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Effects of carbaryl and azinphos methyl on juvenile rainbow trout (*Oncorhynchus mykiss*) detoxifying enzymes

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Abstract

In this study, the effects of sublethal exposures to the anticholinesterase insecticides azinphos methyl (AzMe) and carbaryl on the detoxifying responses of juvenile rainbow trout *Oncorhynchus mykiss* were investigated. Juvenile specimen were exposed to sublethal concentrations of AzMe (2.5 and 5 μg/L) and carbaryl (1 and 3 mg/L) for 24, 48 and 96 h. Carboxylesterase (CbE), catalase (CAT) and glutathione S-transferase (GST) activities as well as reduced glutathione (GSH) and cytochrome P450-1A (CYP1A) levels were monitored in liver and/or kidney. In all exposed groups liver CbE was significantly inhibited. Liver and kidney GSH level was reduced after sublethal exposure to both compounds. Carbaryl induced CAT activity during the first 48 h of exposure, followed by a significant decrease, whereas AzMe continuously decreased CAT activity. GST activity and CYP1A were transiently induced at 24 h by carbaryl exposure (3 mg/L) but sublethal exposure to AzMe did not affect GST activity or CYP1A. Our results show that the *O. mykiss* detoxifying system are a target for carbaryl and AzMe action, probably affecting redox balance. Although the responses showed similar trends in both organs, they were more important in liver than in kidney. The early inhibitory effect in CAT activity and GSH content produced by AzMe may be associated with a high degree of oxidative stress. Early induction of CYP1A, GST and CAT by carbaryl followed by enzyme inhibition suggests a milder or delayed oxidative stress, revealing differences between both pesticides metabolization. CbE inhibition is a good biomarker for AzMe and carbaryl exposure.

Keywords: Fish; Catalase, GSH; Glutathione S-transferase; Carboxylesterase; Insecticides

1. Introduction

Organophosphorus (OP) and carbamate (CB) insecticides are primarily recognized by their anticholinesterase action. However, they have been reported to affect other targets including detoxifying and antioxidant responses in various species [1–4] including fish [5]. These anticholinesterase insecticides have been detected in superficial and groundwater in the Valley of Río Negro and Neuquén, Patagonia, Argentina [6,7], an area with more than 35,000 hectares under intensive agriculture. Pest control in this region requires an intensive use of pesticides, among which two of the most frequently used are azinphos methyl [S(3,4-dihydro-4-oxobenzo-[1,2,3-*d*]triazin-3-ylmethyl) *O*-dimethyl phosphorodithioate] and carbaryl (1-napthyl methylcarbamate). Even though the concentrations of both pesticides in rivers and channels in this region generally range below the lethal concentrations for aquatic organisms, the criteria for protection of aquatic life may be exceeded throughout the growing season (November–March) [8].

The detoxifying system protects aquatic species from endogenous and exogenous aggression and impairment of this function lead to cell damage. In the present work, the objective was to evaluate the effect of two broadly used insecticides: azinphos methyl (AzMe) and carbaryl on the trout (*Oncorhynchus mykiss*) detoxifying system.

Carboxylesterases (EC 3.1.1.1, CbE), an ubiquitous B-esterase [9], participate in the detoxification of these

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insecticides by two main mechanisms: (1) by the hydrolysis of carboxylic esters present in CB and some OP and (2) by their own inhibition reducing the amount of insecticide that effectively reaches the acetylcholinesterase active site [10]. These serine-containing enzymes are phosphorylated or carbamylated by OP and CB, respectively, thus becoming inhibited.

In addition to their anticholinesterase action some OP and CB are capable of altering the antioxidant defence system in diverse organisms [4,11–13]. The sensitivity to oxidants is modulated by diverse antioxidant enzymes and also by low molecular weight compounds such as vitamin E, ascorbic acid, nonprotein thiols, and reduced glutathione (GSH), which participate in the redox balance [14].

GSH is present in high concentrations in most of the cells, participating in diverse metabolic reactions [15], its content is critical for cellular thiol homeostasis and cellular protection against free radicals damage [16]. A 20–30% reduction in GSH levels may affect the antioxidant response and lead to oxidative damage and cellular death [2,3,10].

