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Natural and anthropogenic environmental oestrogens: the scientific basis for risk assessment*

Metabolism and fate of xeno-oestrogens in man

J. G. Liehr[1,†], A. Somasunderam[1] and D. Roy[2]

[1]*Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77555-1031, USA and* [2]*Department of Environmental Health Sciences, University of Alabama School of Public Health, Birmingham, AL 35294-0022, USA*

Abstract: A large variety of chemicals of either natural or synthetic origin possess oestrogen-like activity and are thus called xeno-oestrogens. Some of these chemicals such as the pesticide methoxychlor require metabolic activation for their oestrogenic activity, whereas other compounds may themselves be oestrogenic and may be deactivated by their metabolism. In this chapter, the metabolism of representative examples of environmental oestrogen-like chemicals has been discussed to illustrate common trends in the large structural variety of xeno-oestrogens. The compounds included are zearalenone, methoxychlor, bisphenol A, DDT, β-sitosterol, and genistein and have been selected based on availability of information, the potential of exposure of humans and wildlife to the compounds, their industrial or agricultural importance, and the importance of metabolism for their activation or deactivation. The oestrogenic activity of phenolic xeno-oestrogens, a large class of compounds of natural or synthetic origin or their metabolites, likely is based on the weak oestrogen receptor binding of phenol. These compounds are mainly metabolized by analogy to steroidal oestrogenic hormones, i.e., by aromatic ring hydroxylation (catechol formation), subsequent methylation of the catechol and further phase II metabolism by glucuronide and/or sulfate formation. In contrast, the chlorinated hydrocarbon pesticides, which are weakly oestrogenic, are mainly metabolized by dehalogenation at relatively low metabolic rates. Thus, these compounds may persist in the body, accumulate in fatty tissues and provide a chronic reservoir of oestrogenic chemicals.

INTRODUCTION

Many chemicals, both natural and synthetic, exhibit oestrogen-like activity [recently reviewed by Roy *et al.* (1)]. At present, we know of more than one hundred chemicals with oestrogenic activity (Table 1). Many of these environmental oestrogen-like chemicals are substituted phenols. Phenol itself (hydroxybenzene) has a low but detectable relative binding affinity to the oestrogen receptor. Substituted phenols may bind to the oestrogen receptor with much higher affinity depending on the structural and steric similarity of the compound to the natural hormone oestradiol. This concept is best illustrated by the stilbene oestrogens, which do not resemble a steroidal ring system, but are arranged in such a way that they fit into the oestrogen receptor with exceptionally high affinity. Many other substituted phenols possess intermediate or relatively weak affinity for the oestrogen receptor, but nevertheless may elicit hormonal responses at elevated concentrations.

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† *Present address:* Stehlin Foundation for Cancer Research, 1918 Chenevert, Houston, TX 77003, USA.

Table 1. Examples of known or suspected environmental oestrogen-like endocrine-disrupting chemicals

	Primary use/source(s)
1. Pesticides	
a. Herbicides	
2,4-Dichlorophenol	herbicide and fungicide
Atrazine	triazine herbicide
b. Insecticides	
Dicofol	organochlorine insecticide
Dieldrin	organochlorine insecticide
DDT	organochlorine insecticide
Methoxychlor	organochlorine insecticide
Chlordecone (Kepone)	organochlorine insecticide
Toxaphene	organochlorine insecticide
Heptachlor	organochlorine insecticide
2. Other industrial chemicals	
a. Polychlorinated biphenyls (PCBs)	
Trichloro-4-biphenyl (mixture of isomers)	adhesives, fire retardants, waxes
Tetrachloro-4-biphenyl (mixture of isomers)	adhesives, fire retardants, waxes
b. Pthalates	
Di-n-butyl pthalate	plasticizer in food packaging, PVC cellulosics, elastomers, insect repellants
Butylbenzylpthalate	plasticizer for the production of vinyl floor tiles
Bis(2-ethylhexyl)pthalate	plasticizer
c. Alkylphenols	
4-Nonylphenol	additives to lubricating oil , plasticizers, resins, detergents, surface active agents
4-Octylphenol	detergents, paints, herbicides
Nonylphenoxy carboxylic acid	detergents, paints, herbicides
Bisphenol A	use in manufacture of polycarbonate plastics
Naphthol-1	use in production of antioxidants for synthetic rubber, dyes, perfumes etc.
Naphthol-2	as above
6-Bromo-2-naphthol	as above
5,6,7,8-Tetrahydronaphthol-2	as above
d. Polycyclic Aromatic Hydrocarbons	
Benzo[a]pyrene	combustion of fossil fuels
3,9-Dihydroxy-dimethylbenzanthracene	combustion of fossil fuels
3. Natural Oestrogenic Compounds	
a. Phyto-oestrogens	
Coumestrol	alfalfa
Genistein	soya beans
Daidzein	soya beans
Equol	a metabolite of coumestrol, genistein, and diadzein
Formononetin	red clover, alfalfa
Biochanin A	red clover, alfalfa
Pelargonin	flowers of Pelargonium zonale
Indolo[3,2-b]carbazole	vegetables of the Brassica genus
Mirestrol	natural products
Δ^9-Tetrahydrocannabinol	Cannabis sativa (marijuana)
β-Sitosterol	plant oils, legumes, wood
b. Mycoestrogens	
Zearalenone	moldy (*Fusarium* spp.) corn, wheat, barley, sorghum, and oats in animal rations
Zeranol	Veterinary anabolic agent
c. Microbial oestrogens	
Enterolactone	intestinal microbial metabolite
4. Synthetic Oestrogens (drugs used as oestrogens)	
Diethylstilbestrol (DES)	oral contraceptive, prostate cancer therapy
17 α-ethinyloestradiol	oral contraceptive
Tamoxifen	breast cancer adjuvant therapy
Polyestradiol phosphate	oral contraceptive
Piperazine oestrone sulfate	oral contraceptive
Quinestrol	oral contraceptive
Chlorotrianisene	oral contraceptive
Dienestrol	oestrogenic hormone therapy
5. Drugs with oestrogenic activity not intended for use as oestrogens	
Cimetidine	histamine H2-receptor antagonist
Digitalis glycosides	cardiac glycosides
Sulfonamide	sulfonamide antimicrobials

