

HEPATIC GLUTATHIONE S-TRANSFERASE ACTIVITY IN MOSQUITOFISH (*GAMBUSIA AFFINIS*) AND TOPMOUTH GUDGEON (*PSEUDORASOBORA PARVA*) EXPOSED TO FENITROTHION

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Abstract: Two common fish species, mosquitofish (*Gambusia affinis*) and topmouth gudgeon (*Pseudorasbora parva*) were exposed to different concentrations of fenitrothion in static system for 96 h. Hepatic glutathione S-transferase activity was evaluated after 48 and 96 h pesticide exposure, and was also examined in fish pretreated with pepironyl butoxide and triphenyl phosphate and then exposed to fenitrothion. Results indicated presence of intense glutathione S-transferase activity in both species, mosquitofish exhibiting the higher activity. In both species the activity decreased as the concentration of fenitrothion increased, topmouth gudgeon being more susceptible than mosquitofish. In mosquitofish pretreated with pepironyl butoxide, glutathione S-transferase activity was increased (11.8%) compared with the control but in topmouth gudgeon it was decreased (21.6%) at the end of 96 h. Glutathione S-transferase activity was significantly reduced in both species pretreated with triphenyl phosphate at the end of 96 h exposure, topmouth gudgeon being highly susceptible.

Key words: fenitrothion, hepatic GST activity, glutathione S-transferase, fish

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INTRODUCTION

Fenitrothion (O, O-dimethyl O-(3-methyl-4-nitrophenol) phosphorothioate), the active ingredient in the formulated product sumithion, is an organo-phosphate (O - P) insecticide and used in agriculture for crop protection and control of vector born diseases in various countries (Self et al., 1973; Volpe et al., 1981; Gandahasada et al., 1984; Erns et al., 1991; Erns et al., 1994). Although O - P compounds tend to undergo fairly rapid degradation in the environment, with repeated input, aquatic organisms may be exposed to sublethal concentrations for an extended period of time (Sancho et al., 1997).

According to Begum and Vijayaraghavan (1996), pollution of aquatic environment by pesticides brings changes in the metabolic activities and alters physiological state, thereby changing the biochemical constituents, of aquatic organisms. Glutathione S-transferases (GSTs) are thought to play a physiological role in initiating the detoxification of potentially alkylating

agents (Habig et al., 1974) including pesticides. The presence of such detoxifying enzyme is known to decrease the potential toxicity of pesticides. Furthermore, the different rate of dealkylation and dearylation of O - P pesticides in different species by GSTs provides one example of the potential importance of this enzyme in determining selective toxicity. This enzyme had also been employed as biomarker for the monitoring of environmental pollution and associated toxic manifestations in mammals and aquatic organisms including fish (Stegeman et al., 1991; Martinez-Lara et al., 1996; Otta et al., 1996).

Studies were undertaken to investigate the nature and function of hepatic GST in rainbow trout, *Salmo gaidner/Oncorhynchus mykiss* (Ramage et al., 1984; Lauren et al., 1989); sturgeon, *Acipenser baeri* (Perud-Durand et al., 1989); channel catfish, *Ictalurus punctatus* (Gallagher et al., 1989); and also to investigate GST activity in gill (Al-Ghais et al., 1995) and kidney (Al-Ghais, 1997). But there has

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been little study on the effect of waterborne pesticides on hepatic GST activity in fish species.

According to Qifa et al. (1995), fenitrothion (FNT) is used in paddy fields to control the rice stem borer, *Chilo suppressals Walker*. Fenitrothion has high potential to contaminate aquatic ecosystem and affect common fish species. In addition FNT was shown by Hollingworth (1969) to be demethylated by a glutathione-dependent mechanism in which GST is involved. Piperonyl butoxide (PBO), a synthetic methylenedioxyphenol (MDP) compound, is used as a synergist in pesticidal formulations including FNT. Its inhibitor effect on cytochrome P-450 dependent mixed function oxidase (MFO) has been exploited in various studies involving metabolism and toxicity in different species of organisms including fish (Epstein et al., 1967; Conney et al., 1972; Reinbold et al., 1976; Glinkman et al., 1977; Levin et al., 1977; Melanocon et al., 1977; Erickson et al., 1988; Qigfa et al., 1995). According to Plapp et al. (1963), Plapp and Tong (1966), triphenyl phosphate (TPP) is one of the most active OP compounds used as synergist against some resistant strains of insects, and is a known carboxylesterase inhibitor.

