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Exposition biomarkers to organophosphorus pesticides in *Tigriopus fulvus Fischer* (Copepoda, Harpacticoida)

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Abstract

The effects of the organophosphorus pesticide Malathion, Pestanal® on the activity of the enzymes Acetylcholinesterase (AChE) and Glutathione-S-transferase and on survival were evaluated in* Tigriopus fulvus. *The mortality of the organisms after 96 hours (acute toxicity) and after 7 days (chronic toxicity) of treatment was evaluated. LC_{50} was not calculable after 24 hours of exposition; after 48, 72 and 96 hours it resulted 905.72, 552.78 and 248.94 µg l^{-1} respectively. After 7 days the NOEC value was 25 µg l^{-1}. The LOEC value for the inhibition of AChE was 25 µg l^{-1} and for the stimulation of GST was 12.5 µg l^{-1}.

Introduction

In ecotoxicological tests it is essential to dispose of easy to rear test-species and of sensitive and appropriate experimental methods. As a matter of fact the copepods, owing to their role in the marine trophic chain, are particularly suitable to be used as 'target'organisms. Organophosphorus pesticides are very hazardous xenobiotics for the aquatic environment because they interact with the lytic enzymes of the cholinergic system (AChE and pseudoChE) of the organisms blocking their catalytic site [1]. Into organisms pesticides undergo biotransformation through different enzymes such as GSTs, a family of detoxifying enzymes able to make xenobiotics easily removable through excretion [2, 3].
In this work the effects of the organophosphate Malathion, Pestanal® on *Tigriopus fulvus*, a copepod living in the supra-littoral rocky shores of the Ligurian Sea, were evaluated [4]; ecotoxicological acute and chronic tests were carried out on this organism and some exposition biomarkers such as the variation of the activity of Acetylcholinesterase (AChE) and Glutathione-S-transferase (GST) enzymes were evaluated.

Materials and methods

Copepods were sampled in the supra-littoral splashpools of the Ligurian Sea, acclimatized in the laboratory with filtered (filter porosity=0.45 µm) artificial sea water with 37‰ salinity (Instant Ocean®); the organisms were maintained at 20±0.5°C with a 16:8 hour light:dark cycle, fed with a mixture composed by commercial fry food, phytoplanktonic algae and a little amount of commercial yeast *Saccharomyces cerevisae*. To perform the ecotoxicological tests, 10 same-aged *T. fulvus* carrying-eggs females, obtained as previous reported [5], were set in each replicate of the tested concentrations. Malathion was diluted with analytical grade acetone (Riedel-de Haën, Buchs, Switzerland). The acute and chronic toxicity tests were carried out using respectively the 1000, 500, 250, 125, 62.5 µg l-1 concentrations chosen after preliminary tests, and 50, 25, 12.5, 6.2, 3.1, 1.6 µg l-1 chosen considering the LC_{50} (median Lethal Concentration) value obtained after the acute tests; all dilutions were prepared in seawater. Two controls, the former made of artificial seawater, the latter made of an aqueous solution of acetone (0.1%) were prepared.
For the evaluation of biomarkers further four higher concentrations (between 100 and 800 µg l-1) were added and the organisms were frozen at -20 °C after 24, 48 and 96 hours of exposition. The NOEC (No Observed Effect Concentration) values on the selected enzymes activity were calculated when possible. The evaluation of biomarkers was made only when mortality was less than 10%.
The organisms were homogenized (Polytron PT 300, Kinematica, Bohemia, NY, USA) in Na-phosphate buffer solution 0.01 M pH 7 with 0.1% Triton X-100 (w/v 1/4) [6, 7], centrifuged (centrifuge BR 4i, Jouan, Saint-Herblain, France) at 4°C at 2000 rpm (15 minutes) and then at 12000 rpm (30 minutes) and the supernatant was frozen at -20°C until analysis. Bradford's method [8] was used for the quantitative determination of total proteins. The evaluation of AChE activity was made according to the Ellman *et al.* method [9] and that of GST according to the Habig *et al.* method [10]; both methods were modified considering the different matrix subjected to the analysis.

