism Rat Models

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Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by impaired social communication, social interactions and repetitive behaviors. Up to now, the etiology of autism remains unknown and its molecular basis is not well understood. Both genetic and environmental factors contribute ASD. In this study, we examined social behaviors and related gene expressions using Sprague-Dawley (SD) background of fragile X mental retardation 1 (Fmr1) gene Knockout (KO) rats and valproic acid (VPA)-induced autism model rats. Fmr1 KO male ($n=5$) and female ($n=5$) SD rats (7-week-old) were purchased from SAGE Laboratories (St. Louis, MO, US) and used at the age of 15 to 17-week-old for behavioral test. For VPA-induced autism rat model, SD pregnant rats (Charles River Laboratories Japan, Inc., Japan) were given VPA 600 mg/kg (intraperitoneal injection) on day 12.5 of gestation and male ($n=10$) and female ($n=10$) offspring at the age of 11 to 13-week-old were used for social behavioral test. We performed 3-chamber social interest test including sociability and social novelty preference test and after completion of behavioral test, the hippocampus was collected from each rat to detect social behavior-related genes and neuroimmune biomarkers using real-time RT-PCR method. Male and female of both Fmr1 KO and VPA-induced autism model rats show poor sociability and social novelty preference test compared to the corresponding control SD rats. Expression level of social behavior-related genes such as serotonin receptor 5HT5B, brain-derived neurotrophic factor and neuroligin-3 were significantly reduced and inflammatory marker such as interleukin (IL)-1 beta and tumor necrosis factor (TNF)-alpha were significantly increased in the hippocampus of VPA-induced autism model rats. Our findings indicate that similarities to autism features were observed in Fmr1 KO and VPA-induced rat models and these animal models are valuable experimental models to study neurodevelopmental alterations induced by environmental risk factors.

P363 - 0537 Metabolomic Approach Uncovered Impaired Amino Acid Metabolism in Human Astrocytoma Cells Induced by Manganese Exposure

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Besides the favorable functions of manganese (Mn), excess exposure to Mn emerges adverse effects, such as Parkinson's disease-like symptoms. These neuronal effects seem to result from both accumulation of Mn in the brain and subsequent neural cell death. However, the precise mechanisms of Mn-induced neurotoxicity remain unclear. In the central nervous system, Mn distributes in astrocyte. Therefore, over-exposure to Mn might disrupt astrocyte homeostasis. In this study, we measured the time-course of amino acid contents in culture media using liquid chromatography-mass spectrometer (LC/MS) to identify the crucial metabolic pathways for Mn-induced neural dysfunction. Human astrocytoma CCF-STTG1 cells were cultured in RPMI1640 media supplemented with 10% FBS. Cells were treated for 4 d with manganese chloride ($MgCl_2$, 100–400 μM). Cell proliferation was monitored using live-cell imaging system, and live/dead cell number was counted using Hoechst33342/propidium iodide (PI) staining. Culture media (10 μL) was collected once a day and, metabolites were extracted by adding absolute methanol (140 μL). Amino acids were measured using LC/MS system. CCF-STTG1 cells were treated with $MnCl_2$ (100–400 μM), and cell proliferation was monitored for 4 d using live-cell imaging. Overall, enhanced cell proliferation was observed at 100–200 μM of $MnCl_2$, whereas inhibitory effect was seen at 300–400 μM of $MnCl_2$. To determine the detailed effects of $MnCl_2$ on the live-dead state of CCF-STTG1 cells, Hoechst 33342/PI staining-positive cells were counted at day 4. As observed in live-cell imaging, $MnCl_2$ (100–200 μM) tended to increase the number of live cells. Similarly, $MnCl_2$ (300–400 μM) significantly decreased the number of live cells, although no significant increase was seen in dead cells. These results suggest that a high-dose of $MnCl_2$ modulates cell cycle mechanisms, not the cell death. Next, we determined the contents of amino acids in culture media in the

presence of $MnCl_2$ (400 μM). Interestingly, glutamate, aspartate, and alanine contents were increased significantly at day 3 and day 4 as compared with control. In contrast, lysine, and arginine contents were decreased at day 1 through day 4. This modulation of amino acid contents would reflect the toxic response of astrocyte to Mn exposure, and further/deep analyses of the amino acid network would provide a clue for understanding the mechanisms of manganism.

P364 - 0539 Optical Recording Methods with Voltage-Sensitive Dye (VSD) Is Useful to Evaluate the Impact of Chemicals on Brain Activities in Acute and Late Effects

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The function of the brain depends on a complex interaction between numerous neurons and synapses. The brain is especially vulnerable to environmental disturbances that affect developmental signals. The effects of some of these disturbances can last for a very long time and have an acute effect on developmental processes. Certain types of chemicals have been known to cause such a traumatic impact on the brain function, which has been measured with changes in behavior tests in the rodent model. However, evaluation of the changes in the action of the neural circuits is critical to understand the mechanism of the effect. The electrophysiological tools have been used for that purpose. Here, we introduce that the optical recording methods with voltage-sensitive dye (VSD) are a useful tool to evaluate the modification on the brain function caused by these chemicals. Our technical improvements made it possible to use the method for quantitative evaluation of the neural circuit activity. We will show the benefits of the VSD imaging method for assessing the acute and late effects of the chemicals. The first example is the maternal application of valproic acid (VPA), which is known to cause changed cognitive function and reduced mental ability in the children born to that mother so that it should affect the unborn children's brain development during pregnancy. We aimed to evaluate the changes in functions of neural circuits responsible to those deficits. The VSD imaging is a population signal and can visualize the excitation and inhibitions in a wide area of the neural circuits. The robustness of the method allows us to assess those changes. Using electrical stimulation, we evaluated the neural circuit response to well-known tri-synaptic circuits with VSD imaging. We found that VPA induced reduction in inhibitory components tested at the age of two weeks and after eight weeks. The second example is the similar effect caused by bisphenol A (BPA), which produced a similar impact on the hippocampal circuit. We will also introduce how we can apply the methods to an overall assay of the neuronal signal in the larger neural network.

P365 - 0544 Anti-neurotoxic Effect of *Passiflora edulis* Extracts against Glutamate-Induced Oxidative Damage in Cultured HT-22 Cells

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Alzheimer's disease (AD) is the major case of dementia, which is a devastating age-related neurodegenerative disease. Oxidative stress is one of the pathological risks of AD. Excess glutamate promotes intracellular ROS level, which further causes excitotoxicity and neuronal apoptosis. *Passiflora edulis* Sims (*P. edulis*), a tropical fruit is widely used in traditional medicines, but little is known about its neuroprotective effects. In this study, we aimed to examine the neuroprotective effects of *P. edulis* extracts against glutamate-induced neurotoxicity. The phenols and flavonoids of *P. edulis* pulp and peel extracts were determined using total phenolic content assay and total flavonoid content assay, respectively. The radical scavenging activity of *P. edulis* extracts was examined using both DPPH and ABTS⁺ assay. HT-22 mouse hippocampal cell was used as *in vitro* cell model. The cell viability was assessed using MTT, LDH assay. Intracellular ROS level in HT-22 was determined via H₂DCFDA assay. Moreover, the differentially expression of genes involving *P. edulis* extracts against glutamate-induced neurotoxicity in HT-22 hippocampal cells was assessed by transcriptomic and bioinformatic analysis. Results show that glutamate (5mM) decreased HT-22 cell viability and increased cell apoptosis and intracellular ROS level. However, these results were mediated when HT-22 co-treated with glutamate and selected *P. edulis* extracts. Furthermore, number of glutamate up-regulated genes are down-regulated in *P. edulis* co-treated groups, vice-

verse. Results suggest that *P. edulis* extracts attenuate glutamate-induced intracellular ROS and cell death through regulation of AD related gene expressions, thereby protecting HT-22 cells from glutamate-induced toxicity.

PS P366 - 0558 Cadmium Effects on Memory Impairment and on Brain Neurochemistry: Modulation by *Lepidium sativum* L.

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The aim of our study is to investigate the effect of *Lepidium sativum* L. on memory and on acetylcholinesterase (AChE) activity in different brain structures among cadmium-exposed rats. 21 female rats (155.95±3.99) g, 3 months old were divided into three groups (n=7): 1) Control group (T): received a distilled water orally, 2) Treated group (C): received oral administration dose of CdCl₂ at 10 mg / kg, 3) Treated group (C + LS): received CdCl₂ (10mg/kg) and the aqueous extract of *Lepidium sativum* L. (20mg/kg) orally. To assess cognitive function we used Novel Object Recognition Test, and the measure of AChE activity is realised by Ellerman method. The obtained results showed that subchronic CdCl₂ intoxication at a dose of 10 mg/kg caused a significant decrease in the index of recognition of short-term memory and long-term memory (p <0.05) compared to the control group. In addition, this index increase in the group (C + LS) compared to the group intoxicated by CdCl₂. However, this increase remains less important compared to the control group. A highly significant increase in AChE inhibition rate was found in hippocampus (p<0.0001), cerebellum (p<0.0001), and cortex (p<0.0001) in the Cd-group compared to the control group. The administration of oral CdCl₂ at a dose of 10 mg/kg caused a deterioration of mnesic capacities. However, the administration of CdCl₂ and the aqueous extract of *Lepidium sativum* L. showed a neuroprotective action against the disorders induced by cadmium. Nevertheless, the plant does not seem to have a significant impact on the modulation of AChE activity.

PS P367 - 0562 Developing a QSAR Model for Predicting Neurotoxic Agents

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Chemical-induced neurotoxicity may result in neurodegenerative disorders. Furthermore, neurotoxicity is one of the major causes for withdrawal of pharmaceuticals from the market. Though chemical-induced neurotoxic effects can be evaluated by several standard toxicity assays that are usually time- and resource-consuming, there is a big demand for the development of rapid and early screening testing strategy to assess whether compounds are neurotoxic. Computational methods are potential alternatives to *in vivo* and *in vitro* testing. We developed a Quantitative Structure-Activity Relationships (QSAR) model for the identification of potential neurotoxins based on an *in vitro* neuron toxicity assay. A set of 907 chemicals was utilized to construct the prediction model using the Random Forest with a high accuracy (90.6%) within a defined applicability domain. An independent test yielded a high accuracy (85.7%) showing the robustness of the model. The QSAR model can be used as a screening tool for chemical neurotoxicity prior to safety and efficacy testing.

PS P368 - 0566 Comparative Effects of Two Pyrethroids on Behavioral Reactions in Developmental Neurotoxicity Studies

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Nowadays the problem of distant consequences of pesticides action, in particular synthetic pyrethroids, which are a potential hazard to environment and health of the population is especially relevant. Pregnant women and children are the most vulnerable to adverse effects layer of population. Recent researches demonstrate that impact of synthetic pyrethroids on the development of organism is underestimated and requires thorough investigation. The aim of our study is to compare the impact of zeta-cypermethrin and cypermethrin under their exposition at pre- and postnatal period on rat offsprings.

Developmental Neurotoxicity Studies of zeta-cypermethrin and cypermethrin (ZCYP and CYP) were conducted in accordance with OECD guideline 426 and GLP requirements. Dams Wistar Hannover were exposed daily orally by CYP at doses 0; 17,5; 35,0 and 70,0 mg/kg/bw and by ZCYP at doses 0; 12,5; 35,0 and 70,0 mg/kg/bw from day 6 of gestation to day 21 of lactation. Estimation of behavioral reactions of offsprings was conducted at "open field" test at 13, 17, 21 and 60 postnatal day (PND). As a result of conducted comparative analysis of impact of CYP and ZCYP on behavioral reactions of the pup rats in "open field" test, we established that CYP in doses 17,5 mg/kg/bw and 35,0 mg/kg/bw did not caused changes in behavior of offsprings in the postnatal development. Exposure by CYP at dose 70,0 mg/kg/bw in pre- and postnatal period influenced adaptive abilities and motor activity of male rat offsprings (increasing of latency to move and decreasing number of crossed squares). CYP did not influenced of exploratory behavior and did not cause the anxiety state of rat pups at all tested doses. There were no changes in behavior of pup rats during and after exposure of ZCYP in the dose of 12,5 mg/kg/bw. ZCYP caused changes of emotional state of male and female pup rats (♂ ♀ —decreasing of grooming at dose 35 mg/kg/bw and 70 mg/kg/bw), motor and exploratory activity (♂ —decreasing number of crossed squares and rearings, ♀ —decreasing number of crossed squares at dose 70,0 mg/kg/bw) and adaptive abilities (♂ —increasing of latency to move at dose 70,0 mg/kg/bw). Obtained results has been demonstrated that ZCYP has more neurotoxic effect compared with CYP.

PS P369 - 0571 Early-Life Exposure to Low Levels of Permethrin Exerts Impairments in Learning and Memory Associated with Glial Cell Disturbance in Adult Male Mice

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Permethrin, a pyrethroid chemical, is widely used as a pesticide because of its rapid insecticidal activity. Although permethrin is considered to exert very low toxicity in mammals, the effects of early, low-level, chronic exposure on the adult central nervous system are unclear. In this study, we investigated the effects of low-level, chronic permethrin exposure in early life on the brain functions of adult mice, using environmentally relevant concentrations. We exposed mice to the acceptable daily intake level of permethrin (0.3 ppm) in drinking water during the prenatal and postnatal periods. We then examined the effects on the central nervous system in adult male offspring. In the permethrin group, we detected behavior that displayed incomplete adaptation to a novel environment, as well as an impairment in learning and memory. In addition, immunohistochemical analysis revealed an increase in doublecortin- (an immature neuron marker) positive cells in the hippocampal dentate gyrus in the permethrin exposure group compared with the control group. Additionally, in the permethrin exposure group there was a decrease in astrocyte number in the hilus of the dentate gyrus, and remaining astrocytes were often irregularly shaped. These results suggest that exposure to permethrin at low levels in early life affects the formation of the neural circuit base and behavior after maturation. Therefore, in the central nervous system of male mice, low-level, chronic permethrin exposure during the prenatal and postnatal periods has effects that were not expected based on the known effects of permethrin exposure in mature animals.

PS P370 - 0580 The Transcription Factor REST/NRSF Mediates Protective Effects of 17β-Estradiol and Tamoxifen against Manganese-Induced Toxicity in Astrocytes

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Estrogen (17β-estradiol, E2) and tamoxifen (TX), a selective estrogen receptor modulator, exert protective effects against various neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD) and man-ganism, the latter being caused by overexposure to manganese (Mn), sharing pathological symptoms with PD. Studies have shown that astrocytes are critically involved in E2/TX-induced neuroprotection against various toxicants, including Mn, although the molecular mechanisms involved are not completely understood. The transcription factor RE1-silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF) has been shown to be neuroprotect-

tive in several experimental models including AD, but no studies have been reported on its role in E2/TX-induced protection against Mn toxicity. The REST promoter contains several putative estrogen response element (ERE), prompting us to test if E2/TX exert protective effects against Mn toxicity via REST activation in human H4 astrocytes. The results showed that Mn decreased REST promoter activity, mRNA and protein levels, while E2/TX increased REST and reversed Mn-reduced expression of REST. The estrogen receptor (ER)- α was the primary ER to mediate E2/TX-enhanced REST expression, since overexpression of ER- α , but not ER- β , significantly increased REST promoter activity and mRNA/protein levels in astrocytes. Moreover, E2/TX activated the Wnt- β -catenin signaling, which is known to enhance REST, suggesting that the Wnt- β -catenin pathway also plays a role in E2/TX-induced REST expression. REST overexpression attenuated Mn-induced oxidative stress and impairment of mitochondrial membrane potential in astrocytes. REST reversed Mn-induced TNF- α production and attenuated Mn-reduced antiapoptotic proteins Bcl-2 and Bcl-xL, while inhibiting Mn-increased proapoptotic proteins Bax and Daxx. REST also attenuated Mn-induced apoptosis by flow cytometry with Annexin V/PI staining. Moreover, inhibition of REST with dominant-negative REST (DN-REST) abolished the protective effects of REST against Mn toxicity in astrocytes. Since E2/TX enhanced astrocytic EAAT2 which is a main glutamate transporter to prevent excitotoxic neuronal death, we have tested if REST modulates EAAT2 expression. Our findings reveal that REST increased EAAT2 promoter activity and mRNA/protein levels in astrocytes. Taken together, our findings indicate that REST mediates E2/TX-induced protective effects against Mn toxicity in astrocytes, suggesting that REST can be a potential molecular target of E2/TX to treat Mn toxicity. *Supported by NIH R01 ES024756 (EL) and R01 ES10563 (MA).*

P371 - 0583 Pharmacological Responses in Cultured Human iPSC-Derived Sensory Neurons Using MEA System

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Functional evaluation assays using human induced pluripotent stem cell (hiPSC)-derived sensory neurons are expected to predict the pain-related toxicity of drugs and the pharmacological effects. However, evaluation assays in hiPSC-derived sensory neurons has not been established, and electrophysiological response to pain-related molecules are not known. In this study, we aimed to evaluate the physiological responses against pain-related molecules including anti-cancer drugs in cultured hiPSC-derived sensory neurons using multi-electrode array (MEA) system. hiPSC-derived sensory neurons were cultured on MEA chips (Presto), and the electrophysiological responses against capsaicin, menthol, AITC, anti-cancer drug oxaliplatin were measured by the MEA system. We also confirmed the expression of Nav1.7, TRPV1, TRPM8, and TRPA1 receptors. We observed spontaneous activities and chemical responses. Addition of the capsaicin, menthol and AITC induced significant changes of the firing rate and concentration-dependent responses. We confirmed that the typical response of sensory neurons can be easily obtained using MEA system, and found that time variation of responses are dependent on the type of pain-related molecules, and that there are a lot of type neurons from electrophysiological responses of pain-related molecules. These results suggested that electrophysiological measurements in sensory neurons using a MEA system may be beneficial for clarifying the functions of sensory neurons in pain research and for drug screening applications.

