— Review —

Metabolic Activation of Proestrogens in the Environment by Cytochrome P450 System

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(Received March 14, 2008)

Liver microsome-mediated activation of proestrogens in the environment is reviewed here. Proestrogens such as methoxychlor, *trans*-stilbene, diphenyl, diphenylmethane, 2,2-diphenylpropane, benzo[*a*]pyrene, benzophenone, 2-nitrofluorene (NF), chalcone, *trans*-4-phenyl-3-buten-2-one and styrene oligomers are negative in *in vitro* estrogen screening tests. However, those proestrogens exhibit estrogenic activity after metabolic activation by the microsomal cytochrome P450 system. In these cases, hydroxylated derivatives of the compounds are formed as major metabolites, and these metabolites exhibit significant estrogenic activities. Thus, the estrogenic activities of proestrogenic compounds are a consequence of metabolism of the parent compounds. Various candidates for proestrogens among medicines and insecticides are also discussed.

Key words —— estrogenic activity, proestrogen, metabolic activation, cytochrome P450, *trans*-stilbene, diphenyl, styrene oligomer

INTRODUCTION

Various man-made chemicals mimic the biological activity of hormones such as sex hormones and thyroid hormone, thereby interfering with hormone receptor function. These chemicals are called endocrine disrupters, and include various persistent chlorinated pesticides, such as 1,1,1-trichloro-2,2-bis(2-chlorophenyl-4-chlorophenyl)ethane (*o*,*p*′-DDT), dieldrin, kepone, methoxychlor and some polychlorinated biphenyl congeners, and industrial chemicals such as the plasticizer bisphenol A, the surfactant breakdown product nonylphenol and some polychlorinated biphenyl congeners.[1,2] Quantitative structure-activity relationship (QSAR) studies on the structural features of estrogen receptor ligands show that an unhindered hydroxyl group on an aryl ring and a hydrophobic group attached *para* to the hydroxyl group are essential.[3–6] Ligand binding assay and studies in a reporter/transcriptional system for the estrogen receptor support the requirement for these structural features.[7,8] Among such compounds, those which are lipophilic and persistent may be accumulated through the food web, posing a health threat to humans and animals. Endogenous estrogens have been shown to have multiple sites of activity and to exert biological effects. Many so-called xenoestrogens produce a wide variety of toxic effects in animals.

Xenoestrogens can accumulate in our environment, and may play a role in the increasing incidence of breast cancer, testicular cancer, and other problems of the reproductive system in humans. It is therefore important to screen environmental contaminants for estrogenic activity. Their metabolites also need to be identified and screened in order to identify proestrogens, which are activated to estrogens by metabolic systems. Several reports indicate that proestrogens, which act as xenoestrogens after metabolic activation, exist in the environment.[9–16] The potential of these proestrogens, such as methoxychlor, for endocrine disruption needs to be assessed.

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In this review, we cover environmental compounds that are not directly estrogenic, but which are activated by the liver microsomal metabolic system in the mammalian body after ingestion. These include methoxychlor, *trans*-stilbene, diphenyl, diphenylmethane, polycyclic aromatic hydrocarbons, nitropolycyclic aromatic hydrocarbons, α,β-unsatulated ketone, styrene oligomers and others.

ACTIVATION OF *TRANS*-STILBENE AND RELATED COMPOUNDS BY THE CYTOCHROME P450 SYSTEM

It is well known that *trans*-stilbene derivatives, such as diethylstilbestrol (DES), euvestin, 4,4′-diaminostilbene and pinosylvin, have a variety of biological actions, including hormonal, hypocholesterolemic, sympathomimetic, antifungal, antibacterial, antimalarial and anticancer activities.[17–19] DES, a potential estrogen, has been used medically as a substitute for endogenous estrogen and as a hormonal therapy for prostate or breast cancer, and also to prevent threatened abortions.[20, 21] However, it may induce vaginal adenocarcinoma.[22] Its estrogenic activity is similar to that of β-estradiol in *in vitro* estrogen screening tests. However, the estrogenic activity of *trans*-stilbene, which is the parent compound of stilbene derivatives and is used as an industrial material, has not been extensively examined.

trans-Stilbene is not estrogenic in the estrogen screening tests. However, when *trans*-stilbene was incubated with liver microsomes of 3-methylcholanthrene-treated rats in the presence of NADPH, the extract of the incubation mixture exhibited an estrogenic effect in the concentration range of 10^{-5}–10^{-6} M. In contrast, *cis*-stilbene showed little estrogenic activity after incubation with liver microsomes.[9] When *trans*-stilbene was incubated with liver microsomal enzyme system of 3-methylcholanthrene-treated rats, *trans*-4-hydroxystilbene and *trans*-4,4′-dihydroxystilbene were both formed, though *trans*-4-hydroxystilbene was predominant. Human cytochrome P450 1A1 and 1A2 isoforms expressed in human lymphoblastoid cells catalyzed both oxidations.[23] In the case of *cis*-stilbene, such hydroxylated metabolites were not detected (Fig. 1).

