Topic 2.4

Pharmaceuticals and personal care products: A source of endocrine disruption in the environment?*

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Abstract: A wide variety of chemicals are used in pharmaceuticals. Most of these are already under thorough control for endocrine activity. The main causal agents recognized for endocrine disruption from sewage are substances used in medicine (sex hormones, glucocorticoids, and others), natural substances (estrone and 17β-estradiol), and synthetic estrogens (e.g., 17α-ethinylestradiol). Similar substances are used in anabolic agents (growth hormones) in livestock production in some countries. Although the estimated use of anabolic agents in livestock production is approximately one order of magnitude below the natural release of estrogens from farm animals, their possible significance remains unanswered.

At present, no other medical substances are recognized as endocrine disruptors in the environment. However, candidates may be identified on the basis of simple assumptions regarding their use and activity: (1) Nonestrogenic steroids may react with environmental endocrine receptors or metabolize on their way to the environment and thus form endocrine disruptors. (2) Many high-volume drugs released to the environment have not yet been tested for their endocrine properties, and some of these are known to interact with the human endocrine system. (3) Compared to medicinal substances, personal care products and additives in drugs are used in high amounts; from this group, parabens, siloxanes, and other substances are suspected of causing endocrine disruption in the environment.

INTRODUCTION

Since the discovery that pharmaceutical usage leads to the occurrence of medical substances in natural waters, numerous research activities have focused on revealing the extent and consequences of these increasingly emergent findings [1,2]. The occurrence of these substances in the environment is linked to usage in either a veterinary or human context. While the reasons for the occurrence of medical substances in the environment are well understood, the possible effect they may have is subject to discussion. A number of papers regarding general ecotoxicology show that these substances generally occur at nontoxic concentration levels [3,4]. But of even more interest may be research addressing questions related to specific pharmacological properties of these substances such as antibacterial resistance (antibiotics) [5] and endocrine disruption (endocrine active pharmaceuticals). At present, the knowledge regarding the possible endocrine effects in the environment that is related to pharmaceuticals is sparse.

It is obvious that the use of pharmaceuticals is only acceptable if no side effects occur. Additionally, medical products are accepted if their side effects are tolerable when compared to the benefits associated with their use. In order to avoid safety problems, drugs must undergo a thorough approval procedure that aims to document their safety [6,7]. This approval practically covers all known aspects of health effects and drug behavior in the treated organism over both the short and long term. Approval also takes unwanted hormonal effects into account, and in this light, the risk that approved substances are unsafe to the organism under treatment is limited.

The hormonally related health problems associated with drug administration are under stringent control, however, an opposing situation exists in relation to the control and monitoring of environmental endocrine disruption due to the presence of medical substances. One may argue that it is unlikely that environmental effects can occur considering that health issues are under control. However, there are several reasons to believe that pharmaceuticals could be environmental endocrine-disrupting chemicals (EEDCs). Firstly, some drugs are in fact used with the purpose of controlling hormonal mechanisms (e.g., contraceptives). Secondly, most drugs that are only active in the organism under treatment for a few hours but may after excretion persist in the environment for a much longer period. Thirdly, hormonal systems of environmental target organisms may be different to those found within the organism under treatment and consequently react differently to the presence of a substance. Ultimately, hormonal systems in the environment may be more sensitive than human hormonal systems, thereby reacting to much lower concentrations of a substance.

In the current chapter, these and other aspects related to the possible endocrine effects of pharmaceuticals in the environment will be discussed in more detail. As endocrine effects in the environment have primarily been linked to the estrogen receptor, the term endocrine activity will relate to this receptor if nothing else is mentioned. Initially, a concept for assessing their relevance as EEDC is presented. The medical substances that are relevant in the current context will then be identified, and finally the present information about these substances will be used to identify future research needs.

