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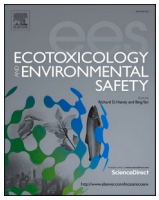

Research paper

A comparison of developmental toxicity of brominated and halogen-free flame retardant on zebrafish

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ABSTRACT

Brominated diphenyl ethers (BDEs) are halogenated flame retardants. Several concerns related to persistence and toxicity of BDEs have been resulted in a growing need of BDEs replacement. The use of halogen-free flame retardants (HFFR) has increased as a safer alternative, but little information is available on their toxic potential for environmental health and for developing organisms. Therefore, the aim of this study was to evaluate and compare the toxicity of three congeners of BDEs (BDE-47, BDE-99 and BDE-154) with an HFFR (aluminum diethylphosphinate, ALPI) on zebrafish (*Danio rerio*) by assessing endpoints of lethality, sub-lethality and teratogenicity at the earlier stages of development. The highest tested concentration of BDE-47 (12.1 mg/L) induced pericardium and yolk sac edemas that first appeared at 48 h post-fertilization (hpf) and then were mostly reabsorbed until 144 hpf. BDE-47 also showed a slight but non-significant tendency to affect swim bladder inflation. The rate of edemas increased in a concentration-dependent manner after exposure to BDE-99, but there were no significant differences. In addition, the congener BDE-99 also presented a slight and non-significant effect on swim bladder inflation, but only at the highest concentration tested. Regarding BDE-154 exposure, the rate of edemas and swim bladder inflation were not affected. Finally, in all ALPI exposure concentrations (0.003 up to 30 mg/L), no sub-lethal or teratogenic effects were observed on developing organisms until 96 hpf. Although further studies are needed, our results demonstrate that when comparing the developmental toxicity induced by flame retardants in zebrafish, the HFFR ALPI may be considered a more suitable alternative to BDE-47.

1. Introduction

Brominated diphenyl ethers (BDEs) are halogenated substances belonging to the group of flame retardants, used as safety criteria to protect people and properties from potential fire hazards (Fromme et al., 2016). BDEs are effective in relatively low amounts, compared to other flame retardants (Alaee et al., 2003), and have been used since 1970s in electronics, furniture, textiles, automotive and construction industries, where they are added in usual materials such as polyurethane foams, plastics, fabrics, household utensils, among several others (Lv et al., 2015; Li et al., 2016; Jinhui et al., 2017). As a result of many years of widespread usage, BDEs concentrations in environmental and human samples have increased. Many of these substances are very persistent and bioaccumulate in the aquatic and terrestrial food chain, being associated with reproductive, neurological and developmental disorders (Hendriks et al., 2014; Díaz-Jaramillo et al., 2016; Gramatica et al., 2016).

Thus, it becomes more evident the importance of conducting ecotoxicological tests to elucidate the mode of action of these compounds. Chen et al. (2012) assessed the BDE-47 toxicity on zebrafish, and showed that neuronal connectivity patterns were altered, which may have contributed to motor behavioral deficits presented by these organisms. Another interesting study evaluated the absorption of BDE-47 by benthic organisms, in order to analyze bioaccumulation and biomagnification in polychaetes and crabs as test organisms. In the bioaccumulation assay, polychaetes absorbed more BDE than crabs. On the other hand, in the biomagnification assay, crabs were fed with polychaetes containing the compound, and a higher concentration was quantified in crabs comparing with the first assay, clearly demonstrating the high accumulation potential of these compounds in the

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trophic chain (Díaz-Jaramillo et al., 2016).

Due to concerns related to persistence, bioavailability and toxicity of BDEs, in 2004 penta and octa BDEs were banned in Europe and discontinued in the U.S (Betts, 2007). Later, they were included as persistent organic pollutants in the Stockholm Convention (UNEP, 2009). Despite that, the levels of BDEs continue growing, as they are still present in many products that were sold before being banned, or are still found in products originated from countries where the ban has not been implemented (Richardson and Kimura, 2016; Jinhui et al., 2017). Therefore, there is an urgent need for the replacement of BDEs by more suitable and harmless flame retardants.

