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Toxicity of Vertimec® 18 EC (active ingredient abamectin) to the neotropical cladoceran *Ceriodaphnia silvestrii*

Maressa P. Casali-Pereira *, Michiel A. Daam [1], Juliana C. de Resende, Ana M. Vasconcelos, Evaldo L.G. Espíndola, Clarice M.R. Botta

NEEA/CRHEA/SHS, São Carlos Engineering School, University of São Paulo, Av. Trabalhador São Carlense, 400, 13.560-970 São Carlos, Brazil

HIGHLIGHTS

- The acute EC50-48 h for immobilization was 1.47 μg a.i./L.
- The chronic NOEC-8 d for survival and fertility were 169 and 84 ng a.i./L, respectively.
- Mesocosm water treated with Vertimec had lower toxicity than expected from laboratory bioassay toxicity data.
- *Ceriodaphnia silvestrii* is a suitable species for ecotoxicity testing in the tropics.

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ABSTRACT

The aim of the present study was to evaluate the toxicity of abamectin to the neotropical cladoceran *Ceriodaphnia silvestrii*. To this end, acute and chronic bioassays were conducted with the commercial formulation Vertimec® 18 EC. In addition, the toxicity of water samples taken from a microcosm experiment evaluating the effects of a single application (144 μg a.i./L) and two applications (2 × 36 μg a.i./L) of Vertimec® 18 EC, in the presence or absence of a tadpole species (*Lithobates catesbeianus*), was also assessed. The acute LC50-48 h for immobilization was 1.47 μg a.i./L and chronic NOEC-8 d for survival and fertility (number of neonates per female) were 169 and 84 ng a.i./L, respectively. Irrespective of the presence of tadpoles, water samples from the microcosms applied with the single concentration of 144 μg a.i./L remained toxic until the end of the experiment, even when samples were diluted 32 times with culture medium. Water in the repeated pesticide treatment showed a similar toxic response after both applications. Toxicity of water samples from the microcosms was lower than that expected based on the generated LC50 values, which is explained by a potential reduced bioavailability of the test compound resulting from absorbance to organic material. Potential side-effects on *C. silvestrii* related with the use of Vertimec® 18 EC in Brazil and the suitability of this species for tropical toxicity testing are discussed.

1. Introduction

Avermectins are natural fermentation products of the soil-dwelling actinomycete *Streptomyces avermitilis* with nematicidal, acaricidal and insecticidal activity (Ali et al., 1997). They include abamectin, ivermectin and doramectin, which are highly effective against a broad spectrum of common pests in agriculture, making avermectins one of the most widely used classes of parasiticides (Wislocki et al., 1989). Avermectins have also been reported to be effective veterinary drug and mosquito control agents (Tišler and Eržen, 2006; Pridgeon et al., 2009).

The avermectin abamectin is a mixture that contains about 80% avermectin B1a and 20% avermectin B1b, which have similar biological and toxicological properties (Campbell, 1989). It has low toxicity to mammals, but can pass the blood-brain barrier of fish and subsequently exert toxic effects (Wislocki et al., 1989; Høy et al., 1990). In laboratory toxicity tests conducted with aquatic organisms, invertebrates have been indicated to be the most sensitive taxonomic group to abamectin (Tišler and Eržen, 2006; Novelli et al., 2012a). Cladocerans and the mysid shrimp *Americamysis bahia* are specially sensitive to abamectin, with acute LC50 and chronic NOEC values for reproduction lower than 1 μg/L and 0.01 μg/L, respectively (Table 1).

* Corresponding author.
E-mail addresses: maressa@sc.usp.br, maressapcp@gmail.com (M.P. Casali-Pereira).
[1] Present address: CESAM and Department of Biology, University of Aveiro, Campus Universitário de Santiago, 3810-191 Aveiro, Portugal.

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Table 1
Selected acute EC50 and chronic NOEC toxicity data of abamectin tech. to aquatic invertebrates. Source data: EC (2006) unless stated otherwise. When more than one toxicity value with the same endpoint was available for a species, the range of values (minimum–maximum) was noted.

