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Toxicity Persistence Following an Experimental Cypermethrin and Chlorpyrifos Application in Pampasic Surface Waters (Buenos Aires, Argentina)

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Abstract The Pampa plain is intensively cultivated, the genetically modified glyphosate-resistant soybean being the main culture. A small first-order stream draining a cultured farm was studied. During the dry 2007 spring, the channel became a wetland with remnant intercalated shallow pools. Toxicity persistence of cypermethrin and chlorpyrifos to the amphipod *Hyallela curvispina* was assessed following a simulated plane application in the recently formed pools. In situ exposures and laboratory toxicity tests were performed. An ephemeral toxicity pulse lasting 4 h after the application and mortality cessation following that was observed.

Keywords Cypermethrin · Chlorpyrifos · Toxicity · Persistence

The Argentine Pampa is an extended plain of mild climate and fertile soils developed over deep loessic sediments. It was originally covered by grasslands, and for a long time farmers employed a mixed system of livestock and crops, mainly wheat and corn. The occurrence of intense showers during the seeding period made erosion a matter of concern. It was not until the genetically modified soybean resistant to glyphosate became available that the no-tillage technique was adopted. Several applications of the cheap and efficient glyphosate herbicide are needed to implement no-tillage practices. Soybean was not a traditional crop in the Pampa. The genetically modified soybean resistant to glyphosate was released to the market in 1996, and was fast adopted by the farmers.

At present, soybean represents roughly one-half of the total harvest and cultivated area (50 million tons and 15 million ha, respectively), and almost all of it is resistant to glyphosate (MAGyP, 2010). Wheat varieties with a short growing period allow two harvests per year: wheat followed by soybean. Livestock have been moved to marginal areas or concentrated in feedlots. The fast implementation of new technological developments has driven soybean-related products (oil, flour, pellets) into the main Argentine production, making Argentina one of the largest transgenic producer after the United States (MAGyP, 2010). Pesticide consumption has also largely increased. The pyrethroid cypermethrin represents more than half of the total insecticide consumption, followed by the organophosphate chlorpyrifos and the organ chlorinated endosulfan (Jergentz et al. 2004b). Cypermethrin is usually sprayed twice during the soybean growing period. When the culture reaches a certain size, airplane application is commonly used to avoid the culture damage caused by terrestrial vehicles. Although some recent publications have dealt with detailed aspects of pesticide effects on the biota, the overall impact of such vast agricultural intensification remains unreported. Jergentz et al. (2004a, b) reported toxicity pulses in the regional streams. Pulses were either related to runoff events or to plane applications.

An extended drought throughout the 2007/2008 growing period resulted in a large discharge reduction in the regional streams, and some of them showed imperceptible water flux, a situation that is not uncommon during dry

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summers. The present study reports a field experiment in which the persistence of the toxicity to the amphipod *Hyallela curvispina* was followed after the experimental application of cypermethrin and chlorpyrifos in the pools formed in a small stream at the low water phase.

Materials and Methods

The experiment was performed in the small first-order Sauce stream, draining a cultured plot inside a private farm of 110 ha, located roughly 15 km south west from La Plata city, Buenos Aires, Argentina (35°01′S, 57°59′W). The stream at a steady-state condition is 2–3 m wide, 20–30 cm deep, and has a 10–20 l/s discharge, with a 2–6 m wide riparian strip. The basin contains Argiudol soils characterized by an A superficial horizon (30 cm deep) of grain-sized silty clay and 4.5% organic matter, followed by a B horizon (80–100 cm deep) with 50–60% clay (Mugni 2009). During the dry 2007 spring, the Sauce stream remained a vegetated channel with almost unperceivable water flux, showing several intercalated shallow water pools of small vegetation growth. Three of these sites of similar size, roughly 2 × 5 m, 10–20 cm deep, were chosen. The upstream pool remained as a control, and the two downstream pools were sprayed with cypermethrine and chlorpyrifos by means of a hand-held sprayer, applying the same amount per unit surface as the usually performed aerial applications (200 ml/ha cypermethrin and 800 ml/ha chlorpyrifos). The commonly available commercial products Galgotrin and Shooter were used, containing 25 and 48 g active ingredient per 100 ml of cypermethrin and chlorpyrifos, respectively, representing 0.2 and 0.8 ml of the commercial products applied in each pool. Water flow ceased completely downstream from the applied pools at a nearby widening that had developed a small wetland system, showing luxuriant macrophyte growth.