Several manufacturers have already voluntarily replaced BDEs by alternative halogen-free flame retardants (HFFRs) (Betts, 2007). Hence, the use of HFFRs, such as organophosphate esters flame retardants, has increased as a suitable alternative, not being persistent or bioaccumulative and rapidly biodegraded in the environment (Van der Veen and Boer, 2012). Many of these HFFRs are already being marketed, although there is a limited knowledge regarding their potential impact on the environment. Several HFFRs have shown low acute toxicity for *Daphnia magna* (EC50>10 mg/L) (Waaijers et al., 2013b), making them promising substitutes. However, after long-term exposure, their toxicity increased to a 21-d LC50 of 3.2 mg/L, affecting life cycle parameters and decreasing the population growth rate of daphnids (Waaijers et al., 2013a). Recent studies also suggested that such flame retardants are more persistent than previously thought, demonstrating that they are also bioaccumulative and subjected to long-range transport (Salamova et al., 2014a, 2014b; Gramatica et al., 2016).

To overcome the need for information on BDEs and their potential substitutes HFFR, a series of tests must be carried out to ensure a reliable level of safety for these substances. As BDEs and also some alternative phosphorus flame retardants are related to developmental defects (Van der Veen and Boer, 2012; Gramatica et al., 2016), it is expected that exposure to them during early development would result in malformations and mortality. Zebrafish (*Danio rerio*) has been widely used in toxicological and ecotoxicological studies in order to analyze the potential effects of chemical compounds on embryogenesis (Scholz et al., 2008; Carlsson et al., 2013). They develop rapidly, achieving most of the organogenesis at 30 h-post fertilization (hpf), and their transparency and small size facilitate the monitoring of the development. In addition, zebrafish has a high genetic homology to humans, and similar organogenesis and functional response mechanisms, reinforcing the use of this test organism to conduct toxicological studies (Scholz et al., 2008; Howe et al., 2013).

Therefore, the aim of this study was to evaluate and compare the toxicities of HFFR and BDEs on zebrafish early development. To this purpose, an organophosphorus HFFR was selected: aluminum diethylphosphinate (ALPI), mostly used in the engineering plastics segment, such as electrical and electronic equipment, connectors, switches and encapsulated electronic components; and three congeners BDEs were selected: BDE-47, BDE-99 and BDE-154, usually added in polyurethane foams, plastics, fabrics, household utensils, among several others (Lv et al., 2015; Li et al., 2016; Jinhui et al., 2017). We investigated endpoints of lethality, sub-lethality and teratogenicity in order to detect whether such compounds trigger a rapid and severe response on zebrafish development.

2. Material and methods

2.1. Chemicals

The brominated congeners BDE-47 (2,2′,4,4′-tetrabromodiphenyl ether, CAS no. 5436-43-1, MW 485.79), BDE-99 (2,2′,4,4′,5-pentabromodiphenyl ether, CAS no. 60348-60-9, MW 564.69) and BDE-154 (2,2′,4,4′,5,6′-hexabromodiphenyl ether, CAS no. 207122-15-4, MW 643.58) were purchased from AccuStandard (New Haven, CT, USA). Manufacturer's certification indicated 99.9% purity. The HFFR ALPI (aluminum diethyl-phosphinate, CAS 225789–38–8, 98.5% purity) was purchased from Clariant (trade name of Exolit® OP 1230). The chemical structures of the flame retardants are shown in Fig. 1..

Stock solutions of BDEs were prepared in 100% dimethyl sulfoxide (DMSO) and further diluted in embryo medium (2 mM $CaCl_2$, 0.5 mM $MgSO_4$, 0.75 mM $NaHCO_3$, 0.07 mM KCl) to the tested concentrations (1% DMSO, v/v). ALPI was directly dissolved in embryo medium and left overnight on a shaker at 50–80 rpm for complete dissolution, and further diluted to prepare the tested concentrations. Embryo medium and 1% DMSO in embryo medium (v/v) were used as negative controls, and 4.0 mg/L 3,4-dichloroaniline (DCA, CAS no. 95-76-1, 98% purity, Sigma-Aldrich) was used as positive control.

2.2. Test-organism

Zebrafish eggs were provided by the facility of the School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (Ribeirão Preto, Brazil). Briefly, adults are kept in ZebTEC system (Tecniplast, Italy) under standard conditions (temperature 26 ± 1 °C, pH 7.5 ± 0.5, dissolved oxygen at 95% saturation, conductivity 750 ± 50 μs/cm, photoperiod ranging between 13:11 and 14:10 h light:dark) and fed with TetraMin® Tropical Granules. For eggs acquisition, males and females fish (2:1 ratio) were placed into 1-L breeding tank or 60-L breeding system (i-Spawn) and the spawning was trigged once the light onset in the morning. Eggs were collected about 90 min after spawning and rinsed in embryo medium. Fertilized eggs were randomly selected under stereomicroscope for the subsequent tests. All protocols were approved by the Commission of Ethics in the Use of Animals of the University of São Paulo (CEUA-USP, protocols 14.1.586.53.1 and 19.1.845.60.4).