P372 - 0595 The Toxic Effects of MnCl₂ on Motor Function Are Reduced in Mice Using a Contaminated Water Sediment Exposure Model

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Manganese (Mn) toxicity results in impaired neuronal function and is associated with neurodegenerative Parkinson's disease (PD). Mn ions in the environment concentrate in the watershed and regularly contaminate drinking water. The relationship between ingestion of Mn in water and PD remains uncertain and mechanistic insights are needed. Animal studies use high concentrations of MnCl₂ added directly to water to study neurotoxicity. This approach does not consider metal-sediment interactions within the watershed that can have a substantial effect on the speciation and bioavailability of Mn. The effect of those interactions on Mn neurotoxicity has not been previously investigated. The goal of this study was to develop an exposure model in mice using water sediment spiked with manganese chloride (MnCl₂) at concentrations synony-

mous with those found in Baltimore City harbor (~1000mg/kg sediment). Adult male and female C57/BL6 mice were exposed for six weeks and compared to untreated mice and mice treated with the same concentration of MnCl₂ added directly to water. Motor impairment was analyzed using the rotarod test, the beam traversal test, and the cylinder test. We observed that female and male mice given water incubated with MnCl₂ spiked sediment (sed-MnCl₂) performed better than mice given MnCl₂ directly in water (water-MnCl₂) in at least two tests ($p < 0.05$). Female sed-MnCl₂ mice performed the same as control mice in all three tests. Female water-MnCl₂ mice had a 35% decrease in time on the rotarod and a 50% reduction in rearing behavior but did not perform worse in the beam traversal test. Male sed-MnCl₂ mice had a 28% decrease in time on the rotarod and a 60% reduction in rearing behavior but did not perform worse in the beam traversal test. Male water-MnCl₂ had lower performance in all three tests with a 40% reduction in the time on the rotarod, a 70% reduction in rearing behavior, and a 234% increase in time to cross the beam. We are analyzing water and sediment samples with mass spectrometry to understand changes in speciation and bioavailability of Mn in this environment. Interestingly, we observed sex dependent effects of manganese on behavior in both treated groups. Males appeared to be more sensitive than females despite having the same estimated daily exposure ($p < 0.05$). This exposure model will improve toxicity testing by simulating complex interactions of contaminants with their surrounding microenvironment. It may be adapted for use with other watershed contaminants such as heavy metals and pesticides. It will be useful for testing mixtures of chemicals within watersheds that require environmental interactions to generate toxic effects.

P373 - 0645 Analysis of Manganese Accumulation in the Pituitary Gland, Olfactory Bulb, and Hippocampus of Smelter Workers Using High-Resolution 3D T1-Weighted MRI

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Chronic exposure to excessive manganese (Mn) has neurotoxic effects which can lead to behavioral and motor deficits. Excessive Mn exposure commonly occurs in the smelting and welding industries and previous studies have found significant Mn accumulation in the basal ganglia and other brain regions in exposed populations. This study investigates the pituitary gland, olfactory bulb, and the CA2 area of the hippocampus as additional potential regions of Mn deposition in the brain. Animal studies have shown Mn accumulation in these regions but there are few human studies investigating these brain regions, as they are very small and not readily segmented from magnetic resonance images (MRI) images. This study analyzed high-resolution 3D T1-weighted (1x1x1.25 mm³) MRI data from a former study on 10 smelters, recruited from a Mn-Fe alloy factory, and 10 controls with no history of Mn exposure from Zunyi, China. Manual regions of interest (ROIs) were placed in the pituitary gland, olfactory bulb, hippocampus, and tongue (for reference) using ITK-SNAP. The relative intensity of each region was determined by calculating the ratio of the intensities of the brain region to the tongue in order to account for natural variability in the MRI. While no significant difference in relative intensity between smelters and controls was found in the olfactory bulb, or the hippocampus, smelters showed a significant increase ($p = 0.002$) in relative intensity in the pituitary gland compared to controls, indicating increased Mn accumulation in this region. Similar findings have been shown previously in non-human primates. Uptake of Mn into the pituitary gland is physiologically plausible due to the lack of blood brain barrier in the pituitary gland and the chemical similarities between Mn and Calcium in the blood. This study provides preliminary evidence of increased Mn accumulation in the pituitary gland in a human exposed population. *This research is supported by NSF Grant DMS-1246818.*

P374 - 0737 Biphasic Effect of the Gliotoxin L- α -Aminoadipate on Astrocytes to Impact on Mouse Hippocampal Synaptic Plasticity

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Astrocytes, the most numerous cells in the brain, are functionally associated with synapses, through their ability to respond to neurotransmitters and release gliotransmitters, but also structurally since astrocytic processes enwrap synapses. To grasp the role of astrocytes, we now detailed the impact in the hippocampus (a brain region encoding memory) of L- α -aminoadipate (L-AA), a proposed gliotoxin in glial Muller cells. Mouse hippocampal slices exposed for 2h to L-AA (100 μ M) displayed lower synaptic plasticity, namely lower long-term potentiation (LTP) amplitude ($43.4 \pm 5.3\%$ vs. $74.3 \pm 8.2\%$ in control, $n=6$), without changes of basal synaptic transmission in Schaffer fiber-CA1 pyramid synapses. This effect was mimicked by two other gliotoxins, trifluoroacetate (100 μ M, a TCA cycle inhibitor) and dihydrokainate (15 μ M, a glutamate transporter-1 inhibitor), both occluding L-AA effects on LTP ($n=5-6$). Acute L-AA exposure caused a hypertrophy of astrocytes, typified by an increased length and complexity of their processes. In contrast, prolonged L-AA exposure, i.e., 72h after *in vivo* intracerebroventricular bilateral injection of 1 μ M L-AA in adult mice, reduced the density of hippocampal astrocytic markers (S100 β and glial fibrillary acidic protein, GFAP), and decreased LTP amplitude ($52.4 \pm 6.2\%$ vs. $77.0 \pm 8.6\%$ in control, $n=6-7$). Finally, the exposure of cultured cortical astrocytes to L-AA (100 μ M; $n=4-7$) for 2h did not affect viability ($104.5 \pm 5.4\%$, assessed by an MTT assay), but increased the extracellular levels of glutamate (by $97.8 \pm 8.5\%$) and ATP ($44.5 \pm 14.6\%$), two neurotoxic signals (Cell Death Dis 9: 297; 2018). The present findings validate L-AA as a selective gliotoxin with an initial astrogliosis and increased release of glutamate and ATP; the prolonged exposure to L-AA then has a subsequent deleterious impact on astrocytes, typified by a reduction of astrocytic markers. Notably, this time-dependent biphasic effect of L-AA on astrocytes systematically reduces the functioning of neuronal circuits, as heralded by the reduction of synaptic plasticity (LTP) both upon acute or chronic exposure to L-AA. *Supported by LaCaixa, CENTRO-01-0246-FEDER-000010 and FCT projects PTDC/NEU-NMC/4154/2014 and POCI-01-0145-FEDER-031274.*

P375 - 0741 Paternal Factors in Neurodevelopmental Toxicology: THC Exposure of Male Rats Causes Long-Lasting Neurobehavioral Effects in Their Offspring

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Developmental neurotoxicity from maternal exposure to a wide variety of toxicants during gestation is very well established. In contrast, the impacts of paternal toxicant exposure before conception on offspring neurobehavioral function are much less well studied. We have previously shown that pre-conception delta-9-tetrahydrocannabinol (THC) exposure in male rats significantly alters sperm DNA methylation (Murphy et al., *Epigenetics*, 13:1208-1221, 2018). We also found altered sperm DNA methylation in human males who smoke cannabis. The question remains what potential impacts this altered sperm DNA methylation might have on offspring development. Persisting epigenetic marks might lead to abnormal genetic expression in the offspring. In the current study we investigated the effects of THC exposure of young adult male rats (0 or 2 mg/kg/day PO for 12 days) to the neurobehavioral development of their offspring. This dose regimen of paternal THC exposure was not found to significantly impact the clinical health of the offspring. However, it did cause a long-lasting significant impairment in attentional performance in the offspring relative to controls when they were tested in adulthood. There was also a significant increase in habituation of locomotor activity in the adult offspring of the males exposed to THC prior to mating. This study shows that pre-conception paternal THC exposure even at a modest dose for a brief period can cause deleterious behavioral effects in their offspring, including attentional impairment. Further research should be conducted to determine the degree to which this type of risk is seen in humans and to investigate the mechanisms underlying these effects and possible treatments to ameliorate these long-term adverse behavioral consequences of paternal THC exposure. *This research was supported by grant number 60564 from the John Templeton Foundation.*

P376 - 0751 Adolescent Toluene Exposure Increases DOI-Induced Behavioral, Molecular, and Electrophysiological Responses in Adult Mice: Effects of Antipsychotic Treatment

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Toluene is one of the most commonly abused inhalants among adolescents. Toluene has been characterized as a non-classical hallucinogen drug through activation of 5-HT_{2A/2C} receptors. It remains unclear whether long-term and intense toluene exposure changes the functional activity of 5-HT_{2A/2C} receptors. The present study aimed to study the functional abnormalities in 5-HT_{2A/2C} receptors after adolescent toluene exposure and to compare the therapeutic effects of antipsychotics, haloperidol and risperidone. NMRI male mice were treated with toluene (750 mg/kg, i.p.) during postnatal days 35-39 and 42-46. Subsequently, a hallucinogenic 5-HT_{2A/2C} receptor agonist 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI)-induced head twitch, c-Fos and Egr-2 expression, and field potentials in the medial frontal cortex were monitored. The levels of 5-HT_{2A} and 5-HT_{2C} in the medial prefrontal cortex were also determined. Furthermore, the effects of post-treatment with haloperidol (0.1 and 0.3 mg/kg) and risperidone (0.1 and 0.3 mg/kg) for 2 weeks on the enhancement of DOI-induced responses following adolescent toluene exposure were compared. Toluene exposure during adolescence enhanced DOI-induced head-twitch response, c-Fos and Egr-2 expression in the infralimbic cortex and increased amplitude and frequency of the field potentials. However, the expression levels of 5-HT_{2A} and 5-HT_{2C} were unaltered. Subchronic treatment with risperidone (0.3 mg/kg), but not haloperidol, could reverse the hyper-responsiveness to DOI after adolescent toluene exposure. These findings suggest that adolescent toluene exposure increases 5-HT_{2A} receptor-mediated signaling responses and recommend the antipsychotics with more selective 5-HT_{2A} receptor antagonism as the first-line treatment for psychiatric symptoms associated with chronic toluene abuse.

P377 - 0775 Evaluation of Anticonvulsant Activity of Methanolic Leaf Extract of *Solanum nigrum* Plant and Its Fractions in Mice

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This study investigated the anti-convulsant effect of *Solanum nigrum* leaf on isoniazid challenged mice; with a view to sourcing safe and potent anti-convulsant lead compound for drug discovery. The leaf of *Solanum nigrum* (150g) was extracted with aqueous-methanol, and partitioned with n-hexane, ethyl acetate and butanol. The fractions and crude were screened for secondary metabolites and further confirmed with thin layer chromatographic (TLC) technique. Albino mice of both sexes (25-30 g) were randomly grouped into five, $n=5$. Groups 1, 2, and 3 were pre-treated with 30, 60 and 120 mg/kg body weight of the extract or fraction. Group 4 served as control and received only distilled water (10 ml/kg IP). Group 5 served as standard and received diazepam (5 mg/kg IP) only. After 30 minutes of pre-treatment, isoniazid was administered at a dose of 300 mg/kg body weight to each mouse, i.p. Ability of the extract to delay seizure or protect the mice from lethality within 30 minutes observation period was considered as an indication for anti-convulsant activity. Both the crude extract and its sub-fractions exerted a significant anticonvulsant activity as compared with the control. The control group 4 recorded 100% mortality. In contrast, Group 5 recorded zero percent mortality. The n-hexane fraction (NHF) and the crude extract exhibited the least anti-convulsant activity. Whereas the ethyl acetate fraction (EAF) exhibited the most potent activity; followed by the n-butanol fraction (NBF). EAF recorded 60% inhibition against mortality at 60 and 120 mg/kg. The NBF recorded 40% inhibition against mortality at 60 and 120 mg/kg. The crude extract and NHF recorded 20% at 30 and 60 mg/kg, respectively. Also there were significant delays in the time of mortality across the treated groups as compared with the untreated. Qualitative screening of all the fractions and crude revealed the presence of flavonoids, phenols, alkaloids, cardiac glycosides, saponins, steroids, and triterpenes. In specific, EAF which exerted the maximum protection against convulsion had much presence of flavonoids, and phenols. NHF which exerted the least activity did not test positive for flavonoids and phenols. The study concluded that the leaf extract of *S. nigrum* possesses anti-seizure activity. However, EAF proved to be the most potent. Further investigation into the phytochemical constituents revealed that, EAF contains unique flavonoids (Rf 0.63) and phenols (Rf 0.26) known to exert an anti-seizure property. It could be surmised therefore that the anti-seizure effect could be mediated by the presence of these phytochemicals.

PS P378 - 0777 A Toxicological Profile of Valproic Acid in Experimental Model of Autism Spectrum Disorder: Developmental, Behavioral, Biochemical, and Histopathological Evidences

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Autism is defined as a group of multifaceted neurodevelopmental disorders of unknown etiology manifesting problems like social interaction, language, communication, behavior deficit etc. The present study aim to evaluate the extent of toxicity in terms of developmental aberration, behavioral, biochemical and histopathological changes in valproic acid (VPA) and iron deficient diet (IDD) induced model of autism spectrum disorder (ASD) in rats. Experimental animals were taken following permission from Institutional Ethics Committee and were grouped into four groups. Group one (Control, received only Normal saline), Group two (VPA on PND 12.5), Group three (Mother received VPA on PND 12.5 and iron deficiency diet), Group 4 (Risperidone 2.5 mg/kg, PND 23 to 43). Parameters assessed in pups were weight on PND 1, Weight on PND 10, Day of one eye opening, day of both eye opening, eye opening score on PND 12, tooth eruption on PND 12, tail length on PND 12, ano-genital distance on PND 12, eye-ear distance on PND 12, and total body length on PND 12. Parameters assessed in pups were developmental, behavioral, biochemical and histopathological and data was analyzed by SPSS version 22 software by considering $p < 0.05$ as significant. Significant difference was found between the groups with regards to weight on PND-1, weight on PND-10, single eye opening PND, both eye opening PND, teeth eruption on PND 12, and total body length at PND 12. Among the different groups VPA + IDA and the VPA alone group showed significant difference when compared to control in parameters like Weight on PND 1, Weight on PND 10, Head body length on PND 12. The VPA + IDD group additionally showed significant difference when compared to control with regards to single eye opening PND, both eye opening PND and teeth eruption PND. Regarding VPA and VPA + IDD model no difference was observed at any of the outcome variables. Risperidone significantly protected against the VPA induced autism related effects. VPA has significantly reduced the levels of antioxidants like GSH, SOD and catalase as compared to control group. Risperidone has reversed the oxidative stress produced by VPA in ASD. The histopathological score was found to be significantly increased in comparison to control group. Developmental parameters were delayed by VPA in ASD. But VPA and IDD model showed additional delay in some developmental parameters like eye opening and tooth eruption. VPA causes increase in cognitive impairment, oxidative stress and neuronal injury in ASD rats.

PS P379 - 0796 Environmental Toxicants and the Melatonin System

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Our hormonal systems can be influenced by environmental pollutants that act as endocrine disruptors (EDs). Studies have shown that exposure to EDs during early life stages may affect the onset of various reproductive, metabolic, immune, and neurological diseases later in life. The pineal gland and the melatonin hormone system have not been researched enough regarding endocrine disrupting effects, despite emerging evidence of melatonin potentially acting as an important epigenetic regulator during development. In addition, melatonin has a neuroprotective role and reduced melatonin levels have been linked with an increased risk to develop neurodegenerative disease. In this study we sought to evaluate the effects of three abundant environmental pollutants that may act as EDs—glyphosate, perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA)—on melatonin synthesis. Primary rat pinealocytes were exposed to 100 nM to 500 μ M of the compounds for 24 hours. Cells exposed to 100 μ M or 500 μ M of glyphosate showed a decrease in cell viability, while the melatonin levels were significantly reduced even at 50 μ M. PFOS and PFOA did not induce any effect on pinealocyte viability or melatonin levels. Subsequently, we used an animal model to investigate the long-term effects of developmental exposure to a glyphosate-based herbicide (GBH) on melatonin synthesis. Rats exposed to 1% GBH (0.36 % glyphosate) via drinking water from gestational day 5 to postnatal day 15, had significantly lower serum melatonin levels compared to controls, at 90-days of age. Our results indicate that perinatal exposure to glyphosate may induce adverse effects that manifest in the adult brain. Further research on the melatonin hormone system and endocrine disruption by environmental pollutants is warranted.

