Estrogenic Activity of Styrene Oligomers after Metabolic Activation by Rat Liver Microsomes

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In this study we examined estrogenic activity of styrene oligomers after metabolic activation by rat liver microsomes. trans,1,2-Diphenylcyclobutane (TCB), cis,1,2-diphenylcyclobutane (CCB), 1,3-diphenylpropane, 2,4-diphenyl-1-butene, 2,4,6-triphenyl-1-hexene, and 1α-phenyl-4β-(1-phenylethyl)tetralin were negative in the yeast estrogen screening assay and estrogen reporter assay using estrogen-responsive human breast cancer cell line MCF-7. However, TCB exhibited estrogenic activity after incubation with liver microsomes of phenobarbital-treated rats in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH). Minor activity was observed when liver microsomes of untreated or 3-methylcholanthrene-treated rats were used instead of those from phenobarbital-treated rats. CCB, 1,3-diphenylpropane, and 2,4-diphenyl-1-butene also exhibited estrogenic activity after metabolic activation by liver microsomes, but the activity was lower than that of TCB. 2,4,6-Triphenyl-1-hexene and 1α-phenyl-4β-(1-phenylethyl)tetralin did not show estrogenic activity after such incubation. When TCB was incubated with liver microsomes of phenobarbital-treated rats in the presence of NADPH, three metabolites were detected by high-performance liquid chromatography (HPLC). One metabolite isolated by HPLC exhibited a significant estrogenic activity. The active metabolite was identified as trans-1-(4-hydroxyphenyl)-2-phenylcyclobutane by mass and nuclear magnetic resonance spectral analysis. These results suggest that the estrogenic activity of TCB was caused by the formation of the 4-hydroxylated metabolite. Key words: trans,1,2-diphenylcyclobutane, endocrine disruption, estrogenic activity, human breast cancer cell line MCF-7, metabolic activation, rat liver microsomes, styrene oligomer, yeast estrogen screening assay. Environ Health Perspect 111:329–334 (2003). doi:10.1289/ehp.5723 available via http://dx.doi.org/ [Online 1 November 2002]

Styrene oligomers—such as trans,1,2-diphenylcyclobutane (TCB), cis,1,2-diphenylcyclobutane (CCB), 1,3-diphenylpropane, 2,4-diphenyl-1-butene, 2,4,6-triphenyl-1-hexene, and 1α-phenyl-4β-(1-phenylethyl)tetralin—are incorporated into polystyrene resin as impurities in the course of manufacture and may have a variety of biologic actions, including hormonal activity (Kawamura et al. 1998b, 1998c). Polystyrene has been used to manufacture food containers for takeout, such as coffee cups, meat trays, salad boxes, and soup bowls, as well as containers in which instant foods such as noodles, pasta, and rice are cooked by adding hot water. Some reports indicate that styrene oligomers migrate from these containers into the food contents (Kawamura et al. 1998b; Sakamoto et al. 2000). Kawamura et al. (1998b) reported that the quantity of migrated styrene trimers was higher than that of bisphenol A transferred from the lacquer coating of vegetable cans to the food contents, as reported by Breton et al. (1995). Some manmade compounds mimic the effect of estradiol; they include chlorinated organics, such as the insecticides kepone, α,p′-DDT (α,p′-dichlorodiphenyldichloroethane), p,p′-DDE (p,p′-dichlorodiphenyldichloroethane), and dieldrin and some polychlorinated biphenyl congeners, as well as nonchlorinated compounds such as the plasticizer bisphenol A and the surfactant breakdown product nonylphenol (Colborn 1995; Andersen et al. 1999). Such compounds can accumulate in our environment, and these so-called environmental estrogens may play a role in the increasing incidence of breast cancer, testicular cancer, and other problems of the reproductive system. Quantitative structure–activity relationship work on the structural features of estrogen receptor ligands shows that an unhindered hydroxy group on an aryl ring and a hydrophobic group attached para to the hydroxyl group are essential (Anstead et al. 1997; Fang et al. 2000; Shi et al. 2001; Hong et al. 2002). The ligand binding assay and studies in a reporter/transcriptional system for the estrogen receptor support the requirement for these structural features (Nishihara et al. 2000; Blair et al. 2000; Branham et al. 2002). Recently, we showed that trans-stilbene, the parent compound of diethylstilbestrol, was not estrogenic but exhibited a potent estrogenic activity after metabolic activation by a liver microsomal oxidation system (Sugihara et al. 2000). In that report, we suggested that the estrogenic activity of trans-stilbene was caused by hydroxylated metabolites. TCB, a styrene dimer, may also be converted to an estrogen by metabolic activation, based on the similarity of its structure to that of trans-stilbene.

