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Different effects of subchronic exposure to low concentrations of the organophosphate insecticide chlorpyrifos in a freshwater gastropod

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ABSTRACT

Chlorpyrifos is an organophosphate insecticide used for pest control on a number of food crops in many parts of the world. In recent years, there has been an important decrease in the number of organisms of *Planorbarius corneus*. Since the presence of pesticides in the water can be one of the reasons for this decrease, it is very important to study the effect of subchronic exposure to environmental concentrations of pesticides on these organisms. The aim of the present work was to investigate different effects of the subchronic exposure to low concentrations of the organophosphate chlorpyrifos in *P. corneus* and the possibility to use these as biomarkers. To this end, we have exposed the organisms to 0.4 and 5 $\mu g\,L^{-1}$ of chlorpyrifos for 14 days and recorded the number of egg masses, the number of eggs per mass, the number of eggs without embryo, the time for hatching, and the % of hatching and survival. We have also determined the activities of cholinesterases, carboxylesterases and glutathione S-transferase in whole organism soft tissue and in the gonads. A 14 days exposure to 0.4 $\mu g\,L^{-1}$ caused an increase in the number of egg masses without eggs and a decrease in carboxylesterases measured with *p*-nitrophenyl butyrate. However the exposure to 5 $\mu g\,L^{-1}$ also caused an increase in the time for hatching, a decrease in the % of hatching and survival and also inhibition of cholinesterases and carboxylesterases with *p*-nitrophenyl acetate and butyrate. In contrast, the glutathione S-transferase has not been modified with the tested concentrations. We concluded that when *P. corneus* exposed to chlorpyrifos for 14 days, the CES determined with *p*-nitrophenyl butyrate proved to be the most sensitive biomarker. However, exposure to environmental concentrations showed a decrease in the reproduction ability which could cause a decrease in the number of organisms of this species.

1. Introduction

Chlorpyrifos is an organophosphate (OP) insecticide widely used in a variety of food crops to control a great number of insects and is frequently detected in surface waters around the world (Palma et al., 2009). The values of chlorpyrifos concentrations estimated for surface waters reported by the Environmental Protection Agency range between 0.026 and 0.4 $\mu g\,L^{-1}$ (EPA USEPA, 2006). However, both direct and indirect applications can cause higher chlorpyrifos concentrations in small streams and wetlands adjacent to agricultural fields than those estimated by EPA (3.7–700 $\mu g\,L^{-1}$) as reported by Moore et al. (2002) and Wood and Starck (2002). Chlorpyrifos has a relatively persistent nature compared to other organophosphorus insecticides, with a half life in water ranging from 29 to 74 days (Racke, 1993; EPA USEPA, 2006; Palma et al., 2009).

OPs are thought to exert their toxicity by binding to acethylcholinesterase, which hydrolyzes the neurotransmitter acetylcholine, inhibiting the action of this enzyme. This causes the accumulation of acetylcholine in synapses and, consequently, an overstimulation of neurotransmission followed by depression or paralysis and eventual death. Cholinesterases (ChEs) are a family of enzymes that hydrolyze choline esters and belong to the B esterases groups: esterases that are inhibited by OPs (Sanchez-Hernandez, 2007). In aquatic invertebrates, it has been reported that ChEs differ in many aspects from either vertebrate acetylcholinesterase (AChE) or butyrylcholinesterase (BChE) (Bocquené et al., 1997; Sanchez-Hernandez, 2007). The ChEs of aquatic invertebrates generally show a preference for acetylthiocholine (AcSCh) as substrate, however, there are some species that hydrolyze propionylthiocoline (PrSCh) faster than

Abbreviations: CES, carboxylesterases; ChE, cholinesterase; CI_{50}, concentration that produce 50% of inhibition; DTNB, 5,5′-dithio-2-bis-nitrobenzoate; G, gonads; GST, glutation S-transferase; OP, organophosphate insecticide; *p*-NPA, *p*-nitrophenyl acetate; *p*-NPB, *p*-nitrophenyl butyrate; T, whole organism soft tissue.

