

Antiandrogenic Activity and Metabolism of the Organophosphorus Pesticide Fenthion and Related Compounds

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We investigated the endocrine-disrupting actions of the organophosphorus pesticide fenthion and related compounds and the influence of metabolic transformation on the activities of these compounds. Fenthion acted as an antagonist of the androgenic activity of dihydrotestosterone (10^{-7} M) in the concentration range of 10^{-6} – 10^{-4} M in an androgen-responsive element–luciferase reporter-responsive assay using NIH3T3 cells. The antiandrogenic activity of fenthion was similar in magnitude to that of flutamide. Fenthion also tested positive in the Hershberger assay using castrated male rats. Marked estrogenic and antiestrogenic activities of fenthion and related compounds were not observed in MCF-7 cells. When fenthion was incubated with rat liver microsomes in the presence of NADPH, the antiandrogenic activity markedly decreased, and fenthion sulfoxide was detected as a major metabolite. The oxidase activity toward fenthion was exhibited by cytochrome P450 and flavin-containing monooxygenase. Fenthion sulfoxide was negative in the screening test for antiandrogens, as was fenthion sulfone. However, when fenthion sulfoxide was incubated with liver cytosol in the presence of 2-hydroxypyrimidine, an electron donor of aldehyde oxidase, the extract of the incubation mixture exhibited antiandrogenic activity. In this case, fenthion was detected as a major metabolite of the sulfoxide. Metabolic interconversion between fenthion and fenthion sulfoxide in the body seems to maintain the antiandrogenic activity. **Key words:** aldehyde oxidase, antiandrogenic activity, cytochrome P450, endocrine disruption, fenthion, fenthion sulfoxide. *Environ Health Perspect* 111:503–508 (2003). doi:10.1289/ehp.5917 available via <http://dx.doi.org/> [Online 15 January 2003]

Fenthion [*O,O*-dimethyl-*O*-(4-methylmercapto)-3-methylphenylthio-phosphate] is an organophosphorus pesticide widely used throughout the world as a broad-spectrum insecticide for numerous crops and also as an ectoparasiticide for farm animals (Roberts and Hutson 1999). Fenthion inhibits acetylcholine esterase and is thought to be a safe pesticide because it is not easily converted to the possibly highly toxic oxon derivative in animal species. However, toxic effects such as ultrastructural change in testes of gobiid fish, reduction in larval production in sand fiddler crabs, chronic toxicity in hens, acute toxicity in birds, reptiles, and fish, and decreases in muscarinic receptor function in rat retina have been reported (Mullie et al. 1999; Schoor et al. 2000; Tandon et al. 1994; Tuler and Bowen 1999; Zutshi and Murthy 2001). Furthermore, it has been reported that fenthion and its oxidation products, fenthion sulfoxide and fenthion oxon, contaminate olives, mosquitos, fish, and the environment (Cavanna and Molinari 1998; Fukushima 1991; Kitamura et al. 2000; Lacorte et al. 1997; Meyer et al. 1998; Peiris and Hemingway 1996; Pereira and Hostettler 1993; Prasad et al. 1997; Tsuda et al. 1996). The relationship between the toxicity and metabolism of fenthion has been examined in human neuroblastoma cell lines, birds, and fish, and either an increase or a decrease in toxicity as a result of metabolism was found (Cova et al. 1995; Kitamura et al. 2000; Roux et al. 1995).

Many environmental xenobiotics exert hormonal effects at the cellular and organismal

levels. These compounds can mimic the biologic activity of sex hormones and thyroid hormone and are called “endocrine-disrupting chemicals.” The estrogenic chemicals include chlorinated insecticides such as kepone, *o,p'*-DDT, dieldrin, methoxychlor, and nonchlorinated compounds used in the plastics and detergent industries, such as alkylphenols and bisphenol A (Andersen et al. 1999; Gaido et al. 1997; Soto et al. 1994). 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. 1999). However, the estrogenic activity of fenthion has not been examined in mammalian cell lines. Further, the antiandrogenic activity of fenthion has not been examined, even though some pesticides are known to have antiandrogenic properties. The pesticide vinclozolin is known to exhibit antiandrogenic activity after metabolic activation (Gray et al. 1994; Kelce et al. 1994). Flutamide, a diuretic agent, must also be metabolically activated before it can exert effects (Wong et al. 1995). The widespread use of these pesticides and fungicides in the environment may generate antiandrogens by metabolic activation and may alter male sexual development and reproductive processes (Kelce and Wilson 1997).

