*Environmental Mutagens & Carcinogens 21-2 : 67-71 (2001)*

# Neurotoxicant (fenitrothion) alters superoxide dismutase, catalase, and peroxidase activities in *Chironomus riparius* Mg. (Diptera, Chironomidae) larvae

Jinhee Choi
*Laboratory of Ecology and Zoology, CNRS UPRESA 8079, Bât. 442, University of Paris-Sud, F91405 Orsay cedex, France*

(Received August 9, 2001 / Accepted September 25, 2001)

***ABSTRACT*** **: Effects of exposure to a neurotoxicant, fenitrothion on antioxidant enzyme activities in *Chironomus riparius* Mg. (Diptera, Chironomidae) larvae were evaluated under laboratory conditions. Exposure to this chemical led to an increase of cupper, zinc type superoxide dismutase and manganese type superoxide dismutase activities and to a decrease of glutathion peroxidase activity. An activation of catalase was observed in the larvae exposed to high fenitrothion concentration. The response of superoxide dismutase was rapid and sensitive to low chemical concentrations, but changes in catalase, total peroxidase and glutathion peroxidase were less sensitive. In this study, antioxidant enzyme activities in *Chironomus riparius* larvae were identified as pertinent biomarkers for environmental monitoring**

**Keywords : *Chironomus riparius*, fenitrothion, superoxide dismutase, catalase, peroxidase, glutathion peroxidase**

## Introduction

Antioxidant enzymes such as superoxide dismutases (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) are involved in the elimination of reactive oxygen species generated by physical, chemical or metabolic processes (Beyer *et al.*, 1991; Ahmad, 1995). In spite of this protection, various forms of intracellular damage such as DNA alteration or lipid peroxidation may occur when the prooxidant-antioxidant balance is disrupted (Lemaire and Livingstone, 1993). Many environmental contaminants may induce oxidative stress either directly or after bioactivation (e.g. phase I reactions) and antioxidant enzyme activities are frequently considered as pertinent biomarkers of such stress, especially in aquatic organisms (Winston and Di Giulio, 1991).

In freshwater ecosystems, chironomid larvae of the subfamily Chironominae are frequently exposed to nearly anoxic conditions due to their sedimentary habits. Among these, *Chironomus riparius* Mg. larvae are well adapted to low oxygen concentration since they contain large amounts of hemoglobin (Green *et al.*, 1986; Osmulski and Leyko, 1986; Williams *et al.*, 1986). Changes in the oxygen content of surrounding water may modify the antioxidant processes in these animals especially through changes in hemoglobin. The presence of highly active respiratory pigments implies that these organisms possess efficient antioxidant enzymatic systems, which prevent an oxidative damage.

Thus, larvae of *Ch. riparius* constitute potential sentinel organisms for the monitoring of an oxidative stress caused by various chemicals (i.e. environmental pollutant). This study presents effects of chemical stress on superoxide dismutase, catalase and peroxidase activities in 4$^{th}$ instar larvae of *Ch. riparius*. In order to verify if the organophosphates, well-known neurotoxicant but insufficiently explored as oxidant stressors, are implicated in the production of reactive oxygen species, fenitrothion was chosen as a chemical stress model.

## Materials and Methods

### Organisms

Fourth instar larvae were used for the present study. The *Ch. riparius* strain was provided by INERIS (Institut National de lEnvironnement Industriel et des Risques, F 60550 Verneuil-en-Halatte, France). Larvae were reared in our laboratory in aerated 25 liter glass aquaria filled with dechlorinated tap water at constant temperature (20 ± 1°C) and photoperiod (14 h day/10 h night). A 5 cm thick layer of washed siliceous sand and cellulose (Sigma S-3504;

*Current address: Dept. of Pharmacology, Seoul National University, College of Medcine 28 Yongon-dong, Chongno-gu, Seoul 110-799, Korea

Sigma-Aldrich, F-38297 St Quentin Fallavier, France) was used as 'sediment'. Adult chironomids were retained using wood cages covered with steel wire mesh (1 mm mesh size) and reproduced continuously.