PS P380 - 0821 Magnoliae Cortex Extract Protects PC12 Cells from Oxidative Stress through Induction of Drug-Metabolizing and Antioxidant Enzymes

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Alzheimer's and Parkinson's diseases are neurodegenerative disorders of unknown cause. Neurodegenerative diseases are generally considered to be incurable diseases in which the nervous system is irreversibly impaired by the death and loss of functional neurons. There is no cure or way of prevention or slow its progression. Oxidative stress is thought to be involved in the onset of neurodegenerative diseases. On the other hand, it is well known that oxidative stress is suppressed by drug-metabolizing enzymes, such as glutathione S-transferase and NAD(P)H: quinone oxidoreductase 1 (NQO1), and antioxidant enzyme, such as catalase. In the present study, we investigated the protective effect of Magnoliae Cortex extract against oxidative stress in neuronal-like PC12 cells. After treatment with *Magnoliae Cortex* extract in serum-free medium for 24 hr, NQO1 and catalase activities in cytosolic fractions of PC12 cells were measured. NQO1 and catalase activities in PC12 cells were significantly increased after treatment with *Magnoliae Cortex* extract. In addition, the cytotoxic effect of H_2O_2 and 6-hydroxydopamine (6-OHDA) was drastically reduced by pretreatment of the cells with *Magnoliae Cortex* extract. In order to isolate active constituents, the powder of *Magnoliae Cortex* was extracted with methanol and then the methanol soluble fraction was separated and analyzed by reversed-phase HPLC. The two potential active constituents in *Magnoliae Cortex* were identified as magnolol and honokiol. NQO1 and catalase activities were induced by pretreatment of PC12 cells with magnolol and honokiol. The cytotoxic effect of H_2O_2 was significantly suppressed by the pretreatment of the cells with magnolol and honokiol. However, only the pretreatment of PC12 cells with magnolol significantly inhibited the cytotoxic effect of 6-OHDA. These results suggested that magnolol play an important role to protect against oxidative stress in PC12 cells through induction of drug-metabolizing and antioxidant enzymes.

PS P381 - 0846 Protective Effect of the Imidazoline I2 Receptor Agonist 2-BFI on Oxidative Cytotoxicity in Astrocytes

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Astrocytes perform a variety of functions that are important for normal neuronal activity and recovery after brain injury. Because astrocytes are very vulnerable to H_2O_2 , protection of astrocytes from oxidative damage in various neurological diseases is important in maintaining brain function and preventing brain damage. In this study, we investigated the characteristics and mechanisms of a specific imidazoline I2 receptor agonist 2-BFI-mediated cytoprotection using a rat astrocyte cultures of H_2O_2 -exposed oxidative stress. Here we show that 2-BFI in H_2O_2 -exposed astrocytes protects cell death through increased lysosomal membrane stability, LC3-II conversion, and subsequently suppresses accumulation of p62. These effects of 2-BFI were significantly reversed after treatment with the lysozyme activity inhibitor Bafilomycin A1. These results suggest that the cytoprotective effects of 2-BFI, which increases lysosomal stability in oxidative stress, may involve regulation of lysosomal-associated membrane protein-dependent autophagy and autolysosome degradation in astrocytes.

PS P382 - 0849 Behavioral and Immunohistological Analysis of Peripheral Nerve Injury in Methylmercury-Exposed Rats

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Methylmercury (MeHg) is an organometallic compound which causes central and sensory nerve disorders known as Minamata disease. Excessive exposure to MeHg induces sensory nerve disorders including the globe and stocking type as one of its early symptoms. However, differences in the sensitivity of MeHg in the several sensory modalities which detect external stimuli such as pain, pressure, heat, and cold have not been well investigated. To evaluate the susceptibility of the sensory modalities to MeHg, we performed the behavioral

analysis of MeHg exposed Wistar rat and the immunohistochemical analysis of the dorsal root ganglion (DRG) where peripheral sensory nerve cells gather. The male Wistar rats were orally exposed to MeHgCl solution (6.7 mg/kg/day) for 5 days and were not exposed for the subsequent 2 days. These rats were treated with this cycle twice. The behavioral analysis of the susceptibility of the sensory modalities to MeHg was quantified by performing the following tests once every day for 70 days since the first day of MeHg treatment. The tail dipping test and the tail flick test were carried out for the thermal sensitivity. Von Frey test and the foot shock test were carried out for the pressure and pain sensitivity, respectively. It is found that in the MeHg exposed rat, significant hypesthesia was observed in the cold sensitivity from day 15 to day 30 and in the hot sensitivity from day 12 to day 33. Both recovered later. The pressure sensitivity became significantly low from day 9 to day 23, recovered after day 23, and then became significantly low again from day 44. The pain sensitivity became significantly low from day 18 to day 46, however, the sensitivity recovered after day 46. In conclusion, each sensory modality expresses responses in different chronological timings which suggest the differences in susceptibility to MeHg. For the further investigation, we are currently conducting the immunohistochemical staining on frozen DRG section and the histological analysis of different types of nerve cells that carry different sensory modalities to clarify the apparent difference from the histological approach.

PS P383 - 0854 Comparison of the Neurotoxicity Associated with Cobalt Nanoparticles and Cobalt Chloride in Wistar Rats

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Cobalt nanoparticles (CoNPs) have been widely used in industry given their physical, chemical and magnetic properties; however, CoNPs may cause neurological symptoms and diseases in human, yet their mechanisms of toxicity remain unknown. Here, we used male Wistar rats to investigate differences in the toxic effects associated with CoNPs and CoCl₂. Upon exposure to CoCl₂ and 96 nm or 123 nm CoNPs at the same concentration, the Co²⁺ content in CoCl₂ group was significantly higher than that in either the CoNPs groups in brain tissues and blood, but lower in liver. Significant neural damage was observed in both hippocampus and cortex of the temporal lobe. Increase malondialdehyde (MDA) content and CASPASE 9 protein level were associated both with CoCl₂ and CoNPs treatments, consistent with lipid peroxidation and apoptosis. Heme oxygenase-1 and (NF-E2) p45-related factor-2 protein levels were elevated in response to 96 nm CoNPs exposure. In PC12 cells, NRF2 downregulation led to reduced cell viability and increased apoptotic rate. In conclusion, both CoNPs and CoCl₂ cause adverse neural effects, with nanoparticles showing greater neurotoxic potency. In addition, NRF2 protects neural cells from damage induced by CoCl₂ and CoNPs by activating downstream antioxidant responses.

PS P384 - 0865 In Vitro and In Vivo Toxicity of Poly-L-arginine

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PLR is a cationic poly-amino acid and one of the several peptide drugs applied to chronic degenerative diseases due to their high uptake rate and decreased side effects, like immune responses. Prion diseases are caused by prions composed of misfolded prion proteins (PrP^{Sc}), which propagate in the mammalian central nervous system. Prior to the investigation of the PLR therapeutic effect on prion diseases, we investigated the toxicity of PLR in cell and mouse models. PLR composed of either 52 and 200 L-arginine monomers, designated PLR52 and PLR200 did not show cytotoxicity in neuroblastoma cells under 0.3 μM, but PLR with 571 L-arginine monomers (PLR571) showed cytotoxicity at 0.1 μM. We also tested the PLR toxicity in a mouse model using PLR200. Ten - forty mg/kg PLR200 was administered to mice twice a week by the intraperitoneal route for four weeks. After 84 days after administration, we collected the serum and tissues to determine the cytotoxicity of kidney and liver. During necropsy, adhesions of the intraperitoneal organs was observed. But in serum biochemistry analysis, AST, ALT, ALB and ALP levels were not changed significantly. Also, histological analysis showed no major changes or tissue necrosis in liver and kidney. In conclusion, mice tolerated PLR under our experimental conditions.

PS P385 - 0883 In Vitro and In Vivo Assessment of SGI-1027 in Toxicological Perepectives

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SGI-1027 is a DNA methyltransferase inhibitor and shows a great potential for cancer therapy because it effectively suppresses cancer cell growth. To investigate toxicity, we performed testes in cell and animal models. The cell viability of SGI-1027 in neuroblastoma cells treated with a range from 0 to 2 μM indicated that SGI-1027 did not show cytotoxicity under 1 μM. For *in vivo* study, ICR mice received 10-50 mg/kg SGI-1027 by the intraperitoneal route twice a week for four weeks. After 28 days after administration, we collected the blood and tissues to determine the cytotoxicity of kidney and liver. In complete blood cell count analysis, WBC, RBC, hemoglobin and platelets levels were not changed significantly. Likewise, serum biochemistry analysis, AST, ALT, ALB and ALP levels were not changed significantly. Also, histological analysis showed no obvious changes in liver and kidney of all treated group. However, in necropsy, we observed accumulation of SGI-1027 that remained unabsorbed in internal organs such as liver and intestine. In the group that received 50 mg/kg SGI-1027, spleen hypertrophy was found.

PS P386 - 0889 Manganese Induced Neurotoxicity Cross-Species due to Protein Biosynthesis Impairment with Putative Implications for Neurodegeneration

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Manganese (Mn) is an essential element; but in animal and humans, chronic and/or acute exposure to this metal can lead to neurotoxicity and neurodegenerative disorders including Parkinsonism and Parkinson's Disease by unclear mechanisms. However, we hypothesized that if these alterations are conserved cross-species, which must improve the understanding of the toxicological mechanisms of this metal. Hence, this study aimed to investigate the mechanisms of the manganese-induced toxicity in *Saccharomyces cerevisiae* (yeast), *Danio rerio* (zebrafish), *Mus musculus* (cerebellar granular neurons) and *Homo sapiens* (human neuroblastoma SH-SY5Y cell line). The biological models were exposed for species of manganese and studied through different bioanalytical approaches: cell viability-MTT-assay; sensitivity-gene-screening assay; b-galactosidase assay; ribosome profiles; transcriptomics (human gene expression microarray kit and qRT-PCR); semi-quantitative proteomics - tandem mass tag (TMT); translation protein efficiency assay; metallomics, bioinformatics (gene ontology - GO and prediction of protein-protein interaction -PPI, which were conducted at the String database (String-db) and the comparative toxicogenomic database (CTD) respectively. The results were expressed as mean ± SEM of at least 3 experiments; LC50 and statistically significant differences were estimated by ANOVA (analysis of variance followed by Bonferroni's tests), using the GraphPad Prism 4.0 Software Inc, San Diego, CA, USA. We verified that independently of the chemical speciation the manganese induces, from yeast to mammalian, impairment of protein biosynthesis and correlate processes as a mechanism of neurotoxicity. Finally, using both String-db and CTD, we discovered that these events are associated with neurodegenerative disorders such as Alzheimer's disease, Amyotrophic Lateral Sclerosis, Huntington's disease, Parkinson's disease, and Parkinsonism.

P387 - 0924 Association of Initial Troponin I Level with the Development of Delayed Neurologic Sequelae in Patients with Acute Carbon Monoxide Poisoning

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The increase of troponin I is frequently observed in acute carbon monoxide (CO) poisoning. However, little is known about the association between increased troponin I level and the development of delayed neurologic sequelae (DNS). The aim of this study was to evaluate the prognostic value of initial troponin I level in relation to DNS in patients with acute CO poisoning. This retrospective observational study was conducted in the emergency department (ED) of a tertiary, university-affiliated hospital and included patients with acute CO poisoning who presented to the ED over 8 years. The baseline characteristics and laboratory findings were compared between two groups; the DNS group and the non-DNS group. We also analyzed the ability of the initial troponin I level on ED admission to predict the development of DNS, defined as newly developed neurologic symptoms or signs within 6 weeks post-discharge. Of the 610 patients finally included in the study, 101 (16.6%) had DNS. Initial troponin I level was higher in the DNS group compared to the non-DNS group (0.5 (0.0-2.4) vs. 0.0 (0.0-0.1) ng/mL; $p < 0.001$). In multivariate analysis, initial troponin I level, the duration of CO exposure, and mental status on ED admission were the only variables associated with DNS, with odds ratio of 1.57 (95% CI, 1.25-1.98; $p < 0.001$), 1.16 (95% CI, 1.10-1.22; $p < 0.001$), and 1.63 (95% CI, 1.20-2.21; $p = 0.002$), respectively. The area under the receiver operating characteristic curve of initial troponin I level for the prediction of DNS was 0.78 (95% CI, 0.72-0.83; $p < 0.001$). The age, sex-adjusted odds ratio of abnormal troponin I level, taken at a cut-off value of 1.5 ng/mL for the prediction of DNS was 26.02 (95% CI, 13.48-50.23; $p < 0.001$). The specificity and negative predictive value of abnormal troponin I level for the prediction of DNS were 96.7% and 89.6%, respectively. Our study demonstrated that the initial troponin I level on ED admission is independently associated with DNS and troponin I level above 1.5 ng/mL is a significant predictor of DNS. These findings suggest that troponin I may be a biomarker that reflects the long-term neurologic prognosis of acute CO poisoning and deserves further prospective investigation.

P388 - 0058 Validation Study of Developmental Toxicity Test Using Embryoid Body's Area

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The embryonic stem cell test (EST) from the European Centre for the Validation of Alternative Methods (ECVAM) evaluates the embryotoxic potential of substances and measures the half inhibition levels in viability of mouse embryonic stem cells (ESCs), fibroblasts (3T3 cells), and ESC cardiac differentiation. In the previous study, we suggested an advanced developmental toxicity test method (the EBT) that assessed the area of embryoid bodies (EBs) instead of assessing cardiac differentiation. EBT used two same endpoints as EST, the half inhibition concentrations for cell viability of mouse ESCs (IC_{50} E14) and 3T3 fibroblasts (IC_{50} 3T3), but replaced the half inhibition concentration for cardiac differentiation (ID_{50} CM) with the half inhibition concentration for EB area (ID_{50} EB). We used the hanging drop method to form embryoid bodies. In order to verify the proposed EBT method in this study, interlaboratory reproducibility (5 substances in common) and predictive capacity (10 substances in each laboratory) tests were performed. To ensure reliability of the study results, the tests were conducted using identity-coded test substances. The results of statistical analysis of the interlaboratory reproducibility test indicated that reproducibility accuracy 87%, sensitivity 78%, and specificity 100%. The results of statistical analysis of the predictive capacity test indicated that the lead laboratory had reproducibility accuracy 80%, sensitivity 86%, and specificity 67%. Participatory laboratory 1 had reproducibility accuracy 80%, sensitivity 71%, and specificity 100%, and participatory laboratory 2 had reproducibility accuracy 80%, sensitivity 86%, and specificity 67%. The results of the intra- and interlaboratory tests were highly accuracy 83%, sensitivity 80%, and specificity 89% when substances were classified using the predictive model. EBT can accurately classify various embryotoxicants in a short time with less effort and greater validation. *This research was supported by a grant (17182MFDS487) from Ministry of Food and Drug Safety in 2017.*

P389 - 0071 Quercetin Ameliorates Cadmium Chloride-Induced Hepatotoxicity in Wistar Rats

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Cadmium, a toxic element exposure to humans in food, air, and water, may result in its bioaccumulation in the liver. This study evaluated the ameliorative effects of quercetin on some serum enzymes in cadmium chloride-treated rats. Thirty female Wistar rats (180 to 220 g) were randomly assigned to 5 groups of 6 rats each as follows: Group A, the normal control, received orally 0.2 ml of 2% DMSO/day; group B received $CdCl_2$ (5 mg/kg b.w./day) according to the methods of [1]; group C rats were pretreated with QE (20 mg/kg b.w./day) 6 h before $CdCl_2$ (5 mg/kg b.w./day) administration; group D rats were pretreated with $CdCl_2$ (5 mg/kg b.w./day) 6 h before QE (20 mg/kg b.w./day) administration; while group E rats were treated with $CdCl_2$ (5 mg/kg b.w./day) and QE (20 mg/kg b.w./day) simultaneously. The cadmium chloride and quercetin were reconstituted in 2% DMSO and the study period was 14 days [1]. Thereafter, the animals were euthanized and blood collected for serum liver enzyme assays. There was a significant decrease ($p < 0.001$) in the serum activities of ALT, AST, ALP, and LDH in groups C and D compared with group B; the activities of the liver enzymes in the quercetin-treated groups compared well to the normal control, suggesting a near total restoration of liver function. We therefore conclude that quercetin may possess potentials to restore hepatic injury induced by cadmium.