To clarify the endocrine-disrupting action of fenthion and related compounds, we examined their androgenic and antiandrogenic activities in this study using androgen-responsive

element (ARE)-luciferase reporter-transfected mouse fibroblast cell line NIH3T3 *in vitro* and the Hershberger assay *in vivo*. We also examined the estrogenic and antiestrogenic activities using estrogen-responsive element (ERE)-luciferase reporter-transfected human breast cancer cell line MCF-7. The influence of the metabolism of fenthion on these endocrine-disrupting activities was investigated using rat liver preparations.

Materials and Methods

Chemicals. Fenthion (98% pure), fenthion sulfoxide (99%), fenthion sulfone (99%), fensulfothion (98%), fenitrothion (98%), trichlorfon (98%), malathion (99%), and EPN (ethyl *p*-nitrophenyl benzenethiophosphonate, 98%) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Dihydrotestosterone (DHT), 2-hydroxypyrimidine, and menadione were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). SKF 525-A, α -naphthoflavone, 17β -estradiol (E_2), and α -naphthylthiourea were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Animals. Male rats were housed at 22°C with a 12-hr light/dark cycle, with free access to tap water and a standard pellet diet MM-3 (Funabashi Farm, Funabashi, Japan). All experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* of Hiroshima University.

Cell culture. NIH3T3 and MCF-7 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma) plus 5% calf serum (Gibco/Invitrogen Corp., Carlsbad, CA, USA) and DMEM plus 5% fetal bovine serum (Gibco/Invitrogen), respectively, containing penicillin and streptomycin. For assays, the medium was changed to phenol red-free DMEM (Sigma) containing the same antibiotics along with dextran-charcoal-treated calf serum or fetal bovine serum for a week.

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Plasmids. The p(ARE)₂-luc plasmid was constructed with pGL3-enhancer (Promega Co., Madison, WI, USA) by inserting a synthetic double-strand oligo-DNA containing the consensus androgen-responsive element 5'-CATAGTACGTGATGTTCTAGGCC-TAGTACGTGATGTTCTCTATAC (with Sac I/Xho I ends). For pSG5-hAR, human androgen receptor cDNA was amplified from human prostate cDNA by polymerase chain reaction with LA-Taq (Takara) and introduced into pCR2.1 with a TA Cloning Kit (Invitrogen). The EcoRI fragment was then inserted into the EcoRI site in the pSG5 plasmid. The p(ERE)₃-SV40-luc plasmid, which contains three tandem copies of the consensus ERE, was a gift from M. Kudoh of Yamanouchi Pharmaceutical Co. (Tsukuba, Japan). pRL-CMV Renilla luciferase derived with the CMV enhancer/promoter (Promega Co., Madison, WI, USA) was used as the internal control.

Liver preparations. Male rats (160–190 g, Slc:Wistar/ST strain) obtained from Japan SLC, Inc. (Shizuoka, Japan) were used. Livers were excised from exsanguinated rats and immediately perfused with 1.15% KCl. The livers were homogenized in 4 volumes of 1.15% KCl. The homogenate was centrifuged for 20 min at 9,000 × g. We further separated the supernatant fraction into cytosol and microsomes by centrifugation for 60 min at 105,000 × g. The microsomes were washed by resuspension in 2 volumes of the KCl solution and resedimentation for 60 min at 105,000 × g. The washed microsomes were suspended in a volume of 1.15% KCl equivalent to the original liver weight. The microsomal fraction was stored at –80°C before use.

Purification of aldehyde oxidase from livers of rats. We purified aldehyde oxidase from rat livers according to the reported method (Sugihara et al. 1995). The cytosol obtained from 14 g of rat liver was used for the purification. The active fractions showed a single band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Assay of androgenic and antiandrogenic activities in vitro. We measured androgenic activity using the ARE-luciferase reporter assay in NIH3T3 cells. Cells were plated at 5 × 10⁴/well in 24-well plates and transiently transfected with 0.1 µg of pSG5-hAR, 0.5 µg of p(ARE)₂-luc with TransFast transfection reagent containing a synthetic cationic lipid (Promega), following the supplied protocol. The weight ratio of TransFast reagent to DNA was 1:1. After 24-hr incubation with samples, cells were harvested with 50 µL of cell lysis buffer (Promega) and the firefly and renilla luciferase activities were determined with the dual luciferase assay kit (Promega) by measuring luminescence with a Wallac MicroBeta scintillation counter (PerkinElmer Life

Sciences, Boston, MA, USA). Firefly luciferase reporter activity was normalized to renilla luciferase activity from pRL-CMV. To assess antiandrogenic activity, the inhibitory effects of test compounds on the androgenic activity of DHT at the concentrations of 10⁻¹¹ and 10⁻¹⁰ M was examined. Because androgen, progesterone, and glucocorticoid receptors share the same hormone-responsive element, the reporter responses to progesterone and glucocorticoid were examined and confirmed to be negative (data not shown). Data are expressed as the means ± SD of four experiments using independent transfected cells.

