Chemosphere 144 (2016) 1408–1414

Contents lists available at ScienceDirect

Chemosphere

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

Impact of runoff water from an experimental agricultural field applied with Vertimec® 18EC (abamectin) on the survival, growth and gill morphology of zebrafish juveniles

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HIGHLIGHTS

- Vertimec® 18EC is a commercial formulation of abamectin.
- Tests were conducted utilizing runoff water contaminated with Vertimec. Ecotoxicological tests with *Danio rerio.*
- No effects of runoff water were recorded on survival of the zebrafish.
- There were significant differences in growth as well as gill morphology of zebrafish.

ARTICLE INFO

Article history:
Received 3 July 2015
Received in revised form
30 September 2015
Accepted 1 October 2015
Available online xxx

Keywords:
Pesticide
Avermectin
Toxicity test
Fish
Gill morphology

ABSTRACT

Edge-of-field waterbodies in tropical agroecosystems have been reported to be especially prone to pesticide contamination through runoff resulting from intensive irrigation practices and tropical rainfall. In the present study, the effects of runoff from an experimental agricultural field applied with Vertimec® 18EC (active ingredient: abamectin) on zebrafish were evaluated. To this end, the experimental field was applied with the Vertimec® 18EC dose recommended for strawberry crop in Brazil, whereas another field was treated with water only to serve as control. No effects of runoff water from either plot were recorded on survival. Water from the treated field led to increased growth and gill alterations. In general, these alterations were of the first and second degree, including proliferation of cells between the secondary lamellae, dilation at the lamellar apex, detachment of the respiratory epithelium and aneurism. These results confirm the high toxic potential of Vertimec® 18EC and provide evidence that environmental risks are likely to occur in areas subject to runoff containing this pesticide.

1. Introduction

Vertimec® 18EC is a commercial formulation containing the active ingredient (a.i.) abamectin. It has been used as an acaricide, insecticide and nematicide on various fruit and vegetable crops as well as ornamental plants in many parts of the world including Brazil (Nunes and Espíndola, 2012; Riga et al., 2014).

Abamectin belongs to the avermectin (AVM) family, a group that also includes ivermectin and doramectin. AVMs are derived from the fermentation of the actinomycete *Streptomyces avermitilis*, and are highly lipophilic compounds, with low solubility in water and high adsorption to organic matter (Campbell, 1989; Ŏmura, 2008). Abamectin is a mixture that contains around 80% avermectin B_{1a} and 20% avermectin B_{1b}, which have similar biological and toxicological properties (Campbell, 1989). Previous laboratory bioassays evaluating the toxicity of abamectin to aquatic organisms conducted at our facilities indicated toxicity values as low as 5.1 ng L^{-1} (EC_{50} 48 h) for *Daphnia similis*, 2.7 μg L^{-1} (LC_{50} 96 h) for

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http://dx.doi.org/10.1016/j.chemosphere.2015.10.004
0045-6535/

Chironomus xanthus and 33 μg L^{-1} (LC_{50} 48 h) for *Danio rerio* (Novelli et al., 2012a). In addition, mesocosms contaminated with Vertimec® 18EC showed acute and chronic effects, especially for species of Copepoda and Cladocera (Novelli, 2010) and on the test-organism *D. similis* (Novelli et al., 2012b) after direct application of 40 μg a.i. L^{-1} as well as after adding runoff water from plots experimentally contaminated with the recommended dose of Vertimec® 18EC for strawberry crop. The AVMs act mainly on the nervous system although their mechanism of action has still not been fully elucidated. In general it is known to be related to gamma-aminobutyric acid (GABA) receptors in both invertebrates and vertebrates, and also to glutamatergic receptors in the chloride channels of invertebrates (Cully et al., 1994; Clark et al., 1994; Martin, 1997). Most authors mention that AVMs may act not only as GABA agonists, but may also stimulate the release of GABA in the presynaptic inhibitory terminals (Mellin et al., 1983; Turner and Schaeffer, 1989; McKellar and Benchaoui, 1996). In both processes, the permeability to chloride ions increases, leading to hyperpolarization of nerve and muscle cells. This interferes with the transmission of neural signals, causing death (Campbell, 1989). Unlike in mammals, AVMs can cross the blood–brain barrier in fish and cause toxicity (Høy et al., 1990).