### Fenitrothion exposure

The organophosphate insecticide fenitrothion (*O,O*-dimethyl-*O*-nitro-*m*-tolyl phosphoro thioate), a well-known neurotoxicant, undergoes oxidation by cytochrome P450 monooxygenases (Eto, 1974). The corresponding oxon forms are able to irreversibly inhibit acetylcholinesterase (AChE) and may therefore severely perturb the functioning of the nervous system.

Glass tanks (20×15×20 cm) containing 2 liters of dechlorinated tap water and 1 cm of sediment layer were used for all the experiments. The effects of fenitrothion on antioxidant enzyme activities were assessed using groups of 4$^{th}$ instar larvae collected in rearing aquaria. Larval instar was determined using head capsule size. Larvae were then randomly introduced in control or treated aquaria.

In order to evaluate the effects of sublethal concentrations of fenitrothion on antioxidative enzymes, intoxication levels were determined using the results of preliminary toxicity tests (Choi, 1998). Intoxication levels corresponded to the range from $LC_{50,24h}/100$ to $LC_{50,24h}/1000$ (i.e. 2, 5, 10 and 20 $\mu g \cdot L^{-1}$) and acetone was used as a solvent. For each experiment, 2 ml of toxic solution were added to the experimental tanks prior to the introduction of larvae. Exposure was carried out under constant temperature (20±1°C) and photoperiod (14 h day/10 h night). Larvae were collected daily in control and experimental tanks and enzymatic assays were performed as described below.

### Sample preparation

A total of 10 larvae were pooled for each set of biochemical analysis. Body fluids (i.e. hemolymph) were collected placing cut larvae in 2 ml plastic containers filled with ice-chilled 0.6% NaCl/distilled water solution for 5 minutes. Larval remains were then collected and placed immediately in liquid nitrogen. Body fluid samples were kept at −80°C until SOD and total peroxidase activities measurement. Larval remains were homogenized in 2.5 ml of Tris-EDTA buffer (40 mM, pH 7.8) using a Potter-Elvehjem homogenizer. Crude homogenate was centrifuged 15 min. at 500 g (4°C) and supernatant was then centrifuged 30 min. at 12 000 g (4°C). The resulting supernatant was used to measure post-mitochondrial enzyme activities (SOD, catalase and GSH-Px). After repeated washing with homogenizing buffer, the pellet was used to measure mitochondrial SOD activity.

### Enzyme assays

SOD activity was determined after hemoglobin precipitation and extraction in ethanol/dichloromethane (2/1, v/v) by the chemiluminescence method of Bensiger and Johnson (1981), using a LKB Wallac type 1250 luminometer. The different forms of SOD were distinguished using subcellular fractionation and specific inhibitors. Arbitrary enzymatic units were used, one unit corresponding to the amount of enzymes, which produced 50% inhibition of chemiluminescence as compared to control. Total peroxidase activity was measured using guaiacol test (George, 1953). For the determination of GSH-Px activity, we employed the method described by Paglia and Valentine (1967). The rate of $H_2O_2$ disappearance (measured at 240 nm) was used to quantify catalase activity (Beers and Sizer, 1952).

### Chemicals

Fenitrothion and potassium dichromate were obtained from Cluzeau Info Labo (F33220 Sainte Foy la Grande, France) and Prolabo (94126 Fontenay-sous-bois, France) respectively. Biochemicals were purchased from Sigma Chemical Company Europe (F38297 Saint Quentin Fallavier, France).

### Statistics

Statistical differences were checked using parametric *t*-test. Correlations were calculated using Pearsons coefficient. Analysis was performed using Statview 4.02 for Macintosh computer (Abacus Concepts Inc., USA).

