CYPERMETHRIN, CHLORPYRIFOS AND ENDOSULFAN TOXICITY TO TWO NON-TARGET FRESHWATER ORGANISMS

Hernan Mugni*, Ariel Paracampo, Natalia Marrochi and 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

ABSTRACT

Pesticide consumption has increased considerably over the last decades in the Argentine Pampa. The persistence of toxicity of three commonly used insecticides to two regionally abundant organisms was assayed at environmentally realistic conditions. To simulate a runoff event, insecticides were added to aquariums containing water and sediment collected from a representative stream. The toxicity of insecticides formulations containing cypermethrin, chlorpyrifos and endosulfan to the amphipod *Hyalella curvispina* and the fish *Cnesterodon decemmaculatus* was assessed with laboratory bioassays conducted on water taken from the aquariums immediately after pesticide addition and every 2 to 3 days thereafter until toxicity ceased. Endosulfan did not cause mortality to *H. curvispina* at a nominal concentration of 1 µg/L. Cypermethrin and chlorpyrifos caused 100% mortality immediately after application at this concentration. Toxicity ceased 4 days after addition of chlorpyrifos, and 9 days after addition of cypermethrin, resulting in a 50% lethal time of 3 and 4 days, respectively.

C. decemmaculatus demonstrated no mortality at 1 µg/L nominal concentration of the formulated insecticides assayed. At a dose of 5µ/L, no mortality was exhibited for cypermethrin and chlorpyrifos, while endosulfan produced 100% mortality immediately after application, but no significant toxicity 2 days after application.

Present results suggest short persistence of toxicity of the commonly used insecticide formulations in Pampasic surface waters.

KEYWORDS: Insecticides, Toxicity Persistence, Cnesterodon decemmaculatus, Hyalella curvispina.

1 INTRODUCTION

The Argentine Pampa is an extensive plain with a mild climate and fertile soils that were covered with grasslands prior to agricultural development. For a long time, farmers employed a mixed system of livestock and crops, mainly wheat and corn. Soy was not a traditional crop in Argentina, with a cultivated area of a few thousand hectares during the seventies. The genetically modified soy resistant to glyphosate was introduced to the market in 1996, and was fast adopted by farmers, along with the no-tillage management practice. The area cultivated with soy increased rapidly to 8,300,000 ha at the end of the last century [1]. At present, soy represents roughly one-half of the total harvest and cultivated area (50 million tons and 18 million ha, respectively). Wheat and soy varieties with a short growing period allow for two harvests per year, wheat followed by soybean. Livestock was moved to marginal areas or concentrated in feedlots. Along with enhanced agricultural production, the amount of agrochemicals consumed increased. Pesticide consumption increased from 6 to 18 million kilograms in the 1992-1997 period alone and has continued to increase at lower rates since then. Cypermethrin, chlorpyrifos and endosulfan represent the most utilized insecticides, cypermethrin accounting for roughly half of the total pesticide consumption [1].

The environmental impact of this agricultural intensification remains largely unreported. Jergentz et al. [1] demonstrated the occurrence of toxic events affecting the invertebrate fauna in streams draining intensively cultivated basins in the Pampasic region. Jergentz et al. [2] and Mugni et al. [3] detected cypermethrin, chlorpyrifos and endosulfan in the water, suspended matter and bottom sediments of first order streams passing through soy cultivated plots.

The objective of the present work was to assess the toxicity of the most commonly used formulated insecticides in simulated field runoff scenarios to two widely distributed freshwater organisms of South America: the amphipod *Hyalella curvispina* and the fish *Cnesterodon decemmaculatus.* A runoff event was simulated by adding the pesticides to soil-water suspensions and applying them to laboratory aquariums filled with water and bottom sediments

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* Corresponding author

collected from a representative Pampasic stream. Toxicity persistence was assessed by performing toxicity tests in successive water samples collected from the aquariums every 2 to 3 days until mortality ceased.

2 MATERIALS AND METHODS

Sediments and water were collected from the Sauce stream, located 15 km southwest of La Plata City, Buenos Aires, Argentina (35º 01´ S, 57º 59´ W). Toxicity of stream water and sediments was tested on *H. curvispina* and C. *decemmaculatus* and no mortality was exhibited.

H. curvispina and *C. decemmaculatus* were originally obtained from an uncontaminated stream. They were later bred in the laboratory aquariums containing water and sediment from the Sauce stream, under the same controlled conditions as those under which the tests were performed. Water surfaces in the aquariums containing *H. curvispina* were covered with the floating macrophyte *Lemna* sp, and *H. curvispina* fed on the periphytic community of the *Lemna* rhizosphere. In addition, a food supplement mixture of fresh lettuce leaves and dried algae was added twice a week. *C. decemmaculatus* were fed with commercial fish food.

