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Accumulation and Elimination of Pentachlorophenol in the Freshwater Bivalve *Corbicula fluminea*

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The La Plata River, which is one of the most important aquatic systems in South America and provides the freshwater supply for Buenos Aires, the capital of Argentina, receives the discharge of a great number of chemicals (Verrengia Guerrero and Kesten 1994).

The response of aquatic organisms has been suggested as a sentinel for the contamination of the hydrosphere. Sedentary species as mussels and other bivalves are desirable as bioindicators of heavy metals and organic compounds since they filter large volumes of water, accumulate a wide range of contaminants and are likely to reflect changes in the pollution status of their environment (Mäkelä and Oikari 1990, Sheehan *et al*. 1995).

Assessment of the toxicological risks posed by the introduction of a chemical into the aquatic environment requires the integration of biological effects with time and concentration in tissues of the living organisms (Widdows and Donkin 1991). Species of the genus *Corbicula* have been used as biological monitors in freshwater environments (Doherty 1990). However, there are no data available about the time course of organic compounds in bivalves of the species *Corbicula fluminea* from the coast of the La Plata River. The accumulation and elimination of organic compounds depend on the species and the system, and it is very difficult to extrapolate results from other authors (Ernst 1979; Tachikawa *et al.* 1991; Tjeerdema *et al*. 1994). The main objective of the present work was to investigate the feasibility to use this bivalve species for bioconcentration experiments. Therefore, we obtained the accumulation and elimination curves at different concentrations of pentachlorophenol (PCP) in *C. fluminea*. While many classes of toxicants could have been selected, PCP was chosen because it is a well-studied organic substance whose widespread use -mainly as fungicide and wood preservative- has led to environmental contamination (IPCS 1987).

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MATERIALS AND METHODS

Pentachlorophenol (PCP) > 99% was obtained from Sigma (St. Louis, MO, U. S. A.) and was free of 2,3,7,8 TCDD. Tetrachloro-p-hydroquinone and tetrachloro-p-benzoquinone were also purchased from Sigma. Tetra and trichlorophenols were a generous gift from Dr. I Gebefügi (GSF-Institute for the Reserch of Environment and Health, Germany). Methanol was HPLC grade. All other reagents and solvents were analytical grade.

PCP was dissolved in methanol to obtain a 1 mg/mL solution. This solution was dissolved in distilled water to obtain the working solutions.

The PCP concentrations were determined by GLC. A Hewlett-Packard gas chromatograph Model 5840A, equipped with ^{63}Ni electron capture detector was used. A glass column, 2 m length x 2 mm I.D. was packed with 3% OV-17 on Chromosorb WAW/DMCS, 80-100 mesh and conditioned. The operating conditions of the gas chromatograph were: column temperature, 175 °C; injector temperature, 220 °C; detector temperature 250 °C; carrier gas nitrogen at 40 ml/min. Chromatograms were recorded using a computing integrator.

In all cases the PCP solutions were acetylated as it is indicated below and extracted with 1 mL of n-hexane. An aliquot of 2 µL was injected into the gas chromatographic system.

Buenos Aires tap water -filtered through animal carbon, glass wool and filter paper, and aerated- was used as dilution water for bioassays (total hardness 67 mg/L as $CaCO_3$; alkalinity 29 mg/L as $CaCO_3$; pH 7.0). PCP was not detected in water before addition of the toxicant.

Bivalves of the species *Corbicula fluminea* were collected on three different occasions between July and August 1995 from the coast of the La Plata River nearby the Buenos Aires harbour. In the laboratory, they were acclimated to test conditions for a week, in aerated glass aquariums containing dilution water (20±2°C) with a 16h/8h day/night photoperiod. During the acclimatization period, the organisms were not fed. The mean length of the bivalves analyzed was 26.1±2.9 mm and the mean weight 0.65±0.10 g.

All bioassays were semi-static and conducted 1 week after collection, according to international standards (APHA-AWWA-WPCF 1980; FAO 1987). The molluscs were placed in 2.5 L aerated glass aquaria containing 3 bivalves/L.

PCP solutions for bioassays were prepared by adding small amounts of PCP-methanol solution to dilution water to obtain the desired PCP concentrations. The final concentration of methanol did not exceed 50 µL/L. The concentrations of PCP were maintained by daily renewal of the exposure solutions and a 100 mL sample was stored for PCP analysis. Mean loss of PCP in the test vessels over each 24 hr period was less than 7%.

