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ACUTE TOXICITY AND EFFECT OF FENITROTHION ON LIVER ESTERASE OF FISH

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ABSTRACT: The acute toxicity of fenitrothion (FNT) alone and combined with piperonyl butoxide (PBO) or triphenyl phosphate (TPP), and their effect on liver esterase (LE) activity of *Gambusia holbrooki*, *Pseudorasobora parva* and *Oncorhynchus mykiss* was studied in aquaria for 96h. The results indicated that fenitrothion is moderately toxic to the three species of fish tested, the toxicity being highest to *O. mykiss* followed by *P. parva* and *G. holbrooki*. Both FNT concentrations and the exposure time on the activity of LE and FNT (two way ANOVA $P<0.05$) caused more inhibition in *G. holbrooki* than *P. parva*. Pretreating fish with either PBO or TPP changed the acute toxicity level and LE sensitivity compared with those exposed to FNT alone. The acute toxicity level and susceptibility of LE to FNT was found to be inversely related. With additional studies *P. parva* and *G. holbrooki* have the potential to be used as bioindicator, and LE as biochemical biomarker of environmental pollution by FNT and other related compounds.

Key words/phrases: Fenitrothion, fish, liver esterase, synergists, toxicity

Abbreviations: FNT (fenitrothion), PBO (piperonyl butoxide), TPP (triphenyl phosphate), LE (liver esterase), OP (organophosphate), ANOVA (analysis of variance), EEC (Economic Commission for Europe), WHO (World Health Organization), Glutathion (GSH), Glutathione S-transferase (GST), AchE (Acetylcholinesterase), MFO (Mixed Function Oxidase).

INTRODUCTION

Apart from the target pests, pesticides also affect the non-target organisms present in the ecosystem. Fish forms one of the important non-target organisms in the aquatic system, and is one of the major sources of protein for human being. It is necessary to examine the toxic effects of pesticides on fish, as they constitute an important link in the food chain and their contamination by pesticides disturb normal flow of energy in the aquatic ecosystem (Begum and Vijayaraghavana, 1995).

Begum and Vijayaraghavan (1996) stated that, pollution of aquatic environment by pesticides brings changes in the metabolic activities and alters physiological state thereby changing the biochemical constituent of aquatic organisms.

It is well known that xenobiotics are enzymatically metabolised in the liver of fish. According to Chambers *et al.* (1994), phosphrothionate insecticides and their active metabolites can be detoxified by a variety of hepatic mechanisms. This includes P-450-mediated dearylation, phosphorylation of aliesterases (carboxylesterases), catalytic hydrolysis of oxon by A-esterases, glutathion (GSH)-dependent detoxification, etc.

Enzymes have been recently used as biochemical biomarkers in order to assess and monitor the degree of pollution. According to Walker (1992), biomarkers, which can measure both exposure and toxic effect, are particularly valuable because they can provide a measurement of the harm that is caused by an environmental chemical.

The active ingredient in the formulated product sumithion, fenitrothion [0,0-dimethyl 0-(3-methyl-4-nitrophenyl) phosphorothioate], is an organophosphate (OP) insecticide which is widely used in agriculture for crop protection, control of vector born diseases of public health and veterinary importance in various countries including China and Ethiopia (Self *et al*., 1973; Volpe and Mallet, 1981; Gandahusada *et al*., 1984; Ernst *et al*., 1991; 1994; Qifa *et al*., 1995). In Ethiopia, it is used to control armyworm (*Spodptera exempta*), African bollworm (*Helicoverpa armigera*), aphids, locusts, grasshoppers, plant bugs, etc. (Personal communication with Ministry of Agriculture). Piperonyl butoxide (PBO) and triphenyl phosphate (TPP) are also used as a synergist in pesticide formulations including fenitrothion (FNT).

Genlin *et al*. (1991) studied the effect of FNT in the aquatic ecosystem of paddy fields in China (in a 'rice-fish system'). According to them, when used at 375-750g active ingredient (a.i.) ha^{-1} (50% EC 500-800 dilution), FNT is safe to fish. However, the effect of its sublethal concentrations on common species of fish has not been studied.

The objectives of the present study were therefore, to 1) determine the relative acute toxicity of FNT alone and combined with synergist to three

fish species, namely, the *Gambusia holbrooki*, *Psuedorasobora parva* and *Oncorhynchus mykiss*; 2) examine the dose-response relationship of liver esterase (LE) activity in the *G. holbrooki* and *P. parva*, exposed to FNT; 3) assess the effect of synergists (PBO and TPP) as well as their respective combinations with FNT on the susceptibility of LE of *G. holbrooki* and *P. parva*; and 4) examine if there is any relationship between acute toxicity level and sensitivity of LE to FNT and its combinations with synergists.

