IN VITRO METABOLISM OF FENTHION AND FENTHION SULFOXIDE BY LIVER PREPARATIONS OF SEA BREAM, GOLDFISH, AND RATS

SHIGEYUKI KITAMURA, TOMOHARU SUZUKI, TOMOKO KADOTA, MAYUMI YOSHIDA, KOJI OHASHI, AND SHIGERU OHTA

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ABSTRACT:

The in vitro metabolism of fenthion and its sulfoxide (fenthion sulfoxide) in sea bream (Pagrus major) and goldfish (Carassius auratus) was investigated and compared with that in rats. Fenthion was oxidized to fenthion sulfoxide and the oxon derivative, but not to its sulfone, in the presence of NADPH by liver microsomes of sea bream, goldfish, and rats. These liver microsomal activities of the fish were lower than those of rats but were of the same order of magnitude. The NADPH-linked oxon- and sulfoxide-forming activities of liver microsomes of the fish and rats were inhibited by SKF 525-A, metyrapone, α-naphthoflavone, and carbon monoxide. The oxidizing activity to fenthion sulfoxide was also inhibited by α-naphthylioure. Several cytochrome P450 isofoms and flavin-containing monooxygenase 1 exhibited these oxidase activities. Fenthion sulfoxide was reduced to fenthion with liver cytosol of the fish and rats upon addition of 2-hydroxyprimidin, N1-methylnicotinamide, or butyraldehyde, each of which is an electron donor of aldehyde oxidase, under anaerobic conditions. The activity was inhibited by menadione, β-estradiol, and chlorpromazine, which are inhibitors of aldehyde oxidase. The activities in the fish livers were similar to those of rat liver. Aldehyde oxidase purified from the livers of sea bream and rats exhibited the reducing activity. Thus, fenthion and fenthion sulfoxide are interconvertible in fish and rats through the activities of cytochrome P450, flavin-containing monooxygenase, and aldehyde oxidase.

Fenthion1 [O,O-dimethyl-O-(4-methylmercapto)-3-methylphenylthiophosphate] is an organophosphorus pesticide, which is widely used throughout the world as a wide-spectrum insecticide for numerous crops (Roberts and Hutson, 1999). We demonstrated that fenthion does not have estrogenic activity, based on the finding that vitellogenin levels in male goldfish were not enhanced when the fish were kept in fenthion solution (Kitamura et al., 1999b). Fenthion was developed as a safe pesticide because it is not easily converted to the possibly highly toxic oxon derivative (fenthion oxon) in mammalian species. However, fenthion oxon was detected in olives and rice sprayed with fenthion (Fukuda et al., 1962; Cabras et al., 1993). Brady and Arthur (1961) reported that fenthion was metabolized to fenthion oxon in rats. Oxidation products of fenthion, including fenthion oxon, were isolated from the tank solution and the bodies of the fish. Some other in vivo metabolic studies of fenthion have been done to identify the metabolites in plants and mammals, as mentioned above. However, no in vitro study has been conducted to identify the metabolizing enzymes, fenthion oxidase and fenthion sulfoxide reductase, in fish and mammals.

Metabolism in fish has been extensively investigated, and drug-metabolizing enzymes acting on a variety of compounds, as in mammalian species, are found. Several reviews have appeared (Stege- man and Hahn, 1994; Buhler and Wang-Buhler, 1998; Livingstone and Goldfarb, 1998). The oxidative metabolism of various xenobiotics is catalyzed by cytochrome P450 (P450; especially CYP1A subfamily) in the liver, and this enzyme is induced by various chemicals, as in mammalian species (Stege- man and Kloepper-Sams, 1987; Livingstone and Goldfarb, 1998). The existence of flavin-containing monooxygenase (FMO) in fish liver was also reported (Schlenk, 1998). We also reported that fish contain drug-reducing enzymes that are responsible for the reduction of nitro compounds such as 1-nitropyrene, 2-nitrofluorene, and veterinary antimicrobial nitrofurans (Tatsumi et al., 1992; Kitamura and Tatsumi, 1997; Ueda et al., 1996). Recently, we examined the in vivo metabolism of fenthion in fish in detail (Kitamura et al., 2000). In that study, goldfish were administered fenthion, and two metabolites, fenthion sulfoxide and fenthion oxon, were isolated from the tank solution and the bodies of the fish. It was also shown that when goldfish were kept in fenthion sulfoxide solution, fenthion sulfoxide was reduced back to fenthion in the fish. Some other in vivo metabolic studies of fenthion have been done to identify the metabolites in plants and mammals, as mentioned above. However, no in vitro study has been conducted to identify the metabolizing enzymes, fenthion oxidase and fenthion sulfoxide reductase, in fish and mammals.