For this report, we examined the estrogenic activities of styrene oligomers in the presence or absence of a liver microsomal metabolic system using a yeast estrogen screening (YES) assay and an estrogen-responsive element (ERE)-luciferase reporter assay in the estrogen-responsive human breast cancer cell line MCF-7. We examined TCB, CCB, 1,3-diphenylpropane, 2,4-diphenyl-1-butene, 2,4,6-triphenyl-1-hexene, and 1α-phenyl-4β-(1-phenylethyl)tetralin in this study (Figure 1). We found that some styrene oligomers, especially TCB, exhibit significant estrogenic activities after activation by rat liver microsomal mixed function oxidase.

Materials and Methods

Chemicals. TCB (99.3%), CCB (99.9%), 1,3-diphenylpropane (98.1%), 2,4-diphenyl-1-butene (96.1%), 2,4,6-triphenyl-1-hexene (99.4%), and 1α-phenyl-4β-(1-phenylethyl)tetralin (99.1%) were obtained from Kanto Chemical Co. Inc. (Tokyo, Japan), and 17β-estradiol (E2; > 98%) and tamoxifen (> 99%) from Sigma Chemical Co. (St. Louis, MO, USA). Chlorophenol red β-galactopyranoside (CPRG) was obtained from Roche Diagnostics (Mannheim, Germany). We obtained trans-1,2-bis-(4-hydroxyphenyl)cyclobutane (96%) by isomerization of cis-1,2-bis-(4-hydroxyphenyl)cyclobutane by the method of Williard and Fryhle (1980), and synthesized cis-1,2-bis-(4-hydroxyphenyl)cyclobutane according to the method of Brown (1968).

Animals. Male Sprague-Dawley rats (190–220 g) were obtained from Japan SLC, Inc. (Shizuoka, Japan). The animals were housed at 22°C with a 12-hr light/dark cycle, with free access to tap water and a standard

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pellet diet (MM-3; Funabashi Farm, Funabashi, Japan). In some experiments, rats were given intraperitoneal administration of phenobarbit al (80 mg/kg) or 3-methylcholanthrene (25 mg/kg) daily for 3 days before use.

Preparation of liver microsomes. The livers were excised from exsanguinated rats and immediately perfused with 1.15% KCl. The livers were homogenized in four volumes of the KCl solution using a Potter-Elvehjem homogenizer (Iwaki Glass Co., Ltd., Tokyo, Japan). The microsomal fraction was obtained from the homogenate by successive centrifugation at 9,000 g for 20 min and 105,000 g for 60 min. The fraction was washed by resuspension in the KCl solution and resedimentation. The pellets of microsomes were resuspended in the solution to make 1 mL equivalent to 1 g of liver. Protein contents in the liver microsomal preparation of untreated, phenobarbital-treated, and 3-methylcholanthrene-treated rats were 13.7–17.6, 13.9–15.4, and 6.18–8.16 mg/g, respectively.

Assay of TCB oxidase activity. An incubation mixture consisted of 0.1 µmol TCB, 0.5 µmol NADPH, and 0.02 mL liver microsomes equivalent to 20 mg liver wet weight (0.2–0.3 mg protein) in a final volume of 1 mL 0.1 M K,Na-phosphate buffer (pH 7.4). The incubation was performed at 37°C for 5 min. After incubation, 0.1 µmol trans-stilbene oxide as an internal standard was added, and the mixture was extracted with 5 mL ethyl acetate. The extract was evaporated to dryness, the residue was dissolved in 0.1 mL methanol, and an aliquot (5 µL) was analyzed by HPLC.

