



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



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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 adsorbance 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.

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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

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(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).

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)
<i>EC50</i>			
<i>Daphnia similis</i>	Cladocera	Static, 48 h, mortality	0.0051 ^c
<i>Daphnia pulex</i>	Cladocera	Static, 48 h, immobility	0.12–0.28
<i>Americamysis bahia</i>	Mysida	Static, 96 h, mortality	0.21 ^a
<i>Daphnia magna</i>	Cladocera	Static, 48 h, immobility	0.26–0.56
<i>Simocephalus</i> sp.	Cladocera	Static, 48 h, immobility	0.3
<i>Daphnia longispina</i>	Cladocera	Static, 48 h, immobility	0.38
<i>Diaphanosoma</i> sp.	Cladocera	Static, 48 h, immobility	0.53
<i>Daphnia galeata</i>	Cladocera	Static, 48 h, immobility	0.55
<i>Aedes aegypti</i>	Insecta	Static, 48 h, mortality	2.2 ^b
<i>Chironomus xanthus</i>	Insecta	Static, 96 h, mortality	2.7 ^c
<i>Thamnocephalus platyurus</i>	Anostraca	Static, 24 h, immobility	2.8–30
<i>Gammarus</i> sp.	Amphipoda	Static, 48 h, immobility	6.2–8.6
<i>Chaoborus</i> sp.	Insecta	Static, 24 h, immobility	41–190
<i>Lymnaea stagnalis</i>	Gastropoda	Static, 48 h, mortality	55
<i>Crassostrea virginica</i>	Bivalvia	Static, 48 h, mortality	430
<i>Brachionus calyciflorus</i>	Rotifera	Static, 24 h, immobility	4000
<i>NOEC</i>			
<i>Americamysis bahia</i>	Mysida	Flow-through, 28 d, reproduction	0.0035
<i>Daphnia magna</i>	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 CaCO₃). The organisms were fed daily with the algae *Raphidocelis subcapitata* (10⁶ 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 polypropylene plastic cup containing five 6–24 h old neonates in 10 mL of test solution.

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