Other environmental chemicals with weak oestrogenic activity are halogenated hydrocarbons. Although the nature of their interactions with the oestrogen receptor is poorly understood, it is thought that the chlorine substituents of these hydrocarbons are capable of playing a similar role as the phenol hydroxyl substituents.

A large number of pesticides and industrial chemicals possessing oestrogen-like activity are ubiquitous in the environment and find their way into the food chain. Some of the pesticides that are oestrogenic include endosulfan, 1-hydroxychlordane, dicofol, and methoxychlor. Some of these pesticides, such as methoxychlor, need to be metabolized before they can bind to the oestrogen receptor, and their metabolites may even be more oestrogenic. The pesticide DDT is banned in the United States, but is still used in developing countries. This compound and its metabolites are still present in the environment due to previous wide-spread use and a relatively long half-life. In addition to pesticides, industrial chemicals, such as some polybrominated and polychlorinated biphenyls (PBBs and PCBs, respectively), phthalates, and styrene have oestrogenic activity and are found as contaminants in the environment. Although most of these compounds are only weakly oestrogenic, they may accumulate in animal or human tissue and exert hormonal effects at elevated concentrations.

The focus of this chapter is on the metabolism of oestrogen-like chemicals. Since large numbers of chemicals possess oestrogen-like activity, it would be very difficult to report and evaluate the metabolism of all known oestrogen-like chemicals. Therefore, we have decided to discuss the biotransformation of a limited number of environmental oestrogen-like chemicals based on: a) the availability of information; b) the potential exposure of humans and wildlife to a specific oestrogenic compound; c) the industrial or agricultural importance of a compound; and d) the importance of metabolism for the compound's activation or inactivation. We have concentrated our efforts on the metabolism of a mycotoxin, zearalenone; two phyto-oestrogens, β-sitosterol and genistein; and three industrial chemicals, DDT, bisphenol A and methoxychlor. Our choices will give the reader a general understanding of the biotransformation processes underlying metabolic activation or inactivation resulting in increases or decreases in hormonal activity. It needs to be kept in mind that metabolic biotransformation reactions may be species- gender-, and tissue-dependent. Such influences have been demonstrated for the natural hormone oestradiol, may also operate with other oestrogen-like chemicals, and add a level of complexity beyond the scope of this overview.

ZEARALENONE

Zearalenone is a mycotoxin, produced by a fungus (*Fusarium* sp.) present on moldy corn, wheat, barley, etc. Its importance is based on the large-scale use of one of its derivatives, zeranol, as a growth promoter with oestrogenic property in the cattle industry in the United States.

Experimental systems

In vitro, zearalenone in the presence of rat liver homogenate is reduced to zearalenol, possibly catalyzed by a hydroxysteroid dehydrogenase (Fig. 1). Both zearalenone and zearalenol may be conjugated with glucuronic acid in the presence of glucuronyl transferase (2). The interconversion of zearalenone and zearalenol is catalyzed by rat erythrocytes *in vitro* (3). The major metabolite obtained by incubating zearalenone, **1,** with erythrocytes or whole blood from SD rats are α-zearalenol, **5**. β-Zearalenol, **4,** is also formed, but at levels several times lower than those of α zearalenol. Conversion of α-zearalenol and β-zearalenol to the corresponding epimer is also observed when incubated with erythrocytes (3). *In vitro*, zearalenone and both α- and β-zearalenol are converted to hydroxylated (catechol) metabolites, **2,** presumably by a cytochrome P450 hydroxylase, and these catechol metabolites are subsequently methylated to **3** by catechol-O-methyltransferase (COMT) (4).