	Taxonomic group	Method, duration, endpoint	Value (μg/L)
EC50			
Daphnia similis	Cladocera	Static, 48 h, mortality	0.0051[c]
Daphnia pulex	Cladocera	Static, 48 h, immobility	0.12–0.28
Americamysis bahia	Mysida	Static, 96 h, mortality	0.21[a]
Daphnia magna	Cladocera	Static, 48 h, immobility	0.26–0.56
Simocephalus sp.	Cladocera	Static, 48 h, immobility	0.3
Daphnia longispina	Cladocera	Static, 48 h, immobility	0.38
Diaphanosoma sp.	Cladocera	Static, 48 h, immobility	0.53
Daphnia galeata	Cladocera	Static, 48 h, immobility	0.55
Aedes aegypti	Insecta	Static, 48 h, mortality	2.2[b]
Chironomus xanthus	Insecta	Static, 96 h, mortality	2.7[c]
Thamnocephalus platyurus	Anostraca	Static, 24 h, immobility	2.8–30
Gammarus sp.	Amphipoda	Static, 48 h, immobility	6.2–8.6
Chaoborus sp.	Insecta	Static, 24 h, immobility	41–190
Lymnaea stagnalis	Gastropoda	Static, 48 h, mortality	55
Crassostrea virginica	Bivalvia	Static, 48 h, mortality	430
Brachionus calyciflorus	Rotifera	Static, 24 h, immobility	4000
NOEC			
Americamysis bahia	Mysida	Flow-through, 28 d, reproduction	0.0035
Daphnia magna	Cladocera	Semi-static, 21 d, reproduction	0.01

[a] LC50-96 h = 0.02 μg/L under flow-through conditions (EC, 2006).
[b] Pridgeon et al. (2009).
[c] Novelli et al. (2012a).

Despite considerable increased pesticide use over the past decades, relatively little research has been done into their fate and effects in surface waters of tropical regions (Daam and Van den Brink, 2010). In line with this, little research had been conducted into the environmental fate and potential side effects of abamectin in Brazil despite its extensive use – According to information on the website of the Brazilian Ministry of Agriculture, Livestock and Supply, the active ingredient abamectin is currently allowed for use in 15 different formulated products against 18 different pests in 24 crops (MAPA, 2015). To address the lack in knowledge of the potential impacts of abamectin on non-target aquatic organisms, laboratory bioassays evaluating the toxicity of technical abamectin to the cladoceran *Daphnia similis*, the midge *Chironomus xanthus* and the fish *Danio rerio* were previously conducted at our research facilities (Novelli et al., 2012a). As anticipated, *D. similis* was the most sensitive species with an LC50 as low as 5.1 ng/L, which is an order of magnitude lower than the most sensitive cladoceran that had been tested until then (*Daphnia pulex*, LC50 0.12–0.28 μg/L; Table 1).

In a subsequent study, experimental soil plots were contaminated with Vertimec® 18 EC at the recommended field dose indicated for strawberry crops in Brazil (16.9 g a.s./L; MAPA, 2015). After application, torrential rainfall was simulated and the collected runoff water was subsequently evaluated in acute and chronic bioassays for potential effects on *D. similis* and *Ceriodaphnia dubia*, respectively. The results showed high acute toxicity of the runoff water on *D. similis* (Novelli et al., 2012b). Chronic toxicity to *C. dubia* could not be assessed since the effect at even the highest dilution tested was lethal, preventing the evaluation of effects on reproduction (Novelli, 2010).

The aim of the present study was to evaluate the toxicity of abamectin (applied as Vertimec® 18 EC) to the neotropical cladoceran *Ceriodaphnia silvestrii*. This species is native and common in Brazilian freshwaters and methods for laboratory rearing and toxicity testing have previously been developed (Fonseca and Rocha, 2004; ABNT, 2005; dos Santos et al., 2006). *C. silvestrii* has also been proven to be a very sensitive species to a wide range of toxicants (e.g. Freitas and Rocha, 2011). In this study, the sensitivity of *C. silvestrii* to Vertimec® 18 EC was evaluated through acute and chronic laboratory toxicity tests to determine LC50-48h values and chronic NOEC-8d values for reproduction. The toxicity of water from a microcosm experiment determining the effects of single and repeated applications of Vertimec® 18 EC in the presence or absence of a tadpole species (*Lithobates catesbeianus*) was also assessed. To this end, water samples were taken to the laboratory and subjected to laboratory testing with *C. silvestrii*. Potential impacts on *C. silvestrii* related with the use of Vertimec® 18 EC in Brazil and the suitability of this species for tropical toxicity testing are discussed.

