HEPATIC GLUTATHIONE S-TRANSFERASE ACTIVITY IN MOSQUITOFISH
( GAMBUSA AFINIS) AND TOPMOUTH GUDGEON
( PSEUDORASOBORA PARVA) EXPOSED TO FENITROTHION

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Abstract: Two common fish species, mosquitofish (Gambusia affinis) and topmouth gudgeon (Pseudorasobora 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 peprinyl 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 peprinyl 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

INTRODUCTION

Fenitrothion (0, 0-dimethyl 0-(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; Gandahusada 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 gairdneri/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 suppressalis 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 ± 1°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