Chemosphere 86 (2012) 36–40

Contents lists available at SciVerse ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Lethal effects of abamectin on the aquatic organisms *Daphnia similis*, *Chironomus xanthus* and *Danio rerio*

Andréa Novelli [a,*], Bruna Horvath Vieira [a], Daniela Cordeiro [b], Luciana Teresa Dias Cappelini [b], Eny Maria Vieira [b], Evaldo Luiz Gaeta Espíndola [a]

[a] *Núcleo de Estudos em Ecossistemas Aquáticos, Programa de Pós-Graduação em Ciências da Engenharia Ambiental, Universidade de São Paulo, Av. Trabalhador SanCarlense, 400, 13.560-970 São Carlos, SP, Brazil*
[b] *Instituto de Química de São Carlos, Universidade de São Paulo, PC 780, São Carlos, SP, Brazil*

ARTICLE INFO

Article history:
Received 29 March 2011
Received in revised form 24 August 2011
Accepted 28 August 2011
Available online 28 September 2011

Keywords:
Abamectin
Toxicity tests
Daphnia similis
Chironomus xanthus
Danio rerio

ABSTRACT

Abamectin is used as an acaricide and insecticide for fruits, vegetables and ornamental plants, as well as a parasiticide for animals. One of the major problems of applying pesticides to crops is the likelihood of contaminating aquatic ecosystems by drift or runoff. Therefore, toxicity tests in the laboratory are important tools to predict the effects of chemical substances in aquatic ecosystems. The aim of this study was to assess the potential hazards of abamectin to the freshwater biota and consequently the possible losses of ecological services in contaminated water bodies. For this purpose, we identified the toxicity of abamectin on daphnids, insects and fish. Abamectin was highly toxic, with an EC_{50} 48 h for *Daphnia similis* of 5.1 ng L^{-1}, LC_{50} 96 h for *Chironomus xanthus* of 2.67 μg L^{-1} and LC_{50} 48 h for *Danio rerio* of 33 μg L^{-1}.

1. Introduction

The avermectins (AVMs) are compounds derived from the fermentation of the soil bacterium *Streptomyces avermitilis*. This group includes 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 (Campbell, 1989; Kövecses and Marcogliese, 2005).

Ivermectin was the first to be commercialized and is commonly used to control ecto- and endoparasites in livestock, aquaculture, pets and humans. Subsequently, abamectin (ABM) was marketed as an acaricide and insecticide for fruits, vegetables, ornamental plants and also as anthelmintic for animals (Campbell, 1989; Ali et al., 1997). Abamectin is a mixture that contains about 80% avermectin B_{1a} and 20% avermectin B_{1b}, which have similar biological and toxicological properties (Campbell, 1989; Lankas and Gordon, 1989; Fisher and Mrozik, 1992).

AVMs act mainly on the nervous system of organisms and although the antiparasitic activity of AVMs was first described in 1979, its action mechanism has not been fully elucidated. However, it is usually related to GABAergic receptors (γ-aminobutyric acid) in invertebrates as well as vertebrates, and glutamate-gated chloride channels in invertebrates. Most authors mention that the AVMs can act not only as GABA agonists, but also can stimulate the release of GABA in the presynaptic inhibitory terminals. In both cases, they increase the permeability of chloride ions, hyperpolarizing the nerve and muscle cells, ultimately interfering with neuromuscular transmission, leading to death (Mellin et al., 1983; Turner and Schaeffer, 1989; Cully et al., 1994). In fish, the AVMs can cross the blood–brain barrier and cause toxicity (Høy et al., 1990).

ABM, on the other hand, shows low levels of toxicity to mammals but is extremely toxic to microcrustaceans and fish (Wislocki et al., 1989). The toxicity levels reported in the literature are 0.25 μg L^{-1} (EC_{50} 48 h) for *Daphnia magna* and 55.1 μg L^{-1} (LC_{50} 96 h) for *Danio rerio* (Tišler and Eržen, 2006). Although abamectin is not directly used in aquatic ecosystems, it may have adverse effects on aquatic biota, since mesocosm studies have shown the effects of Vertimec® 18EC, whose active ingredient is abamectin, on zooplankton (Novelli, 2010) and phytoplankton (Vieira, 2010) due to direct application as well as through runoff from experimentally contaminated plots.

