REDUCTIVE METABOLISM OF \( p,p' \)-DDT AND \( \alpha,\alpha' \)-DDT BY RAT LIVER CYTOCHROME P450

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ABSTRACT:

The in vitro metabolism of \( p,p' \)-DDT \([1,1,1\text{-trichloro-2,2-bis(4-chlorophenyl)}\text{-ethane}]\), an important environmental pollutant, was examined in rat liver, focusing on reductive dechlorination. When \( p,p' \)-DDT was incubated with liver microsomes of rats in the presence of NADPH or NADH, a dechlorinated metabolite, \( p,p' \)-DDD \([1,1\text{-dichloro-2,2-bis(4-chlorophenyl)}\text{-ethane}]\), was formed under anaerobic conditions together with a dehydrochlorinated metabolite, \( p,p' \)-DDE \([1,1\text{-dichloro-2,2-bis(4-chlorophenyl)}\text{-ethylene}]\). \( p,p' \)-DDE was also formed from \( p,p' \)-DDT by liver microsomes. The dechlorinating activity was inhibited by carbon monoxide, metyrapone, and SKF 525-A (proadifen hydrochloride), but the dehydrochlorinating activity was unaffected. The reductase activity toward \( p,p' \)-DDT was induced by the pretreatment of rats with phenobarbital and dexamethasone. The dechlorination was catalyzed enzymatically by recombinant cytochrome P450 2B1, 3A1, 286, and 3A4. When \( p,p' \)-DDT was incubated with liver microsomes of rats in the presence of both a reduced pyridine nucleotide and FMN, \( p,p' \)-DDD was also formed under anaerobic conditions. In this case, the dechlorinating activity was not abolished when the microsomes were boiled. The reductase activities were inhibited by carbon monoxide. Hematin exhibited reductase activity toward \( p,p' \)-DDT in the presence of NADH and FMN. The activity of hematin was also supported by FMNH\(_2\). The reductive dechlorination also seems to proceed nonenzymatically with the reduced flavin, catalyzed by the heme group of cytochrome P450. Similar enzymatic and nonenzymatic reducing activities were observed toward \( \alpha,\alpha' \)-DDT \([1,1,1\text{-trichloro-2,2-bis(2-chlorophenyl-4-chlorophenyl)}\text{-ethane}]\).

\( p,p' \)-DDT \([1,1,1\text{-trichloro-2,2-bis(2-chlorophenyl-4-chlorophenyl)}\text{-ethane}]\) is a broad-spectrum insecticide, which was used from the 1940s in large quantities, but was banned in many countries in the 1970s because of its persistence in the environment. It was replaced in part by \( \alpha,\alpha' \)-DDT because of its persistence in the environment. It was replaced in part by \( \alpha,\alpha' \)-DDT and related compounds, many of which are carcinogenic and mutagenic, are also known to be environmental estrogens. \( p,p' \)-DDT is a phenobarbital-type inducer, which induces the cytochrome P450 (CYP) 2B and 3A subfamily (Lewis and Lake, 1997; Nims et al., 1998). \( p,p' \)-DDT and its metabolites accumulate in animal tissues and induce various enzymes (Esaac and Matsumura, 1980). The higher levels of \( p,p' \)-DDE \([1,1\text{-dichloro-2,2-bis(4-chlorophenyl)}\text{-ethylene}]\) than \( p,p' \)-DDT in tissues reflect its stability (Morgan and Roan, 1971). These compounds mimic hormones and bind to the estrogen receptor and androgen receptor (Robinson et al., 1985; Kelce et al., 1995; Chen et al., 1997). \( p,p' \)-DDT and related compounds, \( p,p' \)-DDD \([1,1\text{-dichloro-2,2-bis(4-chlorophenyl)}\text{-ethane}]\), \( \alpha,\alpha' \)-DDT \([1,1,1\text{-trichloro-2,2-bis(2-chlorophenyl-4-chlorophenyl)}\text{-ethylene}]\), and dicofol \([1,1\text{-bis(4-chlorophenyl)-2,2-trichloroethanol}]\), are known to be xenobiotic estrogens, but \( p,p' \)-DDE is an antiandrogen (You, 2000). To assess the possible risks associated with human exposure to the pesticide, it is essential to thoroughly elucidate its metabolism in mammalian species, birds, and marine and freshwater species.