trans-4-Hydroxystilbene and *trans*-4,4′-dihydroxystilbene both showed estrogenic activity similar to that of DES in estrogen screening tests. Thus, *trans*-stilbene was converted to the active metabolites, *trans*-4-hydroxystilbene and *trans*-4,4′-dihydroxystilbene, by rat liver microsomes, and so the estrogenic activity of *trans*-stilbene might be due mainly to *trans*-4-hydroxystilbene, with some contribution from *trans*-4,4′-dihydroxystilbene. In contrast, *cis*-stilbene was not metabolized to the corresponding hydroxylated metabolites with liver microsomes (Fig. 1). In an *in vivo* estrogenicity test using ovariectomized (OVX) rats, *trans*-stilbene was positive, as well as 4-hydroxylated stilbene.[24] This shows that *trans*-stilbene exhibited estrogenic activity after metabolic activation *in vivo*.

It was demonstrated that resveratrol, which is a derivative of stilbene found in grapes and wine, is an agonist for the estrogen receptor.[25] Among stilbene-related compounds, metabolic activation of *trans*-stilbene oxide, *trans*-α-methylstilbene, tolan, dibenzyl and azobenzene to estrogenic compounds by the cytochrome P450 system was also demonstrated. These compounds did not show

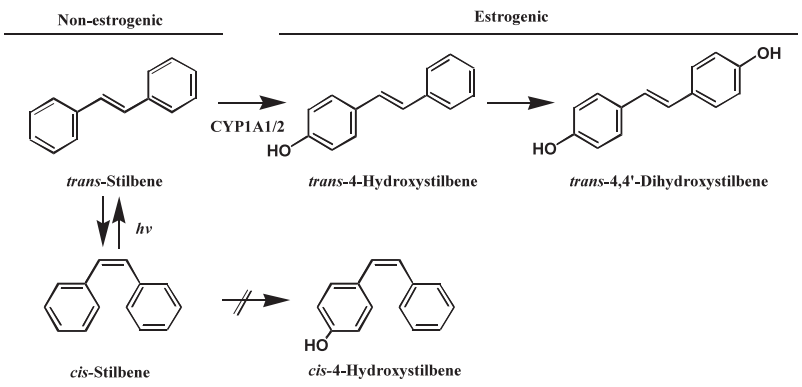

Fig. 1. Metabolic Pathways for the Activation of *trans*-Stilbene to Estrogens by the Cytochrome P450 System

estrogenic activity, but exhibited estrogenic activity after metabolic activation by liver microsomes from 3-methylcholanthrene- or phenobarbital-treated rats (Fig. 2).[26] Furthermore, the estrogenic activities of several stilbene derivatives were compared. The 4-hydroxyl group of the A-ring plays the most important role, but nitro and amino substituents also result in some estrogenic activity. The vinyl linkage is necessary for high activity. The hydrophobic B-ring plays an important role, because the estrogenic activity of hydroxystilbene is higher than that of hydroxystyrene. The structural requirements for the estrogenic activities of stilbene derivatives were proposed to be as shown in Fig. 3. A *p*-hydroxy group in the A-phenyl ring, vinyl linkage, a B-phenyl ring and hydrophobicity of the linkage are necessary for the maximal activity of stilbene derivatives.[26]