2.3. Fish embryo acute toxicity

Tests were performed according to Fish Embryo Acute Toxicity (FET) Test guideline of the Organisation for Economic Co-operation and Development (OECD, 2013) and Nagel (2002). For each tested chemical, 60 eggs were used per treatment, divided into three independent replicates. Twenty wells from a 24-well plate were assigned to each treatment, with one egg individually placed into each well filled-up with 2 mL solution. The remaining four wells were used as internal plate control filled-up with embryo medium only. Treatments consisted of negative control (embryo medium and 1% DMSO in embryo medium), positive control (4 mg/L DCA), and 5 concentrations of BDE-47 (0.05, 0.5, 2.5, 5.0 and 12.1 mg/L), BDE-99 (0.056, 0.56, 2.8, 5.6 and 14.1 mg/L), BDE-154 (0.064, 0.64, 3.2, 6.4 and 16.1 mg/L) and ALPI (0.003, 0.03, 0.3, 3 and 30 mg/L), prepared as described in Section 2.1. These concentrations were chosen based on a previous range finding study to determine the maximum dissolved concentration that could be achieved. In short, the concentration range of BDEs was set in 0.1 up to 25 μM, which were further converted to mg/L unit. The highest concentration of 25 μM (12.1 mg/L for BDE-47, 14.1 mg/L for BDE-99 and 16.1 mg/L for BDE-154) was selected according to their limit of solubility (practically insoluble in water), also respecting the limit of the organic solvent. On the other hand, ALPI is poorly soluble in other solvents than water; therefore, it was directly dissolved in embryo medium. The range finding study of ALPI showed that concentrations from 50 mg/L precipitated, although up to 100 mg/L did not induce any adverse effect (data not shown), therefore the maximum concentration was set in 30 mg/L. Plates were incubated at 27 ± 1.5 °C, with photoperiod ranging between 13:11 and 14:10 h of light:dark.

Zebrafish development was monitored under stereomicroscope at 24, 48, 72 and 96 hpf. BDEs were also monitored at 120 and 144 hpf due to early-induced edemas that were reabsorbed over time (discussed hereafter). Lethality was assessed by egg coagulation, tail not detached, malformation of somites and no heartbeat. Morphology was assessed by malformation of eyes, otoliths, head and tail, body axis defects,

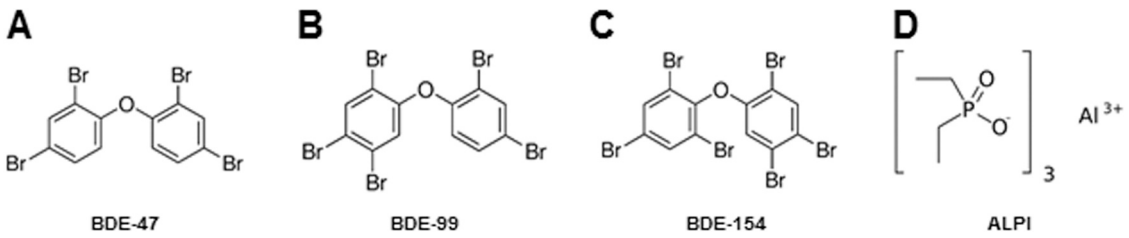

Fig. 1. BDE-47, BDE-99, BDE-154 and ALPI chemical structures.

deformity of yolk sac, spontaneous movement, lack of pigmentation, edemas and delayed growth. For BDEs, the complete inflation of the swimming bladder was also monitored at 144 hpf. The distinction between normal and abnormal embryonic development was established according to the description of zebrafish embryogenesis described by Kimmel et al. (1995). Images of the embryos and larvae at different post-fertilization times were captured by a digital camera (AxioCam ICc 5, Zeiss, Germany) coupled to a stereomicroscope (Stemi 508, Zeiss, Germany).

2.4. Statistical analysis

Statistical analysis of the experimental data was performed using GraphPad Prism 5.01 software (GraphPad Software, San Diego, CA, USA). Data were analyzed by one-way ANOVA, followed by the Dunnett's *post-hoc* test to verify significant differences between treatments and controls. Significance was set at $p < 0.05$ (*). Data are presented as mean and standard error of the mean (SEM). The lowest observed effect concentration (LOEC) was determined when significant differences were found.