\( p,p' \)-DDT is converted by reductive dechlorination to \( p,p' \)-DDT in insects, birds, and animals (Hassall, 1972; Esaac and Matsumura, 1980). Several reports have indicated that \( p,p' \)-DDT is mainly metabolized to \( p,p' \)-DDE by dehydrochlorination in mammalian species, insects, and microorganisms, and \( p,p' \)-DDD is an intermediate of the reaction, which may proceed via \( \alpha \)-hydroxyl-DDD (Benitez et al., 1995; Fox et al., 1998). \( p,p' \)-DDE and \( p,p' \)-DDD are further oxidized to 2,2-bis(4-chlorophenyl)acetic acid (\( p,p' \)-DDA), the major excreted metabolite in animals (Wallcave et al., 1974; Gold and Brunk, 1982). The dechlorination is of considerable significance because this reaction is the first and also is the rate-limiting step in the metabolism of \( p,p' \)-DDT in mammals and microorganisms. A role of CYP in the microsomal reduction of \( p,p' \)-DDT was suggested (Esaac and Matsumura, 1980; Baker and Van Dyke, 1984; Kelner et al., 1986). However, the mechanism of microsomal reductive dechlorination of \( p,p' \)-DDT is not known in detail. Furthermore, the metabolism of \( \alpha,\alpha' \)-DDT, which contaminates technical-grade DDT about 20%, has not been established. In the present study, the in vitro metabolism of \( \alpha,\alpha' \)-DDT and \( p,p' \)-DDT by rat liver microsomes was examined, focusing on reductive dechlorination to DDS isomers.
Experimental Procedures

Materials. p,p'-DDT, p,p'-DDE, and p,p'-DDD were purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). α,p'-DDT, α,p'-DDE, p,p'-DDMU [1-chloro-2,2-bis-(4-chlorophenyl)ethylenel], p,p'-DDA, dicofol, and α,p'-DDE were obtained from Labor Dr. Ehrenstorfer-Schäfers (Augsburg, Germany). FMN and hematin (FeCl₃) were from Sigma Chemical Co. (St. Louis, MO). Reduced FMN was prepared photochemically from FMN by the previously described method (Kitamura et al., 1999a). p,p'-DDT and α,p'-DDT were used after recrystallization from n-hexane. Microsomal preparations from recombinant rat CYP 2B1 and 3A1 expressed in insect cells and from nine recombinant human CYP isoforms (i.e., CYP 1A1, 1A2, 2A6, 2B6, 2C9, 2D6, 2E1, 3A4, and 4A11) expressed in a human lymphoblastoid cell line were purchased from GENTEST (Woburn, MA).

Animals. Male rats (Slc:SD; 180–210 g; Slc:Japan; Shizuoka, Japan) were used. In some experiments, phenobarbital was administered to rats intraperitoneally at a dose of 80 mg/kg for 3 days, 3-methylcholanthrene at 25 mg/kg for 3 days, dexamethasone at 100 mg/kg for 4 days, clofibrate at 250 mg/kg for 3 days, and acetone at 3 g/kg orally for 1 day.

Preparation of Liver Microsomes. Liver microsomes were determined from a large-scale incubation mixture. The large-scale incubation mixture for Liver Microsomes. Liver microsomes were prepared from rats treated with various chemicals. The presence of NADPH, under anaerobic conditions. After incubation, the mixture was centrifuged at 9000 g for 3 days, and the supernatant was centrifuged at 9000 g for 60 min. The pellets of microsomes were resuspended in a solution to make 1 ml equivalent to 1 g of liver. Protein contents in the liver microsomal preparation of untreated, phenobarbital-treated, dexamethasone-treated, clofibrate-treated, acetone-treated, and 3-methylcholanthrene-treated rats were 12.6 to 14.8, 18.5 to 20.9, 14.5 to 16.7, 13.4 to 15.8, 12.5 to 14.4, and 13.4 to 17.2 mg of protein/ml, respectively, as determined by the method of Lowry et al. (1951).