The amphipod *H. curvispina* was originally sampled from the uncontaminated Destino stream, draining the Pearson biological reserve, 45 km south from La Plata. It was later bred in the laboratory in large plastic beakers filled with Destino stream water. The surface water was covered by the macrophyte *Lemna sp*. *H. curvispina* fed on the perifytic community of the *Lemna* rhizosphere, which was supplemented with a mixture of fresh lettuce leaves and dried algae twice a week.

Chambers were constructed for the in situ exposure following Jergentz et al. (2004b), with plastic tubes 15 cm long and 5 cm in diameter, with the ends closed by tight plastic caps. Two rectangular windows of 3 × 5 cm were excavated on opposite sides of the chambers. These windows were covered with a plastic mesh with 1.5 mm pores size.

Before pesticide application to the pools on November 1, 2007, three replicates containing ten *H. curvispina* of 10–15 mm in body length were placed together in each pool on the streambed with one window pushed into the soft bottom sediments to enable contact between organisms and sediments. After 24 h exposure, the chambers were opened, and the surviving organisms were counted and replaced by new ones for another 24 h exposure, followed by a final 3 day exposure, where the experiment finished because no mortality was observed.

Laboratory toxicity tests with *H. curvispina* were performed in water samples taken from the pools immediately after, 30 min after, and 4, 24, and 48 h after pesticide application, following USEPA (US Environmental Protection Agency) (2000). Ten *H. curvispina* of 10–15 mm length were exposed to stream water in 100 ml beakers, in triplicate. Tests were performed without feeding at 22 ± 2°C and with natural photoperiod, assessing mortality after 48 h exposure. As a validity criterion, less than 10% mortality was considered as no effect (USEPA (US Environmental Protection Agency) 2000).

Dissolved oxygen (YSI 51B), pH (Orion 250 A), temperature, and conductivity (Hanna Instruments 8733) in the pool waters were measured in situ throughout the experiment. The texture of the stream superficial sediments was 22% clay, 67% silt, 11% sand, and its organic matter content was 12%.

Pesticides in the water were determined after sample pretreatments: 300 ml of water were extracted in three sequential steps with dichloromethane, previous adjustment to pH 5 with 2 N HCl. Pooled extracts were rotoevaporated and taken to dryness with N_2 flow, and re-suspended in acetonitrile; soil samples (30 g) were extracted into dichloromethane by sonication in three contacts with 50 ml solvent. Extracts were pooled, rotoevaporated and taken to dryness with N_2 flow and finally re-suspended in 1 ml acetonitrile (Marino and Ronco 2005).

Chemical analyses were performed using an LC-MS Agilent 1100 LC system (Agilent Technologies Inc., USA) equipped with a binary pump and a diode array detector (DAD), and coupled with a MSD VL quadrupole (Agilent Technologies, USA) with an electrospray ionization (ESI) interface. A Rheodyne 7725i injector with a 20 µl loop was used. Data acquisition and analysis were performed using LC/MSD Agilent ChemStation. The chromatographic separations were performed on a C-18 reverse phase (150 mm × 4 mm, 3 µm pore size). The column was maintained at room temperature (22 ± 1°C). The mobile phases selected were acetonitrile–water with ammonium acetate 20 mM (50:50), respectively, for 5 min then gradient to 100% acetonitrile to 15 min. The electrospray ionization was performed using nitrogen to assist nebulization, at a flow rate of 10 l/min, and a nebulizer pressure of 40 psi. The capillary temperature and voltage were set at of 330°C and 3,000 V, respectively. Positive scan ion modes were used for identifying the base peaks *m/z* values for each analyte. Subsequently, programmed single ion monitoring (SIM+) was used for analyte quantification. The $[M + H]^+$ ions were fixed to both analytes. Recovery was tested by spiking environmental samples with known concentrations of the assayed pesticides. Sample storage at −20°C was also tested to assess holding time of the tested pesticides. J.T Baker solvents for pesticide analysis were used. Standards of cypermethrin and chlorpyrifos were provided by SENASA (Argentinean National Service for Sanitary and Quality of Agriculture and Food). Detection limits were 0.02 µg/l cypermethrine and 0.01 µg/l chlorpyrifos in water and 1 µg/kg d.w. for both pesticides in sediments.