3. Results

The negative control group presented normal embryonic development as described by Kimmel et al. (1995) and the mortality rate remained below 10% throughout the experiment. DCA 4 mg/L induced mortality above 30%, thereby satisfying the validation criteria of the OECD guideline 236 (2013). No significant mortality was observed in the embryos/larvae exposed to BDE-47 ($F_{(5;12)}$=0.91, p=0.5085), ALPI ($F_{(5;12)}$=0.30, p=0.9036), BDE-99 and BDE-154 (F *values* could not be determined, all samples have a standard error of zero) at any concentration tested until 96 or 144 hpf (Table S1 summarizes the one-way ANOVA analysis). The LC50 values could not be determined due to the lack of mortality up to the highest tested concentration of all BDEs and ALPI, which were limited by their solubility in the test medium. Therefore, the LC50 of BDE-47, BDE-99 and BDE-154 are expected to be higher than 12.1, 14.1 and 16.1, respectively. According to U.S. EPA. Environmental Protection Agency (EPA), 2015, the 96h-LC50 of ALPI for zebrafish adults is greater than 100 mg/L.

Although no significant mortality was observed (Table S1), exposure to BDE-47 induced pericardium and yolk sac edemas at the highest concentration tested (LOEC=12.1 mg/L, $F_{(5;12)}$=4.7, $p = 0.0129$) (Figs. 2 and 3A). When we analyzed the evolution of edemas during development (Table 1), we observed that edemas on embryos exposed to 12.1 mg/L BDE-47 first appeared at 48 hpf, reaching 11.7% of the organisms, whereas no edemas were detected in the control group ($F_{(5;12)}$=6.56, $p = 0.0037$). At 72 hpf, organisms did not present edemas and this might be associated with their ability to reabsorb them. A slight increase in edemas rate was observed again at 120 hpf, reaching approximately 3% of exposed organisms at 144 hpf, but no significant differences were found when compared to control groups (120 hpf: $F_{(5;12)}$=0.8, $p = 0.5705$; 144 hpf: $F_{(5;12)}$=1.0, $p = 0.4582$) (Fig. 3A). As