Identification of Reductive Metabolites of p,p'-DDT Formed by Rat Liver Microsomes. Two reductive metabolites of p,p'-DDT or p,p'-DDD were determined from a large-scale incubation mixture. The large-scale incubation mixture consisted of 1 μmol of the substrate, 5 μmol of NADPH, and 1 ml of liver microsomes of untreated rats in a total volume of 5 ml of 0.1 M Tris-HCl buffer, pH 7.4. After incubation for 20 min, the mixture was extracted with 20 ml of n-hexane. The supernatant was evaporated to dryness, and 0.1 ml of methanol was added. The solution was injected into a high-performance liquid chromatograph and a gas chromatograph-mass spectrometer. Gas chromatograph-mass spectrometer was performed using a Shimadzu GC-17A/QP-5000 (Kyoto, Japan) in the electron impact mode. The column temperature was held at 100 °C for 3 min, then increased at a rate of 15 °C/min to 250 °C. Splitless injection was used. The retention times of p,p'-DDT, p,p'-DDE, and p,p'-DDMU were 13.4, 13.0, and 12.4 min, respectively. The mass spectra of p,p'-DDT, p,p'-DDE, and p,p'-DDMU showed m/z 284 (M⁺), 235, 165, m/z 318 (M⁺), 246, and 176, and m/z 284 (M⁺), 212, and 176, respectively.

Assay of Reductase Activity. The incubation mixture consisted of 0.1 μmol of p,p'-DDT or α,p'-DDT (50 μl of methanol solution), 1 μmol of NADPH or NADH, and 0.2 ml of liver microsomes in a final volume of 1 ml of 0.1 M KNa-phosphate buffer, pH 7.4. Incubation was performed using a Thunberg tube under anaerobic conditions. The side arm contained NADPH or NADH, and the body contained all the other components. The tube was gassed with nitrogen. The reaction was started by mixing the components of the side arm and the body together and was continued for 30 min at 37 °C. The mixture, after addition of 1 μg of phenothiazine as an internal standard, was extracted once with 5 ml of n-hexane, and the extract was evaporated to dryness in vacuo. The residue was dissolved in 0.1 ml of methanol and then subjected to high-performance liquid chromatography (HPLC).

HPLC. HPLC was performed in a Hitachi L-6000 high-performance liquid chromatograph (Tokyo, Japan) equipped with an ultraviolet absorption detector. The instrument was fitted with a 4 × 125-mm LiChrospher RP-Select B (5 μm) (Merck, Darmstadt, Germany). The mobile phase was acetonitrile/H₂O (6:4). The chromatograph was operated at a flow rate of 1.0 ml/min at ambient temperature and at a wavelength of 254 nm. Elution times of p,p'-DDA, dicofol, p,p'-DDD, α,p'-DDD, p,p'-DDT, p,p'-DDMU, α,p'-DDT, p,p'-DDE, and α,p'-DDE were 4.5, 15.4, 16.5, 17.8, 27.7, 29.0, 35.3, and 36.7 min, respectively. The amounts of metabolites formed were determined from the peak areas.

Thin-Layer Chromatography (TLC). Silica gel plates (Kieselgel 60 GF254, 0.2 mm thick; Merck, Darmstadt, Germany) were developed in n-hexane:acetone (9:1, v/v). Spots were visualized under UV light (254 nm). The Rf values of authentic p,p'-DDA, dicofol, p,p'-DDD, p,p'-DDT, p,p'-DDMU, p,p'-DDE, and α,p'-DDE were 0.12, 0.33, 0.36, 0.48, 0.52, and 0.63, respectively.