The reduction of zearalenone by subcellular fractions from hen and rabbit hepatocytes clearly shows species-specific differences in the cofactor requirements, rate of metabolism and production of metabolites (5). The presence of NADH as cofactor in the reaction mixtures enhances only the reducing activity of the microsomal fraction from rabbit hepatocytes, while NADPH enhances the reducing

activities of the cytosolic fraction from rabbit and both the microsomal and cytosolic fractions from hen hepatocytes. Hen hepatocytes metabolize faster and produce β-zearalenol as the major metabolite, whereas rabbit hepatocytes metabolize zearalenone slowly and mainly into α-zearalenol, the more uterotrophic metabolite.

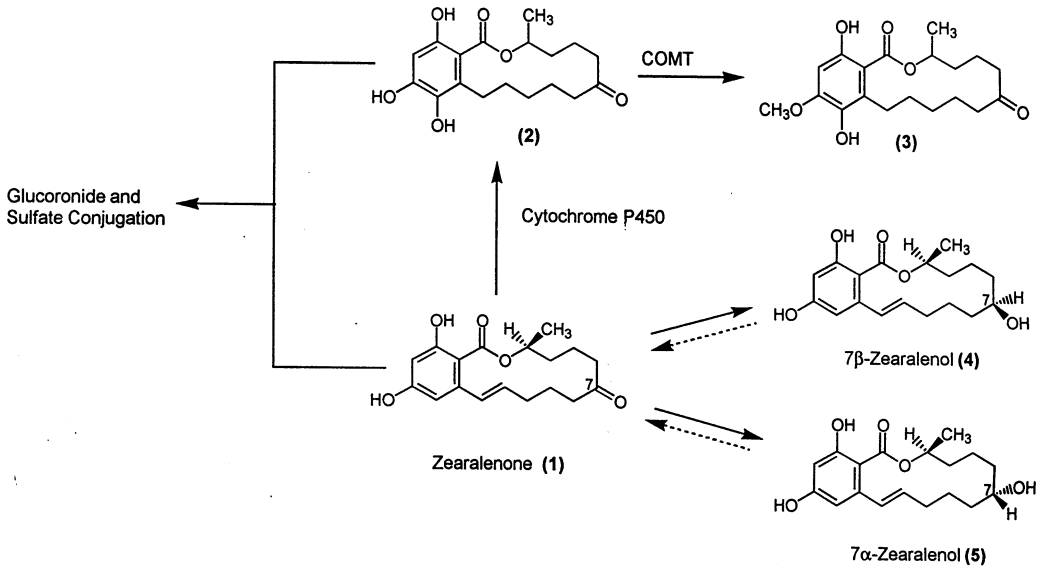

Fig. 1. Metabolism of zearalenone, **1**, Like zearalenone, both 7α- and 7β-zearalenol (**5** and **4**, respectively) may undergo hydroxylation and their hydroxylated metabolites may be further metabolized by catechol-O-methyltransferase (COMT)-catalyzed methylation (4). Zearalenone or its phase I metabolites may also undergo other conjugation reactions.

Animals

In animals, zearalenone is carried in the blood from the gastro-intestinal tract to the target organs. Therefore, the metabolism of zearalenone in hepatic tissues or in the target organs may be modified by the action of enzyme(s) present in red blood cells (3). In rats, zearalenone and metabolites are excreted mainly in the free form, with the production of α-zearalenol, **5,** the most potent oestrogenic metabolite, being greater than 10% of the zearalenone dose (6,7).

In pigs, both free and conjugated zearalenone (63%), α-zearalenol (32%), and β-zearalenol (5%) are present in the urine of the pig following administration of zearalenone to the stomach. α-Zearalenol is the predominant metabolized species in the pig (6). At low doses pigs rapidly conjugate almost all absorbed zearalenone and its metabolite α-zearalenol with glucuronic acid (8). Glucuronidation mainly occurs in the liver and also to some extent in the gastrointestinal tract. Biliary excretion is principally by glucuronide conjugation. The intestinal mucosa is active in reducing zearalenone to zearalenol and conjugating these metabolites with glucuronic acid.

In rabbits, 46% of the urinary metabolites are in the form of zearalenone conjugates, 29% are conjugates of α-zearalenol and 25% are conjugates of β-zearalenol (6).

In cows, free and conjugated (glucuronide and sulfate) zearalenone (29%),α-zearalenol (20%) and β-zearalenol (51%) have been observed. β-Zearalenol is the predominant metabolic species in the cow (6). The distribution of metabolites in the feces is similar to that in the urine. Free and conjugated forms of zearalenone and diastereomeric zearalenols are present in cow's milk. The total concentration of zearalenone and its metabolites in milk has been reported to be 1.3 p.p.m. after administration of 25 p.p.m. dietary zearalenone for 7 days. In the milk, concentrations of zearalenone, α-zearalenol, and β-zearalenol are 35%, 31%, and 34%, respectively (6).