## Results

The activities of SOD and catalase measured in control larvae were very high. Previous experiments have shown that three types of SOD can be measured in *Ch. riparius* larvae : Cu,Zn-SOD in extracellular and postmitochondrial fractions, Mn-SOD in mitochondrial fraction and putative Fe-SOD of an unclear origin (endosymbiotic or parasitic; Choi, 1998). In this study, approximately 70% of total SOD activity was due to Cu,Zn-SOD whereas presumed Fe- and Mn-SOD activities contributed to 17 and 13% of total SOD activity, respectively. Extracellular SOD activity represented 30 % of total SOD activity and consisted exclusively of Cu,Zn-SOD. Selenium-dependent GSH-Px activity was not detected in larvae (results non shown), suggesting that GSH-Px activity was only associated with GST activity. Over 90 % of total peroxidasic activity was located in the hemolymph.

The experiment was performed on 4 different concentrations of fenitrothion (2, 5, 10 and 20 $\mu g \cdot L^{-1}$). The two highest

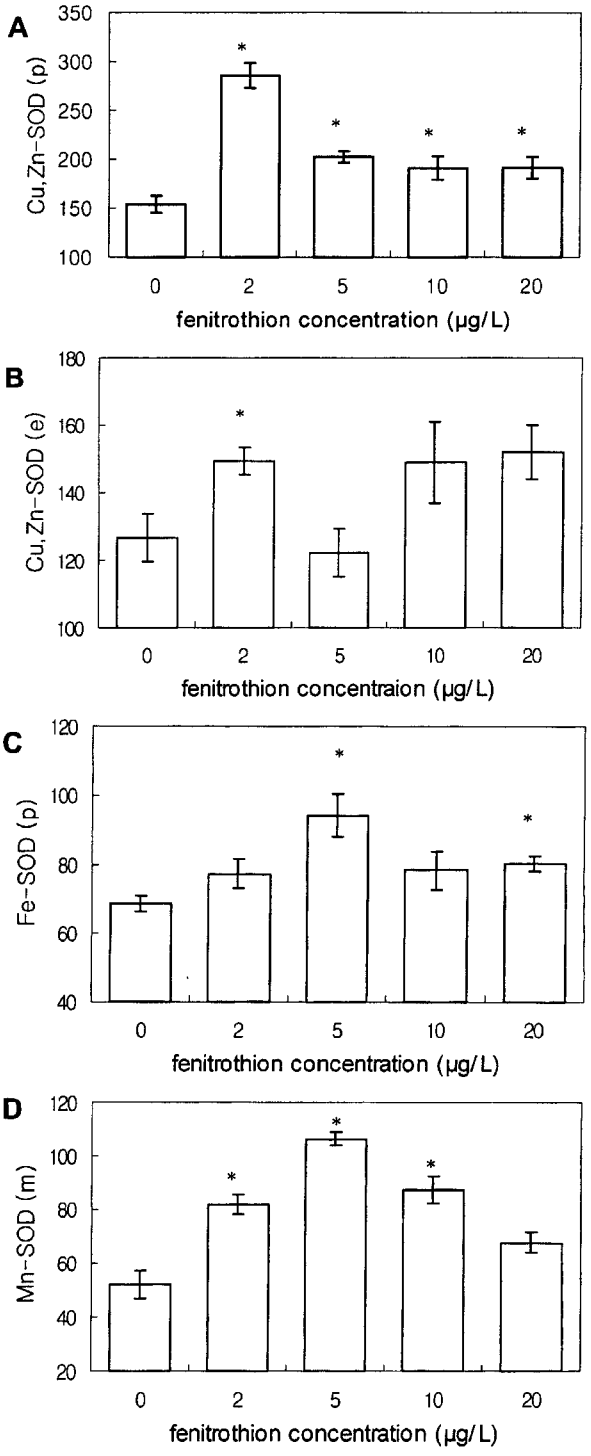


**Fig. 1.** Effects of fenitrothion exposure on Cu,Zn-SOD (A, B), presumed Fe-SOD (C), Mn-SOD (D) activities (mean ± s.e.; $n = 5$) measured in 4th instar larvae of *Ch. riparius*. Enzymatic activities are expressed as unit·mg$^{-1}$ protein.
e: extracellular; m : mitochondrial; p : postmitochondrial.
*: significantly different from control, $p<0.05$.