P390 - 0093 Treatment of Human Choriocarcinoma Cells with Acrylonitrile-Induced Cell Cycle Arrest and Apoptosis by Promoting the Formation of Reactive Oxygen Species

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Acrylonitrile (AN), which is widely utilized in the manufacture of plastics, acrylic-amide, acrylic fibers, and resins, is also one of main components of cigarette smoke (CS). In this study, we examined the effects of AN on the cell viability and apoptosis of JEG-3 and BeWo human choriocarcinoma cancer cell lines. A cell viability assay confirmed that AN decreased the cell proliferation of JEG-3 and BeWo cells in a dose-dependent manner. Additionally, Western blot assay revealed that protein expression of cyclin D and cyclin E decreased, while protein expression of p21 and p27 increased in response to AN treatment for 48 h. The changes in reactive oxygen species (ROS) levels in JEG-3 and BeWo cells exposed to AN were also measured by a dichlorofluorescein diacetate (DCFH-DA) assay, which revealed that ROS levels increased in response to AN treatment for 48 h. Moreover, western blot assay confirmed that AN treatment of JEG-3 and BeWo cells for 4 h promoted the expression of phosphorylated eukaryotic initiation factor 2 alpha protein (p-eIF2 α), C/EBP homologous protein (CHOP), and Caspase 12, which are known to be involved in ROS-mediated endoplasmic reticulum stress (ER-stress) related apoptosis. Overall, the protein expression of p53 and Bax (a pro-apoptosis marker) increased, while the expression of Bcl-xl (an anti-apoptotic marker) decreased and the number of apoptotic cells increased in response to AN treatment for 48 h. Taken together, these results suggest that AN has the potential to induce apoptosis of JEG-3 and BeWo human choriocarcinoma cancer cells by activating ROS. *This work was supported by the Global Research and Development Center (GRDC) Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT 2017K1A4A3014959.*

P391 - 0095 Cigarette Smoke Extracts Induced Apoptosis and Oxidative Stress in Mouse Embryonic Stem Cells via Regulating Apoptotic and Oxidative Stress Related Genes

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The previous studies reported that cigarette smoke or cigarette smoke extract (CSE) have negative effects on embryo development. However, there is no mechanism study on how CSE affects the cellular signaling pathway for apoptosis and oxidative stress in embryonic cells or how the two pathways

cross-link. Therefore, we investigated the effects of CSE on apoptosis and oxidative stress in mouse embryonic stem cells (mESCs). We measured changes in cell viability by CSEs (3R4F and two domestic cigarettes CSE 1 & 2) using the water soluble tetrazolium (WST) assay and the neutral red uptake (NRU) assay, resulting in decreased cell viability in a concentration-dependent manner. In Western blot, the expression of cyclin D1 and cyclin E1 was decreased and the expression of p21 and p27 was increased by CSE. The number of terminal deoxynucleotidyl transferase (TUNEL)-stained cells was increased by CSE, and Bax and Caspase-3 were increased and Bcl-2 was decreased in Western blotting. Finally, we performed 2',7'-dichlorofluorescein diacetate (DCF-DA) assay and ROS-Glo H2O2 assay to confirm the reactive oxygen species (ROS) generation by CSE; we could found the increased ROS by CSE. The increased ROS by CSE was associated with upregulated Keap-1 and CHOP. Overall, we concluded that the CSE inhibited cell proliferation by regulating cell cycle-related protein expression and increased the oxidative stress by regulating the expression of Kelch-like ECH-associated protein 1 (Keap-1) and CCAAT/Enhancer-Binding Protein Homologous Protein (CHOP), then resulted in apoptosis in mESCs. This research was supported by a grant (14182MFD5977) from the Ministry of Food and Drug Safety in 2018.

P392 - 0122 Exposure to Embalment Fluid and Changes in Biomarkers of Oxidative Stress, Reproductive Hormones, and Renal Function in Embalmers in Calabar, Nigeria

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Occupational exposure to formaldehyde in embalment fluid has been linked to chronic health conditions; lipid peroxidations, depletion of antioxidants, and oxidative stress have been implicated as pathologic mechanisms. The biomarkers of oxidative stress, reproductive hormones, and renal functions of embalmers in relation to duration of exposure to embalment fluid were determined in this study. Sixty men (18–60 years) comprising 30 embalmers and 30 nonembalmers were recruited into this case control study. Serum levels of biomarkers of oxidative stress (malondialdehyde (MDA), glutathione (GSH), nitric oxide (NO), total antioxidant capacity (TAC), total plasma peroxides (TPP)) and renal functions (urea and creatinine) were estimated by colorimetry, reproductive hormones (leutinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone) by enzyme linked immunosorbent assay method, and urine formic acid (FA-U) by gas chromatography. Data were analyzed using analysis of variance and Pearson's correlation at $p < 0.05$. Embalmers had higher urine formic acid, lipid peroxides, and lower glutathione, nitric oxide, total antioxidant capacity, and testosterone compared with nonembalmers ($p < 0.05$). Increasing duration of exposure to embalment fluid is associated with increased urine formic acid and decreased FSH levels. MDA correlated negatively with TAC ($r = -0.396$, $p = 0.030$) and positively with OSI ($r = 0.519$, $p = 0.003$) only in embalmers. Chronic exposure to formaldehyde in embalming fluid is associated with increased formic acid excretion, lipid peroxidation, and depletion of antioxidants, testosterone, and FSH, which may result in perturbations in male reproductive functions in embalmers studied.

P393 - 0124 Cadmium Exposure of Female Mice Impairs the Meiotic Maturation of Oocytes and Subsequent Embryonic Development

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Cadmium is one major pollutant that is highly toxic to animals and humans. We have previously reported the mechanisms of cadmium hepatotoxicity and neurotoxicity. However, the mechanism of cadmium toxicity on the female reproductive system, particularly oocyte maturation and fertility, remains to be clarified. In the present study, we used a mouse model to investigate the effects of cadmium in the drinking water on the meiotic maturation of oocytes and subsequent embryonic development, and the underlying mechanisms associated with the impairment of oocyte maturation such as mitochondrial distribution and histone modifications. Our results show that cadmium exposure decreased the number of ovulated oocytes and impaired oocyte meiotic

maturation rate both *in vivo* and *in vitro*. The embryonic development after fertilization was also impaired even when the potential hazards of cadmium on the spermatozoa or the genital tract have been excluded by fertilization and embryonic development in culture. Cadmium exposure disrupted meiotic spindle morphology and actin filament, which are responsible for successful chromosome segregation and the polar body extrusion during oocyte maturation and fertilization. ATP contents, which are required for proper meiotic spindle assembly in the oocyte, were decreased, consistent with altered mitochondrial distribution after cadmium exposure. Finally, cadmium exposure affected the levels of H3K9me2 and H4K12ac in the oocyte, which are closely associated with the acquisition of oocyte developmental competence and subsequent embryonic development. Next, we will test whether cadmium exposure affects the DNA methylation in the oocytes and how cadmium affects the epigenetic reprogramming process in early embryos. In conclusion, cadmium exposure in female mice impaired meiotic maturation of oocytes and subsequent embryonic development by affecting the cytoskeletal organization, mitochondrial function, and histone modifications.

P394 - 0137 Antidepressant Sertraline Induces Cytotoxicity in Human Placental Cells

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Selective serotonin reuptake inhibitors (SSRIs), sertraline and escitalopram, are widely used for the treatment of depression. Depression is one of the most common psychiatric disorders in pregnant women, and SSRIs are the most commonly prescribed antidepressants during pregnancy. Several studies have reported that the early prenatal exposure to SSRIs may increase the risk of malformation, but other studies showed no correlation between the risk of malformation and SSRIs use. The placenta has a critical role in the survival and growth of a fetus, involving the delivery of nutrients and oxygen and removal of waste compounds from the fetal compartment. The placenta forms an interface between the maternal and fetal circulations. In this study, we investigated the effects of sertraline and escitalopram on human placental cells. Human placental choriocarcinoma BeWo cells were cultured in the presence of 0–100 μ M sertraline or escitalopram for 6–48 hours. Cell viability was determined by WST-8 assay, extracellular LDH activity, and cellular ATP content. Cellular reactive oxygen species (ROS) and caspase-3/7 activities were determined by using 2',7'-dichlorofluorescein diacetate (DCFDA) and Caspase-Glo 3/7 Assay System (Promega), respectively. BeWo cell viability was significantly decreased by exposure to 50 μ M sertraline for 24 h, but escitalopram had no effects. Extracellular LDH activities were increased by 25 μ M sertraline, indicating the membrane damage of BeWo cells. Exposure to sertraline decreased the cellular ATP contents in a time-dependent and concentration-dependent manner, showing the sertraline-induced cytotoxicity in BeWo cells. Sertraline increased the cellular ROS and caspase-3/7 activities. These results suggest that antidepressant sertraline induces ROS-dependent apoptosis in human placental BeWo cells.

P395 - 0145 BMAA Alters Striatal Neural Stem Cell Proliferation and Differentiation through Glutamatergic Mechanisms

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The widespread environmental contaminant β -methylamino L-alanine (BMAA) is a developmental neurotoxicant that can induce long-term cognitive deficits. Studies have shown high transplacental transfer of ^{3} H-BMAA and a significant uptake in fetal brain. Therefore, more information on how BMAA may influence growth and differentiation of neural stem cells is required for assessment of the risk to the developing brain. The aim of this study was to investigate the effects of BMAA exposure using primary cultures of striatal neurons and embryonic neural stem cells. Exposure to BMAA for 24 h induced concentration-dependent neurotoxicity in neural stem cells but not in primary neurons. BMAA reduced neural stem cell proliferation through apoptosis and G2/M arrest. To study neurite outgrowth and differentiation, cells were exposed to concentrations that not affected cell proliferation (50–100 μ M) for 24 h and allowed to differentiate for seven days in total. The results showed that BMAA altered neural stem cell neurite outgrowth and their differentiation into neurons, astrocytes, and oligodendrocytes. The co-incubation of neural stem cells with BMAA and glutamatergic receptor antagonists MK-801 (N-methyl-D-aspartate antagonist) and MCPG (metabotropic receptor antagonist) prevented the effects on neurite outgrowth and differentiation caused by BMAA, suggesting that these effects are mediated through glutamatergic mechanisms. This study shows

that striatal embryonic neuronal stem cells are more susceptible to BMAA exposure than primary neurons. The results indicate that short-term, early-life exposure to BMAA impair cellular processes that can affect the development of the nervous system and may result in long-term consequences predisposing for both neurodevelopmental disorders and neurodegenerative disease. More attention should be given to the potential adverse effects of BMAA exposure on brain development.

PS P396 - 0146 The Role of Autism Risk Gene, CHD8, in Chlorpyrifos-Induced Neurotoxicity in iPSC-Derived BrainSphere Model

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The strong increase of autism in recent years worldwide cannot be explained solely by genetics or exposure to environmental chemicals, but rather as interplay between both. A combination of three cutting-edge technologies (the induced pluripotent stem cell [iPSC] technology, CRISPR-CAS9 gene editing, and 3D human relevant microphysiological systems) allows to produce a human brain model with an autistic genetic background, which recapitulates the critical phase of early brain development. Autism is a multifactorial disease that makes it challenging to find disease causation. Mutation in the chromodomain helicase DNA binding protein 8 (*CHD8*) is one of the major genetic risk factors linked to ASD. *CHD8* is important regulator of brain development and regulates a number of ASD-related genes. Here, we identified a potential synergy of two putative autism risk factors (i.e., *CHD8* mutation and the pesticide chlorpyrifos [CPF]). A heterozygous *CHD8*^{+/−}knockout iPSC cell line was generated using CRISPR-CAS9 technology (Wang et al. 2015). *CHD8*^{+/−}mutant iPSC alongside the control iPSC were differentiated in 3D to form BrainSpheres (Pamies et al. 2016). BrainSpheres were exposed for 24h to chlorpyrifos at 4 weeks of differentiation. BrainSpheres derived from *CHD8*^{+/−}iPSC were more sensitive to CPF than control, as identified by viability assay, increased oxidative stress, decreased neurite outgrowth, and synaptogenesis. Both *CHD8* mutation and CPF increased the level of dopamine and tyrosine hydroxylase, which can be associated with increased oxidative stress. Interestingly, exposure to CPF reduced the level of *CHD8* protein, suggesting that chlorpyrifos may target the same pathways as *CHD8* mutation. Further experiments are being conducted using multi-omics approaches (metabolomics mRNA and microRNA profiling) to identify molecular mechanisms of potential synergy between *CHD8* genetic background and chlorpyrifos exposure.

PS P397 - 0152 The Combined Effects of Cadmium and Methylene Blue in Zebrafish Embryos

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Cadmium (Cd) is a nonessential metal and environmental contaminant that is classified as a human carcinogen. It is known to have embryotoxic effects in experimental animals, including zebrafish, a model organism increasingly being used to understand developmentally harmful chemicals. While the mechanisms of Cd embryotoxicity are not established, oxidative stress is likely to play a role. Methylene blue (MB), which also leads to oxidative stress at the cellular level, is a synthetic basic dye that has been used both clinically and industrially. MB is typically used in the rearing solution for zebrafish and other aquatic organisms because of its antifungal properties. There is some evidence that it also has harmful effects on developing organisms, and it was thus of interest to investigate whether exposure to MB would exacerbate the embryotoxic effects of Cd in zebrafish. We investigated whether simultaneous treatment of early embryos with Cd and MB would alter the embryotoxicity observed with either treatment alone. Wildtype zebrafish were maintained at 28°C, with a cycle of 14 hours light and 10 hours dark. Embryos were collected soon after the lights came on and were cultured in E3 medium in the dark, with or without Cd and MB. Cadmium chloride (200 or 500 µM) and/or MB (1 ppm or 5 ppm) were prepared in E3 and were added to the culture medium, alone or in combination, at 8 hours post fertilization (hpf). Additional controls were cultured in E3 alone. Exposure continued until 72 hpf, when the embryos were assessed for survival, malformations, and various aspects of growth. Exposure to either Cd alone or MB alone caused an increase in mortality of embryos, from 23% in the controls to 52% in the high Cd exposure and 60% in the high MB exposure. There was also an increase in malformations from 4% in the controls to 20% in the low

Cd concentration, and 18% in the low MB concentration. In embryos exposed to combinations of the chemicals, the total number of affected individuals (deaths plus malformations) was reduced from 71.4% in the group exposed to 200 µM Cd alone to 40% in the group exposed to 5 ppm MB plus 200 µM Cd. In the 500 µM Cd group, 64% of the embryos were affected, and this was reduced in the group exposed to 500 µM Cd plus 5 ppm MB. The reduction in affected embryos was not observed in the group exposed to 200 µM Cd plus 1 ppm MB. Thus the effects of exposure to both Cd and MB are complex, but the toxic effects are not greater with a combined exposure. It is possible that Cd and MB interact within the culture medium, leading to a reduced embryotoxic effect when both are present. Supported by Trent University Natural Sciences Committee.

PS P398 - 0211 Teratogenicity of Multiwall Carbon Nanotubes and Asbestos Intratracheally Administered to Mice

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The fetal toxicity of multiwall carbon nanotubes (MWCNTs) and amosite, and the synergistic effect of lipopolysaccharide (LPS) on those exogenous fibrous materials were investigated in mice. On day 9 of gestation, the dams were given a single intratracheal administration of the test materials. The non-heat-treated MWCNTs (M) 4 mg/kg b.w markedly increased early fetal death and external or skeletal malformation, but the heat-treated (250°C, 2 hours, LPS-deprived) MWCNTs (HM) 4 mg/kg b.w. did not show fetal toxicity. The HM 8 mg/kg b.w. significantly increased early fetal death and external or skeletal malformation. While the LPS 25 or 50 µg/kg b.w., the HM 1 mg/kg b.w., or the heat-treated (LPS-deprived) Amosite (HA) 4 mg/kg b.w. alone, respectively, had no adverse effects on the dams and their fetuses, the combination of HM 1 or 4 mg/kg b.w.+LPS and the combination of HA 4 mg/kg b.w.+LPS markedly increased early fetal death and external or skeletal malformations. The results indicated that (1) the intratracheal administration of MWCNTs showed fetal toxicity, (2) the fetal toxicity of MWCNTs was reduced by the LPS depriving heat treatment and was strengthened by the combined administration of LPS at the noneffective dose, and (3) the fetal toxicity of Amosite was also strengthened by the combined administration of LPS at the noneffective dose.

PS P399 - 0242 p, p'-DDT Induces Apoptosis in Human Endometrial Stromal Cells through Oxidative Stress

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Bis-[4-chlorophenyl]-1,1,1-trichloroethane (DDT) was originally synthesized in 1874, its insecticide actions were first described in 1939. Initially, it was widely used to protect military personnel from malaria during Second World War. As DDT and its metabolites, bis-[4-chlorophenyl]-1,1-dichloroethane (DDE), were found in human tissues and their long half-lives were revealed, their effects on humans have been studied. DDT is associated with reduced mean luteal-phase length, spontaneous abortion, early age at menopause, and reduced duration of lactation. However, to date, there have been few studies on the effect of DDT on human endometrial cells. In this context, we studied whether DDT causes oxidative stress and apoptosis in human endometrial cells to determine the pathogenesis of diseases involving endometrium. Human endometrial stromal cells were obtained from reproductive aged women with normal menstrual cycle. We determined whether DDT induced oxidative stress through the ROS assay and analyzed gene expression of antioxidative enzymes by real-time RT-PCR and western blot. Annexin-V/propidium iodide labeling flow cytometry assay for apoptosis were performed. Also we have studied association between DDT and estrogen receptors (ER) and NF-κB pathways in human endometrium. From flow cytometric analysis, we found that DDT contributed to apoptosis. Fluorescence microscopy showed that DDT induced formation of apoptotic bodies and DNA fragmentation. After exposure to DDT, Bcl-2 with anti-apoptotic potential decreased and BAX, an apoptotic regulator, showed a dose-dependent increase. The expression of caspase-3, 6, 8, and 10 were also significantly increased. Treatment of endometrial cells with DDT resulted in a significant increase in DCF fluorescence and showed significant decrease in GPX and SOD expression. Regarding ER, DDT downregulated the expression of ER-β and reversed by antagonist treatment. These data showed that the mechanisms of apoptosis in DDT exposed human endometrial cells are associated with the increasing of oxidative stress via estrogen receptors. This effect of DDT

on changes in modulators of antioxidative enzymes and caspase pathway will significantly affect endometrial environment, which can determine the pathogenesis of diseases involving endometrium.