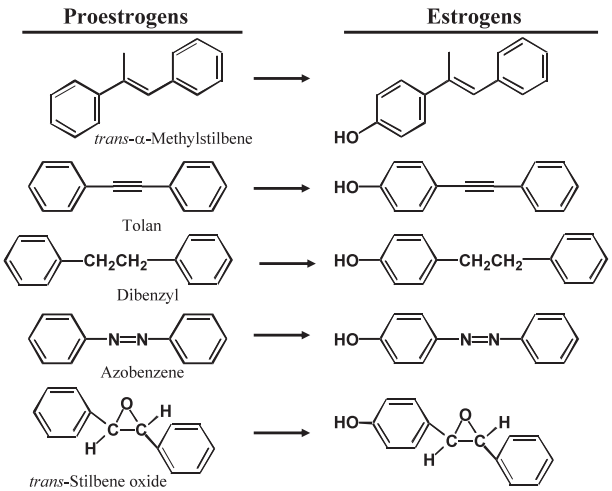

Fig. 2. Metabolic Activation of Stilbene-related Compounds to Estrogens

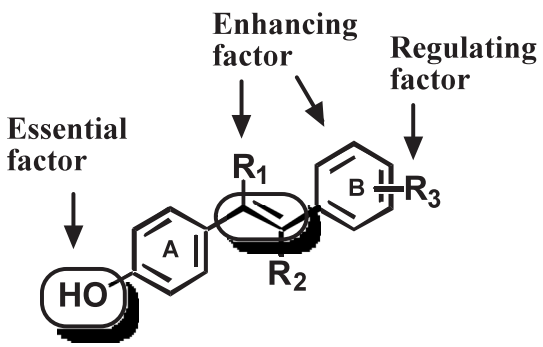

Fig. 3. Structural Requirement of Stilbene Derivatives for Estrogenic Activity

ACTIVATION OF DIPHENYL AND RELATED COMPOUNDS BY THE CYP1A1/2 AND 2B1 SYSTEMS

Diphenyl is used as an antifungal agent for citrus fruits, and also as wrapping paper for impregnated fruit. Under improper conditions, it has been toxic to production workers.[27, 28] Diphenylmethane is also used as a dye carrier and synthetic intermediate, similarly to diphenyl. Diphenyl is metabolized to hydroxylated diphenyls. 4-Hydroxydiphenyl, 4,4′-dihydroxydiphenyl and 3,4-dihydroxydiphenyl were identified as urinary metabolites of diphenyl in experimental animals.[29] 2-Hydroxydiphenyl, 2,4′-dihydroxydiphenyl and 3-hydroxydiphenyl were also identified as *in vitro* metabolites with liver microsomes of various animals.[30] These phenylphenols are used as household insecticides, especially for indoor applications,[31] and as intermediates in the manufacture of rubber and resins.

Diphenyl, diphenylmethane and 2,2-diphenylpropane were negative in estrogen screening tests. However, they exhibited estrogenic activity after incubation with liver microsomes of 3-methylcholanthrene-treated rats in the cases of diphenyl and diphenylmethane, or after incubation with liver microsomes of phenobarbital-treated rats in the cases of diphenyl and 2,2-diphenylpropane.[10] When diphenyl was incubated with liver microsomes of phenobarbital- and 3-methylcholanthrene-treated rats in the presence of NADPH for the detection of the estrogenic metabolites, four metabolites (2-hydroxydiphenyl, 3-hydroxydiphenyl, 4-hydroxydiphenyl and 4,4′-dihydroxydiphenyl) were detected. 4,4′-Dihydroxydiphenylmethane and 4-hydroxydiphenylmethane were also detected as metabolites of diphenylmethane with liver microsomes of 3-methylcholanthrene-treated rats. Bisphenol A [2,2-bis(4-hydroxyphenyl)propane] and 2-(4-hydroxyphenyl)-2-phenylpropane were detected as metabolites of 2,2-diphenylpropane with liver microsomes of phenobarbital-treated rats. The estrogenic activity of bisphenol A, which is an active metabolite of 2,2-diphenylpropane in this case, is well-known. However, the amounts of monohydroxyl derivatives of these compounds were much higher than those of the 4,4′-dihydroxyl derivatives (bisphenol A).

These hydroxylated derivatives all showed estrogenic activity. However, 2-hydroxydiphenyl and 3-hydroxydiphenyl showed lower activities than

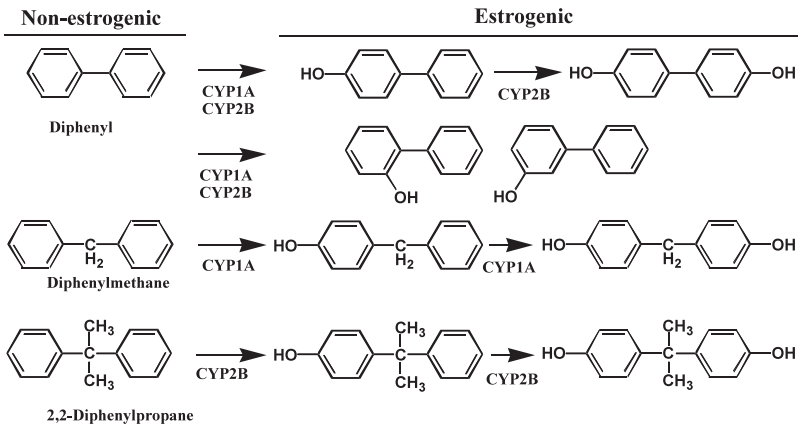

Fig. 4. Metabolic Pathways for the Activation of Diphenyl, Diphenylmethane and 2,2-Diphenylpropane to Estrogens by the Cytochrome P450 System

4-hydroxydiphenyl and 4,4′-dihydroxydiphenyl. Soto *et al.*[32] reported that 2-hydroxydiphenyl, which is used as an antifungal, is a weak estrogen, and the related compounds 4-hydroxydiphenyl and 3-hydroxydiphenyl are also estrogenic. Estrogenic activity of 4,4′-dihydroxydiphenyl has also been reported.[33] A possible metabolic activation pathway of these proestrogenic chemicals with liver microsomes is shown in Fig. 4. The estrogenic activity is likely to be mainly exhibited by the 4-hydroxyl derivatives, because the pathway leading from 4-hydroxyl derivatives to 4,4′-dihydroxyl derivatives does not proceed effectively in this metabolic system.

