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Pharmaceuticals

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Abstract: An inventory is presented of pharmaceuticals with a potential to affect human fecundity via exposure through the human food chain. Pharmaceuticals are reviewed in particular with respect to their mechanism of action, especially in view of endocrine disruption, their use pattern and their detection and persistence in the environment as important indicators of possible human exposure via the food chain. Sections on mechanisms of toxicity to fertility and on nutritional exposure pathways are followed by an extensive review of a series of relevant classes of pharmaceuticals. Finally, risk assessment approaches are reviewed. This work has been conducted as the first phase in the EU-FP6 project Food & Fecundity (F&F) as part of the identification of pharmaceutical compounds of interest for further analysis in food matrices.

Key words: fecundity, fertility, pharmaceuticals, endocrine disruption, environmental exposure.

18.1 Introduction

The volume of pharmaceuticals consumed in the European Union (EU) is large, with the number of different substances used estimated as approximately 3000 in human medicine (Fent et *al.*, 2006), e.g. analgesics, anti-inflammatory drugs, antibiotics, contraceptives. A large number of pharmaceutical products (PP) are also used in veterinary medicine, e.g. antibiotics and anti-inflammatory drugs. Possible accumulation of these pharmaceuticals in the environment has not been of great concern in the past. However, with the improvement of the accuracy and sensitivity of the detection methods the awareness of the presence of pharmaceutically active compounds in the environment has increased. The EU-FP6 project 'Food and Fecundity' was aimed at the identification of possibly hazardous concentrations of pharmaceutical residues in the food chain, specifically with respect to adverse effects on fertility. As a first step in the project, an inventory of relevant pharmaceuticals was made.

The prime relevant characteristic of pharmaceuticals of interest is their known effect on fertility, through an endocrine mechanism of action. We have therefore reviewed pharmaceuticals for causing infertility, sexual dysfunction in men or women, and altered libido. In Section 18.2, a detailed description of disorders falling into each category is given together with evidence reported in the literature.

In Section 18.3 exposure pathways of pharmaceuticals in food are identified. The link between three media (soil, surface water and groundwater) is shown, indicating possible cross-contamination. The classification of main pathways is given together with detailed description of how dispersion of pharmaceuticals in the environment may occur. Finally, the importance of water as PPs transportation media is stipulated.

Sections 18.4–18.13 provide an overview of pharmaceuticals which are suspected of affecting human fecundity. The classes of interest identified are as follows: non-steroidal anti-inflammatory drugs, NSAIDs (ibuprofen, naproxen, diclofenac and indomethacin), antipyretic drugs (acetaminophen), peroxisome proliferators (clofibrate and gemfibrozil), antihypertensive drugs (methyldopa), anticonvulsants (carbamazepine, valproic acid, phenobarbital and phenytoin), selective seratonin reuptake inhibitors (SSRIs) (fluoxetine hydrochloride, fluvoxamine maleate and sertraline), beta-blockers (propranolol, metoprolol and atenolol), progestins (ethynodiol diacetate, norethindrone and levonorgestrel) and estrogens (17α-ethynylestradioi), antibiotics (sulfamethoxazole–trimethoprim combination, tetracycline, doxycycline, minocycline and erythromycin). The key factors which affected inclusion of PPs in the list were: evidence of adverse effects related to fertility and fecundity, production volume, presence and persistence in the environment, and ability to reach target populations through relevant exposure pathways. The results of the findings are included in the description of PPs.

The risk assessment process for compounds present in food is discussed in Section 18.14, starting from the classical components: hazard identification, dose–response assessment, exposure assessment and risk characterisation. Special attention is given to approaches currently accepted by regulatory agencies. Additionally, owing to limitations of adopted techniques, a probabilistic assessment is described for both dose–response and exposure assessment, which allows replacing point estimates with the range of plausible values together with their uncertainties. Finally, {quantitative) structure–activity relationships models are discussed. These models act as a supportive tool in the risk assessment process and are potentially capable of reducing the number of animal tests.