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AcSCh (Basack et al., 1998; Hannam et al., 2008; Mora et al., 1999; Talesa et al., 1990; Varó et al., 2002). Assessment of ChE inhibition in wildlife population has been proposed as a general method for detecting environmental contamination from OPs, particularly since many of these chemicals have relatively short half lives in the aquatic environment and a rapid metabolism in biota (Gagnaire et al., 2008; Lacorte et al., 1995; WHO, 1986). In contrast, after being exposed to Ops, ChE recovery in organisms is very slow. Therefore, enzymatic inhibition can be detected although there is no longer pesticide in the water (Escartin and Porte, 1996; Ferrari et al., 2004; Kristoff et al., 2006, 2011, 2012; Kumar et al., 2010; Rodríguez, 2009). This may offer an advantage in monitoring OPs over the use of chemical analysis alone (Arufe et al., 2007).

Carboxylesterases (CES) are another type of B-esterases. These enzymes catalyze the hydrolysis of a wide range of exogenous and endogenous esters and are assumed to play a protective role in anticholinesterase intoxication by removing a significant amount of pesticide by two main mechanisms: the detoxification by hydrolysis of ester bonds in some of these pesticides and by providing alternative sites of OP binding (Jokanovic, 2001; Sanchez-Hernandez, 2007).

Glutathione S-transferase (GST) belongs to a phase II family of detoxifying enzymes. Mainly by the action of this enzyme, glutathione (GSH) can form conjugates with a wide variety of electrophilic compounds. This conjugation is essential for the detoxification of xenobiotics but also for maintaining the normal physiological metabolism (Strange et al., 2000). For this reason, GST activity can be used as a biomarker of effect.

OPs can produce other adverse effects in exposed organisms. Therefore, other parameters such as oxidative stress, hematological, immunological, genotoxic and reproductive parameters can be studied to give information of the biological effects of pesticides on the test species and to be used as biomarkers.

Chlorpyrifos has been used in different *in vivo* toxicity tests in aquatic invertebrates. It has been reported that chlorpyrifos decreases ChE activity of *Artemia salina, Artemia parthenogenetica, Biomphalaria glabrata*, *Corbicula fluminea*, *Daphnia magna*, *Gammarus pulex*, *Lamellidens marginalis*, *Lumbriculus variegatus*, *Paratya australiensis*, *Planorbarius corneus*, *Potamopyrgus antipodarum*, and *Procambarus clarkii* (Amanullah et al., 2010; Barata et al., 2004; Cacciatore et al., 2011; Cooper and Bidwell, 2006; Gagnaire et al., 2008; Kumar et al., 2010; Rodríguez, 2009; Varó et al., 2002; Vioque-Fernández et al., 2007; Xuereb et al., 2007) and CES activity of *B. glabrata, L. variegatus, P. clarkii* and *P. corneus in vivo* (Cacciatore et al., 2011; Rodríguez, 2009; Vioque-Fernández et al., 2007). However, less is known about the chronic effects of low concentrations of pesticides on more ecologically relevant endpoints such as growth and reproduction (Roex et al., 2003). Some authors have reported toxic effects due to chlorpyrifos on reproduction, survival and embryonic development in vertebrates (De Silva and Samayawardhena, 2005; Farag et al., 2010) and in invertebrates species (Jager et al., 2007; Li-Xia et al., 2009; Palma et al., 2009; Varó et al., 2006; Zaliznick and Nugegoda, 2006). In the case of crustacean, Palma et al. (2009) reported in *Daphnia magna* a reduction in the number of offspring produced per male and abnormalities including arrested eggs; Zaliznick and Nugegoda (2006) have studied the effect of chlorpyrifos on the next two generations of *Daphnia carinata* reporting that the pesticide affected survival and fecundity of animals in the first generation while in the second one, a longer time of hatching was observed. Varó et al. (2006) have studied the effect of chlorpyrifos on capsulated and decapsulated cysts of *Artemia* sp. showing that the pesticide caused a decrease in hatching and survival. However, little is known about the chlorpyrifos effect on the gastropods reproduction. Although gastropods in the case of Ops, are not the most sensitive group of organisms (Van Wijngaarden et al., 2005), freshwater gastropods represent about 20% of recorded mollusk extinctions (Strong et al., 2008).

