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Highlighted Article

Zooplankton community responses to chlorpyrifos in mesocosms under Mediterranean conditions ☆

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Abstract

The effects of chlorpyrifos (organophosphate insecticide) on zooplankton were studied in outdoor experimental tanks (mesocosms) sited in the Mediterranean Region (Madrid, Spain) at two nominal concentrations of chlorpyrifos (0.1 and 1 μg a.s./L applied as Chas® 48) and control were used. Five tanks were used as control and the treatments were performed in quintuplicate. A single chlorpyrifos application simulating spray-drift was conducted. The population and community effects were analyzed by means of univariate statistics and through the multivariate principal response curves (PRC) technique. The most affected zooplankton taxa were cladocerans (*Daphnia* group *galeata*), copepods (cyclopoids and copepod nauplii) and rotifers (*Keratella cochlearis*) showing in all the cases significant decreases in abundance at 1 μg chlorpyrifos/L. The calculated NOEC was 0.1 μg/L for these taxa as well as for the community. The zooplankton community was considered to be recovered after 99 days post-application. The results of this experiment were similar to those derived from mesocosm/microcosm studies performed in temperate regions. This indicates that a chlorpyrifos concentration of 0.1 μg chlorpyrifos/L could be the appropriate safe level for zooplankton community in different climatic regions. However, at treatment level of 1.0 μg/L the time required for full recovery of the affected populations (particularly Cladocera) was longer than in the other experiments performed in temperate regions.

Keywords: Chlorpyrifos; Mesocosm; Mediterranean region; Zooplankton community; PRC; NOEC; Recovery

1. Introduction

Model ecosystems that mimic freshwater environments (i.e. microcosms and mesocosms) are tools often used to assess potential ecotoxicological hazards of pesticides (Touart, 1988; Graney et al., 1994; Hill et al., 1994). A major advantage of these experimental systems is their realistic simulation of ecological effects of pesticide stress on aquatic communities. Thus, effects on and recovery of a wide array of species can be studied while allowing interactions between the community populations. Among the aquatic community, zooplankton includes many different species at different trophic levels. Zooplankton plays a key role in freshwater ecosystems as it occupies a central position in the food chain, transferring energy from primary producers to organisms at higher trophic levels (Chang et al., 2005). The application of a toxicant on a natural or artificial ecosystem can modify the structure and function of the community, thus altering the population densities and affecting prey–predator interactions (Brock et al., 1992; Hanazato, 1998; Preston, 2002; Fleeger et al., 2003).

Historically, higher tier studies have been performed mainly in Atlantic Central Europe and North America due the results have been extrapolated to other climatic regions including the Mediterranean. However, the climatic and ecological conditions of those regions are quite different (i.e. temperature, light intensity, community structure,

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species composition) and it could be expected that the fate, bioavailability and effects of pollutants will be different. In a laboratory study with chlorpyrifos Van Wijngaarden et al. (2005a) showed that critical threshold levels for effects on cladocerans were similar between microcosms-simulating temperate and Mediterranean conditions, but that at higher concentrations indirect effect were more pronounced under Mediterranean conditions and also the rate of recovery of *Daphnia* was slower. The question at stake is whether these laboratory observations can be confirmed under more realistic field conditions.

Chlorpyrifos ((*O*,*O*-diethyl-*o*-(3,5,6-trichloro-2-pyridyl) phosphorothioate) is a broad-spectrum organophosphorus insecticide that displays activity (cholinesterase inhibitor) against a wide range of insect and arthropod pest. The mode of action and physicochemical properties of the insecticide have been previously described by Marshall and Roberts (1978). Since initial product commercialization in the mid-1970s on crops such as corn, cotton and peaches, the use of chlorpyrifos was expanded to include a diversity of agricultural situations (Barron and Woodburn, 1995). Nowadays, chlorpyrifos is used to control pests attacking citrus crops and vineyards that are particularly important in Mediterranean countries. In Spain more than 303.8×10^3 and 1272×10^3 ha are used for citrus crops and vineyards, respectively (*source*: Statistical Office of the European Communities (EUROSTAT)). Moreover, there is a large number of published microcosm/mesocosm experiments performed in other climatic regions with chlorpyrifos; hence, this pesticide was selected to be tested in Mediterranean conditions.

The aims of this study were: (1) to evaluate the effects of chlorpyrifos on the zooplankton community in outdoor experimental ponds in Spain, (2) to evaluate the recovery of affected zooplankton populations under Mediterranean conditions and (3) to compare the threshold levels obtained, with those reported for previous micro/mesocosm experiments performed in other climatic regions.