LIFECYCLE OF ENVIRONMENTAL ENDOCRINE PHARMACEUTICALS

If the use of a medical compound should lead to adverse endocrine effects in the environment, the substance must gain or retain endocrine activity during numerous consecutive processes. These processes are conceptualized in Fig. 1, which shows that the properties of the medical substance determines its fate in three main compartments: (1) the organism under treatment, (2) the environment, and (3) absorption in the target organisms. It must be stressed that no environmental effects will occur if just one of these processes leads to degradation, deactivation, or immobilization of the chemical. Consequently, the assessment of whether or not a medical compound may have impact on natural endocrine systems should ideally include the whole lifecycle of the substance from its initial use to its fate within the target organism. Obviously, this is impossible for all substances, and a number of criteria for identifying relevant substances with potential risk of being EEDC should be considered in succession:

- The predicted environmental concentrations (PECs) or the measured environmental concentrations should be high enough to induce endocrine effects.
- The use of a medical compound should lead either to the excretion of compounds that are hormonally active or to excretion of nonactive substances that can be converted in the environment to such active substances.
- Excreted hormonally active substances should after excretion, be persistent and mobile in the environment.

A number of studies have published methods for estimating worst-case concentrations for human [4] and veterinary medical substances [8,9]. In the simplest form, these assume that no immobilization, degradation, or metabolism of the drugs occurs on their route to the environment. Rough estimates of the dilution in wastewater or manure and in the receiving environments (soil, waters receiving sewage
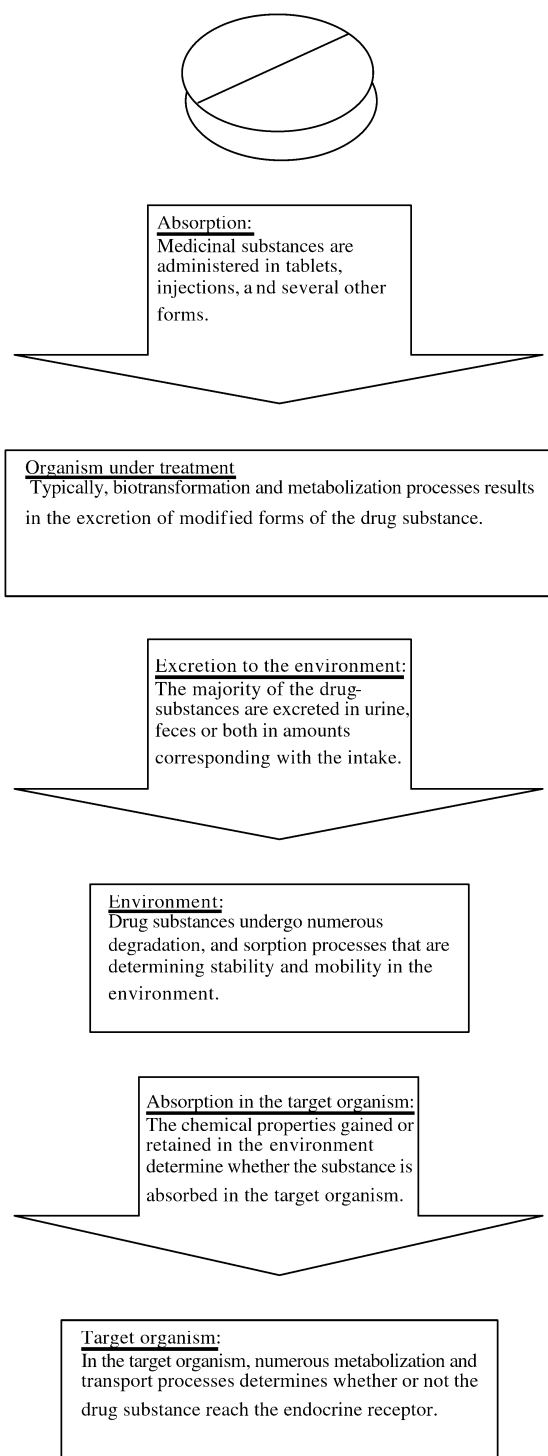
Fig. 1 Description of the route and processes of medicinal substances on their way to the target where they possibly result in an environmental endocrine effect.

effluent) is then used to calculate worst-case environmental concentrations. By implementing these estimation methods on commonly used human medical compounds; concentrations in sewage effluents ranging from a few ng/l (hormones) [10,11] to hundreds of µg/l (high-dose drugs such as painkillers) have been obtained [4,12].

