Chemosphere 84 (2011) 585–591

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Effects of the organophosphate insecticide azinphos-methyl on the reproduction and cholinesterase activity of *Biomphalaria glabrata*

Gisela Kristoff, Luis C. Cacciatore, Noemí R. Verrengia Guerrero, Adriana C. Cochón *

Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Nuñez, 1428 Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 10 November 2010
Received in revised form 21 March 2011
Accepted 24 March 2011
Available online 23 April 2011

Keywords:
Azinphos-methyl
Freshwater snail
Cholinesterase
Reproduction

ABSTRACT

Azinphos-methyl is an organophosphate insecticide used for pest control on a number of food crops in many parts of the world. The snail *Biomphalaria glabrata* is a freshwater gastropod widely distributed in South America, Central America and Africa. The aim of the present work was to investigate whether azinphos-methyl causes alterations in the reproduction of *B. glabrata*. To this end, gastropod pigmented specimens were exposed to various concentrations of the insecticide (0.021, 0.5, 2.5, and 5 mg L^{-1}) for either 2 or 14 d. Along 14 d, several reproduction parameters and cholinesterase (ChE) activity were evaluated. In each group, the number of egg masses, the number of eggs per mass, the number of hatchings, the time to hatching, and the survival of the offspring after one month of treatment was evaluated. The results showed that, depending on the concentration and time of exposure, azinphos-methyl induced alterations in the reproduction of *B. glabrata*. These alterations were mainly represented by a decrease in the number of egg masses, and, in some cases, by a lower number or even the total absence of hatchings. Thus, the gastropods exposed to 2.5 and 5 mg L^{-1} of azinphos-methyl for 14 d showed ChE inhibitions higher than 35% along time and completely lost their ability to reproduce. On the other hand, exposure to high acute concentrations or exposure to low concentrations for 14 d resulted in ChE inhibition equal to or lower than 35% between 7 and 14 d of treatment and similar alterations in reproduction. These were represented by a decrease in the number of egg masses. At low pestice levels, the number of egg masses and the number of offspring resulted to be more sensitive biomarkers than ChE inhibition. It is concluded that the insecticide azinphos-methyl can cause a decline in the reproductive performance of *B. glabrata*.

1. Introduction

Azinphos-methyl is an organophosphate insecticide used for pest control on a number of food crops in many parts of the world. In Argentina, it is the insecticide most used in the fruit-horticultural activity of the Valle de Río Negro and Neuquén in North Patagonia, an area of more than 35,000 hectares devoted to intensive agriculture (Loewy et al., 2003). Azinphos-methyl's half-life is about 26 d at 30 °C and pH 7, and it can reach watercourses via runoff and spray drift (US EPA, 2001; Schulz, 2004; Loewy et al., 2006). In the area of the Alto Valle, this insecticide is repeatedly applied throughout the growing season (November to March). When monitoring the groundwater and surface water of this region, azinphos-methyl was the insecticide found most frequently and in highest concentrations: the concentration in groundwater was found to be 3.22 ± 7.78 μg L^{-1} (Loewy et al., 1999).

The mechanism of action of organophosphate and carbamate insecticides consists in the inhibition of the activity of acetylcholinesterase (AChE). For this reason, the activity of the cholinesterases (ChEs) has been used for decades as a sensitive biomarker of exposure to organophosphate and carbamate pesticides (Timbrell, 2000). However, in order to gain more insights into the adverse effects of these insecticides, and because the toxicological studies require the use of a wide range of biomarkers for the same toxic as well as the identification of new biomarkers that may be more sensitive, it is also important to study other toxic effects which may occur in exposed species (van der Oost et al., 2003).