In the assay of the antiandrogenic activity of the metabolites produced from fenthion or fenthion sulfoxide, substrates (0.1 µmol) were incubated with 0.1 mL rat liver microsomes in the presence of 1 µmol of NADPH, or with 0.2 mL liver cytosol in the presence of 2-hydroxypyrimidine for 30 min in a final volume of 1 mL 0.1 M phosphate buffer. After the incubation, the mixture was extracted with 5 mL ethyl acetate and evaporated to dryness. The residue was dissolved in 1 mL ethanol, and an aliquot was used for the antiandrogenic activity assay described above. We calculated the total concentration of the substrate and its metabolites from the original amount of the substrate.

Assay of androgenic and antiandrogenic activities in vivo (Hersberger assay). We used F344 male rats obtained from Charles River Co. (Kanagawa, Japan). They were surgically castrated at 5 weeks of age. At 7 weeks of age, they were divided into five groups consisting of seven animals each, except six in the control. We treated the rats once a day for 7 days with subcutaneous doses of 0.2 mL of vehicle (Panacetate 810; Nippon Oils and Fats Co., Ltd., Tokyo, Japan), testosterone propionate (500 µg/kg/day), testosterone propionate plus flutamide (6 mg/kg/day), or testosterone plus fenthion (25 or 50 mg/kg/day). We sacrificed animals under anesthesia and dissected and weighed the ventral prostate and seminal vesicles.

Assay of estrogenic and antiestrogenic activities in vitro. We assayed estrogenic activity according to the previously reported method using an ERE-luciferase reporter in MCF-7 cells (Sugihara et al. 2000). Briefly, transient transfections in MCF-7 cells were performed using TransFast in 12-well plates at 1 × 10⁵ cells/well with 1.9 µg p(ERE)₃-SV40-luc and 0.1 µg pRL-CMV as an internal standard. Twenty-four hours after addition of the sample, the assay was performed with a dual luciferase assay kit. We measured antiestrogenic activity in terms of the inhibitory effect of the test compounds on the estrogenic activity of E₂ at the concentrations of 1 × 10⁻¹⁰ and 1 × 10⁻⁹ M. Data are expressed as the means ± SD of four

experiments using independent transfected cells.

Metabolizing activities of rat liver preparations. The incubation mixture consisted of 0.2 µmol fenthion or fenthion sulfoxide, 1 µmol of an electron donor and liver microsomes (2–3 mg protein) or cytosol (1–2 mg protein) 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 37°C. 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. After the incubation, the mixture, after addition of 10 nmol 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). The time-course of the formation of the metabolite in the presence of NADPH with 1.5 mg protein of liver microsomes of rats was linear to 60 min. The dependency on amount of liver microsomes was linear up to 10 mg protein. Data are expressed as the means ± SD of four experiments using independent rats.

HPLC. 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, vol/vol, pH 5.4). 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. We determined the amounts of metabolites formed from the peak areas. In some experiments for the determination of metabolites, a Beckman 168 photodiode array ultraviolet (UV) detector (Beckman Instruments, Inc., Fullerton, CA, USA) was fitted to the HPLC unit to identify the metabolites of fenthion and fenthion sulfoxide.

GC-MS. Gas chromatography–mass spectrometry (GC-MS) was performed using a Shimadzu GC-17A/QP-5000 (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, USA) 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. We injected 1 µL of sample into the injection port at 220°C. Splitless injection was used. The retention

times of fenthion sulfoxide and fenthion were 12.2 and 14.7 min, respectively.

Results

Androgenic and antiandrogenic activities of fenthion and metabolites. Androgenic and antiandrogenic activities of fenthion, fenthion sulfoxide, and fenthion sulfone were examined using an ARE-luciferase reporter in NIH3T3 cells. These compounds had no detectable androgenic activity in the concentration range of 10^{-7} – 10^{-4} M. In contrast, DHT at 10^{-11} – 10^{-9} M exhibited marked androgenic activity (Figure 1A). When fenthion was added to the assay system at 10^{-7} – 10^{-5} M, the activity of DHT at a concentration of 10^{-11} M was inhibited concentration dependently. This antiandrogenic effect of fenthion was also observed at a concentration of 10^{-10} M DHT. The antiandrogenic activity of fenthion is comparable to that of flutamide. However, fenthion sulfoxide and fenthion sulfone did not show such antiandrogenic effects in the concentration range of 10^{-7} – 10^{-5} M (Figure 1B,C).