HPLC and thin-layer chromatography. HPLC was performed in a Hitachi L-6000 chromatograph (Tokyo, Japan) fitted with a 150 × 4.6 mm Inertsil ODS-3 column (GL Science, Tokyo, Japan). The mobile phase was acetonitrile–water (6:4, vol/vol). The chromatograph was operated at a flow rate of 0.5 mL/min at a wavelength of 240 nm. The elution times of trans-1-(4-hydroxyphenyl)-2-phenylcyclobutane, TCB, CCB, and trans-stilbene oxide (an internal standard) were 14.6, 68.0, 55.3, and 25.2 min, respectively. For the detection of trans-1,2-bis-(4-hydroxyphenyl)cyclobutane, the mobile phase was acetonitrile–water (3:7, vol/vol). The chromatograph was operated at a flow rate of 0.5 mL/min at a wavelength of 240 nm. The elution time of trans-1,2-bis-(4-hydroxyphenyl)cyclobutane was 12.2 min.

Silica gel plates (Kieselgel 60 GF254, 0.1 mm thick; Merck, Darmstadt, Germany) were developed in n-hexane–ethyl acetate (9:1, vol/vol). Spots were visualized under ultraviolet light (254 nm). The Rf (rate of extraction) of the metabolite was measured using a Dual Luciferase assay kit (Promega Co.) and the values were calculated as follows: Net OD540 = (OD540 for test – OD620 for test) – (OD540 for blank – OD620 for blank).

Assay of estrogenic activities of styrene oligomers in MCF-7 cells. For the assay, recombinant yeast strain transfected with the human estrogen receptor (ERα) gene and expression plasmids carrying the reporter gene lac-Z preceded by ERE were kindly provided by J. Sumpter (Brunel Laboratories, Tokyo, Japan). The yeast estrogenicity assay was conducted as described by Routledge and Sumpter (1996, 1997), with some minor modifications. In brief, a styrene oligomer mixture was read using a Dual Luciferase assay kit (Promega Co.) as an internal standard. The plates were maintained at 37°C for 20 min and 105,000 g for 60 min.

Isolation of TCB metabolites from the incubation mixture of rat liver microsomes. The incubation mixture consisted of 12 µmol TCB, 30 µmol NADPH, and 1.2 g liver microsomes equivalent to 1.2 g liver (wet weight) in a final volume of 20 mL of 0.1 M phosphate buffer (pH 7.4). The incubation was performed at 37°C for 30 min. Then, the mixture was extracted twice with two volumes of ethyl acetate. The combined extract was extracted with 5 mL of ethyl acetate and the organic solution was evaporated to dryness. The residue was dissolved with 1 mL of ethanol and an aliquot was used for the estrogenic activity assay. The total concentration of the substrate and its metabolites was calculated from the original amount of the substrate. In some experiments, tamoxifen was added at the concentration of 1 × 10–8 M to wells as an antioestrogen.

Figure 1. Structures of styrene oligomers.
flow) values of TCB, trans-1-(4-hydroxyphenyl)-2-phenylcyclobutane, and trans-1,2-bis-(4-hydroxyphenyl)cyclobutane were 0.78, 0.44, and 0.17, respectively.

Mass and nuclear magnetic resonance spectra. A Japan Electron Optics Laboratory HX-100 mass spectrometer (JEOL, Tokyo, Japan) in electron impact mode was used for structure analysis of the metabolites. Mass spectroscopy was carried out at a collision energy of 70 eV and an acceleration voltage of 10 kV.

Results

Estrogenic activity of TCB and CCB with or without a microsomal activation system. Estrogenic activity of TCB and CCB in the presence or absence of a rat liver microsomal oxidation system was examined in the YES assay. These compounds did not show estrogenic activity in this assay. However, when TCB was incubated with liver microsomes of phenobarbital-treated rats in the presence of NADPH, the extract of the incubation mixture exhibited an estrogenic effect on the cells in the range of $10^{-3}$ to $10^{-6}$ M. In contrast, little effect was observed when liver microsomes of untreated or 3-methylcholanthrene–treated rats were used instead of those from phenobarbital-treated rats. CCB showed estrogenic activity after incubation with the liver microsomes of 3-methylcholanthrene–treated rats, but the activity was much lower than that of TCB (Figure 2). These estrogenic activities were inhibited by the addition of tamoxifen at the concentration of $1 \times 10^{-6}$ M (data not shown).

