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# Interconversion between Fenthion and Fenthion Sulfoxide in Goldfish, *Carassius auratus*

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**The *in vitro* metabolism of fenthion, an insecticide, was examined in fish, focusing on the interconversion between fenthion and its sulfoxide (fenthion sulfoxide). When fenthion was incubated with hepatopancreas microsomes of goldfish, *Carassius auratus*, in the presence of NADPH, the oxidized metabolite, fenthion sulfoxide was formed, but not fenthion sulfone. The oxidase activity was inhibited by SKF 525-A, and partly by α-naphthylthiourea. In contrast, fenthion sulfoxide was reduced back to fenthion by the hepatopancreas cytosol of goldfish in the presence of 2-hydroxypyrimidine, an electron donor for aldehyde oxidase. The activity was markedly inhibited by menadione, an inhibitor for aldehyde oxidase. The interconversion appears to be mediated mainly by the cytochrome P450 system and aldehyde oxidase.**

**Key words** —— fenthion, fenthion sulfoxide, goldfish, cytochrome P450 system, aldehyde oxidase

## INTRODUCTION

Fenthion [*O*,*O*-dimethyl-*O*-(4-methylmercapto)-3-methylphenylthiophosphate] is an organophosphorus pesticide which is widely used in the world. Organophosphorus pesticides remain in the environment after being applied, and undergo chemical, physical and biological changes.[1] Contamination by fenthion and its oxidation products has been reported in Japan.[2–4] It is also reported that fenthion accumulates in fish tissues.[5] In order to assess the possible risks associated with human exposure to the pesticide, it is essential to thoroughly elucidate the metabolism in mammalian species, and in marine and freshwater species. The metabolic fate of fenthion has not been extensively examined in mammalian species and fish.

Drug metabolism in mammalian species has been well investigated and comprehensive literature exists. However, metabolic pathways in fish species have received considerably less attention. Early studies of detoxification systems in fish indicated that fish do not have drug-metabolizing enzymes because lipid soluble xenobiotics were rapidly eliminated through the gills.[6] However, it was later demonstrated that fish have the ability to metabolize a variety of compounds.[7–11] These aspects of drug metabolism in fish were also reviewed in detail.[12–14] We also have reported that drug-reducing enzymes exist in fish, similarly to mammalian species, and the enzymes exhibited reductase activities towards a variety of compounds such as *p*,*p*'-DDT, 1-nitropyrene and veterinary antimicrobial nitrofurans.[15–19]

In the present study, the *in vitro* metabolism of fenthion and fenthion sulfoxide by the hepatopancreas of goldfish was examined. The hepatopancreas is an important tissue for drug metabolism in fish such as carp and goldfish, as well as in crustaceans.[20,21]

## MATERIALS AND METHODS

**Materials** —— Fenthion, fenthion sulfoxide and fenthion sulfone were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 2-Hydroxypyrimidine and menadione were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Goldfish (*C. auratus*, a kind of red crucian carp, 9—12 cm length, 12—15 g), which were commercially available, were used.

**Preparation of Hepatopancreas Microsomes and Cytosol** —— Fish hepatopancreases were homogenized in 3 volumes of 1.15% KCl. The homogenate was centrifuged for 20 min at 9000 × *g*, and the supernatant fraction was separated into microsomes and cytosol by centrifugation for 60 min at 105000 × *g*. The microsomes were washed by resuspension in 2 volumes of KCl solution and by resedimentation for

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60 min at 105000 × *g*.

**Assay Method** —— The incubation mixture consisted of 0.2 μmol of fenthion or fenthion sulfoxide, 1 μmol of an electron donor and hepatopancreas microsomes or cytosol in a final volume of 1 ml of 0.1 M K, Na-phosphate buffer (pH 7.4). In the case of the reduction of fenthion sulfoxide, the incubation was performed using a Thunberg tube under anaerobic conditions. The side arm contained an electron donor, and the body contained all other components. The tube was gassed for 3 min with nitrogen, evacuated with an aspirator for 5 min and again gassed with nitrogen. The reaction was started by mixing the components of the side arm and the body together, and continued for 30 min at 37℃. The mixture, after adding 10 nmol of benzophenone as an internal standard and two volumes of acetonitrile, was centrifuged, and an aliquot of the supernatant was subjected to analysis by high performance liquid chromatography (HPLC).