2. Materials and methods

2.1. Test organisms

Neonates of *C. silvestrii* were obtained from an in-house culture at NEEA/CRHEA. The culture was kept under controlled temperature (24 ± 2 °C) and photoperiod (16:8 h light/dark; light intensity ± 1000 lux) in dechlorinated tap water with pH 7.0–7.6, conductivity 160 μS/cm and hardness 40–48 mg/L (as $CaCO_3$). The organisms were fed daily with the algae *Raphidocelis subcapitata* (10^6 cells mL/L) and Vitormonio (1 mL/L), a commercial preparation containing yeast and fish food.

2.2. Laboratory toxicity tests

The acute and chronic tests followed the standards issued by the Brazilian Association of Technical Standards (ABNT, 2004, 2005). The tests were conducted under the same conditions (light, temperature, medium) as those described for the culture. In the tests to determine LC50 (acute laboratory tests) and NOEC (chronic laboratory tests) values of Vertimec® 18 EC, treatment solutions were prepared by dilution of a stock solution with culture medium to achieve concentrations of 0 (control), 1.1, 2.3, 4.5, 9 and 18 μg/L (acute test) and 0 (control), 0.2, 0.3, 0.6, 1.1 and 2.3 μg/L (chronic test). In the acute toxicity test, four replicates were used per treatment. Each replicate consisted of a nontoxic polypropene plastic cup containing five 6–24 h old neonates in 10 mL of test solution.

Test vessels were checked for immobilised individuals of *C. silvestrii* 24 and 48 h after the beginning of the test. The chronic test was conducted using ten replicates each containing one neonate in 15 mL test solution. The duration of the chronic test was 8 days. The test solutions were renewed every two to three days after the number of surviving animals and neonates had been recorded. To account for the variability and hence the reproducibility of the toxicity values, six definitive acute tests and three chronic tests were conducted. Avermectins like abamectin have often been reported to be quickly photo-degradated (Tišler and Eržen, 2006; Campbell, 1989). Subsequently, to verify the influence of light (fluorescent bulbs with wavelength between 400 to 800 nm and 1000 lux of intensity) on Vertimec toxicity, three of the six acute tests were conducted in darkness.

An acute and chronic test with the reference substance sodium chloride were also carried out to evaluate the physiological conditions of the organisms and hence the validity of the tests. In addition, pH (Micronal B374 potentiometer), dissolved oxygen (OD YSI meter) and conductivity (Orion 145A conductivimeter) of the water were measured at the start and end of the toxicity tests.

2.3. Microcosm test

Based on toxicity values of abamectin for cladocerans obtained in the laboratory tests (see Section 2.2.),the Vertimec concentrations tested in the microcosm study were anticipated to lead to 100% mortality of *C. silvestrii*. Subsequently, six dilutions (3.125%, 6.25%, 12.5%, 25%, 50%, and 100%) of the water from all 18 microcosms (see Section 2.4) with culture medium were tested. Water samples from one replicate microcosm of each treatment were taken at various times after the start of the pesticide treatment (3 h before and 3 h after the first and second pesticide application, as well as 4, 8, 12, 16, 20 and 24 days after the first treatment). Water samples were filtered through a nylon net (mesh size 68 μm) before testing to remove zooplankton and filamentous algae. Otherwise the tests were conducted as described above.

