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Acute toxicity of cypermethrin to the non target organism *Hyalella curvispina*

*Hernán Mugni**, *Ariel Paracampo, Natalia Marrochi, Carlos Bonetto*

ILPLA (CONICET-CCT La Plata) – UNLP Instituto de Limnología "Dr. Raúl. A. Ringuelet", Av. Calchaquí Km. 23.5, 1888 Florencio Varela, Buenos Aires, Argentina

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ABSTRACT

The acute toxicity of cypermethrin to the amphipod *Hyalella curvispina* was evaluated by means of a toxicity test under laboratory conditions. Cypermethrin is one of the most widely used insecticides in Argentina. *H. curvispina* is a widely distributed and commonly abundant component of the invertebrate assemblages in shallow waters of southern South America. The experiments were repeated three times. The mean 48-h LC_{50} value for *H. curvispina* was estimated at 0.066 μg/l. *H. curvispina* represents a good model for exotoxicological risk assessment.

1. Introduction

Genetically modified crop production has increased considerably around the world. At present, soy represents roughly one-half both of the total harvest and of the cultivated area in Argentina (53 million tons and 18 million ha, respectively; FAO, 2012). The United States, Brazil, Argentina, China and India, in that order, are the world's five main producing countries of the Roundup-resistant (RR) variety of soybean. In South America, soy is widespread in Brazil, Argentina, Uruguay, Paraguay and Bolivia (Bindraban et al., 2009). Cypermethrin is one of the most widely used insecticides (CASAFE, 2012).

Cypermethrin is applied 2–3 times per growing season at 25–75 g of active ingredient per hectare. Repeated pesticide applications in the fields represent a risk to adjacent surface waters. However, the environmental impact of such agricultural intensification remains largely unreported. Marino and Ronco (2005) detected insecticides in rivers running through the main agricultural districts in the Argentine Pampa. Jergentz et al. (2004a,b) reported the occurrence of toxic events affecting the invertebrate fauna in streams draining intensively cultivated basins in the Pampasic region. Jergentz et al. (2005) and Mugni et al. (2011) detected cypermethrin, chlorpyrifos and endosulfan in the water, suspended matter and bottom sediments of first order streams passing through soy cultivated plots.

Amphipods have often been used as an ecotoxicological model for risk assessment and for testing insecticide toxicity to non target invertebrate fauna (Borgmann et al., 1989; Adam et al., 2009; Xuereb et al., 2009; Dutra et al., 2009; Wheelock et al., 2005). The freshwater amphipod *Hyalella curvispina* has a wide distribution and is often the dominant invertebrate in the benthic and epiphytic communities of shallow environments in southern South America (García et al., 2010).

* *Corresponding author*. Tel.: +54 0221 422 2785/2832; fax: +54 0221 422 9329.
E-mail addresses: hemu@netverk.com.ar, mugni@ilpla.edu.ar (H. Mugni).
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The aim of this study was to determine the acute toxicity (48-hLC_{50}) of cypermethrin to the amphipod *H. curvispina*, the most abundant non-target organism in regional aquatic invertebrate assemblages. The reported information is significant for further evaluation of the environmental impact.

2. Materials and methods

Specimens of *H. curvispina* were originally collected from an uncontaminated stream located 25 km south of La Plata City and transported to the laboratory, where they were reared for several weeks. They were kept in large plastic containers with stream water, which was gradually replaced with unchlorinated tap water to compensate for evaporation losses. The locally abundant macrophyte *Lemna* sp. was placed on the surface of the water. *H. curvispina* individuals fed on the periphytic community of the *Lemna* rhizosphere and received a supplement of a mixture of fresh lettuce leaves and dried algae twice a week.

Procedures for *H. curvispina* toxicity tests were similar to standardized protocols for *Hyalella azteca* (USEPA, 2000). Ten *H. curvispina* individuals, 5–10 mm in length, were exposed to different cypermethrin concentrations diluted in 100 ml of reconstituted, moderately hard synthetic water (APHA, 1995), placed in 250 ml beakers. Three replicates of each concentration were tested. Tests were performed without feeding, at $22 \pm 2\,^{\circ}C$, and natural photoperiod. Mortality was recorded at 24 and 48 h of exposure. Dead individuals were removed immediately. As a validity criterion for the negative control, less than 10% mortality was considered acceptable. Preliminary tests were conducted to choose an appropriate cypermethrin concentration range within which to test lethal effects. Three independent determinations were performed. As a standard laboratory quality control practice, a reference test with copper sulfate (SO_4Cu5H_2O, 99.9% Merck) was performed. The 48-h LC_{50} positive control was 265 μg Cu II/l. This value lies within the acceptable range in the control chart (225 ± 79 μg Cu II/l) conducted by Mugni (2009).

