Aquatic Toxicology 177 (2016) 365–372

Contents lists available at ScienceDirect

Aquatic Toxicology

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

Comparative study of toxicity and biochemical responses induced by sublethal levels of the pesticide azinphosmethyl in two fish species from North-Patagonia, Argentina

Mariana Guerreño[a], María Fernanda López Armengol[a], Carlos Marcelo Luquet[b], Andrés Venturino[a,*]

[a] Centro de Investigaciones en Toxicología Ambiental y Agrobiotecnología del Comahue, CITAAC, UNCo-CONICET, Instituto de Biotecnología Agropecuaria del Comahue, Facultad de Ciencias Agrarias, Universidad Nacional del Comahue, Ruta 151, km 12, 8303 Cinco Saltos, Río Negro, Argentina
[b] INIBIOMA, UNCo-CONICET- Laboratorio de Ecotoxicología Acuática, CEAN, Ruta provincial 61, km 3, 8371, Junín de los Andes, Neuquén, Argentina

ARTICLE INFO

Article history:
Received 18 April 2016
Received in revised form 16 June 2016
Accepted 17 June 2016
Available online 23 June 2016

Keywords:
Organophosphate
Oxidative stress
Odontesthes hatcheri
Jenynsia multidentata
Risk assessment

ABSTRACT

Biochemical effects of azinphosmethyl (AZM), an organophosphate pesticide, were determined in gill, brain and muscle tissues of *Odontesthes hatcheri* and *Jenynsia multidentata*. The 96-h toxicity was first assessed, estimating lethal concentrations fifty (LC50) of 7 and 30 μg L^{-1} AZM for *O. hatcheri* and *J. multidentata*, respectively. Considering the LC50, sublethal 96-h static exposures were designed for *O. hatcheri* (0.1–0.5 μg L^{-1} AZM) and *J. multidentata* (5–10 μg L^{-1} AZM) to determine biochemical endpoints. Brain acetylcholinesterase (AchE) was inhibited by AZM in both species, while the buffer enzyme carboxylesterase (CarbE) was not affected in this tissue. Conversely, muscular AchE was not affected but CarbE was augmented by AZM. The enzymes glutathione reductase, glutathione-*S*-transferase and CarbE were significantly inhibited in *O. hatcheri* gills but none of them was affected by AZM in *J. multidentata* gills compared to control. GSH levels were augmented in gills of both species in exposed fish compared to controls and in addition, lipid peroxidation was significantly increased in *O. hatcheri* gills. *Ex vivo* histochemical analysis of ROS by fluorescence microscopy was also performed in *J. multidentata* gills, indicating a significant increase upon exposure to 10 μg L^{-1} AZM. Principal component analyses (PCA) were applied, both to the species together or separately. The general analysis demonstrated a clear separation of responses in the two species. For *O. hatcheri*, the variable that explains the major variation in PC1 is gill catalase and brain AchE in PC2. In *J. multidentata* in turn, the variable that explains the major variation in PC1 is brain AchE and total oxyradical scavenging capacity in PC2. The toxicity data and biomarker responses obtained for both species were compared to environmental concentrations of AZM detected in superficial water from different points in the Alto Valle region and risk quotients (RQ) were calculated. This approach indicated probable acute effects for *O. hatcheri* in river and irrigation channels (RQ > 0.1), while the risk was unacceptable in drainage superficial water (RQ > 1). In contrast, *J. multidentata* showed minimal risk in river or channel water (RQ < 0.1) and probable risk in drainage water (RQ = 0.75). We conclude that not only the differential susceptibility of both species to AZM is environmentally relevant, but also that the different biomarkers responding in each case underlie particular pathways stressed by this agrochemical.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

The Alto Valle of Río Negro and Neuquén in Northern Patagonia is a region of intensive fruit production. Pesticides are widely used to manage the coddling moth *Cydia pomonella* in crops and orchards, being organophosphate (OP) insecticides the main applied. Organophosphates have been detected in superficial and shallow ground water in this fruit-producing region (Loewy et al., 2011). The mechanism of action of OP is based on inhibition of