Antiandrogenic activities of fenthion-related compounds. Antiandrogenic activities

of fenthion and related compounds were comparatively examined with an ARE-luciferase reporter in NIH3T3 cells. Figure 2 shows the IC_{50} (concentration for 50% inhibition) values of fenthion, fenthion sulfoxide, fenthion sulfone, fenitrothion, fensulfothion, EPN, trichlorfon, and malathion against the androgenic activity of DHT at a concentration of 10^{-10} M. The highest activity among organophosphorus compounds was observed in fenitrothion, followed by fenthion and EPN. However, fenthion sulfoxide, fenthion sulfone, fensulfothion, trichlorfon, and malathion did not show such activity. These results indicate that some organophosphorus insecticides have antiandrogenic but not androgenic activity.

Estrogenic and antiestrogenic activities of fenthion and metabolites. Estrogenic activities of fenthion, fenthion sulfoxide, and fenthion sulfone were examined using ERE-luciferase reporter-transfected MCF-7 cells. E_2 exhibited estrogenic activity at 10^{-12} – 10^{-9} M. In contrast, no estrogenic activity of fenthion, fenthion sulfoxide, or fenthion sulfone was observed in the concentration range of 10^{-7} – 10^{-4} M, except for a marginal effect at 10^{-4} M (data not shown).

When fenthion, fenthion sulfoxide, or fenthion sulfone was added to the assay system of E_2 in the concentration range of 10^{-8} – 10^{-5} M, the estrogenic activity of E_2 at the concentrations of 10^{-11} M and 10^{-10} M was not inhibited (data not shown). Thus, no significant estrogenic or antiestrogenic effect of fenthion and related compounds was observed.

Antiandrogenic activity of fenthion after metabolism with rat liver microsomes. The effect of the metabolism of fenthion by rat liver microsomes on the antiandrogenic activity of the compound was examined. When fenthion was incubated with rat liver microsomes in the presence of NADPH, the inhibitory effect of the extract on the androgenic activity of DHT at the concentrations of 10^{-11} and 10^{-10} M was markedly decreased compared with the original activity of fenthion. The decrease of antiandrogenic activity was minimal when the incubation was performed without liver microsomes (Figure 3A,B). Fenthion sulfoxide was detected by HPLC as a major metabolite in the complete incubation mixture. The mass and UV spectra of the metabolite confirmed that it was fenthion sulfoxide (data not shown).

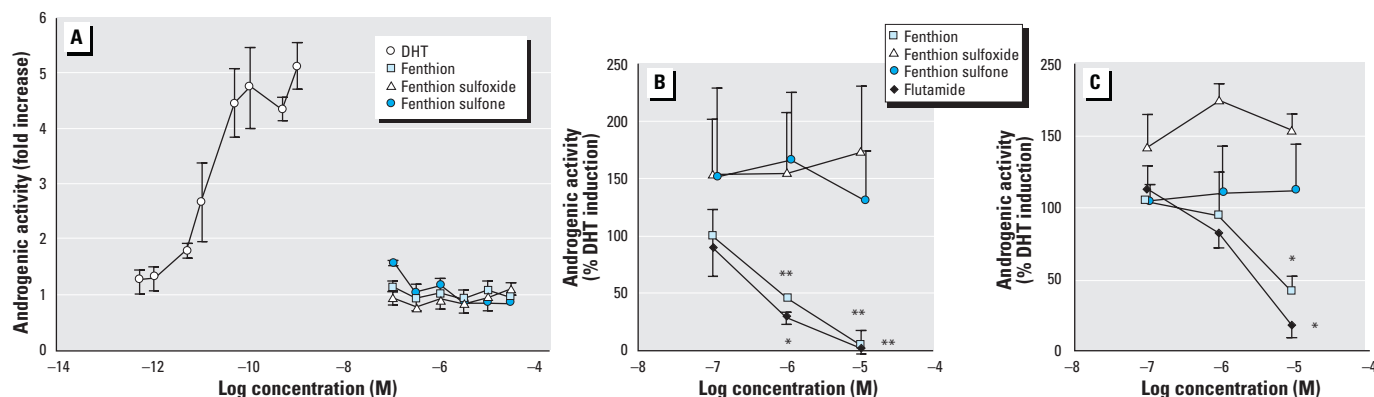


Figure 1. Androgenic and antiandrogenic activities of fenthion, fenthion sulfoxide and fenthion sulfone. (A) Androgenic activity examined with an ARE-luciferase reporter transiently transfected in NIH3T3 cells. Activities are expressed as fold increase in the response relative to the control level. Antiandrogenic activity examined with the same reporter system in the presence of DHT at the concentrations of 10^{-11} M (B) and 10^{-10} M (C). Data indicate percent values of ARE-luciferase activity relative to the level induced by DHT. Each value represents the means \pm SD of four independent experiments.