Several authors have reported that organophosphate insecticides can cause oxidative stress (Peña-Llopis et al., 2002; Özcan Oruç et al., 2004; Shadnia et al., 2005; Verma et al., 2007; Kristoff et al., 2008), immunotoxicity (Eason et al., 1999; Galloway and Depledge, 2001; Galloway and Handy, 2003), DNA damage (Bonfanti et al., 2004; Shadnia et al., 2005) and reproductive alterations (Rodríguez and Pisanó, 1993) in several species. In particular, the effects on reproduction have important ecological significance because the survival of the species is determined by

Abbreviations: AChE, acetylcholinesterase; ChE, cholinesterase; ASCh, acetylthiocholine; DTNB, 5,5′-dithio-2-bis-nitrobenzoate.

* Corresponding author. Address: Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Departamento de Química Biológica, Ciudad Universitaria, Pab. II. 4to piso, 1428 Buenos Aires, Argentina. Tel./fax: +54 11 4576 3342.
E-mail address: adcris@qb.fcen.uba.ar (A.C. Cochón).

0045-6535/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2011.03.058

the success in giving birth to new individuals. The alteration in the reproductive potential resulting from the presence of contaminants in the environment can lead to malformations in the offspring, a decrease in the number of individuals of a population, or even the extinction of a species (van der Oost et al., 2003).

Some authors have studied the reproductive alterations caused by different pollutants in freshwater gastropods. In *Biomphalaria tenagophila,* for example, exposure to the organochlorine insecticide endosulfan leads to an increase in malformations and a decrease in the number of hatched eggs (Oliveira-Filho et al., 2009a), whereas exposure to surfactants leads to a decrease in fecundity (Oliveira-Filho et al., 2009b). In *Physa fontinalis* and *Lymnaea stagnalis*, a significant inhibition of the growth and survival of the offspring was found after exposure to tributyltin, an anti-fouling compound used in paints for ships (Leung et al., 2004 and Leung et al., 2007). Khangarot and Das (2010) reported a significant delay in hatching and a large number of malformations in the embryos of the freshwater *Lymnaea luteola* exposed to copper. Also, Tripathi and Singh (2004) reported that the carbamate insecticide carbaryl induces alterations in the reproduction of the freshwater snail *Lymnaea acuminata.* However, there is still very little information on the effects of organophosphate insecticides on the reproduction of these organisms.

The snail *Biomphalaria glabrata* is a gastropod mollusc widely distributed in various parts of South America, Central America and Africa. It is herbivorous and feeds on wild algae and softer parts of aquatic vascular plants. Since this species is relatively easy to adapt and maintain in laboratory conditions, it has been used in toxicological studies and has been postulated as a bioindicator of water pollution (Münzinger, 1987; Verrengia Guerrero et al., 2000; Abd Allah et al., 2003; Ansaldo et al., 2006; Kristoff et al., 2010). In addition, *B. glabrata* is an intermediate host of the parasite *Schistosoma mansoni*, the causative agent of schistosomiasis in humans. For this reason, some authors have compared the reproductive performance of infected snails with that of uninfected ones (Crews and Yoshino, 1989). However, studies on the effect of pollutants on reproduction are scarce. Münzinger and Guarducci (1988) observed that fecundity was significantly lowered by 500 and 1500 ppb of zinc and completely suppressed by 3000 ppb. Ansaldo et al. (2009) investigated the effects of cadmium, lead and arsenic on oviposition, hatching and embryonic survival of *B. glabrata* and found that arsenic induced the highest impact on the egg-laying process of *B. glabrata* and that cadmium and lead had the highest effect on embryo survival and time to hatching. However, we found no reports addressing the toxic effects of anticholinesterase insecticides on the reproduction of this species.

In previous works, we have reported that azinphos-methyl inhibits cholinesterase activity (Kristoff et al., 2006) and generates oxidative stress in the freshwater gastropod *B. glabrata* (Kristoff et al., 2008). The aim of the present work was to investigate whether azinphos-methyl causes alterations in the reproduction of this species. To this end, gastropod pigmented specimens were exposed to various concentrations of the insecticide for 2 and 14 d (acute and subchronic treatments, respectively). In each group, we evaluated the number of egg masses, the number of eggs per mass, the number of hatchings, the time to hatching, and the survival of the offspring after 1 month of treatment. In addition, we determined the activity of ChE of the total soft body homogenate in order to relate the effects observed on reproduction with the enzymatic inhibitions obtained.

