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ENDOCRINE DISRUPTORS IN THE ENVIRONMENT

(IUPAC Technical Report)

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Endocrine disruptors in the environment

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Abstract: Many chemical substances of natural or anthropogenic origin are suspected or known to be endocrine disruptors, which can influence the endocrine system of life. This observation has led to increased interest on the part of the public and the media, as well as to a steep rise of research activities in the scientific community. New papers and results are presented so fast that it is impossible to give a complete review of this emerging research field. Therefore, this paper tries to give insight into some topics of the great scope of endocrine disruptors in the environment. To get a general idea of the biochemical and biological background, some parts of the endocrine systems of mammalians and nonmammalians are explained. The sections that follow describe important mechanisms of endocrine disruption such as interactions with hormone receptors. Test strategies for anthropogenic chemicals on various organisms are critically reviewed with respect to their problems and gaps concerning endocrine disruptors. The main emphasis of the paper is on the chemical substances suspected or known to be endocrine disruptors. To get a better comprehension of their behavior in the environment, physicochemical data such as water solubility or K_{ow}, as well as information about their use and/or function are reviewed and compared. The main routes of exposure for most chemicals are shortly described, and data about concentrations in the environment (soil/sediment, water) are detailed.

CONTENTS

1. INTRODUCTION

The possibility that some chemicals may disrupt the endocrine systems in humans and animals has received considerable attention in the scientific and public community. Endocrine disruption is on the agenda of many experts' groups, steering committees and panels of governmental organizations, industry, and academia throughout the world. Because the disturbance of the endocrine system is a very sensitive topic, scientific findings or observations are often controversially discussed among scientists, environmentalists, and authorities. Therefore, the aim of this technical report is to provide a science-based interim insight into endocrine disruption caused by chemicals with special emphasis on ecological well-being. Owing to the complexity of this topic and the tremendous scientific research in this field, only a general overview can be given, which might be, however, beneficial and helpful for interested parties of academia who want to be informed on this subject.

1.1 Background

In conjunction with the nervous and immune systems, the endocrine system forms the main regulatory mechanism that controls different pivotal functions in the human or animal body. The messengers of the endocrine system are hormones that are synthesized and excreted at very low quantities from specialized glands and transported to the target organ(s) via the bloodstream. Hormones are transported in the blood in the free state or attached to carrier proteins and bind at the target organs to specialized hormone receptors on the cell surface or within the cell (nuclear receptors). This hormone-receptor complex then activates different cell or organ functions. The binding between hormone and receptor is based on steric complementarities comparable with the "key and lock" principle. Hormones influence several essential regulatory, growth, developmental, and homeostatic mechanisms of the organism, such as reproduction, maintenance of normal levels of glucose or ions in the blood, blood pressure, general metabolism, and other muscle or nervous system functions. Examples of hormones are adrenaline, which helps stimulate physical activity or the male and female sexual hormones, testosterone and estrogen, which are essential for such important reproductive functions as sexual development, growth, and behavior. The balance of the hormones (homeostasis) in the organism is essential in order to prevent functional disorders. Therefore, the endocrine system includes a number of central nervous system-pituitary-target organ feedback mechanisms that enable the body to react very flexibly on internal or external changes of the hormone status. But this complex system is very sensitive toward disturbing influences that can severely impair the whole development of the organism. At present, it is highly uncertain whether the fetus or the young are capable of regulating changes of the endocrine milieu.

1.2 Definitions

In recent years, a growing body of scientific research indicates that substances in the environment may interfere with the normal function of the endocrine system of humans and wildlife. These compounds may be man-made (so-called xenoestrogens), e.g., industrial chemicals, crop protection chemicals, or they may be natural like the phytoestrogens. Some scientists have hypothesized that minute amounts of these chemicals are able to disrupt the endocrine system and cause cancer, harm to male (e.g., reduced sperm counts) and female reproductive systems, and other adverse effects [1,2]. Therefore, those substances are called "endocrine disruptors". There are some definitions of an endocrine disruptor (ED) in place. The European scientific and regulatory community has agreed on the following definition of an endocrine and a potential ED during the Weybridge Conference [3]:

> "An **endocrine disruptor** is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, consequent to changes in endocrine function."

> "A **potential endocrine disruptor** is a substance that possesses properties that might be expected to lead to endocrine disruption in an intact organism."

In May 1997, the U.S. Environmental Protection Agency (EPA) task force on endocrine disruption (EDSTAC) agreed on the following operational definition:

> "An **endocrine disruptor** is an exogenous chemical substance or mixture that alters the functions(s) of the endocrine system and thereby causes adverse effects to an organism, its progeny, or (sub) population."

The inclusion of the word "adverse" effect was controversially discussed. In order to achieve consensus, the EDSTAC finally agreed to the following general description [4]

> "The EDSTAC describes an **endocrine disruptor** as an exogenous chemical substance or mixture that alters the structure or function(s) of the endocrine system and causes adverse effects at the level of the organism, its progeny, the populations, or subpopulations of organisms, based on scientific principles, data, weight-of-evidence, and the precautionary principle."

In a special report on endocrine disruption, the EPA stated that, based on the current state of the science, endocrine disruption is not considered to be an adverse end point per se, but rather to be a mode or mechanism of action potentially leading to other outcomes, for example, carcinogenic, reproductive, or developmental effects [5]. However, only a limited number of causal relationships between exposure to environmental chemicals and adverse effects on human health have been established [6]. However, findings in wildlife increase the concern that such a link may indeed exist also for further substances. At present, it is not clear whether the observed adverse effects are restricted to local areas or if they are a widespread phenomenon, since they are mostly restricted to highly polluted areas (e.g., from accidental spillage) caused by older products. These products have already been identified as "problem substances" and at present are heavily regulated or even banned.

2. REPRODUCTIVE ENDOCRINE SYSTEMS

To allow a better understanding of the mechanisms by which endocrine disruptors might exert their effects of endocrine disruption, a short introduction into the endocrine system of mammalian species and into the sexual differentiation of nonmammalian species shall be presented. More detailed information can be found in recent publications dealing specifically with the topic [7–11].

2.1 Mammalian species

The primary function of an endocrine system is to transform various exogenous stimuli into chemical messengers, hormones, resulting at least in the expression of the appropriate gene and thus in the synthesis of proteins or in the activation of already existing tissue-specific enzyme systems. The endocrine system represents an important tool for the timely coordination of development (e.g., induction of spawning cycles or sexual maturity) and metabolism (e.g., glucose homeostasis). Exogenous stimuli like day length, temperature, light, or pheromones, as well as endogenous stimuli generally known as the "internal" clock, are processed in the central nervous system. After a complex chain of biochemical processes, the hypothalamus secretes releasing hormones or releases inhibition hormones that control the secretion of hormones from the pituitary gland. These secreted glycoproteins—the so-called glandotrophins—induce synthesis and release of tissue-specific hormones in the various glands (thymus, thyroid, parathyroids, adrenals, pancreas, pineal, testes, and ovary). Hormones secreted by these internal glands travel through the bloodstream to their target tissues and target cells where they initiate a change in cellular activity by attaching to a receptor protein. This change is transmitted across the plasma membrane of a cell in different ways depending on the type of hormone. The cascade of different, interdependent physiological processes is regulated by complex mechanisms such as a negative-feedback pathway that is turned on and off in response to fluctuating hormone levels: When hormone production of the glands peaks, the hormone acts as an inhibitor and causes the hypothalamus and/or pituitary to shut down the pathway producing the substance. Although many steps of this sensitive system can be influenced by different external stimuli, most effects of endocrine disruptors observed and explained until now are attributed to the function of the gonads, which control the development of sexual differentiation, secondary sex characteristics, and functioning of sex organs [12,13].

2.1.1 Steroid hormones

Cholesterol is the precursor of the five major classes of steroid hormones. According to their number of carbon-atoms, these classes are:

C21: derivatives of pregnane, so-called progestagens, glucocorticoids, and mineralcorticoids;
C19: derivatives of androstane, so-called androgens; and
C18: derivatives of estrone, so-called estrogens

Because of their common precursor cholesterol, they are all structurally related. The sex steroids are the progestagens, androgens, and estrogens. The major sites of synthesis of the sex steroids are corpus luteum for progestagens, testis for androgens, and ovaries for estrogens.

2.1.2 Female sex steroids

The synthesis of the female sex steroid hormones—estrogens and progestagens—is a very complex cascade-like system. The limbic system of the brain releases specific neurotransmitters or neuropeptides that stimulate the hypothalamus to produce so-called releasing factors. These releasing factors thereon stimulate the pituitary to release specific hormones (gonadotrophins) that are transported via the blood stream to hormone-synthesizing tissues. In the case of mammals, the gonadotrophins from the pituitary are luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Under the influence of these substances, estrogens and progestagens are released from the hormone-synthesizing tissue, the ovaries, into the blood circulation.

Estrogens and progestagens are female hormones with different tasks. Estrogens (estradiol, estrone, estriol, equilin, equilenine) are responsible for female secondary sex characteristics and regulation of reproduction. Estradiol stimulates proliferation and growth in the reproductive tract organs, causes the development of the endometrium of the uterus, and influences libido. Estrogens help maintain pregnancy and prepare the breasts for lactation. Progesterone helps regulate changes that occur during menstruation and influences the development of fetal membranes and mammary glands during pregnancy.

The biosynthesis of sex steroids is catalyzed by a series of enzymes that form the steroidogenic pathway. This pathway causes the conversion of pregnenolone to progesterone, the precursor for androgens. Androgens themselves—male sex steroids—can then be transformed to estrogens. The extent to which this biotransformation takes place depends on the expression of the various enzymes in specific tissues. The enzyme complex 19-hydroxylase-aromatase, which catalyzes the conversion of androgens to estrogens, plays a major role in this biotransformation.

About 98 % of the lipophilic steroid hormones are transported by testosterone-estrogen-binding protein also termed sex hormone binding globulin (SHBG). Albumin contributes also to the transport of these hormones, but to a much lesser extent owing to its lower affinity. Only 2 % of the steroid hormones are circulating free and thus are biologically active. Progesterone is transported bound by the globulin transcortine. Metabolization of estrogens and progestagens mainly takes place in the liver. The lipophilic compounds are conjugated with glucuronic acid or sulfate to increase their water solubility and are excreted through the kidneys. A small amount of conjugates can enter the enterohepatic circulation via excretion into the bile and serve again as active hormone in the body.

2.1.3 Male hormones

The principal androgens or male hormones are testosterone and dihydrotestosterone. They promote development and differentiation of male reproduction organs before and after birth. Androgens determine secondary male sex characteristics. They also contribute to generalized anabolic functions of bone growth and increase protein synthesis, especially in muscles. In adulthood, testosterone is essential for sperm production. The principal androgen produced and released from the testes is testosterone. The gonadotrophic hormones from the pituitary, LH and FSH, also regulate production and secretion of testosterone. Negative feedback from the concentration of testosterone in the blood can lower or block LH production. Pregnenolone is the precursor of the biosynthesis of testosterone that is formed by two

principal ways in the Leydig cells located in the testes: (1) Δ^4-biosynthesis leads to progesterone, 17-α-hydroxyprogesterone, and androstenedione to testosterone and (2) the Δ^5-biosynthesis leads to 17-α-hydroxypregnenolone, dehydroepiandrosterone, and Δ^5-androstendiol to testosterone.

Androgens are transported in the blood mainly bound to a special testosterone–estrogen-binding protein.

Testosterone is metabolized in peripheral tissues (30–50 %) and in the liver (50–70 %). Degradation products, such as androsterone and etiocholanolone, are excreted free or as glucuronide conjugates in the urine.

Testosterone is reduced to the more potent androgen 5-α-dihydrotestosterone (DHT) by the microsomal fraction in target organs (e.g., prostate and epididymis) and is the precursor of estradiol and estrone. The male and female hormones are actually secreted by both sexes. However, male hormones are secreted in higher quantities and are more potent in males, whereas female hormones are secreted in higher quantities and are more active in females.

Sex steroids have very important functions during sexual differentiation of mammals, which is genetically and hormonally regulated. The process of sexual differentiation—which shall not be explained in more detail here—is very sensitive toward external influences because of its enormous complexity.

2.1.4 Intracellular hormone receptors

Many hormones—among these are also the steroid hormones—exhibit their effect in the target tissue by influencing transcription of specific genes leading to an increased or inhibited biosynthesis of specific proteins (e.g., enzymes). For this purpose, the steroid hormones must interact with intracellular receptors. To each class of steroid hormones belongs a specific steroid receptor, but they all have certain common structural features and, therefore, belong to the so-called superfamily of nuclear hormone receptors. The interaction between hormone, hormone receptor, and DNA shall be explained describing the estrogen receptor (ER). As already described, the lipophilic sex steroids released from the gonads into the bloodstream are transported to their target organs or tissues. They enter the cell by passive diffusion through the lipid membrane. The hormone then binds to the specific receptor protein, located within the cytosol. The "free" receptor (i.e., without a ligand) is maintained in an inactive conformation through interactions with a number of associated proteins. After hormone-receptor binding, these associated proteins dissociate, allowing the receptor to alter its conformation from the inactive into the active form and to move into the nucleus. Once activated, the receptor then forms homodimers, which seek out specific DNA motifs, termed "hormone response elements", located in the nucleus, upstream of hormone-responsive genes. Binding of the receptor complex to the hormone-response element of DNA results in chromatin rearrangement, usually allowing the cells' transcriptional machinery increased access to the promoter region of hormone-inducible genes, producing increased mRNA production followed by increased protein expression, resulting finally in observed effects such as increased growth in the reproductive tract organs and mammary glands [14]. Although the steroid structure of sex hormones is highly conserved among many species, the structures of the hormone receptors are varying, despite a high degree of similarities. Steroid receptors of the superfamily of nuclear hormone receptors have two specialized regions called the ligand binding domain (LBD) and the DNA binding domain (DBD). Thus, function and mechanism of the androgen and progesterone receptors (ARs, PRs) are very similar to that of the estrogen receptors.

In 1996, Kuiper et al. reported a novel ER type [15]. The previously known ER was called ERα; the new one was named ERβ. The two ER subtypes are expressed differently in estrogen target organs and seem to mediate different estrogen actions [16]. ERβ is structurally similar to ERα, although amino acid variations have been identified within the DBD and the LBD. These differences indicate that structurally different ligands may be capable of binding to the LBD, causing variable activation of estrogen-responsive genes. Phytoestrogens appear to prefer ERβ for binding, while most endogenous sex steroids do not exhibit such difference. These results suggest that phytoestrogens could exert their actions preferentially through ERβ. But it is not yet clear what this means in vivo. In theory, this preference of ERβ

of exogenous estrogens could allow unique, tissue- or organ-specific actions, different from those of endogenous hormones.

2.2 Nonmammalian species

Although the endocrine system is highly conserved in the animal kingdom, inter-species differences exist that may affect sensitivity and response to endocrine disruptors. These differences include quantitative and qualitative variability in endogenous hormone and receptor levels, differences in the timing and duration of critical periods of development and inter-species differences of sex-determination processes [17].

2.2.1 Birds

As in mammals, the sexual differentiation in birds is based on heterogametic sex chromosomes, but in contrary to mammals, where the heterogametic sex is male (XY), the heterogametic sex chromosome in birds is female (ZW) with a complementary male sex chromosome of ZZ. The homogametic sex is in both, mammals (XX, female) and birds (ZZ, male) the "default" sex, i.e., the phenotype into which the embryo will develop in the absence of sex-specific hormones or signals that cause sex differentiation [18]. This means that in birds the phenotypic differentiation of the embryo into a male will occur unless specific female gene products are expressed and estradiol is synthesized, causing the differentiation of the gonad into an ovary. Owing to these differences in the genetic sex determination as well in the hormonal control of sex differentiation, environmental estrogens have different effects during the embryonic development in birds compared to mammals. It can, therefore, be assumed that the influence of exogenous compounds that might exhibit estrogenic activities is more pronounced in birds than in mammals and vice versa in the case of androgenic activities. Avian embryos are, furthermore, at high risk to lipophilic "estrogenic" xenobiotica because they may selectively accumulate in the high lipid yolk of eggs, especially in raptors and fish-eating birds. The observed feminization of male gulls in the wild might be attributed to the feminization effect of DDT [19] because levels of DDT found in the eggs can cause feminization of male embryos. Findings in rats [20] suggest that abnormalities in male sex development induced by *p,p′*-DDE, a persistent metabolite of DDT, may be mediated at the level of the AR, since this metabolite exhibits an antiandrogenic activity.

2.2.2 Reptiles and amphibians

In many reptiles, individuals lack sex chromosomes and have, therefore, evolved other mechanisms of sexual differentiation. Their sexual differentiation depends on environmental factors by which the undifferentiated embryo is determined. In many reptiles, the temperature of egg incubation determines the sex of the offspring, a process known as temperature-dependent sex determination (TSD) [21]. High temperatures produce dominantly males, and low temperatures produce females in many crocodilians and lizards, whereas this pattern is reversed in most turtles. A mixture of these patterns is evident in the leopard gecko, the snapping turtle, and in crocodiles, where at extreme incubation temperatures females and at intermediate temperatures varying ratios of males and females are produced [22]. Reptiles with TSD lack heterogametic sex chromosomes, and each individual has the equal ability to become a female or a male, depending on the incubation temperature. The specific mechanism behind TSD is unknown, but it is hypothesized that temperature stimulates or suppresses pivotal steroidogenic factors [23] during the sensitive time window of the midtrimester of egg development. The temperature modifies the activity as well as the temporal and spatial sequences of enzymes and hormone receptors such that sex-specific hormone milieus, created in the urogenital system of the developing embryo, determine gonad type. It seems that the steroidogenic aromatase, an enzyme that converts androgens to estrogens, plays a pivotal role in TSD. From the results of several studies dealing with this enzyme in reptiles, it can be suggested that the aromatase is a critical part of TSD and can be modified by extrinsic factors [for an overview, see ref. 24]. Due to the involvement of sexual hormones or enzymes in the sexual development of reptiles, it is therefore not surprising that exogenous chemicals can mimic the ef-

fects of temperature on sex determination. For example, when red-eared turtle embryos are exposed to 17β-estradiol at a male-producing temperature, phenotypically female turtles are produced in a dose-dependent manner [25].