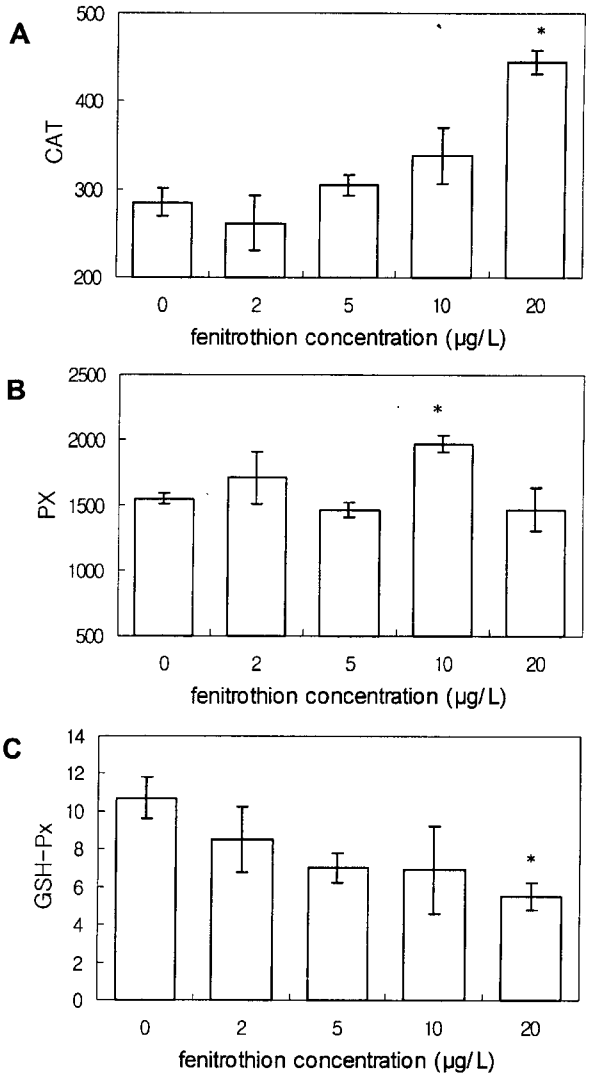


**Fig. 2.** Effects of fenitrothion exposure on catalase (CAT; A), peroxydase (Px; B), glutathion peroxydase (GSH-Px; C) activities (mean ± s.e.; $n = 5$) measured in 4th instar larvae of *Ch. riparius*. Enzymatic activities are expressed as unit·mg$^{-1}$ protein.
*: significantly different from control, $p<0.05$.

concentration values proved to prevent adult emergence in chronic toxicity tests whereas the other two did not (Choi, 1998). Many significant differences were observed between control larvae and larvae exposed to fenitrothion for 24 h (Fig. 1, 2).

Figure 1 presents various types of SOD activities in fenitrothion treated and non-treated *Ch. riparius* larvae. Post-mitochondrial Cu,Zn-SOD was significantly more elevated in every treated larvae than in control (increase of 86.0, 31.9, 24.4 and 24.7 % of control value for 2, 5, 10 and 20 μg·L$^{-1}$ of fenitrothion, respectively), the effect of

intoxication on this enzyme was higher for the lowest concentrations (2 $\mu g \cdot L^{-1}$). Exposure to this toxic compound also caused a significant increase in mitochondrial Mn-SOD activity (increase of 57.7, 103.8, 67.3 and 22% of control value for 2, 5, 10 and 20 $\mu g \cdot L^{-1}$ of fenitrothion, respectively) whereas presumed Fe-SOD activity increased in exposed larvae but differences with control were not always significant.