As previously stated, it is assumed in the calculations above that the drugs in question can be fully recovered from excreta. The "bulk" intake of many compounds is equivalent to their environmental release, however these substances are rarely released in an unaltered state. In most cases, pharmaceuticals are excreted as one or several metabolites [1]. These metabolites are typically the result of either phase I or phase II metabolic reactions. Phase I reactions usually consist of oxidation, reduction or hydrolysis, and products are often more reactive and sometimes more toxic than the parent drug. Phase II reactions involve conjugation, which normally results in inactive compounds. Both phase I and phase II reactions change the physicochemical behavior of the substance and always render metabolites more water-soluble than parent compounds.

The behavior of a medical substance and its metabolic products in the environment should be assessed using the general procedures used for other environmental pollutants. However, some factors complicate this assessment. Firstly, the substances are present at extremely low concentrations, making them difficult to monitor. Secondly, investigation into the behavior of metabolites is complex. Despite this complexity, studies have showed that the phase II metabolites generally can be reactivated into the parent compounds [13]. Furthermore, many unconjugated metabolites have environmental properties similar to the parent compound. These results may justify that complex studies including all metabolites are limited to studies of the parent compound alone.

POTENTIAL ENDOCRINE-DISRUPTING, HIGH-VOLUME PHARMACEUTICALS

In theory, endocrine active pharmaceuticals are drug components that have impact on the endocrine system. This broad definition encompasses any compound possessing activity on endocrine receptors and originating from use in human or veterinary medicine. Substances with such properties are numerous and could be any type of endocrine agonist or antagonist (estrogens, androgens, thyroids, adrenocorticosteroids, etc.). It is, therefore, obvious that only those substances used in high volumes should be concentrated on here. The following will briefly introduce the most important classes of pharmaceuticals, and this will be followed by a discussion of their potential as environmental endocrine disruptors.

From sales statistics for human and veterinary drugs available in many countries, high-volume drugs have been identified by several authors [4,12,14,15]. With regard to endocrine activity, only certain groups are of interest. An overview is shown in Table 1. Generally, steroidal structures are the most relevant; as these are active in the endocrine system in humans, but other substances will also be discussed. Steroids can be characterized as lipophilic, nonvolatile substances. In the human organism, these steroids undergo various biotransformations usually leaving the steroid-structure intact [16]. The substances are then either excreted in urine as water-soluble conjugates or in feces in an unchanged state.

Estrogens and progestogens used for humans

Synthetic analogs of 17β-estradiol or the natural substance itself are primarily used for treatment of postmenopausal syndrome, breast cancer, or in contraceptives [17]. Contraceptives and postmenopausal drugs are extensively used in western countries [10–12]. Estrogen antagonists (e.g., tamoxifen) are also used in small quantities in the treatment of, for example, breast cancer.

The most important use of progesterone is in contraceptives where typical daily doses range from 0.25 to 2.5 mg. Progestogens are also used in the treatment of infertility, various cancer forms, and in combination with estrogens in the treatment of menstrual disorders and in contraceptives.

Table 1 Presentation of medicinal substances that could be relevant as endocrine disruptors.

Pharmaceutical group	Example	Main use in therapy
Estrogens	17β-estradiol (E2), estrone (E1), 17α-ethinylestradiol (EE2)	Contraception and hormone replacement therapy (treatment of postmenopausal syndrome).
Progestogens	Norenthindrone, Progesterone	The main clinical use of progesterons is in oral contraceptives.
Androgens and glucocorticoids	Testosterone, Beclometazone, Hydrocortisone	Androgens (testosterone) are used in small amounts in hormone replacement therapy. Glucocorticoids are used in the treatment of numerous diseases. Among these are treatments of inflammations, infections, asthma, allergy, skin diseases, and rheumatism.
Veterinary growth hormones	Trenbolone acetate, Melengestrol acetate	Used as growth promoters for meat-producing animals (anabolic steroids).
Nonsteroidal pharmaceutical substances	Acetylsalicylic acid, Clofibrate, Ibuprofen, Oxytetracycline	A multitude of nonsteroidal medicinal substances is used in high volumes. The examples given here are an agent against heart diseases (acetylsalicylic acid), a lipid-lowering agent (clofibrate), an antiinflammatoric agent (ibuprofen), and antibiotic (oxytetracycline).