In the present study, in vitro metabolism of fenthion and fenthion sulfoxide by livers of sea bream and goldfish was examined and...
compared with that in rats. For this purpose, the hepatopancreas in goldfish was considered to be equivalent to the liver in sea bream and rats.

Materials and Methods

Materials. Fenthion, fenthion sulfoxide, and fenthion sulfone were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 2-Hydroxy-pyrimidine, benzaldehyde, and menadione were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Fenthion oxon was synthesized by the reported method (Kitamura et al., 2000). Goldfish (Carassius auratus, a kind of red crucian carp, mixed sex, 9–12 cm length, 12–15 g), which are commercially available, were used. Living marine fish, red sea bream, Pagrus major (mixed sex, body weight of 0.9–1.5 kg) were purchased from the shop. Male Sprague-Dawley rats (Slc:SD, 180 g) commercially available, were used. Living marine fish, red sea bream, goldfish was considered to be equivalent to the liver in sea bream and rats.

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Liver Preparations. Fish and rat livers (hepatopancreas in the case of goldfish) were dissected and homogenized in 3 volumes of 1.15% KCl containing 0.1 mM phenylmethanesulfonyl fluoride, 0.1 mM EDTA disodium salt (EDTA), and 0.1 mM EGTA. The homogenate was centrifuged for 20 min at 9000 g. The supernatant fraction was further separated into cytosol and microsomes by centrifugation for 1 hr at 105,000 g.

Purification of Aldehyde Oxidase from Livers of Sea Bream and Rats. Purification of aldehyde oxidase from sea bream liver was fractionated with ammonium sulfate after heat-treatment at 37°C (rats). In the incubation mixture consisted of 0.2 mol of fenthion or fenthion sulfoxide, 1 mol of an electron donor and liver microsomes, or cytosol in a final volume of 1 ml of 0.1 M K.Na-phosphate buffer (pH 7.4).

Materials and Methods

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Assay of Aldehyde Oxidase Activity. The assay of aldehyde oxidase activity was performed using benzaldehyde or p-dimethylaminominaldehyde (DMAC) as a substrate in phosphate buffer (Johns, 1967; Stell et al., 1980). The assay was performed using benzaldehyde or aldehyde oxidase activity was performed by the oxidation of benzaldehyde. The overall purification of aldehyde oxidase from sea bream livers was shown in Table 1. Further purification could not be conducted because the fish enzyme was very labile.

Assay Methods for Fenthion Oxidase and Fenthion Sulfoxide Reductase Activities. The incubation mixture consisted of 0.2 μmol of fenthion or fenthion sulfoxide, 1 μmol of an electron donor and liver microsomes, or cytosol in a final volume of 1 ml of 0.1 M K.Na-phosphate buffer (pH 7.4). The incubation was continued for 30 min at 30°C (fish) or 37°C (rats). In the case of 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. The mixture, after adding 10 nmol of benzophenone as an internal standard and two volumes of acetonitrile, was centrifuged, and then an aliquot of the supernatant was subjected to analysis by high-performance liquid chromatography (HPLC).

HPLC and Thin-Layer Chromatography (TLC). HPLC was performed in a Hitachi L-7110 high-performance liquid chromatograph (Tokyo, Japan) equipped with an ultraviolet absorption detector. The instrument was fitted with a 4 × 125 mm Inertsil ODS-3 column (GL-Science, Tokyo, Japan). The mobile phase was acetonitrile/0.1 M KH₂PO₄ (6:4, v/v, pH 5.4). The chromatogram was operated at a flow rate of 0.5 ml/min and at a wavelength of 254 nm.

Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS was performed using a Shimadzu GC-17AQP-5000 (Shimadzu, Kyoto, Japan) in the electron impact mode. A DB-5 fused-silica capillary column (30 m × 0.25 mm i.d.; J & W Scientific Inc., Folsom, CA) was used. The column temperature was held at 100°C for 5 min, then increased at a rate of 10°C/min to 200°C. One microliter of sample was injected into the injection port at 220°C. Splitless injection was used. The retention times of fenthion sulfoxide, fenthion oxon, and fenthion sulfone were 5.8, 7.9, 8.9, 15.0, and 25.1 min, respectively. The amounts of metabolites formed were determined from these peak areas. In some experiments, a Beckman 168 photodiode array UV detector (Beckman Coulter, Inc., Fullerton, CA) was fitted to the HPLC unit to identify the metabolites of fenthion and fenthion sulfoxide.

Silica gel plates (Kieselgel 60 GF254, 0.1 mm thick; Merck, Darmstadt, Germany) were developed in benzene:acetic acid (9:1, v/v). Spots were visualized under UV light (254 nm). RF values of authentic fenthion, fenthion sulfoxide, and fenthion sulfone were 0.62, 0.41, 0.17, and 0.07, respectively.

Results

Metabolism of Fenthion by Liver Microsomes of Sea Bream, Goldfish, and Rats. When fenthion was incubated with liver microsomes of sea bream, goldfish, and rats in the presence of NADPH, two metabolites, which were designated fenthion metabolite 1 and 2, were detected in the HPLC chromatograms of the extracts of the incubation mixtures (Fig. 1). However, fenthion sulfoxide was not detected. The two metabolites were not detected in the control chromatogram of the mixture incubated without fenthion. The mass spectra of fenthion metabolite 1 showed the molecular ion at m/z 294 and the fragment ion at m/z 279. The mass spectra of fenthion metabolite 2 also gave the molecular ion at m/z 262 and the fragment ion at m/z 247. The UV spectra of fenthion metabolite 1 revealed the absorption maxima at 243 and 280 nm. The UV spectra of fenthion metabolite 2 also gave the...
The mass and UV spectra of fenthion metabolite 1 and 2 were identical to those of authentic samples of fenthion sulfoxide and fenthion oxon, respectively. The HPLC and TLC behaviors of these metabolites were also identical with those of authentic samples.

**Fenthion Oxidase Activity in Fish Liver Microsomes.** The time course of the formation of these metabolites in the presence of NADPH with 1.5 mg of protein of microsomes of these fish was linear up to 60 min. The dependence on amount of liver microsomes was linear up to 10 mg of protein. The oxidase activities increased with temperature at 10 to 30°C and then remained unchanged up to 40°C (Fig. 2). In this study, fenthion was incubated with 1.0 to 1.5 mg of protein for 30 min at 30°C. Table 2 shows NADPH-linked and NADH-linked fenthion oxidase activities of the liver microsomes of sea bream and goldfish. In each case, both fenthion sulfoxide and fenthion oxon were formed from fenthion. In the formation of both metabolites, NADPH was more effective than NADH as an electron donor. The NADPH-linked activity of goldfish liver was similar to that of sea bream. However, NADH-linked activity in goldfish was higher than that of sea bream. The formation of fenthion sulfoxide was higher than that of fenthion oxon in both fish (Table 2).

**Inhibition of the Oxidase Activity in Fish Liver.** The effects of several chemicals on the liver microsomal NADPH-linked fenthion oxidase activity in the fish were examined. The NADPH-linked oxidizing activity in liver microsomes of sea bream and goldfish toward fenthion to oxon derivative was inhibited by SKF 525-A, metyrapone, α-naphthoflavone, and carbon monoxide, indicating that P450 is involved in the oxidation of the fish. The oxidizing activity of fenthion to fenthion sulfoxide was inhibited by SKF 525-A, metyrapone, α-naphthoflavone, carbon monoxide, and α-naphthylthiourhea (Fig. 3). This suggests that the oxidizing activity to the sulfoxide is due to both P450 and FMO in the fish.

**Fig. 1.** HPLC of the in vitro metabolites of fenthion by liver microsomes of sea bream, goldfish, and rats.

The extract of the incubation mixture of fenthion with sea bream liver microsomes (A), goldfish liver microsomes (B) and rat liver microsomes (C). The absorbance unit (full scale = 0.016 AU) was employed at 254 nm. Fenthion metabolite 1 was detected at 5.8 to 5.9 min. Fenthion metabolite 2 was detected at 7.8 to 8.0 min.
Reduction of Fenthion Sulfoxide by Fish Liver Preparations.