2. Materials and methods

2.1. Chemicals

Acetylthiocholine iodide (ASCh) and 5,5′-dithio-2-bis-nitrobenzoate (DTNB) were obtained from Sigma (St. Louis, MO). Azinphos-methyl (98.3% pure) was a gift from Bayer S.A., Argentina. All other chemicals used were of analytical grade.

2.2. Organisms selected

Pigmented *B. glabrata* snails were originally obtained from a laboratory culture established at the Laboratorio de Invertebrados, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. The organisms were then cultured in our laboratory under standard conditions in aerated glass aquaria (17–20 L), at a temperature of 22 ± 2 °C, and under a 14:10 (L:D) h artificial photoperiod regime. Animals were fed lettuce leaves *ad libitum* (Fried et al., 1992). For all the experiments, adult snails of similar size (18 ± 2 mm) were used.

2.3. Bioassays

To perform the bioassays, we used 3-L plastic containers, using dechlorinated water filtered through a carbon column. The organisms were fed every 7 d and all solutions were renewed once a week. Each concentration of azinphos-methyl was obtained by diluting the stock solution of the insecticide prepared in acetone. The concentration of acetone did not exceed 0.05% in any of the groups. The constancy of the azinphos-methyl concentration in the aqueous solution has been previously demonstrated in stability studies conducted in our laboratory (Cacciatore, 2009).

Two independent experiments were carried out, using 240 adult specimens, divided into 10 groups, for each experiment.

Two containers were prepared simultaneously for each group and 12 snails were placed in each container. One of the containers of each group was used to study the reproduction parameters, whereas the other was used to (1) determine the activity of ChE, and (2) when necessary, to replace the dead bodies of the first container so that the number of snails in the container used to study the parameters of reproduction remained constant during the whole experiment. Therefore, all the snails used for reproduction studies were exposed for the same period of time and under identical conditions to those used for ChE studies.

Fig. 1 shows the experimental design. On day -4, all snails were placed in containers with dechlorinated water for 48 h. On day -2, all the egg masses laid in those 48 h were removed and discarded, and the solutions changed as follows: groups (A), (F), (G), (H), (I) and (J) continued in dechlorinated water for additional 48 h, groups (C), (D) and (E) were placed in containers with 0.5, 2.5 and 5 mg L^{-1} azinphos-methyl, respectively, and group (B) was placed in a container with 0.05% acetone. Then, on day 0 all the egg masses laid in the last 48 h were withdrawn and the solutions were changed as follows for 14 d: groups (B), (C), (D) and (E) were placed in dechlorinated water, groups (G), (H), (I) and (J) in containers with 0.021, 0.5, 2.5 and 5 mg L^{-1} azinphos-methyl, respectively, and group (F) in 0.05% acetone. Along those 14 d, the number of egg masses and the number of eggs per egg mass were recorded every 2 d by observing with a magnifying glass. Each egg mass was transferred to individual containers, as described by Tripathi and Singh (2004), where it received the same treatment as adults (groups A, B, C, D and E: water, groups G, H, I and J: medium with pesticide, and group F: 0.05% acetone). For each egg mass, the time to hatching and the number of hatchings per egg mass were registered. The offspring were fed with lettuce and survival was recorded over 1 month.

The concentrations of azinphos-methyl were chosen based on previously published studies about pesticide ability to inhibit *B. glabrata* ChE activity after 48 h of exposure (Kristoff et al., 2006). Thus, 0.021 and 0.5 mg L^{-1} azinphos-methyl correspond to concentrations that would not likely result in enzyme inhibition at