Similar to reptiles, low temperatures during larval development in amphibians are known to result in the differentiation of predominantly females, while at higher temperatures mainly males dominate. Amphibians, like anurans (frogs and toads), have both aquatic and terrestrial life histories and might, therefore, be very vulnerable to environmental exposure to xenoestrogens. The primary androgens in male urodeles (salamanders and newts) are testosterone, 11β-hydroxytestosterone, and 11β-dihydroxytestosterone, whereas the main androgen in anurans is 5α-dihydrotestosterone [26]. There are some paradoxical observations regarding the action of sexual hormones in amphibians. For example, estradiol in larval tiger salamanders acts as an estrogen on the müllerian duct (oviduct), but as an antiandrogen on the wolffian duct (spermatic duct) [27]. Such paradoxical actions (androgens acting as estrogens and vice versa) have been described within all the major vertebrate classes [28], and they show the necessity for studying amphibians separately from mammalians.

As in fish, vitellogenin is the precursor molecule for egg yolk as the source of metabolic energy for the developing embryo [29]. However, there is evidence that in reptiles the presence of a distinct growth hormone concentration is a prerequisite for vitellogenin production [30]. Nevertheless, the induction of vitellogenin in amphibians and reptiles is thought to have some utility as an estrogenic biomarker of exposure to environmental EDs.

2.2.3 Fish

Similar to mammals, the main ovarian steroid in fish is 17β-estradiol. Differences exist with regard to the major androgens, where besides testosterone also 11-ketotestosterone or 11β-hydroxytestosterone are responsible for the development of male secondary sex characteristics and for the induction of spermatogenesis [31]. In contrary to mammals where the female plasma testosterone levels normally do not exceed one-tenth of male levels, in sexually maturing female salmonid fish, testosterone levels can exceed the concentration of endogenous 17β-estradiol. The expression of sexuality in fish is greatly diversified, including various types of sex chromosomal mechanisms, the genetic sex. Unlike mammals and birds, homogamety exists beside heterogamety for male as well as for female fish. Most fish examined karyologically have no morphologically differentiated sex chromosomes (monomorphic), but also heteromorphic chromosomal sex types as well as gynogenetic (triploid) sex do exist [32]. Physiological sex is formed through the biochemical process of ontogenesis under the control of genetic sex. There are several types of differentiation, such as gonochorism (existence of either testes or ovaries) and hermaphroditism (both ovarian and testicular tissue in the same individual fish). Most cultured fish species have the gonochoristic type of sexuality, and among the gonochoristic species of teleosts, there are some that reveal transitory hermaphrodism during a juvenile period. For example, developing gonads of the European eel *Anguilla anguila* pass through an intersexual phase in a certain period of the elver stage. In the Cyprinid *Danio rerio* (zebra fish), a common test species in ecotoxicology, gonads start differentiating as ovaries about 10–12 days after hatching, irrespective of their definitive sex. About half of the ovaries begin to be transformed into testes when the fish reach about 23–25 days of age. The whole process of sex reversal is normally completed within nearly 40 days after hatching, but it tends to be protracted in fish that have undergone a retarded growth in their early life [33]. In salmonids and medaka (*Oryzias latipes*), the undifferentiated gonad directly differentiates into either a testis or an ovary. Several findings have also indicated that, in fish, there is a critical period of development within which hormones are effective in causing sex reversal. This has been shown by serial injections of eggs with sex hormones where the test compounds apparently are retained until sex differentiation begins [34]. In fish breeding, influencing the sex of fish by feeding high doses of sex hormones had been a common method to obtain monosex cultures [32]. Because sex differentiation differs not only between families but also within families, feminization by exposure to estrogenic compounds

is not a common feature of fish, but has been observed in some fish species, such as roach (*Rutilus rutilus*) [35], medaka (*O. latipes*) [36], or guppies (*Poecilia reticulata*) [37].

Vitellogenin or egg yolk production represents a key estrogen process in oviparous vertebrates that is essential for oocyte maturation. Vitellogenin is a phospholipoprotein synthesized in the liver of female oviparous vertebrates under the control of different hormones, but estrogens, typically 17β-estradiol, play a dominant role. Vitellogenin enters the bloodstream and reaches the ovary, where it is transformed into two major types of yolk proteins, lipovitellins, and phosphovitins [6], which serve as a food reserve of the developing embryos. These yolk proteins are responsible for the enormous growth of the oocytes in the month prior to ovulation. In female rainbow trout, the concentration of vitellogenin in plasma increases million-fold during this period and can reach levels of tens of milligrams per liter. In adult male fish (e.g., rainbow trout), circulating levels of endogenous estradiol are nearly undetectable, but there is some evidence that other teleost male fish do synthesize small amounts of estradiol, and that the hormone may regulate the production of the male androgens 11-ketotestosterone and testosterone [38,39]. Although the vitellogenin gene is also present in male fish, no remarkable concentrations of vitellogenin can be detected, presumably due to the very low concentration of plasma estradiol. However, the exposure of male fish to various concentrations of natural and synthetic estrogens or xenoestrogens has shown that vitellogenin can markedly be induced [for an overview, see ref. 40]. Therefore, the induction of vitellogenin can serve as a sensitive biomarker for estrogenic environmental exposure.

2.2.4 Invertebrates

Although invertebrates represent more than 30 different phyla and account for more than 90 % of our animal kingdom, only limited information is available with regard to endocrine disruption to most of the invertebrate species. Therefore, only a brief introduction will and can be made. For those interested in more details, the SETAC publication *Endocrine Disruption in Invertebrates: Endocrinology, Testing, and Assessment* [41] gives an excellent overview on this topic. The best-known phyla of invertebrates are mollusks, annelids, and arthropods (e.g., insects and crustaceans). Many aquatic and terrestrial invertebrates developed complicated life histories with reproduction cycles that are most often very complex in nature. Their reproduction cycles are regulated by sexual hormones (steroids, peptides, or terpenoids) and/or can be controlled by several environmental factors such as temperature, light intensity, desiccation, or diet. In general, many of the processes known to be under endocrine regulation in vertebrates (i.e., development, growth, maturation, reproduction, water, and ion balance) are also under endocrine regulation in invertebrates. In addition, processes unique to some taxa of invertebrates (i.e., molting, limb regeneration, diapause, pheromone production, pigmentation and color change, and metamorphosis) are also under endocrine control [41]. One of the best-investigated endocrine systems of invertebrates is that of arthropods owing to their commercial importance and the need to control agricultural pests. Ecdysone and related compounds, the ecdysteroids, are the most important endocrine regulators in arthropods, which are involved in embryonic development, molting, metamorphosis, reproduction, or pigmentation. Although these ecdysteroids are also found in other groups of invertebrates (e.g., annelids), their specific role is not well established. Besides the ecdysteroids, juvenile hormones in insects and methylfarnesoate in crustaceans (both belong to terpenoids) are further main hormones that are deemed necessary to mediate the different regulatory functions of ecdysteroids. Besides the insects, mollusks are the most diverse phyla of invertebrates with classes such as bivalves (clams, oysters), gastropods (slugs, snails), or cephalopods (octopus, squid). In contrast to other invertebrate phyla (e.g., insects), ecdysteroids seem to play only a minor role, while there is evidence that vertebrate-like sex hormones such as progesterone and testosterone are involved in sexual development [42].

2.2.5 Mollusks

Steroid hormones seem to play important roles in the sexual development of mollusks, although the information is limited to their reproductive physiology or biochemistry. In a snail, *Helix aspersa*, an-

drostenedione metabolism produces several kinds of steroids, including testosterone, estrone, and estradiol-17β [43]. These conversions implicated the presence of several steroid conversion enzymes: dehydrogenases, reductase, and an aromatization system. Also, with some species of gastropods, the transformation of androstenedione into neutral steroids has been reported [44,45]. Cytochrome P450 systems and their function in xenobiotic metabolism in mollusks are reported [42]. In mollusks, there is good evidence for the existence of diverse neuroendocrine systems that are not very different from those of vertebrates, and the majority of the neurohormones (most probably of peptidic nature) of mollusks act directly on target tissues. It is also reported that a release of neural factors from the pleural ganglia induces penis growth [46]. On the other hand, some gonadal activity seems to be controlled by a gonadostimulin or mitogenic substance that is suggested as an androgenic factor [47]. Biosynthetic pathways of steroid hormones are essentially identical to those of other animal species. Thus, inhibition of the conversion of testosterone to estradiol catalyzed by P450-aromatase in the pathway may affect the sexual development of mollusks as suggested for the tributyltin (TBT) action mechanism causing imposex [48–50]. Particularly in the presence of cyproterone, which competitively blocks the AR, TBT activity to cause the imposex is suppressed [50]. Inhibition of the neural factors in mollusks may also seriously affect their sexual development. Another important possibility is that the steroid metabolism itself depends on neurohormones [48]. Therefore, we must carefully examine several possible modes of action before drawing a conclusion about the endocrine-disrupting mechanism of chemicals on mollusks.

MECHANISMS OF ENDOCRINE DISRUPTORS ON HORMONE ACTION

Owing to the complex nature of the endocrine system, it is obvious that external stimuli, e.g., exposure to xenobiotica-mimicking endocrine activity, can influence its important functions. The first observations of endocrine effects in wildlife research activities were focused on the explanation of main mechanisms underlying the observed effects. Several possible modes of actions have been cleared up in recent years, and the most important shall be introduced in brief.

3.1 Direct interactions with hormone receptors

3.1.1 Agonistic action

An exogenous agonist can be defined as a ligand that can bind to a receptor like the natural substrate and "turn it on". The activation of the hormone receptor then finally leads to the same effects that can be caused by endogenous hormone action. The potency of an exogenous agonist depends on its affinity to the receptor as well as on its ability to turn the receptor on. It should be mentioned that different species exhibit different structures of the hormone receptors. Therefore, ligand binding to a specific receptor does not automatically mean that this substance exhibits the same affinity for the respective hormone receptor of another species. Well-known examples for estrogen copycats are the synthetic estrogens diethylstilbestrol (DES) and ethinylestradiol. Also, most of those endocrine disruptors called "xenoestrogens" exert their effect by an agonistic action on the hormone receptor.

3.1.2 Antagonistic action

Other substances are acting on the hormone receptors via an antagonistic mechanism: An antagonist is a ligand that blocks or diminishes responses elicited by agonists because the receptor cannot be activated as usual.

The inhibition of the receptor can be competitive (i.e., the endogenous agonist and exogenous antagonist compete for the same active binding site) or it can be noncompetitive (i.e., the inhibitor binds at the receptor or receptor-hormone complex, but not at the active binding site). Competitive inhibition can lead to total deactivation of the receptor; noncompetitive inhibition can result in slower or reduced reactions performed by the receptor.

Typical antagonists for hormone receptors are the herbicides linuron, vinclozolin, and their metabolites [20,51], or the pharmaceutical tamoxifen, which compete for binding sites at the androgen and estrogen receptors, respectively.

For agonistic as well as for antagonistic reactions between exogenous ligand and hormone receptor, the concentration of the ligand often plays an important role. Concentrations of endogenous hormones are normally very low. If concentrations of xenobiotica in the organism are high, endocrine disruption effects can be caused even if the exogenous ligands exhibit only a low binding affinity to the receptor.

3.2 Indirect interactions with the endocrine system

3.2.1 Hormone concentration

Chemicals can influence hormone metabolism in different ways: Hormone production can be impaired by inhibiting important enzyme-catalyzed reactions. As mentioned above, biosynthesis of estrogens includes the conversion of testosterone to an estrogen catalyzed by the enzyme aromatase. Xenobiotica can inhibit this enzyme, leading to higher testosterone concentrations and to lower estrogen concentrations. The effects observed can be interpreted as antiestrogenic or androgenic, depending on the point of view. This mechanism probably causes the effects of TBT compounds observed in marine neogastropods: Imposex (i.e., females with typical male sex characteristics) is caused by inhibition of the enzyme aromatase, resulting in increased levels of testosterone in females [50,52]. Hormone metabolism can also be influenced by induction of hormone-metabolizing enzymes like the cytochrome P450-group in the liver. These enzymes have a key function in the synthesis and degradation of steroid hormones, and their production or activity can be influenced by various xenobiotica such as PCB congeners and dioxin [53,54].

An influence on the transport of the hormones via the bloodstream to the target tissues and organs can also lead to a disturbance of the endocrine system. Only a small part of the lipophilic compounds is circulating free in the blood, since they are mainly bound to special hormone carrier proteins such as albumin and globulins. But the intensity of the receptor-mediated effect depends on the amount of free hormone substances because only free molecules can bind and activate the receptor. Chemicals that compete with sex steroids for the binding sites of transport proteins may increase the level of free and, therefore, effective hormones.

3.2.2 Hormone receptor concentration

In receptor-mediated processes, both components, endogenous ligand and hormone receptor, own a key function. Every exogenous influence may, therefore, shift this sensitive balance. A so-called "down-regulation" of steroid hormones is discussed for some antiestrogenic compounds, especially for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). TCDD is an exogenous agonist for the arylhydrocarbon (Ah)-receptor. This receptor is not directly involved in hormone metabolism, but its activation can have different influences on the endocrine system by: (1) an increased degradation rate of estrogen receptors (down-regulation), (2) induction of estradiol metabolizing enzymes, and (3) inhibition of gene expression controlled by estradiol or growth promoters [55].

The mechanisms of interactions between xenobiotica and the endocrine system presented in this paper are only a small part of possible modes of action. The increased research in this field will surely elucidate more complex relations between exposed organisms, endocrine disruption, and xenobiotica.

4. TEST SYSTEMS

For any given chemical, there is a complex testing regime in place to characterize the potential toxic hazard for subsequent risk assessment procedures. These testing requirements depend upon different factors, including the chemical's use (industrial chemicals, by-products, pesticides, pharmaceuticals,

food additives, etc.), the quantities produced (e.g., assessed on the tonnage level per annum in case of industrial chemicals), physicochemical parameters, the scientific knowledge and understanding of the class of chemicals to which the compound belongs, and many others. Although many industrial chemicals and all pesticides have undergone extensive toxicological and ecotoxicological testing according to well-established test methods (e.g., OECD, EPA, Federal Insecticide, Fungicide, and Rodenticide Act—FIFRA) some doubts have been raised concerning whether this testing has been adequate to detect the endocrine disrupting potential of a given chemical. The existing regulatory guidelines are being carefully evaluated by OECD [56], EPA [5], industrial associations [57,58], or scientists [59,60] with special emphasis on their reliability to detect endocrine disrupting effects. In the case of pesticides and products for veterinary uses, the OECD Report [56] concluded that: "…a wide range of toxicity studies addressing subchronic, chronic, reproductive and, possibly, carcinogenic end-points are routinely conducted....chemicals of these classes will also be subjected to wildlife toxicity assessments, thus further expanding the knowledge base on their activity profile. Pragmatically, it is reasonable to assume that any inherent endocrine disruptive potential will have been identified for an existing chemical that has been subjected to such a full hazard identification process."

The Scientific Committee on Plants (SCP) of the European Commission evaluated recently the currently used legislation for the placing of plant protection products (PPP) on the market on the background of endocrine disruption and concluded:

> "The SCP is following the scientific progress in the knowledge about ED with attention, but does not consider this problem to be of great concern for the assessment of Plant protection Products (PPP) currently carried out under Directive 91/414/EEC, because the current process of evaluation, if conducted with specific attention to this issue, already permits a rather comprehensive appreciation of the ED-related toxicological risk for mammalians and man. Also ecotoxicological risks arising from ED generally can be captured by the current assessment scheme, although for some species (in particular invertebrates) the test programme is not yet satisfactory. While a further refinement of the protocols in use for the toxicological testing of the active substances of PPP as provided for by the Annex 2 of the Directive 91/414/EEC is deemed to be desirable by SCP in the near future, the SCP considers it appropriate to wait for the conclusion of the ongoing ED test guideline-update and development programme by OECD before recommending to the EU to undertake specific actions aimed at introducing supplementary testing in the Annex 2 of the Directive 91/414/EEC." [61]

However, it is well recognized among legislators, industry, and the scientific community that the existing test guidelines, especially for environmental effects, may not cover all possible adverse effects resulting from the disturbance of the endocrine system. In particular, sensible or vulnerable life stages of organisms are not fully covered by existing testing regimes, and there is a lack of information for distinct groups of organisms (e.g., amphibians).

It was, therefore, concluded that the adequacy of existing data requirements to identify endocrine-disrupting activities, especially of industrial chemicals, may be questioned, and the introduction of additional screening and testing regimes might be advisable. In light of several uncertainties, concerns, and data gaps, regulatory authorities and the OECD have established special task forces to solve this problem. A driving force in this process is the EPA, which mandated recent legislation, i.e., the reauthorization of the Safe Drinking Water Act (SDWA) and the passage of the Food Quality Protection Act (FQPA) in the United States. The EPA established two task forces, the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC) and the Endocrine Disruptor Standardization and Validation Task Force, to evaluate the recent developments and to provide advice to the agency on a strategy to screen and test chemicals and pesticides that may cause endocrine disruption in humans and wildlife.