PS P400 - 0275 Gestational and Lactational Exposure to an Environmentally Relevant Mixture of Brominated Flame Retardants Dysregulates Cell-Cell Interactions, Thyroid Homeostasis, and the Proliferation-Apoptosis Balance in Rat Mammary Glands at Puberty

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Mammary gland development occurs mainly during puberty, pregnancy, and lactation and is characterized by proliferation, differentiation, and apoptosis cycles tightly regulated by hormones. Cell-cell interactions via adherens, gap, and tight junctions are also essential for proper mammary development and homeostasis. Exposure to endocrine disruptors, such as brominated flame retardants (BFRs), is ubiquitous and may alter mammary development and function. BFRs are added to consumer products to satisfy flammability standards. Previously, we showed that exposure of rat dams during gestation and lactation to an environmentally relevant mixture of BFRs, based on the mixture observed in house dust, disrupted proteins of the adherens junctions in the mammary glands. Here, we hypothesized that gestational and lactational exposure to BFRs will disrupt junctional proteins and signaling pathways that control mammary gland development in the offspring. Prior to mating and during pregnancy and lactation, female Sprague-Dawley rats were exposed to this BFRs house dust mixture in diets designed to deliver 0 (control), 0.06, 20, or 60 mg/kg/day. Female offspring were euthanized on post-natal day 46 (post-pubertal) and mammary glands were collected. Exposure to BFRs (0.06 mg/kg/day) significantly down-regulated the levels of adherens junction proteins E-cadherin and β -catenin and of the gap junction protein p-Cx43, while markers of the epithelial to mesenchymal transition remained unaffected. No effects were observed on protein levels of estrogen or progesterone receptors. However, exposure to the low dose of BFRs, but not higher doses, reduced thyroid hormone receptor alpha (TR α) protein levels in the mammary glands of the offspring. Interestingly, the same low dose of BFRs induced a significant decrease in the protein levels of cleaved caspase-3, a marker of apoptosis, while PCNA, a marker of proliferation, tended to increase. Together, our results suggest that gestational and lactational exposure to an environmentally relevant mixture of BFRs disrupts cell-cell interactions as well as thyroid hormone receptor levels and tissue homeostasis during puberty. Since puberty is a sensitive period for mammary gland development, and since dysregulation of cell-cell interactions, signaling, and apoptosis-proliferation balance are all strongly associated with cancer and metastatic progression in the breast, our results raise important questions about the long-term consequences of this dysregulation on breast carcinogenesis. Supported by FRQS, QBCF, NSERC (IP), by CIHR (ML, BFH, BR), by MERSCI, RQR-CIRD (RJG) and by FAF (ED).

PS P401 - 0298 Analysis of Epigenetic Effect of Low-Level Methylmercury on Neuronal Differentiation by Using an *In Vitro* Model

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Methylmercury (MeHg) is a well-known developmental neurotoxicant. Recently, adverse effect of relatively low-level MeHg have become an important issue in the toxicological study of MeHg. Epidemiological studies have reported that low-level prenatal MeHg exposure may be associated with the poorer psychomotor development. Experimental studies have also shown that low-level MeHg exposure adversely affects developing cranial nervous system. However, the underlying toxicological mechanisms remain to be elucidated. Epigenetics is important to consider the molecular mechanisms of effects of MeHg exposure in embryonic period. This study focused on toxicological mechanisms of MeHg exposure on neuronal differentiation from the view of epigenetics, using human fetal brain-derived immortalized cells (LUHMES cells) as a human neuronal differentiation model. LUHMES cells were exposure to MeHg (1 nM), that is no effect on cell viability, for 6 days, from day 2 to day 8 of neuronal differentiation. First of all, we observed a significant reduction of axon length by MeHg

exposure. Furthermore, we determined the level of histone modifications and DNA methylation, which are typical epigenetic modifications. Acetylated histone H3 (AcH3) levels, related to transcriptional activation, were decreased by MeHg exposure. On the other hand, tri-methylation of histone H3 lysine 27 levels (H3K27me3), related to transcriptional repression, were significantly increased by MeHg exposure. In addition, an increase of DNA methylation was observed by MeHg exposure. Next, the level of histone deacetylase (HDAC) was examined, because significant reduction of AcH3 was observed. As a result, the level of HDAC3 and HDAC6 were significantly increased by MeHg exposure. We further investigated whether reduction of axon length by MeHg exposure via deacetylation of histone H3 caused by HDAC. As a result, the reduction of axon length by MeHg exposure was improved by HDAC inhibitor, trichostatin A (TSA), treatment. Therefore, it was thought that neurological effect such as decrease of neurite extension by MeHg exposure was caused by decrease of AcH3 level via HDAC. Our finding is expected to provide useful evidences to novel toxic mechanisms of MeHg exposure on neuronal differentiation.

PS P402 - 0466 Differential Gene Expression Analysis in Mouse Placentae Reveals Association between Preterm Birth Linked Genes and PM_{2.5} Exposure

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Numerous epidemiological and animal studies have demonstrated that exposure to ambient fine particulate matter (<2.5mm in diameter [PM_{2.5}]) during gestation is associated with adverse obstetric outcomes including preterm birth (PTB). Early delivery has been linked to several lifetime health consequences for offspring, including behavioral and psychological abnormalities and reduced immune and respiratory functions. In a previous study performed in this laboratory, B6C3F1 pregnant mice exposed to concentrated ambient PM (CAPs) by inhalation, demonstrated shortened (by 0.4 d) gestational duration compared to filtered air (FA) controls. The mechanisms underlying the association between PM_{2.5} and PTB are not currently well understood. Since the placenta provides a crucial link between the intrauterine environment and fetal growth/development, it is a major target of PM and key for studying the effects on birth outcomes. Therefore, in this study, placentae from the previously developed pregnant mouse model (n=6 each from CAPs and FA groups) were subjected to whole transcriptomic profiling by RNAseq. A bioinformatic RNAseq analysis workflow (tximport, Salmon and edgeR /limma-voom) was used to identify differentially expressed genes between treatment groups. The 648 genes from the curated dbPTB (database Preterm Birth) were used for a candidate gene approach and were examined using gene counts obtained from RNAseq. Following PM_{2.5} exposure, six PTB genes were downregulated in placentae (Ace, Ddah1, Col1a2, Chst15, Akap12, Ephx1) and one was upregulated (Chys3) (p<0.01). Gene Ontology demonstrated that these seven genes are involved in neutrophil-mediated immunity, arterial blood pressure regulation, amino acid binding, cell membrane function, metal ion binding, and aromatic compound catabolism among other functions that could be linked to PTB. Additional computational models, agnostically testing placenta transcriptome-wide differential gene expression, revealed additional genes that were differentially expressed between the two treatment groups. These findings suggest that PM exposure influences the placenta genome thereby mediating PTB. Identification of these differentially expressed genes may contribute to intervention strategies to mitigate these adverse effects. Supported by: March of Dimes, NIEHS P30ES000260, and NIEHS P30ES023515.

PS P403 - 0471 Overabundance of microRNA 133b Induces Oil-Like Cardiotoxicity in Developing Zebrafish (*Danio rerio*) Embryos

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Large-scale oil spills, such as the Deepwater Horizon (DWH) in the Gulf of Mexico in 2010, can have significant ecological impacts on marine populations via exposure to highly toxic polycyclic aromatic hydrocarbons (PAHs). The timing of the DWH spill coincided with the spawning season for many pelagic species in the area, resulting in potential exposure of early life-stage fish to oil. Exposure of early life-stage fish to environmentally-relevant PAH concentrations induces cardiotoxicity, craniofacial malformations, and vision

impairments. Transcriptomic studies have suggested that improper heart development following exposure is correlated with downregulation of genes important for normal heart development during embryogenesis, such as genes regulating delayed rectifier potassium channels. This has further been linked with upregulation of microRNA (miR) 133b, which suppresses gene expression of many cardiac genes. To test the hypothesis that excess miR133b induces oil-like cardiotoxicity in developing fish, cleavage-stage zebrafish embryos were microinjected with ~3 nL of 75 mM negative control miR or miR133b. At 72 hours post fertilization, embryos were assessed for phenotypic effects and transcriptional changes in cardiac genes previously observed to be altered during oil exposure. miR133b-injected fish exhibited several oil-like cardiac malformations, including significantly increased pericardial area, cardiac looping defects, and decreased blood circulation relative to negative control miR-injected embryos. Other phenotypic effects, such as craniofacial malformations, alterations in eye area, and reduced heart rate were not observed. Gene expression analysis using qPCR showed that miR133b decreased the abundance of heart-specific delayed rectifier potassium channel (*kcnh6*) transcripts by ~40% relative to negative control miR-injected embryos. There were no changes in *kcnh2A* and *kcnh2B* transcripts, which are minimally expressed in the zebrafish heart based on prior studies reported in the literature. These results suggest that increased miR133b following oil exposure may contribute to underlying cardiac perturbations in oil-exposed fish by targeting cardiac-specific genes essential for proper heart development. Analyses are currently ongoing to explore other cardiac genes and proteins that may be altered by miR133b and contribute to improper cardiac development. *This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER).*

PS P404 - 0473 Maternal Exposure during Pregnancy to Electronic Cigarette Aerosols Modifies Behavior of the Adult Offspring: Learning Neurodevelopment from a Mouse Model

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Electronic cigarettes (e-cigarettes), battery-powered devices that provide nicotine and other additives to the user in the form of an aerosol, entered the market in 2007 and by 2014 were the most commonly-used tobacco product among adolescents and young adults. Among high school students, current e-cigarette use increased from 1.5% (220,000 students) in 2011 to 20.8% (3.05 million students) in 2018. While more pregnant smokers turn toward e-cigarettes as a safe alternative to smoking, the implications of vaping during gestation on the mother and/or child remain unknown. Indeed, clinicians have mixed opinions regarding use of e-cigarettes during pregnancy and expectant mothers looking for a way out of tobacco smoking are also confused. While it is well-known that prenatal exposure to traditional cigarette smoke impacts neurodevelopment and adversely affects later life behavior, the effects of e-cigarette aerosols on these health parameters, are completely unknown. Thus, a toxicological study was undertaken to determine whether exposure to e-cigarette aerosols (with and without nicotine) during gestation impacted adult behavior in a mouse model. While effects of prenatal exposure to e-cigarette aerosols on adult male and female behavioral characteristics including, anxiety, social interactions, depression and learning/memory are still being evaluated, 6-mo-old male and female offspring exposed prenatally to nicotine-bearing aerosols (16 mg nicotine) demonstrated significantly increased motor activity (as measured by total distance traveled on an elevated plus maze) compared to their filtered air- and solvent-exposed counterparts. These data are similar to those observed in our laboratory with 4-mo-old offspring exposed to nicotine-associated e-cigarette aerosols throughout pregnancy and weaning. Results of the current study demonstrates that use of e-cigarettes with nicotine during pregnancy could be linked with hyperactivity disorders in children later in life.

PS P405 - 0554 Poly(ADP-ribose) Polymerase-1 Inhibitor Restores Neuroimmune Function through RORyt and Stat3 Transcription Factors Signaling in BTBR Mice

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Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by abnormal social interactions, repetitive behaviors and restricted interests. Increasing indication is unveiling a role for poly(ADP-ribose) polymerase (PARP)-1 in the regulation of immune responses. The aim of the present study was to determine the potential neuroprotective effects of PARP-1 inhibitor 5-aminoisoquinolinone to explore the role of PARP-1 inhibitor in a BTBR mouse model of autism. BTBR inbred mice are generally used as a model for autism, as they show repetitive behaviors and social deficits that resemble signs of autism in humans. In this study, we investigated the potential effects of the PARP-1 inhibitor (5-AIQ) on marble burying and self-grooming tests, and expression levels of IL-17A, RORyt, Stat3, and IL-4-producing CD4⁺ T spleen cells in BTBR and C57 mice. We also assessed IL-17A, RORyt, Stat3, and IL-4 mRNA and protein expression levels in the brain tissues. The 5-AIQ-treated BTBR mice showed significantly prevented marble burying and repetitive behavior as compared with the BTBR control mice. The results further revealed that the 5-AIQ efficiently decreased the IL-17A, RORyt, and Stat3, and decreased the IL-4-producing CD4⁺ T cells. In addition, 5-AIQ significantly decreased the IL-17A, RORyt, and Stat3, and increased the IL-4 mRNA and protein expression levels as compared with the BTBR control mice. Our results clearly indicate that the PARP-1 inhibitor may represent a unique target for future therapeutic strategies for neuroimmune dysfunction.

PS P406 - 0565 Investigation of Gonado- and Reproductive Toxicity of Carbendazim on Male and Female Wistar Hannover Rats

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Carbendazim is one of the widely used systemic fungicides of the benzimidazoles class, intended for the protection of plants against various fungal diseases. It is known that this pesticide disrupts the endocrine system and cause reproductive toxicity, which is manifested of changes in hormones levels, adversely affects testicular steroidogenesis and has the anti-androgenic effect, etc. The aim of the study was hazard identification and risk assessment gonado- and reproductive toxicity of two generic pesticides carbendazim with different purity on male and female Wistar Han rats. Three groups of animals, 20 males and female each, were daily treated of carbendazim (C1—98 %, C2—98,1 % purity) by gavage for 5 days/week, at doses of 0,0; 2.5 and 25 mg/kg of body weight for 10 weeks (female) and 11 weeks (male). Control animals received an equivalent amount of vehicle. At the same time with control and experimental groups, there were kept intact animals, intended for mating. The detection of the functional state of the gonads and evaluation of the reproductive ability for males were performed at the end of the exposure period. The duration and the frequency of each stage of the estrous cycle in female rats were registered. The reproductive parameters of females were assessed on the 20th day of pregnancy. The indices of mating, conception, fertility, pregnancy, and the length of pre-coital interval were taken into account. The obtained data testify that at the administration of two carbendazim samples in a dose of 25 mg/kg over 11 weeks were shown a systemic toxicity (weight reduction) in males when exposed by C2. All test substances at the maximum dose reveal signs of reproductive toxicity and has a pronounced antiandrogenic action that appears to change morpho-functional parameters of gonads in experimental groups of males. In addition, there was a negative influence on females duration and the frequency of estrous cycle (C 2). Conclusions. Based on the results we can conclude that all the studied samples of carbendazim have reproductive toxicity in a dose of 25 mg/kg of body weight. No observed effect levels (NOEL) for all test substances is a dose 2.5 mg/kg body weight. The "dose-response" was observed in the range of studied doses.

P407 - 0612 Comparative Analysis of Zebrafish and Planarian Model Systems for Developmental Neurotoxicity Screens Using an 87-Compound Library

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There is a clear need to establish and validate new methodologies to more efficiently screen chemicals for potential toxicity, particularly on development. The emergence of alternative animal systems for rapid toxicology screens presents valuable opportunities to evaluate how systems complement each other. In this study, we compared a chemical library of 87 compounds in two integrated non mammalian systems, developing zebrafish and freshwater planarians, by screening for developmental neurotoxic effects. The chemical library was curated by the US National Institute of Environmental Health Sciences (NIEHS) National Toxicology Program (NTP) and contained negative controls, flame retardants, polycyclic aromatic hydrocarbons (PAHs), drugs, industrial chemicals, and pesticides. Tropical 5D wild-type zebrafish were dechorionated and statically exposed to 8 concentrations in embryo medium from 6 to 120 hours post fertilization (hpf). The embryos were examined at 24 and 120 hpf for 22 morphological malformations, and 2 behavioral endpoints. Overall, zebrafish were more sensitive to this chemical library, yielding 86/87 hits, compared to 50/87 hits in planarians. The difference in sensitivity could not be attributed to molecular weight, Log K_{ow}, or the bioconcentration factor. Of the 87 chemicals, 28 had previously been evaluated in mammalian developmental neurotoxicity (DNT), neuro- or developmental toxicity studies. Of the 28, 20 were hits in the planarian, and 27 were hits in zebrafish. Eighteen of the 28 had previously been identified as DNT hits in mammals and were highly associated with activity in zebrafish and planarian behavioral assays in this study. Only hydroxyurea (out of 28) was a false negative in both zebrafish and planarian systems. Many of the other chemicals in the library had *in vitro* data that suggesting they were hazardous for carcinogenicity or other toxicities. The multi-dimensional zebrafish platform identified all these chemicals, demonstrating its ability to rapidly identify hazardous chemicals. We show that the systems' toxicological profiles are complementary to each other, with zebrafish yielding more detailed morphological endpoints and planarians more behavioral endpoints. Our data from this targeted developmental, neurotoxicity and developmental toxicity chemical library revealed that utilizing the higher vertebrate system and the multi-dimensional endpoints are highly sensitive and concordant with mammalian neurotoxicant models.