MATERIALS AND METHODS

Test chemicals and reagents

Fenitrothion (FNT) of technical grade (93% w/w) was obtained from Ningbo pesticide factory (China). Piperonyl butoxide (PBO) (90%) and triphenyl phosphate (TPP) (99 + %) were products of Aldrich chemical company inc. Alpha-naphthyl acetate was purchased from Shanghai first reagent factory; α-naphthol from Xin-Tin chemical factory (Shanghai, P.R.China) was a product of roth; Fast blue B and Sodium laurylsulphate from Chemical and reagent supply station of Shanghai reagent factory (P.R.China). Bovine serum albumin was a product of Boehringer Company. All other chemicals were of analytical grade and obtained from local commercial sources.

Fish species

For acute toxicity test adults of two common species of freshwater fish namely, the *Gambusia holbrooki* (mosquito fish) (Girard, 1859) and *Pseudorasobora parva* (topmouth gudgeon) (Li and Fan 1996b) and one cold water fish, *Oncorhynchus mykiss* (rainbow trout) (Page and Burr, 1991) were used. The two fresh water fish were also used to study LE activity. These species were used because they are readily available and grow easily under laboratory conditions. *G. holbrooki* (0.2–0.26g) and *P.parva* (0.6–1.0g) were procured from a local pet market and *O. mykiss* (2.5–4.3g) was obtained from fish hatcheries. All fish were 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 at least for 24 hours before use and during the experiment period.

Experimental conditions

For both acute toxicity test and LE analysis, the experiment was carried out in 40 litter glass aquarium and the test diluents consisted of 20 litre aerated tap water. Twenty to thirty fish were grown in each aquarium (the mass/volume ratio did not exceed 1g fish L^{-1}). The test was conducted at water temperatures of 23 ± 1^0 C for *G. holbrooki* and *P. parva*, and 11^0 C for *O. mykiss*.

The stock solutions of test chemicals (FNT, PBO and TPP) were prepared by dissolving in acetone (99.5% purity) and the known volume of each stock was added to 20 litres of diluents in the aquaria to attain the concentration.

The concentrations of FNT used for acute toxicity test were first determined based on tests conducted prior to the actual experiment. Concentrations of FNT used for le activity tests were determined based on the 96h LC_{50} for *G. holbrooki* and *P. parva* (see the result section). The concentration of PBO (1mg L-1) was prepared based on recommendations of previous researchers (Glickman *et al.*, 1977; Melanocon *et al.*, 1977). The highest concentration of TPP (0.2mg L-1) that cause no mortality was considered for the experiment.

A 96h static exposure system was used in this study and fish were exposed to 1) different concentrations of FNT alone and, 2) 0.4 mg L-1 of FNT after 24h pre-treatment of either with PBO (1 mg L-1) or TPP (0.2mg L-1) and, 3) PBO or TPP alone. And the acute toxicity and LE activity in these exposed groups was compared with that of the control. In all cases fish that were not exposed to chemicals served as the control group. In addition, to test the effect of acetone on acute toxicity and LE activity, fish were exposed to acetone (15 µl L-1) alone under similar conditions to the experimental group.

Acute toxicity test

The susceptibility of the fish to FNT was determined according to the method of Macek and McAllister (1970). The end point of bioassay was death of the fish and death was defined as complete immobilization and failure to respond to gentle prodding. As mortality occurred the dead fish were immediately removed from the test aquarium and the median lethal concentration (LC50) was determined based on the method of Finney (1964). This method involves conversion of the concentrations tested and the corresponding observed mortality to log and probit values, respectively. The values were then used for further statistical analysis.

Liver homogenate preparation and esterase analysis

From each treatment group, six fish were used to estimate esterase activity. Liver homogenate was prepared following the method of Van Asperen (1962). Alpha-naphthylacetate was used as a substrate and the stock solution was prepared by dissolving in acetone. The diazoblue-sodium laurylsulphate solution (consisted of two parts of a 1% fast blue B and five parts of a 5% solution of sodium laurylsulphate) was used as an indicator to estimate the naphthol produced in the reaction system. The difference in colour absorbency between the control and the different treatments was measured with double-beam spectrophotometer (Shimazu, Japan) at a wavelength of 600 nm using a 1 cm light path cell. The amount of α-naphthol produced in the reaction was estimated from the standard curve and the LE activity calculated and expressed as nmol of α-naphthol produced min^{-1} mg $protein^{-1}$. Total protein was determined by the method of Lowry *et al.* (1951). Each assay was run in triplicate and the results are presented as the mean ± SD of esterase activity.