Human

In the presence of homogenates of human prostate glands and NADPH, zearalenone, **1,** is converted into several metabolites including α-zearalenol, **5,** α-*cis*-zearalenol, and a minor amount of β-*cis*-zearalenol (9). The *in vivo* metabolism of zearalenone by man is similar to that of pigs with respect to the production of a large proportion of α-zearalenol, and to that of rabbits with both exclusively producing glucuronide conjugates (6). Zearalenone, **1,** and α-zearalenol, **5,** are found in roughly equal abundance after hydrolysis. β-Zearalenol, **4,** was also observed, but its concentration was about one-third of that of α-zearalenol (6).

METHOXYCHLOR

Methoxychlor [1,1,1-trichloro-2,2-bis-(4-methoxyphenyl)-ethane], **6,** a broad-spectrum pesticide, is currently used as a substitute for certain pesticidal activities of DDT, which has been banned in the industrially developed countries (10).

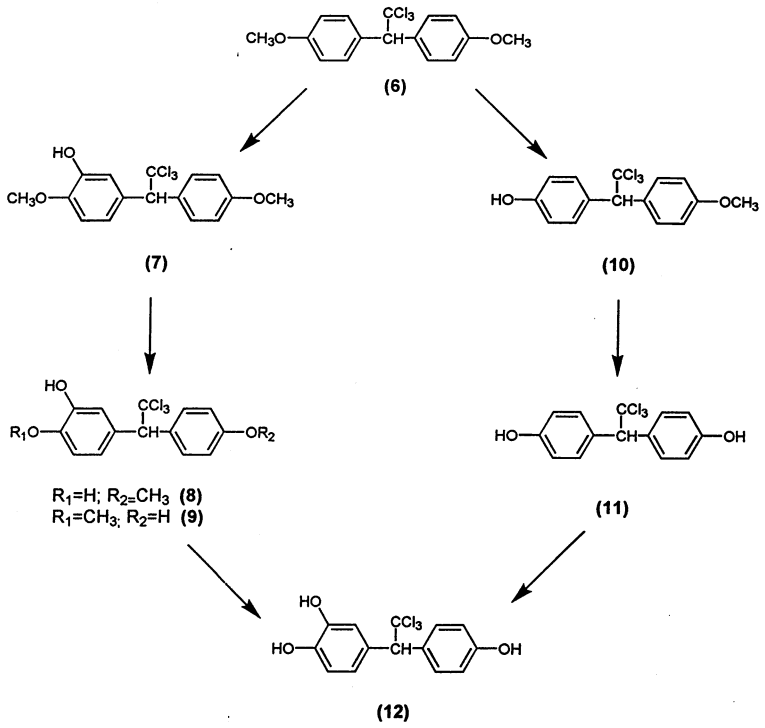

Fig. 2. Metabolism of methoxychlor, **6.** Rat liver microsomes catalyze the formation of the demethylated catechol metabolite **12** by two different pathways as shown above (10,11). In contrast, human liver microsomes only catalyze the demethylation of methoxychlor (formation of metabolites **10** and **11**), but not ring hydroxylation (10,11).

Experimental systems

In vitro, mammalian liver microsomes sequentially demethylate methoxychlor, **6,** yielding two oestrogenic metabolites, monodemethylated, **10,** and bis-demethylated methoxychlor, **11** (Fig. 2). Liver microsomes from phenobarbital (PB) treated rats (PB microsomes) catalyze the conversion of methoxychlor into the trihydroxycatechol metabolite, **12** (11). The ring-hydroxylation of methoxychlor (formation of **7**) is catalyzed by CYP2B. Incubation of **10** with rat liver microsomes yielded only metabolite **11**, whereas incubation of metabolite **7** resulted in monodemethylated compounds **8** and **9** and didemethylated ring-hydroxylated compound **12**. Thus, the formation of the catechol metabolite **12**

involves two metabolic pathways: *via O*-demethylation followed by ring-hydroxylation and *via* ring-hydroxylation and subsequent *O*-demethylation as shown in Fig. 2 (10).

Animals

Multiple twice daily doses of methoxychlor to female rats produce a marked induction of the hepatic microsomal P450 2B1/2B2 and 3A proteins (12). Methoxychlor moderately elevates the enzymatic activity corresponding to CYP2B and 3A catalysis. Methoxychlor belongs to the phenobarbital type of inducers. This exposure to methoxychlor can affect its own metabolism since CYP2B catalyzes the hydroxylation of the aromatic ring (12).

Human

Human liver microsomes catalyze demethylation of methoxychlor but not catechol formation by ring-hydroxylation (10). The products of demethylation in human, **10** and **11**, are the same as those of rats shown in Fig. 2 (10,11).

BISPHENOL A

Bisphenol A (BPA) is widely used as a monomer for the production of plastics, resins, and coatings.