2.4. Experimental design of the microcosm experiment

The microscosm experiment was conducted at the Center for Water Resources and Environmental Studies (CRHEA), located in the municipality of Itirapina, São Paulo state, Brazil (22°01′22″S, 43°57′38″W). Each of the 18 microcosms used in the experiment consisted of a cylindrical enclosure (60 cm diameter × 90 cm height) placed in a large concrete tank (10.24 m^2 and 1.14 m height). Before setting the enclosures, the high-density polyethylene cylinders were washed with 5% nitric acid to remove metal ions and with acetone to remove organic contaminants, and then rinsed with distilled water. The cylinders, open at both ends, were inserted in a 10 cm deep layer of sediment (5–9% organic matter) that covered the base of the tank, and then filled with water to a depth of 80 cm (water volume approximately 200 L). Water was obtained from the nearby Lobo reservoir four months prior to the start of the experiment. The cylinders were pushed in the sediment eight days prior to the start of the treatment and fixed by means of four concrete weights (about 12 kg each) attached to the outer sides of the cylinders to keep them from floating and to prevent water, sediment and organism exchange between the microcosms. The objective of this study was to assess the toxicity Vertimec® 18 EC to freshwater organisms. Both a single and two applications (interval between applications 12 days) were evaluated, since 1–4 applications of the formulated product are allowed in crops with an interval between applications of 7–14 days, depending on the type of crop and target pest. This experiment also intended to test the vulnerability of the tadpole species (*L. catesbeianus*) to Vertimec® 18 EC and the impact of the presence of this amphibian on the freshwater microcosm communities in the cylinders.

Subsequently, the following six treatments were made, all in triplicate:

(1) Control (C): No contamination, tadpoles absent
(2) Control – Tadpole (CT): No contamination, tadpoles present
(3) Single Application (SA): Single application of 144 μg abamectin/L (as Vertimec® 18 EC), tadpoles absent
(4) Single Application – Tadpoles (SAT): Single application of 144 μg abamectin/L (as Vertimec® 18 EC), tadpoles present
(5) Multiple Application (MA): Two applications (with a 12 d interval) of 36 μg abamectin/L (as Vertimec® 18 EC), tadpoles absent
(6) Multiple Application – Tadpoles (MAT): Two applications of 36 μg abamectin/L (as Vertimec® 18 EC), tadpoles present

The test concentrations of the multiple applications of Vertimec® 18 EC were based on the concentration measured in runoff of the microcosm experiment by Novelli et al. (2012b). For the single pesticide treatment, the concentration level was based on the LC50 value of the tadpole tested (*L. catesbeianus*; Vasconcelos, 2014). The microcosms receiving tadpoles were loaded with 80 individuals (0.4 per liter) immediately after the pesticide treatment. To evaluate the effect of Vertimec on the microcosm structure and functioning, samples were also taken randomly between the cylinders in the concrete tank throughout the course of the experiment ("Random" treatment). The samples were taken at 4 days intervals until the end of the experiment (24 days), and on the days of application (day 0 and 12) samples were taken twice (three hours before and three hours after the contamination). The microcosms were covered with transparent plastic sheets at night and on rainy days throughout the experiment to prevent dilution effects from heavy precipitation episodes.

2.5. Physical and chemical parameters

From one day before the treatment onwards, temperature, pH, conductivity and dissolved oxygen in the microcosms were measured almost every morning between 0900 and 1030 h with a portable water quality checker (U-10, Horiba). Water turbidity and nutrients (Hach Model DR/2000 spectrophotometer) as well as bioavailable metals (through Atomic Absorption Spectroscopy with a Varian SpectrAA 220 spectrophotometer) were analyzed at 4-day intervals according to the methods described in APHA (1995).

Chlorophyll-a of the phytoplankton was also sampled at 4-day intervals by filtering a known water sample volume (150 mL) though a Whatman GF/C glass-fiber filter (mesh size, 0.45 μm), followed by extraction with 80% ethanol and quantification through spectrophotometry as described by Moed and Hallegraeff (1987). Suspended solids were measured at the beginning (day 1), middle (day 12) and end (day 24) of the experiment through gravimetry using fiberglass filters (GF/C – 47 mm; Teixeira et al., 1965). Sediment samples were taken at the beginning and end of the experiment and analyzed for organic carbon content (incineration at 550 °C), total phosphorous and nitrogen content, and bioavailable metals (APHA, 1995).