Considering that accidental introductions of ABM can occur in aquatic ecosystems and that contamination from runoff into water bodies from agricultural areas often occurs, representing a potential threat to the aquatic community, in this part of the research we evaluated the effect of this contaminant on aquatic organisms from different trophic levels, namely *Daphnia similis* (a zooplankton), *Chironomus xanthus* (an insect) and *D. rerio* (a fish).

* Corresponding author. Tel.: +55 16 3373 8258; fax: +55 16 3373 8251.
E-mail address: deanovelli@gmail.com (A. Novelli).

0045-6535/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2011.08.047

2. Materials and methods

2.1. Cultivation of test organisms

Neonates of *D. similis* and larvae of *C. xanthus* were obtained from cultures kept at the Laboratory for Ecotoxicology and Ecophysiology of Aquatic Organisms at the Center for Water Resources and Applied Ecology (CRHEA), part of the São Carlos School of Engineering (EESC), University of São Paulo (USP), Brazil.

The *D. similis* cultures were kept under controlled temperature (24 ± 2 °C) and photoperiod (16:8 h light/dark) in reconstituted water with 7.0–7.6 pH, conductivity of 160 μS cm^{-1} and hardness between 40 and 48 mg L^{-1} for $CaCO_3$. The organisms were fed daily with *Pseudokirchneriella subcapitata* chlorophycean algae (10^6 cells mL^{-1}) and Vitormonio® (1 mL L^{-1}), a composition prepared with yeast and fish food. All procedures followed the Brazilian standard ABNT, NBR 12713/2004.

The *C. xanthus* cultures were kept in plastic trays covered with nylon cages to retain the adult organisms. Natural sediment and reconstituted water were placed in these trays (pH between 6.5 and 7.0, conductivity between 25–55 μS cm^{-1} and hardness between 12 and 16 mg L^{-1} for $CaCO_3$). The organisms were kept under constant aeration, temperature-controlled at 23 ± 2 °C and a 12:12 h light/dark photoperiod. The larvae were fed with commercial fish food (0.04 mg mL^{-1}/d) and the alga *P. subcapitata* (10^5 cells mL^{-1}) only on the first day of cultivation (Fonseca and Rocha, 2004).

The *D. rerio* juveniles were obtained commercially and kept in the laboratory for acclimation in 25-L aquaria in reconstituted water (pH between 7.0 and 7.6 and hardness between 40 and 48 mg L^{-1} for $CaCO_3$). The organisms were kept under temperature-controlled constant aeration, (25 ± 2 °C) and a 12:12 h light/dark photoperiod. The food was the commercial feed Tetramin®, given twice a day, which was suspended 24 h before the toxicity tests. The procedures followed the Brazilian standard ABNT, NBR 15088/2004.

Acute tests with the reference substance potassium chloride were carried out during the tests, to evaluate the physiological conditions of the organisms, thus validating the tests.

2.2. Toxicity test

The abamectin (CAS – No. 71751-41-2; 80% avermectin B_{1a} and 4% avermectin B_{1b}) was obtained from Sigma–Aldrich (Brazil). A stock solution of abamectin was prepared for each test by dissolving abamectin in acetone (analytical grade). The stock solution was protected from light to avoid photodegradation.

All test solutions were prepared by serial dilution of stock solution diluted in water reconstituted from the cultures of each respective test organism. Thus, for acute toxicity tests with *D. similis*, the nominal concentrations were 2.5, 5, 10, 20, 40 and 80 ng L^{-1}; for *C. xanthus* they were 0.2, 0.6, 1.8, 5.4, 16.4 and 48.6 μg L^{-1} and for *D. rerio* they were 20, 40, 60, 80 and 100 μg L^{-1}. To exclude the possibility of the solvent's toxic effect influencing the toxicity results, a negative control was prepared, with the highest concentration of acetone used in the toxicity test, never exceeding the maximum concentration of 50 μl L^{-1}. In total, four definitive tests were performed with each test organism, and in the last test the initial and final concentrations of abamectin were quantified by chromatography.