Experimental systems

BPA is extensively metabolized by a Gram-negative aerobic bacterium (strain MV1) *via* oxidative rearrangement of the BPA (13). Oxidation of the aliphatic methyl group of BPA leads to production of both methyl-hydroxylated 2,2-bis(4-hydroxyphenyl)-1-propanol and a skeletally rearranged triol, 1,2-bis(4-hydroxyphenyl)-2-propanol. The major route of this bacterial metabolism (>80%) is through the rearrangement. Thus, 1,2-bis(4-hydroxyphenyl)-2-propanol is dehydrated to 4,4′-dihydroxy-α-methylstilbene, which is rapidly cleaved by oxidation to 4-hydroxybenzaldehyde and 4-hydroxyacetophenone. 4-Hydroxybenzaldehyde is oxidized further to 4-hydroxybenzoic acid. The minor product of BPA hydroxylation, 2,2-bis(4-hydroxyphenyl)-1-propanol, is further oxidized to form both 2,2-bis(4-hydroxyphenyl)propanoic acid and a skeletally rearranged tetraol, 2,3-bis(4-hydroxyphenyl)-1,2-propanediol. 2,3-Bis(4-hydroxyphenyl)-1,2-propanediol is slowly transformed to 4-hydroxyphenacyl alcohol (13). This biotransformation of BPA by this bacterial strain has not been detected in mammalian species.

Animals

In rats, 28% of the BPA is excreted in the urine and 56% in the feces. BPA is primarily excreted as the glucuronide. Less than 1% of the material present in urine is free BPA. In feces 35% of material is free BPA, 35% is a hydroxylated product of BPA, and the remaining 30% is probably present as a conjugate (14).

Recently, Atkinson & Roy have shown that BPA, **13,** undergoes hydroxylation and produces the hydroxylated BPA **14** shown in Fig. 3. In incubations of BPA, rat liver microsomes and NADPH or cumene hydroperoxide cofactor, this compound bound covalently to DNA as determined by ^{32}P-postlabeling analysis (15,16). An identical DNA adduct pattern was generated by incubation of DNA and bisphenol o-quinone **16.** These data suggest that BPA is converted by rat liver cytochromes P450 to this bisphenol o-quinone which is a reactive metabolic intermediate and covalently binds to DNA (15,16). These DNA adducts have been detected *in vitro* and in rats *in vivo*.

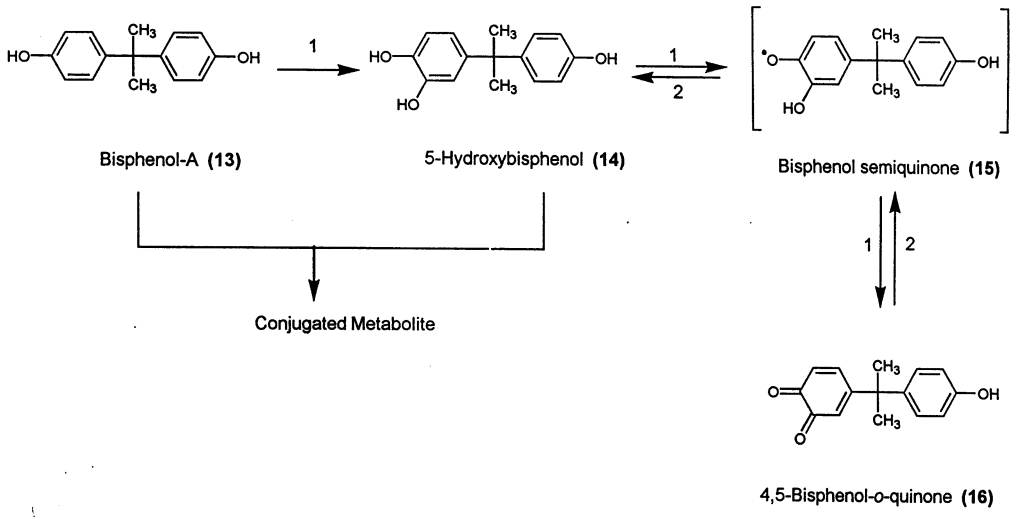

Fig. 3 Metabolism of bisphenol A, **13**. Metabolic conversion of bisphenol to a catechol and further activation to a DNA-reactive semiquinone/quinone intermediate (**15** and **16**, respectively) (15,16). Most of bisphenol A and its metabolites are excreted as glucuronide conjugates (14).

Humans

Not known.

DDT

DDT is a broad-spectrum pesticide, which has been used in large quantities in the 40s, 50s, and 60s, but is now banned in most industrialized countries because of its long-term toxic effects. However, it is still being used in developing countries.