P408 - 0631 Occupational Exposure to n-Hexane Is Associated with Reduced FSH Levels and Also with Prolonged Menstrual Cycles in Mexican Workers of Reproductive Age

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Former studies in rodents and cell lines have demonstrated ovarian toxicity caused by n-hexane and/or 2,5-hexanedione (2,5HD). In women occupationally exposed to solvents, variables "menstrual cycle period" and "time for getting pregnant" have been longer compared with controls, without identifying a compound responsible for those effects. We studied a group of Mexican women laboring in a shoe factory (n=32). Individual environmental levels for seven compounds, included n-hexane, were measured. Also, urinary 2,5HD and serum FSH and anti-Müllerian hormone (AMH) as potential biomarkers of ovarian toxicity, in addition to a gynecologic-obstetric history were obtained. We performed all tests and questionnaires in a reference group as well (n=32). Mean exposure levels to n-hexane (49.2 ± 39.6 mg/m³) and toluene (30.8 ± 24.5 mg/m³) were the highest observed. There were no significant differences in serum FSH and AMH concentrations between groups (p > 0.05). Exposed group showed prolonged menstrual cycles (p = 0.007) and augmented time for getting pregnant compared with controls (p = 0.007). Also in the exposed group, significant correlations were observed between FSH levels and n-hexane (r = -0.34, p = 0.028) as well as FSH and 2,5HD (r = -0.33, p = 0.029). N-hexane exposure may be responsible for a prolonged menstrual cycle. As judged by the correlations between FSH with n-hexane and 2,5HD this affection could be in the endocrine pathway rather than in the ovary itself. N-hexane could act as endocrine disruptor in women of reproductive age.

P409 - 0648 Exposure to Di(2-Ethylhexyl) Phthalate (DEHP) Affects the Maturation and Quality of Blastocysts

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DEHP is one of the most used plasticizers worldwide because it does not covalently bind to the plastic matrix. Thus, it can leach into food or liquids from containers, representing the most frequent route of exposure. Similarly, DEHP is able to leach into air, water, soil, among others, when DEHP-containing plastics are in the environment. Because numerous systemic effects have been reported, the effects at reproductive level have been of special attention because they have been related to decreased quality of the embryo and low implantation rate at low doses. The objective of this study was to evaluate the stage of blastocyst development (early, late and expanded), and morphology of the internal cell mass (ICM) and the trophoctoderm (TM). Female CD-1 mice were orally exposed to 0, 20, 200 and 2000 µg/kg/day for one month by deposition in the mouth. They were superovulated by administering PMSG (5 IU) ip followed by hCG (5UI) ip at 48 hours later. Then, they were mated with males of proven fertility. The females were euthanized in stage-specific times to obtain blastocysts at 96 h post-mating. The data showed that DEHP significantly increased the percentage of blastocysts at late stages (200 µg/kg/day: 28.8% ± 1.2, and 2000 µg/kg/day: 37.4% ± 4.2, respectively) compared to control. Similarly, 2000 mg/kg/day DEHP significantly increased the percentage of blastocysts having deteriorated morphology of the ICM compared to control. Further, the doses of 200 and 2000 µg/kg/day significantly increased the percentage of blastocysts having deteriorated TM (27.5% ± 1.2 and 39.6% ± 3.5, respectively) compared to control. These data suggest that DEHP at doses of 200 and 2000 µg/kg/day may impair the quality of the blastocyst. More studies are needed to characterize this effect.

P410 - 0650 Effect of Pre-pubertal Exposure to Di(n-butyl) Phthalate (DBP) on Ultrastructural and Immunohistochemical Changes in Peritubular Myoid Cells (PTMC) of Adult Japanese Quail Testes

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Endocrine disrupting chemicals (EDCs) such as the phthalate esters, di(n-butyl) phthalate (DBP) are known to possess anti-androgenic effects and may alter reproductive function in both mammalian and avian wildlife species. Previously, we have established that the DBP effect is anti-androgenic and causing dose- and parameter-specific alterations in seminiferous tubular structure, as well as impairment of Leydig cell steroidogenesis in the avian model. However, little is known about the toxic effects of DBP on the peritubular myoid cells (PTMCs) in the avian species. The present study was designed to assess the dose-response of PTMC in adult Japanese quails that were exposed to DBP during the pre-pubertal period of growth and differentiation, using immunohistochemical (i.e., vimentin intermediate filaments) and ultrastructural methods of investigation (TEM). A total of ninety (90) pre-sexed, 10wk-old male quails were randomly divided into six dosage groups. The control received a corn-oil vehicle only (a dose of 1mL/kg), while the other five experimental groups, received intragastric dosage levels of 1, 10, 50, 200, 400mg DBP/kg/body mass dissolved in corn oil, for a period of 30-days. Immunoreactivity to vimentin was absent in the PTMCs in all the DBP-treated birds. Ultrastructurally, there was a derangement of PTMC structure, especially in birds treated with high dosage levels of DBP (200mg- and 400mg/kg/body). There was a preponderance of lipid droplets and dense bodies in the cytoplasm, and the mitochondria were visibly swollen and/or disorganized. It is concluded that repeated administration of DBP during the pre-pubertal phase of growth, for a period of 30-days, has adversely affected the structure and possible function of the peritubular myoid cells (PTMC) in the corresponding adult male Japanese quails. In these birds, in another study, both the Sertoli and Leydig cells were also adversely affected. It is now known that there is a close paracrine, developmental, functional relationship between the Sertoli cells, Peritubular myoid cells (PTMC) and Leydig cells, in this avian specie.

PS P411 - 0704 Sodium Arsenite Induces Inflammation and Reduces Functionality of Ovaries of Wistar Rats

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Arsenic (As) exposure has been a matter of concern for over a century. Unhealthy concentrations of this metalloid have been found in drinking water in countries like Taiwan, India, Chile, United States, Argentina and Mexico. Chronic exposure to As has been associated to several diseases like diabetes, cardiovascular disease, metabolic and endocrine disruption, cancer, and reproductive disorders in both genders. Reproductive female disorders include: polycystic ovary syndrome (PCOS), morphologic changes in the uterus, hormone alterations, infertility, and oxidative stress. However, the relationship between the alterations in the expression of proteins related to inflammation signaling (IL-1, TNF- α , and COX-2), and the functionality loss of the ovaries due to the morphological damage generated by As ingestion has not been evaluated. The aim of this study is to determine if such relationship exist, and if so, what kind of damage is As inducing to the ovaries. Wistar female rats of 8 weeks of age were exposed to 0, 50 and 100 ppm of NaAsO₂ (n=6) for 28 days. Concentrations of the toxic were mixed in commercial low sugar gelatin (10 mL per rat/day) prepared with purified water every third day to avoid oxidation. Rats were sacrificed on the 29th day and organs of interest dissected. The liver was processed for the determination of As by wet digestion and HG-AAS. Ovaries were processed for morphologic analysis by H&E staining, and by IHQ to evaluate the expression of IL-1, TNF- α , and COX-2. Statistical analysis was carried out in STATA 11.0 software (StataCorp LLC 2015, USA), a significant difference was considered when $p < 0.05$. Morphologic analysis showed an increase in atretic follicles, hemorrhage, fibrosis, and cellular damage in a dose-dependent manner. Also, there was a decrease in the number of secondary and antral follicles in the animals of the exposed groups when compared to control. Additionally, an important damage in the cilium of the fallopian tube was observed in rats exposed to 100 ppm of As. Expression of IL-1 and TNF- α was significantly higher in the exposed groups than the control group, and was present mainly in the endothelium, overlapping with the regions of damage found in the morphologic analysis. COX-2 had no significant differences among the groups. Our results suggest that As ingestion produces an important damage to the ovaries, inducing an immune response for tissue repair that can lead to chronic inflammation, and therefore increasing the risk of developing reproductive disorders that can result in infertility in the female population.

PS P412 - 0719 Maternal PCB Exposure and Disinhibitory Behaviors among Adolescent Offspring

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Polychlorinated biphenyls (PCBs) are halogenated aromatics hydrocarbons and lipophilic toxicants persistent in the environment. Due to the continued persistence and ubiquity of these compounds, risk of exposure to those living in highly industrialized countries is still high. Exposures to PCBs can accumulate in human tissues, and research shows they can cross the placenta after maternal exposures and resulting in fetal exposure. PCB exposures may alter reactions to pleasure centers in the brain and potentially influence subsequent inhibitory behaviors. Therefore, disruptions in dopamine synthesis or uptake could potentially enhance desires for pleasurable experiences, which can be induced by PCB exposures. However, the potential toxic effects of PCB exposures in the general population on substance abuse outcomes in adolescent offspring of exposed mothers is not well known. We used The Child Health and Development Studies (CHDS), a prospective cohort study of pregnant mothers recruited in the Oakland and East Bay areas of California between 1959 and 1966 (n=554) and their offspring followed from infancy through adulthood. We investigated associations between *in utero* exposure to PCB congeners (66, 74, 99, 118, 138, 153, 170, 180, 187, 194 and 203), as measured in maternal serum samples taken soon after birth of the child, and later disinhibitory behaviors in adolescents (15-18 years), specifically alcohol consumption and smoking. Outcome measures were type, amount, and frequency of alcohol use and smoking status. We used logistic regression models to evaluate associations between disinhibitory behaviors with maternal lipid-adjusted PCB levels categorized into quartiles, and exposures below the median serving as the reference measure. All statistical analyses were conducted with a 5% level of significance using SAS 9.4 statistical software. The adjusted odds ratio (aOR) for being a current smoker, but not ever smoking or drinking >2 alcoholic beverages

per week, was higher in subjects with the third quartile of maternal PCB 66 levels compared to those below the median (aOR = 1.93; 95% CI 1.05, 3.55). Odds of currently smoking, ever smoking one cigarette per day, and drinking >2 alcoholic beverages per week were higher for adolescents with the third and fourth quartile of PCB 118 levels, though not statistically significant. The study results suggest that prenatal exposure to PCBs is unrelated to disinhibitory alcohol use and smoking behaviors among offspring adolescents. Future replicate analyses with larger sample sizes and different alcohol and substance use measures should be conducted.

PS P413 - 0732 Withdrawn

PS P414 - 0829 Enhanced Histone H3K9 Tri-methylation Suppresses Steroidogenesis in Rat Testis Chronically Exposed to Arsenic

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Arsenic poses a profound health risk including male reproductive dysfunction upon prolonged exposure. Histone methylation is an important epigenetic driver; however, its role in arsenic-induced steroidogenic pathogenesis remains obscure. In current study, we investigated the effect of histone H3K9 tri-methylation (H3K9me3) on expression pattern of steroidogenic genes in rat testis after long-term arsenic exposure. Our results revealed that arsenic exposure down-regulated the mRNA expressions of all studied steroidogenic genes (Lhr, Star, P450scc, Hsd3b, Cyp17a1, Hsd17b and Arom). Moreover, arsenic significantly increased the H3K9me3 level in rat testis. The plausible explanation of increased H3K9me3 was attributable to the up-regulation of histone H3K9me3 methyltransferase, Suv39h1 and down-regulation of demethylase, Jmjd2a. Since H3K9me3 activation leads to gene repression, we further investigated whether the down-regulation of steroidogenic genes was ascribed to the increased H3K9me3 level. To elucidate this, we determined the H3K9me3 levels in steroidogenic gene promoters, which also showed significant increase of H3K9me3 in the investigated regions after arsenic exposure. In conclusion, arsenic exposure suppressed the steroidogenic gene expression by activating H3K9me3 status, which contributed to steroidogenic inhibition in rat testis.

PS P415 - 0179 Involvement of PI3K-Akt Signaling in Antiapoptotic Effect Evoked by 1,2-Naphthoquinone

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The redox-mediated chemical modification occurs via oxidative reaction between electrophile and cysteine thiol in the presence of an electron acceptor. 1,2-Naphthoquinone (1,2-NQ) is one of the environmental pollutants and an electrophile in cigarette ingredients/smoke or metabolite of naphthalene. It has been reported that many proteins including protein-tyrosine phosphatase 1B (PTP1B), Keap1, and IKK β were modified by 1,2-NQ. These proteins play an important role in the intracellular signaling pathway. In addition, it is widely recognized that long exposure to or high concentration of 1,2-NQ results in cytotoxic effect. Whereas, low concentration of 1,2-NQ specifically stimulates Akt signaling involving cell proliferation and antiapoptosis possibly via protein oxidation. In this study, we addressed to clarify the effects of 1,2-NQ on PI3K-Akt pathway involved in cell proliferation and antiapoptosis. We performed western blotting to detect the levels of phosphorylated form of Akt using specific antibodies. Phosphorylation of Akt was observed by treatment with 1,2-NQ in a concentration-dependent manner in A549 cells. This activation was sensitive to both wortmannin (a PI3K inhibitor) and OSU-03012 (a PDK1 inhibitor). To assess cell viability, we then conducted the cytotoxicity assay with WST-8 and investigated nuclear morphology using chromatin dye Hoechst33258. Consequently, we found that 1,2-NQ attenuated serum deprivation-induced cell death. Since Akt signaling contributes to antiapoptosis mediated by phosphorylation of Bcl-2 associated death promoter (BAD), a proapoptotic member of the Bcl-2 gene family, we next checked if 1,2-NQ stimulated the phosphorylation of BAD. Western blot analysis revealed that 1,2-NQ treatment significantly enhanced the levels of phosphorylation of BAD. Moreover, the antiapoptotic effect by

exposure to 1,2-NQ was eliminated by pretreatment with PI3K-Akt signaling inhibitors. Hence, these findings suggested that treatment with appropriate amount of 1,2-NQ leads to antiapoptotic action through PI3K-Akt signaling.

PS P416 - 0233 Chemical Characterization and Toxicological Effects of Emissions Generated from New Tobacco- and Smoking-Related Products: Comparison with Conventional Cigarette Smoke

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Cigarette smoke exposure is responsible for almost 30% of cancer deaths and the cause of nearly 90% of lung cancer. Smoking cessation is, at present, the only effective way to slow down the progression of cancer. Electronic cigarettes (e-cig) and more recently heated tobacco products (HTP) provide an alternative for smokers, as they are generally perceived to be less harmful than conventional cigarettes. These new devices can quickly gain popularity, even before there is sufficient scientific evidence to determine their effects on the user. This work was performed (i) to characterize the chemical composition (carbonyl compounds and polycyclic aromatic hydrocarbons) of these tobacco-related product emissions, (ii) to investigate their potential toxicity in human bronchial epithelial cells (BEAS-2B) cultured at the air-liquid interface, and (iii) to compare their emissions and their respective toxicities to those of cigarette smoke. Aerosols were generated by a smoking machine from HTP, different models of e-cig (low or high power), or conventional cigarette (3R4F). The numbers and concentrations of carbonyl compounds and PAHs emitted by HTP and e-cig are lower than with cigarette smoke. At the cellular level, HTP exposures induce a cytotoxicity higher than the different e-cig models tested but lower than conventional cigarettes. Significant increases in IL-6, IL-8, IFN γ , and GM-CSF secretion are observed in BEAS-2B cells after HTP or 3R4F exposures, while only IL-6 is increased for e-cig. Overall, these data evidence lower emissions of harmful chemicals and lesser *in vitro* toxicity of HTP compared with cigarette smoke. By contrast, HTP generate higher levels of hazardous compounds and result in greater cytotoxicity than e-cig. Moreover, effects observed with e-cig are more marked when they are used at high rather than low power. These first conclusions shall be further comforted by additional experiments conducted in animal models after long-term exposures. *The authors declare that they have no conflict of interest with tobacco, HTP, or e-cig industries. This work benefited from grants from the INCa (Contract n°INCa_11505).*

PS P417 - 0240 TGF-Beta Induced CTGF Expression and Epithelial-Mesenchymal Transition in Human Lung Epithelial Cells through ERK, ADAM17, RSK1, and C/EBPbeta Pathways

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Idiopathic pulmonary fibrosis (IPF) is a type of an interstitial lung disease prevalent in elder smokers. In addition, occupational risk factors including metal and wood dust have been proposed to be damaged lung epithelial cells and activation, and associated with pulmonary toxicity and fibrosis. Several reports indicated that transforming growth factor- β (TGF- β), connective tissue growth factor (CTGF), and epithelial-mesenchymal transition (EMT) formation play important roles in IPF. In the present study, we investigated whether TGF- β -induced expression of CTGF and EMT formation was regulated by the extracellular signal-regulated kinase (ERK)/a disintegrin and metalloproteinase 17 (ADAM17)/ribosomal S6 kinases 1 (RSK1)/CCAAT/enhancer-binding protein β (C/EBP β) signaling pathway in human lung epithelial cells (A549). Our results revealed that TGF- β -induced CTGF expression was weakened by ADAM17 small interfering RNA (ADAM17 siRNA), TAPI-0 (an ADAM17 inhibitor), U0126 (an ERK inhibitor), RSK1 siRNA, and C/EBP β siRNA. TGF- β -induced ERK phosphorylation as well as ADAM17 phosphorylation was attenuated by U0126. TGF- β -induced increase in RSK1 phosphorylation was inhibited by TAPI-0 and U0126. TGF- β -induced C/EBP β phosphorylation was weakened by U0126, ADAM17 siRNA, and RSK1 siRNA. In addition, TGF- β increased the recruitment of C/EBP β to the CTGF promoter. Furthermore, TGF- β enhanced fibronectin (FN) and CTGF mRNA levels and reduced E-cadherin mRNA levels. Moreover, TGF- β -stimulated FN protein expression was reduced by ADAM17 siRNA and CTGF siRNA. In conclusion, the results suggested that TGF- β activates the ERK/

ADAM17/RSK1/C/EBP β signaling pathway, which induces CTGF expression and ultimately mediates the EMT process in human lung epithelial cells. Therefore, suppressing the signaling pathway of TGF- β may have developmental potential for the treatment of IPF.