Results

Results about acute and chronic toxicity tests are respectively shown in Fig. 1 and 2, as well as in Tab. 1; ctr1 and ctr2 refer to the artificial sea water control and to the acetone control. LC_{50} was calculated with the Trimmed

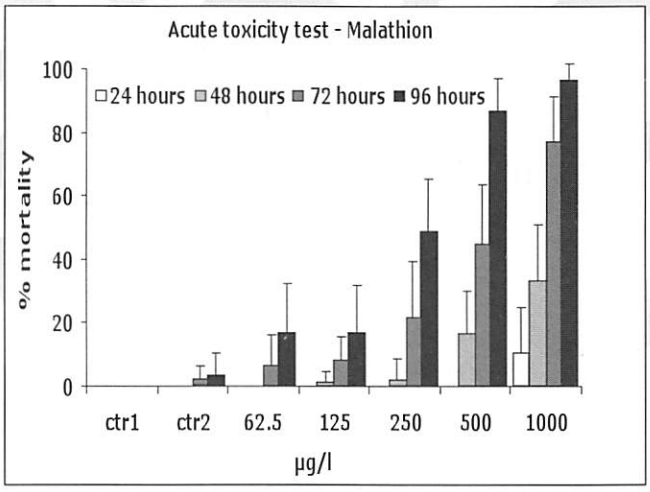

Figure 1. Acute toxicity tests. Percent mortality after 24, 48, 72 and 96 hours

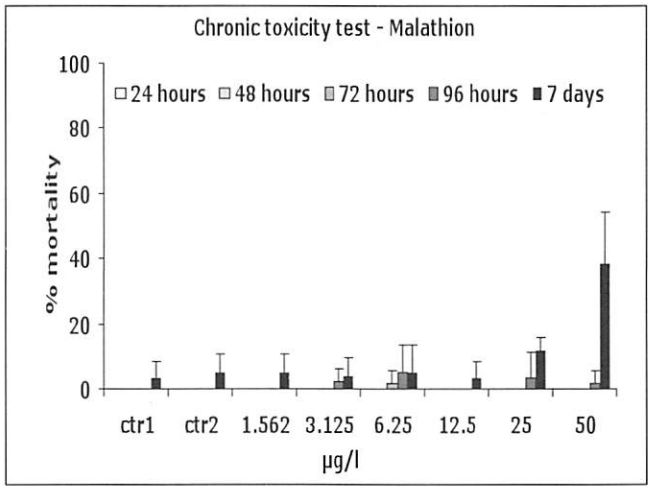

Figure 2. Chronic toxicity tests. Percent mortality after 24, 48, 72, 96 hours and 7 days

Spearman-Karber method (TSK) [11]. For chronic toxicity the NOEC and the LOEC (Lowest Observed Effect Concentration) were calculated by DUNNET test.

EXPOSITION TIME	LC50 (µg l^{-1})	NOEC (µg l^{-1})	LOEC (µg l^{-1})
24 hours	n.c.	n.c.	n.c.
48 hours	905.7 (566.5-1448.1)	n.c.	n.c.
72 hours	552.8 (423.3-727.4)	n.c.	n.c.
96 hours	248.9 (201.0-309.5)	n.c.	n.c.
7 days	–	25	50

Table 1. Acute toxicity test, LC_{50} and confidence limits. Chronic toxicity test, NOEC and LOEC. (n.c.=not calculable)

The results about AChE activity are shown in Figs. 3, 4 and 5. The NOEC (12.5 µg l^{-1}) and the LOEC (25 µg l^{-1}) after 24 hours of exposition were determined according to USEPA [12] that considers significant the 20% inhibition values. After 48 hours NOEC and LOEC values decreased (LOEC=1.6 µg l^{-1}), while after 96 hours the lower concentrations were alike the control and only after an expositions to 12.5 µg l^{-1} (LOEC) the AChE inhibition was evident.

The results concerning GST activity are shown in Figs. 6, 7 and 8. After 24 hours of exposition to Malathion (Fig. 6) GST activity was stimulated (NOEC = 6.2 µg l-1), while after 48 (Fig. 7) and 96 hours (Fig. 8) it decreased with the increase of concentrations as well.