P. corneus is a freshwater hermaphroditic gastropod that is distributed all over the world (Jopp, 2006). In previous works of our laboratory, we have characterized the activities of B-esterases and we have also reported that the exposure for 48 h to chlorpyrifos inhibits ChE and CES of whole organism soft tissue (Cacciatore et al., 2011, 2012). It has been reported that *P. corneus* population declines constantly (Jopp, 2006; Wiese, 2005) so it is very important to study different effects of the subchronic or chronic exposure to low concentrations of contaminants. In particular, the alterations on reproduction which have an important ecological significance because the survival of the species is determined by the success in giving birth to new individuals.

Chronic and subchronic exposures are relative terms especially in relation to the species. They are often designed according to the expected lifespans of the species involved (Barile, 2008). Generally, a chronic exposure implies an exposure of at least 10% of the lifetime. An acute exposure corresponds to until 96 h while the subchronic exposure involves a duration between the acute and the chronic exposure (Barile, 2008). In laboratory conditions, it has been registered that *P. corneus* can live until 2 years (Collins Baker, 1945), so a 14 days exposure should be considered a subchronic one.

The aim of the present work was to investigate different toxic effects of subchronic exposure to low concentrations of the OP chlorpyrifos, in order to find the most senstitive biomarker. To this end, we (1) determined ChE, CES and GST activities in whole organism soft tissue and in the gonads after 14 days of exposure. We chose to work on gonads to study the possible link of biochemical responses to the effects on the reproduction. Also, enzyme sensitivity to the pesticide was compared in whole organisms soft tissue and in the gonads (2) studied the effect of the pesticide on different parameters of reproduction in adult snails and egg masses exposed by recording the number of egg masses, the number of egg masses without eggs, the number of eggs per egg mass, the number of non-embryonated eggs per mass, the time for hatching, the percentage of hatching and the percentage of survival of the offspring.

2. Materials and methods

2.1. Chemicals

Acetylthiocholine iodide (AcSCh), *p*-nitrophenyl acetate (*p*-NPA), *p*-nitrophenyl butyrate (*p*-NPB), 5,5′-dithio-2-bis-nitrobenzoate (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), reduced glutathione and chlorpyrifos were purchased from Sigma-Aldrich of Argentina S.A. All other chemicals used were of analytical reagent grade.

2.2. Organisms

Adult *P. corneus* snails were purchased from Discus Morón S.R.L., Buenos Aires, Argentina. Afterwards the snails were reared in our laboratory in an aerated glass aquaria (17–20 L), at a temperature of 22 ± 2 °C, and under a 14:10 (L:D) h artificial photoperiod regime. For all the experiments, adult snails of similar size (12 ± 2 mm) were used.

2.3. Bioassays

To perform the bioassays, we used 1 L glass vessels, containing 800 mL for each solution (dechlorinated water, 0.001% of acetone in dechlorinated water, 0.4 μg L^{-1} of chlorpyrifos in dechlorinated water and 5 μg L^{-1} of chlorpyrifos in dechlorinated water). During the bioassay animals were fed once a week. No mortality was observed either in the control groups or in the treated groups. All the bioassays were performed at 22 ± 2 °C under a photoperiod of 14:10 (L:D) h. The following physico–chemical parameters were recorded: total hardness $= 67 \pm 3$ mg $CaCO_3$ L^{-1}; alkalinity $= 29 \pm 2$ mg $CaCO_3$ L^{-1}; pH 7.0 ± 0.2 and