Catalase and peroxidase activities were shown in Figure 2. Catalase and GSH-Px activities were significantly affected only by the highest fenitrothion concentrations (20 $\mu g \cdot L^{-1}$), but in opposite ways : the former was increased whereas the latter was decreased following the intoxication. Moreover, a significant correlation between catalase activity and fenitrothion concentration has been demonstrated (Pearsons $r = 0.97$, $p < 0.01$). Total peroxydase activity measured in treated group did not show a significant difference compared to the control group.

## Discussion

Many invertebrates possess efficient enzymatic oxygen radical scavengers (e.g. SOD, catalase, peroxidase, ...) which protect them against oxidative tissue damage and SOD activities are generally higher in hemoglobin containing animals, such as chironomid (Abele-Oeschger, 1996). Our data suggest that SODs are very active in *Ch. riparius* larvae Among the various forms of SOD, post-mitochondrial Cu,Zn- and mitochondrial Mn-SOD were significantly activated by the chemical. Usually, Mn- and Fe-SOD are known not to be induced by stress (Ahmad, 1995). However, intrinsic conditions may have an effect on the metabolism of mitochondria and act directly on mitochondrial enzymes, as shown in fish red blood cells (Roche and Boge, 1993). Se-dependent GSH-Px activity is not present in *Ch. riparius* larvae. Insects exhibit peculiar GST which present Se-independent GSH-Px activity which is involved in lipid hydroperoxide reduction (Ahmad, 1995). Conversely, catalase activity is elevated in insects (Ahamd, 1992; 1995), this seems necessary to compensate for peroxidasic activity deficiency. Steady state accumulation of hydrogen peroxide is higher in insects than in other animals. These short-life animals require high energy levels during their embryogenesis, tissue differentiation and metamorphosis, which in turn causes periodical elevations in hydrogen peroxide contents in mitochondria and cytosol due to active ATP synthesis (Ahmad, 1995). High levels of catalase activity are therefore needed to protect larvae against these periodical increases in reactive oxygen species production.

Oxyradical formation and oxidative stress following xenobiotic exposure have frequently been reported (Hassan and Fridovich, 1979; Canada and Calabrese, 1989; Livingstone *et al.*, 1990). Similar phenomena were observed in *Ch. riparius* larvae intoxicated by fenitrothion. Interactions between organophosphate intoxication and oxidative stress have been poorly investigated. We have observed that fenitrothion exposure causes an increase in Cu,Zn- and Mn-SOD and, to a lesser extent, in presumed Fe-SOD activities, whereas catalase activity was increased and GSH-Px activity was decreased in a concentration-dependent way by the 24 h-fenitrothion exposure. The activated form of fenitrothion, fenitrooxon, is formed by oxidative desulfuration catalyzed by the cytochrom P450-dependent system (Escartin and Porte, 1996). With the mitochondrial respiratory chain, the microsomal cytochrome P450-dependent system is well known as the main oxyradical production source in the living cell (Winston, 1991). The fenitrothion, consequently, would involve the generation of oxyradicals and thus stimulate antioxidant enzyme systems through its detoxification pathways by cytochrome P450-dependent enzymes.

In conclusion, the response of antioxidant enzymes, especially, the activation of Cu,Zn-, and Mn-SOD was rapid, whereas changes in catalase, peroxidase and GSH-Px were less sensitive. Our results showed that as biomarkers antioxidant enzymes systems seem to be interesting parameters in the *Ch. riparius* larvae, probably due to the particularities of this animal (e.g. high amounts of hemoglobin in the hemolymph). However, for a useful multi-level/multi-biomarker approach of environmental monitoring, the calibration and the validation of these biochemical parameters will be necessary for their use *in situ*.

## References

Abele-Oeschger, D. (1996): A comparative study of superoxide dismutase activity in marine bentic invertebrates with respect to environmental sulphide exposure. *J. Exp. Mar. Biol. Ecol.* **197**, 39-49.

Ahmad, S. (1992): Biochemical defense of pro-oxidant plant allelochemicals by herbivorous insects. *Biochem. Syst. Ecol.* **20**, 269-296.