**HPLC** —— HPLC was performed in a Hitachi L-7110 high performance liquid chromatograph equipped with an ultraviolet absorption detector. The instrument was fitted with a 4 × 125 mm Inertsil ODS-3 (GL-Science, Tokyo, Japan). The mobile phase was acetonitrile-0.1 M $KH_2PO_4$ (6 : 4). The chromatograph was operated at a flow rate of 0.5 ml/min and at a wavelength of 254 nm. Elution times of fenthion sulfoxide, fenthion sulfone, benzophenone and fenthion were 5.8, 8.8, 15.2 and 25.4 min, respectively. The amounts of metabolites formed were determined from these peak areas.

**Measurement of Protein Content** —— Protein content was determined by the method of Lowry *et al.*,[22] with bovine serum albumin as a standard protein.

## RESULTS AND DISCUSSION

### Fenthion Oxidase Activity in Fish Hepatopancreas

When fenthion was incubated with hepatopancreas microsomes of goldfish in the presence of NADPH, as described in Materials and Methods, one metabolite, whose retention time corresponded to that of fenthion sulfoxide, was detected by HPLC. The mass spectrum of the metabolite isolated by HPLC showed a molecular ion at *m/z* 294 and fragment ions at *m/z* 279, 169, 153, 125 and 109. The mass spectrum of the metabolite was identical with that of an authentic sample of fenthion sulfoxide (Fig. 1A and B). However, when fenthion or fenthion sulfoxide was incubated with the microsomes, fenthion sulfone was not detected by HPLC.

The ability of the hepatopancreas microsomes of goldfish to oxidize fenthion to fenthion sulfoxide was examined under various conditions. The microsomes exhibited oxidase activity in the presence of NADPH or NADH. In the formation of fenthion sulfoxide, NADPH was more effective than NADH as an electron donor. The NADPH-linked activity of the microsomes was inhibited by SKF 525-A, an inhibitor of cytochrome P450, and partly by α-naphthylthiourea, an inhibitor of flavin-containing monooxygenase. The majority of the NADPH-linked activity was abolished by boiling the microsomes (Fig. 2). The results suggest that the oxidation of fenthion to fenthion sulfoxide is mainly catalyzed by cytochrome P450, but not by flavin-containing monooxygenase.

### Fenthion Sulfoxide Reductase Activity in Fish Hepatopancreas

When fenthion sulfoxide was incubated with hepatopancreas cytosol of goldfish in the presence of 2-hydroxypyrimidine, one metabolite corresponding to fenthion was detected by HPLC. The metabolite was identified by comparison with the mass spectrum of an authentic sample of fenthion (Fig. 1C and D). The cytosol exhibited significant reductase activity toward fenthion sulfoxide under anaerobic conditions, when 2-hydroxypyrimidine, an electron donor for aldehyde oxidase, was added. However, NADH and NADPH, electron donors of DT-diaphorase, and xanthine, an electron donor of xanthine oxidase, were not effective for the sulfoxide reduction. The 2-hydroxypyrimidine-dependent activity was markedly inhibited by menadione, an inhibitor of aldehyde oxidase. The activity was diminished under aerobic conditions, and abolished by boiling the cytosol (Fig. 3). The hepatopancreas microsomes did not exhibit reductase activity, even in the presence of NADPH or 2-hydroxypyrimidine. These results suggest that the reduction of fenthion sulfoxide to fenthion is catalyzed by aldehyde oxidase in the hepatopancreas cytosol of goldfish.

