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Bioaccumulation in Freshwater Crabs. Endosulfan Accumulation in Different Tissues of *Zilchiopsis collastinensis* P. (Decapoda: Trichodactylidae)

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Abstract We examined the bioaccumulation of α- and β-endosulfan and endosulfan sulfate in tissues from the crab *Zilchiopsis collastinensis*. There was more endosulfan accumulated in the hepatopancreas (from <2 to 467.8 ng g^{-1}) than in the gonads (from <2 to 52.1 ng g^{-1}) or muscles (<2 ng g^{-1}). The endosulfan concentrations in the hepatopancreas decreased over time and with the endosulfan dilution ($p < 0.05$). In the gonads there was little bioaccumulation, which did not vary over time ($p > 0.05$). The hepatopancreas is a dynamic organ that is able to depurate itself, whereas the gonads act as a sink for pesticides.

Keywords Bioaccumulation · Bioindicator · Agricultural pollution · Pesticides

Biocides are periodically used in agricultural activities with the goal of minimizing pest damage. The widespread use of persistent organic pollutants has resulted in the contamination of freshwater ecosystems, mainly through drift and runoff. Because of its persistence, endosulfan continues to act long after its application, and affects many nontarget organisms (Ernst et al. 1990; Jergentz et al. 2005).

The biota is able to accumulate persistent pesticides. The wildlife is used as a bioindicator for environmental pollution status and trends for certain regions, specially the sentinel species. The biota that inhabits in polluted areas may act as sentinel organisms. The burrowing crabs of the genera *Zilchiopsis* are territorial animals with an intermediate position in both the aquatic and terrestrial food webs, being occasionally consumed by humans. Despite of it importance, there are few records related with metal accumulation in freshwater crabs and, to the best of our knowledge, there is no available data on their capacity for pesticide accumulation or in the differential accumulation between the different tissues (Gagneten et al. 2008). The aim of this study was to examine the accumulation of two isomers of endosulfan (α- and β-) and their degradation product (endosulfan sulfate) in the hepatopancreas, ovary and muscle tissues of the freshwater burrowing crab *Zilchiopsis collastinensis* when exposed under controlled conditions.

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Materials and Methods

Zilchiopsis collastinensis individuals were collected on the Paraná River floodplain (31°30′S, 60°41′W; Santa Fe, Argentina) in late winter, before pesticide applications occurred, acclimated and placed in 300 liter aquaria filled with dechlorinated water. The mean (±SD) carapace width of the crabs used was 51.45 (±2.86) mm. The largest individual was not more than 1.5 times larger than the smallest. The aquaria were placed in an open greenhouse covered with shade cloth, to avoid the overheating caused by direct sun exposure. The crabs were fed fresh fish muscle 3 times a week. Food was supplied in the evening, and the leftovers were extracted early the next morning. Commercial grade pesticides (Zebra Ciagro®; Red Surcos S. A., Argentina) containing 35 % endosulfan were employed. Three sublethal concentrations of endosulfan were applied, with an initial concentration followed by dilution with pesticide-free dechlorinated water, simulating the typical contaminant

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dilution process for aquatic ecosystems. The initial nominal concentrations used were 0 (control group) 100, 200 and 400 μg endosulfan l^{-1} (C_0, C_1, C_2 and C_3 respectively), with three replicates of each treatment. The dilution rate was 20 L/day in a continuous flow system, and the excess water was drained by overflow, as it was in the acclimation period. Dissolved oxygen, pH, water temperature and conductivity were measured 3 times a week before feeding.