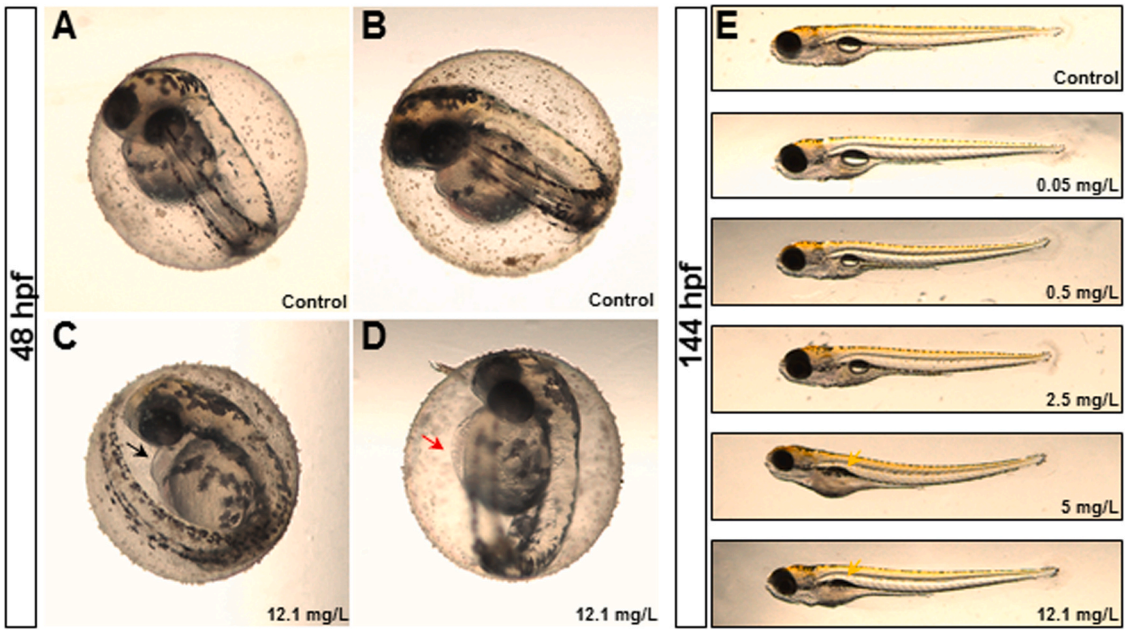

Fig. 2. Effects of BDE-47 on zebrafish embryos/larvae at 48 and 144 h post-fertilization (hpf). Representative pictures of embryos from control group (1% DMSO) (A-B) and exposed to 12.1 mg/L of BDE-47 (B-C). Representative pictures of larvae from control group (1% DMSO) and exposed to different concentrations of BDE-47 (E). The black arrow indicates a pericardium edema, the red arrow indicates a yolk sac edema and the yellow arrow indicate the absence of inflated swim bladder in treated embryos/larvae (3.2/1.25× magnification).