Mortality differences among field treatments were assessed by means of analysis of variance followed by the post hoc multiple-comparison Tukey's honest significance test. The significance level was 0.05.

Results and Discussion

Table 1 shows the measured environmental variables. Air temperature, measured in a field experimental station 5 km from the studied site, showed extended daily amplitude, common during spring. Water temperatures, measured early every morning throughout the experiment, showed a small variation range. Stream water was alkaline and well oxygenated, with an intermediate conductivity. Comparatively high SRP (738 µg/l) and relatively low nitrate (862 µg N/l) concentrations were measured. No significant differences were measured among the measured variables in the control and insecticide-containing pools.

Table 1 Environmental variables measured throughout the experiment

	Mean	Range
Air temperature (°C)	18	9–25
Solar radiation (kWat/m^2)	6.3	5.0–6.7
Water temp (°C)	20	18–21
Water pH	7.8	7.7–8.2
Cond. (µS/cm)	334	312–390
Oxygen (mg l^{-1})	10.3	8.8–12
Suspended matter (mg l^{-1})	89	82–95
Dissolved organic C (mg l^{-1})	13	12–14
Particulate organic C (mg l^{-1})	7.1	5.8–7.6
HCO_3^- (mg l^{-1})	72	36–185

Table 2 shows *H. curvispina* mortality during the in situ exposures. Both assayed pesticides showed the same behaviour, attaining high mortalities, being significantly different from the control only during the first day after application. The following exposures showed low mortalities, not significantly different from the control. Mortality in the control remained among expected figures for in situ exposures. As a validity criterion, less than 20% mortality was considered as no effect in sediment tests developed for *H. azteca* (USEPA (US Environmental Protection Agency) 2000). Jergentz et al. (2004b) reported similar mortalities in the controls of in situ *H. curvispina* exposures in Pampasic streams.

Table 3 shows *H. curvispina* mortality in laboratory exposures to water sampled in the field at different times following pesticide application. Both pesticides showed the same behaviour: mortality was high, significantly different from the control, in the first two samples, from immediately after to half an hour after the pesticide application. The water sampled 4 h after the application already showed a considerably lower mortality than the previous samples without being significantly different from the control. No significant mortality was observed in the subsequent water samples taken 24 or 48 h after pesticide application. No mortality was observed in controls throughout the experiment.

Pesticide concentrations in water were 0.55 µg/l cypermethrin and 0.12 µg/l chlorpyrifos half an hour after the application, and fell below detection limits in the following samplings. Pesticide concentrations in bottom sediments were 4.30 µg/kg cypermethrin and 2.51 µg/kg chlorpyrifos 4 h after application. After 24 h, 0.92 µg/kg cypermethrin was measured in sediment samples, whereas chlorpyrifos fell below detection levels. Both pesticides remained below detection limits the following days.