When fenthion sulfoxide was incubated with liver cytosol of sea bream or goldfish in the presence of 2-hydroxypyrimidine, a metabolite of fenthion sulfoxide was detected by HPLC. The metabolite was identified as fenthion by comparison of its mass and UV spectra, and the metabolite was detected by HPLC. The metabolite was identified as fenthion by comparison of its mass and UV spectra, and the metabolite was detected by HPLC.

The metabolism of fenthion and fenthion sulfoxide by a rat liver preparation was examined in the same way as for livers of sea bream and goldfish. When fenthion was incubated with liver microsomes in the presence of NADPH, fenthion sulfoxide and fenthion oxon were detected in the HPLC chromatogram of the extract of the incubation mixture (Fig. 1). The oxidase activities leading to fenthion sulfoxide and fenthion oxon were determined using HPLC. Other details are described under Materials and Methods. In A, the time course of the activity; B, dependence on amount of liver microsomes; and C, incubation temperature dependence. In A, a mixture containing 0.2 μmol of fenthion and 0.1 ml of liver microsomes from sea bream in 0.1 M phosphate buffer (pH 7.4) was incubated with 1 μmol of NADPH at 30°C for 10, 20, 30, 40, 50, or 60 min. The oxidation products formed were determined using HPLC. Other details are described under Materials and Methods.

Fenthion Sulfoxide Fenthion Oxon Fenthion Sulfoxide Fenthion Oxon

<table>
<thead>
<tr>
<th>Addition</th>
<th>Sea Bream</th>
<th>Goldfish</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.18 ± 0.09</td>
<td>N.D.</td>
</tr>
<tr>
<td>NADH</td>
<td>0.39 ± 0.16</td>
<td>0.59 ± 0.17</td>
</tr>
<tr>
<td>NADPH</td>
<td>9.29 ± 1.89</td>
<td>2.89 ± 0.40</td>
</tr>
</tbody>
</table>

N.D., not detected.

Sulfoxide Reduction by Aldehyde Oxidase. The sulfoxide reduction of fenthion sulfoxide by aldehyde oxidase from liver of sea bream was examined. Aldehyde oxidase from sea bream liver exhibited a significant sulfoxide reductase activity (21.7 nmol/min/mg of protein) in the presence of 2-hydroxypyrimidine under anaerobic conditions. The specific activity of sulfoxide reductase in purification steps increased according as that of aldehyde oxidase. The sulfoxide reductase activity was inhibited by the addition of menadione and was markedly diminished under aerobic conditions. These activities were abolished by the boiling the enzyme (data not shown). The results support the view that liver aldehyde oxidase catalyzes the reduction of the sulfoxide in the fish.

Metabolism of Fenthion and Fenthion Sulfoxide by Rat Liver Preparation. The metabolism of fenthion and fenthion sulfoxide by a rat liver preparation was examined in the same way as for livers of sea bream and goldfish. When fenthion was incubated with liver microsomes in the presence of NADPH, fenthion sulfoxide and fenthion oxon were detected in the HPLC chromatogram of the extract of the incubation mixture (Fig. 1). The oxidase activities leading to fenthion sulfoxide and fenthion oxon were NADPH-dependent and were partially inhibited by α-naphthoflavone, quinidine, and SKF 525-A. The oxidizing activity to fenthion sulfoxide was also inhibited by α-naphthylthioleu, as in the case of fish livers. These activities of rats were higher than those of livers of sea bream and goldfish. However, the facts suggest that aldehyde oxidase in the cytosol fraction, but not P450, functions as fenthion sulfoxide reductase in fish livers. The cytosolic activity was much higher than the NADPH-dependent microsomal oxidase activity.

Figure 2. Oxidation of fenthion to fenthion sulfoxide and fenthion oxon with liver microsomes of sea bream.

A. Time course of the activity; B, dependence on amount of liver microsomes; and C, incubation temperature dependence. In A, a mixture containing 0.2 μmol of fenthion and 0.1 ml of liver microsomes from sea bream in 0.1 M phosphate buffer (pH 7.4) was incubated with 1 μmol of NADPH at 30°C for 10, 20, 30, 40, 50, or 60 min. The oxidation products formed were determined using HPLC. Other details are described under Materials and Methods.

