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Topic 2.7

Role of metabolism in the endocrine-disrupting effects of chemicals in aquatic and terrestrial systems*

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Abstract: This review describes the role of metabolism with endocrine active substances. Many modern synthetic compounds are readily metabolized to more polar forms that often contain hydroxy groups. This presence of polar groups and aromatic moieties in the parent compound or metabolite can play an important role in the mechanism of endocrine disruption. In addition, phase II metabolism (e.g., glucuronidation) can also lead to deactivation of the endocrine properties. In the case of bisphenol A and alkylphenols, metabolism can be considered as a detoxification mechanism as glucuronides decrease of inhibit binding to the estrogen receptors. In the case of phthalate esters, the primary metabolites, the monoesters, and further degraded metabolites do not interact with the estrogen receptor either. In contrast, the demethylation of methoxychlor in fish and other vertebrate species leads to metabolites with an increased affinity for the estrogen receptor. Certain PCB metabolites with hydroxy groups on the para position without vicinal chlorines have estrogenic activity, but these metabolites are not relevant for the environment. PCB metabolites with methylsulfonyl groups are commonly found in environmental biota and have been associated with several endocrine, developmental, and reproductive effects. Some DDT metabolites bind weakly to the estrogen receptor, but the major biotransformation product *p,p*-DDE is an androgen receptor (AR) antagonist. Vinclozolin is an anti-androgen and this effect appears to caused by two of its more water-soluble metabolites. The chloro-*s*-triazines exhibit an in vitro induction of aromatase, but their dealkylated metabolites show a decrease or lack of this effect.

It is recognized that common metabolic processes can differ strongly among species that complicates ecotoxicological risk assessment of endocrine active substances. In conclusion, the testing of metabolites for endocrine-disrupting properties should be encouraged in the future to establish a better risk assessment process.

An appendix containing levels and half-lives of various endocrine-disrupting chemicals in the environment and in wildlife is included at the end of this article.

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INTRODUCTION

Since the middle of the last century, many industrial chemicals and pesticides have been introduced into the environment. Many of these compounds are chlorinated aromatic structures, which are not easily metabolized, resulting in bioaccumulation or biomagnification. This bioaccumulation process has caused unwanted side effects in nontarget species, particularly those at higher trophic levels in the food chain. With growing awareness of these problems, various persistent halogenated compounds have gradually been banned from use. Since then, environmental levels in biota have decreased slowly during the last decades. As a replacement, new products have been developed that are less hydrophobic than organochlorines, and consequently do not accumulate in humans and wildlife.

The capacity of an organism to metabolize a compound to more polar products is often considered to be a detoxification mechanism. Furthermore, the presence of polar hydroxy groups in parent compounds often prevents bioaccumulation. In addition, introduction of even more polar groups such as glucuronides or sulfates further increases the ability of an organism to eliminate the compound [1,2].

A disadvantage of this approach is that the presence of hydroxy groups and complicated structures with aromatic moieties may bear resemblance to steroid structures. As a result, less bioaccumulative compounds such as alkylphenols, phthalate esters, and methoxychlor have shown biological activities similar to estrogens or androgens. These compounds can act as either (partial) agonists or antagonists for steroid receptors such as the estrogen receptor. Such interactions may have consequences for (sexual) development, reproduction, and the formation of hormone-dependent tumors. Thus, subtle differences among molecules, such as the presence or absence of an OH group, can lead to significant changes in their ability to bind to steroid receptors or inhibit steroidogenic enzymes. The presence of an OH and/or aromatic group plays a significant role in both steroid metabolism and receptor binding [3,4]. Thus, biotransformation plays a significant part in the endocrine-disrupting properties of a compound. On the one hand, the introduction of an OH group may bioactivate the parent compound by forming a metabolite that can interact with a steroid receptor or steroidogenic enzyme. On the other hand, a rapid phase II metabolism producing glucuronides or sulfates helps the organism to eliminate the parent compound from the body, reducing the opportunity for adverse effects.

This article summarizes the present state of knowledge of the role of metabolism in endocrine-related effects in aquatic and other terrestrial organisms. Although these effects are not discussed in view of results obtained in experimental laboratory systems with, for example, rodents, a comparison with experimental models is made if appropriate. In this topic, a selection of known endocrine active compounds (EASs) has been made that are presently considered to be most relevant for the environment. This overview focuses on the interactions of metabolites of EASs with the estrogen and androgen receptor or with steroidogenesis, because this has been the main emphasis of research over the last decade. It should however be recognized that these interactions are from a mechanistic point of view most firmly linked to reproduction, development, and hormone-dependent tumors. With increasing research efforts, undoubtedly other endocrine-related effects of metabolites of EASs will be uncovered. In addition, the authors have limited themselves to those EASs that are environmentally relevant. Pharmaceuticals with endocrine-disrupting properties have been excluded from this review. Nevertheless, these pharmaceutical compounds should not be ignored in the future as significant emissions from human and agricultural sources are to be expected.

BISPHENOL A

Most studies regarding the role of metabolism and metabolites in estrogenicity have been performed with rodents. In vitro studies with rat hepatocytes and in vivo studies with rats have shown that the major metabolite of bisphenol A (BPA) is a glucuronide. This phase II metabolite is predominantly formed in the liver and excreted in the bile [5–7]. In addition, at least four metabolites, among others a monosulfate and 3-OH BPA, were also formed in the rat, although quantitatively less important [6].

Experiments with different isoforms of UGT showed that UGT2B1 is probably most important glucuronidation enzyme in the rat [8].

In fish, the glucuronidation of BPA has also been reported to occur easily. This was illustrated by the presence of BPA glucuronide in the bile of caged fish that were exposed to sewage effluent [10]. Toxicokinetic studies with rainbow trout showed that the formation of BPA glucuronide can reach plasma concentrations that are about twice that of the parent compound (see Fig. 1) [11]. Thus, it can be concluded that in both mammalian and piscine systems the formation of glucuronides is the preferred metabolic pathway. This is to be expected as BPA and several of its analogs contain (several) hydroxy groups, which are highly susceptible to phase II metabolism.

Glucuronidation of BPA should be considered a detoxification process, as several investigators showed that these metabolites lacked or had decreased binding affinity for the ERα or ERβ in mammalian systems [7,9,12]. At present, there is no indication that these glucuronides would behave differently in piscine or avian systems.

However, in the rat certain minor metabolites of BPA do possess estrogenic activity, in some cases exceeding that of the parent compound. The biological relevance of these estrogenic metabolites is unknown. Incubations of BPA with rat liver S9 fraction showed that estrogenicity can increase several fold, which is possibly caused by activities of P450 enzymes [13]. Furthermore, hydroxylation of either the 3 or 5 position of BPA produces estrogenic metabolites that are 5 to 10 times less potent than the parent compound [6,9]. As several fish species exhibit significant cytochrome P450 activities the formation of hydroxylated BPA in fish is also likely. In view of the estrogenic activities of BPA observed in several fish species [11,14], it can not be excluded that certain hydroxylated metabolites of BPA may contribute.

Limited information is available about the biodegradation of BPA in the environment. Bacteria from sewage sludge were able to degrade BPA to an intermediary metabolite 4,4′-dihydroxy-α-methylstilbene, that has a structural resemblance to diethylstilbestrol (DES). However, this compound is easily further degraded to 4-hydroxybenzaldehyde and 4-hydroxyacetophenone [13,15]. The ecotoxicological significance of this formation process is presently unknown, but in general, BPA is considered as a readily biodegradable compound according to OECD standards [16].