Animals

In mice exposed for three days to DDT, major metabolites of DDT, **17,** are (2,2-bis(p-chlorophenyl) acetic acid (DDA), **21,** and 1,1-dichloro-2,2-bis(4′-chlorophenyl)ethene (DDE), **22,** with DDA predominating (Fig. 4). However, after 4 months of chronic feeding of DDT to mice, DDE has been found to be nearly as prominent a metabolite as DDA (17). In contrast, the hamster does not efficiently metabolize DDT to DDE (17). It has been proposed that the metabolism of DDT to DDA involves a sequential combination of reductive dechlorination, dehydrochlorination, reduction, hydroxylation and oxidation steps (18). Gold & Brunk (18) found that there is a another pathway from DDD to DDA in the rat. They suggest that DDD(1,1-dichloro-2,2-bis(p-chlorophenyl)ethane, **18**, is metabolized to DDA via enzyme-mediated hydroxylation on the C-1 side chain carbon to form **19** (Fig. 4): DDT ($R_2CH\text{-}CCl_3$, **17**) → DDD ($R_2CH\text{-}CHCl2_2$, **18**) → ($R_2CH\text{-}COHCl_2$,**19**) → ($R_2CH\text{-}COCl$, **20**) → DDA ($R_2CH\text{-}COOH$, **21**).

There is no significant change in the metabolism of DDE, the persistent DDT metabolite with significant oestrogenic activity, after prolonged exposure of mice to DDE , and there is no indication for the existence of metabolic activation of DDE to a reactive electrophilic species (19). In rats, the major route of metabolism of DDE, **22,** involves ring hydroxylation which produces 3′-OH-DDE and 2′-OH-DDE and 3′-chloro-4′-OH-DDE.

There is no significant dermal metabolism in the skins of human, rat, pig, and guinea-pig (20).

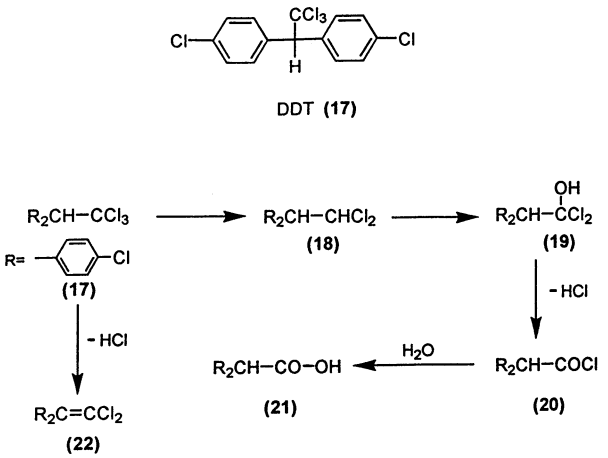

Fig. 4. Metabolism of DDT, **17**. The main metabolic conversions of DDT are reductive dechlorination (formation of **18**), dehydrochlorination (formation of **22**), and further metabolic oxidation to **19, 20,** and **21** (17–19).

Human

Residues of DDT and its metabolites have been reported in leiomyomatous and normal human uterine tissue by gas-liquid chromatography (21). The metabolites detected are: 2,2-bis-(p-chlorophenyl)-1,1-dichloroethene (DDE, **22**), 2-(o-chlorophenyl)-2-(p-chlorophenyl)-1,1,1-trichloroethane (o,p′-DDT), 2,2-bis-(p-chlorophenyl)-1,1-dichloroethane (DDD, **18**), and 2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane (DDT, **17**). Total DDT ranged from 0.245 to 1.982 p.p.m., with a mean value of 0.845 p.p.m. in leiomyomatous tissue. In normal human uterine tissue, total DDT ranged from 0.030 to 0.282 p.p.m., with a mean value of 0.103 p.p.m. (21). It needs to be kept in mind that these DDT metabolite concentrations were reported in 1987, although DDT use was banned in the United States in 1972. In Scandinavia, concentrations of DDE, the most oestrogenic DDT metabolite, have been assayed in human breast milk since 1965 to assess neonatal exposure of infants to this metabolite (22). In the four Scandinavian countries, Finland, Norway, Sweden and Denmark, breast milk concentrations declined from 1.5–3 mg/kg fat in 1965–1970 to approximately 0.3–0.5 mg/kg fat in 1985–1990. These data clearly demonstrate that DDT and its metabolites persist in the environment, have entered the food chain, have accumulated in human tissue and are excreted only very slowly. The major reason for the very slow elimination appears to be the almost complete absence of phase II metabolism of DDT in animals or humans. DDT and most of its metabolites are highly lipophilic and do not contain hydroxy or amino groups, which could undergo glucuronide or sulfate conjugation.

β-SITOSTEROL

Sitosterol occurs widely in the plant kingdom along with other phytosterols.

Experimental systems

Fermentation of sitosterol, **23,** by a Pseudomonas species (SK-25) results in the formation of 5-stigmastene-3 β,7 α-diol, **24**; 5,6 α-epoxy-5 α-stigmastan-3 β-ol; 5,6 β-epoxy-5 β-stigmastan-3 β-ol and 5 α-stigmastan-3 β,5,6 β-triol , **25** (Fig. 5) (23). Sitosterol can be transformed to androsta-1,4-diene-3,17-dione by immobilized Mycobacterium cells. The genetically modified Mycobacterium sp. BCS 396 strain has been used to transform sterols with stigmastane side chains in order to obtain 26-oxidized metabolites (24). β-Sitosterol is transformed to 4-stigmasten-3-one, 26-hydroxy-4-stigmasten-3-one, and 3-oxo-4-stigmasten-26-oic acid. 26-Oxidation generates the R-configuration on C-25. Metabolites obtained by bacterial degradation of sitosterol have not been observed in animals.