There are significant gaps in the knowledge of whether and how pharmaceutical residues reach effective exposure levels and affect human fecundity owing to a lack of dedicated research. However, the growing amount of literature devoted to assessment of PPs in relation to fecundity indicates increased concern and recognition of this class of chemicals as being able to cause problems. There are ample indications of endocrine-modulating effects of these compounds. On the other hand, critical data on many aspects of PPs' mechanism of action, production volumes, environmental concentrations and persistence are missing. This inventory is a compilation of our knowledge of PPs, and priotitises compounds of concern for further study of concentrations in food matrices as a basis for a better informed risk assessment.

18.2 Classification of the mechanisms by which pharmaceuticals affect fecundity

18.2.1 Infertility

Infertility is one element of a spectrum of reproductive disorders that includes miscarriage, congenital abnormality, premature delivery and stillbirth (Gnoth *et al.*, 2005; Waghmarae, 1972). Infertility, defined as the failure to conceive after two years of unprotected intercourse, is fairly common, affecting about 15% of all couples at some time during their reproductive lives (Fernandez *et al.*, 1992; Kolettis, 2003). It is generally detected only when a couple is actively trying to conceive. It can be difficult to draw firm conclusions about trends in infertility rates but the high number of patients currently attending fertility clinics suggests a growing problem. Causes of infertility in women include failure of ovulation, tubal damage, endometriosis and hostile cervical mucus (Olive *et al.*, 2003; Wardle et *al.*, 1985; Zawar et *al.*, 2003). In men, sperm defects, coital factors such as impotence or retrograde ejaculation, and hypogonadism may be implicated (Boyd, 1988; Oehninger & Alexander, 1991). In as many as 30% of cases, a cause cannot be found (Tadokoro *et al.*, 1997). Drugs and other toxins may be responsible in a number of cases, but, in general, the effects of drugs on fertility have been poorly studied. The activity of the gonads (testes or ovaries) is regulated by the pituitary gonadotrophins, follicle-stimulating hormone (FSH) and luteinising hormone (LH) (Knobil, 1988a). Secretion of both hormones is controlled by gonadotrophin-releasing hormone (GnRH) from the hypothalamus (Knobil, 1988b, 1990; Weiner, 1996). FSH regulates the development of Sertoli cells (which are involved in sperm maturation) in the testes, and the Graafian follicle in females. LH controls formation of the corpus luteum in females and testosterone production by the Leydig cells in males. Both FSH and LH regulate estrogen production and ovulation. Decreased amounts of FSH and/or LH reaching the testes can inhibit spermatogenesis.

About 30% of infertile women have anowlatory infertility (Baird, 1979). They may be present with amenorrhoea (primary or secondary), oligomenorrhoea (infrequent or irregular periods) or occasionally with regular menstrual cycles but low or undetectable serum progesterone concentrations in the putative luteal phase. Secondary amenorrhoea is defined as the absence of menstruation for at least six months in a woman with previously normal and regular menses (Marti, 1991). Hyperprolactinaemia is a common finding in women with amenorrhoea or oligomenorrhoea (Godo, 1984; Judd *et al.*, 1976; Molitch, 1992); this can be drug-induced. Drugs known to increase prolactin include methyldopa (Arze *et al.*, 1981), metoclopramide (Anclerson *et* al., 1981; Rossi *et al.*, 2002), cimetidine (Gonzales-Villapando *et al.*, 1980), phenothiazines (Yarkoni *et al.*, 1978) and oestrogens (Furuhjelm et *al.*, 1980). Amenorrhoea is also associated with high-dose corticosteroids (Turkington & MacIndoe, 1972), danazol (Dmowski, 1988) and isoniazid (Klein *et al.*, 1936).

18.2.2 Sexual dysfunction

Sexual function may be divided into three categories reflecting the sexual response cycle: (I) libido or sexual desire; (2) arousal, including erectile function in men and lubrication in women; and (3) release. Drugs can affect one or more areas of the response cycle. Understanding of the sexual response remains incomplete but there is evidence of dopaminergic, adrenergic, muscarinic and serotonergic involvement. In general, increase in sexual behaviour by dopamine (Giuliano & Allard, 2001) and inhibition by serotonin (Barnes *et al.*, 1979) have been reported. Libido is influenced by reproductive hormones and by the emotional and physical health of the individual. Testosterone is necessary for normal sexual arousal, probably in both men and women, and in men testosterone deficiency is associated with impotence (Buvat, 2003).