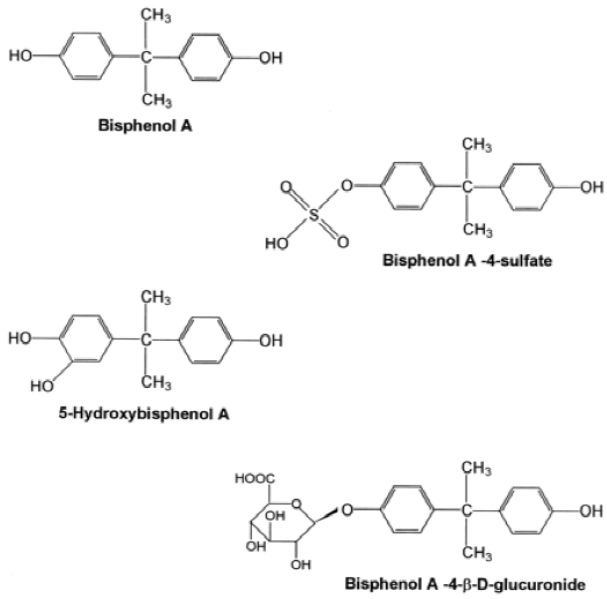


Fig. 1 Structures of BPA and metabolites from [9].

ALKYLPHENOLS

Alkylphenols (APs) are rapidly metabolized to either phase I or II metabolites in mammalian species such as rat and human, which has been reported from in vivo and in vitro studies [17–19]. Hydroxylation of the aromatic ring and the alkyl side chain, followed by glucuronidation are the major metabolic reactions in rat hepatocytes [19] one in vivo study with rats showed that glucuronidation of *p-tert*-octylphenol is the major metabolic pathway [17].

In fish, the metabolic pathways are basically similar to those in the rat. Glucuronidation of either the parent AP or the ω-hydroxylated metabolites is the major route of metabolism [20–22]. In rainbow trout, the major metabolite of 4-*n*-nonylphenol was the glucuronide of the parent compound, but hydroxylation of the ω-1 and 2 position of the alkyl chain also occurred. In addition, the rainbow trout was capable of oxidizing the ω-hydroxylated metabolites into carboxylic acid metabolites [21]. However, in the rainbow trout metabolism of 4-*n*-nonylphenol is also tissue-specific as distinct differences between the metabolic structures were found between the bile and the urine [23].

A study in rainbow trout of the metabolism of another AP congener, 4-*tert*-octylphenol, showed similar biotransformation pathways. The major metabolite was 4-*tert*-octylphenol-β-glucuronide, but hydroxylations of the ω (C2) or ω-3 (C4) positions of the alkyl chain were also found, beside ortho hydroxylation of the aromatic ring [24]. This rapid metabolism to more polar phase I and II metabolites leads to efficient elimination of this compound in the rainbow trout [22,25]. However, some tissue-specific retention of the parent compounds has been observed that may have some toxicological significance at higher exposure levels.

The possible biotransformation of nonylphenol diethoxylate to the more estrogenic nonylphenol was also studied in the rainbow trout, but no evidence could be found for such a metabolic pathway [26].

In Atlantic salmon, 4-*n*-nonylphenol was mainly metabolized to its glucuronide conjugate [27,28]. To a lesser extent, hydroxylated and oxidated metabolites were formed [27]. In vitro experiments with salmon hepatocytes did not show any significance difference in metabolite pattern when compared with in vivo results [28]. The major mono-hydroxylated metabolites were compounds with OH groups on the alkyl chain at the ω, ω-1, and ω-2 positions [29].

Studies with cytochrome P450-selective inhibitors suggest that the CYP2K and CYP2M enzymes play a significant role in the metabolism of 4-*n*-nonylphenol. These fish P450 enzymes show similarities with CYP3A and CYP2B enzymes in mammals [29]. In rats, some of these P450 enzymes are male-dominant and perform specific hydroxylations of testosterone. Several rodent studies have shown that APs can decrease the activity of CYP3A, CYP2B, but also the male-specific CYP2C11 [30–32]. In analogy with the results from rat studies, it may be possible for 4-*n*-nonylphenol to compete with steroids for CYP2K and CYP2M enzymes in fish. Such an interaction could influence the metabolism of steroids such as testosterone and progesterone [29]. In juvenile Atlantic salmon, it has recently been shown that 4-*n*-nonylphenol decreases the rate of the 6β, 16α, and 17α hydroxylation of progesterone [33].

The role of the major (glucuronide) metabolites of the APs was also studied in relation to in vitro binding with the estrogen or androgen receptor, including those in trout hepatocytes. None of the major metabolites of octyl- or nonylphenol showed significant interaction with these steroid receptors. This indicates that the role of metabolism is minor or absent with respect to the estrogenic activities of these compounds [34,35]. This is in agreement with a structure–activity relationships determined between the estrogen receptor and APs, which showed that binding of the para alkylphenols depends on covalent binding of the phenol and alkyl groups resembling the A ring and hydrophobic moiety of the steroids [36]. Thus, glucuronidation and hydroxylation would decrease the ER binding significantly. In summary, it can be concluded that metabolism of alkylphenols in fish, like in mammals, appears to be a detoxification process with respect to the endocrine-disrupting properties of the parent compounds.