Results

Metabolism of p,p'-DDT by Rat Liver Microsomes. p,p'-DDT was incubated with liver microsomes of untreated rats in the presence of NADPH for the detection of metabolites, as described under Experimental Procedures. Two peaks were detected in an HPLC chromatogram of the extract of the incubation mixture (Fig. 1A). These peaks were not detected in the control, which was incubated without the substrate. The metabolites detected at 16.5 and 35.3 min correspond to p,p'-DDE and p,p'-DDE, respectively. In the case of boiled microsomes, these metabolites were not detected. The amounts of metabolites corresponding to p,p'-DDT and p,p'-DDE were isolated as described under Experimental Procedures. They were identified unequivocally as p,p'-DDT and p,p'-DDE by mass spectrometry, TLC, and HPLC comparison with authentic samples. The further metabolized products p,p'-DDMU and p,p'-DDA were not detected by HPLC or TLC as metabolites of p,p'-DDT with liver microsomes (data not shown). When p,p'-DDT was incubated with liver cytosol in the presence of NADPH, p,p'-DDD and p,p'-DDE were not detected in the chromatogram of the extract (Fig. 1B). In contrast, dicofol, an oxidized product of p,p'-DDT, and phenolic metabolites were also not detected.
DDT was catalyzed by the microsomal CYP system but not by disulfiram, sulfaphenazole, and quinidine (data not shown). These facts suggest that the reductive dechlorination of DDT was catalyzed by the cytosolic reductive dechlorination (data not shown). Other details are described under Experimental Procedures. In B, the same procedure as in A was used except that the amount of liver microsomes from rats (0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 mg of protein) was varied and the incubation time was 30 min. p,p'-DDD formed under aerobic or anaerobic conditions was determined by HPLC.

The liver microsomes of untreated rats catalyzed the reductive dechlorination of p,p'-DDT to p,p'-DDD and p,p'-DDE with rat liver microsomes. (data not shown).

Dechlorinating Activity of Rat Liver Microsomes Toward p,p'-DDT. The time course of the dechlorination of p,p'-DDT to p,p'-DDD by liver microsomes from rats in the presence of NADPH was essentially linear for 45 min. The formation of p,p'-DDE from p,p'-DDT was also observed, but the amounts were about one-hundredth of those of p,p'-DDD (Fig. 2A). When the reductase activity was assayed with various amounts of microsomes under anaerobic conditions, the reductase activity increased linearly with increasing amount of liver microsomes up to 4.0 mg of protein. In contrast, very low activities were observed under aerobic conditions (Fig. 2B). In the other experiments of this study, incubations were carried out for 30 min using 0.2 ml of liver microsomes (about 3 mg of protein, equivalent to 200 mg of wet liver weight) under anaerobic conditions.

The liver microsomes of untreated rats catalyzed the reductive dechlorination of p,p'-DDT to p,p'-DDD in the presence of NADPH or NADH under anaerobic conditions. In this case, NADPH was more effective than NADH as an electron donor for the reductive dechlorination. The reductase activity of liver microsomes was enhanced to some extent in the presence of both NADPH and NADH. The NADH- and NADPH-linked dechlorinating activities of the microsomes toward p,p'-DDT were inhibited by SKF 525-A, metyrapone, and carbon monoxide. The NADPH-linked activity was also inhibited by ketoconazole (Fig. 3). However, the activity was not affected by disulfiram, sulfaphenazole, and quinidine (data not shown). These results suggest that CYP 3A1 functions in the dechlorination of p,p'-DDT in untreated rats. The NAD(P)H-linked reductase activity was abolished by boiling the liver microsomes. The dehydrochlorinating activity to p,p'-DDE was unaffected by these inhibitors (data not shown). In contrast, the liver cytosol of rats exhibited no reductive dechlorinating activity toward p,p'-DDT even in the presence of NADPH, as shown in Fig. 1B. Furthermore, NADH, 2-hydroxypryrimidine, N⁰-methylnicotinamide, and xanthine, which are electron donors to DT-diaphorase, aldehyde oxidase, or xanthine oxidase, were not effective for the cytosolic reductive dechlorination (data not shown). These facts suggest that the reductive dechlorination of p,p'-DDT was catalyzed by the microsomal CYP system but not by DT-diaphorase, xanthine oxidase, or aldehyde oxidase.