Statistical analysis

A two-way analysis of variance (ANOVA) was used to determine treatment effect and Duncan's test was used to determine differences between the means. The significance level was set at 0.95 and is expressed as a p-value 0.05.

RESULTS AND DISCUSSIONS

Acute toxicity

Table 1 shows the LC_{50} of FNT, and FNT combined with PBO or TPP for three species of fish. Of the three species tested, the LC_{50} of FNT was lowest for *O. mykiss* and highest for *G. holbrooki*. According to the criteria of Economic Commission for Europe (ECE) pesticides with $LC_{50} \leq 1$ mg L^{-1} are categorized as very toxic and those with 1mg $L^{-1} > LC_{50} \leq 10$ mg L^{-1} are considered moderately toxic to aquatic organisms (Klein *et al*., 1992). Considering this classification, FNT was moderately toxic to all the three species of fish tested. However, relative toxicity of FNT is approximately two-fold more toxic to *P. parva* and *O. mykiss* than *G. holbrooki*.

Compared with the fish species tested in this study, the 96h LC_{50} of a teleost fish, *Anguilla anguilla*, (0.2 mg L^{-1}) was very low indicating that FNT is extremely toxic to this species (Ferrando *et al.,* 1991). Shao-Nan and De-Fang (1996) studied the acute toxicity of malathion to these three species of fish in our laboratory and found the 96h LC_{50} to be 0.7, 14.5 and 0.25 mg L^{-1} for *G. holbrooki*, *P. parva* and *O. mykiss*, respectively. The data show that FNT is approximately five-fold less toxic than malathion to *G. holbrooki* and *O. mykiss*, but nine-fold more toxic to *P. parva*. The results (Table 1) clearly indicate the presence of species related difference in susceptibility of fish to FNT.

In all three species tested, the LC_{50} of FNT for fish pre-treated with PBO had decreased compared with that of FNT alone. Pretreating the fish with PBO increased the toxicity of FNT two-fold, 1.4-fold and 1.2-fold for *O. mykiss*, *P. parva* and *G. holbrooki*, respectively. The present result seems to be consistent with that of Reinbold and Metcalf (1976). These authors found that PBO increased the toxicity of methoxychlor, trifluralin and aldrin to *Lepomis cyanellus* (greenish). They postulated that the increased toxicity was due to the insecticides not being metabolised (detoxified). Shao-Nan and De-Fang (1996) also reported increased malathion toxicity to *P.parva* in the presence of PBO.

Table 1. Acute toxicity of fenitrothion alone and combined with PBO or TPP to three fish species, *G. holbrooki*, *P.parva* and *O. mykissi*. (n=30 for *G. holbrooki* and *P. parva*; n=20 for *O. mykissi*).

Pesticide or synergist mixture	LC_{50} (mg L^{-1})		
	G. holbrooki	*P. parva*	*O. mykissi*
FNT	3.71^{a} (3.49 - 3.93)	1.64^{c} (1.48 - 1.80)	1.31^{d} (0.77 - 1.85)
FNT + PBO	2.99^{b} (2.60 - 3.38)	1.20^{d} (0.33 - 1.94)	0.66^{e} (0.55 - 0.77)
FNT + TPP	1.25^{d} (0.81 - 1.69)	0.73^{e} (0.57 - 0.89)	0.84^{e} (0.76 - 0.92)

Note: FNT, fenitrothion; PBO, pepironyl butoxide; TPP, triphenyl phosphate; Values in parenthesis are 95% confidence intervals; Values with similar letter are not significantly different ($P > 0.05$).

Compared with that of FNT alone, the LC_{50} of FNT to all three species of fish pre-treated with TPP had decreased (Table 1). Triphenyl phosphate (TPP) increased the toxicity of FNT three-fold to *G. holbrooki* and approximately two-fold to *P. parva* and *O. mykiss*. No similar data were found to compare the present results with the previous studies conducted elsewhere. Although the concentration of TPP is greater (1 mg L^{-1}) and the pesticide used is different, Shao-Nan and De-Fang (1996) found higher toxicity of malathion to *G. holbrooki*, *P. parva* and *O. mykiss* pre-treated with TPP. TPP is a known inhibitor of carboxyl esterase (Oppenoorth and Welling, 1976). Carboxyl esterase belongs to a group of hydrolytic enzymes, collectively called non-specific esterases, that are important in detoxification of OP compounds and

synthetic pyrethroids (Dauterman, 1976; Kao *et al*., 1985; Chambers *et al*., 1994). Hence, the observed increase in acute toxicity of FNT to TPP pre-treated fish may be due to inability of the fish to detoxify FNT.