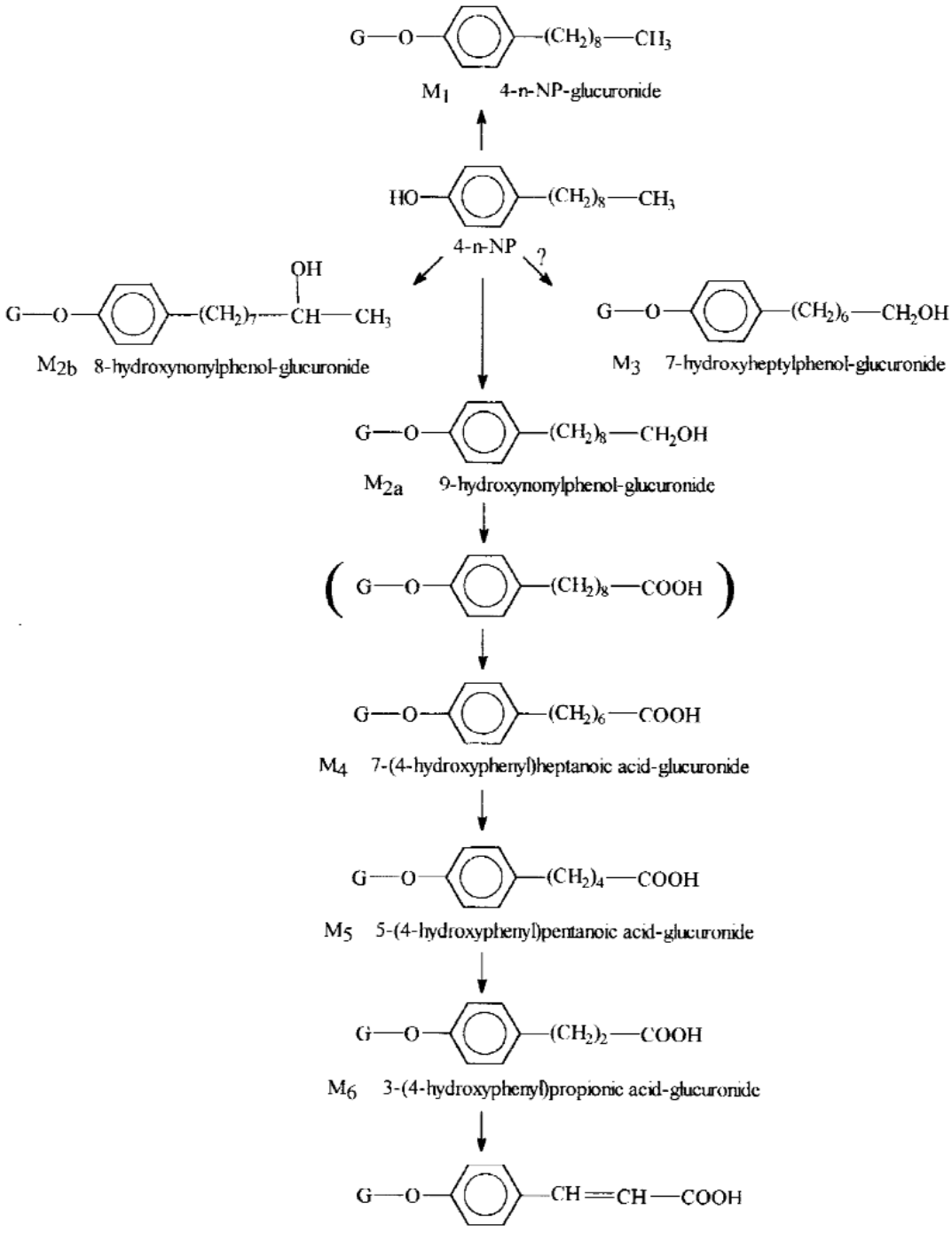


Fig. 2 Proposed biotransformation pathways for the in vivo formation of biliary metabolites from [21].

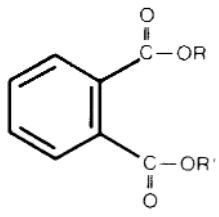
PHTHALATE ACID ESTERS

The esters of phthalic acid, either with dialkyl, aryl, or saturated cyclic hydrocarbon chains are important commercial compounds that are widely spread in the environment. Almost all phthalate acid esters

(PAEs) are not strongly bioaccumulative and metabolism occurs rapidly in all vertebrates [37]. In rats and fish, phthalate diesters are rapidly metabolized in the gastro-intestinal tract and gills, but also in liver and plasma [38–41]. The primary step in the metabolism of phthalate esters is conversion into monoesters, and occurs in mammals and fish (see Fig. 3) [37,42].

In mammals and fish, metabolic breakdown continues readily with phthalic acid as a major biotransformation product [43,44]. In the rainbow trout, extensive metabolism of di-2-ethylhexyl phthalate occurs in the gills where this compound is easily converted by esterases to the corresponding monoester [41]. Experiments with sheephead minnow and di-2-ethylhexyl phthalate combined with selective enzyme inhibitors showed that esterases and not P450 enzymes are responsible for the first step in metabolic breakdown [45]. In addition, the biological breakdown of phthalate esters in aquatic systems, including some invertebrates, is considered to be rapid, and no significant bioaccumulation has been reported [38,46].

As a few phthalate esters show some estrogenic activity [47] in certain in vitro systems, the role of metabolism was further examined. Most studies indicate that monoesters of phthalates and further degraded metabolites, such as phthalic acid, did not interact with the estrogen receptor [48,49]. The exceptions are the 4-hydroxylated metabolites, which have increased binding affinity for the estrogen receptor [50]. However, this metabolic pathway does not appear to be important because cytochrome P450 enzymes play only a minor role in the biotransformation of phthalates [45]. Thus, metabolic pathways in fish of phthalates should be considered as a detoxification process.



Phthalate Esters

Fig. 3 General structure of phthalate esters.

METHOXYCHLOR

In rodent methoxychlor (MXCL) is primarily metabolized to mono and bisphenol demethylated derivatives. In vitro studies with rat hepatic microsomal or S9 fractions in combination with specific P450 inhibitors or inducers implicated the involvement of P450 enzymes in this process (see Fig. 4) [13,51,52].

Information about the metabolism of MXCL in fish is scarce. Liver microsomes of catfish metabolized MXCL to mono- and bisdemethylated metabolites, but hydroxylation on the aromatic can also occur [53]. Thus, similar to mammals, the major metabolic pathway of MXCL in fish appears to be demethylation.

In rats, phenobarbital-inducible P450 enzymes play a role in the demethylation process, but enzymes from the CYP2B family are also responsible for aromatic hydroxylation [52,54]. Studies with

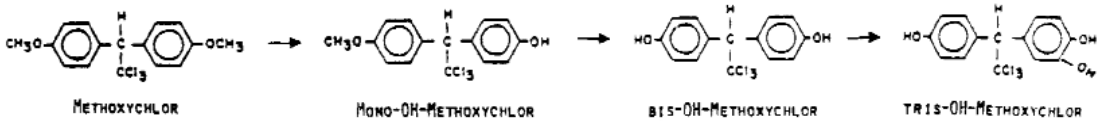


Fig. 4 Metabolism of methoxychlor in mammals (from Kupfer et al., 1990).

hepatic microsomes of the catfish and hepatocytes ruled out the involvement of CYP2K or CYP1A enzymes in the demethylation or aromatic hydroxylation of MXCL [14,53].

Structure–activity studies of the major metabolites of MXCL in rodents have shown that the mono- and bisdemethylated metabolites have agonist activities on the ERα, and antagonist activities on the ERβ and androgen receptor [55–57]. The question is to what extent results obtained with metabolites of MXCL and mammalian ERs are also applicable to other vertebrate groups such as fish, birds, and amphibians. One study in particular showed that different vertebrate species exhibit differential ligand preferences and binding affinities for estrogenic compounds [58].

In summary, it can be concluded that in analogy with the mammalian situation, MXCL most likely has to be bioactivated in fish and other vertebrate species to exert its endocrine-disrupting effects.

CHLORO-*S*-TRIAZINE HERBICIDES (TRZs)

Although the triazines, especially atrazine (ATR), have been shown to influence reproduction and development in multiple species and to stimulate the development of mammary tumors in one rat strain, the possible role of metabolism has not been fully elucidated [59–62]. Metabolism in fish, birds, mammals as well as microbes most commonly involves *N*-monodealkylation and hydroxylation processes [63–68]. For common ATR metabolites, see Fig. 5.

With respect to general toxicity, it is generally assumed that the metabolites of TRZs are substantially less active than their parent compounds [68]. Significant metabolic differences among mammalian species have been reported, but *N*-monodealkylation and isopropyl or ter-butyl hydroxylations followed by sulfoxidation remain the major pathways [63–65]. In rodent species, a distinct involvement of CYP1A and CYP2B enzymes in metabolism has been shown, but a role of CYP 2D1 and 2E1 has

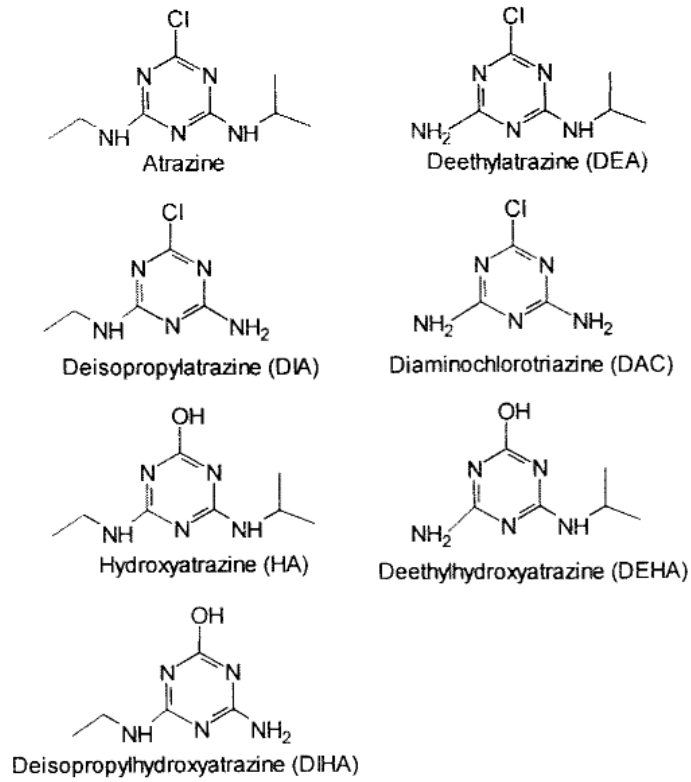


Fig. 5 The chemical structure of atrazine and the most common biodegradation products from [68].

also been found [64,69]. In humans, CYP1A2 specifically has been implicated to play a major role in the hepatic metabolism of atrazine [66]. Pre-induction with 3-methylcholantrene (3-MC) markedly induced the metabolism of TRZs. Therefore, in fish the major hepatic enzyme CYP1A1 that is 3-MC inducible may also be expected to be involved in the metabolism of atrazine. In addition, a study with zebra fish embryos indicated a possible role of GSH conjugation [70].