181.3 Sexual dysfunction in men

The aetiology of erectile dysfunction is often vascular but other contributory factors include drug therapy, endocrine disease and neurological dysfunction (Hafez & Hafez, 2005). Male sexual function depends on the coordination of neurogenic, hormonal and psychological mechanisms and disruption of one or more of these may result in erectile dysfunction. About 25% of cases of erectile dysfunction are believed to be drug-induced (Keene & Davies, 1999; Sidi *et al.*, 1986). The classes of drugs most frequently implicated are antihypertensives (Della *et al.*, *2003;* Dusing, 2005; Kloner, 2003), antidepressants (Labbate *et al.*, 2003; Rosen and Marin, 2003; Rudkin *et al.*, 2004), antipsychotics (Segraves, 1988), (Compton & Miller, 2001) and anti-epileptics (Smaldone *et al.*, 2004). Ejaculation is achieved via stimulation of alpha-adrenergic receptors, leading to contraction of the smooth muscle of the prostate, seminal vesicles and vas deferens. Disorders of ejaculation comprise ejaculatory failure and retrograde ejaculation in which semen passes into the bladder. A number of drugs have also been implicated in these disorders. High rates of erectile dysfunction and ejaculatory failure are associated with the older adrenergic blockers reserpine (Cameron *et al.*, 1996; Dail *et* al., 1987) and guanethidine (Moss & Procci, 1982), which are no longer used. Clonidine (Beeley, 1984; Hedlund & Andersson, 1985) and methyldopa (Melman *et al.*, 1984; Newman & Salerno, 1974) have also caused loss of libido, erectile dysfunction and ejaculatory failure. The alpha-adrenergic blockers indoramin (Holmes & Sorkin, 1986; Pentland et *al.*, 1981) and prazosin (Hedlund & Andersson, 1989; Smith & Talbert, 1986) can cause ejaculatory failure and retrograde ejaculation.

The incidence of sexual dysfunction in men taking diuretics is between two and six times higher than in men taking placebo (Chang *et al.*, 1991). Thiazides may cause reduced libido, erectile dysfunction and problems with ejaculation (Joseph & Schuna, 1990; Muller el *al.*, 1991). The underlying mechanism is unclear as thiazides lack significant hormonal, autonomic or central nervous system effects but a direct effect on smooth muscle is thought to be responsible. Erectile dysfunction is well documented with propranolol and can occur with other beta-blockers (Bathen, 1978; Frances & Kaplan, 1982; Silvestri et *al.*, 2003). The problem is more likely with lipid soluble beta-blockers but has also been reported with atenolol (Morrissette *et* al., 1993; Silvestri *et al.*, 2003) and *with* ophthalmic timolol (Fraunfelder & Meyer, 1985; Katz, 1986). Reduced perfusion pressure caused by a drop in blood pressure or a direct effect on smooth muscle may be responsible. Calcium channel blockers seem to cause fewer problems with sexual function than diuretics or beta-blockers although there are several published case reports of erectile dysfunction (Fövaeus *et al.*, 1987; Sparwasser *et al.*, 1998).

18.2.4 Sexual dysfunction in women

In women, sexual dysfunction has not been thoroughly investigated and the underlying mechanisms are not fully understood. Most reported problems relate to orgasm dysfunction, reduced lubrication or loss of libido. Thioridazine has been known since 1961 to inhibit ejaculation in men but it was not until 20 years later that the first report of inhibition of female orgasm was published (Shen & Park, 1982; Shen & Sata, 1983, 1990).

Failure to achieve orgasm is one of the most common sexual adverse effects of psychotropic drugs in women. This problem has been described with antidepressants (tricyclics or TCAs, monoamine oxidase inhibitors or MAOIs, and SSRIs). Such effects have also been reported with MAOIs (Lesko *et al.*, 1982; Moss, 1983; Pohl, 1983; Shen & Mallya, 1983), TCAs (Cohen & Bartlik, 1998), clozapine (Hummer *et al.*, 1999), risperidone (Kelly & Conley, 2006; Wirshing *et al.*, 2002) and the antihypertensives clonidine and methyldopa (Beeley, 1984; Smith & Talbert, 1986).