Abbreviations: ABAP, 2,2′-azobis 2-methylpropionamidine; AchE, acetylcholinesterase; AZM, azinphosmethyl; CarbE, carboxylesterase; CAT, catalase; CDNB, 1-chloro-2,4-dinitrobenzene; GR, glutathione reductase; GST, glutathione-*S*-transferase; OP, organophosphate; PCA, principal component analysis; RQ, risk quotients; TBARS, thiobarbituric acid-reactive substances; TOSC, total oxyradical scavenging capacity; TROLOX, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

* Corresponding author.
E-mail address: a.venturino@conicet.gov.ar (A. Venturino).

http://dx.doi.org/10.1016/j.aquatox.2016.06.015
0166-445X/© 2016 Elsevier B.V. All rights reserved.

the enzyme acetylcholinesterase (AchE; EC 3.1.1.7), while carboxylesterases (CarbE; EC 3.1.1.1) are OP's main detoxification enzymes (Fukuto, 1990). Carboxylesterases are in turn inhibited by OP, becoming alternative targets to protect the organism from AchE inhibition (Jokanović, 2001).

Redox status reflects the dynamic balance between the antioxidant system and pro-oxidants. Exposure to xenobiotics (*i.e.* pesticides) may produce reactive oxygen species (ROS) as a consequence of their metabolism. When ROS overwhelm the cellular antioxidant defense system, they generate oxidative stress. The direct or indirect ROS-mediated damages include peroxidation of membrane fatty acids, DNA base alteration, carbonyl modification of proteins, and loss of sulfhydryl groups leading to enzymes inactivation and/or increased proteolysis. Lipid peroxidation may occur as a consequence of the imbalance between the antioxidant system and the pro-oxidant state generated by pesticide toxicity (Winston and Di Giulio, 1991). It has been reported that lipid peroxidation has a predictive importance as a biomarker for oxidative stress (Lackner, 1998). Endogenous enzymatic and non-enzymatic antioxidants are essential for the conversion of ROS to harmless metabolites as well as to protect and restore normal cellular metabolism and function (Bebe and Panemangalore, 2003). Like other organisms, fish manage elevated levels of ROS with protective ROS-scavenging enzymes such as glutathione reductase (GR; EC 1.8.1.7), catalase (CAT; EC 1.11.1.6), glutathione-*S*-transferase (GST; 2.5.1.18) and non-enzymatic molecules such as reduced glutathione (GSH) (Sharbidre et al., 2011). Glutathione-*S*-transferase also plays an important role in the detoxification of xenobiotics *via* conjugation with GSH. The induction of these enzymes and elevated levels of GSH are beneficial for cellular redox state and provides useful biomarkers of exposure to oxidative stress-inducing chemical contaminants in fish (Van der Oost et al., 2003; Pereira et al., 2013).

Odontesthes hatcheri is an autochthonous fish from Patagonia with relevance from ecological, commercial and sportive aspects. In turn, *Jenynsia multidentata* is recognized as a good model for toxicological studies as an autochthonous South American fish species because it is widely distributed and easy to handle for culture and maintenance in the laboratory (Ballesteros et al., 2011). The aim of this work was to compare the toxicity and biochemical effects of the OP azinphosmethyl (AZM) in these two fish species inhabiting North Patagonia. We have analyzed the main targets as well as detoxifying and antioxidant activities in brain, muscle and gills at sublethal concentrations of the toxicant, to assess their relevance from an environmental point of view and as ecotoxicological bioindicators.

2. Materials and methods

2.1. Animals

The approval for collection of the specimens at the different places, animal treatment, health care and disclaimer for possible environmental impacts was requested to the Application Authority for Law of Fauna in Neuquén Province, through the management of the Center of Applied Ecology of Neuquén.

2.1.1. Odontesthes hatcheri

Juvenile *O. hatcheri* fish were obtained from a small pond connected to Piedra del Aguila reservoir, Limay river, Neuquén (40°16′36″S, 70°39′36″W) and transported to the aquaculture of the Center of Applied Ecology of Neuquén (CEAN), Junín de los Andes. Fish were acclimated in 100-L artificial ponds constantly receiving filtered water from Chimehuin River, with a mean temperature of 8.0 °C. Fish were daily fed 1% of their body weight with formulated food adapted at CEAN to the Patagonian silverside (Hualde et al., 2011). Fish were maintained with a natural photoperiod of 10.5 h L/13.5 h D, corresponding to autumn season at Junín de los Andes.