In general, triazine herbicides or their major metabolites do not show significant estrogen receptor binding in vitro or in vivo, but experimental results depend on the metabolite studied. The 2-chloro-4-amino-6-isopropylaminotriazine metabolites had a higher binding affinity for the ERα than the parent compounds, although many orders of magnitude lower than 17β-estradiol [63]. However, an earlier study with the fully dealkylated metabolite, diaminochlorotriazine, could not find any in vivo or in vitro estrogenic activity [71,72].

The possible (anti) estrogenic properties of the major metabolites of triazine herbicides were also studied in carp hepatocytes. None of the metabolites had any effect on the estrogen receptor mediated induction of vitellogenesis indicating a lack of interaction with the carp estrogen receptor, either as agonists or antagonists [73].

In vitro studies with the H295R human adrenocortical carcinoma cell line indicated that TRZs could induce the catalytic activity and mRNA expression of aromatase. Although mono *N*-dealkylated metabolites were still capable of inducing aromatase, this effect disappeared with full dealkylation or hydroxylation of the heterocyclic ring [73,74a]. Thus, regarding effects on the enzyme aromatase the metabolism of TRZs results in detoxification.

VINCLOZOLIN

The anti-androgenic activity of vinclozolin in mammals is well known [74b], and the activity depends on the binding of two vinclozolin metabolites, a butenanilide derivative and hydroxybutenoic acid derivative (Fig. 6), to the androgen receptor [74c]. A recent report [74d] on the demasculinization effects of these compounds on the adult male guppy seems to be caused by the similar mechanism. Thus, any endocrine activities displayed by chemicals in mammals via the target site that is ubiquitous among various organism species are likely to be also exhibited in various other species.

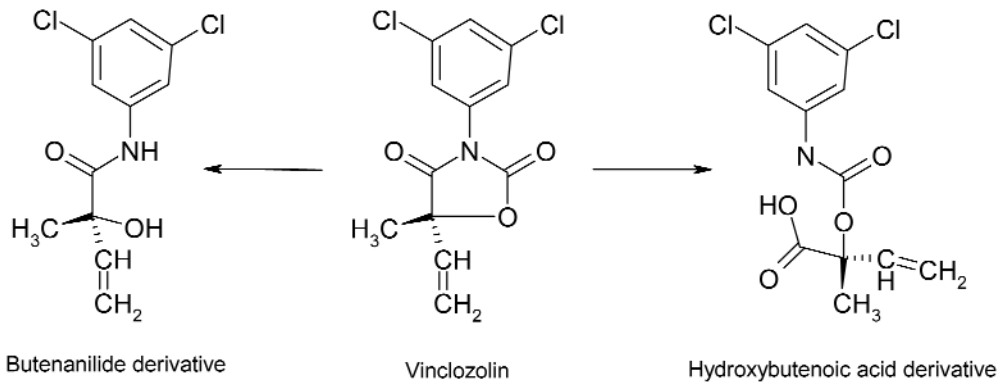


Fig. 6 Structure of vinclozolin and its two anti-androgenic metabolites.

DDT

This is one of the most persistent organohalogen pesticides known and occurs everywhere in the global environment. The major metabolite of DDT in vertebrates, DDE has an extremely long half-life in biota. DDE is formed by enzymatic dechlorination that involves a glutathione-dependent reaction. In addition, other degradation products such as DDD and DDA are formed by series of reductive dechlorination and

oxidative processes in various vertebrate species. The general metabolism pathway of DDT in mammals and avian species is shown in Fig. 7.

The stability of DDT metabolites, including DDE and DDD, is notorious, and the question rises to what extent these metabolites are responsible for observed endocrine-disrupting properties in various vertebrate species. A large number of in vitro and in vivo studies have been performed to identify the endocrine-disrupting properties DDT and its metabolites, but mainly using mammalian systems. Nevertheless, a limited number of these studies used piscine or avian systems to elucidate the role of DDT and metabolites in this process. With respect to binding affinity of these compounds to the ER notable differences have been observed among species for *o,p*-DDT [75]. When evaluating results of these types of studies with DDT metabolites, the interaction appears to be rather species- and compound-specific.

Based on either the interaction with fish ER or the estrogen-mediated vitellogenin synthesis, it can be concluded that DDE is at best a weak ER agonist or antagonist differing many orders of magnitude with E2 in the tested mammalian, fish, amphibian, or reptile species [14,57,77,78]

Although some DDT metabolites bind weakly to the ER, the major biotransformation product *p,p*-DDE has been identified as an androgen receptor (AR) antagonist in mammalian systems [57,79,80]. Experiments with guppies showed that *p,p*-DDE had demasculinizing properties in fish consistent with AR antagonism [81]. At present, it is unclear whether the developmental and reproductive effects of DDT in fish or other vertebrates are caused by the interaction of DDT itself with the ER or that of its metabolite *p,p*-DDE with the AR.

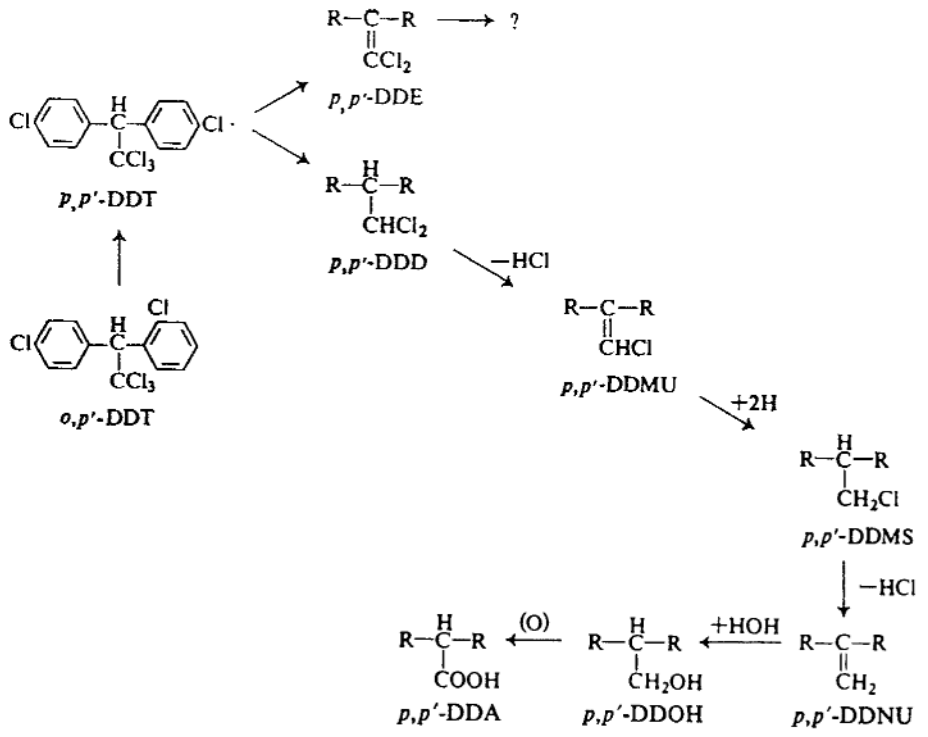


Fig. 7 Degradation of DDT by mammalian and avian tissues from [76].