18.2.5 Altered libido

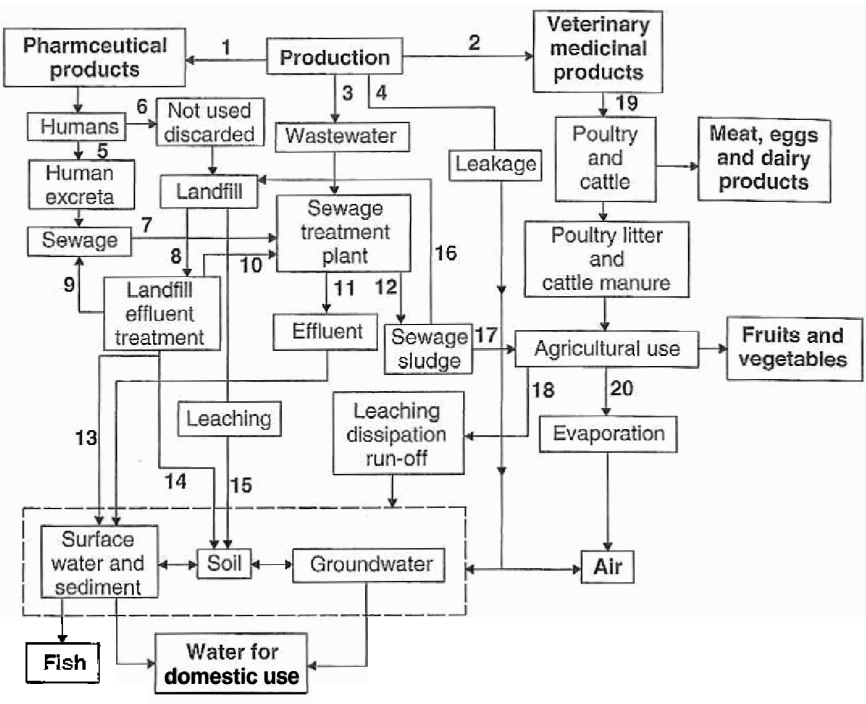
Loss of libido or sexual desire is frequently attributed to medication in both men and women. In women, loss of libido is the commonest reported form of sexual dysfunction; it is extremely difficult to quantify and manage. Changes in desire may be due to illness, stress or fatigue, or may be drug-induced. In controlled studies women have rarely been questioned about the effect of medication on sexual function and therefore most reports of altered libido are anecdotal or case reports.

Several antihypertensives, including clonidine and methyldopa, reduce female libido (Beeley, 1984; Cavalier, 1995; Meston *et al.*, 1997), Studies of both men and women taking methyldopa report an incidence of decreased libido ranging from 7 to 14% (Chowdhury, 1987; Weiss, 2991). Spironolactone has anti-androgenic effects and is clearly linked with decreased libido (Cuttler *et al.*, 1979; Mantero & Lucarelli, 2000). Psychotropic drugs affect sexual desire in men and women by several possible mechanisms, including sedation, effects on central or peripheral neurotransmitters, or effects on hormones (e.g., prolactin) (Clayton & Shen, 1998). Antidepressants have been reported to decrease sexual desire (Rosen *et al.*, 1999). MAOIs, particularly phenelzine, are frequently implicated (Gupta *et al.*, 1999; Warneke, 1994). The SSRIs have all been reported to decrease libido, possibly as a consequence of an indirect effect on dopamine; the incidence in men and women may be as high as 40% (Kanalay & Berman, 2002; Meston, 2004; Montejo-Gonzales *et al.*, 1997; Rosen *et al.*, 1999). In general, rates of sexual dysfunction appear to be greatest with the SSRIs, followed by MAOIs then TCAs. Rates of sexual dysfunction appear to be similar for all the SSRIs and it is not known if switching between them will diminish sexual side effects.