Liver esterase activity

Acetone used to dissolve test chemicals had no effect on LE activity and the values were not different from the control. Liver esterase activity of both *G. holbrooki* and *P. parva*, was found to be very high. In particular, *G. holbrooki* exhibited the highest activity (583.3 nmol $min.^{-1}$ mg protein), which is approximately 1.3-fold higher than that of *P. parva* (451.7 nmol $min.^{-1}$ mg $protein^{-1}$). This result shows differences in the inherent specific activity of LE between the two species. Li and Fan (1996a) also reported a similar result for the two species.

The pattern of LE activity in *G. holbrooki* and *P. parva* exposed to different concentrations of FNT is shown in Figures 1A and B, respectively. A two way ANOVA demonstrated a significant ($P<0.05$) difference in both FNT concentrations and the exposure time of the activity of le in the two species of fish. When exposed to 0.4 and 1.3 mg L^{-1} of FNT for 24h, the maximum activity and the minimum activity observed in *G. holbrooki* were 56 and 49.0% of the control, respectively. A similar concentration-dependent pattern was observed at the end of 48 and 96h. Similarly, in *P. parva*, LE activity progressively decreased with the increase in concentrations (except for 0.2 mg L^{-1}). However, the rate of decline was not similar for the two species, *i.e*., somewhat less for *P. parva* than for *G. holbrooki* (Fig. 1).

Results of this study demonstrated that LE activity was inhibited, at all concentrations used, in both species of fish investigated. This shows the presence of esterase, sensitive to FNT, in the liver of *G. holbrooki* and *P. parva*. Using electrophoresis, Li and Fan (1996b) identified one isoenzyme from *G. holbrooki* and two from *P. parva* LE and found one of the isoenzymes in *P. parva* to be similar to that of *G. holbrooki*. They also studied the effect of malaoxon on the LE and reported the existence of malaoxon-sensitive LE in *P. parva* but not in *G. holbrooki*.

Data concerning the mechanism of fish LEs on pesticides are not available. However, there are related studies that are conducted on the mechanism of LEs in mammals. For example, Lauwerys and Morphy (1969) studied paraoxon binding by rat liver and plasma and concluded that phosphorylation of esterases was probably the primary means of reducing the paraoxon concentration in these preparations. Other studies similarly concluded that AliE (eserin insensitive B-esterases) are an important alternate phosphorylation site for OPs and reduce the concentration inhibition of acetylcholinesterase (AchE) (Funnum and Sterri, 1981; Maxwell *et al*., 1988). Chambers *et al*. (1990) investigated detoxification of the oxons of six phosphorothionate insecticides (methyl parathion, parathion, chlorpyrifos-methyl, chlorpyrifos, EPN and leptophos) in rats and arrived at a similar conclusion. In addition, they stated that the presence of AliE in the liver, in close proximity to where much oxon would be generated by mixed function oxidase (MFO) (cytochrome P-450) - mediated desulfuration reaction, would allow them the opportunity to be readily phosphorylate and prevent much of the hepatically generated oxon from entering the circulation. Furthermore, they concluded that AliE phosphorylation is strongly implicated as a mechanism of noncatalytic detoxification of OPs, which can afford the animal significant protection from intoxication.