POLYCHLORINATED BIPHENYLS

Although many polychlorinated biphenyls (PCBs) are retained as parent compounds in lipid-rich tissues of most vertebrate species, some of their effects have been attributed to the action of hydroxylated

metabolites. In particular, the competitive binding of hydroxylated PCBs (OH-PCBs) to the thyroid hormone transporting protein, transthyretin (TTR), has been shown to have effect on the thyroid hormone levels in rodents. This competitive binding to TTR is most pronounced for those PCB metabolites that have a hydroxy group on the para position with two adjacent chlorine atoms [82]. In addition, some hydroxylated metabolites have been found to interact with the ERα. Certain PCB metabolites with hydroxy groups on the para position without vicinal chlorines have estrogenic activity. However, these OH-PCBs do not occur in the environment, where a preferred retention of metabolites with a para hydroxy group with vicinal chlorine atoms and five to seven chlorine atoms occurs [77,83,84]. The subsequent hydroxylation of intermediate epoxides of PCBs to, for example, catechols can also produce weak estrogenic metabolites that have an activity in the range of nonylphenol and *o,p*-DDT (see Fig. 8) [85].

All these experiments have been done with *either* in vivo or in vitro systems of mammalian origin, and the implications of these results for other groups of vertebrates such as fish or amphibian is unclear. Nevertheless, it should be recognized that fish species, just as mammals, are capable of metabolizing PCBs to hydroxylated forms, with a preference for the para position [86]. Thus interactions between (*di*) OH-PCBs and TTR or estrogen receptor may be expected in other than mammalian vertebrates [75].

Another group of relevant PCB metabolites are those with methylsulfonyl groups. These metabolites are commonly found in several species of fish and mammalian wildlife [87–90] in addition to human milk and plasma [91,92]. Structurally, the 3 and 4-methylsulfonyl metabolites appear to be most commonly found in humans, rodents, and wildlife [88,91–95]. These methylsulfonyl PCBs have been associated with developmental and reproductive effects in the mink [96]. Among the more subtle effects are hepatic P450 induction, modulation of thyroid hormone levels and inhibition of the glucocorticoid synthesis [94,95,97–100].

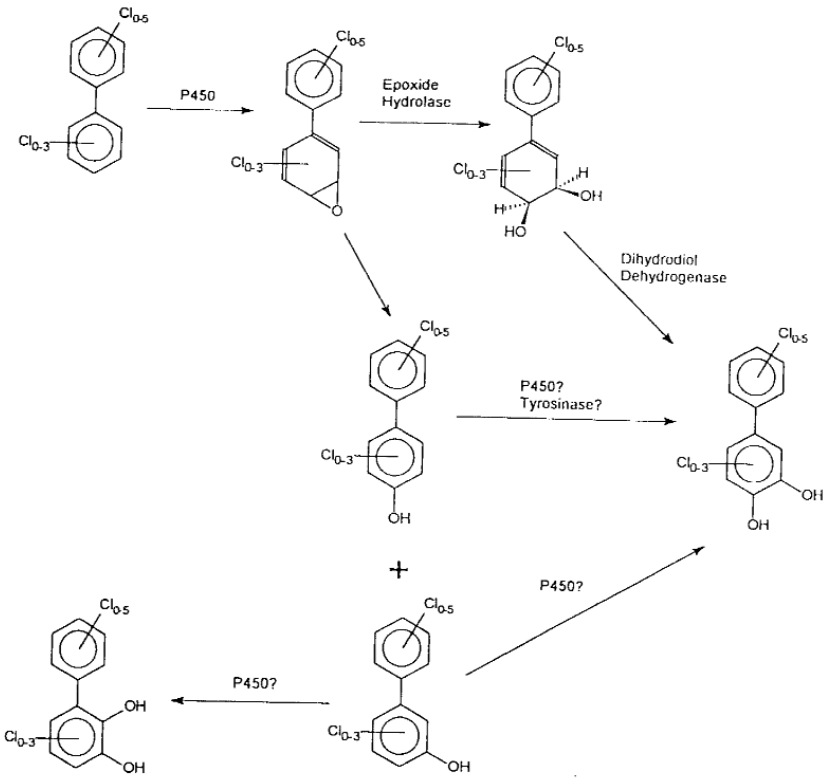


Fig. 8 Formation of monohydroxy and catechol PCB metabolites from [85].

CONCLUSIONS AND RECOMMENDATIONS

The information presented in this chapter illustrates that metabolism plays a significant role in bioactivation and/or deactivation of EASs. The information that is available for most well-known EASs have been derived from either in vivo or in vitro studies using material from humans or rodents. These studies have shown that particularly the presence of hydroxy or keton groups and aromatic moieties play an important role in the mechanism of action of EASs. This is not unexpected as in the steroid biosynthesis pathway these same functional groups are involved in the conversion of cholesterol to progestins, androgens, and estrogens. To prevent persistence and bioaccumulation/magnification, many modern synthetic compounds are readily metabolized to more polar forms often containing one or more hydroxy groups. This enhanced ability to undergo biotransformation also has increased the potential for the formation of more EASs. Consequently, the additional and consistent testing of metabolites for endocrine-disrupting properties should be encouraged in the future in order to establish a better risk assessment process for these types of compounds. Furthermore, it should be recognized that common metabolic processes such as hydroxylation, oxidation, and glucuronidation could differ strongly among species, complicating ecotoxicological risk assessment of EASs.

REFERENCES

1. M. Van den Berg, D. Van de Meent, W. J. G. M. Peijnenburg, D. T. H. M. Sijm, J. W. Tas. In *Risk Assessment of Chemicals: An Introduction*, C. J. Van Leeuwen and J. Hermens (Eds.), pp. 37–102, Kluwer, Dordrecht (1995).
2. A. Parkinson. In *Casarett & Doull's Toxicology: The Basic Science of Poisons*, C. D. Klaassen (Ed.), pp. 113–186, McGraw-Hill, New York (1995).
3. C. L. Williams and G. M. Sancel. In *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, J. G. Hardman, A. Goodman Gilman, L. E. Limbird (Eds.), pp. 1411–1440, McGraw-Hill, New York. (1995).
4. J. D. Wilson. In *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, J. G. Hardman, A. Goodman Gilman, L. E. Limbird (Eds.), pp. 1441 *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, J. G. Hardman, A. Goodman Gilman, L. E. Limbird (Eds.), pp. 1411–1440, McGraw-Hill, New York (1995).
5. H. Inoue, H. Yokota, T. Makino, A. Yuasa, S. Kato. *Drug Metab. Dispos*. **29**, 1084–1087 (2001).
6. Y. Nakagawa and T. Suzuki. *Xenobiotica* **31**, 113–123 (2001).
7. R. W. Snyder, S. C. Maness, K. W. Gaido, F. Welsch, S. C. Sumner, T. R. Fennell. *Toxicol. Appl. Pharmacol*. **168**, 225–234 (2000).
8. H. Yokota, H. Iwano, M. Endo, T. Kobayashi, H. Inoue, S. Ikushiro, A. Yuasa. *Biochem. J*. **340**, 405–409 (1999).
9. R. Elsby, J. L. Maggs, J. Ashby, B. K. Park. *J. Pharmacol. Exp. Ther*. **297**, 103–113 (2001).
10. D. G. J. Larsson, M. Adolfsson-Erici, J. Parkkonen, M. Pettersson, A. H. Berg, P.-E. Olsson, L. Forlin. *Aquat. Toxicol*. **45**, 91–97 (1999).
11. C. Lindholst, S. N. Pedersen, P. Bjerregaard. *Aquat. Toxicol*. **55**, 75–84 (2001).
12. J. B. Matthews, K. Twomey, T. R. Zacharewski. *Chem. Res. Toxicol*. **14**, 149–157 (2001).
13. S. Yoshihara, M. Makishima, N. Suzuki, S. Ohta. *Toxicol. Sci*. **62**, 221–227 (2001).
14. J. M. Smeets, I. van Holsteijn, J. P. Giesy, W. Seinen, M. van den Berg. *Toxicol. Sci*. **50**, 206–213 (1999).
15. J. Spivack, T. K. Leib, J. H. Lobos. *J. Biol. Chem*. **269**, 7323–7329 (1994).
16. R. J. West, P. A. Goodwin, G. M. Klecka. *Bull. Environ. Contam. Toxicol*. **67**, 106–112 (2001).
17. H. Certa, N. Fedtke, H. J. Wiegand, A. M. Muller, H. M. Bolt. *Arch. Toxicol*. **71**, 112–22 (1996).
18. P. C. Lee. *Endocrine* **9**, 105–111 (1998).
19. R. T. Pedersen and E. M. Hill. *Chem. Biol. Interact*. **128**, 189–209 (2000).