The determination of PCP in the exposition water was optimized mainly according to the technique described in Renberg and Lindstrom (1981) . Aliquots of the exposition solutions were adjusted to 3 mL with water from the control aquarium, then 3 mL of K2CO3 0.1 *M* were added (pH = 10) and the solution was shaken for 10 minutes with 100 µL of acetic anhydride at room temperature for complete acetylation. The acetylated PCP was extracted with n-hexane and injected in the gas chromatograph as mentioned before. Standard addition methods were used to compensate for matrix effects. The calibration curves were made by adding known amounts of PCP to water from the control aquarium.

Initial tests determined the acute toxicity of PCP to bivalves (LC50) and established sublethal exposure concentrations to be used for the accumulation studies.

Groups of bivalves were exposed to the PCP test solutions (30, 50, 75 and 100 µg/L). Every day 2 or 3 animals of each concentration were sampled. The whole body was excised from the shell and immediately frozen. The PCP levels in soft tissue were determined within 72 hr by a method based on the studies of Rudling (1970). The entire soft tissue of each bivalve was carefully removed and placed on filter paper to drain extra fluids. Immediately afterwards, it was weighed and homogenized in a Potter Elvehjem with 10 mL of water. Aliquots of this homogenate were treated with H2SO4 50% (v/v) in a 3:1 proportion (pH < 2) during 30 minutes with occasional stirring in a vortex, and extracted with 3 mL of a mixture of n-hexane : ethyl ether 70:30. An aliquot of the organic layer was reextracted with 3 mL of K2CO3 0.1 *M* and then acetylated according to the same procedure used for water. The calibration curves were made using organisms from the control aquarium.

Animals that had been exposed to 50 or 100 µg/L of PCP for 72 hr were transferred to dilution water. The residual PCP in the soft tissue was determined at periods of 0 to 72 hr. PCP excreted into the water was also quantified.

In order to investigate the possible presence of PCP conjugates (sulfate, glycoside), 10 mL of bioassay water from the elimination experiments were put in screw-cap tubes and acidified with 1 mL conc. HCl. The tubes were sealed and placed in a boiling water bath for 1 hr. This procedure is analogous to the one recommended by Renner and Hopfer (1990), and it was optimized in our laboratory for p-nitrophenol (Oneto *et al* ,1995). Once they were cool, the hydrolyzed solutions were treated with K2C03 until pH 10 was reached, and then acetylated by the procedure described previously.

All glassware was thoroughly rinsed with ethanol previous to its use.

RESULTS AND DISCUSSION

The PCP 96 hr lethal concentration LC50 calculated by the noncomputerized method of Lichtfield and Wilcoxon (1949) was of 250 ± 30 µg/L. According

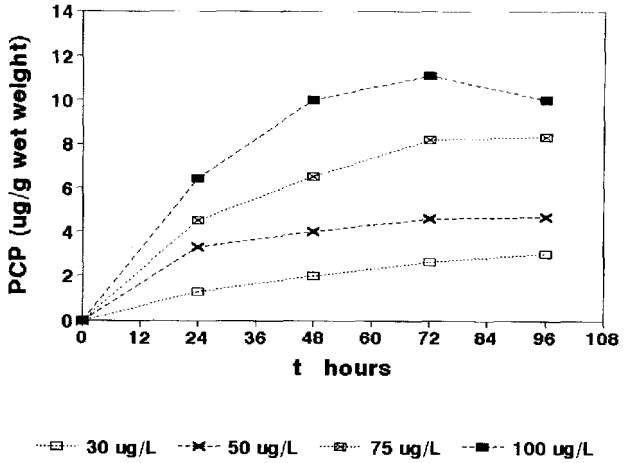

Figure 1. Modification of the PCP accumulation in *C. fluminea* with the exposition time. Each point represents the mean of 15 animals processed separately. Results are the average of three experiments.

to this result, sublethal concentrations of PCP between 30 and 100 µg /L were selected.

No mortality occurred during the sublethal experiments in control and PCP exposed animals.

The chromatogram obtained from the soft tissue of each bivalve of the species *C. fluminea* exposed to PCP, showed a peak at the same retention time as the acetyl derivative of PCP control solutions (4.23 ± 0.05 min). Chromatograms of samples of *C. flumine* showed the absence of any other interfering peak. The detection limit for PCP was 20 µg/L. Recoveries of PCP added to control water or soft tissues of bivalves were greater than 96%.

In the concentration range 0-80 µg/L of PCP, a straight line was obtained with the same slope for control solutions and for the water from the bioassay solutions. The correlation coefficient r^2 was 0.999.

We established using standards that at the retention time of PCP there were no interferences from 2,4,5-,2,4,6- and 3,4,5-trichlorophenols; 2,3,4,6- and 2,3,5,6-tetrachlorophenols; tetrachloro-p-hydroquinone and tetrachloro-p-benzoquinone which are metabolites excreted by mammals and some of them also by aquatic species (Renner and Hopfer 1990; Tjeerdema *et al*. 1994).