PS P418 - 0277 Sex Differences in Respiratory Toxicity Induced by Acute Ozone Inhalation and Sensitive Indicators in Rats

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Epidemiological studies have found that there are gender differences in the incidence and prognosis of respiratory diseases caused by pollution, such as, women are more susceptible to ozone (O₃) induced injuries. However, the gender differences in response to O₃ exposure and their sensitive indicators for respiratory toxicity have not been extensively analyzed. In this study, adult male and female rats were exposed to different concentrations of O₃ (0 ppm, 0.12 ppm, 0.5 ppm, 1.0 ppm, 2.0 ppm, and 4.0 ppm) for 6 h. Then, respiratory function was measured, samples from bronchoalveolar lavage fluid (BALF), blood, urine, and lung tissue were collected, and indexes associated with inflammatory reaction, oxidative stress, pathological changes, and DNA oxidative damage were detected and compared between both gender rats. Our results indicated acute ozone exposure could induce apparent respiratory toxicities, such as increase of respiratory frequency, injury of lung epithelial and permeability, and pathological changes of lung tissue. It also led to systemic inflammatory reaction and oxidative stress. However, gender differences in response to acute ozone exposure were found in some indicators, and the sensitivity indexes were analyzed. Results showed that levels of total protein (TP) and 8-OHdG in BALF, TNF- α and SOD activity in lung tissue, and IL-1 β in blood could be used as a sensitive index for male rats, while for female rats, levels of IL-6 and 8-OHdG in blood were more sensitive than that in male rats after acute ozone exposure.

PS P419 - 0354 Effects of Cigarette Smoking on the Health Status of Workers in Suburban Area of Tokyo

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The effects of cigarette smoking on health status were investigated in 129 males and 136 females working in the suburban area of Tokyo. The subjects were divided into two generation groups, 20 to 39 years old and 40 to 59 years old. Age, body mass index, blood pressures, incidence of hypertensive or diabetic, life-styles (sleeping hours a day, exercise, eating snacks, taking breakfast or drinking habit), standard serum clinical chemistry, standard hematology and white blood cell differentials were compared between smokers (more than 10 cigarettes a day) and non-smokers (never smoking) in each males or females of the generation groups. Among the parameters on health status, most marked in smokers were higher white blood cell (especially in neutrocyte) count and higher mean corpuscular volume in males and females of 40-59 years old group. Also, lower HDL cholesterol level, lower HDL cholesterol/total cholesterol and higher atherosclerotic index were noted in male smokers of 40-59 years old group. Smoking habit may have inflammatory effect, probably in respiratory tract, effect on erythrocyte and serum homeostasis of lipids, especially in senior generation or by chronic smoking.

PS P420 - 0560 Adverse Effects of Real-Time Gasoline Engine Emissions Exposure at Air-Liquid Interface

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Road traffic is a major cause of air pollution-related adverse health effects despite technological improvements and a considerable decrease in type approval limits. Among the problems being faced are the high intensity of traffic within and close to populated areas, a discrepancy between type approval limits and actual emissions in real operation, and limited ability of traditional emissions metrics to reflect relative hazards to human health. *In vitro* toxicity tests of emissions are usually done by the treatment of model cell lines with

extractable organic matter (EOM) under submerged conditions. The aim of our study is to focus on more realistic exposure conditions such as air-liquid interface (ALI) and physical interaction of solid particles and gaseous pollutants with cells. This study reports on an in-house developed air-liquid interface exposure system for direct exposure of lung cell cultures to conditioned exhaust fumes. This approach involves a proportional sampling of exhaust using a partial flow dilution tunnel, conditioning of the sample to stable temperature, humidity and CO₂ content, and its uniform distribution into multiple test cultures for parallel exposure. Computational fluid dynamics models (CFD) were used to arrive at desired velocity profile allowing deposition by diffusion. For this study, we used human lung cell line BEAS-2B grown at the air-liquid interface as a reliable cell model. To investigate the adverse effects, a typical direct injection spark ignition petrol engine was mounted on an engine dynamometer and operated according to a dynamic cycle consisting of speeds and loads matching to those experiences by the engine over the World Harmonized Light Duty Vehicle Test Cycle (WLTC). Additionally, steady-state and high-dynamics tests were run. The results of cell integrity measured as transepithelial electrical resistance (TEER) showed 5-10% decrease for both exposed and control samples. The cytotoxicity measured as lactate dehydrogenase (LDH) release into the apical and basal side of inserts was significantly higher after exposure to dynamic cycles (fold change >2) than steady-state operating conditions (fold change <2). In addition, we maintained the BEAS-2B cells in our exposure device with 0.2 l/min of filtered humidified air supplemented with approximately 5% of CO₂ for >10h with no visible changes. In conclusion, our exposure device is able to maintain cell culture under ALI conditions and it is possible to use it for a wide variety of exposure schemes. *This work was supported by the grant of the Czech Science Foundation (18-04719S).*

PS P421 - 0623 Sex Differences in the Respiratory Response to Multi-Walled Carbon Nanotubes

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Multi-walled carbon nanotubes (MWCNTs) are a versatile engineered nanomaterial of which the production and integration into consumer products is rapidly expanding. Unfortunately, the risk of occupational and environmental exposures continues to increase, yet the long-term consequences and potentially sensitive populations remain undefined. Notably, the respiratory response to MWCNTs closely resembles that seen in asthma, including airway hyper-responsiveness (AHR), Th2-type cytokine production, and eosinophil recruitment to the airways. Based on the similarities in disease pathology and the evidence that asthma occurs most commonly in women of reproductive age, we hypothesized that female animals would be more sensitive to MWCNTs and produce a greater immune response than males. Male and female C57BL/6 mice were exposed to a single dose of MWCNTs (50mg/mouse) via oropharyngeal aspiration and harvested 1- or 7-days later; or exposed once per week for 4 weeks and harvested 8-weeks after the last exposure. Lung lavage fluid was analyzed for cytokine expression and immune cell recruitment; histology was performed on the repeated exposure time point. Comparable to the results seen in asthma, female animals mounted a greater Th2-type immune response and subsequent lung pathology. At all time points airway eosinophil recruitment and IL-33 expression, key mediators in both allergic asthma and MWCNT-induced inflammation, were significantly elevated in females compared to males. Lung pathology in the repeated, long-term exposure study reflected these differences in the acute response: particle accumulation, alveolar inflammation, and epithelial cell hyperplasia were all more severe in female animals than in males. The results of the long-term exposure indicate that differences observed in the acute immune response are not inconsequential nor temporary, but a reflection of an increased sensitivity to MWCNTs in female animals. The next step in this project is to investigate the influence of sex-based biological variables on immune cell recruitment. A likely mechanism is the modification of alveolar macrophage phenotype through steroid hormone signaling. *Work supported by R01 ES023209.*

PS P422 - 0667 The Role of Thrombospondin Type 1 Repeat (TSR) Domain in Multiwalled Carbon Nanotube-Induced Endothelial Dysfunction and Inflammation

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3NIOSH, Morgantown, WV, The United States of America Thrombospondin-1 (TSP-1) is a matrix glycoprotein involved in numerous biological processes, including angiogenesis and inflammation. TSP-1 has been shown to activate immune responses via the activation of the multifunctional cytokine TGFβ1, matrix-metalloproteinases and cell surface receptors CD36 and CD47. The activating motif of TSP-1 is found in the thrombospondin type 1 repeat (TSR) domain of TSP-1. We previously demonstrated that multi-walled carbon nanotube (MWCNT) exposure led to endothelial dysfunction and impaired vasodilation in a CD36 and MMP-9-dependent manner. Additionally, while we observed an ~50% increase in circulating TSP-1 after MWCNT treatment, a 59-mer fragment of TSP-1 containing the TSR domain was elevated ~1000% over control. The mechanism(s) by which lung exposure to MWCNTs leads to CD36 and MMP9-mediated inflammatory activation is unknown. In this study we explore TSP-1/TSR interactions as a possible link between MWCNT exposure and these findings. We hypothesize that the MWCNT exposure induces TSP-1/TSR-mediated immune activation leading to altered endothelial function. To test this hypothesis, we generated a 59 amino acid TSR peptide corresponding to the binding domain of CD36. TSR-peptide was used in *in vitro* studies in mouse brain endothelial cells (MBEC) to assess changes in cell proliferation, migration, angiogenesis and inflammation. Cell proliferation and migration was assessed using the electric cell-substrate impedance sensing (ECIS) system. Angiogenesis was assessed using the Matrigel endothelial cell tube formation assay. Quantitative polymerase chain reaction (qPCR) was used to evaluate changes in angiogenic and inflammatory gene expression. TSR treatment did not significantly alter inflammatory gene expression in MBEC. Treatment of MBEC with TSR peptide led to a dose-dependent decrease in cell migration in ECIS assays. Cell proliferation was unchanged between groups. Studies to evaluate endothelial cell tube formation and angiogenic gene expression changes are ongoing. These preliminary findings suggest that TSP-1 may play a role in MWCNT-mediated endothelial dysfunction via alterations in endothelial integrity.

PS P423 - 0686 Impacts of Inhaled Ozone on Uterine and Placental Function and Maternal Cardiovascular Health

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Exposure to air pollutants such as ozone (O₃) is associated with adverse pregnancy outcomes, including higher incidence of preeclampsia and peripartum cardiomyopathy in epidemiological studies; such effects have been demonstrated in controlled exposure studies in rodents only to a limited extent. Data from our laboratory suggest that the vascular endothelium throughout the body is a target of inflammatory "spillover" from lungs following inhalation exposures. During gestation, proper angiogenesis and vascular supply to the placenta is essential to both maternal and fetal health. Impacts of pollution-induced circulating factors on uterine artery function and placental development are not well understood. The present study examined the mechanisms of how inhaled pollutants may contribute to adverse cardiovascular conditions, specifically, endothelial and smooth muscle reactivity. We began this project by evaluating how serum from O₃-exposed pregnant rats, at gestational days 19 or 20, enhanced uterine artery contractility compared to serum from filtered air exposures. Furthermore, O₃-exposed rats, at both 0.3 and 1.0 ppm, also exhibited ~15-20% net reduction in uterine artery vasodilation (to acetylcholine) as compared to control rats. We further analyzed placental gene expression by quantitative polymerase chain reaction (qPCR). Our genes of interest included: chemokine ligand 1 (CXCL-1), chemokine ligand 2 (CCL2), collagen type 1 alpha 1 chain (COL1A1), transforming growth factor beta 1 (TGFβ1) and frizzled class receptor 1 (FZD1). Altered expression of these genes indicates a disruption in the normal growth and development even in late stage pregnancy. We also evaluated serum concentrations of soluble fms-like tyrosine kinase-1 (sFLT-1), which is a key driver of hypertensive crises in pregnancy. O₃ exposure induced

significant sFLT-1 increases compared to controls. These data suggest that the placenta is an indirect target of inhaled O_3 and that systemic maternal cardiovascular abnormalities may be induced by O_3 at a specific window.

PS P424 - 0805 Breathing beyond the Barrier: The Dynamics of Oxidative Stress and Pro-inflammatory Signaling within a Novel Organotypic Airway Model

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Oxidative stress and release of pro-inflammatory mediators are well established signaling events following inhaled chemical exposure; however, our understanding of the distinct cellular and molecular pathways involved in inhalation toxicity remain limited due to lack of physiologically-relevant studies on diverse cell types beyond the epithelial barrier. Utilizing an *in vitro* organotypic model that recapitulates the "trans-epithelial" nature of analogous exposures *in vivo*, we investigated the effect of the model toxicant (diesel exhaust particulates (DEP)) on human bronchial epithelial cell (HBEC) and lung fibroblast (HLF) oxidative stress and pro-inflammatory signaling. We hypothesized that trans-epithelial exposure to DEP drives oxidative stress/pro-inflammatory response through an imbalance of oxidant/antioxidant gene expression and alternative cellular signaling pathway activation within the airway. Temporal gene expression analysis demonstrated peak HMOX-1 response at 6 hours in HBEC and HLF, while IL-8 and IL-6 peaked at 4 hours in HBEC and 10 hours in HLF, by which time HMOX-1 had declined substantially. Genes involved in glutathione homeostasis and H_2O_2 signaling (NQO1, PTGS2, and GCLM1) were alternatively regulated in response to DEP exposure, and live-cell imaging with a dual-fluorescent biosensor for glutathione oxidation and intracellular H_2O_2 accumulation (roGFP2/HyPER red) showed that DEP treatment increased overall oxidation in HBEC and HLF. Pretreatment with antioxidants (N-acetylcysteine, catalase, and ascorbic acid) attenuated both oxidative stress-responsive (HMOX-1 and NQO1) and pro-inflammatory (IL-8) gene expression and protein signaling. Upregulation of antioxidant response/pro-inflammatory genes in our studies suggested a role for key stress-response pathways (MAPK, NF- κ B, and NRF2) in DEP-dependent signaling. The kinetics of MAPK (p38, JNK, and ERK), NF- κ B and NRF2 pathway activation by DEP exposure were determined via time-course (2-24 hours) immunoblotting and densitometry analysis; confirming DEP-mediated and time-dependent phosphorylation of key cellular targets. Notably, increased DEP-induced neutrophil chemotaxis relative to vehicle validated the impact of trans-epithelial DEP exposure on overall airway microenvironment inflammation. This study is the first to characterize the dynamics of oxidative stress/pro-inflammatory signaling within the airway microenvironment following trans-epithelial chemical exposure and provides novel insight for the development of therapeutic and/or preventative interventions to reduce adverse effects of inhaled chemical exposure.

PS P425 - 0813 Cellular and Epigenetic Effects of Cannabinoid Exposure on Bronchial Epithelial Cells

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Cannabis sativa, commonly known as Marijuana, is one of the most commonly used illicit drugs in the USA, with a reported estimate of 6% of the population aged 26 and older having used marijuana in 2015, for a total of 22.2 million users overall. Therefore, the question of the impact of its use on human health becomes critical. Since inhalation is a major route of exposure, the effect of cannabinoids on bronchial epithelial cells also becomes relevant. The current study investigated the cellular and epigenetic effects of chronic cannabinoid exposure as well as the underlying mechanism of cannabinoid toxicity in bronchial epithelial cells *in vitro*. A time dose gradient was performed using a MTT (3-(4,5-dimethylthiazolyl-2)-2,5 diphenyltetrazolium bromide) assay with concentrations between 60 ng/ml to 2400 ng/ml to determine an effective dose. Delta 9 tetrahydrocannabinol 1800 ng/ml was selected as it significantly decreased cell viability. Bronchial epithelial cells (BEAS2B) were chronically exposed to Delta 9 tetrahydrocannabinol (THC) 1800 ng/ml and the control, consecutively for 9 days. Exposure to Delta 9 tetrahydrocannabinol significantly decreased cellular reduced form of glutathione (GSH), a biomarker of oxidative stress, compared to the control. The expression of gene encoding for DNA methyl transferases and inflammation were significantly decreased as a result of exposure to Delta 9 tetrahydrocannabinol (THC). The expression of gene

encoding histone methyltransferases and allergy did not change. The present results suggest that Delta 9 tetrahydrocannabinol (THC) exposure induces epigenetic changes, alters oxidative stress status and increases inflammatory response in bronchial epithelial cells.

PS P426 - 0871 Subchronic Inhalation Toxicity Study of Mono Methyl Formamide in F344 Rats

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Mono methyl formamide (MMF) is colorless, clear liquid and used as a chemical intermediate including synthesis of pesticides. This substance has the possibility of exposure to workers in workplace by inhalation route. Therefore, we performed this study to evaluate the toxicity of MMF for occupational health safety. Four groups of Fischer 344 (F344) rats were exposed to MMF for 13 weeks (6 hours a day, 5 days per week) at doses of 0 ppm, 10 ppm, 30 ppm, 90 ppm with the whole-body inhalation chamber system with liquid vapor generator. Each dose group consist of 10 animals each sex. Exposure concentrations of inhalation chamber were measured by gas chromatography (GC) over 3 times a day during test period. Clinical signs, mean body weight changes, food consumption, hematology, blood biochemistry and histopathological findings were observed. Soft stool was observed in both male and female rats during experiment period. Significant decrease of mean body weights and food consumptions were observed in the high dose group in both sexes. In the gross findings, the decrease of uterus size was observed in the female rats in the high dose. Absolute weight of liver was increased in the middle and high dose groups compare to the control group. In histopathological findings, hepatocellular hypertrophy of liver at high dose group in male rats, and mucous hyperplasia of nasal cavity at high dose group in both sexes were observed. Based on these results, 30 ppm might be suggested as no observed adverse effect level (NOAEL).