Water samples and samples of the hepatopancreas, ovary and chelae muscle tissues were taken before the endosulfan application, to rule out the baseline presence of pesticides, just after pesticide application (initial concentration) and on days 2, 8, 15 and 22 after the pesticide application. The sampled crabs (3 for each replicate; a total of 9 per concentration assayed) were cryoanesthetized, sized and weighed. The gonad, hepatopancreas and muscle tissues were gently dissected, immediately weighed and conserved at −18°C, blended together and homogenized. Endosulfan levels in the water were measured using the ASTM D 6520-06 method. Samples were subjected to solid-phase microextraction (SPME), and concentrations were measured with a GC—ECD (Gas chromatography—Electron capture detector; GC Hewlett Packard 5890 Series II) using nitrogen as a carrier gas (1 mL/min). The detection limit (LOD) was 2 μg endosulfan l^{-1}. The quantization limit (LOQ) was 6.0 μg endosulfan l^{-1}.

The endosulfan levels (α- and β- isomers and endosulfan sulfate) in the crab tissues (hepatopancreas, gonad and muscle) were measured using the method described in Stoker et al. (2011), with minor modifications. For the lipid tissues of the hepatopancreas and gonads (15.11 ± 4.28 % and 11.49 ± 2.39 %, respectively), a simplified procedure based on the Mills method was employed (Mills et al. 1963). For the extraction procedure of the muscle tissues, which had little or no fat and in which extracted lipids were too low to be measured, a method based on the matrix solid-phase dispersion (MSPD) process was applied (Carro et al. 2005). Gas Chromatography (GC) analysis was performed with a gas–liquid chromatograph (GLC) (Hewlett Packard Model 5890, USA) equipped with a 63Ni electron-capture detector (ECD). Pestanal endosulfan standards (Honeywell Riedel–de Haen Fine Chemicals, Seelze, Germany) were used. All of the solvents used were of pesticide-grade quality (Merck, Darmstadt, Germany). Surrogate crab tissue samples were prepared using known concentrations of α- and β-endosulfan and endosulfan sulfate. The average percentage recoveries varied from 74 % to 117 %. The relative standard deviation (RSD) was lower than 20 % for all residues (n = 6). The detection limit (LOD), which was calculated as three times the average SD of the blank replicates, was 0.8 ng g^{-1} for α- and β-endosulfan and 1.0 ng g^{-1} for endosulfan sulfate. The quantization limit (LOQ), which was calculated as 7.5 times the average SD of the blank replicates, was 2.0 ng g^{-1} for α- and β-endosulfan and 2.5 ng g^{-1} for endosulfan sulfate.

The pesticide concentrations were calculated on a lipid-normalized basis, transformed according to the lipid amounts of each sample and represented as ng endosulfan g^{-1} tissue. Normality was tested using the Kolmogorov–Smirnov test. Analysis of variance (ANOVA) and the Tukey test ($\alpha = 0.05$) were used to compare differences in pesticide accumulation among each time period and concentration and in each tissue (Zar 1996).

Results and Discussion

The values of temperature, dissolved oxygen, pH and conductivity were 14.55 ± 4.32°C; 7.12 ± 1.4 mg L^{-1}; 7.22 ± 1.1; 1231.75 ± 10.53 μS/cm respectively. The temperature varied during the day, but there were no differences between treatments. No endosulfan was detected in the dechlorinated water or in the fish muscles supplied as food. The endosulfan concentrations in the water were highest after pesticide application and decreased with time until no longer detectable by our analytical methods (Fig. 1).

Endosulfan was not detected in the control crabs for any tissue type. Neither β- endosulfan nor endosulfan sulfate was detected at a meaningful concentration, and both compounds were excluded from the statistical analysis. The isomer showing the most accumulation was α-endosulfan.