To confirm the nominal concentrations of abamectin in the tests, stock solutions were analyzed by HPLC/MS/MS. Analysis were made by means of a HPLC/MS/MS Agilent® 6490 series attached to a Zorbax Eclipse plus C18 Agilent column (mobile phase water:acetonitrile = 10:90 V/V). Given the great distance between the laboratories where the toxicity tests and chemical analysis were conducted and the fact that abamectin is known to degrade really quickly in water (Wislocki et al., 1989; Ali et al., 1997), it was not possible to accurately determine the abamectin

concentration throughout the course of the experiments. Subsequently, toxicity values and test concentrations mentioned throughout the manuscript refer to confirmed nominal test concentrations.

2.6. Data analysis

Since non-parametric data was obtained in the acute toxicity tests, data was evaluated by the Trimmed Spearman-Karber method and expressed as LC50-24 h and LC50-48 h with their 95% confidence intervals (Hamilton et al., 1977; US-EPA, 2002). In the chronic tests, ANOVA was used to verify significant differences in the survival of the test organisms in the treatment groups compared with the control group to establish LOEC-NOEC values. All these statistical tests were performed with the Toxstat 3.4 software. Given that the use of EC10 values to express toxicity in chronic tests (besides NOECs) has increased over the past years (e.g. Landis and Chapman, 2011; Jager, 2012), EC10 values were also calculated in the chronic tests by probit regression analysis using IBM SPSS Statistics 20.

3. Results and discussion

3.1. Toxicity thresholds of Vertimec (a.i. abamectin) for C. silvestrii

Control survival in all acute tests and in two out of the three chronic tests was 100%. In the third chronic test, only a single individual did not survive in the control treatment (mortality rate 10%). The reference test using NaCl also indicated that the sensitivity of the test organisms was in the expected range after acute (LC50 ranged between 1 and 1.34 g/L; reference range: 1–1.83 g/L) and chronic (IC25 = 0.15 g/L; reference range: 0.1–0.34) exposure.

Since avermectins like abamectin have often been reported to be quickly photo-degradated (Tišler and Eržen, 2006; Campbell, 1989), the influence of conducting the acute tests under (artificial) light and in darkness was evaluated in the present study. As can be seen from Fig. 1, no difference in LC50 values between the tests with or without light were noted (ANOVA; $p > 0.05$). This either indicates that under the test conditions abamectin could exert its toxic effect before a possible (photo-)degradation or that the resulting metabolite(s) had a toxicity similar to that of abamectin. In the draft assessment report for the Registration of abamectin on the European market, the formation of metabolites in environmentally relevant concentrations in the water phase is not considered likely, except for the photolysis product [8,9-Z]-avermectin B1a (=NOA 427011; EC, 2006). The very different LC50 values (0.082 and 14 μg/L) in the two tests evaluating the toxicity of this metabolite to *Daphnia magna* listed in that report, however, do not allow any solid conclusion on this.

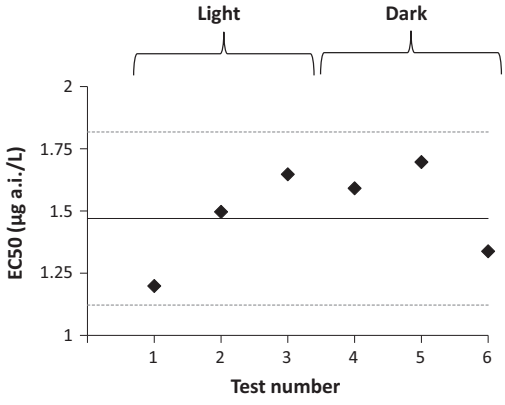

Fig. 1. EC50-48 h values generated for Vertimec with *C. silvestrii* in the present study under dark and light. The dark and dotted horizontal lines indicates the average EC50-48 h value (1.47 μg a.i./L) and 95% confidence interval limits (1.1–1.8 μg a.i./L), respectively. No significant differences in toxicity values obtained under light and dark conditions were detected.