The EPA "Inventory of Chemicals" of the Toxic Substances Control Act (TSCA) consists of about 75 500 chemicals (as of August 1997), and it is estimated that about 15 000 of these chemicals are produced in quantities exceeding 5000 kg per annum [4]. Except for food use and consumer pesticides, biological effects data are lacking for most of these industrial chemicals. Owing to the huge mass of chemicals and the limited resources and capacity, it is foreseen that the existing industrial chemicals and pesticides have to undergo prioritization on the basis of the available scientific database. In addition, a tiered screening and testing approach covering all main types of endocrine disruption have been proposed for these substances. The implementation of automated (bench) technologies, as a so-called high-throughput prescreening (e.g., transcriptional activation and/or receptor binding assays) should help speed up this process.

The OECD is following a similar approach of a harmonized tiered testing strategy based on initial assessment, screening, and testing. The OECD established a working group on "Endocrine Disruptors Testing and Assessment" in March 1998 that considers the existing efforts in the United States, Europe, and Japan [62]. The OECD developed a conceptual framework for the investigation of endocrine disruption. The framework consists of three levels: a screening level for priority setting and characterization of mechanisms of action, a second level for identification and characterization of potential endocrine-disrupting effects, and a third level to provide a definitive answer.

Although the chemical industry believes that their products are safe when used and disposed of as recommended, it shares the public's concern regarding the potential adverse effects of chemicals due to endocrine disruption—as part of its responsible care philosophy. The chemical industry is committed to working with legislative bodies, National Authorities, and the international scientific community to establish a better understanding of the endocrine disruption issue as a basis for managing previously "unknown" risks and, if necessary, modifying existing legislation to incorporate any additional adverse effects that may be identified. For that purpose, the European Chemical Industry Association (CEFIC) established an Endocrine Modulators Steering Group (EMSG) in 1996, which coordinates the activities and efforts of industry as well as the different industry-funded research activities [58].

The Commission Scientific Committee for Toxicity, Ecotoxicity and the Environment (CSTEE) presented its "Opinion on human and wildlife health effects of endocrine disrupting chemicals, with emphasis on wildlife and on ecotoxicology test methods". In March 1999 [63], concerning "Toxicological test guidelines and testing strategies", the CSTEE comes to the following conclusions:

- "present regulatory toxicological test guidelines, in particular the guidelines for ecotoxicity testing, cannot detect all endocrine disrupting effects. Therefore, current test guidelines have to be enhanced or new guidelines developed. In this process, international co-operation (EU OECD, EMSG) is essential to avoid duplication.

- reliance on in vitro assays for predicting in vivo endocrine disruptor effects may generate false-negative as well as false-positive results. Thus, the development of in vitro pre-screening test methods is not recommended. Instead, major emphasis should be put on *in vivo* assays.

- the current enhancement by the OECD of the existing 407 repeated oral toxicity test in rodents and the existing OECD 416 reproduction toxicity test has priority support."

With respect to "Ecological risk assessment and toxicological test guidelines", the committee concluded:

> "Ecological risk assessment is intended to evaluate risks on the structure and functioning of ecosystems. The strategy for ecotoxicity assessment must focus on relevant endpoints for the detection of population-community effects. The analysis of current protocols for ecological risk assessment indicates a concern on the capability of low tier levels to detect the ecological risk of endocrine disruptors because of problems related to the suitability of the test species and the extrapolation from acute lethality to long-term effects."

A common consensus exists among academia, authorities, and industry that further research in this area is urgently necessary to fill the obvious data gaps. These data gaps and research needs were addressed in several workshops [64–66]. Further epidemiological and laboratory studies as well as field tests might be appropriate to better define the scope and nature of the potential problem. Both in vitro and short-term in vivo tests, as well as suitable biomarkers for endocrine disruption, have to be developed and validated as reliable tools for prioritization and further risk assessment.

5. POTENTIAL ENDOCRINE DISRUPTORS IN AQUATIC AND TERRESTRIAL ENVIRONMENTS

Chemical substances enter the environment in different ways. Pesticides are released at their point of application; industrial chemicals are unintentionally released by volatilization, leaking or leaching either during a product's lifetime or after ultimate disposal. Natural hormones are excreted by various organisms and enter environmental compartments directly or after they have passed through wastewater treatment plants. Once a substance has passed through the environment, it can undergo different fates, such as:

- further distribution between the environmental compartments water, air, and soil/sediment, as well as their subcompartments
- degradation and transfer processes in the compartments and subcompartments

In the following sections, the first topic of the fate of selected potential EDs will be reviewed in general, or, if appropriate, in more detail. The second topic will only be considered if degradation or transformation products of native compounds exhibit endocrine-disrupting characteristics.

As useful tools for predicting and understanding the behavior of chemicals in the environment, their physicochemical properties can be used. The most important parameters are:

- Water solubility (WS): The water solubility γ_{sat} is the maximal concentration of a substance solved in pure water at a given temperature. In surface water, solubility strongly depends on parameters such as temperature, pH, salt content, or existence of humic substances and suspended matter in the surface water. Nevertheless, the experimental value is also a helpful tool to assess the hydrophobicity of a chemical. It is expressed as mass concentration of the saturated solution.
- Adsorption coefficient: The knowledge of the adsorption coefficient K_{oc} is useful for the description of the adsorption of a chemical substance to suspended matter in surface water. The K_{oc} value increases with increasing hydrophobicity of a compound, i.e., the higher K_{oc}, the stronger is the adsorption to suspended matter or water. The adsorption coefficient correlates with the octanol/water partition coefficient K_{ow}, a further important physicochemical parameter that describes the partition of a nonpolar organic substance between water and the organic solvent octanol. Octanol serves as a model for the lipid part of animal tissue. The partition coefficient—mainly used as log P_{ow}—is therefore a good descriptor of the bioconcentration.

- Bioconcentration: Bioconcentration and the bioconcentration factor (BCF) refer to the uptake of a chemical substance by an aquatic organism from the water phase. Bioaccumulation refers to uptake from water and food. Of course, not all of the chemical present in the water is necessarily available because of sorption to suspended and dissolved matter, usually organic in nature. This phenomenon is referred to as bioavailability. It should be emphasized that the environmental compartment air is neglected in this paper, which is restricted to the compartments—and transfer processes—in water and soil/sediment.

The following sections deal with potential endocrine disruptors with tables showing the most important physicochemical properties of the substances. Unless otherwise stated, sources of these data are G. Rippen [67] and the Internet, chemfinder.com [68]. For many compounds, data about the chemical properties of interest were not available in the sources used for the review and could not be listed in the tables.

5.1 Gonadal steroids

Estrogens, androgens, and progestins are produced in male and female organisms as well, but in different amounts. Steroid hormones are lipophilic, fat-soluble molecules, which are mainly excreted as water-soluble glucoronates or sulphate conjugates. Under environmental conditions these conjugates are quickly hydrolyzed, leading to the free hormones or their metabolites [69,70]. The behavior of natural sex steroids in the environment was evaluated on the basis of laboratory tests using activated sludge (Table 1) [71]. Microbial degradation was found to depend on addition of nutrients: Under the influence of nutrients, estriol, estrone, and estradiol were nearly totally degraded within four weeks. Without nutrients, degradation occurs, but to a much lesser extent.

Table 1 Estrogens [from 72].

Common name	CAS-no.	γ_{sat}/µg l^{-1}	log P_{ow}
17β-Estradiol	50-28-2	12960	4.01
Estrone	53-16-7	12420	3.13
Estriol	50-27-1	13250	2.45

γ_{sat}: Solubility in water at 25 °C.
P_{ow}: Partition coefficient octanol/water.

Because the water solubility of testosterone and androsterone is lower and the octanol/water partition coefficient is higher than for the estrogens, a different behavior in the environment might be expected (Table 2). However, there is no information at present on the abiotic or biotic degradation.

Table 2 Androgens [from 72].

Common name	CAS-no.	γ_{sat}/µg l^{-1}	log P_{ow}
Testosterone	58-22-0	5570	3.32
Androsterone	53-41-8	8750	3.69

γ_{sat}: Water solubility at 25 °C.
P_{ow}: Partition coefficient octanol/water.

5.1.1 Gonadal steroids: Environmental exposure

Different organisms excrete various amounts of sex steroids, depending on parameters such as age, state of health, diet, or pregnancy. As an example, some values for the human excretion of steroid hormones are presented in Table 3.

Table 3 Human excretion of estrogens [from 71].

Sex steroid	Amount excreted (μg/day)	Sex
17β-Estradiol	ca. 3	Male
17β-Estradiol	0.3–5	Female
Estriol	ca. 3	Male
Estriol	3–65	Female
Estrone	ca. 3	Male
Estrone	2–20	Female
Androgens	2100–23 100	Male
Androgens	800–10 500	Female

The amount of excreted estrogens of pregnant women can be 1000 times higher, depending on the progress of the pregnancy.

A very important source of natural estrogens is livestock in agriculture. Here, hundreds of animals are often living at one site, meaning that sewage and manure probably contain high concentrations of sex steroids. The routes of natural estrogens into the environment are different, depending on the respective source. Potential ways of exposure are presented in Figs. 1 and 2.

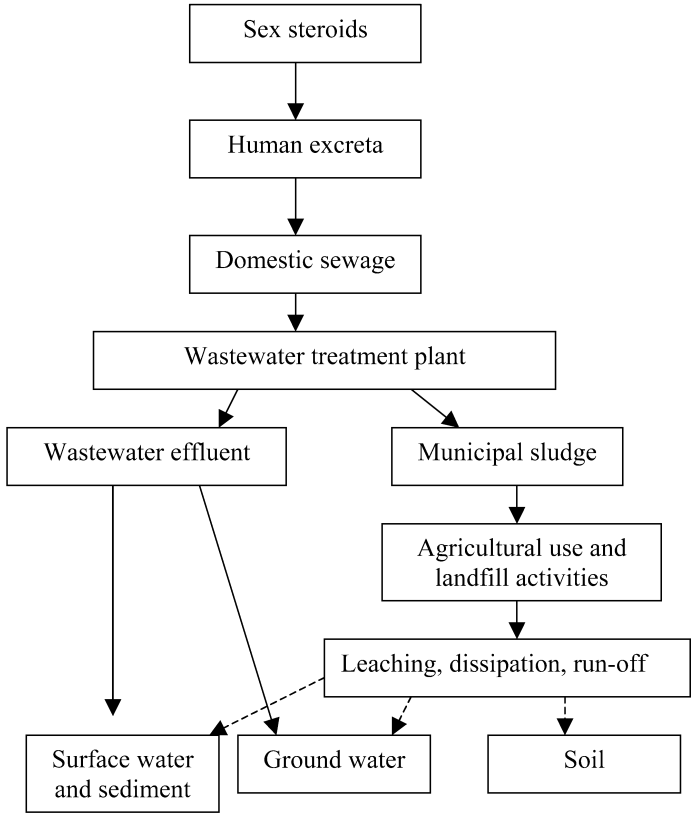

Fig. 1 Potential exposure routes of natural human hormones [modified from 73].

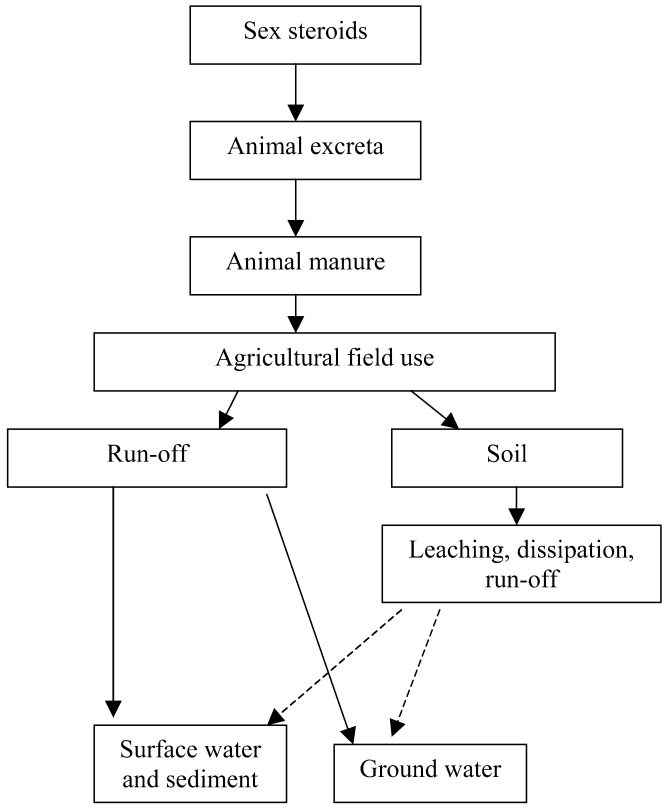

Fig. 2 Potential exposure routes of sex steroids from livestock [modified from 73].

Shore et al. were the first to investigate influence and concentrations of natural sex steroids as environmental pollutants [74]. Recent studies were carried out to determine estrogen and testosterone at different sites and to identify possible sources: 17 streams in the Conestoga River Valley of the Mid-Atlantic region of the United States, sewage treatment plant (STP) effluents as well as run-off and groundwater from fields fertilized with chicken manure were sampled, and the concentrations of estrogen and testosterone were determined applying radioimmunoassays (RIAs). Results can be summarized as follows: For stream sampling, 4 out of 10 sites had testosterone concentrations of >1 ng/l. Three of these sites were in areas with heavy use of chicken manure as fertilizer, and one site received effluent from an STP. Comparison of a stream dominated by forest with a stream dominated by cropland indicated that there was a gradient of estrogen discharge downstream along the stream dominated by cropland (0.54–1.83 ng/l). Raw sewage and effluents of a constructed wetland plant and two activated sludge plants were analyzed, showing testosterone levels between 19–273 ng/l, estrogen levels between 49–73 ng/l for the raw sewage, testosterone levels between 1.6–7.2 ng/l, estrogen levels between 0.8–4.0 ng/l for the effluent. Finally, two sources of pollution were identified—run-off from fields fertilized with manure and discharge into streams from STPs. The levels in freely flowing streams (ca. 5 ng/l estradiol + estrone) mean a potential for environmental effects. Groundwater is not a major route for hormone transport because filtration of sewage water through sand completely removes both hormones [75].

In a survey of STPs in Brazil and Germany, it was found that raw sewage in a Brazilian and a German municipal STP contained 17α-estradiol and estrone with average concentrations of 21 ng/l and 40 ng/l in Brazil and with average concentrations of 15 ng/l and 27 ng/l in Germany, respectively [76].

Elimination in Brazil was higher (99 and 83 %) than in Germany (64 and 68 %). The examination of STP discharges and rivers showed that 17α-estradiol and 16β-hydroxyestrone were frequently detected in discharges of STPs, but the concentrations were in the lower ng/l range. The highest estrogen concentration in a German river was 1.6 ng/l estrone, whereas in most samples no estrogen was detected.

In a survey of STPs [77] in the Netherlands, 17β-estradiol, 17α-estradiol, 17α-ethinylestradiol, and estrone in surface and wastewater were examined. In most effluents of STPs, estrone and 17β-estradiol were detected. The highest concentration observed was 47 ng/l for estrone, while 17β-estradiol concentrations were between 1–12 ng/l. Concentrations of hormones in surface water was generally low (below 1–5 ng/l). Estrone was detected most frequently. In a survey of effluents of German STPs, 17β-estradiol was detected in 8 out of 20 effluents with a maximum concentration of 62 ng/l [78]. In another German study [79], random samples of surface water, sediments, sewage effluents and sewage sludge, and manure were analyzed. In surface waters, 17β-estradiol was detected in 6 out of 117 samples in a concentration range between 0.8–29 ng/l with a median concentration of the positive samples of 1.7 ng/l. In 14 samples, at least one of the metabolites estrone and estriol was determined. Estrone was found in 8 surface waters with a median concentration of the positive samples of 2.3 ng/l, and estriol was detectable in 7 samples with a median concentration of the positive samples of 3.0 ng/l. Twelve lake sediments were analyzed, and 17β-estradiol was found in 3 sediments with a mean concentration of 8.5 ppb dry weight (dry wt). Estrone was only found in one sediment at 13.7 ppb dry wt, and estriol was not detectable. In sewage effluents, at least one of the natural estrogens was analyzable in 17 out of the 52 samples. 17β-Estradiol was found in 8 effluents in a range between 2.6–50 ng/l (median of the positive samples, 21 ng/l), estrone was detectable in 13 samples in concentrations between 1.5–68.6 ng/l (median of the positive samples, 9.2 ng/l) and estriol only in 1 sample with 5.2 ng/l. In 17 out of 38 sewage sludges, natural estrogens could be detected, 17β-estradiol was found in 10 sludges in a concentration range between 4.2 and 111 ppb dry wt (median, 12.7 ppb dry wt), estrone concentrations had been detected between 3.3–328 ppb dry wt in 7 samples, and estriol could be analyzed in 3 samples at 18.1–31.4 ppb dry wt (mean, 26 ppb dry wt). Manure of dairy cattle and pigs had also been investigated. 167–1229 ppb dry wt 17β-estradiol and 254–592 ppb dry wt estrone were found in the 4 samples of dairy cattle, whereas 2 out of 3 pig manures contained only 17β-estradiol (14.8 and 65 ppb dry wt), and in the third sample, only 84.5 ppb dry wt estrone was detectable. Estriol was not detectable in the manures. The effluent concentrations of estrone and estradiol have been measured in Israel and the United Kingdom. In Tel Aviv, estrogens were 24–48 ng/l. In the United Kingdom, concentrations in sewage water plant effluents varied from 1–50 ng/l estrone and 2–50 ng/l 17β-estradiol [74,80].

