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Histopathological effects of endosulfan to hepatopancreas, gills and ovary of the freshwater crab *Zilchiopsis collastinensis* (Decapoda: Trichodactylidae)

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ABSTRACT

In this work, the effects of a pulse exposure of endosulfan on hepatopancreas, gills and ovary of the burrowing crab *Zilchiopsis collastinensis* were evaluated. The crabs were exposed to three sublethal concentrations in a pulse system with controlled dilutions. Water samples for pesticide concentrations measurements and crab tissue samples were taken when applications were made and 2, 8, 15 and 22 days after administering the pesticide. The exposure to endosulfan caused an increase in B cell number and a decrease in F and R cell number ($p < 0.05$). Necrotic tubules, abnormal lumen and other histopathologies were observed in the hepatopancreas of crabs exposed to endosulfan. There was an increase in the proportion of collapsed gills caused by endosulfan effects. Other effects as hyperplasia were also observed. There were no changes in the gonadosomatic index of exposed crabs; however there were changes in the volume of oocytes of exposed crabs in certain days ($p < 0.05$). The increase in B cell number and the consequent reduction in F cell number may be related to the detoxification processes. The changes in cell number within the hepatopancreas and the histopathologies observed both in hepatopancreas and gills might be used as endosulfan exposure indicators.

1. Introduction

Grain-exporting countries are characterised by intensive agricultural activities, which include the use of several biocides to increase crop productivity. The widespread use of persistent organic pollutants has resulted in the contamination of terrestrial and aquatic environments (Ernst et al., 1990; Jergentz et al., 2005). A widely used pesticide is endosulfan (6,7,8,9,10,10-hexacloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-benzodioxathiepin-3-oxide), a broad-spectrum organochlorine insecticide that is used to control boring, chewing and sucking insects and mites (Wan et al., 2005). The recommended application doses of this pesticide ranging from 0.7 to 3 l/10,000 m^2. The Endosulfan acts on the biota by blocking the chloride channels of the gamma-aminobutyric acid (GABA) receptor in the central nervous system, leading to neural excitation and eventually the death of the organism. Like various other organochlorine compounds, endosulfan is stable for several weeks; therefore, it remains effective long after it is applied. Endosulfan is not typically considered mobile and it is known to persist in surface runoff waters (Murray et al., 1993). Endosulfan is highly toxic to fish and aquatic invertebrates, and because of its lipophilicity, it is able to accumulate in these organisms (Howard, 1991; Hose and Van den Brink, 2004; Wan et al., 2005; Silva Barni et al., 2014). Also, endosulfan is an endocrine disrupting chemical that may affect reproductive parameters in animals as fishes, both in males and females (Chakravorty et al., 1992; Rajakumar et al., 2012; Laldinsangi et al., 2014).

During pesticide applications the aerial drift might reach the aquatic ecosystems near the crop areas (Ernst et al., 1990). Moreover, in some crop systems as paddy fields, the pesticide applications occur near rivers and lakes, posing risk to the biota inhabiting them. The decapod crustaceans are found in freshwater environments all over the world, occupying an intermediate position in the food webs. Like all the biota, they are periodically exposed to different pesticides, especially those that inhabit near agricultural areas.

The exposure to pesticides might cause several histopathological effects in animals such as prawn and crabs. Gills are the first organ which comes in contact with environmental pollution. They

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are highly vulnerable to toxic chemicals mainly because their large surface area facilitates the interaction and their absorption. So, the absorption through gills is rapid and therefore the toxic response in gills is also rapid (Pandey et al., 2008). Hepatopancreas is a dynamic organ mainly related with digestive functions. It is also responsible for the major portion of detoxification activities. When crustaceans are exposed to toxicants and pollutants, its functions and structure are likely to be affected by certain xenobiotics (Bhavan and Geraldine, 2000; Wu et al., 2008). The hepatopancreas is also responsible for the production of vitellogenin. During secondary vitellogenesis, the lipids stored in the hepatopancreas are processed into lipoproteins and transported via the haemolymph to the oocytes (Harrison, 1990; Lubzens et al., 1995; Rodríguez et al., 2000).

The crabs of the *Zilchiopsis* genera are active predators and detritus feeders. They are also an important food source for fish, reptiles, birds and mammals, with a central position in both the aquatic and terrestrial food webs (Collins et al., 2007). The burrowing crab *Zilchiopsis collastinensis* is a common crab of the middle Paraná River.

In this crab the gonad gradually develops from spring to summer, when spawning occurs (our laboratory). Pesticide applications occur mainly in the spring, reaching freshwater environments during rainfalls. The females of this species appear to be quite resistant to endosulfan. In previous studies conducted by us, the 96-h LC_{50} of endosulfan was found to be 1902 μg/l (Negro et al., 2014). When *Z. collastinensis* was exposed to endosulfan for 22 days, accumulation of this pesticide was found to have occurred both in hepatopancreas and gonads, although there was a reduction with time of endosulfan concentrations in hepatopancreas (Negro et al., 2012). However, there are few records of the histopathological effects of pesticides in tissues of this crab, and to the best of our knowledge, there is a lack of information about the possible use of these histological biomarkers as a way to estimate the pesticide pollution.