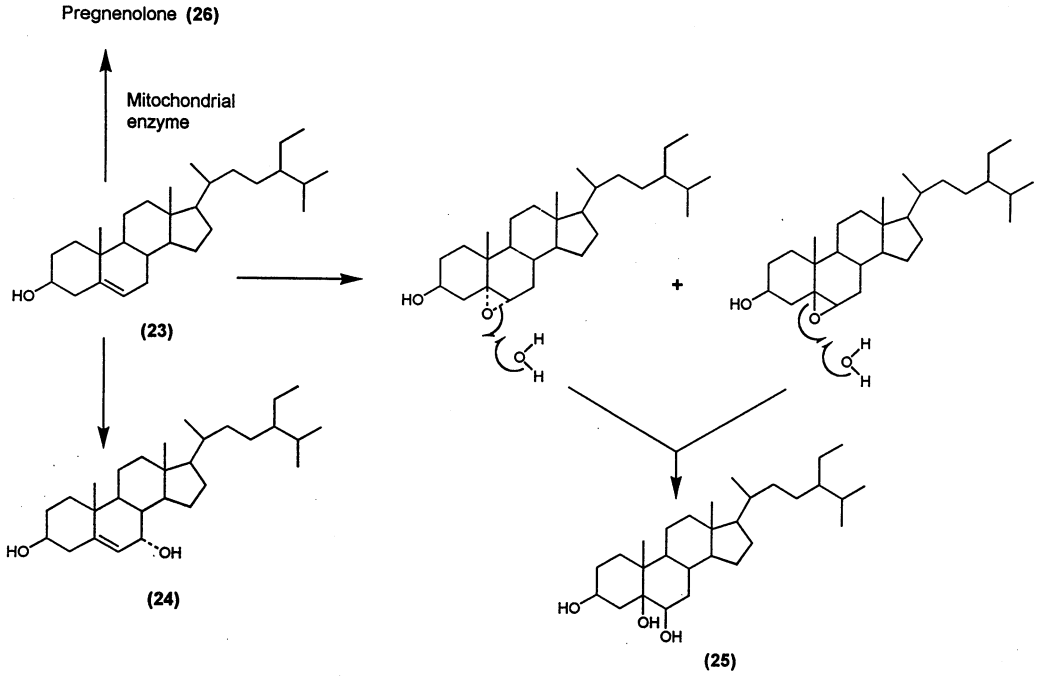

Fig. 5. Metabolism of sitosterol, **23**. Bacterial oxidation of sitosterol to hydroxylated metabolites **24** and **25** (23). Testicular enzymes of rat convert this sterol to pregnenolone, **26** (25).

Animals

A direct conversion of β-sitosterol to steroid hormones by a mitochondrial enzyme system of the rat testes has been reported (25). This enzyme system has been shown to cleave the side chain of cholesterol at C_{20} to yield pregnenolone, **26,** and isocaproic aldehyde. The major oxidation product of β-sitosterol is pregnenolone, **26**.

In rats, two major metabolites of sitosterol have been identified in the bile (26). One metabolite has three hydroxyl groups (3 alpha, 15, and unknown site), and a second metabolite has two hydroxyl groups (3 alpha, 15) and one keto group.

Human

Sitosterol metabolites could not be detected in humans.

GENISTEIN AND OTHER ISOFLAVONES

Genistein and other isoflavones are phyto-oestrogens ingested by humans and animals as part of the diet.

Animals

In rats, genistein, **27,** is rapidly absorbed in the intestine, taken up by the liver and excreted into the bile as its 7-*O*-β-glucuronide conjugate. Genistein undergoes an efficient enterohepatic circulation in the rat (27).