Next, the effect of the pretreatment of rats with various inducers on the reductive dechlorination of p,p'-DDT was examined. When the reductase activities in liver microsomes from untreated, phenobarbital-treated, 3-methylcholanthrene-treated, acetone-treated, clofibrate-treated, and dexamethasone-treated rats were compared in the presence of NADPH, enhanced activity compared with that of untreated rats was observed in phenobarbital-treated and dexamethasone-treated rats (Fig. 4).

Identification of CYP Isoforms Involved in the Dechlorination. Identification of CYP isoforms involved in the dechlorination of p,p'-DDT in liver microsomes was attempted by using recombinant rat and human CYPs. Recombinant rat CYP 2B1 and 3A1 catalyzed this dechlorination in the presence of NADPH at rates of 1.16 and 1.03 nmol/min/nmol CYP, respectively. Recombinant human CYP 3A4 and 2B6 exhibited substantial activities for dechlorination of p,p'-DDT in the presence of NADPH. Human CYPs 1A1, 1A2, 2A6, 2C9, 2D6, 2E1, and 4A11 also showed some dechlorinase activity.
FIG. 4. Dechlorination of \( p,p' \)-DDT by liver microsomes of rats pretreated with inducers of the cytochrome P450 system.

Each bar represents the mean \( \pm \) S.D. of four rats. Incubation was performed at 37°C for 30 min with 200 \( \mu \)l of liver microsomes of rats pretreated with inducers in the presence of NADPH under anaerobic conditions. The \( p,p' \)-DDD formed was determined by HPLC. Other details are described under Experimental Procedures.

(Fig. 5). Control microsomes of human B lymphoblastoid cells exhibited no appreciable dechlorinase activity. These facts suggest that the dechlorination of \( p,p' \)-DDT is catalyzed predominantly by phenobarbital- and dexamethasone-inducible CYPs, but other CYPs also contribute to the dechlorination.

Heme-Based Dechlorination in Liver Microsomes. The NADH- and NADPH-dependent dechlorinating activities in liver microsomes of untreated rats were enhanced by the addition of FMN. Even when the microsomal fraction was boiled, the activities with both NAD(P)H and FMN were not abolished but were enhanced to some extent (Table 1). The activity was sensitive to inhibition by carbon monoxide. When FMN was replaced with flavin adenine dinucleotide or riboflavin, a similar result was obtained in the dechlorination of \( p,p' \)-DDT (data not shown). These facts suggest that CYP is also involved in the nonenzymatic dechlorination of \( p,p' \)-DDT in the liver microsomes of rats.

\( p,p' \)-DDT was also reduced to \( p,p' \)-DDD by rat blood in the presence of both NAD(P)H and FMN at the rate of about 200 nmol/10 min/ml. In this case, the activity was enhanced about 10 times by boiling the blood (data not shown). Hematin showed significant dechlorinating activity in the presence of both NADH and FMN (7.3 \( \pm \) 1.8 nmol/10 min/nmol heme). The dechlorination of \( p,p' \)-DDT by hematin was also observed in the presence of the photochemically reduced form of FMN (10.4 \( \pm \) 1.7 nmol/10 min/nmol heme). These results led us to conclude that \( p,p' \)-DDT can also be reduced to \( p,p' \)-DDD nonenzymatically via the catalytic action of the heme group of hemoproteins, such as CYP and cytochrome \( b_5 \), in the liver microsomes of rats in the presence of both a flavin and a reduced pyridine nucleotide, which can be replaced with a reduced flavin.