ACTIVATION OF STYRENE OLIGOMERS BY THE CYTOCHROME P450 SYSTEM

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 biological actions, including hormonal activity.[34, 35] Polystyrene has been used to manufacture food containers for take-out, such as coffee cups, meat trays, salad boxes and soup bowls, as well as instant food containers, in which instant foods such as Japanese noodles, buckwheat noodles, Chinese noodles, chow mein, spaghetti and rice are cooked by adding hot water. There are reports indicating that styrene oligomers migrate from these containers into the food contents.[36, 37]

These styrene oligomers were negative in the estrogen screening assay. However, TCB exhibited estrogenic activity after incubation with liver microsomes of phenobarbital-treated rats in the presence of NADPH. CCB, 1,3-diphenylpropane and 2,4-diphenyl-1-butene also exhibited estrogenic activity after metabolic activation, but the activities were 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 metabolic activation. After incubation of TCB with liver microsomal system of phenobarbital-treated rats, *trans*-1-(4-hydroxyphenyl)-2-phenylcyclobutane (4-OH-TCB), which exhibited a significant estrogenic activity, was detected. Recombinant human cytochrome P450 2B6 and rat cytochrome P450 2B1 were responsible for the activation. In contrast, cytochrome P450 1A may be mainly responsible for the activation of 2,4-diphenyl-1-butene. Thus, some styrene oligomers, especially TCB, exhibit estrogenic activity after metabolic activation to the 4-hydroxylated metabolite by rat liver microsomes (Fig. 5).[11]

Nobuhara *et al.* reported that styrene oligomers did not induce the proliferation of MCF-7 cells.[38] However, Ohyama *et al.* 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