2. Materials and methods

2.1. Experimental design

The experiment was performed in 15 experimental tanks (mesocosms). The characteristics of each tank were the following: length of 4 m; width of 2 m at water surface; water depth of 1.5 m and total volume of 11 m^3. Zooplankton and phytoplankton from a pond (400 m^3) sited at the National Institute for Agricultural and Food Research and Technology (INIA, Madrid, Spain) were introduced during the pre-treatment period (3 months approximately) but sediment was not added.

Our mesocosms are plankton-dominated systems with low macrophyte densities.

The concentrations used were selected considering that 0.1 μg/L is the Maximum Admissible Concentration Quality Standard (MAC-QS) according to the Water Framework Directive (European Union, 2000) and 1 μg/L is expected to produce relevant effects on aquatic ecosystems based on mesocosm experiments. The tanks were assigned randomly to the different treatment levels. Five tanks were used as control and the treatments were performed in quintuplicate.

2.2. Pesticide application and sampling

The formulated product Chas® 48 EC (48% w/v chlorpyrifos, Agrodan) was applied once on May 16, 2005. The amount of formulated product required to achieve nominal test concentrations in each tank was calculated on the basis of the volume of water in each tank and the active ingredient concentration in the Chas® 48 formulation. Stock solutions of chlorpyrifos (formulated product in deionizated water; total volume 1 L) were premixed in amber glass bottles just before the application.

Chlorpyrifos was applied by means of a spray gun allowing an even distribution of the toxicant over the water surface. To verify initial concentrations, water samples from all tanks were collected immediately after application.

Water samples were collected (0.08, 1, 4, 10 and 21 days post-application) from every tank to measure exposure concentrations of chlorpyrifos. Depth-integrated samples were obtained using a water-sampler that were then transferred into amber glass flask and prefiltered through a Sartorius AG (Goettingen, Germany) nylon syringe filters (diameter 3 cm, mesh size 0.45 μm) to remove larger sample particles.

2.3. Chemical analysis

Chlorpyrifos was extracted from water samples (500 mL) by solid phase extraction (SPE) following the method of the Environmental & Agrochemical Applications Notebook (Waters OASIS sample extraction products, pp. 9, Rev 3, 01/02 2002). Strata-X™ HLB columns (3 mL, 100 mg/mL; Phenomenex® 8B-S100-UBJ) were used for SPE. The columns were conditioned with 6 mL of methyl terbuthyl eter/methanol (MTBE/MeOH; 90/10), 6 mL of methanol and 6 mL of HPLC-water (Milli-Q UV_{185}). Chlorpyrifos was eluted from the extraction columns with 10 mL of MTBE/MeOH (90/10) into glass centrifuge tubes and evaporated to dryness (GENEVAC). The samples were redissolved in 1 mL of mobile phase (MTBE/MeOH (90/10)) and analyzed by Gas Chromatograph with Electron-Capture Detector (GC-ECD).

Dissipation times (DT_{50}) were calculated for each treatment and used to rank dissipation rates from the water within the first 4 days. The course of the dissipation was approximated by first-order kinetics. The dissipation coefficient was calculated by means of linear regression on the *ln*-transformed concentrations.

2.4. Water quality analysis

The physico-chemical properties (water temperature, pH, dissolved oxygen (DO) concentration and electrical conductivity (EC)) of water of each tank were measured at the same time as the collection of zooplankton samples. The measurements were carried out in the morning (between 8 am and 10 am) at mid-water depth. All the parameters were measured using HACH portable apparatus (Hach Company).

2.5. Zooplankton sampling and identification

Zooplankton was sampled from each experimental tank on days −17, −1, in the pre-treatment period, and on days 2, 8, 15, 22, 29, 43, 57, 78 and 99 after the application using a water-sampler (volume 1 L). Several depth-integrated sub-samples were collected until a 10-L sample was obtained. Five liters of each sample were used for zooplankton analysis. The sample was concentrated through a plankton net (mesh size, 55 μm; Hydrobios Kiel, Germany) and preserved with formalin (final volume 4%).

Micro-zooplankton (i.e. Rotifera) was counted and identified under an inverted microscope (Olympus; magnification 400 ×) using a subsample of known volume. Macro-zooplankton (i.e. Cladocera, copepod nauplii and copepodit stadia of Copepoda) was quantified by counting the entire sample using a stereomicroscope (Olympus; magnification 90 ×). Rotifera and Cladocera were identified to the lowest practical taxonomic level (genus–species), whereas Copepoda were classified as calanoids or cyclopoids.