Reductive Metabolism of \( o,p' \)-DDT by Liver Microsomes. \( o,p' \)-DDT, the isomer of \( p,p' \)-DDT, is known to contaminate technical-grade DDT and to have a higher estrogenic activity than \( p,p' \)-DDT. In this study, the dechlorinating activity toward \( o,p' \)-DDT by rat liver microsomes was compared with that toward \( p,p' \)-DDT. The liver microsomes exhibited reductase activity toward \( o,p' \)-DDT in the presence of NADPH, NADH, or both NADPH and NADH. The reductase activity in the presence of these cofactors toward \( o,p' \)-DDT was similar to that in the case of \( p,p' \)-DDT. Similar reducing activity of boiled liver microsomes was observed in the presence of NADPH and FMN (Fig. 6).

Metabolism of \( p,p' \)-DDD by Liver Microsomes. Metabolism of \( p,p' \)-DDD by rat liver microsomes was also examined. When \( p,p' \)-DDD was incubated with liver microsomes in the presence of NADPH under anaerobic conditions, two peaks corresponding to authentic \( p,p' \)-DDE and \( p,p' \)-DDMU were detected in the HPLC chromatogram of the extract of the incubation mixture (Fig. 7). The metabolites isolated by HPLC were identified unequivocally as \( p,p' \)-DDE and \( p,p' \)-DDMU by comparison with authentic samples (data not shown). When \( p,p' \)-DDE was incubated similarly with liver microsomes in the presence of NADPH, \( p,p' \)-DDMU was not detected (data not shown).

Discussion

It is known that halogenated hydrocarbons, including \( p,p' \)-DDT, are metabolized to dehalogenated metabolites by reductive dehalogenation. Previous studies showed that the dehalogenation of carbon tetrachloride, hexachloroethane, halothane, or (\( \alpha \)-bromoisovaleryl)urea occurred in the liver microsomal fraction of mammalian species and that cytochrome P450 was involved in the microsomal dehalogenation (Fujii et al., 1981; Kubic and Anders, 1981; Ahr et al., 1982; Nastainczyk et al., 1982; Oka et al., 1996). Recent experiments have demonstrated that rat liver microsomes can directly transform halothane to dehalogenated metabolites in a reductive reaction involving the formation of a halothane-free radical intermediate (Baker and Van Dyke, 1984). In contrast, it has been shown that \( p,p' \)-DDT is mainly metabolized to \( p,p' \)-DDE and \( p,p' \)-DDD by reductive dechlorination and dehydrochlorination, respectively. In the reduction of \( p,p' \)-DDT...
to p,p′-DDD, Kelner et al. (1986) demonstrated that thiols stimulated the reductive dechlorination in liver microsomes. It was also reported that the pesticide covalently bound to microsomal protein and lipids owing to the formation of a DDT free-radical intermediate that led to the formation of p,p′-DDD (Baker and Van Dyke, 1984). However, the CYP isoform(s) catalyzing the reduction have not been identified.

Some studies on CYP isoforms involved in the reductive dehalogenation of halogenated hydrocarbons have been conducted. Spracklin et al. (1996) demonstrated that the dehalogenation of halothane is mainly catalyzed by CYP 2A6 and 3A4. Reductive dehalogenation of hexachloroethane is catalyzed CYP 1A2 (Yanagita et al., 1998). Oka et al. (1996) also demonstrated that debromination of (α-bromoisovaleryl)urea is catalyzed by CYP 1A1 and 2B1. In the present study, the dechlorination of p,p′-DDT was demonstrated to be mainly mediated by CYP 2B1, 3A1, 2B6, and 3A4. It is known that p,p′-DDT induces CYP 2B1 and 4A1 in rat livers (Lewis and Lake, 1997; Nims et al., 1998). This suggests that the dechlorinating activity toward p,p′-DDT would be enhanced by the pretreatment of rats with p,p′-DDT. Indeed, in a preliminary study in which rats received intraperitoneal p,p′-DDT at a dose of 100 mg/kg for 3 days before sacrifice, the liver microsomes exhibited 5-fold higher activity than those of untreated rats. In contrast, in the liver microsomal reduction of p,p′-DDT, NADH was about half as effective as NADPH. We previously observed that NADH was about half as effective as NADPH in N-oxide reduction, nitro reduction, and zonisamide reduction by liver microsomes of animals (Kitamura and Tsutsumi, 1984; Sugihara et al., 1996; Tsutsumi et al., 1986). NADH seems to be generally effective in drug-reductive metabolism with liver microsomes. In the NADH-dependent reaction, the electron may be transferred through cytochrome b5.