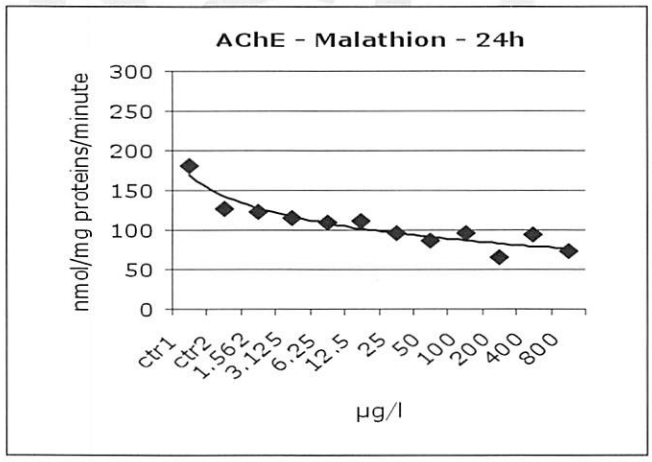

Figure 3. AChE activity, 24 hours

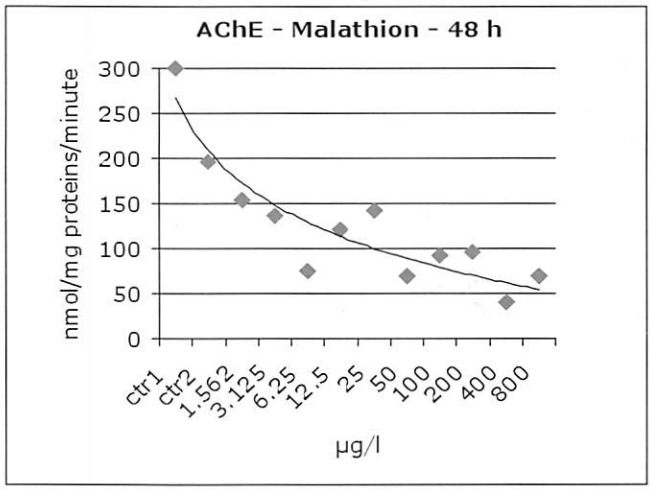

Figure 4. AChE activity, 48 hours

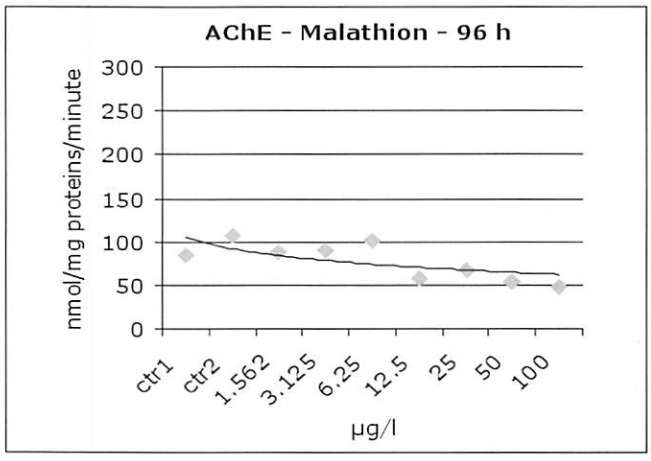

Figure 5. AChE activity, 96 hours

Discussion

Tigriopus fulvus proved to be particularly sensitive to the analysed pesticide; similar results were obtained with other copepods of

the genus *Tigriopus* such as *T. brevicornis* that indeed showed to be more sensitive [13, 14]. The different interspecific sensitivity was confirmed also for other xenobiotics such as heavy metals (Hg, Cd): for these substances *T. fulvus* seems to be more resistant than *T. brevicornis* [5, 15] and more sensitive than *T. japonicus* [16].