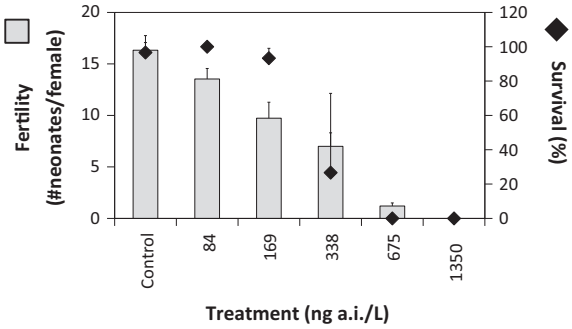

Fig. 2. Effects of Vertimec on survival (%) and fertility (#neonates per female) in the chronic toxicity test. No observed effect concentrations (NOEC) after 8 days of exposure were 169 and 84 ng a.i./L for survival and fertility, respectively.

The LC50-48 h values for acute effects calculated in the present study ranged between 1.2 and 1.7 μg a.i./L (min–max), with an average LC50-48 h of 1.47 a.i. μg/L (Fig. 1). Regarding chronic effects, NOEC-8 d values of 169 ng a.i./L and 84 ng a.i./L were noted for survival and fertility (number of neonates per female), respectively (Fig. 2). The average chronic EC10 value for survival (157 ng a.i./L) was comparable with its corresponding NOEC value. For fertility, the EC10 values obtained in one of the tests (69 ng a.i./L) was also in line with its corresponding NOEC value. SPSS calculations of EC10 values for fertility in the other two tests did not result in reliable EC10 estimates, possibly due to the lack of low percentage effects at the concentrations tested.

When comparing the LC50 values of *C. silvestrii* with those listed for other aquatic invertebrates, it can be deducted that *C. silvestrii* is less sensitive to abamectin than other cladocerans (Table 1). This may be related with the relatively small body size of *C. silvestrii*, since smaller cladoceran species have been found to be generally less sensitive to insecticides compared with larger species (Hanazato, 1998). For example, Daam et al. (2008) showed that the small cladoceran species *Ceriodaphnia cornuta* was less sensitive than larger *Moina micrura* in tropical outdoor microcosms evaluating the insecticide chlorpyrifos under tropical conditions. Nevertheless, there does not appear to be a consistent trend of a lower sensitivity of smaller-sized cladocerans (Sánchez-Bayo, 2006). In line with this, Moreira et al. (2014) reported that *C. silvestrii* and another small cladoceran species naturally occurring in Brazil, *Macrothrix flabelligera*, were more susceptible to the herbicide atrazine than *D. magna*. Similarly, *C. silvestrii* and *C. dubia* were concluded to be more sensitive test organisms than *D. magna* and *D. similis* to the majority of reference substances tested by Freitas and Rocha (2011).

Comparison of the EC10 and NOEC values obtained in the present study with those listed in Table 1 is hampered due to difference in test duration (8 d in the present study as compared to 21 d and 28 d for *A. bahia* and *D. magna*, respectively). Subsequently, the higher EC10 and NOEC values obtained in the present study may also be related with this lower exposure duration, rather than the possible reasons outlined above. On the other hand, given that *C. silvestrii* has a much shorter life cycle than e.g. *D. magna*, the animals were exposed for a similar part of their life cycle (Fonseca and Rocha, 2004; ABNT, 2005).

Using the data obtained in the present study and those listed in Table 1, the acute-to-chronic ratio (ACR; as LC50-48 h/NOEC repro-

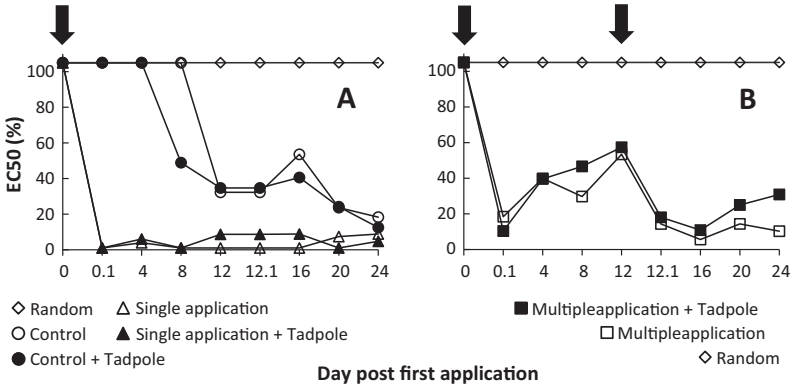

Fig. 3. Percentage dilutions of the microcosm water leading to 50% immobilization of *C. silvestrii* in the acute toxicity tests over the course of the experiment. Effects of the single application (A) and repeated application (B) are provided in separate subfigures to allow a clear visualization of effects. An EC50 greater than 100% indicates that the undiluted sample exerted an effect less than 50%.