Other steroids with impact on the endocrine system used in human medicine

Drugs with androgen activity consist of various testosterone formulations. These formulations differ in their ability to pass membranes, in their resistance to hydrolysis processes and in other factors related to their distribution in the organism. Modifications of androgenic substances (anabolic steroids) are used in large amounts in many countries as anabolic growth promoters for meat-producing animals. However, in comparison to estrogens, their use in human therapy is limited.

The adrenal cortex naturally secretes a number of steroids. The glucocorticoids are the most important of these due to the ability to affect carbon and protein metabolism. In addition, the glucocorticoids have anti-inflammatory and immunosuppressive activity. Therefore, both synthetic modifications and natural substances are widely used in drugs to control inflammatory diseases (hydrocortisone), asthma (e.g., beclometazone), infections, rheumatism, and other illnesses. The steroidal asthma products are extensively implemented and are typically among the 10 most used prescription drugs in industrialized countries [12]. The actions of the different steroids are overlapping indicating a broader spectrum than that observed for, e.g., sex hormones, which react very specifically with their respective hormone receptors.

Veterinary anabolic agents

In veterinary medicine, the two major classes of pharmaceuticals are antibiotics and anabolic growth hormones. As antibiotics are considered irrelevant in the context of this text, only anabolic agents appear to be relevant as environmental endocrine disruptors. At present the six hormones, 17β-estradiol, testosterone, zeranol, progesterone, trenbolone acetate (TBA), and melenogestrol acetate (MGA) are approved for use in the United States for enhancement of meat production in cattle and sheep [18]. Typically, these substances are administered by the use of growth implants as mixtures of steroidal androgens (progesterone, testosterone, TBA, and MGA) and estrogens (17β-estradiol and zeranol).

Nonsteroidal medical substances, personal care products, and drug additives

The number of substances from this group of substances is also numerous. Moreover, usage patterns differ highly between different countries. Therefore, a description of all the environmentally important substances is not possible here, however, several excellent reviews exist [1,2]. In these works, the most important medicinal substances are presented. These are from the following classes: agents used on the blood and blood-forming organs (e.g., acetylsalicylic acid), agents for the treatment of heart and circulatory diseases (e.g., clofibric acid), dermatological drugs (e.g., hydrocortisone), antibiotics (e.g., penicillin, amoxicyllin, tetracyclines), analgesics (e.g., paracetamol), anti-inflamatorics (e.g., ibuprofen), and agents used in treatment of allergy and asthma (e.g., budenoside).

Pharmaceuticals are administered in many different formulations and often in combination with various other chemicals that are included for purposes such as preservation, or as vehicles to aid administration/uptake in tablets, or for adjusting the ion-strength and pH in solutions. Many of these agents are also used in personal care products, and this contributes to the major release of these substances to the environment. In an environmental context, the major compounds are disinfectants, conservation agents, sun screens, musks, and other substances [2].

DISCUSSION OF PHARMACEUTICALS AS ENDOCRINE DISRUPTORS

Estrogens and progestogens used for humans

The most widely used pharmaceutical products for controlling the endocrine system in humans are the oral contraceptives and preparations used in human estrogen-replacement therapy (primarily for relieving postmenopausal syndrome and osteoporosis). Oral contraceptives contain typically 20–40 μg ethinylestradiol (EE_2) and 0.25–2.5 mg of various gestagens. Using these doses, the total consumption of contraceptives has been estimated for various countries (see Table 2). The active estrogenic components in hormone replacement drugs are conjugated estrogens, which are primarily the sulfate esters of estrone, equilin, and other naturally occurring compounds (in doses ranging from 0.3 to 2.5 mg). Depending on the individual circumstances, the substances are often used in combination with 2.5–5 mg progesterone [17]. In the United States, it is estimated that 12.5 to 33 % of the 40 million postmenopausal women are prescribed hormone replacement drugs [19]. In Table 2, estimated total annual consumption of estrogens in these products are reported for the United States and Denmark. As the population in the United States is only 55 times higher than in Denmark, it is clearly seen that the use of hormone replacement drugs per inhabitant varies considerably between different countries.