In the acute toxicity tests with *D. similis*, five organisms (<24 h old) were used in four replicates, exposed in nontoxic plastic cups containing 10 mL of test solution. The specimens were kept under controlled temperature (24 ± 2 °C) and photoperiod (16:8 h light/dark) for 48 h.

For the tests with *C. xanthus*, six larvae were added (IV instar – 7/8 d) to 200 mL of test solution with 50 g of natural sediment, in three replicates. The sediment was composed of sterilized fine sand (sterilized at 550 °C for 2 h) from the surface layer of freshwater lakes. The organisms were kept at room temperature of 23 °C ±2, photoperiod of 12:12 h light/dark with food only the first day. The test lasted 96 h, after which the living organisms were counted.

In the acute tests with *D. rerio* (static), five organisms were used in two replicates. The test lasted 48 h, kept under controlled temperature (25 °C ±2) and photoperiod (12:12 h light/dark). The fish used had average length of 1.14 cm (±0.08) and wet weight of 0.02497 g (±0.006).

The measurements of pH (Micronal B374 potentiometer), conductivity (Orion 145A conductivimeter) and dissolved oxygen (OD YSI meter) of the water samples tested were performed at the beginning and end of all toxicity tests.

2.3. Analysis of abamectin in the water

The initial and final concentrations of abamectin in the test solutions were quantified, for the last test performed, determining the major avermectin component in the samples (avermectin B_{1a}) which showed higher signal intensity for analysis with a HPLC/FLU apparatus (high performance liquid chromatograph/fluorometric detector) (Agilent® 1200 series).

Before sample extraction, 200 mL was previously filtered in a cellulose acetate membrane (0.45 μm). Next, the sample was percolated through a cartridge containing the adsorbent C-18 (SampliQ, Agilent Technologies®, 30 × 6 mL, 500 mg). To extract the water sample, the adsorbent was conditioned with 10 mL of acetonitrile (P.A.) and 5 mL of acetonitrile (P.A.): organic-free water: triethylamine (P.A.) (30:70:0.2, v/v/v). After this stage, the sample was percolated in the cartridge, dried for 3 min and then the analyte was eluted with 20 mL of dichloromethane (P.A.). The full procedure described above was performed under 15 kPa pressure.

The extract eluted from the cartridge containing the adsorbent C-18 was dried under a gentle stream of nitrogen and reconstituted with 1 mL of acetonitrile (P.A.). Next, derivatization was performed in a dark amber bottle with 200 μL of 1-metilmidazol (>99%) and 200 μL of acetonitrile solution: trifluoacetic anhydride (2:1). After 1 h, 100 μL of the sample was injected into the HPLC/FLU device.

For the analysis of abamectin by HPLC/FLU the following chromatographic conditions were used: isocratic mobile phase (organic-free water, acetonitrile and tetrahydrofuran p.a. at a ratio of 14:80:6, respectively); flow (1.0 mL min^{-1}); injection (100 μL); column temperature (25 °C); column (Partisil 5 ODS-3, 4.6 mm × 250 mm, 5 μm, Whatman®); fluorescence detector (excitation at 364 nm and emission at 475 nm) and running time (14 min).

The parameters used to validate the method were selectivity, limits of detection and quantification, linearity, accuracy, precision and recovery (Lanças, 2004). The chromatogram generated showed that the analysis method for abamectin in water was selective. For the limit of detection (LOD), the concentration at which the abamectin signal was three times larger than the noise signal was considered, and for the limit of quantification (LOQ) the concentration at which the analyte was five times higher than noise was considered. The results for LOD were 0.1 μg L^{-1} and for LOQ they were 0.2 μg L^{-1}. The concentrations used to calculate linearity were between 0.2 and 10.0 μg L^{-1}, with seven points in triplicate. The result of the linear correlation coefficient of the curve was 0.994, and the relative standard deviation results of precision and accuracy of the method were 112.46% and 103.64%, respectively. The average recovery was 108.70%.

2.4. Data analysis

The acute toxicity test results were analyzed using the trimmed Spearman-Karber method and expressed as EC_{50} 48 h (*D. similis*), LC_{50} 96 h (*C. xanthus*) and LC_{50} 48 h (*D. rerio*) (Hamilton et al., 1977).