This study aimed to examine the effect of fenitrothion exposure on GST activity in two common freshwater fish species, the mosquitofish (*Gambusia affinis*) and topmouth gudgeon (*Pseudorasobora parva*). The effects of pretreating these fish species with PBO and TPP on the GST activity were analysed.

MATERIALS AND METHODS

1. Test chemicals

Fenitrothion (FNT) of technical grade (93% w/w) was obtained from the Ninbo Pesticide Factory (P. R. China). Piperonyl butoxide (PBO) (90%) and triphenyl phosphate (TPP) (99 + %) were products of Aldrich Chemical Company Inc. 1-chloro-2, 4-dinitrobenzene (CDNB) (98%) was a product of Sigma Chemical Company and bovine serum albumin was a product of Boehringer Company. All other chemicals were of analytical grade and obtained from local commercial sources.

2. Fish species

Two common species of freshwater fish namely, the mosquitofish (*Gambusia affinis*) and topmouth gudgeon (*Pseudorasobora parva*) were used in this study. The mosquitofish (0.2 – 0.26 g) and topmouth gudgeon (0.6 – 1.0 g) were procured from a local pet market and acclimatized to the laboratory conditions for at least seven days prior to use. During the acclimatization period the water was changed daily and the fish were fed on commercially prepared fish food. The fish were judged to be in good physiological condition for use when no mortality was observed in the acclimatizing population. The fish selected for use in the study were starved for at least 24 hours before use and during the experiment period.

3. Experimental conditions

Exposure of the fish to the test chemicals was carried out in a 40L-glass aquarium and the test diluent consisted of 20l aerated tap water. Each test aquarium contained 20 – 30 fish (the mass/volume ratio did not exceed 1g fish/L). The test was conducted at water temperature of $23 \pm 1^\circ\text{C}$.

The stock solutions of test chemicals (FNT, PBO and TPP) were prepared by dissolving in acetone and appropriate volume of these stocks was added to 20l diluent in the aquaria to attain the required concentration. The test concentrations of FNT used were based on the 96 h LC50 values, 3.71 mg/L for mosquitofish and 1.64 mg/L for topmouth gudgeon (Solomon and Fang, 1998, unpublished data). The concentration of PBO used (1 mg/L) was recommended by Glickman et al. (1977) and Melanocon et al (1977). The concentration of TPP used (0.2 mg/L) was determined to be the highest concentration not causing mortality in both species used in this study.

The experiments were conducted in a 96 h static exposure system. Three experiments were carried out on each species of fish, i.e.; fish was exposed to FNT alone; pretreated with PBO and with TPP 24 h before exposure to FNT. In all three experiments two controls were used, i.e., untreated fish and fish treated with a solvent (acetone).

4. GST analysis method

From each treatment group, six fish were

used to analyze GST activity. The enzyme source 9000 g supernatant called S9 was prepared and the GST activity was analyzed and calculated according to the method of Mao et al., (1986). GST was determined spectrophotometrically (double-beam, Shimadzu, Japan) following the formation of GSH conjugate with CDNB. The absorbency difference between the control and the treatments was monitored in a quartz cuvette with 1 cm light path at 340 nm. Non-enzymatic increase in absorbency was corrected by running a parallel control in all experiments. The GST activity was calculated using an extinction coefficient of $9.6 \text{ mmol}^{-1} \cdot \text{L}^{-1} \cdot \text{cm}^{-1}$. The total protein concentration of tissues from test fish was determined by the folinphenol reagent method of Lowry et al. (1955) using bovine serum albumin as standard. The activities of GST were expressed as $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \text{ protein}^{-1}$. All assays in this study were performed in triplicate, each experiment was repeated three times and with results presented as the mean \pm SD of the means. Percentage activity was calculated taking into account the activity of the control group.

5. Statistical analysis

Two-way analysis of variance (ANOVA) was used to determine treatment effects. Duncan's multiple range test was used for mean separation and the significance level was set at 0.05.