The aims of this work were: 1 – to simulate a single contamination pulse of endosulfan in a system with controlled dilution, 2 – to recognise the histopathological effects caused in hepatopancreas, gills and ovaries of the freshwater burrowing crab *Z. collastinensis*, used as an ecotoxicological model and 3 – to determinate if there are histological biomarkers of exposure to endosulfan after water concentrations have decreased.

2. Materials and methods

2.1. Animal collection and acclimation conditions

Adult crabs (*Z. collastinensis* Pretzmann, 1968) were collected on the Paraná River floodplain (31°30′S, 60°41′W; Santa Fe, Argentina) in late winter. This river has a mean discharge of 16,000 m^3/seg and a peak discharge of up to 60,000 m^3/seg (Iriondo, 2004). It is located within an alluvial valley that ranges from approximately 13 to 56 km in width, with a slope of 0.036 m/km. The crabs were collected by hand or by sampling with a hand net (area of 0.9 m^2 with a mesh size of 1 mm) below the aquatic vegetation (*Eichornia crassipes*) (Williner and Collins, 2002, 2013). Only females were used in the experiments. They were acclimated for 14 days in natural light and temperature conditions. One hundred and eighty intermoult crabs were placed in twelve 300 l aquaria filled with dechlorinated water (15 crabs per aquarium). The water was renewed at a rate of 20 l/day in a continuous flow system and the excess water was drained by overflow. Dissolved oxygen, pH and conductivity values of the dechlorinated water were 6.84 ± 1.28 mg/l; 7.12 ± 1.32 and 1228.68 ± 21.42 μS/cm respectively. The aquaria contained plastic shelters to simulate the crab burrows. The mean (± SD) carapace width of the crabs used was 51.45 (± 2.86) mm. Additionally, the largest individual was not more than 1.5 times larger than the smallest individual, a criterion that has been proposed for fish assays (Reish and Oshida, 1987). The aquaria were placed in an open greenhouse covered with shade cloth, to avoid the overheating caused by direct sun exposure (photoperiod 12:12; temperature 14.03 ± 4.26 °C). The crabs were fed fresh fish muscle ad libitum. Food was supplied once in the evening, and the leftovers were removed early the next morning.

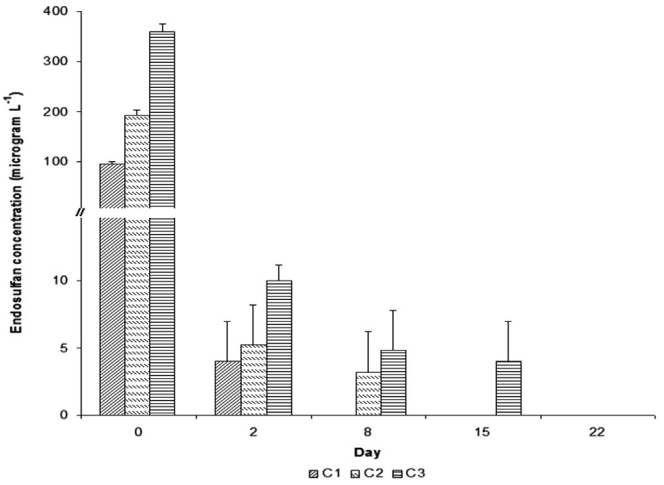

Fig. 1. Mean endosulfan measured concentrations (+SD) in water in each treatment at initial time (day 0) and in the different sampling days. C_1: 94 ± 6 μg endosulfan l^{-1}; C_2: 192 ± 10 μg endosulfan l^{-1}; C_3: 360 ± 15 μg endosulfan l^{-1} (initial concentration).

2.2. Assay conditions

After the acclimation period, each aquarium received a single pulse of commercial grade pesticide (Zebra Ciagro®; Red Surcos S. A., Argentina) containing 35% endosulfan. The commercial product was diluted in dechlorinated water, added to each aquarium and gently homogenised throughout the tank. Based in the LC_{50} of 1902 (1679–2124) μg endosulfan l^{-1}, obtained in a 96 h exposure period test, three sublethal concentrations (about 1/20, 1/10 and 1/5) were used (Negro et al., 2014). Initial concentrations were 94 ± 6; 192 ± 10 and 360 ± 15 μg endosulfan l^{-1} (C_1, C_2 and C_3 respectively). Those concentrations were applied in a dynamic system with a high initial concentration followed by dilution with pesticide-free dechlorinated water, simulating the dilution process of aquatic ecosystems. The dilution rate was 20 l/day in a continuous flow system and the excess water was drained by overflow. A control group was subjected to the same conditions as the exposed group, but without the addition of pesticides. Three replicates of each treatment were performed. The plastic shelters used to simulate the crab burrows were kept during the exposure period. Food was supplied to the crabs in the same manner in which it was supplied during the acclimation period. Dissolved oxygen, pH and conductivity were measured 3 times a week before feeding. The water temperature was recorded twice a day, at 9:00 and 16:00 h.

Water samples were taken before the endosulfan application, to rule out the baseline presence of pesticides, just after pesticide application (initial concentration) and 2, 8, 15, and 22 days after pesticide addition. Endosulfan levels in the water were measured using the ASTM D 6520-06 method. Samples were subjected to solid-phase microextraction (SPME) and concentrations of endosulfan were measured with gas chromatography-electron