TABLE 2

Oxidation of fenthion to fenthion sulfoxide and fenthion oxon by liver microsomes of sea bream and goldfish

<table>
<thead>
<tr>
<th>Addition</th>
<th>Sea Bream</th>
<th>Goldfish</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.18 ± 0.09</td>
<td>N.D.</td>
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<tr>
<td>NADH</td>
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<td>0.59 ± 0.17</td>
</tr>
<tr>
<td>NADPH</td>
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<td>2.89 ± 0.40</td>
</tr>
</tbody>
</table>

N.D., not detected.

Reduction of Fenthion Sulfoxide by Fish Liver Preparations. When fenthion sulfoxide was incubated with liver cytosol of sea bream or goldfish in the presence of 2-hydroxypyrimidine, a metabolite of fenthion sulfoxide was detected by HPLC. The metabolite was identified as fenthion by comparison of its mass and UV spectra, and TLC and HPLC behaviors with those of an authentic sample as described under Materials and Methods. In A, the same procedure as in (A) was used except that the amount of liver microsomes from sea bream was varied, and the incubation time was 30 min. In C, the same procedure as in (A) was used except that the incubation temperature was varied, and the incubation time was 30 min.

Table 2 shows the fenthion sulfoxide formed when 2-hydroxypyrimidine, a metabolite of fenthion sulfoxide in the presence of NADPH, fenthion sulfoxide and fenthion oxon were detected by HPLC. Other details are described under Materials and Methods.

Each value represents the mean ± S.D. of four fish. Fenthion was incubated at 30°C for 30 min with liver microsomes (2–4 mg protein) in the presence of a cofactor. The oxidation products were determined by HPLC. Other details are described under Materials and Methods.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Sea Bream</th>
<th>Goldfish</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.18 ± 0.09</td>
<td>N.D.</td>
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</table>

N.D., not detected.

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Table 2 shows the fenthion sulfoxide formed when 2-hydroxypyrimidine, a metabolite of fenthion sulfoxide in the presence of NADPH, fenthion sulfoxide and fenthion oxon were detected by HPLC. Other details are described under Materials and Methods.
order of the activities was the same as observed in the fish (Fig. 5A). The P450 isoforms of rats, CYP1A1, 2B1, 2C6, 2D1, and 3A1 oxidized fenthion to fenthion sulfoxide with activities of 34.3, 0.2, 2.8, 1.9, and 4.0 nmol/min/mg of protein, respectively, and CYP1A1, 1A2, 2B1, 2C6, 2D1, and 3A1 oxidized fenthion to fenthion oxon with activities of 0.53, 0.15, 0.21, 0.18, 0.16, and 0.24 nmol/min/mg of protein, respectively. In contrast, FMO 1 exhibited marked sulfide oxidase activity at the rate of 62.4 nmol/min/mg of protein. When fenthion sulfoxide was incubated with the liver cytosol in the presence of 2-hydroxypyrimidine or butyraldehyde under anaerobic conditions, fenthion sulfoxide was also reduced to fenthion with liver cytosol of rats. However, xanthine, NADH, or NADPH was not effective. The cytosolic reductase activity was inhibited by menadione at the concentration of $1 \times 10^{-3}$ M. The fenthion formed was determined by HPLC. Other details are described under Materials and Methods.

![Fig. 3](https://example.com/fig3.png)

**FIG. 3.** Influence of various compounds on the oxidase activities of fenthion to fenthion sulfoxide and fenthion oxon by liver microsomes of sea bream and goldfish.

Each bar represents the mean ± S.D. of four fish. Incubation was performed at 30°C for 30 min with 100 µl of liver microsomes of sea bream or goldfish (about 1.2 mg of protein) under aerobic conditions. SKF 525-A, metyrapone, α-naphthoflavone, or α-naphthylthiourea was added at the concentration of $1 \times 10^{-4}$ M. Fenthion sulfoxide and fenthion oxon formed were determined by HPLC. Other details are described under Materials and Methods. *,$ p < 0.05$; **,$ p < 0.01$ compared with control.