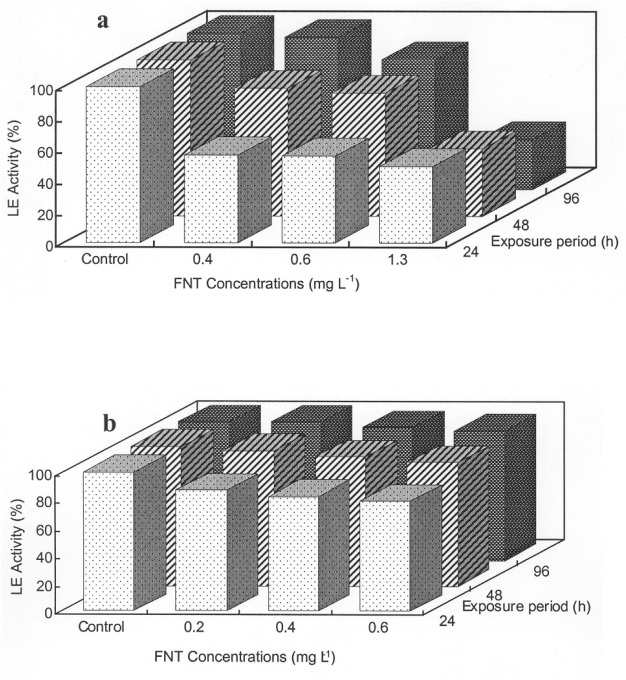

Fig. 1. Effect of fenitrothion on liver esterase activity of A) *G. holbrooki* B) *P. parava*. (Note that LE activity is expressed as percentage of the control).

Earlier results described above suggest that inhibition of LE results from binding to oxons of OP pesticides, which as a result become phosphorylated and decreases the concentration of esterase in the liver. Therefore, inhibition of LE activity

observed in *G. holbrooki* and *P. parva* in this study may be due to the binding of fenitroxon to esterase. This shows that the livers of *G. holbrooki* and *P. parva* are capable of sequestering or noncatalytically detoxifying FNT.

As shown in Figs 1a and b the activity of LE of both species is strongly inhibited within the first 24h exposure period and progressively decreased inhibition with time. This decreased inhibition (increased activity or recovery) with time may be due to decrease in the concentration of FNT and its active metabolite (fenitroxon) in the test system and/or in the fish with time. The exceptionally lowered LE activity with time observed in *G. holbrooki* exposed to highest concentration (1.3 mg L^{-1}) may be due to concentration-dependent permanent inactivation of the enzyme.

Comparison of the response in the two species exposed to similar concentrations (0.4 and 0.6 mg L^{-1}) revealed that FNT caused more inhibition of LE in *G. holbrooki* than *P. parva*. This difference in susceptibility between the two species is expected to cause difference in the rate of detoxification and consequently contribute to difference in the acute toxicity of FNT (Fig. 1).

To investigate the effect of PBO on the susceptibility of LE, fish were pre-treated with 1 mg L^{-1} of PBO for 24h before adding FNT to the test system. The result indicated that in *G. holbrooki* exposed to PBO alone, LE activity was higher than the controls. However, in *P. parva*, it produced a 6.6, 2.4 and 1.1% decline at 24, 48 and 96h exposure periods, respectively (Table 2).

The LE activity in fish exposed to FNT alone (0.4 mg L^{-1}) and pre-treated with PBO was also compared. In both species pre-treating with PBO caused more activity than those exposed to FNT alone (Table 2). This may be explained by considering inhibition of MFO by PBO and the subsequent reduction of fenitroxon production. Inhibition of MFO might have significantly reduced production of fenitroxon and this decreased phosphorylation of LE, thus increasing activity in these than in those exposed to FNT alone. Therefore, this suggests that detoxification action of LE is reduced by PBO treatment.

In both species investigated, liver esterase was strongly inhibited by TPP alone (0.2 mg L^{-1}). As shown in Table 2, LE activity of *G. holbrooki* is more sensitive to TPP inhibition than that of *P. parva*. This agrees well with the result reported by Li and Fan (1996b). These authors studied susceptibility of LE isoenzymes to TPP inhibition and found that both *G. holbrooki* and *P. parva* LEs contain TPP sensitive isoenzymes. Their report also indicated that LE of *G. holbrooki* is more sensitive to TPP inhibition than that of *P. parva*.

The influence of TPP on the sensitivity of LE to FNT was studied and the result compared (Table 2). In both species the activity was significantly decreased ($P<0.05$) in those pre-treated with TPP. Furthermore, the previous study (Solomon Sorsa *et al.*, 1999) also demonstrated that, in *G. holbrooki* exposed to FNT concentrations ranging from 0.4 to 1.3mg L^{-1} and pre-treated with TPP after exposure to 0.4mg L^{-1} of FNT, AchE activity was significantly depressed. This shows that TPP had increased the susceptibility of fish LE to FNT. This finding is supported by three facts 1) LEs of both species was inhibited by FNT alone, 2) both species of fish contain TPP sensitive LEs (23), and 3) TPP is a known specific inhibitor of carboxyl esterase (Maxwell *et al.*,1988) which is one of the LEs.

