Research Article

Integrated Use of Biomarkers (O : N Ratio and Acetylcholinesterase Inhibition) on Aulacomya ater (Molina, 1782) (Bivalvia: Mytilidae) as a Criteria for Effects of Organophosphate Pesticide Exposition

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The effect of residual concentrations of organophosphate pesticide chlorpyrifos (Lorsban 4E) on the activity of the acetylcholinesterase enzyme and oxygen : nitrogen ratio in the mussel Aulacomya ater was analyzed. Toxicity tests show a sensitivity to the pesticide in the bivalve estimated at 16 µg L⁻¹ (LC₅₀ 96 hours). Concentrations between 0.2 and 1.61 µg L⁻¹ were able to inhibit significantly the AChE activity, and concentrations between 0.8 and 1.61 µg L⁻¹ stimulate ammonia excretion and decrease oxygen : ammonia-N (O : N) ratio, with respect to the control group. A. ater proved to be a species sensitive to pesticide exposure and easy to handle in lab conditions. Thus, it is recommended as a bioindicator for use in programs of environmental alertness in the Eastern South Pacific coastal zone.

1. Introduction

For decades, residuals of several compounds of anthropogenic origin have entered the aquatic systems, such as heavy metals, pesticides made from a range of nonnatural compounds, and other synthetic organic compounds [1]. Among these, the organophosphate insecticides have been widely used in the latter years, because of their higher level of biodegradability compared with their predecessors, the organochlorinated pesticides. Therefore, it is important to understand the occurrence of pesticides in aquatic ecosystems and their potential impact [2].

Marine bivalves like mussels are extensively used as biological indicators with the aim of quantifying the potential effects of xenobiotics, as filter feeders are able to accumulate a wide range of xenobiotics in their tissues [3]. One way to quantify the possible effects is through biomarkers which have proved a useful tool for assessing the deleterious effects of pesticides in water bodies [4]. One of these biomarkers is the quantification of the inhibition of the enzyme activity acetylcholinesterase (AChE). Acetylcholine (ACh) is considered a neuroexcitatory neurotransmitter and is involved in neuromuscular stimulation and locomotion. This neurotransmitter is regulated by AChE, which is rendered inactive by hydrolysis into choline and acetate [5]. The AChE is located in neuromuscular junctions and in bivalves, and prosobranch mollusks in particular have high levels of AChE activity in the hemolymph [6].

The organophosphate pesticides (OPs) are extremely neurotoxic and proved to be effective inhibitors of AChE activity. OP pesticides generated in mussels a hyperactivity
syndrome in the nerve cells, resulting in a cell disruption product of oxidative stress and inflammation [7].

The inhibition of AChE activity has been used as a specific biomarker for the presence of organophosphorus compounds [8–11].

Another biomarker used to assess stress is the quantification of oxygen-nitrogen ratio (O : N), which indicates the physiological state of the organism in this case exposed to xenobiotic [12, 13].

This biomarker is the result of the quantification division of oxygen uptake (which is reflected in the metabolic proportions of the bivalves) and the quantification of ammonia excretion. This division of both biomarkers (oxygen uptake/ammonia-N excretion) generates an index which shows the metabolic changes in the organisms and the amount of energy available in them during periods of stress produced by pesticide contamination [14].

A. ater (ribbed mussel) is a commercially important, sessile species, which is long-lived and a filter-feeder bivalve. Its characteristics allow it to accumulate a wide range of xenobiotics in its tissues. This bivalve presents a continuous gamete release over the year, their spawn being related to food availability. Ribbed mussel has a wide latitudinal distribution in the Eastern South Pacific (20° to 56° LS), that is, Callao in Peru to the Strait of Magalanes in Chile [15].

This study responds to the need to identify native species off the coast of the eastern South Pacific that can be used in environmental monitoring programs and to assess the feasibility of implementing the integrated use of biomarkers (AChE activity and O : N ratio) to determine the presence of possible deleterious effects of chlorpyrifos-type organophosphate pesticides.