5.2 Phytoestrogens

Phytoestrogens are naturally occurring substances with estrogenic activity found in plants. They are non-nutritional phytochemicals and are some of the at least 12 000 natural chemicals in plant foods. A large diversity of action has been attributed to naturally occurring weakly estrogenic compounds in the large flavinoids family of plant secondary metabolites, as well as to plant lignans. The flavinoids can be divided into several structurally and biosynthetically related classes such as flavones, flavonols, anthocyanins, flavanones, isoflavonoids (isoflavones, coumestans), and chalcones. The chemical structure of the flavinoids gives hints about their function in the environment in which they occur. As typical phenolic compounds, they can act as antioxidants. As conjugated aromatic compounds, they can protect plants against destructive UV-light, and they are attenuators of physiologically active visible light. Isoflavones mimic steroidal and other controllers of growth and development in their potential predators. Lignans are essential plant constituents because they can bind polymers like proteins, including enzymes, polysaccharides, and nucleic acids in their polymeric form [81]. The main known phytoestrogens are the isoflavones daidzein, genistein, formononetin, biochanin A, equol, the coumestan, coumestrol, and the lignans *seco*-isolariciresinol (SECO) and matairesinol (MAT). It should be mentioned that plant lignans can be precursors of mammalian lignans, which are not present in the diet as

such. But, plant lignans like SECO and MAT are abundant in plants and can be modified by the mammalian gut microflora to mammalian lignans exhibiting estrogenic effects. The two major mammalian lignans enterodiol and enterolactone are the products of colonic bacterial metabolism of the plant lignans SECO and MAT [82]. The pathway of isoflavone metabolism includes ring oxidation and hydrogenation, as well as a ring-opening of a chromanone ring. The metabolic conversion route of formononetin and biochanin A in sheep and cattle includes an oxidative demethylation, reduction of a double bond, ketone reduction, and cleavage of a ring to produce 4-ethylphenol and dihydrogenistein [83]. Formononetin is converted via daidzein to *O*-desmethylangolensin, equol, and further metabolites. The isoflavones and lignans seem to undergo an efficient enterohepatic circulation. After absorption in the intestine, they are conjugated with mainly glucuronic acid besides a small amount of sulphate. These conjugates are excreted in urine and bile, the latter allowing entrance into the enterohepatic circulation. These observations confirm the species specificity of the biotransformation of phytoestrogens.

Estrogenic activity of plants was observed for the first time more than 50 years ago in sheep in Western Australia that had been fed with a special strain of clover and therefore developed infertility syndromes. It was later found that this clover contains the isoflavones genistein, biochanin A, and formononetin [84]. In vivo studies with ovariectomized rats and in mice were carried out, which confirmed their estrogenic potential [85]. More recent studies showed that genistein and coumestrol exert the typical estrogen-like actions in female and male experimental animals [86,87]. In vitro binding assays showed that many phytoestrogens bind to estrogen receptors as agonists similarly to steroidal estrogens, but with a lower affinity than the endogenous steroidal estrogens [88]. The relative binding affinity of isoflavones to estrogen receptors is between 10^{-3}–10^{-4} relative to that of 17β-estradiol. Exposure to phytohormones depends directly on the uptake of plants containing these natural hormones. This is especially important for animals in areas with large monocultures of the respective crops. An impressive example is again the infertility of sheep in Western Australia: In Western Australia, a Mediterranean-type climate favors the growth of a special kind of clover, the subterranean clover, which seems to be the optimal annual legume for this area. But, this clover contains the isoflavones genistein, biochanin A, and formononetin. Together, these compounds count for about 5 % of the dry wt of green clover. The sheep in this area are almost exclusively fed with clover, and although the estrogenic potency of the phytoestrogens is much lower than that of 17β-estradiol, the high amount of these compounds in the diet can result in estrogenic stimulation in those sheep. This disturbance can even be higher than is ever achieved by endogenous steroidal estrogen and can lead to syndromes of infertility [84].

The possible health benefits of dietary consumption have been extensively examined. Mazur and Adlercreutz [81] analyzed the phytoestrogen content in many food plants such as legumes, oilseeds, nuts, grains, cereals, vegetables, berries, fruits, and others. The highest amount of the isoflavones daidzein and genistein was found in soybeans with 105–841 ppm dry wt. The highest concentrations of the lignans SECO and MAT were determined in flaxseed with 3690 ppm dry wt SECO and 10.87 ppm dry wt MAT, respectively. Also, some berries and fruits contain measurable amounts of these lignans. Some beverages—especially green tea—contain SECO (5.61–28.90 ppm dry wt) and MAT (0.56–4.13 ppm dry wt), but only low levels of isoflavonoids [80,81]. Dietary phytoestrogens are very interesting for humans because they may offer protection against a wide range of diseases, such as hormone-dependent breast, prostate, bowel, and other cancers; cardiovascular disease; osteoporosis; and menopausal symptoms. But, most of the mechanisms leading to these effects are still unknown, and the possibility that other plant ingredients are responsible for the observed health effects cannot be excluded.

Because environmental exposure to isoflavones has been expected to be negligible, little data are available. Genistein was found in five effluents at mass concentrations between 2.7–38.1 ng/l with an average concentration of 17.4 ng/l [89]. Out of the flavinoids, only genistein, norringenin, and daidzein were detected in German surface waters [90]. Norringenin and daidzein were detected in a limited number of samples and at low concentrations (very low to 17 ng/l). However, genistein was present in higher concentrations (400–1300 ng/l). Effects on fish are observed by far higher concentrations: in a full life

cycle test with zebra fish (*D. rerio*) genistein caused a significant effect on reproduction endpoints at 4.2 μg/l. In addition, a slight retardation of juvenile growth resulting in a slight prolongation of the time until first reproduction was observed [91].

At least two other important classes of natural products exhibit estrogenic activity, zearalenone and related fungal metabolites and plant sterols such as β-sitosterol. Zearalenone is a mycotoxin, produced by a fungus present on moldy corn, wheat, barley, etc. Its importance is based on the large-scale use of one of its derivatives, zeranol, as a growth promoter in the cattle industry in the United States. β-Sitosterol is a plant sterol found in plant oils, legumes, and wood. It is used as a lipid lowerer in medication, and it can be detected in wastewaters from the fat industry and paper mills in high concentrations [92]. β-Sitosterol is capable of inducing vitellogenin in male fish in the μg/l range, and it provokes in vitro an estrogenic response in MCF-7 and T47D cells [93,94]. β-Sitosterol containing paper mill wastewater showed androgenic effects in fish. Female live-bearing Poecilid fish of the genus *Gambusia* developed male reproductive organs. These androgenic effects only appeared after biotransformation of the sterols in wastewater. Because β-sitosterol is used in the biotechnological production of androgens with mycobacteria, it can be assumed that β-sitosterol in paper mill wastewater was converted into androgens under environmental conditions [92]. In animals, β-sitosterol can be directly converted to steroid hormones such as pregnenolone [96].

β-Sitosterol was determined in effluents of STPs in concentrations up to 402 ng/l [92]. In German rivers and tap water, β-sitosterol concentrations were found to be between 20–56 ng/l [76]. In another study, β-sitosterol was found in all analyzed German surface waters, in concentrations between 37–1405 ng/l with a median value of 319 ng/l (n = 110) [78]. Maximum concentration in sewage effluents was 7105 ng/l, and the median concentration of the 51 positive samples was 519 ng/l (n = 53). β-Sitosterol was also detected in sediments and sewage sludge with median concentrations of 2483 and 9.1 ppb dry wt, respectively. High concentrations of this phytoestrogen up to 221 ppm dry wt were found in manure of dairy cattle and fattening of bulls. Owing to its occurrence in plants, β-sitosterol was also detectable in the ppm range in leachates from landfills and composts [79].

5.3 Synthetic steroids: Pharmaceuticals

This group of pharmaceuticals mainly consists of oral contraceptives (ovulation-inhibiting hormones) as well as steroids used for substitution therapy during menopause. Other applications of steroids (e.g., breast cancer therapy) are rare and can be neglected in this report. Natural hormones like estradiol or progesterone are not suitable for oral applications—or only at higher dosage—because they are quickly metabolized (i.e., deactivated) and excreted. Therefore, mainly synthetic steroids are used for oral application. Ethylination or alkylation of the natural compound prevents metabolization and guarantees the desired effect. The most common estrogen-like synthetic steroids are ethinylestradiol and mestranol, whereas the progestagenic component in oral contraceptives can be norgestrel or norethisterone. A further important group of pharmaceutical hormones are steroids used in agriculture during cattle farming. In the mid-20th century, DES, a synthetic steroid, was also used for fattening of cattle as well as in humans to prevent miscarriages, which led to devastating health consequences (see below). In the EU countries, application of growth-promoting hormones is banned for meat production. In other countries, natural hormones like estradiol, testosterone, and progesterone as well as synthetic or modified phytohormones like trenbolone and zeranol (derivative of zearalenone) are regularly applied [97]. The natural hormones show water solubilities between 5.75–13.25 mg/l, whereas the synthetic steroids showed lower water solubilities between 0.16–4.83 mg/l, respectively [72] (Table 4).

Table 4 Common synthetic and natural estrogens used for their pharmacological properties.

Common name	CAS-no.	γ_{sat}/μg l^{-1}	log P_{ow}
Mestranol	72-33-3	320	
Ethinylestradiol	57-63-6	483	3.67*
Trenbolone	10161-33-8		
Zeranol	26538-44-3		
Diethylstilbestrol (DES)	56-53-1		5.07*

γ_{sat}: Water solubility at 25 °C.
P_{ow}: Partition coefficient octanol/water.
*Estimated using KOWWIN, Version 1.65, SRC Syracuse Research Corp.

Laboratory experiments with optimized cultures and added nutrients in activated sludge have shown that it takes several weeks before estrogens are nondetectable. The most stable molecule under these conditions was ethinylestradiol [96]. Early studies by Norpoth et al. [97] showed that for natural steroids a fast degradation was found, whereas ethinylestradiol and mestranol were degraded to a much lesser extent.

Synthetic sex steroids for pharmaceutical use—ovulation inhibitors, estrogen surrogates during menopause—are nearly exact copycats of natural sex steroids. This is valid for the effects caused as well as for their potency. Besides their advantages in therapeutic uses, some unwanted estrogenic side effects can occur. Owing to their high estrogenic potency, unwanted effects like increased growth of the endometrium and a higher risk of breast cancer can accompany the desired effects during menopause. The exposure of developing organisms to exogenous sex steroids is extremely dangerous. This has been proved tragically by the application of the synthetic sex steroid DES. DES was regularly prescribed in the 1960s to pregnant women to prevent miscarriages resulting in a high exposure of the fetus to the synthetic sex steroid. The offspring of these women had to fight with some severe diseases: daughters developed vaginal cancer, sons suffered from malfunctions of the sexual organs such as sperm anomalies, hypospadias, and ectopic testes. It could be proven that these diseases were caused by perinatal exposure to DES during sensitive stages of sexual differentiation of the developing fetus [100,101].

5.3.1 Synthetic estrogens: Environmental exposure

Routes of exposure for synthetic steroids are nearly identical to those of natural sex steroids (cf., Figs. 1 and 2). According to Velagaleti [73], one additional exposure branch, spillage during production and transport can play a role. In the Ruhr district of Germany, 17α-ethinylestradiol had been detected in surface water in concentrations between <1–4 ng/l [78]. In further studies in Germany, in 116 surface water samples, 17α-ethinylestradiol was found in 6 samples and mestranol in 2 samples [79]. The median concentration of the positive samples for 17α-ethinylestradiol was 3.4 ng/l (median of all, 1 ng/l). Mestranol concentrations in two rivers were 2 and 3 ng/l. In 10 out of 53 STP effluents, the synthetic estrogens could be detected. In 8 effluents, median concentration of the positive samples of 17α-ethinylestradiol was 10.3 ng/l with a maximum value of 35 ng/l. Mestranol was found in 2 samples with nearly 10 ng/l and in one with 43 ng/l. In sludges, 17α-ethinylestradiol was not detectable, whereas mestranol was found in three samples in the range of 1.2–4.5 ppb dry wt. Investigations in the United Kingdom revealed concentration of 17α-ethinylestradiol in effluents of STPs up to 7 ng/l, whereas concentrations between 2–15 ng/l could be determined in river water [80]. Additional German studies found concentrations of 17α-ethinylestradiol in the range of 0.3–0.5 ng/l in effluents of STPs and below the detection limit of 0.2 ng/l in surface water [100]. In the Netherlands [77], 17α-ethinylestradiol was only detected on one occasion in two effluent samples. The highest concentration was 7.5 ng/l. In surface water, concentrations were very low, with values between 0.4–4.3 ng/l. Investigation of the behavior and occurrence of estrogens in municipal sewage plants [76] found that in

general, there was a lower removal rate for 17α-ethinylestradiol and estrone than for the 17β-estradiol and 16α-hydroxyestrone. The removal efficiency probably depends on parameters like temperature, microbial activity, or rain events. 17α-Ethinylestradiol was frequently detected in effluents of STPs. However, the concentrations were mainly in the lower ng/l range, which might be an indication of low amounts entering the receiving waters. Only few data were available for concentrations of synthetic hormones applied in cattle and pig breeding, although this seems to be an interesting point owing to the high amounts used and the treatment of the excreta of animals, which differs strongly from that of human excreta. In Germany, 17α-ethinylestradiol and mestranol could not be detected in random samples of manure from bulls, dairy cattle, and pigs; limit of detection was 2 ppb dry wt [79]. Environmentally relevant concentrations of 17α-ethinylestradiol of 1.1 ng/l had been shown to affect fish reproduction owing to a significant reduction (up to 50 %) of the fertilization rate in a multigeneration study with zebra fish (*D. rerio*) [102].

6. PESTICIDES

Pesticides are a huge class of chemicals with several hundreds of active ingredients in use. Although pesticides are one of the most investigated chemical classes, there are several indications that some pesticides—mostly older chemicals—are potential endocrine disruptors. Some of these examples are discussed in more detail for their potential endocrine effects (Table 5).

Table 5 Pesticides suspected of being endocrine disruptors.

Common name	CAS-no.	γ_{sat}/μg l^{-1}	log P_{ow}
p,p′-DDT	50-29-3	3.4	6.20
p,p′-DDD	72-54-8	90	5.86
p,p′-DDE	72-55-9	24	5.76
Methoxychlor	72-43-5	45	4.68–5.08
Kepone, Chlordecane	143-50-0	76	5.41
Linuron	330-55-2	75 000	3.20
Diuron	330-54-1	42 000	2.68
Vinclozolin	50471-44-8	3400	3.10
3,4-Dichloroaniline	95-76-1	600 000	2.69

γ_{sat}: Water solubility at 25 °C.
P_{ow}: Partition coefficient octanol/water.

6.1 DDT and its metabolites

DDT [1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane], a derivative of diphenylethane, was produced for the first time almost 60 years ago. Since then, it has been intensively used as an insecticide especially against insects communicating diseases like malaria or sleeping sickness. In the 1970s, use and production was prohibited in most industrial developed countries. In tropical areas of developing countries, however, it is still needed and used as a cheap alternative to fight against malaria. It has been estimated that until the ban of DDT, approximately 2 million tons have entered the environment. Technical DDT is a mixture of mainly *p,p′*-DDT and *o,p′*-DDT. Besides these main components, some DDD [1,1-dichloro-2,2-di(4-chlorophenyl)ethane] and DDE [1,1-dichloro-2,2-di(4-chlorophenyl)ethylene] isomers can be found. All these compounds are highly lipophilic, nearly insoluble in water and very persistent with half-life periods for microbial degradation of 3 to 20 years. The dehydrochlorination of DDT—the main step during biotic as well as abiotic transformation—leads to the principal DDT metabolite, DDE. DDE is considerably persistent in the environment and shows various residual effects

for a long period of time. DDT and its metabolites accumulate in organisms—especially in fat tissues—as indicated by its high hydrophobicity and high log P_{ow}. Bioconcentration factors in fish are between 1900–330 000 for *p,p′*-DDT, 64 000 for *p,p′*-DDD, and 2700–81 000 for *p,p′*-DDE, respectively. Because DDE is the most persistent metabolite, its amount within the total DDT burden increases with increasing trophic steps [67]. Depending on the solid material, adsorption coefficients vary between 81 200 l/kg (soil) and 1 780 000 l/kg (suspended matter) for DDT, for DDT and metabolites an adsorption coefficient of 21 900 l/kg on sediment was found. It has been known since 1968 that DDT shows estrogenic activity [103]. This property of DDT was later intensively investigated by use of different in vivo test systems such as the influence of DDT on weight and glycogen content of immature uteri and oviducts or the sexual differentiation of rodents [104,105]. Several in vitro tests on the binding affinity to estrogen receptors likewise revealed differences of the estrogenic potential of *o,p′*-DDT compared with DES or estradiol as in vivo experiments [106,107]. In vitro tests to determine binding affinity to estrogen receptors were performed by various groups showing similar results concerning differences of estrogenic potential of *o,p′*-DDT compared with DES or estradiol as in vivo experiments. It was found that *o,p′*-DDT has a binding affinity to the estrogen receptor, which is 5×10^{-3} and 2.5×10^{-3} relative to that of estradiol and DES, respectively [108]. In vivo as well as in vitro studies demonstrated that *o,p′*-DDT is the most active component of technical DDT. Although *p,p′*-DDE and *p,p′*-DDD do not reveal any estrogenic potency, antiestrogenic effects are reported for *p,p′*-DDE.

6.2 Methoxychlor and its metabolites

Methoxychlor [1,1,1-trichloro-2,2-di(4-methoxyphenyl)ethane] is a further diphenylethane derivative that was introduced in 1944 as an insecticide exhibiting similar capabilities as DDT. It shows a lower persistence and toxicity for homoisotherms than DDT and was thus used as a surrogate substance for DDT. In mammalian organisms, as well as in fish, methoxychlor is rapidly metabolized. The main metabolites are the dechlorination product MDDE [1,1-dichloro-2,2-di(4-hydroxyphenyl)ethylene] and its mono- and dihydroxyderivates. Further metabolic products are the mono- and dihydroxylates of methoxychlor itself [109,110]. Technical methoxychlor contains 90 % methoxychlor, the metabolites mentioned above, and more than 50 further components. Methoxychlor and MDDE are so-called proestrogens, which means that primarily metabolic activation to the mono- and dihydroxy metabolites results in endocrine active substances. This observation was first made by Tullner [111]. Further studies supported this by showing that pure methoxychlor has a lower estrogenic activity than the technical product [108]. Dihydroxy-MDDE and dihydroxymethoxychlor increased uterine weight in rats and stimulated uterine ornithine decarboxylase activity. Their potency was much higher than that of purified methoxychlor [112]. However, even highly purified methoxychlor increased uterus weight and stimulated MCF7-cell proliferation in an E-screen test with a potency of 10^{-6} relative to that of estradiol [113]. In wildlife, there are reports of a possible impairment of bird reproduction [5]. Furthermore, male seagulls were feminized after injecting methoxychlor into seagull eggs [113].