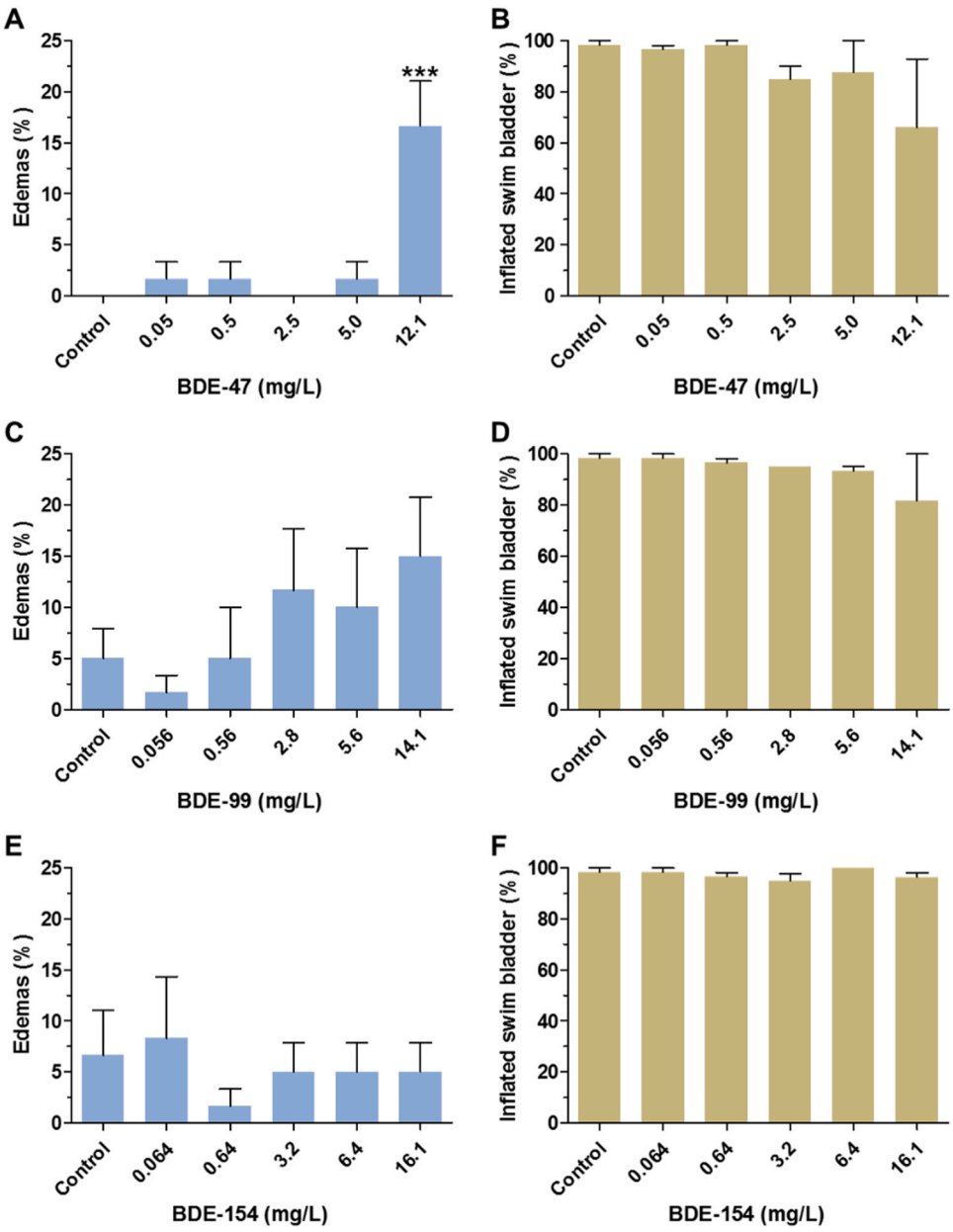

Fig. 3. Developmental alterations induced by different concentrations of polybrominated diphenyl ether congeners (A and B) BDE-47, (C and D) BDE-99 and (E and F) BDE-154 in zebrafish. (A, C, E) Cumulative frequency of zebrafish embryos/larvae (48–144 h post-fertilization) with pericardium and yolk sac edemas; (B, D, F) Frequency of zebrafish larvae at 144 h post-fertilization with inflated swim bladder. The bars represent means and the error bars represents standard error of the means (SEM) (n = 60). * **One-way ANOVA followed by Dunnett's test, $p = 0.001$ for significant differences between exposed and control groups.

shown in Figs. 2E and 3B, the effects of BDE-47 on swim bladder inflation were also evaluated. Although no significant effect was found in any tested concentration ($F_{(5;12)}$=1, $p = 0.4424$), BDE-47 presented a slight trend to affect swim bladder inflation (from 2.5 to 12.1 mg/L) (Table S1 summarizes the one-way ANOVA analysis).

In contrast to BDE-47, neither sub-lethal nor teratogenic effects were detected after exposure to the congeners BDE-99 and BDE-154. In treatments with BDE-99, the rate of edemas showed a trend to increase in a concentration-dependent manner, but there were no significant differences ($F_{(5;12)}$=1.1, $p = 0.4209$) (Fig. 3C, Table 1). There was a statistically significant edema effect in larvae at 96 hpf exposed to the lowest concentration of BDE-154 ($F_{(5;12)}$=4.0, p = 0.0228), however the effect did not reach at least 10% of organisms, thus it is unlikely to be a treatment effect. As suggested by OECD guideline 236 (2013), if the effect at the tested concentration exceeds the negative control by 10%, therefore the effect might be investigated, which is not the case. So, this punctual effect might be a random variation rather than a treatment effect. On the other hand, as observed for BDE-47, the congener BDE-99 also presented a slight and non-significant effect on swim bladder inflation, but only at the highest tested concentration (14.1 mg/L) ($F_{(5;12)}$=0.68, $p = 0.6440$) (Fig. 3D). The rate of edemas ($F_{(5;12)}$=0.35, $p = 0.8704$) and swim bladder inflation ($F_{(5;12)}$=0.95, $p = 0.4842$) were not affected after exposure to BDE-154 (Fig. 3E and F, Table 1) (Table S1 summarizes the one-way ANOVA analysis).

For all ALPI exposure concentrations, wherein the highest tested concentration was approximately 2-fold higher than those used for BDEs exposure, no sub-lethal or teratogenic effects were observed on embryos/larvae development until 96 hpf compared to controls (Fig. 4, Table 1) (Table S1 summarizes the one-way ANOVA analysis).

4. Discussion

In this study, we performed a comparison of the adverse effects induced by exposure to BDE congeners and a new alternative flame retardant on zebrafish embryo/larvae model. Three BDE congeners (BDE-47, BDE-99 and BDE-154) and the HFFR ALPI were individually evaluated using endpoints of lethality, sub-lethality and teratogenicity as recommended by Nagel (2002). BDEs treatments were conducted

Table 1
Frequency of edemas occurrence in zebrafish early life stages exposed to polybrominated diphenyl ether (BDE) congeners and to aluminum diethylphosphinate (ALPI). The edemas were located in the pericardium and/or yolk sac (n = 60).

		% Organisms with edemas *hours post-fertilization					
Compound	[mg/L]	48	72	96	120	144	TOTAL
BDE-47	0	0	0	0	0	0	0
	0.05	1.7	0	0	0	0	1.7
	0.5	0	0	0	1.7	0	1.7
	2.5	0	0	0	0	0	0
	5.0	1.7	0	0	0	0	1.7
	12.1	12***	0	0	1.7	3.3	17***
BDE-99	0	3.3	0	1.7	0	0	5.0
	0.056	0	0	0	0	1.7	1.7
	0.56	5.0	0	0	0	0	5.0
	2.8	6.7	1.7	1.7	0	1.7	12
	5.6	10	0	0	0	0	10
	14.1	13	0	0	1.7	0	15
BDE-154	0	5.0	1.7	0	0	0	6.7
	0.064	5.2	0	3.4*	0	0	8.6
	0.64	1.7	1.7	0	0	0	3.3
	3.2	5.0	0	0	0	0	5.0
	6.4	5.1	0	0	0	0	5.1
	16.1	3.5	1.8	0	0	0	5.3
ALPI	0	0	–	5.1	–	–	5.1
	0.003	0	–	0	–	–	0
	0.03	1.7	–	1.7	–	–	3.3
	0.3	0	–	0	–	–	0
	3	1.7	–	0.0	–	–	1.7
	30	0	–	1.7	–	–	1.7