20. A. C. Meldahl, K. Nithipatikom, J. J. Lech. *Xenobiotica* **26**, 1167–1180 (1996).
21. R. Thibaut, L. Debrauwer, D. Rao, J. P. Cravedi. *Xenobiotica* **28**, 745–757 (1998).
22. N. G. Coldham, S. Sivapathasundaram, M. Dave, L. A. Ashfield, T. G. Pottinger, C. Goodall, M. J. Sauer. *Drug Metab. Dispos*. **26**, 347–354 (1998).
23. R. Thibaut, L. Debrauwer, D. Rao, J. P. Cravedi. *Sci. Total Environ*. **233**, 193–200 (1999).
24. R. T. Pedersen and E. M. Hill. *Xenobiotica* **30**, 867–879 (2000).
25. A. M. Ferreira-Leach and E. M. Hill. *Mar. Environ. Res*. **51**, 75–89 (2001).
26. J. P. Cravedi, G. Boudry, M. Baradat, D. Rao, L. Debrauwer. *Aquat. Toxicol*. **53**, 159–172 (2001).
27. A. Arukwe, T. Celius, B. T. Walther, A. Goksoyr. *Aquat. Toxicol*. **49**, 159–170 (2000).
28. A. Arukwe, A. Goksoyr, R. Thibaut, J. P. Cravedi. *Mar. Environ. Res*. **50**, 141–145 (2000).
29. R. Thibaut, L. Debrauwer, E. Perdu, A. Goksoyr, J. P. Cravedi, A. Arukwe. *Aquat. Toxicol*. **56**, 177–190 (2002).
30. N. Hanioka, H. Jinno, Y. S. Chung, T. Nishimura, T. Tanaka-Kagawa, M. Ando. *Arch. Toxicol*. **73**, 625–631 (2000).
31. N. Hanioka, T. Tanaka-Kagawa, Y. S. Chung, T. Nishimura, H. Jinno, M. Ando. *Bull. Environ. Contam. Toxicol*. **64**, 804–810 (2000).
32. N. Hanioka, H. Jinno, Y. S. Chung, T. Tanaka-Kagawa, T. Nishimura, M. Ando. *Xenobiotica* **29**, 873–883 (1999).
33. A. Arukwe, F. R. Knudsen, A. Goksoyr. *Environ. Health Perspect*. **105**, 418–422 (1997).
34. G. J. Moffat, A. Burns, J. Van Miller, R. Joiner, J. Ashby. *Regul. Toxicol. Pharmacol*. **34**, 182–187 (2001).
35. T. Madigou, P. Le Goff, G. Salbert, J. P. Cravedi, H. Segner, F. Pakdel, Y. Valotaire. *Aquat. Toxicol*. **53**, 173–186 (2001).
36. Y. Tabira, M. Nakai, D. Asai, Y. Yakabe, Y. Tahara, T. Shinmyozu, M. Noguchi, M. Takatsuki, Y. Shimohigashi. *Eur. J. Biochem*. **262**, 240–245 (1999).
37. N. P. Moore. *Reprod. Toxicol*. **14**, 183–192 (2000).
38. J. A. Thomas and M. J. Thomas. *Crit. Rev. Toxicol*. **13**, 283–317 (1984).
39. I. R. Rowland, R. C. Cottrell, J. C. Phillips. *Food Cosmet. Toxicol.* **15**, 17–21 (1977).
40. R. D. White, D. E. Carter, D. Earnest, J. Mueller. *Food Cosmet. Toxicol.* **18**, 383–386 (1980).
41. M. G. Barron, I. R. Schultz, W. L. Hayton. *Toxicol. Appl. Pharmacol*. **98**, 49–57 (1989).
42. M. J. Melancon and J. J. Lech. *Drug Metab. Dispos*. **5**, 29–36 (1977).
43. T. Mettang, D. M. Alscher, C. Pauli-Magnus, R. Dunst, U. Kuhlmann, A. W. Rettenmeier. *Adv. Perit. Dial*. **15**, 229–233 (1999).
44. C. Nativelle, K. Picard, I. Valentin, J. C. Lhuguenot, M. C. Chagnon. *Food Chem. Toxicol*. **37**, 905–917 (1999).
45. A. H. Karara and W. L. Hayton. *Drug Metab. Dispos*. **16**, 146–150 (1988).
46. R. L. Metcalf, G. M. Booth, C. K. Schuth, D. J. Hansen, P. Y. Lu. *Environ. Health Perspect*. **4**, 27–34 (1973).
47. S. Jobling, T. Reynolds, R. White, M. G. Parker, J. P. Sumpter. *Environ. Health Perspect*. **103**, 582–587 (1995).
48. C. A. Harris, P. Henttu, M. G. Parker, J. P. Sumpter. *Environ. Health Perspect*. **105**, 802–811 (1997).
49. K. Picard, J. C. Lhuguenot, M. C. Lavier-Canivenc, M. C. Chagnon. *Toxicol. Appl. Pharmacol*. **172**, 108–118 (2001).
50. D. Asai, Y. Tahara, M. Nakai, Y. Yakabe, M. Takatsuki, T. Nose, T. Shinmyozu, Y. Shimohigashi. *Toxicol. Lett*. **118**, 1–8 (2000).
51. G. D. Charles, M. J. Bartels, C. Gennings, T. R. Zacharewski, N. L. Freshour, B. Bhaskar Gollapudi, E. W. Carney. *Reprod. Toxicol*. **14**, 207–216 (2000).
52. D. Kupfer, W. H. Bulger, A. D. Theoharides. *Chem. Res. Toxicol*. **3**, 8–16 (1990).