The dissolved oxygen and temperature in the aquariums were measured with a Yellow Spring Instrument (YSI 51B), pH was measured with an Orion 250 A meter and conductivity was measured with a Hanna Instruments 8733 meter. All meters were calibrated prior to each use, utilizing appropriated standards.

Twelve glass aquariums 60 cm long, 20 cm wide and 40 cm high were filled with 9 liters of stream water. The bottom was covered with a layer of approximately 2 cm of stream sediments representing approximately 1.5 kg of wet sediment. Three aquariums were kept as controls. The remaining nine received a single insecticide application. Three received cypermethrin; 3 received endosulfan and 3 received chlopryrifos. Insecticides were added to a suspension of 50 g dry soil and 1 L of deionized water, and the mixture was added to the aquariums to simulate a runoff event. The soil was collected from a field adjacent to the Sauce stream, close to the site where sediment and water were collected. The land surrounding the stream at this site was known to have natural grassland without any insecticide application for several years [3]. Soil toxicity to *H. curvispina* had been previously tested using a 10 day sediment bioassay USEPA [4] with no toxicity observed. Insecticide was added to the suspensions in order to attain a 10 µg/L concentration mixed thoroughly, and immediately added to each aquarium resulting in a nominal concentration of 1 µg/L. This concentration was chosen based on concentrations measured in Pampasic streams by Jergentz et al. [1-3]. The control received the soil suspension without insecticide addition. The aquariums were gently stirred after insecticide addition to attain a homogeneous distribution. Water samples were taken from all the aquariums for bioassays immediately and 2, 4, 7, and 9 days after pesticide addition. Commonly available commercial products Galgotrin, Shooter and Brometan were used, containing 25 g of cypermethrin, 48 g of chlorpyrifos and 35 g of endosulfan per 100 ml, respectively. Brometan commercial formulation consists of a mixture of 70% alfa and 30% beta endosulfan isomers.

Laboratory toxicity tests with *H. curvispina* were performed in the water samples taken from the aquariums following standardized protocols recommended for *Hyalella azteca* [4]. Ten *H. curvispina* of 5-10 mm length were exposed to 100 ml aquarium water in 250 ml beakers, in triplicate. A reference test with copper sulfate (SO_4Cu5H_2O, 99.9% Merck®) was performed. The 48 h LC50 positive control was 265 µg CuII/L. This value lies within the acceptable range in the control chart (225 ± 79 µg CuII/L) conducted by Mugni [5].

Tests with fish were carried out following USEPA [6]. Exposures were performed in 3 L beakers, containing 1 L aquarium water and 10 *C. decemmaculatus* of 20-25 mm length, in triplicate. Tests were performed without feeding, at 22 ± 2ºC, and natural photoperiod, assessing mortality after 48 h exposure for *H. curvispina* and 96 h for *C. decemmaculatus*. As a validity criterion for the negative control, less than 10% mortality was considered as acceptable [4, 6]. In addition to conducting bioassays on both species using a nominal concentration of 1 µg/L, fish bioassays were conducted with a nominal concentration of 5 µg/L of each pesticide in aquarium water prepared with the same method.

Mortality in the successive samplings from each aquarium following pesticide application was used to estimate the 50% lethal time (LT50) by means of Probit analysis. Differences among treatments were assessed by means of the t test for independent samples or analysis of variance (ANOVA) followed by the post hoc all pair wise multiple comparison procedure (Tukey test). Whenever the required conditions (homoscedasticity, normality) for ANOVA utilization were not attained, the equivalent non parametric methods were used, such as Kruskal Wallis tests. The significance level for all the applied tests was 0.05.

The LC50 values of cypermethrin, endosulfan and chlorpyrifos to *H. curvispina* under standardized conditions were determined in laboratory synthetic and Sauce stream water. The laboratory synthetic water was prepared following the APHA [7] recommendation for a moderately hard water typology. Ten *H. curvispina* of 5-10 mm length were exposed to 100 mL water in 250 mL beakers, in triplicate. Seven different insecticide concentrations assayed were prepared using dilution series from a stock solution of 1 mg/L nominal concentration in deionized water. The stock solution was prepared using the same commercial product used in the experiment. No mortality was registered in the controls. The LC50 was estimated by Probit analysis.

3 RESULTS AND DISCUSSION

The Sauce stream had high organic and suspended matter content: total organic carbon was 17 mg/L and suspended solids were 86 mg/L [5]. The texture of the bottom sediments was 22% clay, 67% silt and 11% sand, and organic matter content was 12% [5]. Water temperature, pH, dissolved oxygen and conductivity were measured throughout the experiment. No significant differences were observed among treatments, or among successive samplings. Mean measurements in the aquariums were: pH 7.6 ± 0.3, temperature 18 ± 1 ºC, dissolved oxygen 4.5 ± 0.1 mg/L and conductivity 306 ± 66 µS/cm.