Note: *time when edema was first observed. Edema was counted when it was first observed, then was not recounted until the end of the test, to avoid counting the same organism more than once. In organisms having two types of edema (pericardium and yolk sac), only one was considered. -not evaluated.
* $p < 0.05$,
*** $p \leq 0.001$.

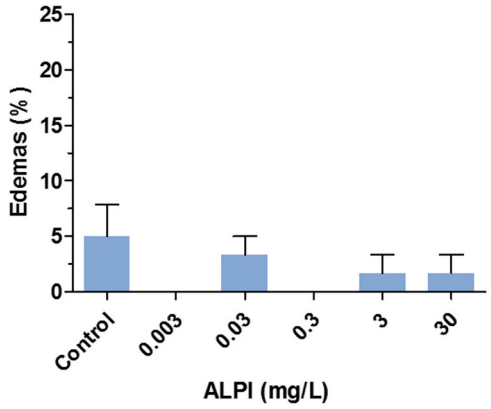

Fig. 4. Cumulative frequency of zebrafish embryos/larvae (24–96 h post-fertilization) with pericardium and yolk sac edemas after exposure to aluminum diethylphosphinate (ALPI). The bars represent means and the error bars represents standard error of the means (SEM) (n = 60).

until 144 hpf in order to assess the evolution of edemas that became evident after 48 hpf, also enabling the assessment of the swim bladder inflation rate. Because some organisms presented pericardium edema at 24 hpf after ALPI exposure, edemas were monitored from that time on. However, as no acute and significant effects were observed until 96 hpf, tests were finished at the end of this period.

It has been reported that some BDEs congeners induce lethal effects on zebrafish larvae after 144–168 hpf, as previously reported for BDE-28, BDE-47, BDE-99 and BDE-100 at concentrations above 18, 26, 13 and 16 mg/L, respectively (Usenko et al., 2011). Despite our study did not observe lethal effects, lethality may only be displayed at even higher concentrations, which are unrealistic in the environment, since only concentrations of BDEs ranging from pg/L to ng/L have been found in water samples according to previous reports (Hites, 2004; Sacks and Lohman, 2012; Moon et al., 2012; Wang et al., 2011; Pei et al., 2018; Liu et al., 2018). Although few studies for determination of BDEs in the environmental water samples have been conducted, it should be considered that the low concentrations reported may be due to their absorption by organisms from the water column or deposition on sediments (Ju et al., 2016; U.S. EPA, 2010). In this way, it is known that BDEs uptake by organisms (e.g. algae) can be considered an important contamination source to higher trophic levels (Qiu et al., 2016). For this reason, even low concentrations in the environment must be carefully considered. Regarding ALPI, data on its lethality have been reported by the United States Environmental Protection Agency (U. S. EPA) and were based on the highest concentration tested. Despite of adequate toxicity values have not been determined, it was estimated that the LC50 value for ALPI in adult *D. rerio* after 96 h of exposure is higher than 100 mg/L (U.S. EPA, 2014).

Sublethal effects of BDEs on zebrafish development have been observed in previous studies (Han et al., 2013; Qin et al., 2014; Macaulay et al., 2017). Here, at sublethal concentrations, zebrafish embryos/larvae were more sensitive to BDE-47 exposure, which induced significant morphological deformities at the highest tested concentration, such as pericardium and yolk sac edemas. In previous studies, the development of pericardium edemas induced by exposure to BDE-47 has been reported, but no information has been provided regarding its reabsorption after 96 h of exposure (Liu et al., 2015; Parsons et al., 2019; Zezza et al., 2019). In addition, BDE-47 has also been associated with vascular toxicity (Xing et al., 2018) and cardiac arrhythmia in exposed zebrafish embryos/larvae (Lema et al., 2007). The edema can be related to several factors: kidney failure, permeability defects, circulatory failure, ionic imbalance. In the early stages of the fish, the organs have not been developed and the influence of an external factor can induce edemas by disruption one of these functions (Hill et al., 2003). As the organs become more functional, if the damage caused by the xenobiotic is reversible, they start to respond and reverse the edema, which might be the case of BDEs.