Case reports of decreased libido with anxiolytics have been published; centrally mediated sedation and muscle relaxation are thought to be responsible. Cimetidine has been reported to cause loss of libido, possibly because of its anti-androgen activity (Biron, 1979; Pierce, 1983; Webster, 1979). This is likely to be dose-related. The influence of testosterone on libido is well recognised and any drug that reduces serum testosterone may lead to a loss of sexual desire. In men, this includes drugs such as estrogens (Matuszkiewics-Rowinska *et al.*, 1999), anti-androgens (Bancroft *et al.*, 1974; Holzbeierlein *et al.*, 2004) and gonadorelin analogues (Falkson *et al.*, 1993; Holzbeierlein *et al.*, 2003; Kher & Kalla, 1996).

38.3 Exposure pathways of pharmaceutical products in food

The main exposure pathways for pharmaceuticals ending in human food chain are shown in Fig. 18.1. In Fig. 18.1 the links between soil, surface water and groundwater are emphasised, indicating that any contaminant in one of these media may eventually contaminate the other two. There are three



Pig. 18.1 Exposure **pathways** of pharmaceutical products that **may end** up in **human** food chain.

main **pathways through** which dispersion of pharmaceuticals in the environment may occur: (i) the **local pathway in** the vicinity of the production line; (ii) the distribution/dispersion through farmed animals; (iii) the distribution/dispersion due to consumption by patients. Pathways (ii) and (iii) are named distribution/dispersion because initially the distribution of the pharmaceuticals takes place to patients/farmed animals and in the next stage the dispersion in the environment occurs through human excreta or poultry litter or cattle manure.

The local pathways would depend on the level of security implemented at the production line and would be represented by the main pathways consisting of **(4)** in Fig. 18.1 in the form of Ieakage in the soil, groundwater, surface water or air and by (3) in the form of wastewater which is discharged directly or after pre-treatment into the sewage treatment system. Another pathway **would be** related to disposal, incineration or treatment of waste products, which is not shown in **the** figure. In this study we presume that the local exposure pathways are negligible due to proper safety mea**sures** implemented at the production **site.**

The second exposure pathway is through veterinary medicinal products administered to food-producing animals, in Fig. 18.1 represented by (2). This link provides a quite short and direct link between the drug and supply food chain for human consumption. As can be seen in the figure, after the veterinary medicinal product has been administered to the animal it can end up in the meat products, eggs and through milk into dairy products. In the EU Directive 2001/82/EC of the European Parliament and of the Council on the Community code relating to veterinary medicinal products amended by Directive 2004/28/EC of the European Parliament regulate the use of veterinary medicinal product in the EU and if these directives are followed no adverse effects should occur in consumers. The main cause for concern is the possibility that some substances are used by farmers without proper control, in which case the residues of the drugs may end up in food for human consumption. This pathway can lead to a less direct link to human and environmental exposure through animal excreta which may contaminate the soil, surface waters and groundwater around farms. In some cases poultry litter and cattle manure are used in agriculture and it may be that in some cases the residues and metabolites end up in agricultural products for human consumption. However, more research in this area is required, There is a possibility that the drug residues are dispersed due to leaching and run-off, while dispersion through air due to evaporation from fields where poultry litter and cattle manure are applied is likely to be negligible.

The third pathway, denoted as route 1 in Fig. 18.1, considered in this study is through pharmaceutical products consumed by patients. It is presumed that there are two main routes for the drugs to end up in the environment. The first one is by patients discarding the unused drugs in domestic waste which further may end up in landfills (route 6). Leaching from landfills may contaminate the soil and groundwater, though the soil would represent a filter which would reduce the amount of drug residues that would reach groundwater. However, in some cases the groundwater table may rise to the bottom of the landfill, establishing a direct link to the leachate. The landfill effluent after treatment may be discarded in surface waters or soil from where it can contaminate groundwater. Also, in some cases the landfill effluent may be discharged in the Iocal sewage system or directly into the sewage treatment plant for further treatment. After the passage through the sewage treatment plant when part of the drug residual would be removed, it may be discharged in surface waters or soil. Also, it may be present in the sewage sludge which may further be used in agriculture. Such application is regulated within the EC by the Council Directive 86/278/EEC on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture.