Chlorpyrifos and cypermethrin were highly toxic to *H. curvispina* resulting in 100% mortality at the beginning of the experiment, while endosulfan did not produce any mortality (Fig 1). A significant decrease of chlorpyrifos toxicity was observed on the second day (20% survival, $p<0.02$, ANOVA) and no mortality was observed from the fourth day onwards. The treatment exposed to cypermethrin showed increased survival from the fourth day onwards (47% survival, $p<0.001$, ANOVA) attaining a maximum of 87% at the end of the experiment, nine days after application. Mortality on days 2 and 4 was significantly different for chlorpyrifos and cypermethrin treatments ($p<0.001$, Kruskal Wallis test).

The chlorpyrifos LT50 was 3.4 days (95% confidence limits 2.3-5.1) and that of cypermethrin 5.4 days (95% confidence limits 4.5-6.2). Differences were not statistically significant ($p=0.11$; t test).

As these compounds are highly hydrophobic ($\log k_{ow}$ 6.6 and 4.7 for cypermethrin and chlorpyrifos, respectively), fast toxicity dissipation likely resulted from extensive insecticide sorption to the bottom sediments and suspended matter. Maund et al. [8] reported the partitioning, bioavailability and toxicity of cypermethrin in water-sediment systems. They found that 98% of the added cypermethrin was adsorbed to the sediments within 2 hours of application. The sediment LC50 values for *H. azteca* and *Chironomus tentans* increased with the organic carbon content of the sediments. Mazanti et al. [9] reported 70–75% chlorpyrifos dissipation during the first 6 h after experimental application to outdoor mesocosms. Farmer et al. [10] observed fast cypermethrin dissipation in outdoor mesocosms, decreasing to 13% of the initial nominal concentration 24 h after the application. Chemical and microbial degradation might also represent major routes of pesticide loss [11]. Water pH influences chemical degradation. Hydrolysis of cypermethrin [12], chlorpyrifos [13] and endosulfan [14] increases as pH increases. The half-life of endosulfan in water decreased from 28, to 5.7, and 0.7 days as pH increased from 5, to 7, and 9, respectively [14]. It seems plausible that the high water pH in the stream water contributed to the observed fast dissipation of the assayed insecticides in the present study. Mugni et al. [15] reported fast toxicity dissipation after experimental pesticide application to pools formed on the Sauce stream bed during a drought. Chlorpyrifos and cypermethrin concentrations of 0.5 and 0.2 µg/l respectively were measured half an hour after spraying. Laboratory exposure of *H. curvispina* to successive stream water samples showed that acute toxicity ceased 4 hours after application. Castro et al. [16] studied the persistence of chlorpyrifos and endosulfan in soil under field conditions and found that their half-lives were lower than those obtained under laboratory conditions.

Pablo et al. [17] assessed chlorpyrifos fate and toxicity to the cladoceran *Simocephalus vetulus* and the mayfly *Atalophlebia australis* in outdoor stream mesocosms. Water flux was stopped for 6 h after pesticide addition. An initial dose of 1 µg/L produced 100% mortality of *S. vetulus* and 97% mortality of *A. sustalis*. Chlorpyrifos rapidly partitioned between sediments and overlaying water, with 31% of the total added amount found in the sediment compartment 6 h after insecticide addition.

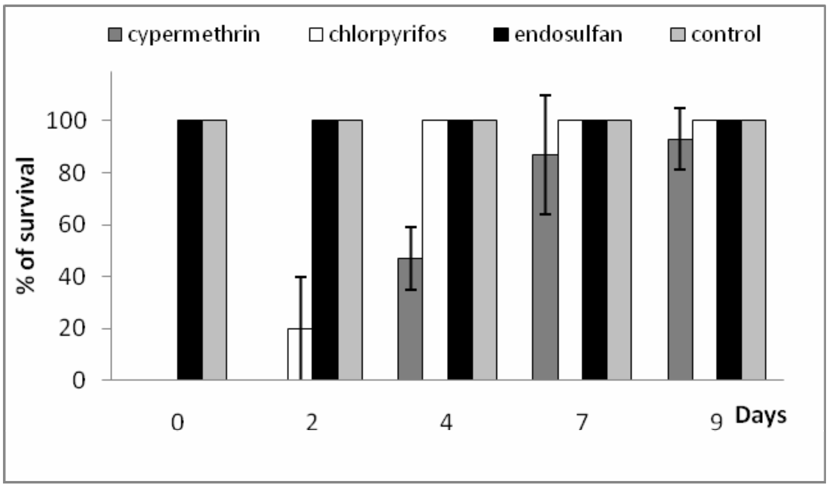

FIGURE 1 - *H. curvispina* survival in 48-h exposures to aquaria water at successive samplings following insecticide addition at 1 µg/L. Bars represent standard deviation