Clark and Shamaan (1984) reported that p,p′-DDT was metabolized to p,p′-DDE by glutathione-dependent dehydrochlorinase in the housefly. They demonstrated that the enzyme was identical to glutathione S-transferase. However, the identity of dehydrochlorinase and glutathione S-transferase has not yet been established. Dehydrochlorination of lindane was also reported to be catalyzed by glutathione-dependent dehydrochlorinase in the housefly (Tanaka et al., 1981). In our experiment, CYP 1A1, 2B1, 3A1, and 2E1 did not catalyze the dehydrochlorination (data not shown). We could not confirm a role of CYP in the dehydrochlorination of p,p′-DDT to p,p′-DDE. In contrast, p,p′-DDD was enzymatically metabolized to p,p′-DDE by rat liver microsomes or cytosol, but only to a small extent. Thus, p,p′-DDE may be formed via p,p′-DDD by an unknown enzyme system in rat liver. p,p′-DDMU was also formed from p,p′-DDD by liver microsomes. This dehydrochlorination may be catalyzed by liver dehydrochlorinase, similar to the case of p,p′-DDD. In contrast, it was reported that p,p′-DDE was dechlorinated to p,p′-DDMU by marine sediment microcosms (Quensen et al., 1998). However, this dechlorinating metabolism of p,p′-DDE to p,p′-DDMU did not occur in rat liver (Fig. 8). It is reported that the major metabolite of p,p′-DDT remaining in tissues of mammalians is p,p′-DDE (You, 2000). It remains to be determined whether p,p′-DDE is formed via p,p′-DDD, as suggested above or by dehydrochlorinase, as has been demonstrated in insects. We are conducting a further study on this point.

Recent experiments have demonstrated that rat blood can transform p,p′-DDT to p,p′-DDD in a reductive reaction involving the heme...
moiety of hemoglobin (Sugihara et al., 1998). The microsomal NAD(P)H-dependent reduction seems to proceed enzymatically, catalyzed by the CYP system. However, when a flavin was added to the mixture, the reducing activity was enhanced and was nonenzymatic in character. The nonenzymatic dechlorination of p,p'-DDT in liver microsomes presented in this study seems to proceed in two steps. The first step is the reduction of a flavin by NADPH-cytochrome P450 reductase with NADPH or NADH-cytochrome b5 reductase with NADH, whereas in boiled microsomes, the flavin seems to be reduced nonenzymatically by NADPH or NADH, as reported by Singer and Kearney (1950). The second step is the nonenzymatic reduction of p,p'-DDT to p,p'-DDD by the reduced flavin catalyzed by the heme group of hemoproteins, such as CYP and cytochrome b5 (Fig. 7). In boiled microsomes, the activity was increased, possibly because the substrate and cofactor have easier access to the heme moiety of denatured hemoproteins. We proposed a similar mechanism for the dechlorination of (α-bromoisovaleryl)urea in the blood of rats (Kitamura et al., 1999a). We also demonstrated that the major route of dechlorination of p,p'-DDT in fish was a nonenzymatic reaction by blood catalyzed by heme (Kitamura et al., 1999b). Other investigators demonstrated that reduced iron porphyrins catalyze the reductive dechlorination of p,p'-DDT (Castro, 1964; Zoro et al., 1974). Here, we suggest that the dechlorination of p,p'-DDT is also mediated by nonenzymatic reduction in liver microsomes separately from the enzymatic reduction by the CYP system.