6.3 Chlorinated cyclodienes and camphenes

Various chemical reactions on hexachlorocyclopentadiene led to the insecticides aldrin, dieldrin, mirex, kepone, chlordane, heptachlor, endosulfan, and toxaphene. These compounds were investigated in several in vitro test systems like the E-screen, binding affinity to the estrogen receptor, YES (yeast estrogen system), and the results showed a different estrogenic potency for these pesticides [114,115]. With the exception of kepone, these results could not be supported by in vivo tests [113]. For this reason, only kepone will be described in brief. It was used as insecticide against insects in buildings (e.g., ants, cockroaches, silverfishes). Kepone was exclusively produced in the United States until its production stopped in 1976. In 1988, application of kepone was also forbidden, owing to its high persistence in the environment and its very low biotic and abiotic degradation. Kepone was investigated in many in vivo

and in vitro studies. The increase of uterine weight in juvenile rats with a lowest observed effect level (LOEL) of 10 mg/kg, and a no observed effect level (NOEL) of 1 mg/kg is remarkable [116,117]. It could, furthermore, be shown during the same investigations that kepone is about 2×10^{-3} less potent than 17β-estradiol. Other binding experiments of kepone to estrogen receptors conducted in different laboratories resulted in relative binding affinities between 10^{-4}–5×10^{-3} relative to that of 17β-estradiol [118,119]. The pesticides listed above are weak estrogens owing to their binding to the estrogen receptor. However, recently it has been shown that vinclozolin, the metabolite 3,4-dichloroaniline (3,4-DCA) of the pesticides linuron and diuron, as well as the already discussed DDT metabolite, *p,p′*-DDE, can act as antiandrogens via an antagonistic mechanism at the AR.

6.4 Linuron, diuron, and their metabolites

Besides linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea], the pesticide diuron is used and possesses a similar structure as linuron. Linuron and diuron are broadly applied herbicides used to protect, for example, potatoes, corn, vegetables, fruits, ornamental shrubs, and others. Diuron is mainly applied in nonagricultural areas such as sporting grounds and railroads. Water solubility of diuron is moderate with 42 mg/l, 25 °C. It is lipophilic and strongly adsorbs on sediment. In soils, diuron is very persistent, with half-lives between 90 and 180 days; also, in water, it is slowly degraded biologically. The main metabolite of diuron is 2,4-DCA, besides 1-(3,4-dichlorophenyl)-3-methylurea and 1-(3,4,dichlorophenyl)urea. Photolytic degradation was also observed. Linuron has a higher water solubility (75 mg/l, 25 °C) than diuron, with half-life values between 16 and 42 days [120]. Linuron also degrades in soils more quickly than diuron, with half-life values between 16 and 42 days. Linuron is also biologically degraded to 2,4-DCA, 1-(3,4-dichlorophenyl)-3-methylurea, 1-(3,4,dichlorophenyl)urea, and 1-(3,4-dichlorophenyl)-3-methoxyurea. A high bioaccumulation of linuron and its metabolites is not to be expected owing to their low log P_{ow} values of 3.2 and 2.68, respectively. Cook et al. [121] investigated effects of linuron and its metabolites on juvenile and adult rats. A loss of weight of sexual organs of the animals was observed within two weeks after daily doses of 100 or 200 mg/kg of rat. In short-term in vivo studies, linuron treatment reduced testosterone- and DHT-dependent tissue weights in the Hershberger assay (oral 100 mg/kg daily dose for 7 days) and altered the expression of androgen-regulated ventral prostate genes (oral 54 days, 100 mg/kg daily dose) [122]. Linuron and some of its metabolites (e.g., 3,4-DCA) probably act as competitive antagonists at the AR. For diuron there are no experimental data available that would support endocrine disruption properties of this pesticide. However, owing to the structural similarities between linuron and diuron and the common metabolite 3,4-DCA, an intrinsic endocrine potential may be expected. 3,4-DCA is not only the principal degradation product of linuron and diuron, but also an intermediate product during synthesis of herbicides, dyes, and pharmaceuticals. Worldwide production of 3,4-dichloraniline is estimated to be between 42 000–47 000 tons/year. 3,4-DCA has a water solubility of 600 mg/l and is moderately lipophilic (log P_{ow} of 2.7 [113]). Accordingly, 3,4-DCA has a low tendency to bioaccumulate. A bioconcentration factor of 30 had been measured for zebra fish [113]. 3,4-DCA is adsorbed to particles and sediment where covalent bindings to organic substances are formed. It is only slowly biotically degraded, and photodegradation plays the main role [123]. As mentioned above, 3,4-DCA acts as competitive antagonist at the AR in mammals [121]. However, there is also evidence that 3,4-DCA may disturb the endocrine system in fish. Inhibitory effects were observed at 0.2 mg/l on the synthesis and metabolism of androgens in breeding males of sticklebacks [92].

6.5 Vinclozolin and its metabolites

Vinclozolin [3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-oxazolidine-2,4-dione] is used as a fungicide for fruits, wine, vegetables, ornamental shrubs, hops, and rape. Water solubility is low. It is not persistent

and hydrolyzed in soil and water. Main hydrolysis products are 2-[(3,5-dichlorphenyl)-carbamoyl-oxy]-2-methyl-3-butenoic acid (M1) and 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide (M2). Mammalians also produce M1 and/or M2, which are excreted as glucuronides. Vinclozolin alters sexual differentiation in male rats. In male rat offspring, perinatal exposure to vinclozolin causes hypospadias, ectopic testes, vaginal pouch formation, agenesis of the ventral prostate, and nipple retention. Because the female offspring appears phenotypically normal, the effects observed probably result from an antiandrogenic potential of vinclozolin, respective its metabolites [124]. The molecular mechanisms responsible for the antiandrogenic effects of vinclozolin were investigated. The two primary metabolites M1 and M2 compete for binding at the AR and inhibit dihydrotestosterone-induced transcriptional activation by blocking binding of the AR to androgen response element DNA. Vinclozolin itself is only a poor inhibitor, which means that the metabolization is a prerequisite for the observed antiandrogenic effects [125]. Endocrine-disrupting effects of vinclozolin on wild living species are not reported so far [24].

6.6 p,p′-DDE

p,p′-DDE is the principal metabolite of the pesticide DDT, which has been discussed above. Intensive in vivo and in vitro investigations with rats led to the following results: When administered to pregnant rats (100 mg/kg daily dose) from days 14–18 of gestation, *p,p′*-DDE reduces anogenital distance and causes retention of thoracic nipples in male progeny. Juvenile rats fed with 100 mg/kg daily dose *p,p′*-DDE reached puberty delayed, and adult male rats fed with 200 mg/kg daily dose *p,p′*-DDE lost androgen-dependent weight of semen bladder and ventral prostate. It could be shown that *p,p′*-DDE binds to the androgen receptor in vitro and inhibits dihydrotestosterone-induced transcriptional activation with a potency similar to that of the antiandrogenic drug hydroxyflutamide [20]. In studies of binding affinities to the AR of different compounds, it was found that vinclozolin metabolite M2 and *p,p′*-DDE bind to the receptor with a 10^2–10^3-fold lower affinity than the receptor agonist dihydrotestosterone. DDT and M1 have a 10^3–10^4-fold lower affinity, whereas linuron and vinclozolin have a 10^4–10^5-fold lower affinity than dihydrotestosterone [126].

7. PESTICIDES: ENVIRONMENTAL EXPOSURE

Pesticides are intentionally released at their point and time of application. Plants can either absorb these chemicals directly from their leaves or indirectly from the soil through the roots. The plants may then be eaten by herbivores, and the pesticides can be accumulated under worst-case conditions to high levels in meat and animal dairy products. Furthermore, they can drain or run-off into surrounding surface water bodies during and after their application and then move into the aquatic food chain. The degree of biodegradation, transformation, accumulation in organisms, and distribution in the environmental compartments mainly depends on the physicochemical properties of the substances. DDT, DDE, methoxychlor, and kepone are highly lipophilic and nearly insoluble in water. Besides other properties, these characteristics result in their classification as a special set of organic compounds—the persistent organic pollutants (POPs). POPs possess toxic characteristics, are persistent, are liable to bioaccumulate, are prone to long-range atmospheric transport and deposition, and can result in adverse environmental and human health effects at locations near and far from their sources [127,128]. One specific problem of POPs is their global extent of pollution, i.e., they can be detected in areas such as the Arctic where they have never been used or produced, at concentration levels posing risks to both wildlife and humans. Since the manifestation of this problem, typical representatives of POPs have been determined in many areas and environmental compartments. The data presented in this paper only can give a short insight into the data existing worldwide.

7.1 DDT and its metabolites

DDT concentration (as the sum of *p,p′*-DDT, *p,p′*-DDE, and *o,p′*-DDT) were determined in surface sea water with less than 0.1 ng/l, except for the northern 20 to 40 latitudes, where 1–2 ng/l of DDT were detected in the 1970s. In general, the concentration of DDT in air and sea water decreased from 1974 to 1985 [129]. In the rain above Canada, in 1985 and 1986, the major isomer was found to be *p,p′*-DDE, showing an average concentration of 0.09 ng/l (n.d. to 0.13 ng/l). The estimated average deposition in Canada was 0.036 μg/m^2 yearly [130]. Shallow water at 1034 sites in the United States was investigated between 1993 and 1995. A maximum concentration of 6 pg/l of *p,p′*-DDE was detected with a frequency of 3.9 % [131]. In a survey of rivers and estuaries (eight different places) carried out by the Environmental Agency Government of Japan in 1995 and 1997, *p,p′*-DDT, *p,p′*-DDD, and *p,p′*-DDE were not detected at all in water, but sediments contained *p,p′*-DDT (0.154–13 ppb dry wt), *p,p′*-DDD (0.128–18 ppb dry wt), and *p,p′*-DDE (0.161–28 ppb dry wt) [132]. The concentration of DDT ranged from 0.6 to 15.8 ng/l in water at eight sampling stations along the Palos Verdes Peninsula in California, where the sediments had been contaminated with DDT and PCBs. The results suggested that DDT was released from the sediments at a mass flow rate of 418 kg/year across the Palos Verdes Shelf [133]. The depositional trend of organochlorines was determined in a mid-latitude temperate glacier in Alberta, Western Canada (52° N, 117° W). The concentrations of DDT and its isomers reached a peak concentration of 2.57 ng/l and a maximum mass flow rate to this cold high elevation environment in the 1980s, one decade after the ban of their use in North America. Melted snow from glaciers may contribute to a high concentration of DDT in cold aquatic ecosystems [134]. DDA [bis(dichlorophenyl)acetic acid] was detected in the range from lower than the detection limit (5 ng/l) to 760 ng/l in surface water in the Teltowkanal in Berlin, Germany, where the concentrations of DDT, DDE, and DDD were less than the detection limit (5 ng/l), trace, and n.d.-140 ng/l, respectively [135].

7.2 Methoxychlor and its metabolites

Methoxychlor is detected often in air, river water, rain water, and oceans with other insecticides such as DDT [136]. Methoxychlor as well as DDT, DDE, and PCBs were detected at trace levels in air and water (<10 pg/m^3 air; <10 pg/l water), but it was relatively abundant in melted snow (up to 100 pg/l) [137]. In 1986, methoxychlor was one of the significant pesticides detected in rain or snow in the Great Lakes basin in North America at the range of 2–7 ng/l [138].

7.3 Linuron and its metabolites

Linuron, vinclozolin, and their metabolites show increased water solubility, low persistence, and a higher degradation rate compared with the POP pesticides. Their distribution is not worldwide, but more or less limited to the areas of their application. Nevertheless, they are distributed among the environmental compartments.

In regions of intensive agriculture in Canada, linuron concentrations of 1100 μg/l and 2800 μg/l have been detected in surface waters and groundwaters, respectively [139]. In some sediment samples of the German Wadden Sea, linuron concentrations exceeded 500 ppt fresh wt [140]. In contrary, linuron was not detected in an intensively agricultural district of Italy [141].

7.4 Vinclozolin and its metabolites

The fate of vinclozolin, dimethoate, and cyproconazole in plums, from field treatment to the drying process, was studied. Only vinclozolin showed measurable residue concentrations at harvest. In the drying process of prunes, the residues were not reduced during the fruit washing stage, but the drying stage led to complete elimination of vinclozolin residues [142]. Vinclozolin residues were present in the dis-

tilled spirits of wine. During wine distillation, 5 % of the initial residues of vinclozolin were transferred to the distilled spirit. Low percentages (0.1 % for vinclozolin as active ingredients) also passed from the lees to the final distilled spirit, when samples were fortified at 26.1 ppm for vinclozolin [143]. Surface concentration of vinclozolin (applied as the formulated product Ronilan FL) was 0.87 μg/cm^2 on leaves one day after spraying in the greenhouses. The concentration was higher on floors than on leaves. Air concentrations of vinclozolin after three days of application were below the detection limit (0.4 μg/m^3) [144].

8. INDUSTRIAL CHEMICALS

8.1 Biphenyls

Biphenyls are precursors for the synthesis of hydrocarbons with hydroxy- or chlorine groups used for different industrial purposes. The dihydroxybiphenyls 2,2′-dihydroxybiphenyl and 4,4′-dihydroxybiphenyl are used as educts for plasticizers, pesticides, and disinfectants. Mono- and dihydroxybiphenyls are also generated during the degradation of biphenyl. Polychlorinated biphenyls (PCBs) were produced since 1929 for many purposes. Because PCBs are synthesized via chlorination of biphenyl, mixtures of different congeners are always obtained, which are characterized by the mass percentage of chlorine. Two hundred and nine congeners are possible, ranging from the monochlorinated isomers to the fully chlorinated decachlorobiphenyl isomer. (Some isomers—the so-called coplanar and monoorthocoplanar PCBs—are not considered here because they have a dioxin-like structure and show a different behavior.) It is estimated that since 1929, approximately 1.5 million tons of PCBs were produced. The hydrophobic PCBs are classified as POPs and are extremely persistent owing to their chemical and physical properties, which depend strongly on the chlorination degree: With increasing chlorination degree, the sorption tendency and hydrophobicity increase (log P_{ow} 4.5–log P_{ow} 10) while water solubility γ_{sat} (6000 μg/l^{-1}–0.1 μg/l^{-1}) and biodegradability decrease. The persistence of PCBs and their toxic potential resulted in an almost international production stop in the 1970s and 1980s. However, these properties, such as chemical and thermal stability, noninflammability, high boiling points, high viscosity, and low vapor pressure are the reason for their enormous distribution, for example, as cooling and hydraulic liquids in closed systems. They were also used as dielectric fluids in transformers and large capacitors, as pesticide extenders, plasticizers in sealants, heat exchange fluids, hydraulic lubricants, cutting oils, flame retardants, dedusting agents, and in plastics, paints, adhesives, and carbonless copy paper. Even after the ban of PCB production in most countries, the current world inventory of PCBs is estimated at 1.2 million tons with about one-third of this quantity circulating in the environment [145]. In mammalian organisms, PCBs are mainly hydroxylated and excreted in the urine as conjugates. As a general rule: the higher the degree of chlorination, the lower is the metabolism rate. Due to their high persistence, PCBs accumulate within the food chain with an increasing part of higher chlorinated compounds within the trophic steps [113]. Among the nonchlorinated, hydroxylated PCBs, 2,2′-dihydroxybiphenyl and 4,4′-dihydroxybiphenyl were found to be uterotrophic (i.e., the uterus glycogen content of the immature rat was increased) [103,104]. For chlorinated biphenyl mixtures of PCB, congeners exhibiting a chlorine amount of <48 % have similar effects on the uterus [146]. Only a few investigations on the estrogenic potency of PCBs were carried out. It can be assumed that some congeners, as well as technical mixtures, have an LOEL of 160 mg/kg rat (uterotrophic effect) and they seem to be 4–5 orders less potent than estradiol [106]. It is remarkable that synergistic effects could be observed in the development of turtles. Two of the biphenyl compounds led to an increased percentage of newborn females, especially if they were applied as a mixture [147]. In vitro tests using the E-screen [148] found results comparable to the in vivo tests. It is assumed that PCBs act via the estrogen receptor, and some binding studies demonstrated that among 10 substances tested, the chlorinated compounds had an affinity to the receptor, whereas the nonchlorinated PCBs exhibit only a very weak binding affinity [149].

8.1.1 Environmental exposure to polychlorinated biphenyls

Large amounts of PCBs were released due to inappropriate disposal, accidents, and leaks from industrial facilities. Leakage from old equipment, building materials, stockpiles, and landfill constitutes a continued threat of PCB emission. Some countries with economies in transition still produce and emit PCBs. As mentioned above, PCBs are typical representatives of POPs. This means that their widespread applications in combination with their extreme persistence and mobility results in a worldwide environmental distribution. PCBs have been identified in nearly every environmental compartment or matrix. Because exposure routes and behavior of PCBs are well documented and many reviews have been published [150,151], they will not be described in detail here.