Estrogenic activity of 1,3-diphenylpropane, 2,4-diphenyl-1-butene, 2,4,6-triphenyl-1-hexene, and 1α-phenyl-4β-(1′-phenylethyl)tetralin with or without a microsomal activation system. 1,3-Diphenylpropane, 2,4-diphenyl-1-butene, 2,4,6-triphenyl-1-hexene, and 1α-phenyl-4β-(1′-phenylethyl)tetralin were negative in the YES assay, except for marginal activity at higher concentrations. However, 1,3-diphenylpropane exhibited estrogenic activity after incubation with rat liver in the presence of microsomes of untreated and 3-methylcholanthrene–treated rats in the presence of NADPH. Lower activity was observed after incubation with liver microsomes of phenobarbital-treated rats. 2,4-Diphenyl-1-butene showed estrogenic activity after incubation with liver microsomes of 3-methylcholanthrene–treated rats. Little effect was obtained when liver microsomes of untreated or phenobarbital-treated rats were used instead of those from 3-methylcholanthrene–treated rats. In contrast, 2,4,6-triphenyl-1-hexene and 1α-phenyl-4β-(1′-phenylethyl)tetralin did not show estrogenic activity after similar incubation (Figure 3).

These facts suggest that some styrene oligomers, especially TCB, exhibit estrogenic activity after metabolic activation by rat liver microsomes.

Metabolism of TCB by rat liver microsomes. TCB was incubated with liver microsomes of phenobarbital-treated rats in the presence of NADPH for the detection of the metabolites as described in “Materials and Methods.” Three peaks were detected in an HPLC chromatogram of the extract of the TCB incubation mixture (Figure 4). These peaks were not detected in the control, which was incubated without the substrate. The metabolites of TCB detected at 7.2, 10.9, and 14.6 min were designated TCB metabolites 1, 2, and 3 (TD-M1, -M2, and -M3, respectively).

Identification of estrogenic active metabolites of TCB generated by rat liver microsomes. After the incubation of TCB with liver microsomes of phenobarbital-treated rats in the presence of NADPH, HPLC fractions were collected for the identification of the active metabolites. The fraction corresponding to TD-M3 exhibited estrogenic activity (Figure 4). The mass spectrum of TD-M3 showed signals at 6.75 (doublet, 2H, J = 8 Hz, phenyl groups), 7.09 (doublet, 2H, J = 8 Hz, phenyl groups), 7.24 (multiplet, 5H, phenyl groups), and 8.00 (singlet, 1H, OH). The further oxidized metabolite, trans-1,2-bis-(4-hydroxyphenyl)cyclobutane, could not be detected by HPLC or thin-layer chromatography as a metabolite of TCB. These facts suggest that TCB is mainly metabolized to trans-1-(4-hydroxyphenyl)-2-phenylcyclobutane by rat liver microsomes.

Oxidase activities of rat liver microsomes toward TCB. The oxidative activity of rat liver microsomes transforming TCB to TD-M3 was examined using liver microsomes of phenobarbital-treated rats. The time course of formation of the hydroxylated metabolite was essentially linear for 10 min (Figure 5A). The oxidative activity of the liver microsomes increased linearly with increasing amount of liver microsomes up to 0.5 mg of protein (Figure 5B).

Liver microsomes of phenobarbital-treated rats exhibited the highest oxidative activity in the presence of NADPH. The NADPH-linked activity was inhibited by the addition of SKF 525-A (1 × 10^{-4} M). Little oxidative activity was observed when liver microsomes of untreated or 3-methylcholanthrene–treated rats were used instead of those of phenobarbital-treated rats (Figure 5C). Only marginal activity was observed with reduced nicotinamide adenine dinucleotide (NADH) instead of NADPH (data not shown). This suggests that TCB was mainly metabolized to trans-1-(4-hydroxyphenyl)-2-phenylcyclobutane by cytochrome P450 2B1 in rats.