Cypermethrin and chlorpyrifos toxicity showed similarly short persistence in the stream water in both field and laboratory exposures. The laboratory toxicity tests, made at shorter time intervals, showed that toxicity in water decreased 4 h after the application and ceased after that. An order of magnitude higher content in bottom sediments than in water suggests that adsorption of the tested pesticides likely represents the main fate. Fast dissipation was

Table 2 Percentage of mortality observed in situ *H. curvispina* exposures after pesticide application

Date	Control Mean ± SD	Cypermethrin Mean ± SD	Chlorpyrifos Mean ± SD
01–02.11.07	23 ± 25	80 ± 17[a]	97 ± 6[a]
02–03.11.07	10 ± 17	13 ± 12	27 ± 21
03–06.11.07	10 ± 17	20 ± 12	17 ± 6

[a] Significant differences from the control

Table 3 Percentage of mortality in laboratory *H. curvispina* exposures at different times following pesticide application in the field

Time since application	Control	Chlorpyrifos Mean ± SD	Cypermethrin Mean ± SD
Initial	0	100[a]	100[a]
½ h	0	100[a]	80 ± 35[a]
4 h	0	27 ± 23	20 ± 20
24 h	0	13 ± 23	0
48 h	0	13 ± 23	0

[a] Significant differences from the control

expected because the highly hydrophobic pesticides (log k_{ow} 6.6 and 4.7 for cypermethrin and chlorpyrifos, respectively) are known to be extensively adsorbed by both inorganic and biological surfaces. Maund et al. (2002) reported that 98% of added cypermethrin was adsorbed by the sediments within the first 2 h of incubation in laboratory water-sediment systems. Cypermethrin concentrations in subsurface waters of a stream adjacent to vineyards in France decreased from 0.4 to 1.7 µg/l soon after spraying to undetectable values (<0.1 µg/l) a few hours later (Crossland et al. 1982). Cypermethrin concentrations in surface pond water samples adjacent to potatoes and sugar beets decreased from 6 to 23 µg/l immediately after application to undetectable values in 13 of 14 surface water samples collected after 24 h, and attained 6 µg/l in only one sample (Crossland et al. 1982). Mazanti et al. (2003) reported 70–75% chlorpyrifos disappearance during the first 6 h after experimental application to outdoor mesocosms. Farmer et al. (1995) observed fast cypermethrin dissipation in outdoor mesocosms, decreasing to 13% of the initial nominal concentration 24 h after the application.

The in situ *H. curvispina* exposures in the chambers installed with windows pushed into the sediment surface in the present study showed lack of toxicity in the sediment compartment 24 h after the application, consistent with the observed decreased in the pesticide content of the sediments. Bondarenko and Gan (2004) reported chlorpyrifos degradation half-lives of 14–24 days in laboratory sediment-water incubations with sediments from urban streams in California (USA). In soils, half-life values for chlorpyrifos range from less than 1 week to more than 24 weeks, depending on soil moisture, microbial activity, clay and organic content, and temperature (Odenkirchen and Eisler 1988). The major routes of chlorpyrifos loss from soils are chemical hydrolysis in moist soils, clay-catalyzed hydrolysis in dry soils, and microbial degradation and volatilization. Several processes contributed to the observed fast toxicity decrease in the sediment in the present study. Fang et al. (2008) showed that repeated chlorpyrifos application to soil samples increased degradation rates. A bacterial strain capable of utilizing chlorpyrifos as a sole C source was isolated 21 days after three successive chlorpyrifos applications. The studied stream upper basin is intensively cultivated, receiving several pesticide applications per year. It seems likely that repeated exposures might have developed sediment-resident microbial assemblages with increased degradation ability. The eventual implications of the laboratory results reported by Fang et al. (2008) to field conditions deserve further attention. Hydrolysis strongly increases with pH for both chlorpyrifos (Odenkirchen and Eisler 1988; Bondarenko and Gan 2004) and cypermethrin (Laskowski 2002); the high alkalinity prevailing in the stream water (Table 1) enhanced hydrolysis in the water itself, and likely in superficial sediments. Photolysis and volatilization also represent important losses. Because the pools were extremely shallow, both processes might have contributed to the observed fast dissipation. The high irradiation rates prevailing during the experiment also enhanced pesticide losses.

The studied surface waters are representative of the Pampasic streams. The main environmental conditions that favoured fast dissipation rates seem 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. The water phase is alkaline attaining high pH values (Carriquiriborde et al. 2007).

