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

Environmental exposure, species differences and risk assessment

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Abstract: Field observations and laboratory studies suggest that, under certain environmental conditions, xenobiotics such as DDT, polychlorinated biphenyls (PCBs), tetrachloro dibenzodioxin (TCDD), tributyltin (TBT) and nonylphenol (NP) may cause endocrine disrupting effects. Although there are specific examples of established or suspected endocrine-mediated effects in the environment, such effects cannot be considered of broad, general importance because of wide ecological and species differences. Such species differences may arise because of differences in the basic mechanisms of sex differentiation, differences in receptor structure and function and differences in metabolism. In some cases, natural hormones may be responsible for alleged adverse effects since, despite their minute concentrations, they are particularly potent. Additional in-depth comparative studies are required to assess more precisely the potential endocrine disrupting action of natural and synthetic chemicals in the environment and to develop effective screens for their detection.

INTRODUCTION

Since environmentally relevant hormonally active chemicals, oestrogens, androgens or gestagens, are numerous and structurally diverse and since they are ubiquitously distributed in the environment, environmental risk assessment must consider the following facts:

- The demonstration that a given chemical is endocrinologically active either *in vitro* or *in vivo* in any particular organism does not necessarily mean that it is an environmental endocrine disruptor;
- In environmental risk assessment, the objective is somewhat different from assessing the potential risks to human health. In environmental risk assessment, the primary objective is not to protect each individual organism but to protect populations of organisms and species. Consequently, it is important to determine whether a population effect will occur;
- Since there are a wide variety of both naturally occurring (mammalian and plant constituents) and synthetic endocrine-active chemicals released into the environment, it is important to assess the relative potency of these materials;
- Natural fluctuations (background levels) of field populations must be understood and considered in assessing the actual risks of endocrine-active chemicals. It is often very difficult to measure the magnitude of these natural fluctuations;
- Since there can be no effect without exposure, a thorough evaluation of releases and environmental fate (monitoring) is a critical component of environmental risk assessment;

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- Risk assessment is complicated by the fact that there is often simultaneous exposure to several chemicals and that interactions (synergistic, antagonistic) may occur.

Because of these and other problems, a scientifically sound and practical approach to evaluating the environmental impact of potential endocrine disruptors is to focus initially on those with the greatest potency and those present in the environment at the highest concentrations. It is not appropriate to try to assess in detail the more than 250 substances currently listed as being hormonally active.

Having established that a given chemical is hormonally active, exposure assessment is a critical component of any risk assessment. Exposure is governed by a multitude of factors relating to the release of a chemical into the environment and subsequently its environmental fate. The latter depends on the intrinsic properties of the chemical and a variety of environmental factors. Major physicochemical properties of a chemical that dictate its environmental fate and distribution are its water solubility, vapour pressure, UV-absorption, oil–water partition coefficient and Henry's Law Constant. Environmental factors include the presence of particles in water or dust in the atmosphere, organic matter and moisture content of soil, sunlight intensity and the metabolic capacity of a wide variety of organisms. The interaction of these and other factors result in volatilization and deposition, direct and indirect photolysis and other abiotic interactions, metabolism and biodegradation, bioconcentration and bioaccumulation, transfer between compartments (including biota) and long-distance transport.

EXPOSURE ASSESSMENT AND ENVIRONMENTAL FATE

Natural hormones

Natural hormones from mammals and other organisms are typically effective at extremely low concentrations. Oestradiol, for example, has a Low Observed Effect Concentration (LOEC) for some effects of 10^{-12} M. The total daily excretion of natural oestrogens by a million people is about 200 g (1). While this is minute compared with environmental releases of many synthetic chemicals, the high potency of oestrogen makes it a significant release.

As an example of the kinds of interactions that can occur, 17β-oestradiol undergoes biodegradation when released into the environment. It is readily biodegraded during sewage treatment and does not bioaccumulate. Its metabolic pathways include several oxidation steps. Based on these properties and the low levels of release into the environment it might be concluded that environmental exposure to oestradiol would be low. In recent analyses, however, 17β-oestradiol has been detected in sewage treatment effluents in both the UK and Germany at levels of 2.7–48 ng/L and >1 ng/L, respectively (2). Considering the potency of oestradiol these concentrations represent significant levels of environmental exposure (3).

Synthetic hormones

Synthetic hormones such as ethynyloestradiol, diethylstilbestrol and mestranol are major contraceptive agents and diethylstilbestrol is used in cattle breeding. While information on the quantities of these materials used and released into the environment are not available, they have a potency similar to that of natural hormones. Although the synthetic hormones generally have a lower rate of biodegradation than the natural ones (4), information on this is contradictory. Thus, for ethynyloestradiol and mestranol, both ready biodegradability and persistence have been reported (5,6). Considering the low water solubility and possible persistence of mestranol (310 μg/L), bioaccumulation needs to be investigated.

Synthetic hormones have been detected in some environmental samples (3,7). In the previously referenced UK study, neither ethynyloestradiol nor mestranol were frequently found. In the German study, however, all 20 samples analysed contained ethynyloestradiol (median concentration 17 ng/L) and some rivers contained concentrations of 1–4 ng/L. In an earlier study, river water in the Netherlands was found to contain 0.3 ng/L of ethynyloestradiol. In the German study, effluents from 12 of 20 sewage treatment effluents contained mestranol at a median concentration of 4 ng/L (2). Although these results do not represent a systematic monitoring effort, they do serve to indicate a relevant exposure in the aquatic environment.

Phyto-oestrogens

Phyto-oestrogens are well known constituents of food and feed (8) and include several groups of natural products such as isoflavones, coumestans, etc.

Major phytohormones discussed in the context of endocrine disruption are the isoflavones daidzein and genistein and the coumestan, coumestrol. The genistein and daidzein concentrations in green soybean have been reported to be 729 mg/kg wet wt and 576 mg/kg wet wt., respectively. The highest concentration of coumestrol (27 mg/kg wet wt.) is present in alfalfa sprouts (9).

Although data on their biodegradation are not available, it can reasonably be assumed that phyto-oestrogens neither persist nor bioaccumulate in the environment. Consequently, significant environmental exposures are likely restricted to wildlife feeding on specific plant types. This could have an impact in areas where there are large monocultures of certain crops and where wildlife are restricted in their feeding habits.

Pesticides

A number of pesticides and their metabolites, DDT, diuron, atrazine, endosulfan, methoxychlor, toxaphene, kepone, linuron and vinclozolin have been reported to have endocrine activity. The *o,p′*-isomer of DDT (an impurity in technical DDT) is oestrogenic at a potency of 1 mg/kg (LOEL in rat) and DDE, the major metabolite of DDT, has an androgenic potency of about one thousandth that of dihydrotestosterone. Vinclozolin, a fungicide, and its metabolite (M_1) are also anti-androgenic and have potencies of 10^{-5} and 1.2×10^{-4} that of dihydrotestosterone, respectively (2,10).

The persistence, bioaccumulation and global dispersion of DDT (log P_{OW} of DDT and DDE ≥ 6) have been extensively reviewed and reported and its metabolism and fate has been investigated in a number of aquatic and terrestrial environments. Although the use of DDT has now been restricted or banned in most countries since the early 1970s, it is still considered to be a Persistent Organic Pollutant (POP)(11, 12, 13).

As a consequence of its early heavy use and persistence, DDT and its metabolites (DDE, DDD) are still detectable even in areas where its use was discontinued almost 30 years ago. With the exception of a few 'hot spots', environmental concentrations are now quite low. In Germany, for example, maximum concentrations of *o,p′*-DDT in surface water were 220 ng/L in 1993/1994; currently, concentrations of both DDT and DDE are typically < 0.1 ng/L (2). These values, combined with the relatively low endocrinological potency of DDT and its analogues indicate that these materials have little or no relevance with respect to hormone-like hazard.