53. D. Schlenk, D. M. Stresser, J. C. McCants, A. C. Nimrod, W. H. Benson. *Toxicol. Appl. Pharmacol*. **145**, 349–356 (1997).
54. S. S. Dehal and D. Kupfer. *Drug Metab. Dispos*. **22**, 937–946 (1994).
55. K. Sumida, N. Ooe, H. Nagahori, K. Saito, N. Isobe, H. Kaneko, I. Nakatsuka. *Biochem. Biophys. Res. Commun*. **280**, 85–91 (2001).
56. K. M. Waters, S. Safe, K. W. Gaido. *Toxicol. Sci*. **63**, 47–56 (2001).
57. K. W. Gaido, S. C. Maness, D. P. McDonnell, S. S. Dehal, D. Kupfer, S. Safe. *Mol. Pharmacol*. **58**, 852–858 (2000).
58. J. Matthews, T. Celius, R. Halgren, T. Zacharewski. *J. Steroid Biochem. Mol. Biol*. **74**, 223–34 (2000).
59. J. C. Eldridge, M. K. Tennant, L. T. Wetzel, C. B. Breckenridge, J. T. Stevens. *Environ. Health Perspect*. **102** (Suppl 11), 29–36 (1994).
60. C. Wiegand, E. Krause, C. Steinberg, S. Pflugmacher. *Ecotoxicol. Environ. Saf*. **49**, 199–205 (2001).
61. J. T. Stevens, C. B. Breckenridge, L. Wetzel. *J. Toxicol. Environ. Health A* **56**, 69–109 (1999).
62. R. L. Cooper, T. E. Stoker, L. Tyrey, J. M. Goldman, W. K. McElroy. *Toxicol. Sci*. **53**, 297–307 (2000).
63. N. Hanioka, H. Jinno, T. Tanaka-Kagawa, T. Nishimura, M. Ando. *Xenobiotica* **29**, 1213–1226 (1999).
64. N. Hanioka, H. Jinno, T. Tanaka-Kagawa, T. Nishimura, M. Ando. *Toxicol. Appl. Pharmacol*. **156**, 195–205 (1999).
65. D. Lang, D. Criegee, A. Grothusen, R. W. Saalfrank, R. H. Bocker. *Drug Metab. Dispos*. **24**, 859–865 (1996).
66. D. H. Lang, A. E. Rettie, R. H. Bocker. *Chem. Res. Toxicol*. **10**, 1037–1044 (1997).
67. S. U. Khan and T. S. Foster. *J. Agric. Food Chem*. **24**, 768–771 (1976).
68. K. R. Solomon, D. B. Baker, R. P. Richards, K. R. Dixon, S. J. Klaine, T. W. La Point, R. J. Kendall, C. P. Weiskopf, J. M. Giddings, J. P. Giesy, W. J. J. Hall, W. M. Williams. *Environ. Toxicol. Chem*. **15**, 31–76 (1996).
69. N. Hanioka, H. Jinno, K. Kitazawa, T. Tanaka-Kagawa, T. Nishimura, M. Ando, K. Ogawa. *Chem. Biol. Interact*. **116**, 181–198 (1998).
70. C. Wiegand, S. Pflugmacher, M. Giese, H. Frank, C. Steinberg. *Ecotoxicol. Environ. Saf*. **45**, 122–131 (2000).
71. M. K. Tennant, D. S. Hill, J. C. Eldridge, L. T. Wetzel, C. B. Breckenridge, J. T. Stevens. *J. Toxicol. Environ. Health*. **43**, 197–211 (1994).
72. M. K. Tennant, D. S. Hill, J. C. Eldridge, L. T. Wetzel, C. B. Breckenridge, J. T. Stevens. *J. Toxicol. Environ. Health*. **43**, 183–196 (1994).
73. J. T. Sanderson, R. J. Letcher, M. Heneweer, J. P. Giesy, M. van den Berg. *Environ. Health Perspect*. **109**, 1027–1031 (2001).
74. (a) J. T. Sanderson, W. Seinen, J. P. Giesy, M. van den Berg. *Toxicol. Sci*. **54**, 121–127 (2000); (b) W. H. Bulger, V. J. Feil, D. Kupfer. *Mol. Pharmacol*. **27**, 115–124 (1985); (c) C. Wong, W. R. Kelce, M. Sar, E. M. Wilson. *J. Biol. Chem*. **270**, 19998–20003 (1995); (d) E. Baatrup and M. Junge. *Environ. Health Persp*ect. **109**, 1063–1070 (2001).
75. K. Fent. *Toxicol. in Vitro* **15**, 477–488 (2001).
76. D. J. Ecobichon. In *Casarett & Doull's Toxicology: The Basic Science of Poisons*, C. D. Klaassen (Ed.), pp. 643–689, McGraw-Hill, New York (1995).
77. V. J. Kramer and J. P. Giesy. *Sci. Total Environ*. **233**, 141–161 (1999).
78. E. J. Clark, D. O. Norris, R. E. Jones. *Gen. Comp. Endocrinol*. **109**, 94–105 (1998).
79. W. R. Kelce, C. R. Stone, S. C. Laws, L. E. Gray, J. A. Kemppainen, E. M. Wilson. *Nature* **375**, 581–585 (1995).
80. P. Sohoni and J. P. Sumpter. *J. Endocrinol*. **158**, 327–339 (1998).

81. E. Baatrup and M. Junge. *Environ. Health Perspect*. **109**, 1063–1070 (2001).
82. A. Brouwer, U. G. Ahlborg, M. Van Den Berg, L. S. Birnbaum, E. R. Boersma, B. Bosveld, M. S. Denison, L. E. Gray, L. Hagmar, E. Holene, M. Huisman, S. W. Jacobson, J. L. Jacobson, C. Koopman-Esseboom, J. G. Koppe, B. M. Kulig, D. C. Morse, G. Muckle, R. E. Peterson. *Eur. J. Pharmacol., Environ. Toxicol. Pharmacol. Sect.* **293**, 1–40 (1995).
83. V. J. Kramer, W. G. Helferich, A. Bergman, E. Klasson-Wehler, J. P. Giesy. *Toxicol. Appl. Pharmacol*. **144**, 363–376 (1997).
84. D. D. Vakharia and J. F. Gierthy. *Toxicol. Lett*. **114**, 55–65 (2000).
85. C. E. Garner, W. N. Jefferson, L. T. Burka, H. B. Matthews, R. R. Newbold. *Toxicol. Appl. Pharmacol*. **154**, 188–197 (1999).
86. G. Mehrtens and F. Laturnus. *Chemosphere* **38**, 2995–3002 (1999).
87. R. Lawung, V. Prachayasittikul, L. Bulow. *Protein Expr. Purif*. **23**, 151–158 (2001).
88. R. J. Letcher, R. J. Norstrom, A. Bergman. *Sci. Total Environ*. **160–161**, 409–420 (1995).
89. H. M. Stapleton, R. J. Letcher, J. E. Baker. *Environ. Sci. Technol*. **35**, 4747–4752 (2001).
90. K. Wiberg, R. Letcher, C. Sandau, J. Duffe, R. Norstrom, P. Haglund, T. Bidleman. *Anal. Chem.* **70**, 3845–3852 (1998).
91. K. Noren, A. Lunden, E. Pettersson, A. Bergman. *Environ. Health Perspect*. **104**, 766–772 (1996).
92. K. Noren, C. Weistrand, F. Karpe. *Arch. Environ. Contam. Toxicol*. **37**, 408–414 (1999).
93. Y. Kato, K. Haraguchi, K. Tomiyasu, Hiroyuki Saito, M. Isogai, Y. Masuda, R. Kimura. *Environ. Toxicol. Pharmacol*. **3**, 137–144 (1997).
94. Y. Kato, K. Haraguchi, T. Shibahara, Y. Masuda, R. Kimura. *Arch. Toxicol*. **72**, 541–544 (1998).
95. Y. Kato, K. Haraguchi, T. Shibahara, S. Yumoto, Y. Masuda, R. Kimura. *Toxicol. Sci*. **48**, 51–54 (1999).
96. B.-O. Lund, J. Orberg, A. Bergman, C. Larsson, A. Bergman, M. Backlin B.-H. Hakansson, A. Madej, A. Brouwer, B. Brunstrom. *Environ. Toxicol. Chem*. **18**, 292–298 (1999).
97. Y. Kato, K. Haraguchi, T. Shibahara, Y. Shinmura, Y. Masuda, R. Kimura. *Chem.-Biol. Interact*. **125**, 107–115 (2000).
98. M. Johansson, C. Larsson, A. Bergman, B.-O. Lund. *Pharmacol. Toxicol*. **83**, 225–230 (1998).
99. M. Johansson, S. Nilsson, B. O. Lund. *Environ. Health Perspect*. **106**, 769–772 (1998).
100. B.-O. Lund. *Environ. Toxicol. Chem*. **13**, 911–917 (1994).