Fish are widely used to evaluate the health of aquatic ecosystems and the endpoints most often measured include survival, growth (length and weight) and histological changes (Hoffman et al., 2003; Fanta et al., 2003; Freiry et al., 2014; Braz-Mota et al., 2015). The gill epithelium is the main contact surface with the environment and is an important target of pollutants, which may pass through the gill lamellae, penetrate the blood stream and cause various levels of damage to the animal (Evans et al., 2005). Because of this, the gill epithelium has been used in various studies to assess the toxic potential of xenobiotics both in natural aquatic ecosystems and in the laboratory (Richmonds and Dutta, 1989; Campagna et al., 2008; Fracácio et al., 2008; Costa et al., 2009). The main histological changes that occur in the gills because of direct contact with contaminants are edema, epithelial hyperplasia of the secondary lamellae, infiltration of epithelial cells, lamellar fusion and necrosis of the secondary lamellae (Takashima and Hibiya, 1995). Pesticides such as Vertimec® 18EC may enter the aquatic environment surrounding agricultural fields via spray drift, runoff, drainage and/or accidental spills. Various studies have shown that surface runoff from farms is one of the main means of contamination of surface water, especially in tropical agro-ecosystems due to intensive irrigation practices and tropical rainfall (Daam and Van den Brink, 2010; Novelli et al., 2012b). Chemicals in the environment are influenced by many processes that determine their persistence, mobility and bioavailability (Gravilescu, 2005). Among the environmental characteristics that most influence the dynamic of pesticides are climate (temperature, rainfall, insolation and winds), physical and chemical soil properties (organic matter and clay contents, pH and moisture), microbiological activity, compaction and plant cover (Waxman, 1998). Thus, experiments conducted in the field along with those in the laboratory can generate more ecologically relevant information than laboratory tests evaluating single-peak or constant exposure scenarios alone, because the former simulate real situations reducing the degree of uncertainty on the risk of agricultural chemicals in actual use (Graney et al., 1995).

In light of this context, the purpose of this study was to assess whether the exposure to Vertimec® 18EC (a.i. abamectin) affects the survival, growth and gill morphology of *D. rerio* juveniles. To evaluate the risk of an environmentally realistic exposure level, short-term chronic toxicity tests were conducted utilizing runoff water from experimental field plots applied with the recommended dose for strawberry crop in Brazil (MAPA, 2015). The choice of strawberry was due to the intensive use of this pesticide on this crop in Brazil (Nunes et al., 2009).

2. Materials and methods

2.1. Experimental design

Vertimec® 18EC (Syngenta Proteção de Cultivos Ltda., Brazil) is an emulsifiable concentrate containing 18 g L^{-1} of the active ingredient abamectin. In Brazil, Vertimec® 18 EC is registered for use on 23 different crops (MAPA, 2015). For strawberry crops, it is recommended in dosages from 50 to 75 mL 100 L^{-1} of water with applications of 1.000–1.250 L spray mix/ha (equivalent to 9–16.88 g a.i. ha^{-1}; MAPA, 2015).

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), two agricultural plots with a slope of 6% and measuring 8 m^2 each were set out 6 m apart from one another. Before the experiment, the plots were weeded and plowed. The soil was predominantly sandy, mostly composed of fine sand (45.6%) and with an organic matter content of 13.45% (for more details, the reader is referred to Braun et al., 2012).

One of the two plots was contaminated with Vertimec® 18 EC using a backpack sprayer following the manufacturer's instructions provided for strawberry plants on the pesticide label. To test the recommended dosage for strawberry crop, a volume of 0.75 mL Vertimec® 18 EC was mixed with 1 L distilled water. Subsequently, this was applied to the plot. The other plot was only sprayed with water to serve as a control. To prevent contamination, the control plot was covered with a plastic tarp during pesticide application to the treatment plot. Then a torrential rainfall event was simulated upstream of the plots. The water used as rainfall came from the Lobo Reservoir next to CRHEA (pH: 7.02; conductivity: 16.1 μS cm^{-1}; suspended solids: 2.08 mg L^{-1}; turbidity 10 NTU; hardness: 6 mg L^{-1} $CaCO_3$ $L^{-1}$and dissolved oxygen: 8.74 mg L^{-1}). The intensity (19 mm) was based on the historical records obtained from the weather station of CRHEA/EESC/USP for the same period of the year as the experiment was conducted. The experiment was conducted during the summer season in Brazil, and on the day of application the temperature was 24 °C and there was no wind.

The runoff water samples were collected downstream from the experimental plots by placing plastic tarps in depressions dug when setting up the test system. Part of the runoff water collected was taken to the laboratory for physicochemical characterization and to conduct toxicity tests with the *D. rerio* juveniles.

2.2. Physicochemical parameters of the runoff water

The water samples were analyzed for the parameters pH (Micronal B374 potentiometer), conductivity (Orion 145A conductivimeter) and dissolved oxygen (OD YSI meter). The turbidity was measured using a spectrophotometer (Hach DR/2000; APHA, 1995) and the organic/inorganic suspended solids were measured by gravimetry in fiberglass filters (GF/C – 47 mm; Teixeira et al., 1965). The abamectin concentration was quantified in the 100% runoff sample by liquid chromatography (Shimadzu model SCL-10A) with an SPD-10A UV detector, and was confirmed by GC–MS (Shimadzu model QP2010) using the operating parameters as provided and validated in Lanças (2004).

2.3. Short-term chronic toxicity tests

The *D. rerio* juveniles were obtained commercially and kept in the laboratory for acclimation for 7 days in 25-L aquaria, with replacement of 1/3 of the water volume with fresh reconstituted