* $p < 0.05$ and ** $p < 0.01$ indicate significant differences from control experiments to which only DHT was added.

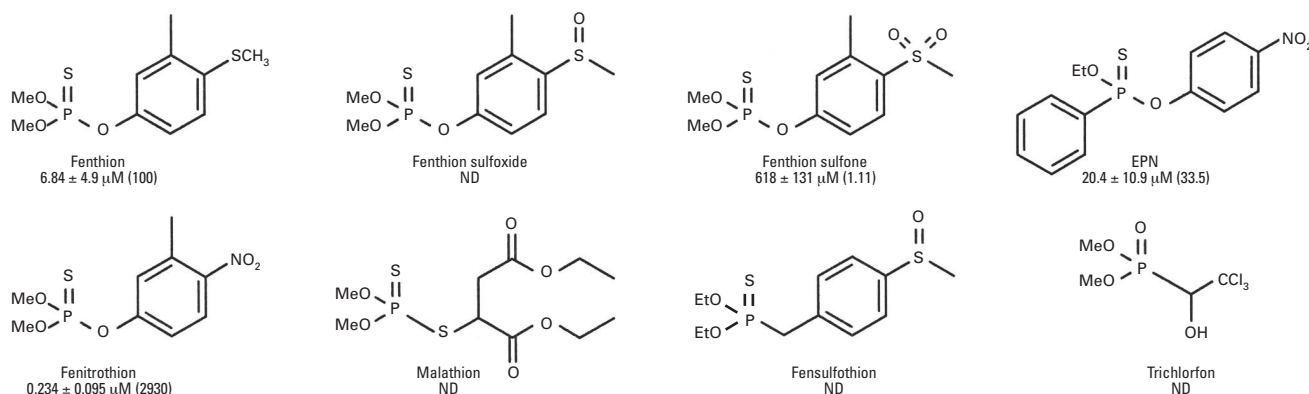


Figure 2. Antiandrogenic activity and structures of fenthion-related compounds. ND, not determined. Each value represents the IC_{50} of the inhibitory effect on the activity of DHT (means \pm SD of four independent experiments); relative activities are shown in parentheses.

Antiandrogenic activity of fenthion sulfoxide after metabolism with rat liver cytosol.

When fenthion sulfoxide was incubated with rat liver cytosol in the presence of 2-hydroxypyrimidine, an electron donor of aldehyde oxidase, under anaerobic conditions, the extract of the incubation mixture inhibited the androgenic activity of DHT on NIH3T3 cells in the range of 10^{-7} – 10^{-5} M. The antiandrogenic activity was not observed after incubation without liver cytosol (Figure 3C, D). Such metabolic activation did not occur after the incubation of fenthion sulfoxide with liver microsomes in the presence of NADPH. When fenthion sulfoxide was incubated with liver cytosol of rats in the presence of 2-hydroxypyrimidine, one metabolite, of which the retention time corresponded to that of fenthion, was detected in HPLC of the extract. The metabolite was identified by comparison of the mass and UV spectra with those of authentic fenthion, as described in “Materials and Methods.” Fenthion was not detected in HPLC of the incubation mixture of fenthion sulfoxide with rat liver microsomes (data not shown). These results suggest that the oxidation of fenthion acts as a detoxification pathway and the reduction of fenthion sulfoxide to

fenthion as an activation step, and this interconversion between fenthion and fenthion sulfoxide in the body functions to maintain the antiandrogenic activity of fenthion.

Metabolism of fenthion and fenthion sulfoxide by rat liver preparations.

In the conversion of fenthion to fenthion sulfoxide by rat liver microsomes, NADPH was effective as an electron donor. The NADPH-linked oxidizing activity in liver microsomes of rats toward fenthion was significantly inhibited by α -naphthylthiourea and partially inhibited by SKF 525-A, α -naphthoflavone, and quinidine (Figure 4A). This suggests that the oxidizing activity to the sulfoxide is due to both cytochromes P450 (CYPs) and flavin-containing monooxygenase (FMO) in the rats.

Fenthion was formed from fenthion sulfoxide by rat liver cytosol when 2-hydroxypyrimidine or benzaldehyde, which are electron donors of aldehyde oxidase, was added under anaerobic conditions. However, xanthine, an electron donor of xanthine oxidase, was not effective for the reduction. In the cytosolic reduction, NADPH and NADH were also much less effective than 2-hydroxypyrimidine. The 2-hydroxypyrimidine-dependent activity in rat liver was inhibited by menadione, which

is an inhibitor of aldehyde oxidase, but not by oxypurinol, an inhibitor of xanthine oxidase (Figure 4B). This suggests that the reducing activity is due to aldehyde oxidase in rat liver cytosol. Indeed, purified aldehyde oxidase from rats exhibited a significant sulfoxide reductase activity (64.7 nmol/min/mg protein) in the presence of 2-hydroxypyrimidine. In contrast, liver microsomes exhibited no reductase activity even in the presence of NADPH, indicating that CYP was not involved in the fenthion sulfoxide reduction (data not shown). The cytosolic reductase activity toward fenthion sulfoxide was higher than the NADPH-dependent microsomal oxidase activity toward fenthion.