### Interconversion between Fenthion and Fenthion Sulfoxide in Fish

Several reports have indicated that sulfide

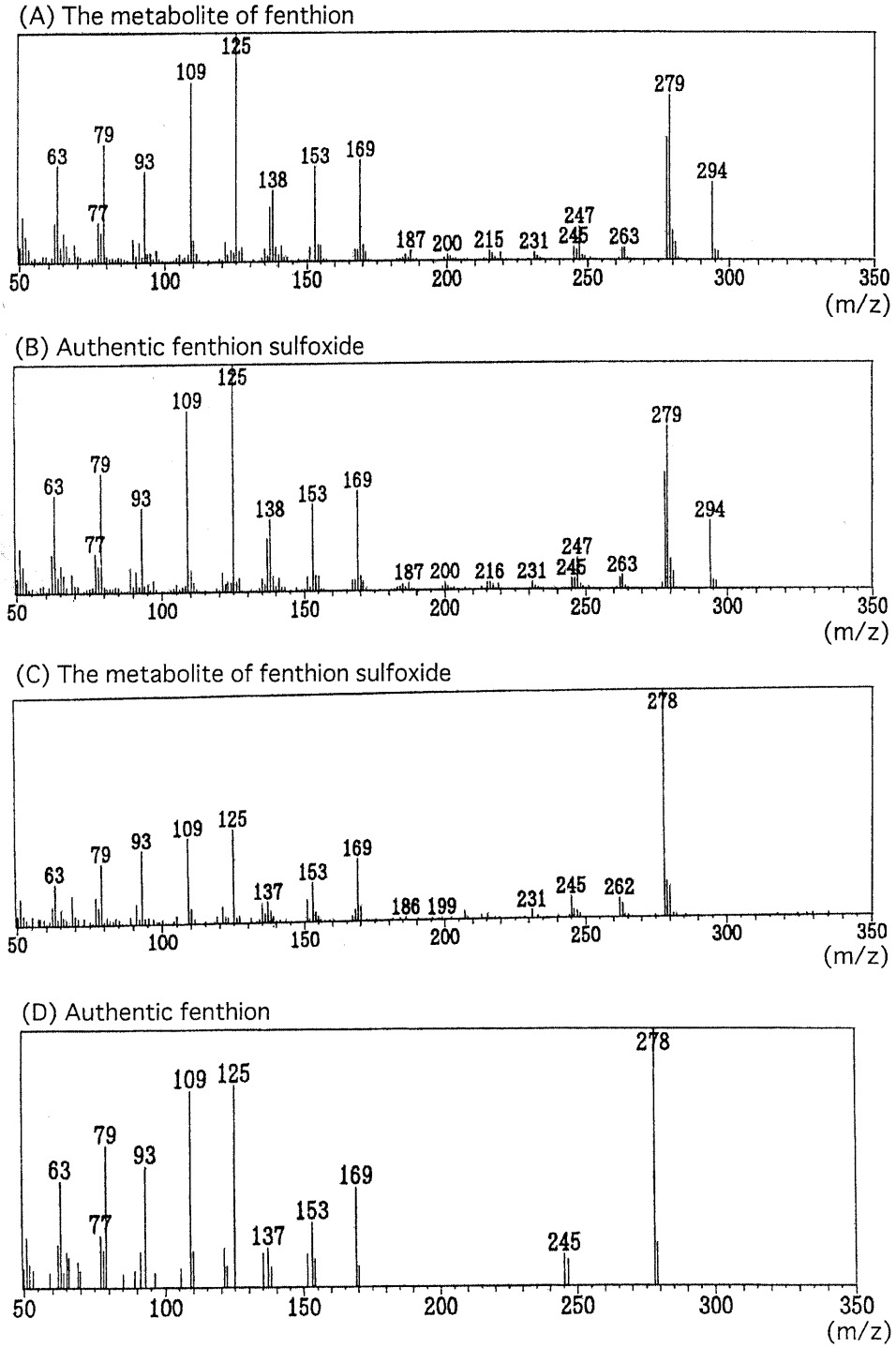


**Fig. 1.** Mass Spectra of the Metabolites of Fenthion and Fenthion Sulfoxide by Goldfish Hepatopancreas

compounds are metabolized by oxidation to corresponding sulfoxide compounds, and some sulfoxide compounds are reduced to sulfide compounds in mammalian species.[23,24] It is known that the oxidation of sulfide compounds is mediated by cytochrome P450 and flavin-containing monooxygenase.[25,26] Furthermore, we demonstrated that aldehyde oxidase is responsible for the reduction of sulfoxide in mammalian species.[27] However, the metabolism of sulfide and sulfoxide compounds has not been extensively examined in fish, except for the oxidation of thiobencarb, an herbicide, to the corresponding sulfoxide. Cashman and Olsen[28] reported that the

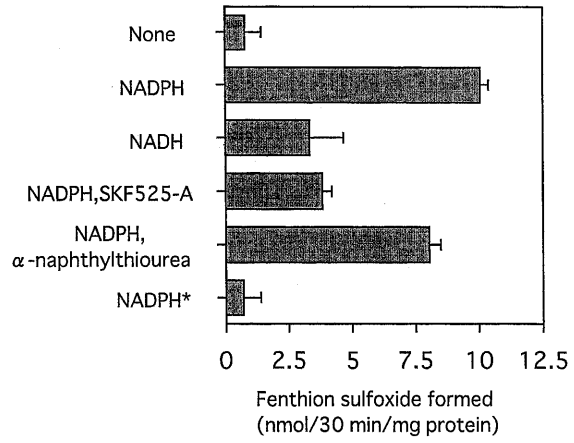


**Fig. 2.** Oxidation of Fenthion to Fenthion Sulfoxide by Hepatopancreas Microsomes of Goldfish

*Boiled microsomes were used in this incubation.