Direct plane fumigation in the small headwater streams represents the worst toxicity scenario, because the pesticide is applied directly over the water at the same load as that used for the culture. *H. curvispina* is a widespread organism very common in the regional environments. Concentrations of cypermethrin and chlorpyrifos lethal to *H. curvispina* are similar to those reported for *H. azteca* (Mugni 2009). Both are among the most sensitive organisms reported (Laskowski 2002; Farmer et al. 1995). Once toxicity ceases for *H. curvispina*, small acute effects would further be expected in the faunal resident community. It is therefore concluded that the ongoing agricultural practices result in ephemeral acute toxicity pulses of a few hours' duration in the regional surface Pampasic waters.

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References

Bondarenko S, Gan J (2004) Degradation and sorption of selected organophosphate and carbamate insecticides in urban stream sediments. Environ Toxicol Chem 23(8):1809–1814

Carriquiriborde P, Diaz J, Mugni H, Bonetto C, Ronco AE (2007) Impact of cypermethrin on stream fish populations under field use in biotech-soybean production. Chemosphere 68:613–621

Crossland NO, Shires SW, Bennett D (1982) Aquatic toxicology of cypermethrin III. Fate and biological effects of spray drift deposits in freshwater adjacent to agricultural land. Aquat Toxicol 2:253–270

Fang H, Yu YL, Wang XG, Chu XQ, Pan XD, Yang XE (2008) Effects of repeated applications of chlorpyrifos on its persistence and soil microbial functional diversity and development of its degradation capability. Bull Environ Contam Toxicol 81(4):397–400

Farmer D, Hill IR, Maund SJ (1995) A comparison of the fate and effects of two pyrethroid insecticides (lambda-cyhalothrin and cypermethrin) in pond mesocosms. Ecotoxicol 4(4):219–244

Jergentz S, Mugni H, Bonetto C, Schulz R (2004a) Runoff-related endosulfan contamination and aquatic macroinvertebrate response in rural basins near Buenos Aires, Argentina. Arch Environ Contam Toxicol 46(3):345–353

Jergentz S, Pessacq P, Mugni H, Bonetto C, Schulz R (2004b) Linking in situ bioassays and dynamics of macroinvertebrates to assess agricultural contamination in streams of the Argentine Pampa. Ecotoxicol Environ Saf 59:133–141

Laskowski D (2002) Physical and Chemical Properties of Pyrethroids. Rev Environ Contam Toxicol 174:49–177

MAGyP (2010). Ministerio de Agricultura, Ganadería y Pesca Series y Estadísticas: http://190.220.136.179/index.php/series-por-tema/agricultura. Accessed on July 2009

Marino D, Ronco A (2005) Cypermethrin and chlorpiryfos concentration levels in surface water bodies of the Pampa Ondulada, Argentina. Bull Environ Contamd Toxicol 75(4):820–826

Maund SJ, Hamer MJ, Lane MCG, Farrelly E, Rapley JH, Goggin UM, Gentle WE (2002) Partitioning, bioavailability, and toxicity of the pyrethroid insecticide Cypermethrin in sediments. Environ Toxicol Chem 21(1):9–15

Mazanti L, Rice C, Bialek K, Sparling D, Stevenson C, Johnson WE, Kangas P, Rheinstein J (2003) Aqueous-phase disappearance of atrazine, metolachlor, and chlorpyrifos in laboratory aquaria and outdoor macrocosms. Arch Environ Contam Toxicol 44:67–76

Mugni HD (2009) Nutrient concentration and pesticide toxicity in superficial water of rural basins. PhD dissertation (in Spanish). La Plata Univ. Argentina. 140 pp

Odenkirchen E, Eisler R (1988) Chlorpyrifos hazards to fish, wildlife, and invertebrates: a synoptic review. US Fish Wildl Serv Boil Rep 13. 28 pp

USEPA (US Environmental Protection Agency) (2000) Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, 2nd edn. EPA 600/R-99/064, March