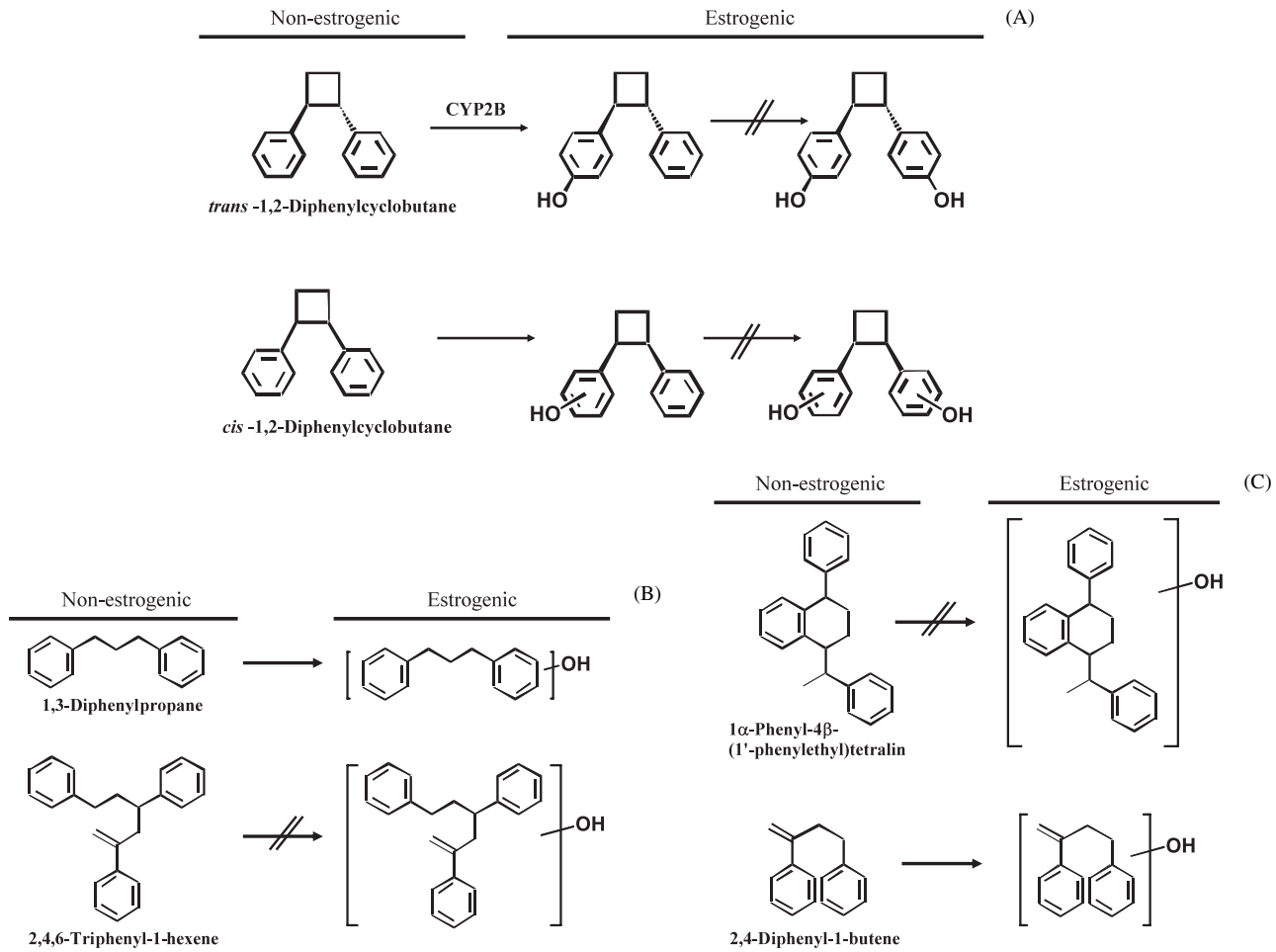

Fig. 5. Metabolic Pathways for the Activation of Styrene Oligomers to Estrogens by the Cytochrome P450 System

metabolic activation.[39] The difference from the above results was not clearly explained. 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.

A metabolic activation pathway of proestrogenic styrene oligomers with liver microsomes is shown in Fig. 5. TCB is converted to hydroxylated derivatives by rat liver microsomes. In the microsomal system, the estrogenic activity of TCB is thought to be mainly exhibited by 4-OH-TCB. The further oxidized metabolite, *trans*-1,2-bis-(4-hydroxyphenyl)cyclobutane, could not be detected. However, the activity of CCB after metabolic activation was lower than that of the *trans*-isomer. *cis*-1-(4-Hydroxyphenyl)-2-phenylcyclobutane may exhibit lower estrogenic activity than the *trans*-isomer. The activity of CCB after metabolic activation may be due to 4-OH-TCB formed after *cis-trans* isomerization.

ACTIVATION OF POLYCYCLIC AROMATIC HYDROCARBONS AND NITROPOLYCYCLIC AROMATIC HYDROCARBONS BY THE CYTOCHROME P450 SYSTEM

Some reports suggest that hydroxylated polycyclic aromatic hydrocarbons act as xenoestrogens.[40–42] Many polycyclic aromatic hydrocarbons present as environmental pollutants are negative in the estrogen screening test, but the possibility should be considered that these compounds may also be metabolically activated to xenobiotic estrogens via hydroxylation in the body. It was demonstrated that hydroxylated metabolites of benzo[*a*]pyrene exhibit estrogenic activity. In this case, the 1-, 3-, 7- and 9-hydroxylated metabolites had binding affinity for estrogen receptor, but the 7,8- and 9,10-dihydroxy metabolites did not.[40–42] De Wiele *et al.* also reported that polycyclic aro-

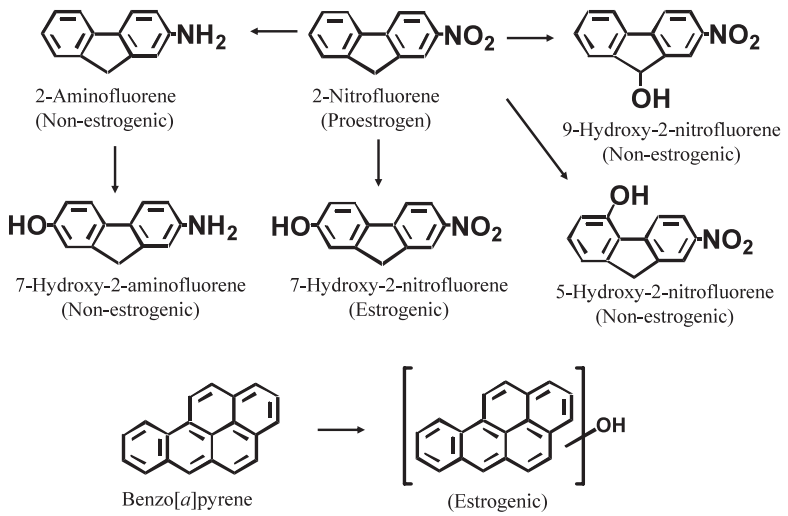

Fig. 6. Metabolic Pathways for the Activation of 2-Nitrofluorene and Benzo[*a*]pyrene to Estrogens by the Cytochrome P450 System

matic hydrocarbons were transformed to estrogenic hydroxylated metabolites by human colon microbiota (Fig. 6).[43)]

Nitropolycyclic aromatic hydrocarbons (nitro-PAHs), which are carcinogenic and mutagenic, enter the environment from diesel engine exhaust, urban pollution sources, cigarette smoking and so on.[44–46)] Several reports have indicated that nitro-PAHs are mainly metabolized by nitro reduction, ring hydroxylation, acylation and conjugation in mammalian species.[47)] Nitro-PAHs should also be examined to see whether nitro reduction or hydroxylation of the aromatic rings activates these compounds to xenobiotic estrogens, as is the case for their carcinogenicity. 2-Nitrofluorene (NF) is a typical carcinogenic nitro-PAH.[48)] NF was detected in diesel exhaust particles as a major component, together with nitropyrenes.[49)] NF was also detected as a major pollutant in the atmosphere.[50)] NF is a potent mutagen and forms DNA adducts in the animal body.[51–53)] It was reported that NF is converted to 2-aminofluorene and its acylamino metabolites, and their oxidative metabolites.[54–57)]