Each bar represents the mean±S.D. of three experiments. Incubation was performed at 37℃ for 30 min with 100 μl of hepatopancreas microsomes of goldfish (about 1.2 mg protein) under aerobic conditions. SKF 525-A or α-naphthylthiourea was added at the concentration of $1 \times 10^{-4}$ M. The fenthion sulfoxide formed was determined by HPLC. Other details are described in Materials and Methods.

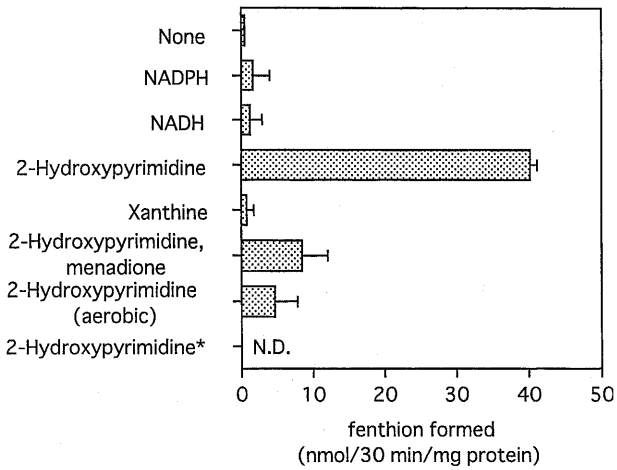


**Fig. 3.** Reduction of Fenthion Sulfoxide to Fenthion by Hepatopancreas Cytosol of Goldfish

*Boiled cytosol was used in this incubation, N.D.: not detected.

Each bar represents the mean±S.D. of three experiments. Incubation was performed at 37℃ for 30 min with 200 μl of hepatopancreas cytosol of goldfish (about 1.5 mg protein) under anaerobic conditions. Menadione was added at the concentration of $1 \times 10^{-4}$ M. The fenthion formed was determined by HPLC. Other details are described in Materials and Methods.

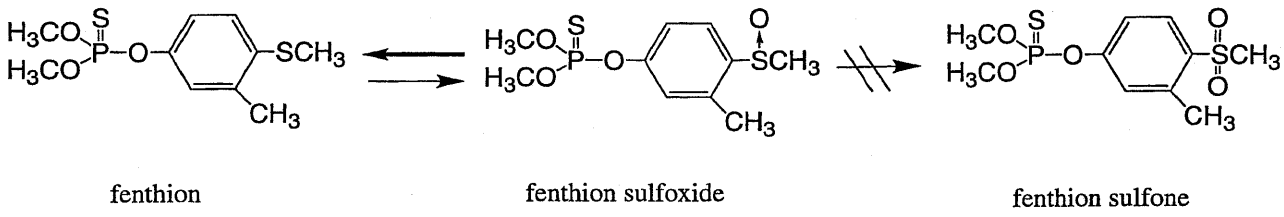


**Fig. 4.** Metabolic Fate of Fenthion in Goldfish

oxidation of thiobencarb was mainly catalyzed by flavin-containing monooxygenase of livers in Striped Bass, *Morone saxatilis*. In this study, we provided a metabolic pathway that fenthion was oxidized to fenthion sulfoxide, and the sulfoxide was reduced back to the parent fenthion. In contrast, fenthion sulfone was not formed from fenthion or fenthion sulfoxide in the fish (Fig. 4). The interconversion between fenthion and fenthion sulfoxide in goldfish is assumed to be mediated by the cytochrome P450 system and aldehyde oxidase in the hepatopancreas.

Tsuda *et al.*[5] reported that fenthion sulfoxide was detected in the body of killifish (*Oryzias latipes*) exposed to fenthion. However, it is possibile that fenthion was chemically oxidized to fenthion sulfoxide in the fenthion solution in which the fish was kept, because fenthion was easily oxidized in water.[4,29] The *in vitro* experiment conducted in this study provides evidence that fenthion can be enzymatically converted to fenthion sulfoxide in the fish body. When the activities of hepatopancreas microsomes and cytosol were expressed per g liver, fenthion oxidase and fenthion sulfoxide reductase activities were about 110 and 2400 nmol/30 min/g liver, respectively. This suggests that the fenthion sulfoxide formed is easily reduced back to the parent fenthion *in vivo*.

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