Estrogenic activity of styrene oligomers in estrogen reporter assay using MCF-7 cells. Estrogenic activity of styrene oligomers in the presence or absence of a rat liver microsomal oxidation system was also examined by ERE–luciferase reporter assay using MCF-7 cells. TCB, CCB, 1,3-diphenylpropane, and 2,4-diphenyl-1-butene were negative in this estrogen screening test, except for a marginal effect at $1 \times 10^{-5}$ M. When TCB was incubated with liver microsomes of phenobarbital-treated rats in the presence of NADPH, the extract of the incubation mixture exhibited estrogenic activity at the concentration of $1 \times 10^{-3}$ M, as in the YES assay. Lower activity was obtained in this assay when liver microsomes of untreated or 3-methylcholanthrene–treated rats were used instead of those from phenobarbital-treated rats. In contrast, CCB showed estrogenic activity after incubation with the liver microsomes of untreated, phenobarbital-treated, and 3-methylcholanthrene–treated rats, but these activities were lower than that of TCB. 1,3-Diphenylpropane exhibited estrogenic activity after incubation with liver microsomes of untreated and 3-methylcholanthrene–treated rats in the presence of NADPH.

2,4-Diphenyl-1-butene...
showed estrogenic activity after similar incubation with liver microsomes of 3-methylcholanthrene–treated rats (Figure 6). These estrogenic activities were completely inhibited by the addition of tamoxifen at the concentration of 1 × 10⁻⁶ M (data not shown).

Thus, the results of the estrogen screening test with MCF-7 cells confirm that some styrene oligomers exhibit estrogenic activity after metabolic activation by rat liver microsomes.

**Discussion**

Styrene oligomers such as TCB are by-products in the manufacture of polystyrene and may later be released from the resin (Kawamura et al. 1998b; Sakamoto et al. 2000). Nobuhara et al. (1999) reported that styrene oligomers did not induce the proliferation of MCF-7 cells. We also showed in this study that these compounds do not exhibit estrogenic activity in the YES assay or estrogen screening assay using MCF-7 cells. However, some of them yield estrogenic metabolites after metabolic activation. Ohyama et al. (2001) reported that some styrene dimers and trimers were estrogenic without metabolic activation in a cell proliferation assay with estrogen-responsive MCF-7 cells. They reported that TCB, CCB, 1,3-diphenylpropane, and 2,4-diphenyl-1-butene were positive without metabolic activation. We cannot explain the difference from our present results. However, one possibility is that because the substrates were in contact with MCF-7 cells for a long time in their assay, estrogenic metabolites were generated in the cells.

We present here the first evidence that TCB, CCB, 1,3-diphenylpropane, and 2,4-diphenyl-1-butene are converted to metabolites with estrogenic activity by liver microsomal enzymes. It has been reported that some styrene oligomers do not give a positive response in uterotrophic assay (Bachmann et al. 1998; Fai et al. 1998; Prinsen and Gouko 2001). However, the levels of active metabolites in the body would be an important factor determining the effects of the oligomers in vivo. The apparent affinities of such xenoestrogens for the estrogen receptor are lower than those of endogenous estradiol or phytoestrogens. Nevertheless, endogenous estrogens are tightly regulated in the body. We speculate that phytoestrogens, which are present in foods that have been consumed from the earliest times, may also be subject to regulation. This may not be the case for xenoestrogens, which may act directly on the endocrine organs. Further, xenoestrogens may be accumulated in the body; their intake amounts vary considerably among consumers, and the amounts released from polystyrene resin are variable. Styrene oligomers may exhibit estrogenic activity in combination with other xenoestrogens. Further work is necessary to assess the in vivo endocrine-disrupting action of styrene oligomers, taking into account the activities of the metabolites produced from the parent compounds.

We showed in previous reports that trans-stilbene and trans-stilbene oxide were oxidized to hydroxylated derivatives by rat liver microsomes (Sugihara et al. 2000; Sanoh et al. 2002). In those studies, we obtained evidence that trans-stilbene is oxidized to the 4-hydroxy and 4,4'-dihydroxy derivatives by cytochrome P450 1A1/2 in rat liver microsomes and is thereby activated to exhibit estrogenic activity. In contrast, cis-stilbene and cis-stilbene oxide exhibited only weak estrogenic activity even after metabolic activation. TCB structurally resembles trans-stilbene and trans-stilbene oxide, and this may be the reason why its metabolites exhibited the highest activity among the compounds tested in this study. As in the case of cis-stilbene and cis-stilbene oxide, the activities of the cis-isomers were much lower than those of the trans-compounds (Sugihara et al. 2000). The activity of CCB after activation is also lower than that of the trans-isomer.