Hershberger assay of fenthion.

Antiandrogenic potential of fenthion *in vivo* was further investigated by means of the Hershberger assay using Fischer 344 rats according to the Hershberger protocol (Hershberger et al. 1953). Body weight was decreased by 25% in the group administered the higher dose of fenthion, but there were no significant differences among other groups. Testosterone treatment significantly increased

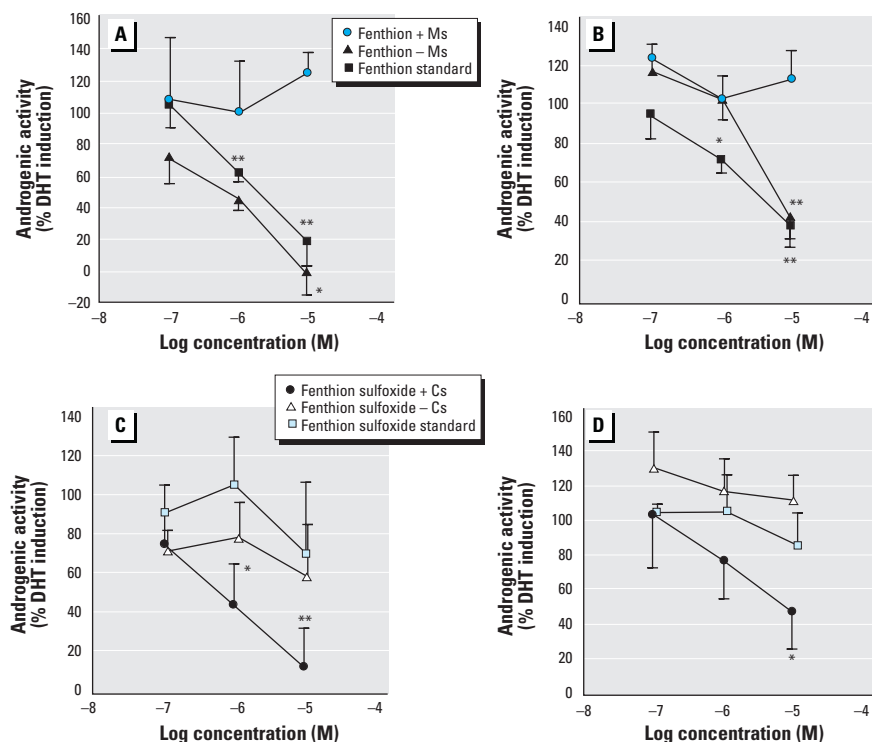


Figure 3. Antiandrogenic activity of fenthion (A,B) and fenthion sulfoxide (C,D) after incubation with rat liver microsomes or cytosol. Abbreviations: Ms, microsomes; Cs, cytosol. Antiandrogenic activity was examined with the ARE-luciferase reporter system in the presence of DHT at the concentrations of 10^{-11} M (A,C) and 10^{-10} M (B,D). The data indicate percent values of the ARE-luciferase activity to the level induced by DHT. In A and B, fenthion was incubated with rat liver microsomes in the presence of NADPH. In C and D, fenthion sulfoxide was incubated with liver cytosol in the presence of 2-hydroxypyrimidine. The extract of the incubation mixture was subjected to the assay. Each value represents the mean \pm SD of four independent experiments.

* $p < 0.05$ and ** $p < 0.01$ indicate significant differences from control experiments to which only DHT was added.