APPENDIX

Table 1 Half-lives of some endocrine active substances in air, water, and soil[a].

Chemicals	Environments		
	Air	Water	Soil/sediments
2,3,7,8-TCDD	1.2–9.6 h		1.5–10 years (O)[b]
2,3,7,8-TCDF	2.1–11.5 h		–20 years (O)
2,3,3′,4,4′-PCB	8–80 days	>56 days	0.91–7.25 years (O)
p,*p*′-DDT	<3 days (P)[b]		45–420 days (O), 7–45 days (R)[b]
Gamma-HCH			1–10 months (O), 10–20 days (R)
Chlodane	12 h–5 days	239 days	0.4–8 years (O)
Benzo(b)fluoranthene	3.4 h–1.4 days	>100 days	14.2 years–87 days (O)
Benzo(a)pyrene	2.4 h	5.4–17.3 years	14.6 years–151 days (O)
Benzo(g,h,I)perylene	0.31–10.0 h	>100 days	1.8 years–173 days (O)
Tributyltin		3–20 days	1–6 months (O), >2 years (R)
Triphenyltin		180 days	Longer than tributyltins
Bisphenol A	0.74–7.4 h (P)	2.5–5 days	
Nonylphenols	10–15 h (P)	2.5–20 days	10–104 days (O), >60 years (R)
4-*tert*-Octylphenol		7–50 days	>60 years (R)
Ethinylestradiol	10 days (P)	20–40 days	Much longer than estradiol
17β-Estradiol	10 days (P)	0.2–9 days	0.11 days (O), 0.37–0.66 days (R)
Estrone		0.2–9 days	0.4 days (O), 11–14 days (R)

[a]It should be noted that half-lives shown here can be varied widely due to the environmental conditions, such as light intensity, temperature, initial concentration of compounds, soil, and water properties, etc., although representative data was collected from the references.
[b]Symbols, (P) denotes photolysis (not always in air), (O) aerobic degradation, (R) anaerobic degradation.

TABLE 1 REFERENCES

- M. Ahel, F. E. Scully Jr. et al. *Chemosphere* **28**, 1361–1368 (1994).
- I. Angelidaki, A. S. Mogensen et al. *Biodegradation* **11**, 377–383 (2000).
- R. Ekelund et al. *Environ. Pollut.* **79**, 59–61 (1993).
- C. A. Staples, P. B. Dorn et al. *Chemosphere* **36**, 2149–2173 (1998).
- S. Kuwatsuka. In *Pesticides: Design and Development Guide* (in Japanese), I. Yamamoto and J. Fukami (Eds.), p. 1222, Softscience, Tokyo (1979).
- J. Kanazawa. In *Environmental Science of Pesticides* (in Japanese), Godo Shuppan, Tokyo (1992).
- S. Prakash, G. S. Tandon et al. *Biochem. Biophys. Res. Commun.* **199**, 1284–1288 (1994).
- D. Y. Shang, R. W. Macdonald et al. *Environ. Sci. Technol.* **33**, 1366–1372 (1999).
- C. A. Staples, J. B. Williams et al. *Chemosphere* **38**, 2029–2039 (1999).
- U.S. Environmental Protection Agency. Persistent bioaccumulative toxic (PBT) chemicals; Final rule, Federal Register, 29 Oct. 1999, pp. 58665–58753 (1999).
- M. Hesselsoe, D. Jensen et al. *Environ. Sci. Technol.* **35**, 3695–3700 (2001).
- C. A. Staples, C. G. Naylor et al. *Environ. Sci. Technol.* **20**, 2450–2455 (2001).
- M. D. Jurgens, K. I. E. Holthaus et al. *Environ. Sci. Technol.* **21**, 480–488 (2002).

Table 2 Selected results of Japanese 1998–1999 survey of EDs in the environment.

EDs	Environmental samples	Sample numbers	Detected numbers	Concentration range
PCDDs + PCDFs + coplanar PCB	Air	100	100	0.0017–0.70 pg TEQ/m^3
	Surface water	204	204	0.0014–13 pg TEQ/l
	Ground water	188	–	ND-5.4 pg TEQ/l
	Sediments	205	–	ND-260 pg TEQ/kg-dry
	Soil	286	286	0.0015–61 pg TEQ/kg
	Fish & aquatic invertebrates	368	368	0.0022–30 pg TEQ/kg
	Carp	48	48	0.20–5.9 pg TEQ/kg
	Frogs	80	80	0.20–7.5 pg TEQ/kg
	Pigeons	8	8	0.99–10 pgTEQ/kg
	Kites	20	20	22–220 pg TEQ/kg
	Predators (mainly owls)	9	9	14–530 pg TEQ/kg
	Whales	22	22	1.3–200 pg TEQ/kg
	Seals	13	13	8.6–27 pg TEQ/kg
	Apodemus speciosus	37	37	0.52–120 pg TEQ/kg
	Japanese monkey	6	6	0.82–9.4 pg TEQ/kg
	Bears	6	6	0.18–1.1 pg TEQ/kg
	Raccoon dogs	5	5	13–100 pg TEQ/kg
Tributyltin	Surface water	170	23	ND(<0.002)–0.008 ug/l
	Sediments	48	44	ND(<0.2)–170 ug/kg-dry
	Soil	7	0	ND(<20) ug/kg
	Fish	141	113	ND(<1)–120 ug/kg
	Carp	145	92	ND(<0.3)–75 ug/kg
	Whales	26	18	ND(<20–50)–330 ug/kg
	Seals	19	1	ND(<20–50)–110 ug/kg
	Pigeon	31	0	ND(<200) ug/kg
	Kites	26	2	ND(2–200)–8 ug/kg
	Owls	5	0	ND(<2) ug/kg
	Predators	30	0	ND(<200) ug/kg
	Wild mice	30	0	ND(<200) ug/kg
	Rhesus monkey	41	0	ND(<200) ug/kg
	Bears	17	0	ND(<50–200) ug/kg
	Raccoon dogs	15	0	ND(<50–200) ug/kg
Nonylphenol	Surface water	431	80	ND(0.1)–4.6 ug/l
	Sediments	51	23	ND(<0.1)–2700 ug/kg-dry
	Soil	94	0	ND(<50) ug/kg
	Fish	141	42	ND(<15)–780 ug/kg
	Carp	145	0	ND(<50) ug/kg
	Pigeons	31	16	ND(<15)–113 ug/kg
	Apodemus speciosus	30	22	ND(<15)–190 ug/kg
	Raccoon dogs	15	14	ND(<15)–2000 ug/kg
17β-estradiol	Water	130	79	ND(<0.001)–0.035 ug/l
	Sediments	20	19	ND(<0.001)–0.3 ug/kg-dry
Ethinylestradiol	Water	197	2	ND(<0.0001)–0.0002 ug/l
	Sediments	20	4	ND(<0.01)–0.34 ug/kg-dry

Data was obtained from the following Japanese Ministry of Environment publications (in Japanese):

- *Advanced Report of Survey on Environmental Active Substances in Water* (1999)
- *Results of Survey on Accumulation of Dioxins in Wildlife* (1999)
- *Results of Survey on Effects of Endocrine Active Substances on Wildlife* (1999)
- *Results of 1999 Survey of Chemical Emission into the Environment* (2000)
- *Results of Survey on Dioxin-Related Compounds in Arable Soils and Crops* (2000)