The slight cardiotoxic effects of BDE-47 may be related to the mode of action of this compound on the cardiovascular system, which has a direct influence on the organogenesis of other biological systems. Cardiovascular malformations in zebrafish embryos/larvae are known to lead to blood circulation failure, and that is a key factor in normal development of the swim bladder, a teratogenic effect (Yue et al., 2015). As mentioned before, most edemas induced by BDE-47 were reabsorbed by the embryos/larvae until 144 hpf (approximately 33.3% of edemas were reabsorbed), which could explain the absence of significant defect on swim bladder inflation. Despite the resorption of edemas and absence of significant teratogenic effects on the swim bladder, these observations indicate that zebrafish embryos/larvae were able to absorb the BDE-47, which is consistent with previous studies (Alzualde et al., 2018).

It is known that there is a pattern of increased toxicity caused by polybrominated BDEs with the amount of bromine atoms. BDEs with less bromination, with bromine atoms in the ortho position and without bromine atoms in the meta position, have higher toxic potential, according to the structure-activity relationship (Harju et al., 2007). Usenko et al. (2011) observed that BDEs with less bromine atoms proved to be toxic to zebrafish (BDE-28, BDE-47 and BDE-100), while polybrominated BDEs with more bromine atoms did not induce problems (BDE-153 and BDE-183). This higher toxicity is linked to the facility of less brominated BDEs to permeate through biological membranes (Pazin et al., 2015; Pereira et al., 2013; Souza et al., 2016, 2013). In the environment, fish have particularly high concentrations of BDEs with less bromine atoms as a result of absorption and bioaccumulation on their fat tissue, thus leading to higher concentrations at higher trophic levels by biomagnification processes (Hites, 2004; Tlustos et al., 2005; Knutsen et al., 2005; Botaro and Torres, 2007). Moreover, congeners

with less bromination are more likely to be found in surface water, such as tetra and penta congeners (Sacks and Lohman, 2012).

After 96 h-exposure to ALPI, our results indicate that the HFFR does not induce morphological abnormalities during the development of zebrafish at the range of concentrations tested. To date, the occurrence and environmental levels of ALPI are unknown. Besides that, with the exception of reports from U.S. EPA, no data from literature about ALPI toxicity to early life stages of zebrafish are available. In mammals, sub-chronic and single oral exposure to ALPI was associated with mild effects related to hepatic injury (Bao et al., 2017) and neurodevelopment and synaptic plasticity, respectively (Hendriks et al., 2014). One of the few studies in aquatic organisms has shown that ALPI induces low toxicity to *Daphnia magna* in an acute toxicity test (Waaijers et al., 2013b). However, in a 21-days test, ALPI was able to affect mobility, survival and fecundity of daphnids, also showing an increase in toxicity in a time-dependent manner, suggesting that the duration of exposure may be an important factor to be considered when ALPI toxicity is evaluated (Waaijers et al., 2013a). Thus, it should be noted that the lack of information regarding ALPI toxicity turns comparisons very limited. Although further studies are needed, this fact demonstrates the relevance and contribution of our research in evaluating the toxicity of ALPI and, consequently, in the selection of new safer chemicals suitable for the replacement of some BDEs.

5. Conclusions

The present study showed that BDE-47 can affect the embryonic-larval development of zebrafish by inducing pericardium and yolk sac edemas, whereas the congeners BDE-99 and BDE-154 did not present acute toxicity to zebrafish larvae/embryos at sub-lethal concentrations. When comparing the developmental toxicity induced by the flame retardants tested in this work, the HFFR ALPI may be considered a more suitable alternative regarding to the use of BDE-47.

CRediT authorship contribution statement

Flávia Renata Abe: Investigation, Formal analysis, Writing - original draft; **Andréia Ávila Soares De Oliveira**: Investigation, Formal analysis, Writing - original draft; **Renan Vieira Marino**: Investigation, Formal analysis, Writing - original draft; **Taisa Carla Rizzi Rialto**: Investigation, Formal analysis, Writing - original draft; **Danielle Palma de Oliveira:** Conceptualization, Methodology, Resources, Writing - review & editing, Project Administration, Funding aquisition; **Daniel Junqueira Dorta:** Conceptualization, Methodology, Resources, Writing - review & editing, Project Administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2020.111745.

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