Vinclozolin is anti-androgenic in rats at high doses (100–200 mg/kg/day), the major effects probably caused by two metabolites (M_1 and M_2) (2). Following agricultural application, vinclozolin may enter the aquatic environment by spray drift or run-off. The water solubility of vinclozolin is 3.4 mg/L, the vapour pressure at 20°C is 1.3×10^{-4} Pa and the log P_{OW} is 3.0. For fish, the bioaccumulation factor has been determined to be 241 at 15 days. Hydrolytic stability decreases with increasing pH, the DT_{50} (time required for 50% disappearance) being 31 hours and 38 min., respectively, at pH 7 and pH 9. In the environment, vinclozolin is degraded oxidatively to products showing some persistence. The DT_{50} of the parent compound in soils ranges up to about 8 days although mineralization is low (14). According to standard biodegradability tests, vinclozolin is not readily biodegradable but in sediment water systems the DT_{50} is only about 0.4 days because of hydrolysis. In surface waters mineralization is very low (2.3–3.4% in 99 days). Due to its low volatility vinclozolin does not partition into the atmosphere (13, 14). The most sensitive aquatic species in acute testing *is Daphnia magna* with an EC_{50} of 4 mg/kg (13).

In Germany, monitoring of surface waters between 1990 and 1994 has indicated vinclozolin concentrations of < 0.01 – < 0.5µg/L (detection limit 0.01µg/L). Although no data on concentrations of the endocrinologically more potent metabolite M1 are available, the analytical data on the parent combined with the rapid disappearance of residues from treated crops, suggest that vinclozolin has no relevance as an environmental endocrine disruptor.

Environmental chemicals

The following section includes just a few examples of the approximately 250 chemicals listed as potential endocrine disruptors. Furthermore, although information from the U.S., Japan and parts of the world are available, this discussion will focus primarily on the European situation.

Polychlorinated biphenyls (PCB)

The use and fate of PCBs have been extensively reviewed (12) and several isomers, hydroxylated metabolites and commercial PCB mixtures have been reported to be oestrogenic (2,10). The potency of the active PCB isomers is around 1 mg/kg (rat LOEL) and the metabolites are slightly more potent. 2,4,5-Trichlorobiphenyl (PCB29) and Arochlor 1242 reportedly influence the moulting time of *Daphnia* as shown by the standard 21 day *Daphnia* test (15).

Like DDT, PCBs are POPs. Prior to use restrictions or bans, they were used heavily as plasticizers and as dielectric fluids in transformers, capacitors and hydraulic fluids. Major routes of release into the environment were from treated materials or from waste treatment, incineration and spills (12, 16).

The commercial products contain a wide range of isomers with different degrees of chlorination. Lipophilicity increases and water solubility decreases as the degree of chlorination increases. Environmental persistence and bioaccumulation increases with increasing chlorination and bioaccumulation factors of 10 000 have been reported (12, 16,17). They partition into soils, sediments and biota according to their lipophilicity. In the years following use restrictions (after 1990) concentrations of single PCB isomers in surface water and sediments are usually < 20 ng/L and < 50 μg/kg dry wt., respectively.

Considering the ability of PCBs to undergo significant biomagnification in food webs, some predatory species could have potentially relevant exposures.

Polychlorinated dibenzodioxins

TCDD and other polychlorinated dibenzodioxin isomers have been reported to be oestrogenic at dose levels of 1–100 μg/kg (rat, mouse LOEL).

As with other POPs, all aspects of the environmental fate and persistence of TCDD and related compounds have been extensively reviewed (12,16). The chlorinated dibenzodioxins are highly persistent and highly bioaccumulating. They are, however, strongly sorbed to organic matter and exhibit a low level of bioavailability. Consequently, data on environmental concentrations are not as important as concentrations in the tissues of organisms (18). Since, for the most part, tissue levels in various organisms are only available as they relate to potential human exposures, it is difficult to determine their significance with respect to potential environmental endocrine effects. On the other hand, since environmental concentrations of dioxins are typically at pg levels and animal LOELs in the μg range, it seems unlikely that chlorinated dibenzodioxins will result in significant environmental endocrine disruption.

Alkylphenol and alkylphenolethoxylates (APEOs)

Nonylphenol and octylphenol have been reported to be oestrogenic, many of the reports relating to their influence on vitellogenin synthesis in fish; LOEL values for this effect for nonylphenol and octylphenol are 20 μg/L and 4.8 μg/L, respectively. The oestrogenic effect of nonylphenol in mammals occurs at about 20 mg/kg (rat LOEL), approximately three orders of magnitude higher (less potent) than that required to cause an ecotoxicologically relevant effect. Octylphenol also reportedly influences the moulting time in *Daphnia* (15).

Technical nonylphenol is a mixture of isomers, mainly 4-nonylphenol (about 90%) and 2-nonylphenol (about 10%) and minor amounts of di-nonylphenols; the alkyl chain consists of several isomers. Nonylphenol is an intermediate for many industrially important products. About half of the use in Western Europe (64 000 tons in 1995) is in phenol resin production, antioxidants, polymerization accelerators, etc. A decreasing amount is used for the production of nonylphenolethoxylates that have

surfactant and emulgator properties and are used as detergents, pesticide formulation additives and additives in textile and leather manufacture. Environmental degradation of APEOs leads to the corresponding alkylphenols (2,19).

Technical nonylphenol has a water solubility of 3000 mg/L (20 °C), a vapour pressure of 10 Pa (20 °C) and a slightly pH-dependent P_{OW} of 3.28 (pH 7). Consequently, the bioaccumulation potential of nonylphenol will increase in more acidic environments. Bioaccumulation factors from controlled experiments are in the region of 1000 (19). The biodegradability of nonylphenol during sewage treatment is reported to be between 0 and 100%; it is most likely about 70%. Very high concentrations (g/kg) of nonylphenol were found in sewage sludge prior to industry agreements in several countries to reduce the use APEOs in household detergents. The APEOs still represent a major source of nonylphenol in aquatic environments and despite risk mitigation measures, this still poses a potential threat to fresh and marine water organisms because of its relatively high acute toxicity [LC_{50}(EC_{50}) range 0.13–5 mg/L] (2).

Based on the properties of nonylphenol and the use and release patterns of APEOs, it is estimated that the environmental distribution of nonylphenol is > 60% in sediment, 25% in water and 10% in soil (10).

There is evidence that environmental levels of nonylphenol are decreasing. In England, total extractable nonylphenol concentrations do not generally exceed 330 μg/L in sewage treatment effluents and in Scotland have been reported up to 1500 μg/L (including the APEO). In England and Wales, river waters contain up to about 180 μg/L total extractable nonylphenol (20). Typically, median levels in sewage sludge are in the range < 1–10 μg/L, in river sediments < 1–13 mg/kg and in river water, for example, in Bavaria in 1995 up to about 1.5 μg/L. Concentrations of nonylphenol plus APEOs in fish have recently been reported up to 7 mg/kg and in algae up to 80 mg/kg. The occurrence of nonylphenol in soils treated with sewage sludge over several years is low (< 0.1–0.7 mg/kg dry wt.) reflecting efficient biodegradation in soils (2,21).

Although, at the present time, the information available on the occurrence of nonylphenol in aquatic environments is insufficient for an environmental risk assessment, a comparison of measured concentrations with the LOEC for vitellogenin synthesis in fish gives some reason for concern.

Phthalate esters

Although dibutylphthalate (DBP) and benzylbutylphthalate (BBP) have been found to be oestrogenic in *in vitro* test systems, corresponding *in vivo* effects have not yet been confirmed (2,22). In the standard 21-day *Daphnia* test, di(2-diethylhexyl)phthalate (DEHP) has been reported to influence moulting (15). DBP and BBP are both very toxic towards algae, aquatic invertebrates and fish with EC_{50}/LC_{50} values of 0.1–10 mg/L. Eggshell thinning in birds and gonadal effects in other vertebrates have also been reported (18, 23, 24).