Ahmad, S. (1995): Oxidative stress and antioxidant defenses in biology. Chapman & Hall, New York, USA

Beers, J.R. and Sizer, I.W. (1952): A spectrophotometric method for measuring the breakdown of hydrogen peroxide. *J. Biol. Chem.* **195**, 133-140.

Bensinger, R.E. and Johnson, C.M. (1981): Luminol assay for superoxide dismutase. *Anal. Biochem.* **116**, 142-145.

Beyer, W., Immay, J. and Fridovich, I. (1991): Superoxide dismutase. *Prog. Nucl. Ac. Res. Mol. Biol.* **40**, 221-253.

Canada, A.T. and Calabrese, E.J. (1989): Superoxide dismutase:

its role in xenobiotic detoxication. *Pharmac. Therapeut.* **44**, 285-295.

Choi, J. (1998): Etude des effets biochimiques et écophysiologiques du bichromate de potassium et du fénitrothion sur *Chironomus riparius* (Meigen) (Diptère, Chironomidae) en vue de lidentification expérimentale de biomarqueurs. Thèse of Université de Paris-Sud, Orsay, France.

Escartin, E. and Porte, C. (1996): Bioaccumulation, metabolism and biochemical effects of the organophosphorus pesticide fenitrothion in *Procambarus clarkii. Environ. Toxicol. Chem.* **15**, 915-920.

Eto, M. (1974): *Organophosphorus pesticides : Organic and Biological Chemistry*, CRS press, Cleveland, USA

George P. (1953): The guaiacol test. *J. Biol. Chem.* 201: 413-423.

Green, D.W.J., Williams, K.A. and Pascoe, D. (1986): Studies on the acute toxicity of polluants to freshwater macroinvertebrates. 4. Lindane (γ-Hexachlorocyclohexane). *Arch. Hydrobiol.* **106**, 263-273.

Hassan, H.M. and Fridovich, I. (1979): Intracellular production of superoxide radical and hydrogen peroxide by redox active compounds. *Arch. Biochem. Biophys.* **196**, 385-395.

Lemaire, P. and Livingstone, D.R. (1993): Pro-oxidant/antioxidant processes and organic interactions in marine organisms, in particular the flounder *Platichthys flesus* and the mussel *Mytilus edulis. Trends in Comp. Biochem. Physiol.* **1**, 1119-1147.

Livingstone, D.R., Garcia-Martinez, P., Michel, X., Narbonne, J.F., OHara, S., Ribera, D. and Winston, G.W. (1990): Oxyradical production as a pollution-mediated mechanism of toxicity in the common mussel, *Mytilus edulis* L., and other molluscs. *Funct. Ecol.* **4**, 415-424.

Osmulski, P.A. and Leyko, W. (1986): Structure, function and physiological role of *Chironomus* haemoglobin. *Comp. Biochem. Physiol.* **85B**, 7016722.

Paglia, D.E. and Valentine, W.N. (1967): Studies on the quantitative and qualitative characterization of erythrocyte glutathion peroxidase. *J. Lab. Clin. Med.* **70**, 158-169.

Roche, H. and Bogé, G. (1993): *In vitro* effects of Cu, Zn and Cr salts on antioxidant enzyme activities of red blood cells of marine fish *Dicentrarchus labrax. Toxicol. in vitro.* **7**, 623-629.

Williams, K.A., Green, D.W.J., Pascoe, D. and Gower, D.E. (1986): The acute toxicity of cadmium to different larval stages of *Chironomus riparius* (Diptera : Chironomidae) and its ecological significance for pollution regulation. *Oecologia (Berlin).* **70**, 362-366.

Winston G.W. (1991): Oxidants and antioxidants in aquatic animals. *Comp. Biochem. Physiol.* **100C**, 173-276.

Winston, G.W. and Di Giulio, T.D. (1991): Prooxidant and antioxidant mechanisms in aquatic organisms. *Aquatic Toxicol.* **19**, 137-161.