Toxicity tests were performed using a certified cypermethrin standard (98% active ingredient) from Accustandard. A stock solution of 2 mg/l was prepared with analytical grade methanol (J.T. Baker). Different exposure concentrations were prepared by diluting the stock solution in reconstituted moderately hard water (APHA, 1995). The dosing volume of the stock solution never exceeded 0.3 ml, for the highest exposure concentration. A set of control replicates were performed adding methanol in the same amount as present in the highest exposure treatment (0.3 ml). No mortality was observed in the controls. Nominal assayed cypermethrin concentrations were 0.3, 0.2, 0.1, 0.05, 0.025, 0.01 and 0.005 μg/l. Cypermethrin concentrations in replicates of the three highest doses were determined after 2 h exposure; measured concentrations were 0.295, 0.236 and 0.09 μg/l, respectively.

The exposure solutions were passed through C18 columns (Agilent, solid phase extraction SPE) and frozen until analysis. Extracts were eluted from C18 columns with 2 ml hexane followed by 2 ml dichloromethane. The sample extracts were injected into a gas chromatograph by GC-ECD (Hewlett Packard, HP 6890), equipped with an HP1 column, 30 m and 0.25 mm column, N_2 carrier, ramp and detector temperatures of 190–250 and 320 °C, respectively.

Recovery from the C18 columns was tested by passing through a solution of known concentration. The C18 columns showed a 92 ± 17.0% cypermethrin recovery.

Sample storage at −20 °C was also tested to assess the holding time of the tested pesticides; 89 ± 15% cypermethrin recovery after two weeks. Solvents used for pesticide analysis were J. T. Baker. The detection limit was 0.025 μg/l.

Mortality data obtained from the 48 h cypermethrin exposures were used to estimate the LC_{50} and 95% confidence limits by means of the Probit statistical analysis method.

3. Result and discussions

An important aspect in determining the suitability of a test for routine use is reproducibility (Sucahyo et al., 2008). The 48-h LC_{50} of cypermethrin to *H. curvispina* was determined on three independent occasions during a 6 month period between November 2010 and April 2011. Results from the 3 determinations are shown in Table 1. The overall mean 48-h LC_{50} was 0.065 ± 0.03 μg/l. The 24-h LC_{50} values for cypermethrin were very similar: 0.07 ± 0.03 μg/l.

Cypermethrin toxicity to other aquatic organisms is reported in Table 2. *H. curvispina* lies among the most sensitive species to cypermethrin, along with shrimps, copepods and cladocerans. Present results confirm previous studies (Farmer

Table 1 – Acute toxicity of cypermethrin to the amphipod *Hyalella curvispina*.

	November 2010		February 2011		April 2011	
Point	Conc. (μg/l)	95% Conf. Lim.	Conc. (μg/l)	95% Conf. Lim.	Conc. (μg/l)	95% Conf. Lim.
LC 1.00	0.002	0.001–0.004	0.022	0.013–0.029	0.042	0.03–0.05
LC 5.00	0.005	0.002–0.008	0.030	0.021–0.037	0.053	0.042–0.061
LC 10.00	0.008	0.004–0.011	0.035	0.026–0.043	0.061	0.05–0.068
LC 15.00	0.010	0.006–0.014	0.040	0.030–0.047	0.066	0.057–0.074
LC 50.00	0.033	0.025–0.046	0.064	0.056–0.072	0.096	0.087–0.108
LC 85.00	0.108	0.071–0.219	0.103	0.089–0.129	0.139	0.121–0.177
LC 90.00	0.144	0.090–0.324	0.115	0.098–0.151	0.152	0.13–0.2
LC 95.00	0.218	0.125–0.581	0.136	0.112–0.189	0.173	0.144–0.24
LC 99.00	0.477	0.232–1.754	0.187	0.145–0.293	0.221	0.175–0.338
Slope ± SE	7.97 ± 0.51	6.98–8.96	10.97 ± 0.89	9.22–12.71	11.55 ± 1.06	9.46–13.63
Intercept ± SE	2.0 ± 0.31	1.4–2.6	4.99 ± 0.75	3.5–6.5	6.44 ± 0.99	4.5–8.4

Table 2 – Lethal concentrations of cypermethrin to different sensitive, non-target invertebrate species.