PCBs were detected in three out of five sediment samples from different locations in Japan with dry sample fractions in the range from less than 1.1 ppb to 3.7 ppb [152]. In the Baltic Sea region, the concentration of total PCB congeners in air increased with the temperature. Low-volatility congeners were more temperature-dependent than the high-volatility PCB congeners [153]. The concentration of total PCBs ranged from 0.06–1.14 ng/l in water at eight sampling stations along with the Palos Verdes Peninsula, California, where the sediments had been contaminated with DDTs and PCBs. The results suggested that PCBs were released from the sediments at a mass flow rate of 49 kg/year across the Palos Verdes Shelf [131]. During an investigation in 1994 and 1995, it was found that the gaseous concentration of total PCB congeners at a site above the lake 15 km from Chicago ranged from 132 to 1120 pg/m^3. PCB concentration was higher in warm periods and during winds coming from Chicago. PCB concentrations in surface waters ranged from 48–302 pg/l, whereas amounts in winter were found to be up to 2.5 times higher than the concentrations in summer. Instantaneous daily net air–water exchange mass flow rates ranged from –32 (absorption) to +59 ng/m. The absorptive mass flow rate was highest in summer. It was estimated that the mass flow rate of total PCB congeners in the southern quarter of Lake Michigan was –18 mg m^{-2} (net absorption) in 1994, corresponding to a yearly net input of 140 kg [154]. Sediments in the harbor of New Bedford in the United States have been contaminated during the production of capacitors. House dust in the houses near the sediment site contained PCBs in the range from 260–23 000 ppb. Yard soil contained 23–1800 ppb. The concentration of PCBs in yard soil increased with the decrease in distance between the sediments and the houses. On the other hand, there was no significant change in the concentration in house dust, suggesting the other sources of PCBs in house dust [155].

8.2 Alkylphenol ethoxylates

Alkylphenol ethoxylates (APEs) are used during the production of phenol resins, as plastic additives, emulsifiers, wetting agents, dispersing agents in household products, in agricultural and industrial applications, and as spermicides in contraceptive applications. The principal use is as nonionic surfactants that comprise 6 % of the total surfactant production and 25 % of the total nonionic surfactant production in the United States. [9]. The annual worldwide production of APEs is about 390000 tons. The typical structure of the APEs—an alkylphenol hydrophobe and the *p*-substituted long chain of repeating ethylene oxide units as the hydrophilic moiety—is responsible for the surfactant activity. The ethoxylate chain can have 1–100 repeating (2-yloethoxyl) units. The longer the chain, the more water-soluble (hydrophilic) is the compound. The alkyl group is a branched nonyl, octyl, or dodecyl chain, whereas the nonylphenol ethoxylates (NPEs) are the most common of the APEs, constituting approx. 82 % of the world production.

In wastewater treatment plants and in the environment, APEs can be microbially degraded, and toxic metabolites such as *p*-nonylphenol and *p*-nonylphenol ethoxylates are formed. These transformation products—not the parent compounds themselves—exhibit estrogenic activity. The degradation products have a higher hydrophobicity than the parent compounds, resulting in log P_{ow} values between 3.9 and 5.99 (Table 6). Owing to these high octanol/water partition coefficients, the metabolites show a tendency toward bioaccumulation in organisms. Algae, fish, ducks, mussels, and crabs all bioaccumu-

late nonylphenol and derivatives from fresh water environments [156,157]. Bioconcentration factors between 1.4 and 230 were found. The aquatic toxicity of APEs increases with decreasing number of ethoxylate units and increasing hydrophobic chain length, meaning that the toxicity of the original compounds is lower than that of the metabolites. For example, the LC50 (48 h) of nonylphenol 16-ethoxylate (NP16EO) is 110 mg/l for fish (*O. latipes*) and decreases to 11.2 mg/l and 1.4 mg/l for nonylphenol 9-ethoxylate (NP9EO) and nonylphenol (NP), respectively [158]. The no observed effect concentration (NOEC) of NP for reproduction for *Daphnia* is in the range of 24 μg/l [159]. Therefore, the acute aquatic toxicity of NP is considerably high. Some metabolism studies were recently carried out for a better understanding of the metabolic pathways of *p*-nonylphenol. No ring hydroxylation was observed. In vitro experiments using trout liver microsomes suggested an oxidative metabolism because the reaction requires NADPH as an essential cofactor and is inhibited by piperonyl butoxide [160]. Industrial *p*-nonylphenol is produced by alkylation using propene trimer. Thus, the product is a mixture of isomeric *p*-nonylphenols [161,162]. The technical product is composed of 22 isomers of *p*-nonylphenols, all of which have a branched portion at the α-position from the aromatic ring, and the most abundant types of the compound are α,α-dimethyl-heptyl-derivative isomers (= 10 isomers are included, group 1) (48.6 %). Some others: group 2: α-methyl-α-ethyl-β-primary type; group 3: α-methyl-β-methyl type; group 4: α-methyl type; group 5: α-methyl-α-propyl type, etc. are also present. Endocrine-disruptive activities of these metabolites are not reported. However, such activity studies of the metabolites are seen as necessary and appropriate because these metabolites have a more structural similarity to an estrogen than the substrate [163].

Table 6 Physicochemical properties of alkylphenol ethoxylates.

Common name	CAS-no.	γ_{sat}/μg l^{-1}	log P_{ow}
4-Nonylphenol, branched	84852-15-3	5430	4.48
4-*n*-Nonylphenol (unbranched)	104-40-5		5.99**
Nonylphenol -ethoxylate (NP1EO)	27986-36-3	3020	4.17
Nonylphenol -diethoxylate (NP2EO)	9016-45-9	3380	4.21
Octylphenol, mixture of isomers	27193-28-8	12600	3.9*
4-*tert*-Octylphenol	104-66-9	12600	4.12**
4-*n*-Octylphenol (unbranched)	1806-26-4		5.50**
Octylphenol-monodiethoxylate		8000	4.1*
Octylphenol-diethoxylate		13200	4.0*

γ_{sat}: Water solubility at 25.0 °C [164].
K_{ow}: Partition coefficient octanol/water [164].
*Mathematical estimation [156].
**Mathematical estimation KOWWIN, Version 1.65, SRC Syracuse Research Corp.

In 1991, Soto et al. reported that a contaminant released from polystyrene—identified as para-nonylphenol (NP, the same as 4-nonylphenol)—was capable of promoting the proliferation of estrogen-dependent breast cancer cells (MCF-7; E-screen) [165]. The authors concluded that NP has an estrogen-like behavior, because it was able to mimic the effects of estradiol in the MCF-7 cell line, i.e., induction of the PR and of cellular proliferation. In vivo experiments with rats supported the suspicion that nonylphenol could be estrogenic: Ovariectomized adult rats (120–150 g body wt) were treated with *p*-nonylphenol. Doses of 20 and 50 mg led to a significant increase of the mitotic index of the endometrium [166,167]. Using E-screen, *p*-octylphenol exhibits the highest activity with a relative potency compared to 17-β-estradiol of 10^{-4} followed by *p*-nonylphenol, butyl- and pentylphenols with relative potencies of 10^{-5} and 10^{-6}, respectively [168]. Also noteworthy are studies which were carried out with fish: Using caged male rainbow trout, it was found that exposure to 4-octylphenol (4-*tert*-OP), 4-nonylphenol, 4-nonylphenol acetic acid, and 4-nonylphenoldiethoxylate induced the production of vitellogenin, a protein usually only found in sexually mature females. The production of this substance

is regulated by endogenous estradiol. Male fish downstream from STPs produce vitellogenin [169], and a high increase of vitellogenin synthesis in fish was found after one to three weeks exposure in drain channels of STPs [170]. Further experiments on vitellogenin synthesis showed that OP had an NOEC and a lowest observed effect concentration (LOEC) of 1.6 and 4.8 μg/l, and the values for nonylphenol were 5 and 20 μg/l, respectively. Concerning fish reproduction, 4-*tert*-OP was investigated in a full life cycle test with zebra fish (*D. rerio*). 4-*tert*-OP caused significant reduction in juvenile growth and prolongation of the time until first reproduction at the highest test concentration of 38 μg/l. At this concentration, egg-laying capacity of the females and fertility of the males were significantly affected. Retardation and reduction of egg production is assumed to be a result of missing mating behavior of the males, normally giving the stimulus for the deposition of eggs [102]. Several in vitro studies were performed to investigate the affinities of alkylphenols to estrogen receptors. It could be shown that 4-nonylphenol, for example, has a relative binding affinity of 2.1×10^{-4} compared to estradiol [168]. The following conclusions can be drawn based on the most important studies: (1) Alkylphenols only have estrogenic activity if the alkyl chain is in the para position. (2) Alkylphenols with alkyl chains having less than four C-atoms are inactive. (3) Alkylphenolethoxylates must be degraded to alkylphenols to gain endocrine activity. For invertebrates, owing to the limited knowledge of their endocrine systems, effects of 4-*tert*-OP on integrative parameters of invertebrate development (hatching, molting, etc.) and reproduction (behavior, egg number, etc.) were investigated [102]. Certain endpoints were found to be affected at rather high, although sublethal, concentrations. A hormone-mediated mechanism remains to be established.

8.2.1 Environmental exposure to alkylphenol ethoxylates

Owing to the widespread use of APEs as detergents and their water dispersal properties, discharge to the environment—to the compartment water—occurs mainly from industrial effluents, STPs, and septic tanks [169–171]. Depending on legal regulations, usage, or voluntary bans, and agreements with industry in Europe, the main sources may be different in different countries. In the environment, as well as in STPs, the APEs are microbially degraded. The first rapid step consists of the hydrolytic removal of ethoxylate groups, resulting in intermediates like alkylphenol (AP), alkylphenol monoethoxylate (AP1EO), and alkylphenol diethoxylate (AP2EO). These metabolites have no surfactant ability and are more lipophilic than the native compounds (cf., Table 6). Therefore, the ultimate biodegradation of the interim products to CO_2 and H_2O is much slower and does not always take place. A part of the metabolites can also be microbially carboxylated to yield alkylphenoxy acetic acid and alkylphenoxyethoxy acetic acid. The slow ultimate biodegradation and the fact that the intermediates are more lipophilic than their parent compounds result in their deposition in environmental sinks, namely, sediment and sewage sludge [9,113,172]. Approximately 50 % of the APEs occurring in the wastewater are estimated to reach the sludge as NP [173]. Under aerobic conditions, oxidation of NP1EO and NP2EO to NP1EC and NP2EC appears to be favored over formation of nonylphenol. However, under anaerobic conditions, much larger amounts of NP appear to be formed from NP1EO and NP2EO. From the available data, reasonable worst-case assumptions from the fate of NPEs during anaerobic wastewater treatment were undertaken in the Draft of the European Union Risk Assessment Report on Nonylphenol [174]: mineralized/highly degradable 45 %, released as NP1EO/NP2EO/NpnEC in effluent 25 %; released as NPnEO ($n > 3$) 8 %, released as NP in effluent 2.5 %, NP in anaerobically digested sludge 19.5 % (based on % weight). The NPEs released to the environment will undergo further degradation.

In wastewater, the concentrations of nonylphenol ethoxylates (NPnEO) and their metabolites, NP, and nonylphenoxy carboxylic acids (NPnEO), as measured in a Canadian STP, were 526 nmol/l in raw sewage and 248 nmol/l in the final effluent. In the raw sewage, 85 % of the total alkylphenols are ethoxylates, while in the final effluent, the major component (nearly 80 %) was in the form of carboxylic acids [175]. In sludge samples collected from nine STPs across Canada, 28–304 ppm of nonylphenol mono-ethoxylate and 4–118 ppm of di-ethoxylate were detected. The concentrations of poly-ethoxylates (from 3–17 ethoxy units) were much lower. Nonylphenoxyacetic and nonylphenoxy

ethoxyacetic acids were detected in only three of the seven samples, with concentrations ranging from 4–38 ppm [176]. In a report from Greece, the mean values of NPE in influents and effluents were 1406 and 62 μg/l, respectively. The removal of NPE ranged from 92–97 % [177]. In rivers of England and Wales, the concentration of total NP was found to be in the range of 0.2–12 μg/l, with an exception of 180 μg/l in one river. OP concentrations were under the limit of detection (1 μg/l). Estuarine concentrations were considerably lower, and the highest was 5.2 μg/l of NP and 13 μg/l of OP [178]. In the Great Lakes, USA, the highest maximum concentrations of NP was 0.92 μg/l and of OP 0.013 μg/l. NP1EO and NP2EO could also be detected up to 7.8 and 10 μg/l, respectively. The St. Lawrence River had lower NPEO concentrations, up to 0.023 μg/l, NP was not detectable [179]. In the sediments of the lower Great Lakes, concentrations of *p*-nonylphenol were in the range of 37 μg/g in sediments and 300 μg/g in the sewage sludge. Concentrations of 4-*tert*-OP were up to 23 μg/g in sediment and up to 21 μg/g in the sewage sludge [180]. In a survey of 109 river waters across Japan in 1998, 4-*n*-octylphenol was not detected with one exception (detection limit: 0.1 μg/l). 4-*tert*-OP was detected in the range from lower-than-detection limit to 0.7 μg/l in 3 out of 109 rivers. NP was detected in 47 out of 109 rivers, and the highest concentration reached 3.0 μg/l. In dry sediments of 20 locations, 4-*tert*-OP was detected in the range from less than 1 ppb to 21 ppb at 5 locations. NP was detected in the range from less than 3 ppb dry wt to 880 ppb at 18 locations. 4-*n*-Octylphenol was not detected at all. In influent water of 10 STPs across Japan, 4-*n*-octylphenol was detected in two plants. The concentration was less than 0.5 μg/l. In the effluent water, no 4-*n*-octylphenol was detected (detection limit: 0.1 μg/l). 4-*tert*-OP was detected in the influent water of five plants in the range from less than 0.1 μg/l to 3.3 μg/l. In the effluent, no 4-*tert*-OP was detected, although trace levels (about the same as detection limit: 0.1 μg/l) were observed. NP was detected in all 10 locations in the range from 1.7–75 μg/l. By the STP, 94 % of NP was removed, and the concentration in the effluent was in the range from the detection limit to 0.9 μg/l [152]. A survey of 65 surface waters in Berlin, Germany [181], resulted in NP concentrations up to 2.7 μg/l in 31 samples with median concentration of all samples less than 0.08 μg/l. OP concentrations of the 9 positive samples were up to 0.27 μg/l, and maximum concentrations of NP1EO and NP2EO were 3.3 (n = 26) and 0.8 μg/l (n = 3), respectively. NP was detectable in the 12 STP effluents investigated; the maximum value was 2.1 μg/l, and the median concentration was 0.5 μg/l. NP1EO was found in 9 samples and NP2EO in 6 effluents with median concentrations of 0.47 and 0.08 μg/l, respectively, maximal values were found to be 2.2 and 1.4 μg/l. Furthermore, 23 sediments were analyzed, and OP could not be detected (<0.01 ppb dry wt). NP was found in 22 samples up to 12.7 ppm dry wt (median, 2.51 ppm dry wt). In these samples, NP1EO and NP2EO could also be analyzed with median concentrations of 0.77 and 0.19 ppm dry wt, maximal values were 1.9 and 0.48 ppm dry wt, respectively. After NP entered the sediments, the compound was little degraded. The half-life was estimated greater than 60 years. The length of chain of ethoxylates did not affect the half-life. It was estimated that in the entire Strait of Georgia (British Columbia, Canada), sediments contain over 170 t of NPEs [182]. NP and NP1EO were found in discharges from a major sewage treatment works in northeast United Kingdom (3 μg/l NP, 45 μg/l NP1EO). The sediments in highly industrialized Tees contained the highest levels of alkylphenols (1600–9050 ppb dry wt NP, 125–3970 ppb dry wt NP1EO, 30–340 ppb dry wt OP) and also in industrialized/urbanized Tyne estuary lower levels (30–80 ppb dry wt NP, 160–1400 ppb dry wt NP1EO, 2–20 ppb dry wt OP) [183]. Concentration of nonylphenol polyethoxylates (NPs) in the coastal atmosphere of the New York–New Jersey Bight ranged from 2.2 to 70 ng/m^3. The source was water-to-air volatilization of NPs to the estuarine atmosphere [184].

8.3 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are generated during all incomplete combustion processes of organic matter (e.g., coal, oil, petrol, wood). Emissions from anthropogenic sources like traffic, heating, as well as aluminum production or steel industry predominate, but some PAHs in the environment arise from natural combustion such as forest fires and volcanoes. PAHs are very lipophilic substances.

As demonstrated in Table 7, their water solubility and thus hydrophilicity decreases with increasing number of aromatic rings. That means that in water bodies most PAHs—especially the larger ones like benzo[*a*]pyrene and benzo[*g,h,i*]perylene—are found bound to particles in sediment and suspended matter. Only the smaller PAHs like naphthalene, phenanthrene, or fluoranthene can be found in higher amounts dissolved in water.

Table 7 Physicochemical data of selected PAHs.

Common name	CAS-no.	γ_{sat}/μg l^{-1}	log P_{ow}
Fluoranthene	206-44-0	220	5.13
Benzo[*b*]fluoranthene	205-99-2	1.2	5.78
Benzo[*k*]fluoranthene	207-08-9	0.55	
Benzo[*a*]pyrene	50-32-8	3.3	6.13
Benzo[*g,h,i*]perylene	191-24-2	0.26	7.23
Indeno[1,2,3-*cd)*]pyrene	193-39-5	62	

γ_{sat}: Water solubility at 25 °C.
P_{ow}: Partition coefficient octanol/water.