We have shown here that trans-1-(4-hydroxyphenyl)-2-phenylcyclobutane is a major active metabolite of TCB in a rat liver microsomal system. Furthermore, formation of the metabolite was stimulated by the pretreatment of rats with phenobarbital. This suggests that cytochrome P450 2B isofrom is responsible for this activation. In a preliminary study, when TCB was incubated with microsomal preparations from cells expressing recombinant human cytochrome P450 2B6 and rat cytochrome P450 2B1 expressed in a

![Figure 3](image-url)
human B lymphoblastoid cell line (Gentest Corp., Woburn, MA, USA), cytochrome P450 2B6 exhibited a higher oxidase activity than did cytochrome P450 2B1. Significant oxidase activity was also observed in human liver microsomes (Gentest Corp.) (data not shown). Thus, cytochrome P450 isoforms other than cytochrome P450 2B may contribute to the metabolic activation in humans. In contrast, 2,4-diphenyl-1-butene was metabolically activated to an estrogenic derivative by liver microsomes of 3-methylcholanthrene–treated rats. In this case, cytochrome P450 1A may be mainly responsible for the activation. The oxidation of 2,4-diphenyl-1-butene was catalyzed by rat cytochrome P450 1A1/2. These isozymes are also known isoforms of cytochrome P450 in humans. It is thus possible that metabolic activation of these styrene oligomers to active estrogens occurs in humans. In contrast, activation of 1,3-diphenylpropane proceeded with liver microsomes of untreated and 3-methylcholanthrene–treated rats but not those of phenobarbital–treated rats. Possibly, the oxidative metabolism of 1,3-diphenylpropane by liver microsomes of phenobarbital–treated rats preferentially generated nonestrogenic metabolites. It is interesting that different styrene oligomers are activated by different cytochrome P450 isoforms, and the potencies of estrogenic activity of the metabolites formed are different.

A possible metabolic activation pathway of proestrogenic TCB and CCB with liver microsomes is shown in Figure 7. TCB is

![Figure 7](Image 224x71 to 565x230)

Figure 7. Proposed metabolic pathways for the activation of TCB and CCB in rats. CYP, cytochrome P450.
converted to hydroxylated derivatives by rat liver microsomes (pathways 1 and 2). In the microsomal system used in this study, the estrogenic activity of TCB is thought to be mainly exhibited by 2-tans-1-(4-hydroxyphenyl)-2-phenylcyclobutanone, because pathway 2 does not proceed effectively in this system. The further oxidized metabolite of trans-1-(4-hydroxyphenyl)-2-phenylcyclobutanone, butane, could not be detected. In contrast, CCB is thought to be metabolized to the corresponding hydroxylated metabolites with liver microsomes. However, the activity of CCB after metabolic activation was lower than that of the trans-isomer. cis-1-(4-Hydroxyphenyl)-2-phenylcyclobutanone may exhibit lower estrogenic activity than the trans-isomer. The activity of CCB after metabolic activation may be due to trans-1-(4-hydroxyphenyl)-2-phenylcyclobutanone formed after cis-trans isomerization (i.e., pathway 3). In our preliminary study, when CCB was incubated with liver microsomes without NADPH, a peak corresponding to TCB was detected by HPLC. The 2–3-hydroxylated metabolite of TCB may also be formed. However, relatively high activity was exhibited only by TD-M3 among the metabolites generated in the present oxidative system, as shown in Figure 4.