The endogenous excretion of hormones by healthy premenopausal women ranges from 10 to 100 μg estrogens per day. After menopause, women only excrete between 5 to 10 μg daily. The values for normal men average from 2 to 25 μg [17]. Pregnant women can excrete up to 30 mg per day, but average values are around 250 μg/day [20]. Using these data, total amounts of excreted estradiol in western countries were estimated using a method where the populations sex distribution was assumed as 50:50 male:female. Furthermore, it was assumed that 60 % of the female population was menstruating and that one out of every 75 females was pregnant. From these numbers the median excretion data and

the amount of endogenous estrogens could be estimated. Table 2 lists such data for the world and a number of industrialized countries. From this table, it is generally seen that estrogenic pharmaceutical products may significantly contribute to the total estrogenic load from humans in the environment. Based on such consumption data, a number of authors have estimated worst-case concentrations of 17ß-estradiol up to approximately 100 ng/l in sewage effluents [19,21] and at levels from 0.01 to 0.1 ng/l in rivers [22]. In comparison, 17ß-estradiol has been shown to induce vitellogenin and inhibit testicular growth in male trout at concentrations of 2 ng/l [23] and other authors have obtained lowest observed effect concentrations of EE_2 at concentrations of 0.03 ng/l [24].

Table 2 Comparison of estimates of human consumption of pharmaceutical estrogens and the excretion of endogenous 17ß-estradiol. Data with no references are estimated as described in the text.

		World	US	UK	Denmark
Consumption of pharmaceuticals	E_2		1700[a]		0.7[b]
	EE_2	528[c]	88[a]	29[d]	1.9[b]
Endogenous excretion of E_2		54 504 kg	2575 kg	531 kg	45 kg

References: [a][10], [b][4], [c][17], [d][12].

These theoretical estimates have been validated in many research projects reporting monitoring data on estrogens in sewage water and effluents in many western countries [e.g., 19,25]. Concentrations of natural hormones (E_1 and E_2) range from 0 to 82 ng/l (E_1) and from 0 to 64 ng/l (E_2). For EE_2, typical concentrations are 5 to 10 times lower. E_1, E_2, and EE_2 have all been identified as the main causal agents explaining feminization observed on male fish in waters receiving sewage effluents [26]. Although, environmental concentrations of EE_2 suggest that it is less important than the natural hormones (E_1 and E_2), the relevance of this substance as an environmental endocrine disruptor is significant, because it has been shown that EE_2 is more persistent than E_2 [27,28] and often more potent [24]. Additionally it is well known that the ethinylation of the estradiol molecule in EE_2 decreases its affinity to the steroid binding proteins and as discussed by [29] this may lead to increased male sexual neural imprinting in female fetuses/larvae of vertebrates. In conclusion, it can stated there is strong evidence indicating that adverse endocrine effects on environmental organisms are a result of the presence of estrogens and additionally that the use of these substances in pharmaceuticals significantly adds to this pollution.

Although exogenous and endogenous estrogens are continually causing adverse effects in the environment, many details about their fate remain to be investigated. Early studies on the biodegradation of 17ß-estradiol at very high concentrations in sewage simulations systems showed that activated sludge microorganisms were able to mineralize estrogens at rates much slower than typical retention times in sewage treatment [28,30]. Although the primary degradation step for biodegradation of E_2 and EE_2 have been shown to occur within a few days and that mineralization occur within a week for E_2 [31], detailed studies on the biodegradation of estrogens at realistic concentrations are needed. In sewage, the source of these agents is excreta from humans containing sulfuric and glucuronic acid conjugates of estrogens. These conjugates do not possess a direct biological activity but there is strong evidence suggesting that they can be converted back to free estrogens by bacteria in the environment [32,33]. The kinetic details of these processes are practically unknown, and, furthermore, little is known about the other metabolic products formed as a part of the excretion of estrogens.