PAHs can be degraded biotically and abiotically. They are mainly metabolized in mammalian organisms, as well as in water and soil by microorganisms via oxidative processes. Main metabolites are epoxides, phenols, dihydrodiols, quinones, and catecholes. Degradation depends on several factors like temperature, soil characteristics, and molecular weight of PAHs. As the size of the PAH increases, the degradation rate decreases. Owing to their high hydrophobicity, PAHs should accumulate in the organism, but as a result of metabolization and excretion an accumulation in the food chain is not observed. Already very early—in the 1930s—Cook et al. found that derivatives of PAHs were found to be estrogenic in the Allen-Doisy test [185]. The binding affinity of 3,9-dihydroxybenz(a)anthracene—a metabolite of benz[*a*]anthracene—to estrogen receptors in rats is several orders less than for estradiol (in vitro: 1.12×10^{-2}; in vivo: 4×10^{-5} relative to that of estradiol) [186]. It should be mentioned that all experiments we were aware of showed positive results for derivatives of the PAHs, but not for the native parent compounds. The PAHs investigated have mainly estrogenic properties. Only some compounds like methylcholanthrene, benzo[*a*]pyrene, 1,2-benz[*a*]anthracene, and 7,12-dimethyl-1,2-benzo[*a*]anthracene showed antiestrogenic effects in in vitro tests [187].

8.3.1 Environmental exposure to PAHs

Because of their physicochemical properties and their behavior in the environment, PAHs are further representatives of POPs. Emitted PAHs are particle-bound transported, and they are distributed into all environmental compartments (including surface and even groundwater) via dry or wet deposition. PAHs are relatively reactive in the environment, but they can persist long enough to become transported over very long distances. Intake, behavior, and exposure data for PAHs have been evaluated for many years and are well documented [188,189]. Concentrations of PAHs in the Seine River and its estuary in October 1993 (1–14 μg/l) were correlated to the suspended PAH concentrations in general, which ranged from 2–687 ng/l. Concentrations of dissolved PAHs were 4–36 ng/l [190]. In a survey of rivers and estuaries (eight sites), by the Environmental Agency Government of Japan in 1995 and 1997, benzo[*a*]pyrene was detected in the range from 8.8 to 1700 ppb dry wt in sediments, but it was not found in water [191]. In contaminated soils used by manufactured gas plants, 2061–14 650 ppm dry wt of PAHs were present [192]. Air/water exchange fluxes of 13 PAHs were determined based on a survey in 1996 and 1997. In the north Chesapeake Bay, all the fluxes of individual PAHs (fluorene, phenanthrene, fluoranthene, anthracene, pyrene, and chrysene) had net absorption. Phenanthrene was always net absorbed in rivers and the bay [193].

8.4 Bisphenol

Bisphenol A (CAS-no. 80-05-7; γ_{sat} 120 mg/l; log P_{ow} 3.4) belongs to the bis(hydroxyphenyl)methanes, for which several endocrine effects are reported. Bisphenol A is one of the most important chemicals worldwide. In Germany, for example, about 210 000 tons were produced in 1995 [67,194], while worldwide the production was 640 000 tons in 1993. It is synthesized for diverse applications: Bisphenol A is, for instance, an intermediate in the manufacture of polymers; epoxy resins; polycarbonates; fungicides; antioxidants; dyes; phenoxy, polysulfone, and certain polyester resins; flame retardants; and rubber chemicals [195]. Other uses of bisphenol A are as a resin in plastic dental fillings (polycarbonate plastics—consisting of bisphenol A monomers—are used to coat teeth, especially children's teeth), in the packing industry, as well as in the inside of food cans. At this last use, the bisphenol may migrate to food [196]. Bisphenol A is widely used as a component in the manufacture of phenoxy resins and corrosion-resistant unsaturated polyester-styrene resins. Furthermore, bisphenol A serves as a stabilizer for plasticizers in PVC, a thermal stabilizer for PVC resins, an antioxidant in rubber and plastics, a fungicide, and raw material in the production of tetrabromobisphenol A and other compounds used in the manufacture of flame retardants [197].

Upon discharge to the environment, bisphenol A is distributed between air, water, soils, sediments, and biota compartments [197]. Based on the moderately high water solubility, the very low vapor pressure (5.32×10^{-6} Pa at 25 °C), and the low Henry's Law constant (10^{-5}–10^{-6} Pa m^3/mol) [99], it is concluded that bisphenol A may have a tendency to partition into water and that the rate of evaporation from soil and water will be low [197] On the basis of the log K_{oc}, of 2.5–3.2 and the high log P_{ow} value of bisphenol A, it can be assumed that the chemical is adsorbed to organic materials [197]. Therefore, transportation of bisphenol A in the aquatic environment is considered to be the predominant pathway for distributing the compound between environmental compartments. In the receiving water, bisphenol A is expected to partition into particulate matter, sediments, and biota. It is expected that bisphenol A will have a low-to-moderate mobility in terrestrial soils. The half-lives in sediment, surface water, and groundwater indicate that bisphenol A is persistent. The biodegradation half-lives in soil are 1–180 days, in air 0.74–7.4 days, in surface water 1–150 days, and in groundwater 2–360 days. The measured BCF in fish is between 5 and 68 and indicates low bioaccumulation, whereas the calculated BCFs from 42–196 indicate medium bioaccumulation [113]. The abiotic degradation of bisphenol A in water is assumed to be negligible, because the molecule contains no hydrolyzable functional groups. Bisphenol A is, for the most part, biologically degraded, with a half-life of <4 days. The primary metabolites of the microbial degradation have been identified as 4-hydroxybenzoic acid and 4-hydroxyacetophenone, which, for the most part, are further mineralized. Minor amounts of bisphenol A are hydroxylated to 2,2-bis(4-hydroxyphenyl)-1,2-propanediol [113]. No information has been found concerning the metabolization of bisphenol A in aquatic and terrestrial invertebrates or vertebrates. In mammals (rats), bisphenol A metabolism occurs through a partial conversion into phenols, which are excreted via urine in a free and bound form. Furthermore, bisphenol A is excreted unaltered and in the form of glucuronides in the urine and feces [197].

As early as 1936, Dodds and Lawson [198] described the estrogenic activity of bisphenol A. Recently, in vitro studies were performed to investigate thoroughly estrogenic activity of bisphenol A. It can stimulate proliferation and synthesis of PRs of MCF-7 cells, displace estradiol from estrogen receptors, induce synthesis of vitellogenin in cultivated trout liver cells and induce transcription of recombinant yeast cells [199–201]. The estrogenic potency is in vitro 10^{-3} to 10^{-4} relative to that of estradiol, and 4×10^{-4} to 10^{-5} in vivo relative to that of DES. Recent reports have indicated that bisphenol in low concentrations in rats can affect secondary sexual organs (daily dose: 2 μg/kg) and reduce sperm count in rats (daily dose: 20 μg/kg) raising concerns for "low-dose effects" of this compound [202,203]. However, extensive large-scale, multicenter studies attempting to reproduce these results have indicated that low-dose effects of bisphenol are not a significant health risk at this time [204]. The effect of bisphenol A on reproduction of fish was investigated in a multigenerational study, in which fathead

minnows (*Pimephales promelas*) were exposed to 1–1280 μg/l. Overall, effects based upon the survival, and reproductive fitness of fathead minnows exposed from F0 breeding adults to F2 offspring occurred at concentrations of 640 μg/l and higher, with hatchability of F2 eggs reduced at 160 μg/l. An LOEC of 160 μg/l and an NOEC of 16 μg/l was reported for vitellogenin induction [Sohini et al., 2000, unpublished observations]. In a full life cycle test with zebra fish (*D. rerio*) exposed to 0.9–1500 μg/L bisphenol A, a decrease of fertilization capacity could be observed with an EC10 value of 390 μg/l and an EC50 value of 1450 μg/l. The LOEC for vitellogenin induction in male fish was found to be 375 μg/l [102]. In studies with adult rainbow trout (*Oncorhynchus mykiss*), the LOEC for induction of vitellogenin was estimated to be 23 μg/l after 96 h exposure [200] and 40 μg/l during 12 days of exposure [205]. Concerning amphibians, the results obtained with the African clawed frog (*Xenopus laevis*) are controversial. Kloas et al. [206] reported an increase of female frogs after exposure toward 23 μg/l. Effects of bisphenol A on integrative parameters of invertebrate development (hatching, molting, etc.) and reproduction (behavior, egg number, etc.) were investigated [102]. Certain endpoints were found to be affected at rather high, although sublethal, concentrations. A hormone-mediated mechanism remains to be established. Further studies are needed and are currently being performed to investigate the effects of bisphenol A on amphibians and invertebrates.

8.4.1 Environmental exposure to bisphenol A

Based on the high worldwide production volume of bisphenol A, and the fact that it is used at many sites and in many types of products, it is likely that bisphenol A enters the environment in substantial quantities. Both diffuse sources (products in use, rest and waste products) and point sources (accidental spills, industrial wastewater discharges) may contribute to the emission of bisphenol A to the environment. Emissions of bisphenol A during production of the pure chemical are considered to be minimal because the production occurs in a closed system. However, inadvertent accidents may occur during manufacturing, processing, handling, and distribution of the chemical [197]. Important point sources for the emission of bisphenol A to the surrounding environment may be the large volumes of wastewaters from industries manufacturing epoxy-, polycarbonate-, and polysulphone hardeners and from industries involved in rubber. But actually no estimates of emissions of bisphenol A from point sources were found in the literature [197]. Emission of bisphenol A from products in use has been reported, determined by the migration of bisphenol A into water from epoxy coatings during a 7-day period to be 4 μg/l at 37 °C. Bisphenol A and other components from epoxy resins used as corrosion-resistant coatings in ship water tanks migrated into the water if the epoxy had not been properly hardened. Based on the concentration of bisphenol A in percolates from Danish waste dump sites (30 μg/l) and a yearly percolate estimate of approximately 1 million m^3, the emission of bisphenol A from waste disposal sites in Denmark is estimated to be 30 kg per year [197].

Bisphenol A-diglycidyl-ether [2,2-bis(4-hydroxyphenyl)propane bis(2,3-epoxypropyl) ether, BADGE] is used as lacquers of polycarbonate for coating the inside of cans. The migration of BADGE from can coatings into foods can be observed [207]. Bisphenol A is also used in dental sealants and composites and leaches from the treated teeth into saliva up to 950 μg of bisphenol A during the first hour after polymerization [208].

The half-life of bisphenol A in acclimated wastewater treatment plants and receiving waters was 2.5 to 4 days [209]. BADGE was detected at levels exceeding 1 ppm in 7 of 15 canned anchovy samples and 5 of 22 sardine samples purchased during the period September 1995–July 1996 in the United Kingdom [210], and it was determined in oil of canned fish from 11 out of 16 samples (detection limit: 0.02 ppm) [211]. Twenty-four brands of plastic baby feeding bottles, made of polycarbonate, did not show detectable migration of bisphenol A (detection limit: 0.03 ppm mg/kg) [211]. There are many other reports on the presence of BADGE or bisphenol A in food (especially cans and baby bottles). According to the reports, about 1 ppm of bisphenol A has been found in cans coated with polycarbonate. Migration of bisphenol A into water phase from polycarbonate materials has been reported in the range of nondetectable to 5 ppb [212]. In the region of Cape Cod, MA, USA, bisphenol A was detected

with phenylphenols in septage and wastewater at about 1 μg/l. In some drinking water wells, bisphenol A was detected with other phenol derivatives such as nonyl/octylphenol tetraethoxycarboxylate at concentrations ranging from below the quantitation limit to 32.9 μg/l [213]. According to the survey of Japanese government in 1997, bisphenol A was detected in the range from 0.010–0.268 μg/l (detection limit: 0.008 μg/l) in 18 water samples out of 50 locations of river and estuaries. Of 55 locations of sediments, bisphenol A was detected in 33 locations in the range from 5.9–600 ppb dry wt (detection limit: 0.9 ppb dry wt). Air (detection limit: 1.7 ng/m^3) was sampled at 6 locations, but did not contain bisphenol A [214]. In the survey of water in 109 locations of rivers across Japan in 1998, 57 % of river water contained bisphenol A. The maximum concentration detected was 1.4 μg/l. In 20 sediments from different locations in Japan, 19 sediments contained bisphenol A with the concentration from the detection limit 0.2–11 ppb dry wt. In 10 STPs, the influent water contained bisphenol A in the range from 0.34–2.0 μg/l. 95 % of bisphenol A in the influent water was removed by the treatment plant and the concentration in the effluent water was 0.01–0.51 μg/l [152]. Bisphenol A was measured in 116 surface waters, 35 sediments, 37 sewage effluents, and 38 sewage sludges in Germany [212]. The result shows that bisphenol A is only present in surface waters in low concentrations between 0.0005–0.41 μg/l (median, 0.009 μg/l). No difference was seen between rivers, lakes, and channels. Concentrations of the sewage work outlets ranged between 0.02–0.7 μg/l bisphenol A, only 1 sample showed concentrations below the limit of detection (0.0001 μg/l). Bisphenol was detected in all sewage sludge samples between <1–1360 ppb (median, 137 ppb). In 30 out of the sediment samples, bisphenol was analyzed between 0.01–0.19 ppm dry wt (median, 0.49 ppm).

8.5 Polychlorinated dibenzodioxins and -furans

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are two chemically similar groups of chlorinated aromatic compounds. PCDDs can have up to 8 chlorine substituents leading to 75 possible congeners; the PCDF group comprises 135 possible congeners. PCDDs and PCDFs are not commercially produced, but are formed unintentionally as by-products of various industrial processes (e.g., chlorine synthesis, production of hydrocarbons), during pyrolysis and uncompleted combustion of organic materials in the presence of chlorine. All these processes form mixtures of different PCDD and PCDF congeners, which are characterized by a special pattern typical for the respective generation process. PCDD/Fs exhibit low vapor pressures and low water solubilities, and they are highly lipophilic (cf., Table 8). These properties depend on the degree of chlorination: the more chlorine the compound contains the lower the water solubility and vapor pressure and combustion processes. As can be seen regarding their physicochemical characteristics and their high persistence, it is obvious that PCDD/Fs belong to the POPs. They can be detected in all environmental compartments. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most active, best-known, and investigated representative of this class of substances. In the PCDF group, also the 2,3,7,8-substituted compounds have the highest biological activity. During the last 20 years, an enormous public and scientific interest was focused on these substances, resulting in many publications on generation, input, and behavior in the environment [215–217]. Mammalians and fish metabolize PCDD/Fs, leading to polar substances, which can be excreted. The rate of biotransformation is also determined by the respective congener. PCDD/Fs are accumulated in water organisms and the food chain, showing BCFs in fish of 1000–86 000 [67].

Table 8 Tetrachlorodibenzo-*p*-dioxin.

Common name	CAS-no.	γ_{sat}/µg l^{-1}	log P_{ow}
2,3,7,8-Tetrachloro-dibenzo-*p*-dioxin	1746-01-6	0.0013	6.76
2,3,7,8-Tetra-chloro-dibenzofuran	51207-31-9	0.42	6.22

γ_{sat}: Water solubility at 25 °C.
P_{ow}: Partition coefficient octanol/water.

The antiestrogenic effects of TCDD have been investigated in numerous in vivo studies [for review, see ref. 218]. TCDD influences uterus weight and reduces the number of estrogen and progesterone receptors in organisms [218,219]. In in vitro tests, primarily with MCF-7 cell lines, different antiestrogenic effects have been observed: TCDD inhibits the estradiol-induced cell proliferation and the estradiol-induced synthesis of progesterone [220,221]. Furthermore, TCDD can cause the so-called "down-regulation" of estrogen receptors [222]. For the polychlorinated dibenzofurans, in vivo data are mainly available for 2,3,4,7,8-pentachlorodibenzofuran. Effects are similar to those of TCDD [217]. In vitro investigations showed that also some other PCDF congeners can exhibit antiestrogenic effects [223]. The NOEL and LOEL for PCDD/Fs are between 0.001–14 mg/kg body weight and between 0.001–43 mg/kg body weight, respectively. However, these values are strongly dependent on the used compound, the test species (e.g., mouse or rat), the test system, and the route of application [113]. In vitro tests showed similar results, and in all investigations performed, TCDD had the highest antiestrogenic potency.

8.5.1 Environmental exposure to polychlorinated dibenzo-para-dioxins

PCDDs are produced primarily from insufficient combustion processes (e.g., incineration of municipal, hospital, and hazardous wastes) and they are emitted with exhaust gas into the environment, where they are long-range distributed. In 1995, the PCDD/F air emissions in Europe are estimated to have been 6500 g I-TEQ (international toxic equivalents) per year, leading to an average estimated daily deposition rate of about 5 pg I-TEQ/m^2 [224]. Owing to their physicochemical properties, PCDD/Fs can be mainly be found particle bound in the environment. In aquatic systems, they are mostly detected in sediment. PCDDs are also spread into the environment as unwanted impurities of various chlorinated organic chemicals, including pesticides such as pentachlorophenol, 2,4-D, and chloromethoxynil. Multivariate statistical methods for the profiles of dioxin mixtures enable the source of dioxin to be determined. For example, dioxin in the river Elbe, Germany, originated from the dioxin-contaminated region [225]. The wastewater from pulp plant contained TCDD/TCDF, which was reduced to 6 % on an I-TEQ basis from 1988 to 1996. The concentrations in the wastewater decreased to a level that can be hardly differentiated from the background level [226]. In 1998, concentration of dioxins in Japanese surface soil ranged from 0.012–330 ppt I-TEQ dry wt in Nose, Osaka Prefecture and from 3.1–51 ppt I-TEQ dry wt in Saitama Prefecture. The concentration in air was in the range from 0.034–0.19 pg I-TEQ/m^3 in Nose, Osaka Prefecture and from 0.012–0.029 pg TEG/m^3 in Saitama Prefecture. In the groundwater of Nose, Osaka Prefecture, the concentration ranged from less than detection limit to 0.32 pg I-TEQ/l [227]. In 1998, concentration of coplanar PCBs in surface soil ranged from 0.0004–3.1 ppt I-TEQ dry wt in Nose, Osaka Prefecture and from 0.21–5.7 ppt I-TEQ dry wt in Saitama Prefecture. The concentration in air was in the range from 0.001–0.010 pg I-TEQ/m^3 in Nose, Osaka Prefecture, and from 0.012–0.029 pg TEG/m^3 in Saitama Prefecture. In the groundwater of Nose, Osaka Prefecture, the concentration ranged from less than detection limit to 0.001 pg I-TEQ/l [227]. In a high cancer-causing area close to a batch-type incinerator for municipal solid wastes in Japan, the concentration of dioxin reached to 52 ppm I-TEQ dry wt [228]. It is estimated that about 5.2 kg of dioxin have been released in Japan, and 80 % of dioxin have been emitted from incineration of municipal solid wastes and 10 % from industrial solid wastes [229]. Dioxins were present at concentration ranging from 0.005–0.1 ng/l (from 0–0.0001 ng I-TEQ/l) in river water of Japan. In the estuaries, the concentrations

of dioxins were 0.41 ng/l (0.001 ng I-TEQ/l) in 1990, 0.049 ng/l in 1992, and 0.02 ng/l in 1995. Effluent water of wastewater treatment plants contained dioxins in the range from 0.015–0.055 ng/l (equivalent to less than 0.0001 ng I-TEQ/l) [230]. Half-lives of dioxin have been estimated. The values of estimation ranged from 1–25 years in surface soil [231]. In subsurface soil, half-life was estimated longer than 100 years [232].