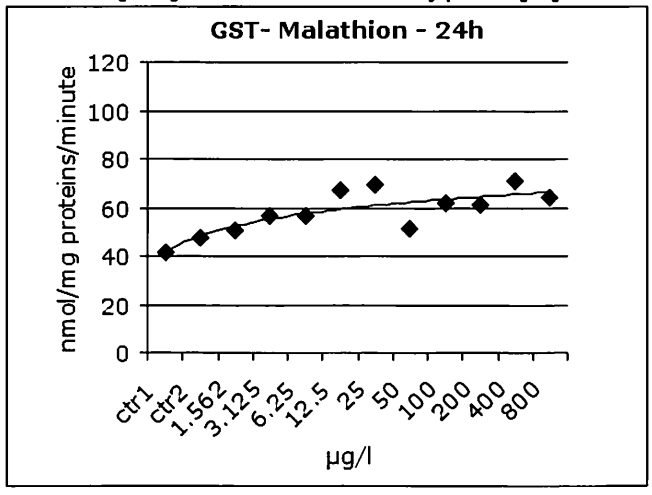

Figure 6. GST activity, 24 hours

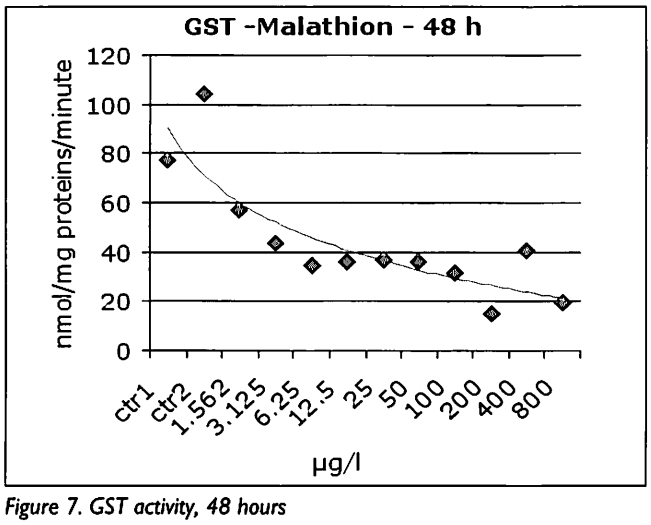

Figure 7. GST activity, 48 hours

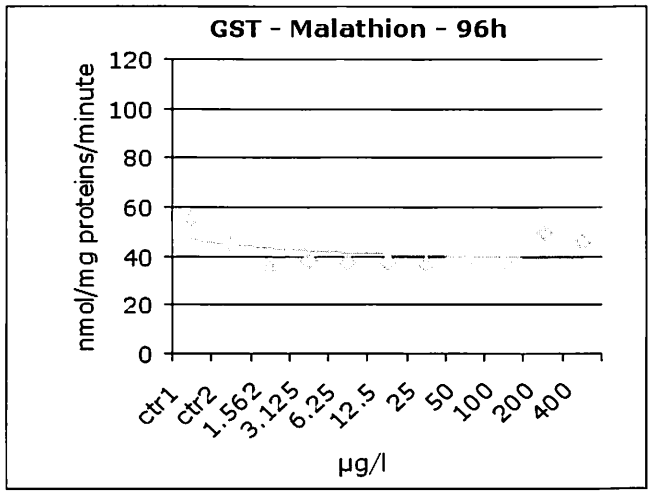

Figure 8. GST activity, 96 hours

About the chronic toxicity, Malathion proved to be moderately toxic [17] and showed NOEC values significantly lower than those obtained with heavy metals [16] and surfactants [18].

For AChE an evident decrease of the enzyme activity with the increase of Malathion concentration was emphasized at each time of treatment; however, after 96 hours of exposition, the inhibition of the enzyme was significant at higher concentration in comparison to those noticed after 24 and 48 hours: this phenomenon could be due to "bioadaptation or tolerance" processes that the exposed organisms seem to show after a prolonged exposition. A similar reduction (from 254 to 194 nmol/mg proteins/minute) of the AChE activity, after 24 hours of exposition to 2 mg l^{-1} of another organophosphorus pesticide (Dimethoate), was obtained in *Carcinus maenas* [19]. The increase of GST activity with the increase of the pesticide concentrations appeared after 24 hours of exposition (Fig. 6) while an opposite behaviour was observed after 48 and 96 hours (Figs. 7, 8); this result was supposed to be due to a widespread stress status of the organism which affects the enzymatic activity. An increase of GST activity was demonstrated also for the planktonic marine copepod *Eurytemora affinis* exposed to other organic substances such as PCBs and PAHs [20] and in *T. japonicus* after 96 hours [16] and 7 days [21] of exposition to heavy metals (Cu, Cd, As, Ag). With this study it was possible to underline a good sensitivity of *Tigriopus fulvus* to organophosphorus pesticide and an appropriate response of the selected biomarkers. Since also other xenobiotics can have similar effect on the enzymatic activities of marine copepods, the assessment of their action mechanisms is a matter of concern. On the whole, *Tigriopus fulvus* showed to be a suitable model organism in ecotoxicology and in the environmental monitoring and for the application of the European Regulation REACH [22].

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