Considering that the most significant use of progestogens is in combination with EE_2 in contraceptives where the typical doses of progestogens are 5 to 15 times higher than EE_2, the detection of these substances in sewage effluent may be expected. However, these substances have only been de-

tected in sewage in a few cases [34,35]. It has been proposed that because these substances are extremely lipophile, large portions are removed via sorptive processes in sewage treatment [20]. Another explanation may be that the significance of these substances as endocrine disruptors is still unclear and consequently they have been given little attention. [36].

Other steroids

It has been speculated that glucocorticoids may be relevant as environmental endocrine disruptors due to their chemical and biochemical links to the hormone system. The eventual transformation of cholesterol to estrogenic steroids due to activities of sewage bacteria has been investigated. However, these studies proved that this substance underwent rapid mineralization and no transformation to estrogens [37]. Considering that the glucocorticoids do not have the hydroxy group of the estrogens, it may be argued that the transformations may more likely result in the formation of substances with androgenic or other endocrine activities. This has, however, not yet been investigated.

The environmental relevance of steroids used in the treatment of asthma, skin diseases, and other diseases could be assessed by simple estimates of consumption. Based on the number of doses and estimates of the size of these doses the total consumption in Denmark was calculated as being 361 kg/year of glucocorticoids in pharmaceuticals. The significance of these substances as environmental endocrine disruptors seems important when compared to E_2 (45 kg/year) and EE_2 (0.7 kg/year) that have both been detected in ng/l-levels. However, as the natural release of steroids is at a level much higher (2–10 mg/l of cholesterol in sewage, [38]) it appears that the glucocorticoids used in medicine are relatively unimportant. However, their relative potency on environmental endocrine receptors is unknown and may differ strongly as it has been shown for receptors in the human organism [39].

Anabolic agents used in livestock production

The use of anabolic agents in meat production is banned in the European Union [40], however, in the United States and many other countries they are still used intensively. Similar to the excretion patterns observed in humans, studies on the metabolization and excretion of estrogens and androgens from agricultural animals have shown that the parent compound or its active metabolites are excreted mostly in urine as glucuronide conjugates [41]. After excretion, these substances may be cleaved, and, thus, the hormones may have impact on the environment when manure is used as fertilizer. The importance of hormones released from livestock farming is not yet clarified [42,43]. Although results are conflicting, it cannot be ruled out that this source of pollution with environmental endocrine disruptors is insignificant. Regarding the anabolic agents, the question of whether they contribute significantly to the total release of hormones from livestock can be answered by using simple mass balances. In cattle, implants with anabolic agents are typically used once or twice in a lifetime in doses of 100 to 200 mg androgens and 20 to 40 mg estrogens [44]. Using these numbers and assuming that all cattle slaughtered in the United States (33.5 million cattle were slaughtered in 2000 [45]) are treated once with growth implant, the total use of hormones can be roughly estimated to 671 to 1342 kg of estrogens and 3356 to 6712 kg of androgens. In comparison, concentrations of endogenously released E_1 and E_2 have been reported in cattle manure from animals used in both milk and beef production. In the six different farms studied, manure concentrations of E_1 and E_2 ranged from 52 to 640 and from 167 to 1229 µg/kg dry matter, respectively [46]. Using these data, and a typical manure production of 17.7 tons/year/animal with a dry matter content of 11.9 % [47], approximate releases of E_1 and E_2 from the 33.5 million beef cattle in the United States can be estimated to 4–45 and 12–87 tons, respectively. Although these numbers are significantly higher than the amount of anabolic agents used the present knowledge about the environmental fate of anabolic agents is too sparse to be used to conclude that these substances are environmentally unimportant. For example, it is possible that the synthetic anabolic agents, behave in similar

ways as it shown for EE_2, and that they are more persistent than their respective endogenous hormones possibly possessing greater environmental impact than expected according to the concentrations in manure. Another example is that data exist showing that cattle subjected to certain anabolic agents generate urine with E_2 concentrations five- to six-fold over cattle not given these substances [48].