Figure 1 shows the PCP concentrations in whole soft tissue for every test solution at the beginning of the test and after 24, 48, 72 and 96 hr. The curves show that the levels of PCP in tissues increase with the concentration of toxicant in the bioassay water, and with time until a steady state is reached,

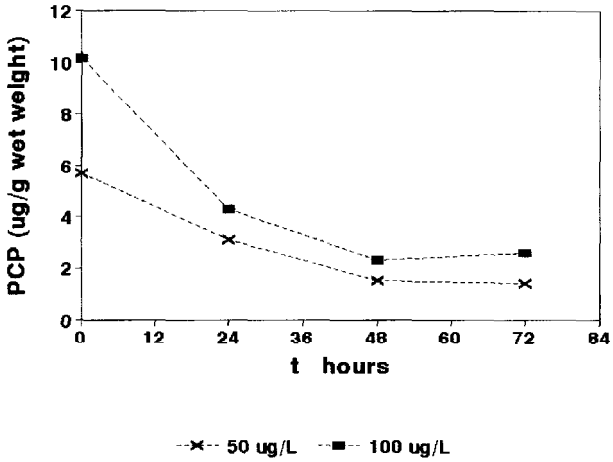

Figure 2. PCP elimination curves in *C. fluminea* after a 72 hr exposition. Each point represents the mean of 12 animals processed separately.

approximately after 72 hr. It is evident that there is a more rapid uptake of PCP during the first 24 hr. These experiments showed that the *Corbicula* species absorb and bioconcentrate PCP from water and this fact motivated the elimination studies. Before starting these studies, the organisms were treated during 72 hr with 50 and 100 µg/L of PCP in order to allow the toxicant to reach a constant concentration in the soft tissue.

Exposition to dilution water clearly diminishes the concentration of PCP in bivalves, as shown in Figure 2. Over the first 24 hr these organisms were able to depurate 50% of retained residues of PCP. This elimination is faster at higher concentrations. Owing to the exponential nature of the processes, the largest chemical movement occurs early. The elimination continues, but at a much slower rate until 48 hr, reaching afterwards an equilibrium.

Table 1. Observed PCP levels eliminated from the soft tissue of 12 bivalves at different times and the corresponding PCP levels quantified in water. Initial treatment: 100 µg/L during 72 hr.

Time (hr)	PCP from soft tissue (µg)*	In water (µg)
24	44 ± 6	38 ± 7
48	63 ± 6	65 ± 8
72	60 ± 7	69 ± 7

* Mean weight of each bivalve 0,65 g

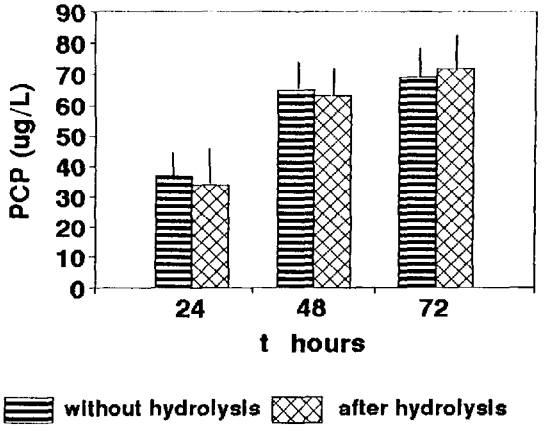

Figure 3. PCP levels in water without hydrolysis and after hydrolysis at different times during the elimination process. The bars represent the mean of 3 experiments ± SD.

After the exposure to 100 µg/L during 72 hr, the concentration of PCP in water determined at the end of the elimination experiments of 24,48 and 72 hr, accounted for the PCP eliminated from soft tissues during the same time periods, which would suggest that Phase I metabolism is not extensive ('Table 1). Other authors have found similar results in many other aquatic organisms (Tjeerdema *et al*. 1994).

Figure 3 shows that after acid hydrolysis, which should liberate phenols from the sulfate and glycoside conjugates, the PCP concentrations were not modified according to the Student's t test ($p < 0.05$). Although it has been demonstrated that PCP and other phenolics are in a limited number of cases detoxified in aquatic organisms by sulfate and/or glycoside conjugation, (including both glucuronidation and glucosidation - Phase II metabolism) that would not be our case (Ernst 1979; Kobayashi 1970; Nagel 1983; Tjeerdema *et al*. 1994).

This study shows that in acute bioassays, *C. flumine* temporarily incorporates high levels of PCP from the exposition water, which seem to be eliminated without any biotransformation within a short time, once the organisms have been transferred to clean water.

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