It was shown that NF exhibits a significant estrogenic activity after activation by rat liver microsomal mixed function oxidase. When the compound was incubated with the liver microsomes of 3-methylcholanthrene-treated rats in the presence of NADPH, 7-hydroxy-2-nitrofluorene (7-OH-NF) was formed as a major metabolite. However, little of the metabolite was formed by liver microsomes of untreated or phenobarbital-treated rats. Rat recombinant cytochrome P450 1A1/2 exhibited a significant oxidase activity toward NF, affording 7-OH-NF. 7-OH-NF exhibited a significant estrogenic activity, while the activity of 5-hydroxy-2-nitrofluorene was much lower.[12)]

The estrogenic activity of NF was due to formation of the hydroxylated metabolite at the 7 position by liver microsomes. 2-Aminofluorene and 2-acetylaminofluorene did not exhibit estrogenic activity. These compounds had weak estrogenic activity after metabolic activation by liver microsomes of 3-methylcholanthrene-treated rats. NF is metabolized to hydroxylated derivatives, mainly 7-OH-NF, and is also converted to a reduced metabolite, 2-aminofluorene. In the microsomal system, the estrogenic activity of NF is thought to be mainly due to 7-OH-NF, because reductive metabolism of NF proceeds effectively only under anaerobic conditions. Further, it is possible that 7-OH-NF and 5-OH-NF are reduced to 7- and 5-hydroxy-2-aminofluorene, and acetylated to 7- and 5-hydroxy-2-acetylaminofluorene. However, these reactions are likely to be inactivation routes as regards estrogenic activity (Fig. 6).

ACTIVATION OF METHOXYCHLOR AND RELATED COMPOUNDS BY THE CYTOCHROME P450 SYSTEM

Methoxychlor is a proestrogen which requires demethylation by liver microsomal mixed function oxidase in animals prior to eliciting estrogenic ac-

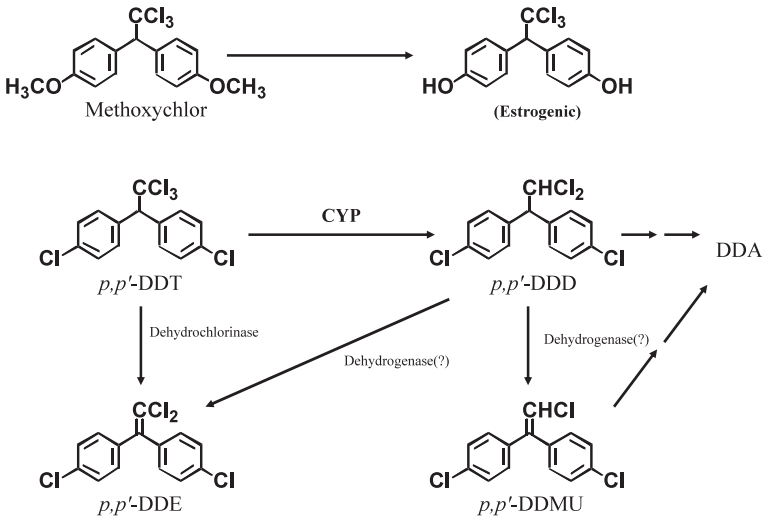

Fig. 7. Metabolic Pathways of Methoxychlor and *p*,*p*′-DDT in Rat Liver Microsomes

tivity.[13] Methoxychlor requires demethylation by liver microsomal mixed function oxidase, involving CYP 1A2 and 2C19, to elicit estrogenic activity.[58] Elsby *et al.* also reported that methoxychlor was activated through demethylation by human liver microsomes.[14] Schlenk *et al.* reported that methoxychlor was activated to estrogen in fish (Fig. 7).[59]

1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (*p*,*p*′-DDT) is metabolized to 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane (*p*,*p*′-DDD) and 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (*p*,*p*′-DDE) by reductive dechlorination and dehydrochlorination, respectively, and *p*,*p*′-DDD is further oxidized to 2,2-bis(4-chlorophenyl)acetic acid (*p*,*p*′-DDA) in animals and fish (Fig. 7).[60–62] *p*,*p*′-DDD shows estrogenic activity and *p*,*p*′-DDE shows anti-androgenic activity.[63] Other DDT isomers exhibited similar endocrine-disrupting activity. The metabolites of *p*,*p*′-DDT and *o*,*p*′-DDT described above exhibited estrogenic activity (Fig. 7).[64–66] Nelson reported an *in vivo* uterotropic effect of some of these DDT metabolites.[67] Gray *et al.* reported that *p*,*p*′-DDT exhibited anti-androgenic activity, like *p*,*p*′-DDE.[68]