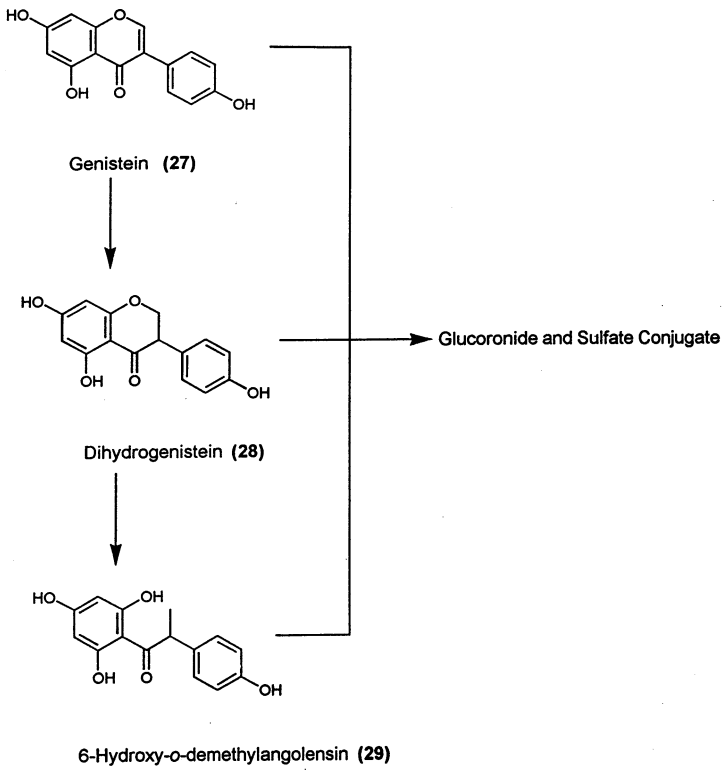

Fig. 6. Metabolism of genistein, **27**. Genistein and its reduced metabolites **28** and **29** are excreted mainly as phase II metabolites.

Human

In omnivorous and vegetarian women only 3.8% of genistein is found free and in the sulfate fraction in plasma, while as much as 21-25% of genistein are in the enterolactone and enterodiol fractions (28). In urine, the phyto-oestrogens daidzein, enterodiol, and equol are excreted mainly as sulfates and glucuronides and genistein as diglucuronide. The general pattern of lignan and isoflavonoid conjugates in urine is similar to that of the endogenous oestrogens (29). Genistein may be metabolized to dihydrogenistein, **28,** and then to 6′-hydroxy-O-demethylangolensin, **29**.

SUMMARY AND CONCLUSION

Although the sites of metabolic biotransformation reactions and their rates depend on individual structures of the xeno-oestrogens, general trends in metabolism are already discernible, despite the limited number of compounds reviewed in this text. A large group of xeno-oestrogens shares a substituted phenolic ring structure with the natural hormone oestradiol. Some of these compounds such as bisphenol A have such a phenol moiety and are oestrogenic based on that structural feature. Other compounds are metabolically activated to a phenol as is the case with methyoxychlor which is demethylated and thus converted to a metabolite with hormonal activity. Many of these phenolic xeno-oestrogens share metabolic pathways with the natural hormones oestradiol and oestrone. These shared metabolic biotransformations include hydroxylation of the aromatic ring system vicinal to the phenolic hydroxyl group (catechol formation) followed by phase II conjugation reactions such as methylation of the catechol metabolite and glucuronide and/or sulfate conjugation of the catechol and/or methoxymetabolites. These metabolic pathways are also shared by the synthetic oestrogen drugs including diethylstilbestrol (DES), ethinyl-oestradiol, dienestrol and the anti-oestrogen Tamoxifen. These oestrogens have not been discussed here, because they have been reviewed in detail elsewhere in the

context of their carcinogenic activities (30–32). Similarly, benzo[a]pyrene and dimethylbenzanthracene metabolism have been excluded, because it has been the subject of extensive reviews (33). The influence and biological effects of metabolites of xeno-oestrogens on overall hormonal regulation in humans are difficult to assess and are still poorly researched and understood. The catechol and conjugated catechol metabolites have decreased oestrogenic activity compared to the parent phenolic (xeno)oestrogens. However, it is largely not known to what extent xeno-oestrogens or their metabolites influence the metabolism of natural hormones and thus affect their hormonal effects by modulating their concentrations. For instance, catechol-containing flavonoids such as quercetin are powerful inhibitors of catechol-O-methyltransferase (34) and may thus interfere with the metabolic deactivation of the natural hormones oestradiol or oestrone, because their catechol metabolites are inactivated by this same enzyme.

A second important but poorly understood issue is the concentration difference and its effects on overall hormonal regulation. For instance, how do higher concentrations of weakly oestrogenic xeno-oestrogens or their metabolites affect interactions between the oestrogen receptor and low concentrations of the natural hormone? It is possible that at least some of the xeno-oestrogens, specifically the phyto-oestrogens, may exert antagonistic rather than agonistic effects. However, more research is needed to gain clarity in this area.

The metabolic fate of halogenated compounds with xeno-oestrogenic activity such as DDT, PCB's, etc. markedly differs from that of the phenolic compounds. These halogenated compounds undergo mainly reductive metabolism by dehalogenation. Unless hydroxyl, carboxyl or amino substituents are metabolically introduced into the parent halogenated compounds or their metabolites, these compounds can not be further inactivated by phase II conjugation. Phase I metabolic rates may also be lower than the hydroxylation rates of phenolic xeno-oestrogens. As a result, the dehalogenated metabolites may largely retain their lipophilicity and accumulate in tissues including human tissues. The influences of persistent high concentrations of halogenated xeno-oestrogens or their metabolites on human pathology such as breast cancer is the subject of discussion elsewhere in this book.

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