![Fig. 4](https://example.com/fig4.png)

**TABLE 3**

**Sulfoxide reduction of fenthion sulfoxide by liver cytosol of sea bream and goldfish**

Each value represents the mean ± S.D. of four fish. Fenthion sulfoxide was incubated at 30°C for 30 min with liver cytosol in the presence of a cofactor under anaerobic conditions. Fenthion formed was determined by HPLC. Other details are described under Materials and Methods. None, ND, not detected.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Sea Bream Fenthion Formed (nmol/30 min/mg protein)</th>
<th>Goldfish Fenthion Formed (nmol/30 min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.43 ± 0.31</td>
<td>0.49 ± 0.21</td>
</tr>
<tr>
<td>2-Hydroxy pyrimidine</td>
<td>26.65 ± 1.16</td>
<td>40.21 ± 0.94</td>
</tr>
<tr>
<td>N1-Methyl nicotinamide</td>
<td>14.47 ± 1.20</td>
<td>27.82 ± 3.40</td>
</tr>
<tr>
<td>Benzoaldehyde</td>
<td>8.27 ± 0.94</td>
<td>22.24 ± 3.44</td>
</tr>
<tr>
<td>Xanthine</td>
<td>0.34 ± 0.24</td>
<td>0.73 ± 0.10</td>
</tr>
<tr>
<td>NADH</td>
<td>0.71 ± 0.30</td>
<td>1.21 ± 0.17</td>
</tr>
<tr>
<td>NADPH</td>
<td>1.02 ± 0.38</td>
<td>1.64 ± 0.23</td>
</tr>
</tbody>
</table>

![Fig. 5A](https://example.com/fig5a.png)

**FIG. 5A.** Influence of various compounds on the reduction of fenthion sulfoxide to fenthion by liver microsomes of sea bream.

![Fig. 5B](https://example.com/fig5b.png)

**FIG. 5B.** Influence of various compounds on the reduction of fenthion sulfoxide to fenthion by liver microsomes of goldfish.

Each bar represents the mean ± S.D. of four fish. Incubation was performed at 30°C for 30 min with 100 µl of liver microsomes of sea bream (about 1.4 mg of protein) under anaerobic conditions. Inhibitors were added at the concentration of $1 \times 10^{-4}$ M. The fenthion formed was determined by HPLC. Other details are described under Materials and Methods. *,$ p < 0.05$; **,$ p < 0.01$ compared with control.
aldehyde oxidase in rat liver cytosol was about the same as those of the fish livers. The reductase activity was higher than the microsomal oxidase activity, as in the case of the fish.

Substrate Specificity of P450 and Aldehyde Oxidase in Livers of Sea Bream and Rats. Substrate specificity of P450 and aldehyde oxidase in sea bream liver was examined using general substrates for these enzymes. In the case of the microsomal oxidase activity, significant EROD and MROD, but not PROD, activities were observed in sea bream liver microsomes. EROD and MROD activities of the fish were lower than those of rat liver. In fish, the microsomal oxidase activity based on CYP1A subfamily is usually reported (Stegeman and Hahn, 1994; Livingstone and Goldfarb, 1998). However, the activity in sea bream liver is remarkable. In contrast, high DMAC and benzaldehyde oxidase activities were observed in liver cytosol of sea bream. The activities were comparable with those of rats (Table 4). It is noteworthy that significant aldehyde oxidase activity was observed in the fish.

From these results, we concluded that fenthion was oxidized to fenthion sulfoxide and fenthion oxon by P450 and FMO, and fenthion sulfoxide was easily reduced to fenthion by aldehyde oxidase, so that these metabolites are interconverted in fish in a similar manner to that in mammalian species.

Discussion

Parathion and malathion are oxidatively desulfurated to their oxon derivatives in insects and mammalian species (Neal et al., 1977). The metabolites showed much higher anticholinesterase activity than the parent pesticides (Eto, 1979); paraoxon has two hundred-fold higher activity than parathion. Fenthion was also easily converted to fenthion oxon by mixed function oxidase in fish and rats, as shown in this study. The conversion of fenthion to the oxon in fish is of considerable toxicological significance. Furthermore, Lacorte et al. (1997) demonstrated that fenthion was converted to fenthion oxon in the environ-

FIG. 5. Oxidation of fenthion and reduction of fenthion sulfoxide with rat liver preparations.

A, liver microsomal oxidase activity; B, liver cytosolic reductase activity. Each bar represents the mean ± S.D. of four rats. Incubation was performed at 37°C for 30 min with liver microsomes or cytosol of rats. In the case of fenthion sulfoxide reduction, incubation was conducted under anaerobic conditions. Inhibitors were added at the concentration of 1 × 10^{-4} M. The products formed were determined by HPLC. Other details are described under Materials and Methods. *p < 0.05, **p < 0.01 compared with control.