ACTIVATION OF α,β-UNSATULATED KETONES BY THE CYTOCHROME P450 SYSTEM

Naturally occurring phytoestrogens also show estrogenic activity *in vitro* in receptor binding assay, in spite of their beneficial effects, such as anticarcinogenicity.[69] Some flavonoids are phytoestrogens. Chalcones are a source of phytoestrogens, acting as C15 precursors in plant flavonoid biosynthesis.[70] *trans*-4-Phenyl-3-buten-2-one (PBO) also has a flavonoid skeleton. Chalcone (*trans*-1,3-diphenyl-2-propen-1-one) is an α,β-unsaturated ketone that has the skeleton of so-called "chalcones." They are also found in naturally occurring compounds, such as plant allelochemicals, insect hormones and pheromones.[71] PBO (also called *trans*-phenyl styryl ketone or benzalacetone) has a wide range of uses as a industrial material for synthesis of chemicals and drugs, and as a flavoring additive for cosmetics, soaps, detergents, cigarettes and foods.[72] α,β-Unsaturated ketones, in which the double bond is adjacent to the carbonyl group, are reactive compounds due to their electrophilic properties, and undergo nucleophilic attack, *e.g.*, with SH-groups in proteins. They exhibit genotoxicity and mutagenicity, as well as having anti-carcinogenic effects.[73–78]

Chalcone was converted to estrogenically active hydroxylated derivatives by rat liver microsomes. 4-Hydroxychalcone exhibited the highest activity. 4′-Hydroxychalcone and 2-hydroxychalcone were minor metabolites of chalcone, and 2′-hydroxychalcone was not formed. Their estrogenic activities were lower than that of 4-hydroxychalcone. The estrogenic activity of chalcone is thus thought to be mainly due to 4-hydroxychalcone, which is the major metabolite. PBO was also metabolically activated to an estrogen by a

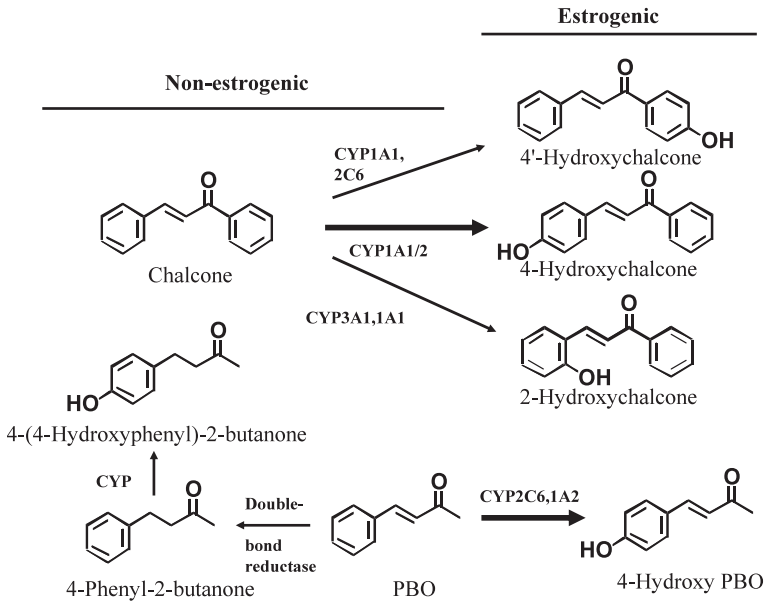

Fig. 8. Metabolic Pathways for the Activation of Chalcone and PBO to Estrogens by the Cytochrome P450 System

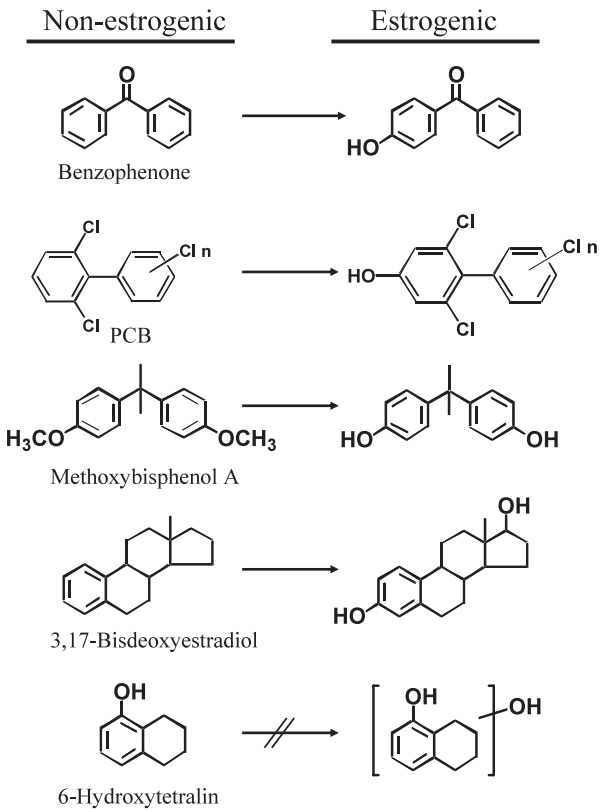

Fig. 9. Metabolic Activation of Other Proestrogens to Estrogens by the Cytochrome P450 System