In the hepatopancreas tissues of crabs exposed to concentration level C_3, the endosulfan concentration was highest on day 2 ($p < 0.05$) and slowly decreased over time, possible because of the decreasing endosulfan concentration in the water over time. The endosulfan accumulation in the hepatopancreas tissues of the crabs exposed to concentration levels C_1 and C_2 were similar for all days

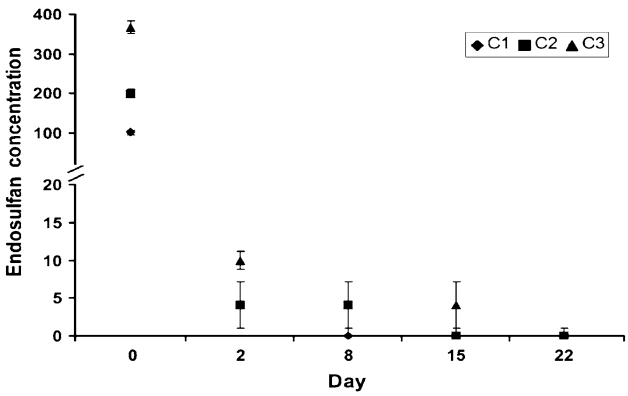

Fig. 1 Mean endosulfan concentrations (±SD) in water in each treatment at initial time (day 0) and in the subsequent days

($p > 0.05$) (Fig. 2). For all days, the crabs exposed to C_3 accumulated more endosulfan than those exposed to C_1 or C_2 in all the days ($p < 0.05$) (Fig. 2). The accumulation of endosulfan in the muscle tissues was not quantifiable. Some endosulfan concentrations were detected on day 2, but these result were isolated and were not included in the statistical analysis.

Because the commercial pesticide has a proportion of 70 % α-endosulfan—30 % β-endosulfan, there was a greater accumulation of the α-isomer, which is present in the pesticide in a higher concentration. In crustaceans, the hepatopancreas plays a major role in food digestion. It is composed of two half organs linked to either side of the stomach. Each half consists of several hundred blind-ended tubules bathed in hemolymph. These tubules fuse to form collecting ducts that end in the stomach. The functions of the hepatopancreas are the synthesis of digestive enzymes and lipids emulsifiers and the absorption, metabolization and storage of food. The digestion products are mobilized by the hemolymph, which transports compounds from the hepatopancreas to the rest of the body, and back to the hepatopancreas. The hepatopancreas also participates in detoxification of xenobiotics (Vogt 2002). Endosulfan travels through the hemolymph to the hepatopancreas, where the lipophilic properties of endosulfan cause it to bind to the lipids present.

The crabs that were exposed to the two lowest concentrations of endosulfan showed no differences in accumulation in the hepatopancreas. However, that was not the case for the crabs exposed to the highest concentration. The accumulation of xenobiotics is the difference between intake and elimination. Several physiological processes regulate intake and elimination and, therefore, accumulation. The accumulation of a xenobiotic substance is not always proportional to the exposure concentration. Detoxification processes may increase pesticide elimination, reducing bioaccumulation. However, detoxification processes may not be completely effective at high exposure concentrations, and the ability to control the accumulation may be compromised (Newman and Unger 2003; Gagneten et al. 2008). Exposure to pesticides may also cause changes in the normal distribution of the cells in the hepatopancreas, increasing the number of cells involved in detoxification. At higher concentrations of pesticides, the resulting histological damages, such as necrosis, could reduce the detoxification capacity of the hepatopancreas (Bhavan and Geraldine 2000). Necrosis and other histopathological effects were observed in crabs exposed to endosulfan (unpublished data).

In the gonad tissues, the concentrations accumulated did not vary with time, as there were no differences for any day ($p > 0.05$). Additionally, the accumulation was not concentration-dependent, as there were no differences among the exposure concentrations for any day ($p > 0.05$) (Fig. 3). During exogenous vitellogenesis, the lipids stored in hepatopancreas are processed to lipoproteins and transported via the hemolymph to the oocytes (Lubzens et al. 1995). Lipophilic pesticides may be attached to these lipoproteins and transported into the yolk. Because the yolk of the ovary is an energy reserve, and because there are no removal mechanisms, there is no renewal of lipids.