RESULTS AND DISCUSSION

An experiment conducted to test if the solvent (acetone) used with test chemicals had any effect on hepatic GST activity indicated that both mosquitofish and topmouth gudgeon GST activity did not exhibit any significant difference from the control (data not indicated). Furthermore, at test concentrations of FNT selected for experim-

ents no mortality of fish was observed except that a few of them became moribund at highest concentrations.

Results of this study revealed intense GST activity in the liver tissue of both mosquitofish and topmouth gudgeon, with mosquitofish exhibiting higher GST activity ($5465.91 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$) 1.3 times that of topmouth gudgeon ($4204.55 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$) (data not indicated in the tables). This shows that interspecific difference in GST activity exists in the two species. Although the method of enzyme preparation was different, the presence of hepatic GST activity and species difference was shown to exist in channel catfish (*Ictalurus punctatus*) and bullhead (*Ameiurus nebulosus*) (Hasspieler, et al. 1994). However, the GST activity observed in their study was much lower than that observed in our study.

Two-way ANOVA indicated significant effect ($P < 0.05$) of both FNT concentrations and exposure period on hepatic GST activity in the two species, wherein the GST activity varied significantly in a dose-dependent fashion as the concentrations of FNT increased (Tables 1 and 2). In the case of mosquitofish, for the lowest concentration used (0.4 mg/L of FNT), there was 11% decline and for the highest concentration (1.30 mg/L), FNT produced a 33.5% decline in the GST activity relative to control at the end of 48 h exposure (Table 1). In topmouth gudgeon, the lowest concentration (0.2 mg/L) resulted in 21% decline and the highest (0.6 mg/L) resulted in 66.9% decline (Table 2). In both species and concentration's exposures, the maximum decline was observed at the end of 48h; the significant decline being at the highest concentrations i. e., 1.30 and 0.6 mg/L in mosquitofish and topmouth gudgeon, respectively (Tables 1 and 2). These results indicate that

Table 1. Hepatic glutathione S-transferase activity ($\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$) of mosquitofish (*G. affinis*) exposed to different concentrations of fenitrothion ($n = 6$)

Exposure period(h)	Concentrations (mg/L)			
	0 (control)	0.4	0.6	1.3
48	5416.67 \pm 6.70 (100)	4791.67 \pm 5.29* (88.5)	4525.46 \pm 4.28* (83.5)	3602.08 \pm 4.46* (66.5)
96 (100)	5486.80 \pm 6.11 (90.6)	4971.04 \pm 5.54* (88.4)	4850.33 \pm 5.40* (84.7)	4647.32 \pm 5.17*

Activities are mean (SD of three experiments. Values in parenthesis are percentage activity.

* Significantly different from control at $P < 0.05$.

Table 2 Hepatic glutathione S-transferase activity ($\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$) of topmouth gudgeon (*P. parva*) exposed to different concentrations of fenitrothion ($n = 6$)

Exposure period(h)	Concentrations (mg/L)			
	0 (control)	0.20	0.30	0.60
48	4133.67 \pm 8.14 (100)	3232.53 \pm 9.19* (78.2)	2641.41 \pm 4.55* (63.9)	1368.24 \pm 2.64* (33.1)
96	4202.08 \pm 8.89 (100)	4122.24 \pm 8.73 (98.1)	3491.93 \pm 7.39* (83.1)	3256.61 \pm 6.89* (77.5)

Activities are mean SD. Values in parenthesis are percentage activities.

* Significantly different from control at $P < 0.05$.

there was an apparent susceptibility difference between the two species to FNT concerning GST activity, the topmouth gudgeon being highly susceptible. Considering the significant role of GST in the detoxification of xenobiotics, the low intrinsic GST activity and high susceptibility observed in topmouth gudgeon to FNT indicates this species may possibly be at disadvantage when exposed to deleterious environmental pollutants.

An experiment was conducted to assess the effect of PBO alone and combined with FNT. A static PBO exposure of 1 mg/L was used 24 h before adding FNT in this experiment wherein this concentration did not result in death in both species of fish during the exposure period. Results indicated that static exposure to PBO alone inhibits the GST activity in both species tested. At the end of 48 h, the GST activity in both species (75.2% in mosquitofish and 77.5% in topmouth gudgeon compared with control) was almost the same, i.e. almost equal inhibition was observed, but after 96 h exposure, GST activity of topmouth gudgeon remained inhibited (79.2%) while that of mosquitofish increased (89.4%) compared with that of 48 h activity (Table 3).