Phthalate esters are used as plasticizers in a wide variety of products. For example, up to 75% of some polyvinylchloride (PVC) products consist of phthalates and in 1990, the total use of phthalates in Europe was 877 000 tons, the major one being DEHP. Major environmental releases (up to about 1% annual use) occur during manufacture, use and disposal of many products. There have been numerous reviews on the environmental fate of phthalates but much variability in the published data (18, 22, 23).

Physicochemical properties of DBP are: water solubility 8–18 mg/L, vapour pressure 2.6×10^{-3} Pa, log P_{OW} 4.6–4.8. Corresponding properties for BBP are: water solubility 0.7–2.9 mg/L, vapour pressure 1.1×10^{-3} Pa, log P_{OW} 3.5–5.6 (24). These properties suggest that DBP and BBP may volatilize from surfaces, that they have some bioaccumulation potential and that they will partition into the solid phases in the environment (soil, particulate matter). Although measured bioaccumulation factors for DBP and BBP in aquatic organisms are generally above 100, there is no biomagnification since organisms at higher trophic levels efficiently metabolize phthalates. DBP and BBP are also readily biodegradable during sewage treatment and in soil and surface water (e.g., DT_{50} in soil is about 100 days).

Distribution calculations show that about 90% of phthalates in the environment partition into soil and only about 10% into the gaseous and water phases. Because of their widespread use, phthalate esters are found ubiquitously in the environment. Rhine river sediment contains up to 30 mg/kg dry wt. of DEHP and in 1991/92 Rhine river water contained DBP and BBP at concentrations of 0.15–0.34 μg/L and

0.08–5.17 μg/L, respectively. Since sewage sludge may contain several hundred mg/kg dry wt. of phthalate esters, it is not surprising that frequent land applications of sewage sludge can lead to soil levels of up to 5 mg/kg dry wt. (21).

Since there are no data on the endocrinological potency of DBP, BBP and DEHP *in vivo*, no estimates of their potential environmental impact as endocrine disruptors are possible.

Bisphenol A

Bisphenol A (BPA) is reportedly oestrogenic with a rat LOEL of 5 mg/kg in the uterotrophic assay (2). Its acute aquatic toxicity (LC_{50}/LD_{50}) ranges from 1–10 mg/L with algae being the most sensitive species.

BPA is a high volume intermediate used in the production of polycarbonates, epoxide resins and other polymers; the BPA monomer content of these products is usually less than 25 mg/kg. It is also used in building materials, as a developer in dyes and as an additive in surface coatings and thermo-fax paper and in dentistry. These widespread uses lead to substantial releases into terrestrial (via sewage sludge) and aquatic environments.

The water solubility of BPA is 120–300 mg/L, its vapour pressure is about 10^{-8} Pa and depending on the publication used, its log P_{OW} is 2.2–3.8 (most probably about 3.4). The latter suggests a tendency of BPA to bioaccumulate and to partition into soils and sediments. Modelling indicates an environmental distribution into water, sediments and soil of 43%, 32% and 24%, respectively (10). It is frequently detected in surface waters at about 5 ng/L although levels may be higher than this in some rivers, particularly around sewage treatment effluents (25). Bioaccumulation factors are reportedly greater than 100 although studies in fish conducted according to OECD Guidelines suggest lower values of 5.1–13.8 (25). BPA is readily biodegraded in water via oxidative pathways.

Tributyltinoxide (TBTO)

TBTO is androgenic and its NOEL in the snails *Nucella lapillus* and *Hinia recticulata* is 5 ng/L. Positive correlations have been observed between the occurrence of TBTO in the aquatic environment and the development of imposex females (26, 27). TBTO is highly toxic to micro-organisms and other aquatic species. As a result, an acceptable water quality objective (standard) of 1 ng/L TBTO has been formally suggested.

Organotin compounds are used as fungicides, biocides in water purification and desalination systems, as wood protection agents and anti-fouling agents. Direct release into aquatic environments is possible from several of these uses (28).

Water solubility of TBTO ranges between 3 and 60 mg/L depending on salinity, vapour pressure is 10^{-3} Pa at 20 °C and log P_{OW} values of 3.2–3.8 have been reported. TBTO is slowly auto-oxidized to tin oxide. Due to its microbiocidal activity, TBTO is persistent in non-adapted aquatic environments and sewage treatment systems; it is readily mineralized in adapted sewage treatment populations.

There is much information available on the concentration of TBTO in aquatic and terrestrial environments. Recent analyses show levels in rivers and the marine environment in the 5 ng/L range, whereas in lake water they may be as high as 10 ng/L and in lake sediments may be up to several 100 mg/kg. Recent investigations in Germany show concentrations in sewage sludge of up to about 10 mg/kg dry wt (2). These levels suggest that, in some areas, concern over the possible adverse environmental effects of TBTO is justified.

ENVIRONMENTAL EFFECTS OF ENDOCRINE DISRUPTORS

The following section will focus on a number of examples where endocrine-mediated effects of xenobiotics on fish or other species (mostly non-mammalian) of wildlife have either been demonstrated or suggested. In each case, the environmental as well as intrinsic biological factors responsible for the development of the effects will be evaluated. In addition, species and sex differences in metabolism and in the interactions of xenobiotics with hormone receptors associated with differentiation, development, growth and homeostatic regulation will be discussed. Information of this type is essential to

understanding the potency of endocrine disrupting chemicals in different species and to enable the prediction of possible adverse environmental effects.

Examples of endocrine disruption effects in the field

Observation 1. American alligators in Lake Apopka, Florida showed low hatching rate, increased mortality in juveniles, decreased blood testosterone levels, micropenis and poor testicular development (demasculinization) in males and increased blood 17β-oestradiol levels and abnormal multi-ovulation (superfeminization) in females.

> Probable Cause: Since these symptoms were not observed in alligators in nearby Lake Woodruff, a search to establish a possible cause for the problems encountered in Lake Apopka was made. This resulted in establishment of a correlation of the adverse effects with pollution resulting from a pesticide spill (mainly DDT, DDE and dicofol) and with contaminants and nutrients derived from extensive agricultural activities (31). This is an example of the potential local hazard from excessive exposure to contaminants.

Observation 2. Several species of birds (woodcock, European sparrow hawk, osprey, rook, shag, etc.) in England and North America, showed decreased egg-laying, delayed reproduction, hatching failure, decreased egg size, eggshell thinning, increased number of broken eggs, increased mortality in egg.

> Probable Cause: DDT and DDE were identified as two of the causal substances based on their ubiquitous presence in the environment (from long-term widespread use in agriculture) and the results of laboratory studies. The inhibition of Ca^{+2}-ATPase by p,p′-DDE may be the critical mechanism of eggshell thinning, possibly combined with inhibition of carbonic anhydrase. The embryotoxicity of organochlorine residues may also be involved (32, 33). The reproductive effects of various pesticides and industrial chemicals to birds have recently been reviewed (34).

Observation 3. Male roach (*Rutilus* spp.) and rainbow trout in some rivers in England have increased blood levels of vitellogenin and males show femininization including delayed development of testis. This is particularly true in parts of the river close to effluents from sewage treatment plants (STP).

> Probable Cause: Initially, these effects were thought to be due to the presence of APEO and alkylphenols (mainly nonylphenol) from their microbial degradation (35) in effluents from STP. Until recently, APEO have been important components of detergents and other industrial products. More recently, however, as discussed earlier, the possible contribution of other oestrogenic chemicals present in river water has been considered, particularly the deconjugated form of 17α-ethynyloestradiol (deconjugation occurs during sewage treatment) used in contraceptive pills and excreted by human females; this is at least 1000-fold more oestrogenic than nonylphenol (36, 37). Both ethynyloestradiol and 17β-oestradiol are now thought to constitute two of the causal substances responsible for femininization of male fish in U.K. rivers.