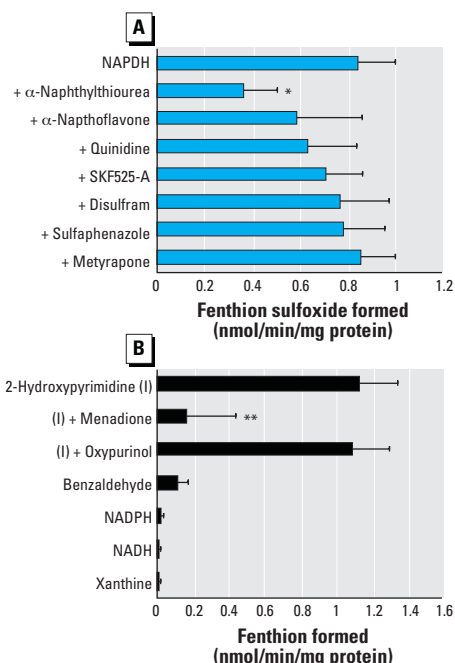


Figure 4. Metabolism of fenthion or fenthion sulfoxide by rat liver preparations. (A) Oxidase activity of rat liver microsomes toward fenthion in the presence of NADPH. (B) Reductase activity of rat liver cytosol toward fenthion sulfoxide in the presence of 2-hydroxypyrimidine. A mixture containing 0.1 μ mol of fenthion or fenthion sulfoxide, 0.5 μ mol of an electron donor and liver preparation in 0.1 M phosphate buffer (pH 7.4) was incubated at 37°C for 30 min. Inhibitors were added at the concentration of 10^{-4} M. The fenthion sulfoxide or fenthion formed was determined using HPLC as described in Materials and Methods. Each value represents the mean \pm SD of four rats.

* $p < 0.05$ and ** $p < 0.01$ indicate significant differences from control experiments to which an electron donor was added.

the weights of the prostate and seminal vesicles, and these increases were completely blocked by flutamide administration. Fenthion also significantly suppressed the effect of testosterone on the weight of both glands (Figure 5). Thus, the antiandrogenic effect of fenthion was also observed *in vivo*.

Discussion

In this study we examined the endocrine-disrupting action of fenthion and its metabolites, focusing on antiandrogenic and estrogenic actions. No estrogenic or antiestrogenic activity of fenthion was detected using estrogen-responsive reporter assay. However, we demonstrated that fenthion is a potent antiandrogen, though fenthion sulfoxide and fenthion sulfone lack this activity. Antiandrogenic activity of fenthion using ARE-luciferase reporter assay in NIH3T3 cells was comparable to that of the reference antiandrogen, flutamide. The Hershberger assay has been used to screen chemicals for androgenic and antiandrogenic activities *in vivo*. In the Hershberger assay used in this study, fenthion could block androgen-dependent tissue growth. Fenthion's effect on androgen-dependent tissue weights was significant. The antiandrogenic activity shown by the reporter assay in NIH3T3 cells is supported by the result of this *in vivo* assay. It is known that fenthion is accumulated in the environment (Fukushima 1991; Lacorte et al. 1997; Pereira and Hostettler 1993). Fenthion and its metabolites were detected in rice and olive oil at concentrations of about 10 and 1 ppm, respectively (Cabras et al. 1993; Fukuda et al. 1962). These compounds were also detected in river water near Osaka, Japan at 20–70 ng/L (Yamaguchi and Fukushima 1994). The concentration detected in the

river water is three orders of magnitude lower than the concentration of fenthion that causes antiandrogenic activity. However, the concentrations detected in rice and olive oil are sufficient to exert antiandrogenic action. Fenthion may accumulate to a level in the body sufficient to cause antiandrogenic action. Further, fenthion in combination with other pesticides may exhibit antiandrogenic activity. Lacorte et al. (1997) reported that fenthion was oxidized to the sulfoxide in the environment. This conversion would contribute to the inactivation of fenthion in terms of antiandrogenic action. However, the effect of fenthion on reproductive tissues in rats *in vivo* shown in this study cannot be neglected.

In this study, we demonstrated that fenitrothion and EPN, in addition to fenthion, are antiandrogenic, but fensulfthion, trichlorfon, and malathion are not. Antiandrogenic compounds so far reported include vinclozolin, linuron, iprodione, chlozolinate, procymidone, flutamide, *p,p'*-DDE, and ketoconazole (Gray et al. 1999a, 1999b; Lambright et al. 2000; McIntyre et al. 2000; Ostby et al. 1999; You et al. 1999). In contrast, both positive and negative results on the antiandrogenic activity of fenitrothion, a relative of fenthion, have been reported (Ashby and Lefevre 2000; Curtis 2001; Sohoni et al. 2001; Sunami et al. 2000; Tamura et al. 2001). The significant antiandrogenic activity of fenitrothion found in our study supports previous findings that fenitrothion has antiandrogenic properties. The structures of fenthion and fenitrothion are the same except for their *para* substituents. These structures closely resemble that of flutamide. It is difficult to understand the structural differences between antiandrogenic and non-antiandrogenic organophosphorus insecticides. Further study of the structure requirements for, and the mechanism of action of, antiandrogenic activity of organophosphorus insecticides is needed.