In Germany, the concentration of dioxins in the surface soil of industrial areas often exceeded 100 ppt I-TEQ dry wt. In a residential area, the concentration was lower than 30 ppt I-TEQ dry wt soil, although some soils in parks exceeded 1000 ppt I-TEQ dry wt. Agricultural soils contained dioxin at concentrations lower than 32 ppt I-TEQ dry wt, except for pasture of an urban area where a level of 100 ppt I-TEQ dry wt was found. The concentration in forest soils reached often up to 100 ppt I-TEQ dry wt, which were slightly higher levels than for residential areas and agricultural soils [233]. In Michigan, USA, the concentration of dioxin in surface soil of industrial areas ranged from lower-than-detection limit to 52 ppb I-TEQ dry wt, and in residential areas, the highest concentration was 270 ppt I-TEQ dry wt. In the agricultural areas, the concentration ranged from 0.02 to 30 ppt I-TEQ dry wt [234,235].

8.6 Organotin compounds

Organotin—mostly TBT (Table 9)—compounds are used as molluskicides; antifoulants on boats, ships, quays, buoys, crab pots, fish nets, and cages; wood preservatives; slimicides on masonry; disinfectants; biocides for cooling systems, power station cooling towers, pulp and paper mills, breweries, leather processing, and textile mills. TBT in antifouling paints was first marketed in a form that allowed free release of the compound to the environment. More recently, controlled-release paints, in which the TBT is incorporated in a copolymer matrix, have become available. Rubber matrices have also been developed to give long-term slow release and lasting effectiveness for antifouling paints and molluskicides. In this form, much of the TBT remains in the matrix of the rubber, although the effectiveness lasts for several years.

Table 9 Organotin compounds suspected of being endocrine disruptors.

Common name	CAS-no.	γ_{sat}/μg l^{-1}	log P_{ow}
Bis(tributyltin)oxide	56-35-9	8000–10 000	3.62
Triphenyltinhydroxide	76-87-9	400	
Tributyltin fluoride	1983-10-4		

γ_{sat}: Water solubility in seawater at 22 °C.
P_{ow}: Partition coefficient octanol/water.

In this document, the term TBTO (tributyltin oxide) is used when that specific chemical is intended. In the environment, however, TBT compounds are expected to exist mainly as TBT hydroxide, TBT chloride, and TBT carbonate. In those cases or when the identity of the specific chemical is not clear, the general term TBT is used [236]. Water solubility and lipophilicity are dependent on pH and ion content of the water. For TBTO, for example, a pH-dependent water solubility of between 0.75 and 60 mg/l was measured. For TBT compounds in general, a water solubility of <1 to >200 mg/l has been calculated. TBT dissociates in water and forms hydrated cations (TBT^+), which can react with various anions (e.g., OH^-, Cl^-, HCO_3^-). The type and percentage of TBT species found in water is again dependent on pH and the ion content of the water. In seawater, for example, a pH-dependent balance between TBT^+ and TBTCl, TBTOH, $TBTHCO_3$ has been found. At pH <7, TBT^+ and TBTCl are primarily present, at pH 8, a mixture of TBTCl, TBTOH, and $TBTCO_3$ predominate [113]. The values given for sediment/water distribution coefficients ($0.34–64 \times 10^4$) [237] indicate that TBTO is adsorbed to the surface of particles in water, sediment, and soil and then accumulated in sediment. Type and content of suspended particles in water determine the percentage of particle-bound TBT in the water. The degra-

dation of TBT in the environment is performed by a stepwise cleavage of the carbon–tin bonds and completed by release of oxidized tin in water. The cleavage of the carbon–tin bonds can be performed by physicochemical (hydrolysis, photodegradation) and biological (degradation by micro- and higher organisms) as well. In sediment, the degradation of TBT is slower than it is in water. The half-life in water ranges from a few days to weeks. In sediment, half-life times of 4–5 months have been reported for the aerobic layer, but more than 500 days for the anaerobic layer [113], meaning that TBT can persist in sediments for several years. The high log P_{ow} indicates that TBTO has a bioaccumulative potential. This is confirmed by experimental BCFs reported of 1000–11 000 for oyster, 4400 for snails, 9900–133 000 for mussels, and 30 000–100 000 for gastropods [67]. Little definitive information is available on the pharmacokinetics of TBTO: TBTO is absorbed from the gut and via the skin of mammals. It can be transferred across the blood–brain barrier and from the placenta to the fetus. The TBTO is adsorbed rapidly and widely distributed among tissues (principally the liver and kidney). The metabolism in mammals is rapid; metabolites are detectable in blood within 3 h of TBTO administration. The principal metabolite appears to be the hydroxybutyl compound, which is unstable and rapidly splits to form the dibutyl derivative and butanol [236]. In lower organisms, tributyl compounds are also metabolized, but here biotransformation is slower—particularly in mollusks—than in mammals. Therefore, the capacity for bioaccumulation is also much greater in lower organisms than in mammals. A metabolic study of ^{14}C-labeled TBT chloride showed that in neogastropods an oxidative debutylation is the principal degradation pathway. The metabolites are, thus, dibutyltin chloride and then monobutyltin chloride [238]. Until now, organotin compounds are the only known nonsteroidal substances showing androgenic effects. TBT is found to be responsible for imposex (i.e., females that show typical sexual characteristics of a male, such as a penis) in many species of neogastropods including dog-whelks, *Nucella lapillus*. The anticipated underlying mechanism is the inhibition of the enzyme aromatase, which catalyzes the conversion of testosterone to estradiol and was discussed previously. Several field studies provided a correlation between TBT concentration in water and intensity of imposex. The LOEL was determined to be 5 ng/l as Sn [239]. An autoradiographic study demonstrated that a substantial portion of the labeled compound accumulated in the nervous tissue of the dog-whelk: the finding may support a view that the imposex is caused through the compound-action on some neurohormones [240]. A previously discussed mechanism of action (i.e., inhibition of aromatase in the course of steroid metabolism) is reported to be caused also through some neurohormones [241].

8.6.1 Environmental exposure to organotin compounds

Organotin compounds are mainly used to protect ship bottoms, but are also applied as insecticides in orchards. They are distributed by the water stream into the environment and taken up by aqueous organisms in river, sediments, and oceans. The enhancement of TBT concentrations in the surface microlayer may present a hazard to littoral organisms, neustonic species (including benthic invertebrate and fish larvae), and surface-feeding seabirds and wildfowl. Accumulation and low biodegradation of TBT in sediment may pose a hazard to aquatic organisms when these polluted sediments are disturbed by natural processes or dredging activities [236]. There are a number of reports on the occurrence of TBT residues in marine organisms. Levels of total butyltin residues [the sum of detected TBT, dibutyltin (DBT), and monobutyltin (MBT)] of 5–230 ppb in muscle of fish; 300 ppb in liver and kidney of marine birds; and 13–395 ppb in muscle of marine mammals have been reported [243,244]. In marine mammals, much higher total butyltin residues were reported for blubber (48–744 ppb), kidney (25–3210 ppb), and liver (40–11340 ppb) [245]. Geographical comparisons showed greater accumulation of residues close to coasts compared with the open sea and in the vicinity of developed compared with developing countries [236]. A high concentration of TBT was detected off shore in southern British Columbia, indicating commercial shipping as a significant source of TBT [246]. In raw municipal wastewater in Zurich, Switzerland, MBT, DBT, and TBT were detected in the range of 140–560 ng/l, 130–1030 ng/l, and 60–220 ng/l, respectively. Most of the organotins (81–92 %) were associated with suspended solids and removed by sedimentation and adsorption during treatment. After treatment,

the concentration in the effluents decreased to 1–17 ng/l, 0.5–0.2 ng/l, and 1.5–0.5 ng/l, respectively. In the sludge, concentrations of 1.1–0.4 ppm dry wt were found [247]. All butyltin compounds were detected in surface sediments, reaching 158 ppb in marinas, with TBT as the predominant compound, while away from pollution sources, MBT was found to be the most abundant substance [248]. In water sediment of freshwater marinas (Lake Lucerne, Switzerland), triphenyltin (TPT), diphenyltin (DPT), TBT, DBT, and MBT were detected. Aqueous TBT concentrations increased to 752 ng/l in summer, slowly decreasing to about 100 ng/l in the winter months during 1988 to 1990 [249]. After the ban of organic tin compounds, the concentrations decreased from 1991 to 1993 to about 40–50 ng/l, and to 9.2 ppm of TBT and 0.7 ppm of TPT in the surface of sediments. The concentrations of metabolites did not increase with increasing depth, indicating high persistence of organotin compounds [250]. Analysis of TBT in core sediments, for which an approximate chronology is known, indicated a half-life for the first-order degradation of TBT of about 8.7 years [251]. Analysis of TBT in sediment core taken near Auckland, New Zealand indicated that degradation occurs with first-order kinetics and that TBT in marina sediments have a half-life time of about 2.5 years [252]. In estuaries, low concentrations of TBT (<5 ng/l) were found along the Georgia coast [253]. In pecan orchards in central Georgia (USA), which were previously sprayed with commercial TPT hydroxide (TPTH) mixtures at concentrations of 8.5 to 37 ppm dry wt of foliage and 1.2–12 ppm dry wt of soil, TPT, DPT, and monophenyltin (MPT) were present in the leaves and soil, with MPT generally the predominant compound. TPT was absent from the subsurface soils (2–15 cm depth) even though it was sprayed 8–10 times a year at a rate of 850 g/ha for the past 10 years [254]. In a survey of 36 locations (river, lake, and estuaries) in Japan from 1990 to 1996, the concentration of TBT had been maintained at constant or slightly decreasing. The concentration in water in 1996 was in the range from lower-than-detection limit (3 ng/l) to 14 ng/l and in sediments from lower-than-detection limit (0.6 ppb) to 930 ppb. Frequency of the detection was 13 out of 36 locations in water and 32 locations in sediments. TPT was not detected in water with one exception at trace level (detection limit: 10 ng/l). In sediments, TPT was detected in 15 out of 36 locations in the levels from lower-than-detection limit (1 ppb dry wt) to 220 ppb dry wt [255].

9. CONCLUSIONS

Natural steroid hormones as well as synthetic sex steroids are very potent endocrine-disrupting compounds, and they are found in water sources in physiological concentrations. Indeed, one of the best-documented and widespread environmental hormonal effects—gonadal abnormalities in fish in the United Kingdom—has been shown to be due to steroidal estrogens. To make a risk assessment of the exposure of aquatic organisms to naturally occurring sex steroids requires, in addition to regular concentration monitoring in water sources, requires a more substantial understanding of the degradation of these potent substances. To date, there exist almost no data, even though elucidation of the degradation routes of sex hormones in the environment is well within the range of available technologies and could be accomplished within a short time span. Therefore, we recommend that such studies should be given special emphasis.

For many industrial chemicals and pesticides (PCBs, PAHs, bisphenol A, PCDDs, PCDFs, TBT compounds), the effect levels determined in in vitro tests are some magnitudes lower compared to physiological hormones like estradiol. These compounds are often recognized to have other toxicities and are, therefore, often well monitored. In the environment, they are not found in concentrations that could be demonstrated in the laboratory to cause endocrine effects. Nonetheless, this is no reason to lower the guard. Although xenoendocrine disruptors generally appear to be less potent than endogenous hormones, many of them—especially the POPs—are persistent and/or accumulate in the environment as well as in the organisms living there. Even if the use of many chemicals is restricted (e.g., DDT, TBT compounds), they are still readily detected in water, sediment, suspended soils, and wildlife, so concerns for continued exposure cannot be disregarded. However, their long-term effects are difficult to determine owing to the long time period involved and to the fact that they usually occur in mixtures.

Environmental endocrine disruptors have varying routes of exposure depending on their inherent physicochemical properties, as well as external conditions such as their specific use, and environmental conditions such as temperature, UV-radiation, and microbial content. As mentioned above, many environmental compartments are well monitored for pollutants. This is particularly true for pesticides and various industrial chemicals with known toxic properties. However, additional potential sources need to be specifically evaluated for endocrine disruption, and such sources should be a target of future investigation. This is demonstrated by some prominent examples of endocrine disruption in wildlife: (1) lake trout (Great Lakes, USA) having decreased gonadotropin levels, steroid levels, decreased egg and gonadal size due to bleach mill effluent; (2) fish (roach) in United Kingdom, displaying intersex and having high percentage of gonadal dysfunction owing to sewage water treatment effluents containing steroidal estrogens and nonylphenols; and (3) marine neogastropods displaying imposex due to tributyl tin compounds. These examples demonstrate that some sources—namely, effluents of water treatment plants, effluents of some industrial processes (e.g., paper mill effluents), and effluents of animal manure—often contain compounds (e.g., sex steroids, β-sitosterol) in concentrations that can cause endocrine-disrupting effects in wildlife and as such must be regularly monitored and the content of harmful compounds lowered if necessary.

The report tries to highlight the main difficulties in dealing with putative endocrine disruptors. The systems involved are very complex, and the number of possible compounds and organisms affected reach proportions that only large-scale and long time investigations can determine if indeed a serious ecological problem is present. As can be seen, such studies are being undertaken by a number of governments and multinational groupings. It should be noted, however, that large systems such as the ecosystems we live in are inherently chaotic even though they may appear to be in equilibrium. Therefore, any technological fix of one variable will change all other variables in a totally unpredictable manner. It is, therefore, inadvisable to make large-scale changes to correct pollution without numerous small-scale studies. Additionally, reliable and standardized test procedures (in vivo and in vitro) that can be applied easily are still lacking for many substances. The development of such methods should be supported and coordinated internationally.

What is also needed is ecological "common sense", e.g., if the fish in rivers used for drinking water display sex reversal, action should be take immediately. If minor or localized disturbances are seen in wildlife ecology, the best course of action would be long-term data collection.

Some areas merit additional comments. Although surface and groundwater have been extensively surveyed in several geographical areas (Europe, United States, Japan, and Brazil), there is a paucity of data from Africa and Asia. It should also be noted that, although the environmental exposure is well documented, there is a scarcity of literature on point source contamination, i.e., how much pollution results from contamination during production and transportation of the suspected compounds.

A comparatively new area of investigation is the contribution of pharmaceutical drugs, particularly synthetic estrogens present in contraceptive pills, to the estrogen "load". The additional effects of the synthetic estrogens need to be determined against the "normal" estrogen load that results from the natural steroids produced by human and animal sources.

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11. ABBREVIATIONS

4-*tert*-OP	4-*tert*-octylphenol
APE	alkylphenol ethoxylate
AR	androgen receptor
BADGE	bisphenol A-diglycidyl-ether
BCF	bioconcentration factor
CSTEE	Commission Scientific Committee for Toxicity, Ecotoxicity and the Environment
DBD	DNA binding domain
DBT	dibutyltin
DCA	dichloroaniline
DDD	1,1-dichloro-2,2-di(chlorophenyl)ethane
DDE	1,1-dichloro-2,2-di(chlorophenyl)ethylene
DDT	1,1,1-trichloro-2,2-di(chlorophenyl)ethane
DES	diethylstilbestrol
DPT	diphenyltin
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ED	endocrine disruptor
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee (U.S. Environmental Protection Agency)
EMSG	Endocrine Modulators Steering Group
ER	estrogen receptor
EPA	U.S. Environmental Protection Agency
FSH	follicle-stimulating hormone
I-TEQ	international toxic equivalent
K_{oc}	adsorption coefficient
K_{ow}	octanol/water partition coefficient
LBD	ligand binding domain
LC	lethal concentration
LH	luteinizing hormone
LOEC	lowest observed effect concentration
LOEL	lowest observed effect level
MAT	matairesinol
MBT	monobutylin (butyltin)
MDDE	1,1-dichloro-2,2-di(4-hydroxyphenyl)-ethylene
MPT	monophenyltin
NOEC	no observed effect concentration
NOEL	no observed effect level
NP	nonylphenol
NPE	nonylphenol ethoxylate
OECD	Organisation for Economic Cooperation and Development
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDF	polychlorinated dibenzofurans
POP	persistent organic pollutant
P_{ow}	octanol/water partition coefficient
PR	progesterone receptor
SCP-PPP	Scientific Committee on Plants—Plant Protection Products
SECO	*seco*-isolariciresinol

SETAC	Society of Environmental Toxicology and Chemistry
SHBG	sex hormone binding globulin
STP	sewage treatment plant
TBT	tributyltin
TBTO	tributyltin oxide
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TPT	triphenyltin
TPTH	triphenyltin hydroxide
TSD	temperature-dependent sex determination
UN-ECE	United Nations Economic Social Council, Economic Commission for Europe
WS	water solubility