Observation 4. The rock shell (*Thais clavigera*, *T. bronni*, etc.) and several *Buccinidae* species in the coastal seas of Japan, Singapore and Indonesia have shown the development of ‘imposex’ in females. This is characterized by the formation of a penis and a proliferation of the vas deferens tissues. Development of the ‘imposex’ state prevents normal breeding activity and causes a decline in the population.

> Probable Cause: Laboratory experiments have shown that organotin compounds, particularly tributyltin (TBT) and its oxide (TBTO) and triphenyltin (TPT) used in anti-fouling agents can cause adverse effects of this type. Environmental monitoring has confirmed that water concentrations of TBT and TBTO reach toxic levels and cause identical symptoms in the environment. Although binding of TBT and TPT to hormone receptors has not so far been documented, it has been shown that TBT competitively inhibits aromatase activity and increases titers of testosterone. The increased androgen titer is apparently the cause of imposex (38, 39) although despite the ubiquitous nature of aromatase not all shellfish species appear to be affected.

Observation 5. Populations of otters and minks in the Great Lakes and U.K. are declining.

Probable Cause: Possible due to accumulated PCBs in food (40).

Other field observations suspected of being, but not definitely established to be, related to endocrine-mediated effects include the following (the suspected causative agent is indicated in parentheses):

Salmonid fish (*Onchorhynchus* Sp.) in Great Lakes exhibit hyperplasia of the thyroid gland, lack of secondary sex character in males and early maturity—[numerous but unidentified goitrogenic substances (41)].

White Sucker fish in Great Lakes (Lake Superior) exhibit delayed maturity, contracted gonads, decreased fertility, lack of secondary sex character in males, decreased blood levels of oestradiol and testosterone—[paper and pulp factory effluent; numerous unidentified compounds; plant sitosterol may be converted microbiologically to androgen (42, 43)].

Females of common gambusia (Cyprinodontiformes) in Florida exhibit masculinization (elongated anal fin)—[paper and pulp factory effluents; numerous but unidentified compounds (43)].

Femininization of Western gull in Santa Barbara Islands (California) indicated by fewer males in population, appearance of lesbian females and degeneration of female reproductive organs—[no causal agent identified (40, 44)].

Herring gulls in Great Lakes with abnormal thyroid gland (hypertrophy, hyperplasia of epithelial tissue, etc.)—(possibly due to halogenated organics (45)].

Decreased hatching rate of American bald eagle in Great Lakes—[PCBs or possibly DDE and other DDT-related products (40)].

Decreased sperm count in Florida panthers with increased incidence of cryptorchidism, increased blood oestrogen in males and decreased female infertility—[oestrogenic pesticide suspected (40, 46)].

In 1988, approximately 20 000 harbour seals died in Europe. The ultimate cause was suspected to be an infection with the measles virus (PDS-1) combined with immunosuppression resulting from bioaccumulation of polyhalogenated aromatic hydrocarbons such as PCBs, polychlorinated dibenzodioxins and dibenzofurans (47, 48). Young seals fed for two years on severely polluted Baltic Sea herring showed damage to the immune system and PCBs rather than dioxins or furans were implicated.

Immunotoxic effects of organochlorine and other chemicals have also been indicated with birds (49,50).

POSSIBLE MECHANISMS OF SPECIES DIFFERENCES IN ENDOCRINE DISRUPTOR EFFECTS

Sex differentiation

While the following information is not new, it is relevant to understanding possible differences in the effects of xenobiotic endocrine disruptors in different classes of vertebrates.

Animal sex is initially determined genetically at fertilization and this dictates the sex of the developing gonads. Subsequently, sexual phenotypes are determined by acquisition of accessory sex organs, secondary sexual characteristics and brain function and behaviour. Substances produced by the gene as well as the hormones produced by the gonad (androgens and oestrogens) greatly influence sexual development. The involvement and importance of each factor differs widely in different species as shown in Table 1 (51).

Although no chromosome differentiation is observed in most species of fish, a few have the XX-XY type of chromosomal composition (killifish, carp, goldfish and rainbow trout) and some the ZZ-ZW type (mosquito fish, Japanese eel, etc.). Sex conversion following exposure to sex hormones is observed in killifish, goldfish and guppy, that is, females can be masculinized by testosterone and males femininized by oestradiol. Many species of marine fish exhibit natural sex conversion during growth and population ranking and, in males or immature individuals, exposure to oestrogen induces the biosynthesis of hepatic vitellogenin.

Table 1. Composition of sex chromosome and determination and differentiation of sex in different classes of vertebrates

Class	Composition of sex chromosome	Factors affecting determination and differentiation of sex	Description
Fishes	(+)	Hormone, growth temperature, rank in population	Sex conversion possible (M→F and F→M)
Amphibians	(+)	Hormone, temperature	Sex conversion possible (M→F or F→M)
Reptiles		Hormone, temperature, environmental factors	No sex conversion
Crocodiles	(–)	"	"
Lizard	(–)	"	"
Turtles	(+)	"	"
Snake	ZZ-ZW	No effect of environmental factors	No sex conversion
Birds	ZZ-ZW	No effect of environmental factors	No sex conversion
Mammals	XX-XY	No effect of environmental factors	No sex conversion

(+): XX-XY type or ZZ-ZW type) in some species.

(–): Unclear differentiation of hetero-sex chromosome.

Like fish, most species of amphibians show no chromosomal differentiation although there are different chromosome types such as XX-XY (newt, salamander) and ZZ-ZW (*Xenopus* and Mexican salamander). While natural sex changes do not occur in amphibians, sex changes can occur following exposure to sex hormones. The change can only occur in one sex however. Thus, in species with the XX-XY chromosome type, juvenile females can be converted to males by exposure to androgen and in those with the ZZ-ZW type, juvenile males are converted to females following exposure to oestradiol. In the latter type, however, conversion of females to males by treatment with testosterone never occurs. When male or female amphibians are treated with oestrogen, synthesis of vitellogenin in liver is activated and blood levels of vitellogenin increase.

Vitellogenin is also synthesized in the cultured liver of *Xenopus* exposed to oestrogen.

The chromosome composition of reptiles is quite variable. In snakes only the ZZ-ZW type is found while in lizards and turtles both ZZ-ZW and XX-XY types occur. Chromosome differentiation is not, however, observed in the crocodile or cameleon.

In reptiles, reproductive nodules of male germ develop into two hemipenis, but when males are castrated in the early period of development, female reproductive nodules are maintained without atrophy. Penis, Wolff tubules and Mueller tubules are developed by exposure to testosterone in both sexes of the Mississippi alligator.

Different sexes of some species of lizard, turtle and crocodile are determined by the temperature at which the eggs are incubated. In general, the temperature range over which sex conversion can occur is 27–31 °C, but almost complete sex conversion typically occurs over a narrow range of only 2–4 °C [The red-eared slider turtle (*Trachemys scripta*), for example, produces all females at 31°C and all males at 26 °C. PCBs, notably 2′, 4′, 6′-trichloro-4-biphenyl and 2′, 3′, 4′, 5′-tetrachloro-4-biphenyl produces females at 26 °C (52).] The sex determining period is the same as the period of gonad formation. In species like snakes that have chromosome differentiation, the sex ratio is not influenced by temperature.

The ZZ-ZW type of chromosome composition is observed in most species of birds. The ovary and uterine tube in birds develop only in the left side; the right side ovary degenerates and only traces of the right side uterine tube remain. Although no effect is observed on germ of a chicken following treatment with sex hormones, the exposure of male germ with oestrogen causes the development of an ovary from

the left testis. In contrast to mammals, the male is the prototype sex in birds and oestrogen from the ovary converts the male to the female. While males and immature males and females are normally incapable of synthesizing vitellogenin in the liver, vitellogenin biosynthesis is observed following administration of oestrogen.