duction) for *C. silvestrii* (8 d), *D. magna* (21 d) and *A. bahia* (28 d) are 17.5, 26–56 and 60, respectively. Subsequently, negative effects on fertility increase markedly with time of exposure, since the difference between acute and chronic toxicity becomes larger as the exposure time increases from 8 to 28 days. This hence indicates that there may not be a dose threshold for this compound in regard to fertility, because no matter how small abamectin concentrations in water may be, they can produce toxic effects given sufficient time of exposure (Hoang et al., 2007; Tennekes and Sánchez-Bayo, 2011; Vogs et al., 2013).

3.2. Toxicity of water samples from the microcosm experiment

The LC50 values calculated as % dilution of the water samples from the microcosms over the course of the experiment are visualized in Fig. 3. Irrespective of the presence of tadpoles, samples from the microcosms applied with the single concentration of 144 µg a.i./L remained toxic until the end of the experiment, even at the highest dilution (3.1%; Fig. 3). From both laboratory and field studies, it is known that abamectin rapidly degraded in water, with reported half-lives of 4–6 h at the water surface and 4 days in the water column (Wislocki et al., 1989; Ali et al., 1997). Subsequently, *a priori* it was expected that in the later stages of the experiment at least the greater dilutions would have showed less toxicity.

In the previous microcosm experiment evaluating Vertimec by Novelli et al. (2012b), toxicity also persisted for more time than was anticipated based on toxicity tests with the active ingredients abamectin. The authors attributed this to the presence of other (more persistent) components in the formulation besides abamectin. It has often been discussed that the toxicity of pesticides in their commercial formulation may be greater than that of the active ingredient alone (e.g. Pereira et al., 2009; Beggel et al., 2010). However, after comparing their respective toxicity values, EC (2006) concluded that "the toxicity of the 18 g a.s./L EC formulation to *D. magna* and *Oncorhynchus mykiss* is comparable to that of abamectin when expressed on the basis of active substance". Another possibility is that the degradation rate of abamectin (and/or eventual other substances in the Vertimec formulation) was slower than the 4 days recorded in Wislocki et al. (1989) and Ali et al. (1997). It is often believed that degradation in tropical freshwaters is faster than in their temperate counterparts. Daam and Van den Brink (2010), however, discussed several reasons why this may not be the case, including (i) decreased photolysis due to turbid water conditions in the tropics; (ii) lower organic content of the sediment; (iii) differences in prevailing pH regime; and (iv) influence of the dimensions of the (artificial) ecosystem under study. In line with this, the two model ecosystem studies using different experimental designs that are listed in the draft assessment report of abamectin revealed half-lives for the water compartment of 4.9 days and 9–10 days (EC, 2006). Since abamectin concentrations could not be measured throughout the course of the experiment, the persistence of abamectin in the microcosms could not be determined. Similarly, (persistent) toxic metabolites may or may not have been formed as discussed above.

Physico-chemical water quality in general may also have played a role in the greater effects on *C. silvestrii* than was expected. For example, the death of the tadpoles after the single high application was accompanied with a drop in dissolved oxygen from around 6–7 mg/L to 0.1 mg/L in the first week post application. More persistently throughout the course of the experimental period was the increase in ammonium concentrations from levels around 50 µg/L before application to levels of 300–600 µg/L from 4 days post application onwards. Although the toxicity of ammonia to aquatic organisms has primarily been linked to the un-ionized form NH_3, the ionized NH_4^+ form may be transformed to NH_3 especially at higher pH and water temperatures (Andersen and Buckley, 1998). Increased ammonium levels may also be related with the toxicity encountered in the control groups from the second week p.a. onwards (Fig. 3). This increase in ammonia levels over time is likely to have originated from microbial metabolism in the sediment of the microcosms related with the denitrification process known to be carried out by bacteria (Camargo and Alonso, 2006; Barry and Logan, 1998).