Relationship Between Acute Toxicity and Liver Esterase Activity

FNT is more toxic to *P. parva* (LC_{50} 1.64 mg L^{-1}) than *G. holbrooki* (LC_{50} 3.71 mg L^{-1}) (Table 1). This toxicity difference observed between the two species may be caused by several factors combined together. According to Chambers *et al.* (1990), the acute toxicity of any poison is governed by its inherent potency at the target site and the effective concentration reaching that target. Moreover, factors such as the ability of noncatalytic detoxification, AchE sensitivity, rate of phosphorothionate activation, and the rate of catalytic detoxification can determine the phosphorothionate's acute toxicity level.

The intrinsic LE activity of *G. holbrooki* is higher (approximately 1.3-fold) than that of *P. parva* and this shows that LEs of *G. holbrooki* are more susceptible to FNT inhibition than that of *P. parva*. Chambers *et al.* (1990) stated that, inhibition of LEs is mainly due to phosphorylation of aliesterases by OP compounds. Moreover, liver aliesterases occupy an alternative phosphorylation site for OPs and are capable of appreciably reducing the concentration of OPs required to inhibit the AchE. They also suggested that low inhibition of aliesterases by phosphrothionates is because of low sensitivity, thus the pesticide could more easily escape the hepatic aliesterases and cause inhibition. Thus, it can be suggested that detoxification of FNT by LEs in *G. holbrooki* was higher than that of *P.parva*. This suggestion agrees with the higher acute toxicity of FNT to *P. parva* than *G. holbrooki*.

Table 2. Liver esterase activity (%) of *G. holbrooki* and *P. parva* pre-treated with PBO (1 mg L^{-1}) and TPP (0.2 mg L^{-1}), and then exposed to fenitrothion (0.4 mg L^{-1}).

Treatments	24h[a]		48h[a]		96h[a]	
	G. holbrooki	*P. parva*	*G. holbrooki*	*P. parva*	*G. holbrooki*	*P. parva*
Control	100	100	100	100	100	100
FNT	56.2	82.3	81.9	92.7	97.5	96.3
PBO	101.2	93.4	109.5	97.6	119.3	98.9
FNT + PBO	78.9	90.7	84.7	92.3	110.2	98.1
TPP	45.7	59.3	46.2	68.6	52.9	82.2
FNT + TPP	51.8	54.7	58.7	57.1	67.4	61.1

[a] Exposure time. For PBO and TPP treatments, exposure period does not include pre-treatment time, i.e., 24h.
Note: FNT= fenitrothion, PBO= Piperonyl butoxide, TPP= triphenyl phosphate

Furthermore, results of a study conducted by Solomon (1999) revealed that hepatic glutathione S-transferase (GST) activity of *G. holbrooki* is 1.3-fold higher than that of *P. parva*. In addition, GST of *P. parva* was found to be more sensitive to inhibition by FNT than *G. holbrooki*. The presence of higher amount of inherent GST activity and lower sensitivity to FNT observed in *G. holbrooki* is indicative of higher detoxification capability of this species. Therefore, it seems likely that GST-dependent detoxification of FNT might have also contributed to low acute toxicity to *G. holbrooki*.

CONCLUSION

The study revealed that fenitrothion is moderately toxic to the three species of fish tested, the toxicity being highest to *O. mykiss* (LC_{50} 1.31 mg L^{-1}) followed by *P. parva* (LC_{50} 1.64 mg L^{-1}) and *G. holbrooki* (3.71 LC_{50} mg L^{-1}). Pre-treatment with two common synergists, PBO (1 mg L^{-1}) and TPP (0.2 mg L^{-1}), significantly increased the toxicity of FNT to *O. mykiss*, *P. parva* and *G. holbrooki*.

Liver esterases of both *G. holbrooki* and *P. parva* were inhibited by FNT, the *G. holbrooki* LEs being highly sensitive to inhibition than *P. parva*. However, in PBO and TPP pre-treated group, it was *P. parva* that become susceptible to FNT at the end of the exposure period. These findings could be indicative of the participation of LEs in detoxification of FNT.

The inhibitory effect of LE activity caused by FNT increased with concentration. Although the level of effect could be different under field condition, the present study revealed that field application rates that leave FNT concentrations of 0.4 mg L^{-1} and more in aquatic environment could be dangerous for the survival of common fish, such as *G. holbrooki* and *P. parva* and related species.

With further characterization, therefore, LE seems to be a candidate biochemical biomarker of environmental pollution by FNT and other related compounds. Furthermore, with additional comparative studies, *P. parva* and *G. holbrooki* may be used as bioindicator for monitoring and assessment of environmental chemicals.

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