PS P427 - 0880 In Vitro Testing for Respiratory Sensitizers Using a Genomic Biomarker Signature and Machine Learning

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Exposure to chemicals with sensitizing properties may result in priming of allergic hypersensitivity reactions in either the skin or the respiratory tract. In recent years, many efforts have been made to develop predictive assays for prospective identification of compounds capable of inducing such reactions. To date, several assays for assessment of skin sensitizers have been proposed, but the need for an assay that specifically identifies chemical respiratory sensitizers remains unfulfilled. The absence of predictive methods for chemical respiratory sensitizers is partly described by a lack of understanding of the exact immunobiological mechanisms giving rise to the toxicological outcome. However, a key mechanistic difference is represented by a preference of skin- and respiratory sensitizers to induce qualitatively different T-cell populations, which in turn is assumed to be dependent on dendritic cells (DCs). Subsequently, a genomics-based testing platform used in combination with an *in vitro* cell system of DCs has the potential to generate readouts with high informational content, enabling for discovery of mechanistic information and identification of novel and highly predictive biomarkers. Therefore, by looking for unique mechanistic differences in dendritic cells, it should be possible to not only identify potential respiratory sensitizers, but also distinguish them from skin sensitizers. Here, using a dendritic cell line, multiple genomic biomarkers and state-of-the-art machine learning, we now present the first approach for identification of respiratory sensitizers. The genomic biomarker signature was identified using a data-driven approach from genome-wide transcriptomic profiling following exposure of a DC model to a panel of reference chemicals, including 10 known respiratory sensitizers and 20 known non-respiratory sensitizers (including some known skin sensitizers). Performance data on external test sets has thus far demonstrated an accuracy of 90%, with a sensitivity of 100%. Most importantly, except for a single test substance, all skin sensitizers tested thus far have been correctly predicted as non-respiratory sensitizers. In conclusion, we here present the first *in vitro* approach capable of identifying and distinguishing respiratory sensitizers from both true non-sensitizers and known skin sensitizers. This poster will not only present data on how this strategy was developed but will also demonstrate its potential utility for addressing public and personal health issues that have not been previously addressed due to lack of an adequate testing approach for this toxicological endpoint.

P428 - 0131 Analysis on the Trend of the Preservative System in Commercial Infant Cosmetics and Anti-sensitive Cosmetics

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Contact allergy caused by ingredients in cosmetic products is a well-known problem. Approximately 6% of the general population has a cosmetic-related contact allergy, mainly caused by preservatives. Infants, as well as adults with damaged skin, are more vulnerable. Therefore, the safety of infant and anti-sensitive cosmetics are more important. Effectively building the preservative system of cosmetics is a difficult problem to be solved in the development of cosmetics. This review focuses on compositional analysis of the preservative system in 29 commercial infant cosmetics, and 13 antisensitive cosmetics were detected by High Performance Liquid Chromatography (HPLC) and gas chromatography (GC). It provides reference for the development of infant and antisensitive cosmetics. Results show that safer even preservatives-free system applied in these products becomes a trend. Phenoxyethanol, with a content of 0.01%–0.99%, was found in 53% of infants and antisensitive cosmetics; CaprylylGlycol, with a content of 0.1%–0.6%, was used in 39% of these products; 15% of products are using polyalcohol and removed the traditional preservatives. Most of cleaning products are using traditional preservatives while skin care products are more inclined to use traditional preservatives combined with polyalcohol. Nipagin esters derivatives were still found in a few products, and no formaldehyde releasers were found. Moreover, each brand has its own special preservative system. (For example, HLS prefer to use the combination of sodium benzoate and phenoxyethanol; LFQ tend to use polyalcohol; SB is more inclined to use the combination of phenoxyethanol, sodium benzoate, and benzyl alcohol; and HSXX is apt to use the combination of phenoxyethanol and P- anisic acid).

P429 - 0163 Suppression of TARC Expression by Impressic Acid through Blockade of ERK1/2/STAT1 and Improvement of p62/Nrf2/HO-1 in Human Keratinocyte

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Acanthopanax koreanum has traditionally been used to rheumatism, hepatitis, diabetes, and inflammatory properties. However, the suppressive effect of impressic acid (IPA), a lupane-type triterpenoid isolated from *Acanthopanax koreanum* in atopic dermatitis-like skin lesions is not fully understood. The present study investigated the inhibitory mechanisms of an IPA on TNF α /IFN γ stimulated thymus and activation-regulated chemokine (TARC) expression in human keratinocytes. IPA strongly decreased the TNF α /IFN γ -increased TARC mRNA expression in a concentration-dependent manner. IPA inhibited TNF α /IFN γ -induced NF- κ B activation through reduction of ERK1/2 pathway. Importantly, IPA increased expression of HO-1, NQO1, and GCLC via enhancing p62/Nrf2 and inhibited level of Keap1 in a time- and concentration-dependent manner. These results suggest that IPA decreases the TARC expression by suppressing the ERK1/2/NF κ B activation and enhancing the p62/Nrf2-mediated antioxidative enzymes level. Consequently, IPA may potentially a useful chemotherapeutic agent that may contribute to improvement to the atopic dermatitis-like skin lesions.

P430 - 0314 Aggravation of Acute Dermatitis Symptoms by Cosmetic Colorants Mediated through the Production of TSLP in Skin

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Safety issues for cosmetic ingredients are major concerns for consumers since cosmetics are repeatedly used on a daily basis. Especially, cosmetic colorants are widely used in various types of products and reported to induce contact dermatitis or allergy. However, it has not been fully elucidated whether cosmetic colorants would cause skin diseases. Thymic stromal lymphopoietin

(TSLP) plays a critical role in the development of atopic dermatitis by switching immune balance towards Th2 immunity. Therefore, we investigated whether cosmetic colorants affected the level of TSLP production in skin, thereby inducing atopic dermatitis-like symptoms. Lithol Rubine B (LR-B, also known as Pigment Red 57) and its calcium salt (LR-BCA) are widely used cosmetic colorants. LR-B and LR-BCA treatment potentiated TSLP production in phorbol-12-myristate-13-acetate-stimulated keratinocytes. Topical application of LR-B or LR-BCA on mouse ear potentiated TSLP production induced by a TSLP inducer (MC903). Topical application of LR-B or LR-BCA aggravated ear edema and histopathological changes in MC903-treated ear skin. In addition, serum levels of IgE and histamine were potentiated by topical application of LR-B or LR-BCA. The expression of Th2 cytokines such as IL-4 and -5 in ear skin was increased by LR-B or LR-BCA. The results show that LR-B and LR-BCA induce TSLP expression in skin, thereby skewing to Th2 immune responses and aggravating atopic dermatitis-like symptoms. The results suggest that certain cosmetic colorants may exacerbate dermatitis symptoms by upregulating TSLP production.

P431 - 0688 Molecular Pathogenesis Underlying Exposure to Cutaneous Warfare Vesicants, Arsenicals

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Arsenicals-based chemical warfare agents such as lewisite cause severe cutaneous injury. We show that both murine and porcine skin respond to these chemicals and lesions similar to those occur in humans are recapitulated in these models. We characterized dose and time responses in skin pathogenesis. Early responses at 6h after lewisite exposure include severe inflammation and pain. Inflammatory mediators IL-1 β , IL-6 and PGE2 besides other cytokines/chemokines were highly elevated. These pro-inflammatory signals are generated by the continuous leukocytes infiltration. The first wave of infiltrated cells were macrophage. Later, neutrophil infiltration also contribute to the injury. These responses are regulated via impaired expression and binding of RNA-binding proteins Roquin and Reganase-1. These proteins regulates degradation of acute phase mRNA both at endoplasmic reticulum and stress granules (SGs). SGs are formed initially as a protective response to chemical injury, later are disassembled and the expression of Roquin and Reganase-1 is diminished. By 24h, we observe severe injury (micro-blistering in mouse and fluid filled blisters in porcine skin). The early tissue damage is progressed by apoptosis in skin keratinocytes followed by massive tissue necrosis, which is orchestrated by the activation of RIP kinases in skin cells. Activation of RIPK1 followed by phosphorylation-dependent activation of RIPK3 and MLKL characterize the injury. The pain responses are regulated by the activation of ion channels particularly, TRPV1 and impaired cannabinoid signaling due to alterations in the expression of cannabinoid receptors CB1 and CB2 as well as the reduced production of their ligands, anandamide and 2AG. Mechanistically, these responses are regulated by the activation of unfolded protein response (UPR) signaling, particularly involving ATF4-eIF2 α axis. Arsenicals-induced ROS was found to be the mediator of UPR activation. Chemical chaperones such as 4-phenylbutyric acid or inhibitor of eIF2 α phosphorylation ISRIB were effective in diminishing inflammation, tissue disruption and pain. RNA-Seq and CHIP-Seq data confirm a role of ATF4 in the pathogenesis of these lesions. We identified ATF4 regulated Klf7, Ppp1r15b, Trim39 and Peli1, which play key role in the tissue's immune regulation. In summary, we have developed murine and porcine models of arsenicals vesicant action, identified mechanisms by which these lesions are developed and found overarching master regulator signaling that underlies pathogenesis of these lesions. We also show efficacy of mechanism-based antidotes, which partially or fully attenuate arsenicals-mediated damage.

P432 - 0791 Mannosylerythritol Lipids Ameliorate UVA-Induced Aquaporin-3 Downregulation by Suppressing c-Jun N-Terminal Kinase Phosphorylation in Cultured Human Keratinocytes

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Mannosylerythritol lipids (MELs) are glycolipids and have several pharmacological efficacies. MELs also show skin-moisturizing efficacy through a yet-unknown underlying mechanism. Aquaporin-3 (AQP3) is a membrane protein that contributes to the water homeostasis of the epidermis, and decreased AQP3 expression following ultraviolet (UV)-irradiation of the skin is associ-

ated with reduced skin moisture. No previous study has examined whether the skin-moisturizing effect of MELs might act through the modulation of AQP3 expression. Here, we report for the first time that MELs ameliorate the UVA-induced downregulation of AQP3 in cultured human epidermal keratinocytes (HaCaT keratinocytes). Our results revealed that UVA irradiation decreases AQP3 expression at the protein and messenger RNA (mRNA) levels, but that MEL treatment significantly ameliorated these effects. Our mitogen-activated protein kinase inhibitor analysis revealed that phosphorylation of c-Jun N-terminal kinase (JNK), but not extracellular signal-regulated kinase or p38, mediates UVA-induced AQP3 downregulation, and that MEL treatment significantly suppressed the UVA-induced phosphorylation of JNK. To explore a possible mechanism, we tested whether MELs could regulate the expression of peroxidase proliferator-activated receptor gamma (PPAR- γ), which acts as a potent transcription factor for AQP3 expression. Interestingly, UVA irradiation significantly inhibited the mRNA expression of PPAR- γ in HaCaT keratinocytes, whereas a JNK inhibitor and MELs significantly rescued this effect. Taken together, these findings suggest that MELs ameliorate UVA-induced AQP3 downregulation in HaCaT keratinocytes by suppressing JNK activation to block the decrease of PPAR- γ . Collectively, our findings suggest that MELs can be used as a potential ingredient that modulates AQP3 expression to improve skin moisturization following UVA irradiation-induced damage.

PS P433 - 0847 Human Steroid Sulfatase Regulates Keratinization through Inducing the Expression of E-cadherin

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X-linked ichthyosis (XLI) is a recessive genetic disorder of the human skin caused by the genetic deletion of the steroid sulfatase (STS) gene. Deficiency of STS activity in XLI leads to intracellular lipid barrier malformation and delays keratinocyte degradation resulting in increasing corneodesmosomes. To understand how STS can regulate keratinization, the expression of cadherin proteins was determined because the cadherin proteins including E-cadherin may control keratinization-related proteins such as involucrin, loricrin and TGM-1. In this study, we observed up-regulation of E-cadherin at mRNA and protein levels by knockdown of STS in human keratinocyte HaCat cells. Activation of E-cadherin promoter was also observed in cells treated with STS siRNA. On the other hand, overexpression of STS inhibits E-cadherin expression. In addition, inhibition of STS enzymatic activity by STX64, a specific inhibitor significantly enhanced E-cadherin expression. Furthermore, EMT-related factors, N-cadherin and vimentin, were significantly down-regulated in STS knockdown cells. When cells were treated with TNF- α to induce STS expression, E-cadherin level was strongly repressed. In summary, these results suggest that down-regulation of STS may contribute to desquamation by inducing the expression of E-cadherin.

PS P434 - 0852 An Ingredient of Cosmetic Hair Dye, Para-phenylenediamine, Increases TSLP and Inflammatory Cytokines and Causes Acute Dermatitis Symptoms

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Cosmetic hair dyes consist of many chemicals for coloring. One of the chemicals, para-phenylenediamine (P-PD) is widely used and preferred in many dyes because P-PD attach the color to the hair permanently by reacting with hydrogen peroxide. However, many reported cases show that P-PD causes allergic contact dermatitis as a contact allergen. P-PD sensitization is well-correlated with the prevalence of dermatitis in patients. However, the mechanism has not been well clarified. Therefore, we studied whether P-PD exposure to the skin increased thymic stromal lymphopoietin (TSLP) and inflammatory cytokines production inducing the potentiation of dermatitis. P-PD increased TSLP production in mouse keratinocyte cell line, KCMH-1 and potentiated TSLP production induced by phorbol 12-myristate 13-acetate (PMA) in mouse keratinocyte cell line (PAM212). Topical application of P-PD (2.5%) on mouse ear skin resulted in dermatitis symptoms such as skin redness and ear edema. The levels of TSLP mRNA and protein were increased by P-PD in ear skin. In addition, P-PD increased the production of IL-1 β and CCL2. Furthermore, P-PD potentiated the IL-6 and IL-25 production stimulated by TSLP inducer MC903. Our results show that P-PD application to skin induces TSLP expression and proinflammatory cytokines production which may enhance the risk of cosmetic dyes to induce contact dermatitis.

PS P435 - 0945 Toxicity and Mechanisms of Vesicating Chemical Warfare Agent Skin Exposure

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Phosgene Oxime (CX), an urticant categorized as a vesicating agent, is a potential chemical warfare and terrorist weapon. CX exposure can result in devastating toxic effects resulting in high mortality due to its fast penetration and ability to cause immediate severe cutaneous injury. The ability to synthesize CX easily outside of laboratory settings makes it a dangerous chemical with both military and terrorist potential. It could be weaponized with other chemical warfare agents to enhance their deleterious effects and cause prompt incapacitation and death. CX is one of the least studied chemical warfare agents with no effective treatments available. The erythema, necrosis and inflammation of the skin following cutaneous exposure to CX and mustard vesicants, e.g., sulfur mustard (SM) and nitrogen mustard (NM), is similar; however, CX also causes severe skin injury with immediate urticaria and blanching, along with mortality. The skin urticaria from CX resembles urticaria caused by allergic and non-allergic reactions to various environmental substances and can occur alone or can be associated with lethal allergic reaction, anaphylaxis. Data from our studies in SKH-1 hairless mice demonstrated that CX dermal vapor exposure leads to mast cell degranulation, release of histamine, tryptase, and TNF α that potentially activate inflammatory pathways, causing an increase in inflammatory mediators, along with increased neutrophil infiltration. These molecular consequences could be associated with observed acute skin lesions including edema, erythema, necrosis, urticaria and blanching that progress to chronic lesions (desquamation and eschar formation), or mortality, following longer-duration CX exposure. The mice pulse oximetry data showed a CX-related decrease in heart and respiration rate, decrease in temperature, vasculature dilation and blood congestion in multiple organs indicative of anaphylaxis. An increase in tryptase and histamine levels is a measure of anaphylaxis; our studies showed an increase in the blood plasma levels of both of these following CX exposure. The results from our completed studies demonstrate that mast cells could be important players in CX-and potentially other vesicating agent-induced toxicity. Hence, therapies that can counteract vesicant-induced mast cell activation and release of inflammatory mediator histamine could be beneficial in mitigating skin urticaria and mortality induced by CX and other vesicating agents via cutaneous exposure.

PS P436 - 0525 Correlation of Epithelial-to-Mesenchymal Transition-Related Genes Produces Sex-Biased Differences Specifically in Liver Cancer Cell Lines

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There is a wide disparity in cancer incidence, malignancy, and mortality across gender. The sex-specificity of cancer seems to be dependent on cancer types. Cancer incidence and mortality have been shown to be sex-specific in various cancers such as liver cancer, while noticeable sex-specificity was not detected in some cancers including colon and lung cancers. The present study aimed to elucidate the molecular basis for sex-biased gene expression in cancer. Here, we investigated the mRNA expression of the epithelial-to-mesenchymal transition (EMT)-related genes including E-cadherin (CDH1), vimentin (VIM), discoidin domain receptor (DDR1), and zinc finger E-box binding homeobox (ZEB1) in female- and male-derived cancer cell lines by RT-PCR analysis and the Broad-Novartis Cancer Cell Line Encyclopedia (CCLE) database analysis. Negative correlation between DDR1 and ZEB1 was observed only in female-derived cancer cell lines by RT-PCR analysis. Negative correlation between DDR1 index (defined by the logarithmic value of DDR1 divided by ZEB1, based on the mRNA data by RT-PCR analysis) and invasive phenotype was observed in cancer cell lines in a sex-specific manner. Analysis of CCLE database demonstrated that correlation of DDR1 and ZEB1 showed a negativity in female-derived cell lines, but not in male-derived cell lines, of liver cancer which was known to be sex-biased. In contrast, cell lines of colon and lung cancers did not show any sex-dependent difference in correlation of DDR1 and ZEB1. Kaplan-Meier survival curves using the transcriptomic datasets such as GEO, EGA and TCGA databases suggested a sex-biased difference in correlation between DDR1 expression pattern and overall survival in liver cancer patients. The results implicate that sex factors may affect regulation of gene expression, contributing to sex-biased progression of cancers, especially liver cancer. Taken together, our findings suggest that analysis of the correlation between DDR1 and ZEB1 may have a potential applicability for investigating sex-biased cancers.

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