Species	Exposure time (h)	Toxicity LC_{50}(μg/l)	References
Shrimp *Palaemonetes pugio*	96-h LC_{50}	0.016	Yilmaz et al. (2004)
Shrimp *Paratya autraliensis*	96-h LC_{50}	0.019	Kumar et al. 2010
Copepod *Eudiaptomus graciloides*	96-h LC_{50}	0.03	Wendt-Rasch et al. (2003)
Copepod *Diaptomus forbesi*	48-h LC_{50}	0.03	Saha and Kaviraj (2008)
Cladoceran *Daphnia cucullata*	48-h LC_{50}	0.05	Wendt-Rasch et al. (2003)
Amphipod *Hyalella curvispina*	48-h LC_{50}	0.07	This paper
Amphipod *Gammarus pulex*	96-h LC_{50}	0.09	Adam et al. (2009)
Insect *Ranatra filiformis*	48-h LC_{50}	0.09	Saha and Kaviraj (2008)
Cladoceran *Ceriodaphnia dubia*	48-h LC_{50}	0.23	Shen et al. (2012)
Amphipod *Gammarus pulex*	24-h LC_{50}	0.24	Ashauer et al. (2011)
Copepod *Oithona similis*	48-h LC_{50}	0.24	Willis and Ling (2004)
Cladoceran *Daphnia magna.*	48-h LC_{50}	0.72	Ashauer et al. (2011)
Copepod *Temora longicornis*	48-h LC_{50}	0.74	Willis and Ling (2004)
Shrimp *Macrobrachium lar*	48-h LC_{50}	1.07	Bajet et al. (2012)
Copepod *Acartia clausi*	48-h LC_{50}	2.67	Willis and Ling (2004)
Rotifer *Brachionus calyciflorus*	24-h LC_{50}	80	Sánchez-Fortún and Barahona (2005)
Crustacean *Thamnocephalus platyurus*	24-h LC_{50}	670	Sánchez-Fortún and Barahona (2005)

et al. (1995) and Laskowski (2002) suggesting that amphipods are among the most sensitive organisms reported. Giddings et al. (2001) performed mesocosm studies on the toxic effects of cypermethrin to the invertebrate assemblages and ranked the sensitivity of the different organisms, with amphipods being among the most sensitive species.

Yilmaz et al. (2004) reported a cypermethrin 96–h LC_{50} of to the grass shrimp *Palaemonetes pugio* as low as 0.016 μg/l, the lowest recorded in Table 2. Kumar et al. (2010) determined the toxicity of 6 insecticides (carbaryl, chlorpyrifos, cypermethrin, dimethoate, diuron and fenarimol) to the freshwater shrimp *Paratya australiensis*, with cypermethrin being the most toxic (96-h LC_{50}: 0.019 μg/l). Adam et al. (2009) determined the 96-h LC_{50} of propiconazole, tebuconazole, 3-iodo-2-propinyl butyl carbamate (IPBC, fungicide) and cypermethrin to the freshwater amphipod *Gammarus pulex*, with cypermethrin being the most toxic (0.09 μg/l).

Friberg-Jensen et al. (2003) examined the effects of cypermethrin exposures on a natural freshwater community in small *in situ* enclosures in the eutrophic Lake Frederiksborg Slotssø, Denmark, in June 1999. Crustaceans were extremely sensitive to cypermethrin. The estimated median effect concentration (EC_{50}) for the total crustacean community and cladoceran and copepod subgroups ranged between 0.02–0.07 and 0.04–0.17 μg/l, respectively, with copepods being less sensitive than cladocerans.

Saha and Kaviraj (2008) determined the acute toxicity of cypermethrin to five non-target freshwater organisms (the crustacean *Diaptomus forbesi* the aquatic insect *Ranatra filiformis* the freshwater carp *Cyprinus carpio* the tadpole larva of the toad *Bufo melanostictus* the oligochaet worm *Branchiura sowerbyi*). The crustacean *Diaptomus forbesi* was the most sensitive, with a 48-h LC_{50} of 0.03 μg/l, followed by the aquatic insect *Ranatra filiformis* (0.09 μg/l).

Bajet et al. (2012) assessed the acute toxicity of cypermethrin to several non target aquatic species native from Laguna Lake, Philippines. The organisms used in the toxicity tests were early life stages of *Tilapia* sp. which included *Tilapia* embryos less than 12 h old, newly hatched *Tilapia*, 22 mm *Tilapia* fingerlings, and freshwater shrimp *Macrobrachium lar*. The freshwater shrimp *Macrobrachium lar* was the most sensitive: 48-h LC_{50}: 1.07 μg/l).

Agra and Soares, 2009 assessed the effects of cypermethrin on the survival, growth and emergence of *Chironomus riparius* determined in a long term exposure test. Cypermethrin exposures as low as 0.1 μg/l caused a significant reduction in larval growth.

Sánchez-Fortún and Barahona (2005) compared the 24-h LC_{50} of rotifers and crustaceans of estuarine and freshwater environments. The freshwater rotifer *Brachionus calyciflorus* was more sensitive (0.08 mg/l) than the estuarine *Brachionus plicatilism* (0.3 mg/l). Similarly, the freshwater crustaceae *Thamnocephalus platyurus* was more sensitive (0.67 mg/l) than the estuarine *Artemia fransiscana* (4.72 mg/l).