Indeed, ammonium levels in the control group with tadpoles followed the same trend as that found in the single application treatment above. A significant correlation between the measured NH_4^+ concentrations and calculated LC50 values was encountered

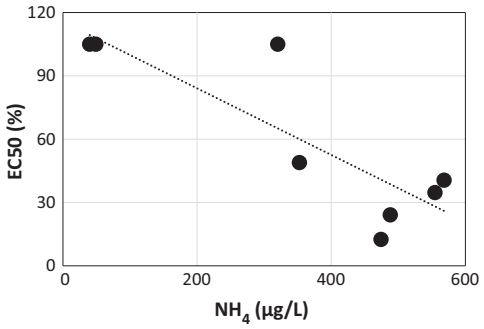

Fig. 4. Correlation between ammonium concentration (in µg/L) and EC50 (in % dilution) in the control treatments with tadpoles.

for this treatment ($r = 0.85$; $p < 0.05$; Fig. 4). Although this could not be verified for the control group without tadpoles, ammonium levels in this treatment increased from approx. 5 μg/L on day 0–231 on day 12.

Confinement of ecosystems and the experimental design adopted have been reported to have a profound effect on the enclosed ecosystem structure and functioning (e.g., Stephenson et al., 1986; Petersen and Hastings, 2001; Caquet et al., 2001; Brock et al., 2008). Based on the classification system by Lamparelli (2004) using total phosphorous (mean ± SD: 280 ± 168 μg/L) and chlorophyll-a (mean ± SD: 26 ± 56 μg/L) values, the ecosystems in all treatments were classified as eutrophic to hyper-eutrophic throughout the experiment. These highly productive and hence unstable systems may have been especially prone to the effects of enclosing part of the ecosystem in the surrounding tank. Ammonia has indeed been identified as the most variable physico-chemical parameters in control microcosms (Caquet et al., 2001 and references therein). The relatively low surface to volume ratio of the tanks used may have impeded air-water exchanges and light penetration, leading to an insufficient nutrient recycling. Due to high temperatures and light levels, model ecosystems in tropical countries are especially prone to inter-tank variability and ecosystem instability (Daam and Van den Brink, 2011).

As discussed above, the effect of the single high Vertimec treatment prolonged for a longer period than was anticipated. The magnitude of the effect directly after application, however, was as expected. The nominal test concentration (144 μg a.i./L) indicates a concentration of 4.5 μg a.i./L at the greatest dilution (3.125%), which is 3 times higher than the LC50 value determined in the present study for *C. silvestrii* (1.47 μg a.i./L). The magnitude of effect immediately after the first and second application of 36 μg a.i./L, however, was less than expected based on the concentrations estimated from the % dilutions leading to 50% effect and the nominal concentration. After the first and second application, estimated concentrations leading to 50% effect were 6.6 and 5.2 μg a.i./L (no tadpoles present) and 3.7 and 6.5 μg a.i./L (tadpoles present), respectively. This may be due to the fact that abamectin is known to be readily adsorbed by organic matter, soil and sediment particles (Tišler and Eržen, 2006) as well as algal cells (OECD, 2000). Subsequently, a part of the applied abamectin may not have been bioavailable. The magnitude of effect after the second application was the same as after the first application, which supports the hypothesis that abamectin disappeared fast from the water column.

4. Conclusion

C. silvestrii is a native and widely distributed cladoceran in Brazil and methods for laboratory toxicity testing have previously been developed. It had been shown sensitive to a wide range of compounds and also the present study indicated toxicity values in the ng/L to low μg/L range for abamectin. The concentration that may be expected in runoff water after application of Vertimec at the recommended dose for strawberry crop in Brazil indicated clear toxic effects on *C. silvestrii*. Previous studies also indicates that acute and chronic effects at this exposure level may be expected on *D. similis* and *C. dubia*, respectively. This thus indicates a potential risk of Vertimec to aquatic life in edge-of-field waterbodies in Brazilian strawberry regions at recommended agricultural practices.

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