H. curvispina was more tolerant to endosulfan than to cypermethrin and chlorpyrifos (Fig. 1, Table 1); the endosulfan LC50 was orders of magnitude higher than those of cypermethrin and chlorpyrifos. Similarly, Woods et al. [18] reported that *Ceriodaphnia dubia* was more tolerant to endosulfan (LC50: 53.3 (35.6-79.8) µg/L) than to chlorpyrifos: (LC50: 0.048 (0.032-0.072) µg/L).

H. curvispina was more sensitive to cypermethrin than chlorpyrifos (Table 1). The estimated LC50 of cypermethrin and chlorpyrifos to *H. Curvispina* was roughly 3 times higher in stream than in laboratory synthetic water, the differences being statistically significant ($p<0.008$ and $p<0.019$ respectively, t test).

TABLE 1 - 48h LC50, and, in brackets, 95% confident limits, of chlorpyrifos, endosulfan and cypermethrin to *H. curvispina* in synthetic and stream water.

	Synthetic water	Stream water
chlorpyrifos (µg/L)	0.06 (0.02-0.1)	0.17 (0.14-0.2)
cypermethrin (µg/L)	0.01(0.007-0.012)	0.024(0.02-0.031)
endosulfan (µg/L)	17.2 (12.7-21.6)	----

No mortality was observed of any of the assayed insecticides to the fish *C. decemmaculatus* at the applied dose of 1 µg/l nominal concentration. *C. decemmaculatus* was more tolerant to cypermethrin and chlorpyrifos than *H. curvispina*. A second assay was immediately performed with a nominal pesticide concentration of 5 µg/l added in the same way. At this concentration, 100% mortality was observed for endosulfan while no mortality was observed for cypermethrin or chlorpyrifos immediately after application. Carriquiriborde et al. [19] reported that the 96h LC50 of cypermethrin to juveniles (10 mm length) of *Cnesterodon decemmaculatus* increased from 0.43 µg/l in laboratory synthetic water to 2 µg/l in filtered stream water and 5.2 µg/l in unfiltered stream water. The filtered stream water represented the toxicity attenuation contributed by the dissolved organic matter, while the unfiltered stream water also included the toxicity attenuation of the particulate fraction, both being important. Carriquiriborde et al. [19] did not observe mortality in caged *C. decemmaculatus* fishes exposed in a first order stream when cypermethrin was sprayed in the surrounding plot, nor in the following runoff events, even though cypermethrin concentrations in streams were higher than the LC50 determined in laboratory synthetic water. Yilmaz et al. [20] reported the 96h LC50 of alpha-cypermethrin 9.4 µg/L to adults (5-6 cm) of *Poecilia reticulata,* which belongs to the same family as *C. decemmaculatus.*

Giddings et al. [21] reported a trend in cypermethrin sensitivity from amphipods (most sensitive) to fish (less sensitive): the reported LC50 values for cypermethrin were 0.021 and 2.7 µg/L respectively. Amphipods are usually among the most sensitive taxa to toxic substances. Peluso et al. [22] suggested the utilization of *Hyalella curvispina* as a centinel organism in South America.On the other hand, *C. decemmaculatus* was more sensitive to endosulfan than cypermethrin and chlorpyrifos. Nalecz-Jawecki et al. [23] studied the toxicity 8 insecticides, 6 fungicides and 10 herbicides to the protozoan *Spirostomum ambiguum*, the cladoceran *Daphnia magna* and the fish rainbow trout. The importance of comparing toxicity to different organisms was emphasized.

Present results emphasize the ephemeral nature of reported toxicity events producing non target invertebrate mortality. Fast dissipation would be expected in lotic environments simply because of downstream transport. However, our experiment showed that toxicity pulses in still waters (ponds, lagoons, riparian habitats) are also brief.

The studied stream was representative of the Pampasic surface waters. The main environmental conditions that favoured fast dissipation rates seem to be a regional feature. The parental soil material provides fine grain textures, and the gentle slopes favour clay settlement in the bottom sediments. High nutrient concentrations favour luxuriant macrophyte growth, which in turn provides high organic matter content in the water and bottom sediments.

No lethality was observed for the three most commonly used pesticides at field realistic concentrations to the common, abundant, and widely distributed fish *C. decemmaculatus.*

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CORRESPONDING AUTHOR

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

Phone: +54 011-4275-8564 /4275- 7799; Ext. 34&48
E-mail: mugni@ilpla.edu.ar; hemu@netverk.com.ar

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