Eggs covered with a hard shell to protect the yolk and white are characteristic of birds and reptiles and the structure and function of the uterine tube in birds are markedly different from those of other vertebrates. The eggshell is formed mainly by the eggshell gland of the uterine tube. Calcium, released from calcium-adenosine triphosphatase (Ca^{2+}-ATPase) in the eggshell gland is converted into crystalline calcium carbonate after combining with CO_3^{2-} and is precipitated on the outside of the eggshell membrane.

The XX-XY type of chromosome is observed in mammals. Mammals are initially differentiated as females but changed to males by the Y chromosome. The secretion of androgen in male rats begins during embryonic development and leads to the development of the sexual behaviour centre in the brain. If males are treated with oestrogen during the embryonic period, the gonads develop into testes that are retained intraperitoneally and the external sex organs degenerate and become femininized.

The mechanism of sex determination in vertebrates can be briefly summarized as follows. In poikilothermic fish, amphibia and reptiles, the regulatory mechanism of sex determination and differentiation extends from the embryonic period throughout juvenile stages of development and longer; both internal (hormonal) and external (environmental) factors strongly affect sexual development. The sex hormones are important factors and, in some cases, complete sex conversion can occur following exposure to exogenous hormones. In these animal species, genetic control of sex determination is weak.

In warm-blooded birds and mammals, the critical regulatory mechanism for sex determination occurs only during the early period of ontogenesis, because sex has already been established genetically. Consequently, it is not possible to determine sex by hormone treatment although sex hormones do play an important role in subsequent sexual differentiation (e.g., the development of accessory sex organs and the sexual behaviour centre in the brain).

The actions of androgens and oestrogens are not equal in different species. In XX-XY type animals, masculinization by androgen plays a dominant role in sexual development whereas, in ZZ-ZW type species, femininization by oestrogen is predominant. Table 1 provides a brief summary of factors affecting sex determination and differentiation.

Thyroid hormone

Thyroid hormones also have important effects on different species at the level of both the individual animal and the population. Examples of these effects are as follows.

Thyroid hormones promote guanine deposition on the skin of juvenile salmon and often cause a metamorphosis called argentation.

Thyroid hormone stimulates anadromous and catadromous migration of many species of fish that move between seawater and freshwater environments.

Treatment of tadpoles with thyroid hormone causes involution of the tail fin and stimulates metamorphosis to the adult stage.

Removal of the thyroid gland from lizards inhibits exuviation and causes a thickening of the skin suggesting that thyroid hormone stimulates exuviation in reptiles. The hormone is also known to stimulate moulting in birds, a process that corresponds to exuviation in reptiles.

In many species of vertebrates (reptiles, turtles, crocodiles, lizards, snakes, amphibians and fish), a positive correlation exists between thyroid function and ambient temperature. Thus, in cold seasons, the release of thyroid hormone is decreased to induce a state of hibernation and suppress the level of metabolism.

In mammals, thyroid hormone promotes growth of hair and a deficiency in the hormone induces weak hair and nails. In most mammals, cold winter weather increases the secretion of thyroid hormone to

accelerate metabolism and increase body temperature. Interestingly, in hibernating mammals, as in reptiles, the secretion of thyroid hormone is decreased.

Thyroid deficiency during embryonic, foetal and juvenile development can lead to decreased water content in the brain, decreased myelination of nerve fibres and delayed differentiation and development of nerve cells. The size of the nerve cells in the cortex may be diminished, the numbers of nerve fibres decreased and the growth of the entire brain may be delayed. Since thyroid hormones play an important role in regulating metabolism, hypothyroidism in humans and other mammals causes a decrease in oxygen consumption and a general decrease in protein, sugar and fat metabolism. Thyroid hormones are particularly important in brain development and the most severe symptoms of thyroid deficiency are psychological disturbances such as mental retardation or Schwashsinn. Children receiving thyroid hormones from early infancy have no or slight acataleptic symptoms whereas irreversible acatalepsy occurs if treatment is delayed (51).

Based on the foregoing summary, it is obvious that the environmental effects of exogenous endocrine disruptors may be quite different from one species to another and should be carefully evaluated individually on a case by case basis. Mechanisms underlying the similarities and differences between species are complicated, attributable not only to the intrinsic mechanism of action of the xenobiotics in various species but also to differential metabolism and a number of environmental factors.

Species differences in receptors

The oestrogen receptor (ER), androgen receptor (AR) and thyroid receptor (TR) as well as the aromatic hydrocarbon receptor (AhR) are ubiquitously distributed throughout many species of animals. The nucleotide sequences coding for these receptor proteins have been documented in several species (Table 2) (51). Although the function of a given receptor is quite similar from one species to another, the precise details of its interaction with the natural hormone and with xenobiotics are not necessarily identical.

Table 2. Species with published cDNA sequences of various receptors.

Species	ER	AR	TR	AhR
Mouse	X	X	X	X
Human	X	X	X	X
Sheep	X		X	
Salmon	X			
Xenopus	X	X	X	
Chicken	X		X	
Rat	X	X	X	X
Cow	X	X		
Camelus	X			
Rabbit	X	X		
Sus	X			
Killifish	X			X
Trout	X			
Dog		X		
Pan		X		
Hylobates		X		
Gorilla		X		
Drosophila			X	
Cairina			X	
Flounder			X	

For example, although the activities of oestrogen and the function of the ER receptor are highly conserved in different species, the amino acid sequence of the regions responsible for ligand binding and

ligand-dependent gene expression (i.e., domains D, E and F) is not as well conserved (Table 3) (53). The percent homology between species is even less obvious when the D, E and F domains are considered as a single functional unit because all three domains contribute to both ligand binding and gene expression (Table 3). This raises serious doubts about the possibility of using one surrogate species to predict responses in other species especially when using structurally diverse xeno-oestrogens (53). As a corollary, the genetic differences in the susceptibility of mouse strains to certain aromatic hydrocarbons is governed by the polymorphism of AhR, the AhR in the responder strain (C57BL6) being structurally and functionally distinct from that in the non-responder strain (DBA/2J) (54). Other factors such as binding to the DNA binding domain (domain C) of the receptor and the possible involvement of interactions with other accessory proteins may also be involved in the action of endocrine disruptors. The nucleotide sequences known as oestrogen responsive elements (EREs) are not identical in all species and may result in species differences in response to environmental oestrogens (55). There may also be differences between tissues in a single species so that tamoxifen, for example, is an oestrogen antagonist in the breast and an oestrogen agonist in the uterus (56). The situation is further complicated by the fact that some chemicals exhibit multiple endocrine activities; 3, 3′, 4, 4′-tetrachlorobiphenyl, for example, elicits both oestrogenic and AhR-mediated anti-oestrogenic activities in MCF-7 cells (57). However, no experimental findings have been reported to relate these receptor-mediated activities with effects in living organisms.

Table 3. Comparison of the amino acid sequences of domains D, E and F of the oestrogen receptor in different species with that of humans.

		Per cent similarity			
Animal	Source	D, E, F	D	E	F
Human	MCF-7 cells	100	100	100	100
Pig		89	76	95	65
Sheep		89	79	95	63
Rat	Sprague–Dawley	89	78	96	61
Mouse	Schneider	89	78	96	61
Bird	Chicken	79	42	93	19
	Zebra Finch	79	39	93	30
Frog	African clawed frog	63	11	82	37
Fish	Rainbow trout	47	18	60	19
	Killifish	47	9	62	16
	Japanese eel	45	21	56	14
ERβ	Leucocyte	47	24	57	12

Differential metabolism

The essential role of metabolism in homeostasis in living organisms cannot be overemphasized and two aspects of this, the biosynthesis and degradation of natural hormones and the activation and degradation of xenobiotics, can play an important role in determining species differences in response to xenobiotic endocrine disruptors. The pathways of hormone biosynthesis (from cholesterol to testosterone, from testosterone to dihydrotestosterone or oestradiol, from thyroglobulin to T_3 and T_4, etc.) and the enzyme systems responsible have been well documented in a variety of species.