2. Materials and Methods

2.1. Organophosphate Pesticide. The trademark name of the chlorpyrifos organophosphate insecticide used in this study was Lorsban 4E by Dow AgroSciences Chile S.A. The insecticide composition is active ingredient: 48% of chlorpyrifos and registered emulsifiers: 52%.

2.2. Biological Material. Juvenile specimens of ribbed mussel were collected (49.92 ± 4.7 mm long and 16.6 ± 4.18 g, of mass) from a low intervention area in the Coliumo Bay (36°50′S 72°55′W). Then, they were taken to the Lenga Coast lab (36°45′S 73°10′W) where it continued the acclimatization in aquariums of 500 L for seven days (15±1°C; 33 ± 1 ups; 8.1 ± 5.5 mg L−1 dissolved oxygen; pH 8.2 ± 0.2, 14 : 10 photoperiod, microalgae mixed cropping food). In this period of acclimatization, ribbed mussels showed exposed gills and no observed valvar closing during this period.

2.3. Acute Toxicity Test. Preliminary assays were carried out (i.e. LC50–96hour) with the pesticide over the ribbed mussel. The assays were performed in identical conditions to the acclimatization, but without feeding. Firstly, work was carried out in a wide range of concentrations between 0 (control) and 1000 µg L−1 (six concentrations) and then between 0 (control) and 50 µg L−1 (five concentrations). Six individuals were used per cuvette with three replicates per concentration. The assays were static type.

2.4. Tissue Selection for Enzymatic Assays. For selection of the tissue for the enzymatic study, gills and hemolymph samples were extracted from the organism control group. For an enzymatic study, the gills were macerated in a homogenizer WiseStir HS-30E to 60 Hz for 10 seconds, in 1 : 1 (grams of tissue-buffer) with phosphate buffer + Triton × 100 (pH 7.4; 0.2 M) under cold conditions. The homogenized sample was centrifuged at 10.000 g, 4°C in an Eppendorf 5804 R for 20 minutes, and the supernatant was used to measure the enzymatic activity. To avoid lethal damage to the bivalve, the hemolymph extraction was performed by perforating one side of the anterior abductor muscle and the hemolymph was extracted with a 1 mL (26 G × 1/2”) tuberculin syringe. Then it was placed in a 1.5 mL Eppendorf and centrifuged at 9000 g 4°C for a period of 10 minutes. To measure the enzymatic activity in both cases, 40 µL of supernatant was used in a UV mini-1240 Shimadzu spectrophotometer by the Ellman method [16].

2.5. Chronic Toxicity Test. In chronic toxicity assays, tested chlorpyrifos concentrations were 0 (control) 0.20, 0.40, 0.80, and 1.61 µg L−1, and the exposition time was 21 days. For each concentration of chlorpyrifos, there were 30 individuals, distributed in six replicates. Reexchanges of the test solution were carried out every 48 hours, adding 6 mL of a microalgae-mixed cropping food every time. Measurements of valvar closing were performed frequently in all tested concentrations during the 21 days of exposition. The results were expressed in percentage of valve closing.

2.6. Biomarkers

2.6.1. Enzyme Activity. Hemolymph was selected for enzymatic assays to assess AChE enzyme activity. To this end, 10 individuals were selected at random per concentration. The AChE enzyme activity was measured through the Ellman method [16]. Each measurement was performed in triplicate, and the results were expressed in AChE activity (µmol acetylthiocholine min−1 mL hemolymph).

Quantitation of proteins in the gill homogenized was measured by the Bradford method using 5 µL of the homogenized sample before being centrifuged [17].

2.6.2. Oxygen Consumption. The determination of oxygen uptake was performed by respirometry in individual chambers of 200 mL at the end of the experiment, determining the concentration of dissolved oxygen through the modified Winkler method [18].