Pesticides such as endosulfan, which have an octanol/water partition coefficient favoring solubility in lipid-rich tissues over solubility in water, can be attached to and transported with the lipid building blocks of the embryo into the egg and passed on to subsequent generations. The accumulation of lipophilic pesticides in the ovaries is postulated as one of the reasons that females are more resistant than males to the effects of pesticide and PCB exposures (Wirth et al. 2001). Because the pesticides reach the reproductive cells, and because the exposure of an embryo to pesticides begins with fertilization, there may be harmful consequences for the offspring. The hepatopancreas is a dynamic organ that depurates itself as the environmental concentration of pesticides decreases. In

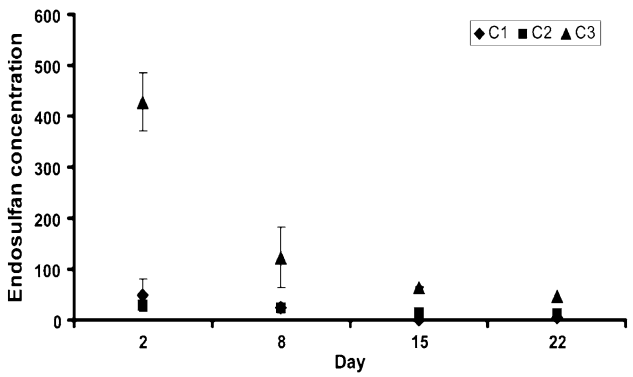

Fig. 2 Mean endosulfan concentrations (±SD) in hepatopancreas of crabs exposed to different endosulfan concentrations in water

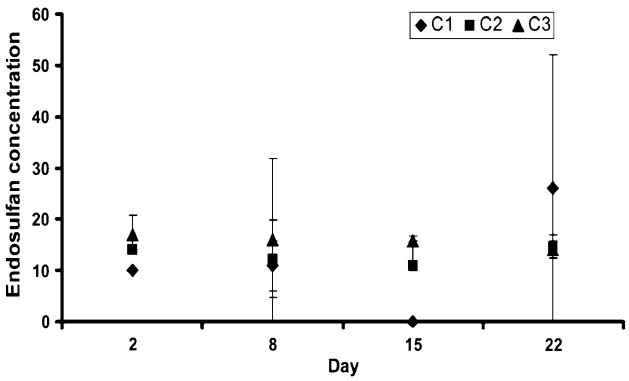

Fig. 3 Mean endosulfan concentrations (±SD) in gonads of crabs exposed to different endosulfan concentrations in water

contrast, the gonads are incapable of depuration and act as a sink for pesticides.

In the muscles, the endosulfan concentrations were too low to be detected and quantified. Muscle tissue is known to be a site of accumulation for heavy metals. However, for organochlorine pesticides muscle tissue is not an accumulation site, possibly because of the lipophilicity of endosulfan and the absence of lipids in the muscle tissue. These results are consistent with previous studies, in which persistent pesticides were found in higher concentrations in the hepatopancreas and gonads than in the muscles (Mortimer 2000).

In some studies endosulfan was found mainly as endosulfan sulfate, with minimal accumulation of the endosulfan parent compounds, indicating that the endosulfan may have been metabolized. Cobb et al. (2003) founded mainly the metabolized species in oocytes, which was transferred via maternal transfer. In this study, we observed mostly α-endosulfan and almost no endosulfan sulfate, indicating the accumulation of the parental isomers in the hepatopancreas and their transfer without metabolism to the oocytes and, perhaps, to the crabs' predators. Crabs are linked to different parts of the food webs in freshwater and terrestrial ecosystems. They are active predators and detritus feeders, occupying an intermediate position in both aquatic and terrestrial food webs. They are also an important food resource for fish, reptiles, birds and mammals (Collins et al. 2006). Because of their capacity for bioaccumulation and their relatively elevated resistance to pesticides, crabs could transfer these pesticides to organisms in a higher trophic level, and eventually to humans.