Bitman et al. (1968) reported that indicators of estrogenic action increased after o,p'-DDT administration to rats and birds, the responses resembling those to 17β-estradiol, whereas p,p'-DDT exhibited only slight activity. Metcafe et al. (2000) also reported that gonadal development and endocrine responses were altered in fish exposed to o,p'-DDT. Furthermore, the relative potencies for intersex induction are o,p'-DDT > nonylphenol. However, the metabolism of o,p'-DDT has not been investigated except for a study in pigeon liver preparations (Hassall and Manning, 1972). In this study, we demonstrated that o,p'-DDT is dechlorinated by rat liver microsomes via enzymatic and nonenzymatic reactions in a similar ratio to p,p'-DDT. This fact suggests that o,p'-DDT is metabolized and degraded in the environment similar to p,p'-DDT.

It is reported that dicofol, α-hydroxy-p,p'-DDT, has a relatively high estrogenic activity among DDT analogs. Kapoor et al. (1972) reported that dicofol was detected as a minor urinary metabolite of dicofol using rat liver microsomes as a metabolic activation pathway. In our experiments, we could not detect any oxidized metabolite of p,p'-DDT. The bridge site of p,p'-DDT may be protected from attack because of steric hindrance. The possibility remains that aromatic rings of DDTs may be hydroxylated by liver microsomal enzymes. However, Sundström et al. (1977) reported that only small amounts of 3-hydroxy-p,p'-DDE were formed from p,p'-DDT in pigs. In our experiments, it was demonstrated that the metabolism of p,p'-DDT or o,p'-DDT in rats is mainly reductive.

References


Kitamura S and Tatsumi K (1984) Reduction of tertiary amine (α-bromoisovaleryl)urea in the blood of rats (Kitamura et al., 1999a). We also demonstrated that the major route of dechlorination of p,p'-DDT in fish was a nonenzymatic reaction by blood catalyzed by heme (Kitamura et al., 1999b). Other investigators demonstrated that reduced iron porphyrins catalyze the reductive dechlorination of p,p'-DDT (Castro, 1964; Zoro et al., 1974). Here, we suggest that the dechlorination of p,p'-DDT is also mediated by nonenzymatic reduction in liver microsomes separately from the enzymatic reduction by the CYP system.

Bitman et al. (1968) reported that indicators of estrogenic action increased after o,p'-DDT administration to rats and birds, the responses resembling those to 17β-estradiol, whereas p,p'-DDT exhibited only slight activity. Metcafe et al. (2000) also reported that gonadal development and endocrine responses were altered in fish exposed to o,p'-DDT. Furthermore, the relative potencies for intersex induction are o,p'-DDT > nonylphenol. However, the metabolism of o,p'-DDT has not been investigated except for a study in pigeon liver preparations (Hassall and Manning, 1972). In this study, we demonstrated that o,p'-DDT is dechlorinated by rat liver microsomes via enzymatic and nonenzymatic reactions in a similar ratio to p,p'-DDT. This fact suggests that o,p'-DDT is metabolized and degraded in the environment similar to p,p'-DDT.

It is reported that dicofol, α-hydroxy-p,p'-DDT, has a relatively high estrogenic activity among DDT analogs. Kapoor et al. (1972) reported that dicofol was detected as a minor urinary metabolite of p,p'-DDT in mice. We also examined the oxidation of p,p'-DDT to dicofol using rat liver microsomes as a metabolic activation pathway. However, we could not detect any oxidized metabolite of p,p'-DDT. The bridge site of p,p'-DDT may be protected from attack because of steric hindrance. The possibility remains that aromatic rings of DDTs may be hydroxylated by liver microsomal enzymes. However, Sundström et al. (1977) reported that only small amounts of 3-hydroxy-p,p'-DDE were formed from p,p'-DDT in pigs. In our experiments, it was demonstrated that the metabolism of p,p'-DDT or o,p'-DDT in rats is mainly reductive.

References