Several of the intermediary steps in hormone biosynthesis are known to be influenced by xenobiotics (PCBs, TCDD) that act, for example, as inducers of cytochrome P-450-mediated reactions. Thus the P-450 isozymes involved in the biosynthesis of androgens and oestrogens including those involved in cholesterol side-chain cleavage (CYP11A), C11 β-hydroxylation (CYP11B1), C11 α-hydroxylation and C 17–20 cleavage (CYP17), aromatase (CYP19) and C21 hydroxylation (CYP21) can all be expected to

be affected to some extent by xenobiotic inducers. TCDD, acting through the AhR, reportedly regulates genes coding for CYP1A1, CYP1A2, UDP glucuronyltransferase, glutathione S-transferase (Y unit) and aldehyde dehydrogenase (58). Enzyme inhibition may also play a role as in the inhibition of rock shell aromatase by TBT. Some xenobiotics potentially have adverse effects by changing the pattern of natural hormone metabolites. Thus, in the in vitro assay using MCF-7 cells, several xenobiotics such as *o, p′*-DDT, *o, p′*-DDE, kepone, atrazine, 2, 2′, 4, 4′, 5- PCB and γ-BHC (lindane) stimulate the formation of 16α-hydroxyoestrone from 17β-oestradiol (59). While still possessing oestrogenic activity, 16α-hydroxyoestrone is capable of damaging DNA and is regarded as a possible biomarker of breast cancer.

While the metabolism of xenobiotics typically leads to biodegradation and detoxication some reactions can give rise to products with enhanced endocrine disrupting potential. Examples are the metabolism of DDT to DDE, the hydroxylation of PCBs (e.g., from 2, 4, 6, 2′, 6′-PCB to its 4-hydroxy analogue) (53), the formation of two anti-androgenic metabolites (M_1 and M_2) from vinclozolin (60) and the formation of 4-nonylphenol (35) and equol and daidzein (61). These biotransformations may occur directly within the individuals of a single species or may involve effects on populations of one species caused by the biodegradation by other species or abiotic reactions (e.g., hydrolysis), e.g., the possible oestrogenic effects of ethynyloestradiol glucuronide in British rivers.

The comparative aspects of absorption, distribution, biotransformation and elimination of xenobiotics in mammals, including humans, have been extensively evaluated with regard to similarities and differences in enzyme make-up and metabolic pathways and are discussed elsewhere (62). Table 4 shows the large species differences that exist in the activity (relative to the rat) of several enzymes that play an important role in xenobiotic metabolism (63). There exists a multiplicity of enzymes in different species and this emphasizes the need for extreme caution in extrapolating metabolic information across species even with structurally similar chemicals. Clearly, these differences can lead to significant species differences in the action of xenobiotic endocrine disruptors.

Table 4. Species differences in enzyme activity towards xenobiotics*

		Animal species (% relative to rat)			
Enzyme	Substrate	Cat	Quail	Trout	Cow
P450		65	45	65	90
Microsomal oxidase	Benzo(a)pyrene	20	50	<10	511 000
	Ethoxyresorufin	800	<<100	600	
Epoxide hydrolase	Styrene oxide	60	40	50	330
N-Acetyltransferase	2-Aminofluorene	<<100	3000	3500	<<100
UDP-Glucuronyl-	1-Naphthol	<<100	200	<<100	40
transferase	Oestrone	600	1500	200	13 000
	Testosterone	20	200	450	30
Glutathione S-transferase	Chloronitrobenzene	75	<10	<10	40
Sulfotransferase	Oestrone	600	350	<<100	100

*Approximation of activity relative to rat estimated from Figures in Ref. (63).

Aquatic ecosystems probably represent those at most risk because of the number and variety of biota present and the fact that they represents major sinks for large volumes of pollutants. Consequently, the potential impact of xenobiotic endocrine disrupting chemicals in aquatic ecosystems should be given special attention. An understanding of the *in vivo* biotransformation reactions occurring in aquatic organisms is essential for identifying bioactive (toxic) metabolites leading, for example, to the onset of endocrine disruption in these organisms. Metabolism studies in aquatic organisms will also furnish valuable information on bioaccumulation potential of endocrine disruptors. Even very lipophilic compounds like the synthetic pyrethroid with log K_{OW} values around 6 does not bioaccumulate because it is readily metabolized to hydrophilic compounds (65).

Biotransformation pathways in aquatic organisms have been summarized and discussed elsewhere and, at least qualitatively, have been shown to be similar to those present in mammals (oxidation, reduction, hydrolysis, conjugation, etc.) (66). Even though the information currently available is not sufficient to predict the metabolic fate of xenobiotics in a given species, it demonstrates that aquatic

species (including phytoplankton and invertebrates) do have a metabolic capability even though it is usually lower than in mammals. There are significant differences between taxonomic phyla. In general, green algae do not show appreciable degradation capability but most others show some activity. Microsomal oxidation occurs in fish, crustaceans and blue-green algae, reduction of nitro groups occurs in crustaceans and molluscs, and hydrolysis by carboxyester and aryl esterases and demethylation by glutathione S-transferases appear to be common to all aquatic organisms (66). In addition, conjugation with glucuronic acid, glycine and glutathione occurs in fish, sulfate conjugation occurs in fish, crustaceans and molluscs, acylation and methylation occur in fish and molluscs, taurine conjugation occurs in fish and crustaceans and glucoside conjugation occurs in crustaceans and molluscs.

ENVIRONMENTAL RISK ASSESSMENT OF POTENTIAL ENDOCRINE DISRUPTORS

In contrast to human risk assessment that involves estimating risks to individuals (non-cancer) or probabilities of risk within the population (cancer), environmental risk assessments do not typically involve stochastic concepts. For environmental risk assessment, exposure assessments using release, fate and occurrence data are typically used to estimate a Predicted Environmental Concentration (PEC) for specific scenarios. According to the EU-Risk Assessment Directive 93/793 and its Technical Guidance Document, the scenarios are selected to reflect probability. Predicted No Effect Concentrations (PNEC) are derived from effects data and ecological assessments are made using PEC/PNEC ratios in a manner similar to that developed by the U.S. EPA (11, 67, 68, 69, 70, 71).

In principle, the PEC/PNEC approach could also be used for assessing the risks of endocrine disruptors. However, decisions on what assessment factors to use, for example, are complicated by the fact that these will depend on toxicological information from a number of different organisms as well as species specific mechanistic information. Unfortunately, many aspects of environmental endocrine disruption by xenobiotics are not yet sufficiently well understood and more research is needed with many species. To date, mammals have been studied more than other species and there is an urgent need for more information on the molecular structure of receptors, biosynthesis and degradation of natural hormones, regulatory mechanisms and xenobiotic interactions in other classes of organisms. Xenobiotics known to be endocrine disruptors in some species (DDT, PCBs, TCDD, NP and TBT) can be used as model compounds to highlight certain types of endocrine effects.

One of the problems that exists is the enormous difference in potency (up to 6 orders of magnitude) between many endocrinologically active chemicals. It is possible that, in some cases, observed environmental effects are being caused by highly potent natural or synthetic hormones that are present in the environment at levels below those that can be detected analytically. Consequently, it is necessary to develop highly sensitive multi-residue analytical procedures for detecting potential disruptors in environmental matrices. If this is not done, it is likely that some environmental effects will be attributed to other much less potent materials present at levels that can readily be detected.