2.1.2. Jenynsia multidentata

Adult females of the freshwater fish *J. multidentata* were obtained from Pellegrini Lake, Río Negro (38°40′S, 68°00′W) and transported to the laboratory for acclimation in 20-L storage tanks with filtered, dechlorinated, and constantly aerated tap water (mean temperature of 24 °C, photoperiod 12 h L/12 h D). Fish were daily fed with food for cold freshwater fish.

2.2. Toxicity tests

All the toxicity tests were performed in static conditions up to 96 h, with a unique application of AZM in acetone as vehicle at the beginning of the experiments. A high purity-certified standard of azinphosmethyl (98.3% AZM, *S*-(3,4-dihydro-4-oxobenzo[d]-[1,2,3]-triazin-3-ylmethyl) *O*,*O*-dimethyl phosphordithioate, Chem Service Inc. West Chester, PA, USA) was first dissolved in acetone to prepare a stock standard solution that was then diluted in water to the desired concentrations. The exact concentration of AZM in the standard solution was checked by capillary gas chromatography and N-P detection. Final concentration of acetone was kept at 0.05% in all the treatments. Control acetone treatment was included, verifying no toxic effects. Feeding was omitted 24 h prior to and during the exposure period of all toxicity tests.

2.2.1. Acute toxicity tests

Ten fish (approximately 0.8 g total body mass per liter) were housed in each aquarium with a 12 h L/12 h D photoperiod and continuous aeration. The mortality was monitored daily up to 96 h according to the Ecological Effects Test Guidelines (USEPA, 1996). For *O. hatcheri*, AZM concentrations of 0.0, 0.1, 0.3, 1.0, 3.0 and 10 $\mu g\,L^{-1}$ were prepared in aquaria filled with filtered water from Chimehuin River containing 0.3 $g\,L^{-1}$ NaCl, at 20 °C. For *J. multidentata*, AZM concentrations of 0, 5, 10, 15, 25 and 50 $\mu g\,L^{-1}$ were prepared in aquaria filled with dechlorinated tap water at 24 °C.

A logistic model was fitted to 96 h-mortality data using a non linear regression method (Venturino et al., 1992). Data from two experiments with duplicates were used together for fitting the model equation. The LC50 was directly estimated from the fitted equation as one of the model parameters. To estimate the AZM concentrations causing minimal or no lethal effects, the LOEC and NOEC for lethality as endpoint were assessed by a probabilistic approach, calculating the LC10 and LC1 respectively as endpoints from the fitted equation (Crane and Newman, 2000; Liendro et al., 2015).

2.2.2. Sublethal exposure tests for biomarkers assessment and tissue preparation

Odontesthes hatcheri juvenile fish (0.678 ± 0.157 g) were transferred into aquariums with aerated filtered water from Chimehuin River and exposed to 0.0, 0.1 and 0.5 $\mu g\,L^{-1}$ AZM (0.8 g of fish/L of media, USEPA, 1996). Four fish were included in each aquarium, performing treatments by triplicate. The exposures were repeated in two independent assays. *Jenynsia multidentata* adult females (0.45 ± 0.05 g) were transferred into aquariums containing aerated tap water and 0, 5 and 10 $\mu g\,L^{-1}$ AZM in 0.05% acetone (0.8 g of fish/L of media, USEPA, 1996). Four fish were included in each aquarium, performing treatments by quadruplicate. The exposures were repeated in two independent assays.

After exposure, fish of both species were weighed and brain, gills and muscle tissues were extracted from the four fish in each batch and gathered as unique sample for biochemical biomarkers determinations in the respective organs. Tissues were homogenized

Download English Version:

https://daneshyari.com/en/article/6382036

Download Persian Version:

https://daneshyari.com/article/6382036

Daneshyari.com