Several reports have indicated that sulfide compounds are metabolized mainly by oxidation to the corresponding sulfoxide and sulfone compounds, and some sulfoxide compounds are reduced in the sulfide compounds in mammalian species (Benoit et al. 1992; DeBaun and Menn 1976; McLane et al. 1983; Mitchell and Waring 1986; Tatsumi et al. 1983). However, the metabolism of fenthion and fenthion sulfoxide has not been extensively examined in mammalian species. Brady and Arthur (1961) briefly reported that fenthion was transformed to the

oxidized metabolites in rats *in vivo*. In the current study, we showed that fenthion was oxidized to fenthion sulfoxide, and the sulfoxide was reduced back to the parent fenthion. However, fenthion sulfone was not formed from fenthion in rat liver. We also demonstrated the inactivation of the antiandrogenic activity of fenthion upon metabolic oxidation and the activation of fenthion sulfoxide by reductive metabolism, and we suggest that the interconversion between fenthion and fenthion sulfoxide in the body functions to maintain antiandrogenic activity (Figure 6).

Fenthion was oxidized to fenthion sulfoxide by rat liver microsomes. The oxidizing activity to the sulfoxide appears to be exhibited by both CYP and FMO in rats. In our preliminary study, microsomal preparations from cells expressing recombinant human CYP 1A1, 1A2, 2B6, 2C9, and 3A4 and FMO I expressed in a human B lymphoblastoid cell line, exhibited sulfoxide oxidase activities toward fenthion amounting to 3.6, 0.4, 0.4, 0.3, 1.0, and 62.4 nmol/min/mg protein, respectively. In contrast, the reductive metabolism of fenthion sulfoxide was catalyzed by a cytosolic enzyme, aldehyde oxidase but not CYP. The velocity of this reverse reaction is about the same as that of fenthion oxidation (1.1 and 0.8 nmol/min/mg protein, respectively).

Metabolic modification of the activity of endocrine disruptors is an important factor influencing the toxicity of these compounds. For example, vinclozolin, an antifungal agent, was activated to antiandrogenic compounds by hydrolysis of the amide linkage in animals (Kelce et al. 1994). *p,p'*-DDT is metabolized to *p,p'*-DDD and *p,p'*-DDE by reductive dechlorination and dehydrochlorination, respectively (Esaac and Matsumura 1980; Kitamura et al. 2002). *p,p'*-DDD shows estrogenic activity and *p,p'*-DDE shows antiandrogenic activity (Chen et al. 1997; Kelce et al. 1995). Polychlorinated biphenyls are converted to hydroxylated metabolites in animals, and some hydroxylated polychlorinated biphenyls show estrogenic and/or antithyroid hormonal activity (Connor et al. 1997; Garner et al. 1999; Korach et al. 1988). Further, methoxychlor, *trans*-stilbene, benzophenone, and benzo[*a*]pyrene are proestrogens that require metabolic activation by liver microsomal mixed-function oxidase in animals (Charles et al. 2000; Fertuck et al. 2001; Gaido et al. 1999; Nakagawa and Suzuki 2002; Sugihara et al. 2000). In contrast, the estrogen screening

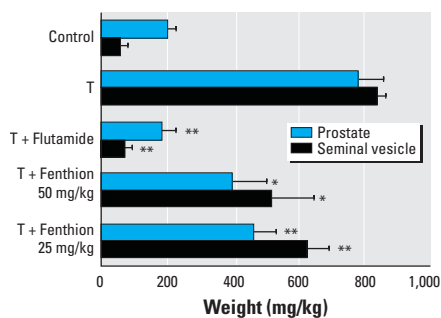


Figure 5. The Hershberger assay of fenthion and flutamide. Weights of the reproductive organs of rats were expressed. T, testosterone propionate. Castrated male F344 rats were treated with T (500 µg/kg/day) and/or fenthion (25 or 50 mg/kg/day) or flutamide (6 mg/kg/day) for a week. One day after the final administration, animals were sacrificed and the prostate gland and seminal vesicle were weighed. Each group consisted of seven rats, except the control group with six animals. Each bar indicates mean ± SD of six or seven rats. **p* < 0.05 and ***p* < 0.01 indicate significant differences from testosterone-dosed rats.

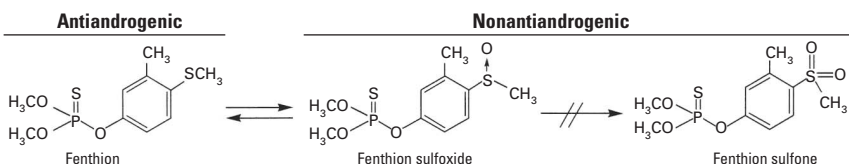


Figure 6. Interconversion between fenthion and fenthion sulfoxide with rat liver preparations.

test of fenthion and related compounds in this study was negative, and these compounds were not metabolically activated to estrogens (data not shown). Much further work is needed to examine the metabolic modification of potentially hazardous endocrine disrupters in the environment.

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