In view of the lack of scientific data on the fate of anabolic agents, the relevance of a recent study where the fate of the androgens trenbolone acetate and melengestrol acetate was studied after administration in cattle is obvious [49]. After eight weeks, approximately 10 ng/g of trenbolone acetate was detected in the manure collected during the experiment. During storage, the half-life of trenbolone was 260 days, and after spreading on a field the hormones were degraded more quickly. The question regarding the environmental importance of these compounds addressed by this study has been followed up by preliminary reports of endocrine effects on fish and Daphnia Magna due to trenbolone acetate [50].

Nonhormone pharmaceuticals

Apparently, no reports on nonhormonal drugs as environmental endocrine disruptors exist. This is not surprising, considering the low environmental concentrations and the thorough approval procedures that exist. On the other hand, drugs are biological active on many levels and are often used in the treatment of diseases related to the endocrine systems (e.g., metabolic disorders). Therefore, it is no surprise that they may have an impact on the endocrine system in the human body. However, as will be shown in the following the relevance of such data for environmental risk assessment is highly questionable and a number of examples will be given to illustrate some of these aspects.

Ibuprofen is an anti-inflammatory agent, which, due to its high use, has been the subject of studies on ecotoxicity [51], degradation [52], and environmental occurrence [53,54]. Based on such studies, the general conclusion is that ibuprofen is environmentally safe [55]. This conclusion may need to be reassessed considering that ibuprofen has been shown to block the effects of the estrogen agonist tamoxifen and partially to block the effects of 17ß-estradiol in a study of bone metabolism in rats [56] and thus may possibly be an environmental endocrine disruptor.

Clofibric acid, the active metabolite of the lipid-lowering agent, clofibrate is relevant because it is bound to a nuclear protein, the peroxisome proliferator activated receptor (PPAR). PPAR is thought to belong to the steroid receptor superfamily and therefore possibly influences the proliferation of cell organelles and lipid metabolism in organisms [29]. The endocrine effects of clofibric acid have been observed in different assays, however, results are often dubious. One example is a rat uterine assay where endocrine effects of clofibric acid were obtained; however, later studies could not confirm these data [57]. This compound is used in high volumes in many countries and is also a major pharmaceutical contaminant in the environment [58], therefore, it seems reasonable to investigate whether this substance may be an environmental endocrine disruptor [29].

In all the previous examples, the substances occurring in the environment are questioned regarding their safety due to results from the medical/pharmaceutical literature. Similarly, data revealing endocrine activity of nonsteroidal drugs can be found for other commonly used drugs. Examples are acetyl salicylic acid, which has been shown to affect the binding capacity of the estrogen receptor from MCF-7 cells [59] and oxytetracycline, which can interact with hormone metabolism [60]. In all cases, the data regarding the endocrine activity of the substance in question is based on assays relevant to human toxicology using concentrations much higher than those found in the environment. In other words, the comparability of these results is highly questionable, and results can only be used if they are confirmed in studies using more environmentally relevant assays. On the other hand, this information could be used as a starting point in the search of environmental endocrine disruptors and further, that future research should focus on extrapolating data from assays for human toxicology to environmental problems.

The final example of a pharmaceutical product being linked to adverse endocrine effects is a dental sealant showing estrogenic activity in the E-screen cell assay [61]. This effect was shown to be due to release in saliva of the well-known endocrine disruptor bisphenol A [62]. In this case, the substance in question is a well-known endocrine disruptor and its effects in nature is out of discussion. On the other hand, the question of whether it may reach the environment in concentrations high enough to cause damage in the receiving environments remains unanswered.

Personal care products and additives in pharmaceuticals

Among the personal care products (e.g., cosmetics or sunscreen agents), several substances are suspected as endocrine disruptors. A Swiss investigation [63] used the in vitro E-screen assay to prove the estrogenic activity of five UV screens [benzophenone-3 (Bp-3), homosalate (HMS), 4-methyl-benzylidene camphor (4-MBC), octyl-methoxycinnamate (OMC) and octyl-dimethyl-PABA]. These substances are lipophilic nonsteroidal substances that have been detected in fish from the Swiss Meerfelder Maar Lake at total concentrations of 2 mg/kg. Furthermore, there is evidence that Bp-3 is excreted in urine after dermal use [64] and in a rat uterotrophic estrogen assay, estrogenicity was observed in vivo after exposure to 4-MBC, OMC, Bp-3 [63]. In vitro estrogenicity of Bp-3 has been observed using the MCF-7 assay [65]. The interpretation of the data is unclear, and, therefore, the results have been subject of intense discussions in several environmental protection agencies.