Stephenson (1982) studied cypermethrin acute toxicity to ten freshwater invertebrates (*Daphnia magna*, *Asellus aquaticus*, *G. pulex*, *Cloeon dipterum*, *Gyrinus natator*, *Chironomus thummi*, *Aedes aegypti*, *Cheoborus crystallinus*, *Corixa punctata*, and *Piona carnea*). The 24-h LC_{50} values for *G. natator*, *C. thummi*, and *C. punctata* were > 5 μg/l. For the seven more susceptible species the 24-h LC_{50} values ranged from 2 to 0.05 μg/l.

H. curvispina is also sensitive to chlorpyrifos and endosulfan (Mugni, 2009). Mugni et al. (2012) simulated a runoff event by adding soil-water slurries spiked with cypermethrin and chlorpyrifos to stream water in laboratory aquariums. A concentration of 1 μg/L in the aquariums resulted in 100% mortality to *H. curvispina* for both pesticides. Mugni et al. (2010) performed an experimental application of cypermethrin and chlorpyrifos to a stream pool in coincidence with a draft. A chlorpyrifos concentration of 0.12 μg/l caused 100% motrality to *H. curvispina*. Mugni et al. (2012) simulated successive rain events following chlorpyrifos application in an experimental soybean plot. Rain events were simulated by means of irrigation sprinkler equipment. Chlorpyrifos concentrations in runoff decreased with time since application attaining 0.06 μg/l 19 days after application, the last event in which toxicity was observed.

Crustaceans have been widely used in aquatic toxicity testing (Graca et al., 2002; Sánchez-Bayo, 2006; Barata et al., 2008; Dahl and Breitholtz, 2008; Adam et al., 2010; Ding et al., 2011;

Shen et al., 2012). Among aquatic crustaceans, *Daphnia* sp., *Ceriodaphnia* sp., *Gammarus* sp. and *Hyalella* sp. have been used most often in aquatic toxicity testing for a variety of reasons, including their widespread distribution in aquatic environments and ease of culture under laboratory conditions. Familiarity with the organism and the availability of a large database may have contributed to their popularity (Hickey, 1989). Standard protocols have been developed for acute toxicity water tests with cladocerans and amphipods typically applying 24–96 h exposure periods (APHA, 1995; USEPA, 2000). Because of its wide distribution in México and USA, *H. azteca* is routinely used as a test organism for toxicity assessment in aquatic environments of North America. *H. azteca* is not present in South America. *H. curvispina* is commonly the most abundant species in amphipod assemblages in a wide area of South America, extending from Rio de Janeiro, Brazil, in the Atlantic coast (22°S, 43°W) to Punta Arenas, Chile, in the Pacific (53°S, 70°W; Somma et al., 2011). Such wide distribution overlaps with most of the agricultural areas in southern South America including the most important crop producer countries Brazil, Argentina, Uruguay, Paraguay and Bolivia.

Several researchers have already used *H. curvispina* as a test organism. Jergentz et al. (2004a,b) and Mugni (2009) detected toxicity pulses to *H. curvispina* in streams adjacent to crops and detected cypermethrin, chlorpyrifos and endosulfan in the stream and runoff water and in sediments. Jergentz et al. (2005) reported pesticide concentrations in a first order stream born in a soybean plot on a farm following common management practices. Cypermethrin was detected in the stream water in coincidence with the rain events following the application, at concentrations ranging from 0.05 to 0.71 μg/l. Cypermethrin was also measured in the runoff events that followed the application, ranging from 0.13 to 0.49 μg/l. Marino and Ronco (2005) surveyed pesticide concentrations in streams and rivers from intensively cultivated areas of Buenos Aires, Argentina. Cypermethrin concentrations were detected in several monitoring events ranging from 0.2 to 3.6 μg/l. Mugni et al. (2011) evaluated toxicity to *H. curvispina* in a first-order stream running through a cultivated farm. Ephemeral toxicity pulses were observed as a consequence of pesticide applications.

4. Conclusion

H. curvispina is highly sensitive to cypermethrin exposure. Being widely distributed and often attaining high densities in shallow South America water bodies, *H. curvispina* seems suitable for use as a sentinel organism for environmental impact assessment. The 48-h LC_{50} determined in the present work lies below most of the reported concentrations and is consistent with the previously reported toxicity pulses observed in Argentine streams draining agricultural areas. Resident amphipod populations of streams draining agricultural areas are exposed to recurrent acutely toxic cypermethrin pulses.

Conflict of interest

Nothing declared.

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