With only a few exceptions (DDT, PCBs, etc. in specific localized situations) it is very difficult to derive realistic PEC/PNEC ratios and most of the ones currently used almost certainly represent worst case scenarios.

For DBP, for example, a PEC/PNEC ratio of between 0.02 and 0.2 has been estimated under worst case conditions (10) suggesting that, under more realistic conditions, it is highly unlikely that a PEC/PNEC of 1would be attained. Consequently, DBP should not be considered an endocrine disruptor under environmental conditions. On the other hand, for nonyl- and octylphenol and APEOs there are probably realistic environmental situations where the PEC/PNEC ratio exceeds 1. The concentrations of nonylphenol and NPEOs frequently exceed the LOEC value for nonylphenol vitellogenin effects in trout although the ecological relevance of this effect has still to be determined. For TBT, the occurrence of the imposex phenomenon has also been confirmed in the real environment. In this case, the PNEC for some snail species is well below 80 ng/L, a concentration that has been found in the environment. Once again, however, neither the ecological relevance of imposex in terms of population effects, nor the likely frequency of exceeding a PEC/PNEC ratio of 1 in surface waters have been established.

THE DEVELOPMENT OF ENDOCRINE DISRUPTOR SCREENS

The development of a series of screening tests to identify potential endocrine disruptors is currently being evaluated by the U.S. EPA Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC, draft report, 1998) and OECD [Draft Detailed Review (DDRP) April, 1997 and Test Guideline Programmes, December, 1997]. As the elaboration of such test batteries are now in their final stages, it is probably not a good time for international organisations such as IUPAC to propose additional systems. The following, however, are a few general points that should be incorporated into the development of any such a test system:

1 The test should be simple, easy to conduct and reproducible and should have a relatively low cost. The results of the test should be applicable to a wide range of organisms and an array of xenobiotics. Any test should only be recommended after appropriate validation. Although *in vitro* studies may be acceptable as an initial step, the findings should be accepted with caution since they are often not well correlated with *in vivo* results. QSAR is of limited value at this time unless both structural and activity parameters have been carefully selected.

2 The selection of test organisms to use as surrogates for other species is difficult and criteria should be based on detailed comparative investigations of the activities of different endocrine disruptors. Possible *in vivo* tests for fish and wildlife include: fish early stage test, fish partial life cycle test, avian subacute test and avian reproduction test. *Xenopus* might be used in some tests. Even if such tests are accepted for fish and avian species, the next question that arises is how can they be expanded to include reptiles and amphibians. Consequently, comparative studies on species specificity need to be encouraged in the hope that tests developed for one species can be extended to cover other species.

3 Any test results in fish and wildlife species should be as complementary as possible to those in mammalian species.

4 A xenobiotic compound that has been demonstrated to be an endocrine disruptor in controlled laboratory tests does not necessarily cause adverse endocrine effects under actual environmental conditions. It is important that other factors such as habitat and trophic level be taken into account in evaluating the relevance of the results observed screening tests to those likely to be seen in the environment.

5 International collaboration is required to avoid overlapping and to maximize available resources. Agreements on quality assurance among laboratories should be made and a supply of authentic reference compounds made available.

REFERENCES

1 M. Rathner and M. Sonneborn. *Biologisch wirksame in Trink- und Abwasser*, Vom Wasser: Sonderheft Institut für Wasser-, Boden- und Lufthygiene des Bundesgesundheitsamtes Berlin, 1978.

2 M. Gülden, A. Turan and H. Seibert. *Substanzen mit endokriner Wirkung in Oberflächengewässern*, Umweltbundesamt Texte 46/97, ISSN 0722–186X, 1997.

3 M. Stumpf, T.A. Ternes, K. Haberer and W. Baumann. *Nachweis von natürlichen und synthetischen Östrogenen in Kläranlagen und Fliessgewässern*, Vom Wasser, 1996, **87**, 251.

4 R.E. Ranney. *J. Toxicol. Environ. Health* 1977, **3**, 139.

5 H. Tabak and R.L. Bunch. In *Development in Industrial Microbiology*, pp. 367–376, Washington, D.C., 1970.

6 K. Norpoth, A. Nehrkorn, M. Kirchner, H. Holsen and H. Teipel. *Zbl. Bakt. Hyg., Abt. Orig.* 1973, **B156**, 500.

7 R.D. Rurainski, H.J. Theiss and W. Zimmerman. *Gwf-Wasser/Abwasser*, 1977, **118**, 287.

8 A.E. Harper. In *Toxicants Occurring Naturally in Foods*, p. 130. National Academy of Sciences, Washington, D.C., 1973.

9 K. Reinli and G. Block. *Nutrition and Cancer*, 1996, **26**, No. 2.

10 Nordic Council of Ministers. Chemicals with Estrogen-like Effects. *TemaNord* 1996, **580**, p. 278.

11 J. Miyamoto. *Pure and Appl. Chem*. 1996, **68**, 1737.

12 H. Fiedler and C. Lau. *Environmental Fate of Chlorinated Organics*. In: G. Schüürmann and B. Markert (eds.), *Ecotoxicology - Ecological Fundamentals, Chemical Exposure and Biological Effects*. Chapter 11, A. John Wiley and Sons, 1997.

13 L.J. Blus. *Organochlorine Pesticides*. In: *Handbook of Ecotoxicology*, D. Hoffmann, B.A. Rattner, G.A. Burton Jr. and J. Cairns Jr. (eds.), CRC Press, Inc., 1995.

14 Biologische Bundesanstalt für Land- und Forstwirschaft. Vinclozolin. Wirkstoffdatenblatt.

15 E. Zou and M. Fingerman. *Ecotoxicol. and Environ. Safety* 1997, **38**, 281.

16 C.P. Rice and P. O'Keefe. *Sources, Pathways and Effects of PCBs, Dioxins and Benzofurans*. In: *Handbook of Ecotoxicology*, D. Hoffmann, B.A. Rattner, G.A. Burton Jr. and J. Cairns Jr.(eds.), CRC Press, Inc., 1995.

17 I. Scheunert and W. Klein. Polychlorinated Biphenyls in the Environment, GSF-Bericht Ö-501. GSF München, 1979.

18 G. Kreysa and J. Wiesner. *Kriterien zur Beurteilung organischer Bodenkontaminationen: Dioxine (PCDD/F) und Phthalate*. Internationale Expertenbeiträge und Resümee, DECHEMA, ISBN 3-926959-51-7, 1995.

19 Beratergremium für umweltrelevante Alstoffe, *BUA-Stoffebericht*, 13, Nonylphenol, VCH Weinheim, 1988.

20 M.A. Blackburn and M.J. Waldock. *Water Research* 1995, **29**, 1623.

21 W. Schnaak, T. Küchler, M. Kujawa, K.-P. Henschel, D. Süssenbach and R. Donau. *Chemosphere* 1997, **35**(1/2), 5.

22 C.A. Harris, P. Hentuu, M.G. Parker and J.P. Sumpter. *Environ. Health Persp.* 1997, **105**, No. 8.

23 K. Furtmann. *Phthalate in der aquatischen Umwelt. Analytik, Verbreitung, Verbleib und Bewertung*. LWA Materialien, Düsseldorf, p. 1–177, 1993.

24 Beratergremium für umweltrelevante Alstoffe. *BUA-Stoffbericht* 172. Di(2-ethylhexyl)phosphat/Tri(2-ethylhexyl)phosphat, VCH Weinheim, 1995.