There are various other examples of metabolic activation to an estrogen, besides that of trans-stilbene, p,p′-DDT is metabolized to p,p′-DDE, p,p′-dichlordiphenyldichloroethyleno] by reductive dechlorination and dehydrochlorination, respectively (Esaca and Matsumura 1980; Kitamura et al. 2002). p,p′-DDE shows estrogenic activity and p,p′-DDE shows antiestrogenic activity (Kcel et al. 1995; Chen et al. 1997). Further, methoxychlor is a proestrogen that requires demethylation by liver microsomal mixed function oxidase in animals before eliciting estrogenic activity (Kupfer and Bulger 1987; Stresser and Kupfer 1998). It is known that PCBs are converted to hydroxylated metabolites in animals (Koga et al. 1999). Some hydroxylated PCBs show estrogenic activity (Korach et al. 1988; Connor et al. 1997; Garner et al. 1999). p-Hydroxybiphenyl, which is formed from benzo[a]pyrene, is an antifungal agent, in rat hepatocytes, is also estrogenic (Nakagawa and Tayama 2001; Nakagawa and Suzuki 2002). Hydroxylated metabolites of benzo[a]pyrene also exhibit estrogenic activity (Charles et al. 2000; Fertuck et al. 2001). Elsby et al. (2000, 2001) predicted estrogenicity by a two-stage approach coupling human liver microsomes and a yeast estrogenicity assay. Methoxychlor, methoxybiphenyl A, and 3,17β-bisdesoxyestradiol were positive, but 6-hydroxysteratin was negative, in this screening system. Much further work is needed to identify potentially hazardous prooestrogens in our environment.

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Anderssen HR, Andersen A-M, Arnold SF, Autrup H, Barfod M, Berrefoed NA, et al. 1999. Comparison of short-term estrogenic activity of TCB is thought to be mainly exhibited by trans-1-(4-hydroxyphenyl)-2-phenylcyclobutanone, because pathway 2 does not proceed effectively in this system. The further oxidized metabolite of trans-1-(4-hydroxyphenyl)-2-phenylcyclobutanone, butane, could not be detected. In contrast, CCB is thought to be metabolized to the corresponding hydroxylated metabolites with liver microsomes. However, the activity of CCB after metabolic activation was lower than that of the trans-isomer. cis-1-(4-Hydroxyphenyl)-2-phenylcyclobutanone may exhibit lower estrogenic activity than the trans-isomer. The activity of CCB after metabolic activation may be due to trans-1-(4-hydroxyphenyl)-2-phenylcyclobutanone formed after cis-trans isomerization (i.e., pathway 3). In our preliminary study, when CCB was incubated with liver microsomes without NADPH, a peak corresponding to TCB was detected by HPLC. The 2–3-hydroxylated metabolite of TCB may also be formed. However, relatively high activity was exhibited only by TD-M3 among the metabolites generated in the present oxidative system, as shown in Figure 4.

There are various other examples of metabolic activation to an estrogen, besides that of trans-stilbene, p,p′-DDT is metabolized to p,p′-DDE, p,p′-dichlordiphenyldichloroethyleno] by reductive dechlorination and dehydrochlorination, respectively (Esaca and Matsumura 1980; Kitamura et al. 2002). p,p′-DDE shows estrogenic activity and p,p′-DDE shows antiestrogenic activity (Kcel et al. 1995; Chen et al. 1997). Further, methoxychlor is a proestrogen that requires demethylation by liver microsomal mixed function oxidase in animals before eliciting estrogenic activity (Kupfer and Bulger 1987; Stresser and Kupfer 1998). It is known that PCBs are converted to hydroxylated metabolites in animals (Koga et al. 1999). Some hydroxylated PCBs show estrogenic activity (Korach et al. 1988; Connor et al. 1997; Garner et al. 1999). p-Hydroxybiphenyl, which is formed from benzo[a]pyrene, is an antifungal agent, in rat hepatocytes, is also estrogenic (Nakagawa and Tayama 2001; Nakagawa and Suzuki 2002). Hydroxylated metabolites of benzo[a]pyrene also exhibit estrogenic activity (Charles et al. 2000; Fertuck et al. 2001). Elsby et al. (2000, 2001) predicted estrogenicity by a two-stage approach coupling human liver microsomes and a yeast estrogenicity assay. Methoxychlor, methoxybiphenyl A, and 3,17β-bisdesoxyestradiol were positive, but 6-hydroxysteratin was negative, in this screening system. Much further work is needed to identify potentially hazardous prooestrogens in our environment.