There are many requirements that a species must meet to be a good biological indicator of pollution, according to Sures (2004). The species must play a key role in the community, transporting the pollutant from the lower to the upper links of the food webs, or participating in the food chain of a species that is economically important to human. The use of native species as sentinel organisms is proposed as a more appropriate way to obtain information about a specific site. *Zilchiopsis collastinensis* is a local abundant crab that is widely distributed in the Paraná-La Plata hydro system, and it is territorial, easy to collect and resistant to pollutants. The endosulfan bioaccumulation capacity of this species contributes to its suitability as a bioindicator for the presence of endosulfan in aquatic systems, although more studies are needed (Sures 2004; Gagneten et al. 2008). Because the biota may accumulate persistent lipophilic organic pollutants, the transfer of these contaminants in the food web, which eventually may reach humans, must be continually observed.

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References

Bhavan PS, Geraldine P (2000) Histopathology of the hepatopancreas and gills of the prawn *Macrobrachium malcolmsonii* exposed to endosulfan. Aquat Toxicol 50:331–339

Carro AM, Lorenzo RA, Fernandez F, Rodil R, Cela R (2005) Multiresidue screening of chlorinated and brominated compounds from aquaculture samples using matrix solid-phase dispersion—gas chromatography–mass spectrometry. J Chromatogr A 1071:93–98

Cobb GP, Bargar TA, Pepper CB, Norman DM, Houlis PD, Anderson TA (2003) Using Chorioallantoic Membranes for Non-lethal Assessment of Persistent Organic Pollutant Exposure and Effects in Oviparous Wildlife. Ecotoxicology 12:31–45

Collins P, Williner V, Giri F (2006) Trophic relationships in Crustacea: Decapoda of river with a floodplain. In: Elewa A (ed) Predation in organisms: a distinct fenomenon. Springer, Heidelberg, p 326

Ernst WR, Jonah P, Doe K, Julien G, Hennigar P (1990) Toxicity to aquatic organisms of off-target deposition of endosulfan applied by aircraft. Environ Toxicol Chem 10:103–114

Gagneten AM, Imhof A, Gervasio S (2008) Accumulation and elimination of Cr in gills and eggs by the freshwater crab *Zilchiopsis collastinensis* after experimental exposure. Fresen Environ Bull 17:182–187

Jergentz S, Mugni H, Bonetto C, Schulz R (2005) Assesment of insecticide contamination in runoff and stream water of small agricultural streams in the main soybean area of Argentina. Chemosphere 61:817–826

Lubzens E, Khayat M, Ravid T, Funkenstein B, Tietz A (1995) Lipoproteins and lipid accumulation within the ovaries of penaeid shrimp. Isr J Aquacult-Bamid 47:185–195

Mills PA, Onley JH, Guither RA (1963) Rapid method for chlorinated pesticide residues in nonfatty foods. J AOAC Int 42:734–740

Mortimer MR (2000) Pesticide and trace metal concentrations in Queensland estuarine crabs. Mar Poll Bull 41:359–366

Newman MC, Unger MA (2003) Fundamentals of Ecotoxicology. CRC press, Boca Raton

Stoker C, Repetti MR, García SR, Zayas MA, Galoppo GH, Beldoménico HR, Luque EH, Muñoz-de-Toro M (2011) Organochlorine compound residues in eggs of broad-snouted caimans (*Caiman latirostris*) and correlation with measures of reproductive performance. Chemosphere 84:311–317

Sures B (2004) Environmental parasitology: relevancy of parasites in monitoring environmental pollution. Trends Parasitol 20:170–177

Vogt G (2002) Functional Anatomy. In: Holdich DM (ed) Biology of freshwater crayfish. Blackwell Science, London, p 512

Wirth EF, Lund SA, Fulton MH, Scott GI (2001) Determination of acute mortality in adults and sublethal embryo responses of *Palaemonetes pugio* to endosulfan and methoprene exposure. Aquat Toxicol 53:9–18

Zar JH (1996) Biostatistical analysis. Prentice Hall, New York