25 C.A. Staples, P.B. Dorn, G.M. Kleck, S.T. O'Block and L.R. Harris. *Chemosphere* 1998, **36**, 2149.

26 J. Oehlmann, U. Schulte-Oelmann, B. Bauer, P. Fiorini and B. Markert. *Fresenius J. Anal. Chem*. 1996, **354**, 540.

27 C. Bettin, J. Oehlmann and E. Stroben. *Helgol. Meeresunters*, 1996, **50**, 299.

28 K. Fent. *Critical Reviews in Toxicol.* 1996, **26**(N1), 1.

29 Beratergremium für umweltrelevante Alstoffe. *BUA-Stoffbericht* 36. Tributylzinnoxid. VCH Weinheim, 1988.

30 F. Bro-Rasmussen *et al*. *Rev. Environ. Contam. Toxicol*. 137. Springer-Verlag, Inc., New York, 1994.

31 L.G. Guillette, Jr., T.S. Gross, G.R. Masson, J.M. Matter, H.F. Percival and A.R. Woodword. *Environ. Health Persp*. 1994, **102**, 680.

32 D.A. Ratcliffe. *J. Appl. Ecol*. 1970, **7**, 67.

33 C.H. Walker. *Aquatic Toxicol*. 1990, **17**, 293.

34 D.M. Fry. *Environ. Health Persp*. 103 (Suppl. 7), 165, 1995.

35 M. Ahel, D. Hrsak and W. Giger. *Arch. Environ. Contam. Toxicol*. 1994, **26**, 540.

36 J.E. Harris, S. Jobling, P. Mathiessen, D.A. Sheahan and J.P. Sumpter. *Effects of trace organics on fish - Phase 2*, FR/D 0022 Allen House, The Listons, 1995.

37 G.P. Daston, J.A. Gooch, W.J. Breslin, D.A. Shuey, A.I. Nikiforov, T.A. Fico and J.W. Gorsuch. *Reprod. Toxicol*. 1997, **11**, 465.

38 P. Mathiessen and P.E. Gibbs. *Environ. Toxicol. Chem*. 1998, **17**, 37.

39 T. Horiguchi, H. Shiraishi, M. Shimizu and M. Morita. *J. Mar. Biol. Ass. U.K*. 1994, **74**, 651.

40 J. Raloff. Science News 1994, **145**, 24.

41 J.F. Leatherland. *Endocrine and reproductive function in Great Lakes salmon*. In: *Chemically-Induced Alteration in Sexual and Functional Development: The Wildlife/Human Connection*, T. Colborn and C. Clement (eds.), Princeton Scientiofic Publishing Co., Inc., pp. 129, 1992.

42 K.R. Munkittrick, C.B. Portt, G.J. van der Kraak, I.R. Smith and D.A. Rokosh. *Can. J. Fish Aquat. Soc*. 1991, **48**, 1371.

43 W.P. Davis and S.A. Bartone. Effects of kraft mill effluent on the sexuality of fishes: an environmental early warning. In: *Chemically-Induced Alteration in Sexual and Functional Development: The Wildlife/Human Connection*, T. Colborn and C. Clement (eds.), Princeton Scientific Publishing Co., Inc., pp. 113, 1992.

44 D.M. Fry and C.K. Toone. *Science* 1981, **231**, 919.

45 R.D. Moccia, G.A. Fox and A. Britton. *J. Wild Dis.* 1986, **22**, 60.

46 C. Facemire, T. Gross and L. Guillette. *Environ. Health Persp*. 1995, **103**, 79.

47 P.S. Ross, R.L. DeSwart, P.J. Reijnders, H. van Loveren, J.G. Vos and A.D.M.E. Osterhaus. *Environ. Health Persp*. 1995, **103**, 162.

48 R.L. DeSwart, P.S. Ross, J.G. Vos and A.D.M.E. Osterhaus. *Environ. Health Persp*. 1996, **104** (Suppl. 4), 829.

49 R.J. Kavlock, G.P. Daston, C. DeRoss, P. Fenner-Crisp, L.E. Gray, S. Kaattari, G. Lucier, M. Luster, M.J. Mac, C. Maczka, R. Miller, J. Moore, R. Rolland, G. Scott, D.M. Sheehan, T. Sinks and H.A. Tilson. *Environ. Health Persp*. 1996, **104** (Suppl. 4), 715.

50 K.A. Grasman, G.A. Fox, P.F. Scanlon and J.P. Ludwig. *Environ. Health Persp*. 1996, **104** (Suppl. 4), 829.

51 J. Miyamoto, M. Matsuo, H. Tanaka, T. Yokoyama, M. Yasuda, K. Yamashita, M. Morita, H. Shiraishi and T. Horiguchi. Japan Chemical Industry Association/Japan Chemical Industry Ecology - Toxicology and Information Center, 1997.

52 J.M. Bergeron, D. Crews and A, McLachlan. *Environ. Health Persp*. 1994, **102**, 780.

53 M.R. Felden, I. Chen, B. Chittim, S.H. Safe and T.R. Zacharewski. *Environ. Health Persp*. 1997, **105**, 1238.

54 M. Ema, N. Ohe, M. Suzuki, J. Mimura, K. Sogawa, S. Ikawa and Y. Fujie - Kuriyama. *J. Biol. Chem*. 1994, **269**, 27337.

55 G.M. Stancel, H.O. Boettger-Tong, C. Chiappitta, S.M. Hyder, J.L. Kirkland, L. Murthy and D.S. Loose-Michell. *Environ. Health Persp*. 1995, **103** (Suppl. 7), 29.

56 B.E. Gillesby and T.R. Zacharewski. *Environ. Toxicol, Chem*. 1998, **17**, 3.

57 S. Safe, K. Connor, K. Ramamoorthy, K. Gaido and S. Maness. *Regul. Toxicol. Pharmacol*. 1997, **26**, 52.

58 A, B, Okey, B.S. Riddick and P.A. Harper. *Toxicol. Lett*. 1994, **70**, 1.

59 H.L. Bradlow, D.L. Davis, G. Lin, D. Sepkovie and R. Tiwari. *Environ. Health Persp*. 1995, **103**, 147.

60 Anonymous. Vinclozolin. In: *1986 JMPR Evaluation, Part II, Toxicology*. World Health Organization, 1987.

61 T.J.O. Lundi, H.I. Petterson and K. Martinsson. *J. Agr. Food Chem*. 1990, **38**, 1530.

62 J. Miyamoto, H. Kaneko, D.H. Hutson, H.O. Esser, S. Gosbach and E. Dorn. *Pesticide Metabolism: Extrapolation from Animals to Man*. Report from IUPAC. Blackwell Scientific Publications, 1988.

63 J.B. Watkins III and C.D. Klassen. *J. Animal Sci.* 1986, **63**, 933.

64 T. Shimada, H. Yamazaki, M. Miura and F.P. Guengerich. 23rd Symposium on Xenobiotic Metabolism and the Efficacy/Toxicity of drugs (Kyoto). Abstract 29-S-5, 1992.

65 H. Ohkawa, R. Kikuchi and J. Miyamoto. *J. Pesticide Sci.* 1980, **5**, 11.

66 J. Miyamoto, N. Mikami and Y. Takimoto. *The fate of pesticides in aquatic ecosystems*. In: *Environmental Fate of Pesticides: Progress in Pesticide Biochemistry and Toxicology*, Vol. 7, T.R. Roberts and D.H. Hutson (eds.), John Wiley and Sons, pp.123, 1990.

67 P.M. Chapman, A. Firebrother and D. Brown. *Environ. Toxicol. Chem*. 1998, **17**, 99.

68 L.W. Barnthouse. In: *Ecotoxicology - Ecological Fundamentals, Chemical Exposure and Biological Effects*, Chapter 24, A. John Wiley and Sons, 1997.

69 Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) 1488/94 on Risk Assessment for Existing Substances.

70 Technical Guidance Document in support of Commission Directive 93/67/EEC for New Notified Substances and on Risk Assessment for New Notified Substances; Commission Regulation (EC) 1488/94 on Risk Assessment for Existing Substances. EC Catalogue No. CR-48-96-002-EN-C.

71 K. Fent. *Ökotoxikologie*. Georg-Thieme Verlag, Stuttgart, New York, 1998.