Other key cellular defence against hydroperoxides that may be the target of pesticide action is the enzyme catalase (E.C. 1.1.1.6, CAT). CAT activity has been modified in different organisms by the exposure to diverse pollutants, including insecticides [17–20].

Glutathione S-tranferases (EC 2.5.1.18, GST) are metabolizing phase II enzymes which are involved in the biotransformation of both xenobiotics and endogenous substances. Besides their function in conjugating xenobiotics with GSH, some isoforms play an antioxidant role by conjugating cleavage products of lipid peroxides. These enzymes may be induced by several anticholinesterase insecticides [2,21] as well as other pesticides [22]. The change is preceded in several cases by ROS increase generated by Phase I detoxifying system induction [23,24]. The expression of cytochrome P450-1A (CYP1A) is another interesting biomarker which is dose-responsive to environmental concentrations of different xenobiotics and it is also related to oxidative stress [1,25,26].

Rainbow trout, the sensitive indicator of aquatic pollution [27] selected for this study, inhabits rivers and streams of the Neuquén and Rio Negro Valley, being significant for sport fishing and food industry purposes. Thus, it is worth studying potential biomarkers that allow the detection of early biological changes produced by contaminants before physiological disturbances could be observed. For these reason CbE, CAT and GST activities, and CYP1A and GSH levels were evaluated in the liver and kidney of the juvenile rainbow trout *O. mykiss,* at different time of exposure, as early indicators of pesticide impact at sublethal and/or environmental levels.

2. Materials and methods

2.1. Fish

Juvenile rainbow trout, *Oncorhynchus mykiss*, (2.7 ± 0.8 g) were purchased from the Universidad Nacional del Comahue fishery, Bariloche, Argentina, and acclimatized for at least 15 days before exposure. Fish were kept at low densities (1.5–2.0 g fish/L) in glass tanks ($50.0 \times 40.0 \times 40.0$ cm) with charcoal-filtered dechlorinated tap water. Water quality characteristics were: pH 7.4–7.6; dissolved oxygen 8 ± 1 mg/L. A 12 h light–12 h dark cycle was applied. The temperature ranged around 16 ± 1 °C. Commercial food was given once a day until satiation.

2.2. Test conditions

Experiments were carried under static conditions, placing 10 fish in 20 L aquaria (1 fish/2 L). The fish were exposed to 2.5 and 5.0 μg/L of AzMe, or to 1 and 3 mg/L of carbaryl, considering about 1/5 and 1/3 of the LC_{50}s, respectively [28], during 24, 48 and 96 h. AzMe (99.0% purity) and carbaryl (99.0% purity) were purchased from Chem Service (West Chester, Pennsylvania, USA). Aqueous solutions containing the insecticides were prepared by dissolving the insecticides in acetone, and diluted with an appropriate amount of water. The concentration of acetone was kept at 0.05% in all insecticide and control solutions used. Each of the test concentrations and the carrier control were assayed by triplicate, except for CYP1A analysis where duplicate exposures were performed. Animals were fasted 24 h before the experiment and during testing.

2.3. Enzymatic determinations

Fish were collected on ice, thoroughly washed with ice-cold water and killed by decapitation. Whole livers and kidneys were excised and homogenized in 20 volumes of 66 mM phosphate buffer, pH 7.0 with sucrose 25 mM. The homogenates were centrifuged at 3000*g* for 20 min at 4 °C [11]. All assays were performed at least in triplicate homogenates. The enzymatic activity was corrected in all cases by spontaneous substrate hydrolysis.

Protein concentration in the supernatants was determined according to the method of Lowry et al. [29], using bovine serum albumin as standard.

2.3.1. Carboxylesterase (CbE) activity

Carboxylesterase (CbE) activity was determined according to Caballero et al. [30] with modifications. Reactions were performed in 2.5 mL 100 mM phosphate buffer pH 8.0 containing: 5% acetone, 1 mM *p*-nitrophenylbutyrate, and 50 μg total proteins of liver supernatant. Activity was continuously recorded at 400 nm. Specific activity was calculated using the molar extinction coefficient for *p*-nitrophenol, $18.6\ mM^{-1}\ cm^{-1}$.

2.3.2. Catalase (CAT) activity

Catalase (CAT) activity was determined recording the continuous decrease in H_2O_2 absorbance at 240 nm [31]. The reaction was performed in 3 mL buffer 50 mM pH 7.0