The hepatic GST activity in fish treated with FNT (0.4 mg/L) alone and pretreated with PBO was also compared. In mosquitofish pretreated with PBO and then exposed to FNT, GST activity decreased (9.6%) at the end of 48 h compared with those exposed to FNT alone. However, at the end of 96 h, it increased by 21.2% and 11.8% compared with those exposed to FNT alone and the control, respectively (Table 3). This result shows that inhibition of MFO activity by PBO caused induction of GST activity. The increased GST activity may be responsible for catalyzing more conjugation reactions in mosquitofish. Our result seems to be consistent with the suggestion of Kamienski and Murphy (1977) that, a large proportion of methyl

parathion, a dimethyl phosphrothionate like FNT, undergoes GSH-dependent metabolism during inhibition of oxidative metabolism by PBO. In the case of topmouth gudgeon, the reverse condition was observed, i.e., an increase (34%) at 48 h and decrease (4.7%) at the end of 96 h exposure compared with GST activity of fish exposed to FNT alone. In this species PBO alone and also combined with FNT, caused almost the same (approximately 11%) inhibition of GST activity compared with the control. The two treatments (PBO alone and PBO + FNT) caused higher inhibition than FNT alone (Table 3). Therefore, PBO or its metabolites might have inhibitory effect on GST activity in particular and GSH-dependent detoxification system in general in topmouth gudgeon.

The effect of TPP (0.2 mg/L) alone and combined with FNT (0.4 mg/L) on the GST activity of the two species of fish was examined. In both species TPP alone as well as combined with FNT resulted in decrease in GST activity in time-dependent fashion, the highest decline being at the end of 96 h exposure. TPP alone caused the higher inhibition (51%) of GST activity in topmouth gudgeon than in mosquitofish (21.6% inhibition). As far as the combined effect was concerned, the GST activity of mosquitofish declined by 22.6% relative to FNT alone while that of topmouth gudgeon declined 30.6% after 96 h exposure (Table 3). These results show that GST activities of the two fish species was more strongly inhibited by TPP alone than by FNT alone, the topmouth gudgeon being highly susceptible. According to Jakob, and Keen (1977), one of the actions of GST is binding covalently with more reactive agents in a so-called 'sacrificial' reaction. This indicates that in such reactions, GSTs are not recovered, i.e., they are permanently inactivated. Therefore, significantly increased inhibition of GST activity with exposure period observed in fish species pre-

treated with TPP may suggest depletion of GST by participating in such 'sacrificial reaction'.

Table 3 Percentage glutathione S-transferase activity of mosquitofish (*G. affinis*) and topmouth gudgeon (*P. parva*) with PBO (1 mg/L⁻¹) and TPP(0.2 mg/L⁻¹), and then exposed to fenitrothion (0.4 mg/L⁻¹)

Treatments	48 h ^a		96 h ^a	
	<i>G. affinis</i>	<i>P. parva</i>	<i>G. affinis</i>	<i>P. parva</i>
Control	100	100	100	100
FNT ¹ (0.4 mg/L)	88.5	63.9	90.6	83.1
PBO ² (1 mg/L)	75.2	77.5	89.4	79.2
FNT + PBO	78.9	97.9	111.8	78.4
TPP ³ (0.2 mg/L)	80.4	99.7	78.4	49.0
FNT + TPP	71.9	74.0	68.0	52.5

FNT = fenitrothion, PBO = Piperonyl butoxide, TPP = triphenyl phosphate.

^aFor PBO and TPP treatments, exposure period does not include pretreatment time, i.e., 24 h.

CONCLUSIONS

This study indicated intensive hepatic GST activity in the two species examined. Genlin et al. (1991) reported that FNT is safe to carp when used in paddy fields at the application rate of 375 – 750 g per ha. However, in both species of fish exposed to sublethal concentrations (as low as 10% of the LC50) of fenitrothion inhibition of GST activity was observed. Difference in activity of Glutathione-S-transferase in liver of the two species investigated suggests species variation in GSH-mediated detoxification of xenobiotics. With additional comparative studies these species may be used as bioindicators of environmental pollution by xenobiotics.

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