Approximately 12 parabens are used commercially as preservatives in cosmetics, food, and pharmaceutical products [66]. The major source explaining the occurrence of parabens in sewage effluent at ng/l concentration levels is the use of these substances in shampoo and cream. Estrogenic effects of parabens have been demonstrated in vivo in mice [67], rats [68], and fish [69] and in various in vitro assays [68]. Using the E-screen assay, the estrogenic potency of the parabens was 5500 to 230 000 times lower than 17β-estradiol [66] indicating that this substance is unimportant as an environmental endocrine disruptor.

Certain phenyl-methyl substituted siloxanes used in cosmetics have been shown to exhibit high estrogenic potencies. The environmental significance of these substances has been questioned as these substances are considered as high-volume chemicals [70]. However, very little is presently known about these compounds as their analysis represents a major problem.

CONCLUSIONS

A few uses of human pharmaceuticals are directly linked to environmental endocrine disruption. Estrogens used in hormone replacement therapy of postmenopausal women (17β-estradiol and estrone) and synthetic hormones used in contraceptives (particularly 17α-ethinylestradiol) are recognized as the main causal agents for sexual disruption in fish in waters receiving effluents from sewage. It remains unclear whether or not the use of natural estrogens in human therapy adds significantly to the background level of natural hormones released from humans. However, due to their higher stability, the impact of synthetic estrogens compared to natural estrogens is greater than would be expected from the environmental concentrations. Technologies for removing these substances from wastewater should be developed further and implemented in waste management strategies.

The environmental importance associated with the use of veterinary anabolic agents for meat-producing animals in some countries is an emerging research subject. The endocrine effect of these synthetic steroids in the environment remains unanswered. Their relevance in comparison to naturally released hormones should be investigated.

In general, pharmaceutical compounds used in normal human therapy are not likely to be environmental endocrine disruptors. Firstly, most of the substances are used in very small amounts. Secondly, a range of processes determining the fate of the substance on its way from the use in medicinal products to the target should coincide. Thirdly, as a part of the approval procedure, medicinal sub-

stances undergo thorough testing for unwanted side effects including endocrine disruption on numerous levels.

On the other hand, there are many arguments suggesting that pharmaceutical compounds used in normal therapy may be environmental endocrine disruptors. Firstly, the medicinal substance may be transformed to endocrine active metabolites on its way to the target in the environment. Secondly, the endocrine system in the target may react differently than that of the patient where drug was used, and finally many drugs are used with the purpose of treating diseases related to the endocrine system (e.g., steroidal hormones).

In the search of whether other medicinal substances may be environmental endocrine disruptors, the following preliminary conclusions can be drawn:

- Even though the chemical structures of several widely used steroids are similar to estrogens (used in the treatment of asthma, skin diseases, etc.), no evidence indicating that these substances are endocrine disruptors in the environment exists. A hypothesis that such substances may metabolize in the environment and form estrogenic substances has been tested with negative result. This should however be investigated further especially with regard to nonestrogenic hormonal activity.
- In pharmaceutical and medical literature, numerous examples of substances with agonistic or antagonistic impacts on endocrine processes due to nonsteroidal drugs exist. These studies may be used as preliminary indicators of whether these substances could be environmental endocrine disruptors.
- Although many medical substances occur in the environment, they have generally not been tested for their endocrine activity. Such studies should be made using environmentally relevant assays.
- A number of substances used as the active substance in personal care products or as additives in medicinal products are of relevance in the current context. Of these substances, parabens and a number of sunscreens have been subject to concern due to their estrogenic activity. In addition, siloxanes (used in cosmetics) have been questioned as potential endocrine disruptors.

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