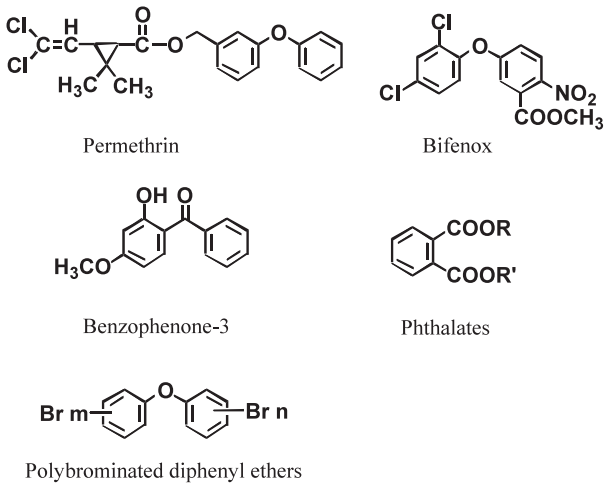

Fig. 10. Candidates for Proestrogens

microsomal enzyme system, and 4-OH-PBO, which is a major metabolite in the microsomal system, exhibited estrogenic activity (Fig. 8).[16] 4-Hydroxyphenyl-2-butanone, a double-bond-reduced derivative of 4-OH-PBO, was not estrogenic. A 4-hydroxyl group on the phenyl moiety is an essential factor for the estrogenic activity, and a double bond conjugated with the phenyl ring may also be necessary.

ACTIVATION OF OTHER PROESTROGENS IN ENVIRONMENTS BY THE CYTOCHROME P450 SYSTEM

There are other examples of metabolic activation of proestrogens to estrogens, besides

the above examples. Various pesticides might be converted to active estrogens by microsomal oxidase systems, though Sumida *et al*. showed that perimethrine was not metabolically activated.[79] *p*-Hydroxybenzophenone, which is formed from benzophenone, an antifungal agent, in rat hepatocytes, is also estrogenic.[80,81] It is also reported that anethole, a flavor agent, is not estrogenic, but 4-hydroxy-1-propenylbenzene, the desmethylated metabolite of anethole, exhibited estrogenic activity.[15] Some hydroxylated polychlorinated diphenyls (PCBs), which are metabolites of PCB, show estrogenic activity.[82–84] In the case of PCB, the presence of adjacent chloride substituents decreases the estrogenic activity.[85] A catechol-type metabolite was also shown to have estrogenic activity.[32] Elsby *et al.* predicted estrogenicity by a two-stage approach, using a human liver microsome assay and a yeast estrogenicity assay.[14,86] Methoxychlor, methoxybisphenol A and 3,17-bisdesoxyestradiol were positive, but 6-hydroxytetralin, a degreasing agent, was negative in this screening system (Fig. 9).

Other environmental compounds may be proestrogenic. Candidate proestrogens are illustrated in Fig. 10. They include pyrethroids,[87] diphenyl ether herbicides (bifenox), polybrominated diphenyl ethers, a flame-retardant, and some benzophenone sunscreens. Some insecticides and medicines are also possible proestrogens. Hydroxylated derivatives of sunscreen,[88] and phthalate esters[89] show positive in estrogenicity tests. Thus, the parent compounds may be proestrogens. There may be a variety of other potentially hazardous proestrogens in our environment, too.

CONCLUSION

We have reviewed environmental proestrogens. The estrogenic activity of *trans*-stilbene in rats *in vivo* seems to be a typical example of the metabolic activation of a proestrogen.[24] It is clearly necessary to consider the activity of metabolites produced from the parent compounds for the assessment of the toxicity of environmental chemicals.

There are also pro-antiandrogen and pro-antithyroid hormonal chemicals.[85,90] It is therefore necessary, when assessing the potential *in vivo* endocrine-disrupting action of chemicals, to take into account the activities of all the metabolites produced from the parent compounds. For example, bisphenol A, a typical xenoestrogen, is further activated, when it is incubated with rat S-9 mix.[91] In this case, dimer-type metabolites, which show higher activity than bisphenol A itself, are formed. Such further activation of xenoestrogens must also also considered in the risk assessment of xenoestrogens. Much further work is needed to identify potentially hazardous proestrogens in our environment.

For the activation of proestrogen to estrogen, it is necessary to introduce a hydroxyl group, often at the 4-position of an aromatic ring, in the absence of bulky groups at the adjacent 3,5-positions. When formation of a phenolic hydroxyl group is possible after aromatic ring hydroxylation or dealkylation of chemicals, we should consider the possibility of metabolic activation to estrogens.

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