![Diagram](image-url)

TABLE 4

<table>
<thead>
<tr>
<th>Activity</th>
<th>Sea Bream</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>EROD (nmol/min/mg protein)</td>
<td>27.4 ± 3.37</td>
<td>100.34 ± 8.61</td>
</tr>
<tr>
<td>MROD (nmol/min/mg protein)</td>
<td>9.44 ± 1.26</td>
<td>48.25 ± 5.22</td>
</tr>
<tr>
<td>PROD (nmol/min/mg protein)</td>
<td>0.28 ± 0.03</td>
<td>18.74 ± 1.04</td>
</tr>
<tr>
<td>Benzaldehyde oxidase (nmol/min/mg protein)</td>
<td>4.35 ± 0.53</td>
<td>5.10 ± 0.29</td>
</tr>
<tr>
<td>DMAC oxidase (nmol/min/mg protein)</td>
<td>7.30 ± 0.55</td>
<td>4.83 ± 0.33</td>
</tr>
</tbody>
</table>

Further study on the metabolism of organophosphorus compounds to the oxon derivatives in fish should be conducted. It is also necessary to evaluate further the toxicity of fenthion oxon in fish and mammalian species.

Several reports have indicated that sulfide compounds are mainly metabolized by oxidation to the corresponding sulfoxide and sulfone compounds, and some sulfoxide compounds are reduced to the sulfide compounds in mammalian species (McLane et al., 1983; Mitchell and Waring, 1986; Benoit et al., 1992). It is known that the oxidation is mediated by P450 and FMO (Jakoby and Ziegler, 1990), and aldehyde oxidase catalyzes the reduction of sulfoxides such as sulindac, diphenyl sulfoxide, and phenothiazine sulfoxide in mammalian species (Tatsumi et al., 1983). These enzymes are also known to exist in many species of fish (Krenitsky et al., 1974; Pohl et al., 1974; Bend and James, 1978; Livingstone, 1998). However, the metabolism of sulfide and sulfoxide compounds has not been extensively examined in fish. Cashman et al. (1990) reported that the oxidation of thiobencarb, a
herbicide, was mainly catalyzed by FMO in the liver of striped bass, *Morone saxatilis*. Schlenk and Buhler (1991) also demonstrated a role of FMO in the oxidation of aldicarb in rainbow trout. In this study, we established that fenthion was oxidized to fenthion sulfoxide, and the sulfoxide was reduced back to fenthion. Fenthion sulfoxide was not oxidized to fenthion sulfone in fish and rats (Fig. 6). We previously demonstrated that fenthion and fenthion sulfoxide were interconverted in goldfish (Kitamura et al., 1999a; 2000). In this current study, we demonstrated that these reactions are mediated by P450, FMO, and aldehyde oxidase in the liver.

Aldehyde oxidase is known to be distributed widely in fish (Krenitsky et al., 1974). However, no information was available concerning fish liver enzymes responsible for the reduction of sulfoxide compounds. The present study suggests for the first time that in fish species as well as mammalian species, liver cytosolic aldehyde oxidase is mainly involved in the sulfoxide reduction by liver preparations. When the reductase activity of fenthion sulfoxide to fenthion was compared with fenthion oxidase activity, the reductase activity was about 4-fold higher than the oxidase activity. This indicates that fenthion sulfoxide formed would be easily reduced back to fenthion.

We reported that fenthion was accumulated at high level in the body of goldfish bathed in a solution of fenthion or fenthion sulfoxide, whereas accumulation of fenthion sulfoxide was much less (Kitamura et al., 2000). Tsuda et al. (1996) also reported that fenthion was accumulated at high concentration in the body of killifish exposed to fenthion. This high activity of the reverse reaction may be a reason for the accumulation of fenthion at a high level.

To our knowledge, the metabolism of organophosphorus insecticides have not been extensively studied in aquatic species, either in vivo or in vitro. The present study has provided evidence that oxidation of fenthion and reduction of fenthion sulfoxide are mediated by similar enzymes to mammalian drug-metabolizing enzymes. Fish liver microsomes containing P450 and FMO exhibited oxidase activities converting fenthion to fenthion sulfoxide and fenthion oxon. These oxidase activities were slightly lower than those of rat liver. However, the sulfoxide reductase activity of rat liver cytosol was not very different with that in fish livers. As fish can come into contact with environmental chemicals, further examination of the metabolism of these chemicals in fish is important.

**References**