From the above considerations it is evident that water is a very important medium for transport of PPs, since the PP residues can potentially end up in the sewage system and sewage treatment works through different pathways and from there can be dispersed in the environment. Also, water is essential for sustaining life on our planet and therefore any contaminant ending up in surface water or groundwater can have a large impact on the safety of humans and environment.

18.4 Pharmaceutical products potentially affecting human fecundity and their mechanism of action

One of the objectives of this work was to create a prioritisation list of PPs bearing a potential of affecting human fecundity by entering the food chain. The list is based on an **extensive** Literature search while considering the following criteria:

- Does the available data indicate existence of a mechanism of action with an effect on fecundity?
- Is the production volume sufficiently large to cause concern?
- Has the PP been detected in food and/or environment?
- Is the PP sufficiently persistent in the environment?

In this chapter pharmaceutical compounds which have been selected according to the above selection criteria are evaluated in detail, prior to further investigation in the EU-FP6 project Food & Fecundity (F&F). The compounds investigated belong to the following groups of pharmaceutical products: NSAIDs, antipyretic drugs, peroxisome proliferators, antihypertensive drugs, anticonvulsants, SSRIs, beta-blockers, steroid contraceptives and antibiotics.

18.5 Non-steroidal anti-inflammatory drugs

The production volume of NSAIDs is high and NSAIDs are prescribed in high amounts. In addition, they have been detected in environmental samples, albeit at low concentrations. Mechanistically, NSAIDs may play a role in at least one type of female infertility involving disruption of sex hormone homeostasis. Prostaglandin inhibition appears to increase the incidence of Iuteinised unruptured follicle syndrome, a condition in which normal ovarian follicular development is followed by an elevation of serum progesterone compatible with ovulation, but the cycle remains anovulatory because the follicular wall remains unruptured (Killick & Elstein, 1987; Marik & Hulka, 1978). Rat and rabbit studies have reported ovulation inhibition in association with the prostaglandin inhibitor, indomethacin (Armstrong & Grinwich, 1972; O'Grady *et al.*, 1972; Espey *et* el,, 1982). The currently available **animal** data have raised an as-yet unresolved dispute about the possible fertility effects of NSAIDs. In women, ultrasound scans of follicular development have been used to show **a** fivefold increase in the incidence of this syndrome in the presence of some NSAIDs (Killick & Elstein, 1987). The prolonged use of **NSAIDs,** which may occur in the

Table 18.1 The consumption of NSAIDs in the Netherlands in defined daily dose (DDD)

Pharmaceutical,	2000	2001	2002	2003	2004
Ibuprofen	29093500	30426100	31550800	31926100	24283400
Naproxen	36432400	36715000	35531500	33585600	29445500
Indomethacin	4404800	3934600	3574500	3316100	2890100
Diclofenac	54018300	53731200	52343500	51529900	48853700

treatment of chronic pain and inflammation of rheumatological conditions, is most likely to be associated with this antifertility effect. Similar findings have been reported for both COX-1 and COX- 2 NSAIDs (Killick & Elstein, 1987; Pall *et al.*, 2001).

In conclusion, the low environmental levels argue against an actual risk of NSAID residues for human health. The high production and use, in addition to possible fertility effects with a mechanistic plausibility argue towards the opposite.

NSAIDs may be considered part of PCPP (pharmaceuticals and personal care products), which is a large and very varied group of chemicals for which it is not possible to make general statements on their relevance for F&F. The consumption of NSAIDs in the Netherlands in defined daily dose (DDDs) was estimated as given in Table 18.1.

18.5.1 Ibuprofen

Ibuprofen may inhibit follicular collapse, bul this effect is only seen in a small group of study subjects (Uhler *et al.*, 2001). Ibuprofen has been detected in the environment:

- STP effluent, Italy, 0.121 μg/L (Zuccato *et al.*, 2005)
- STP effluent, Finland, 0.004–0.064 μg/L (Lindqvist *et al.*, 2005)
- STW effluent, UK, median 3.086 μg/L (Ashton *et al.*, 2004)
- STP effluent, Källby, Sweden, 0.15 μg/L (Bendz *et al.*, 2005)
- STP effluents, survey, 0.05–7.11 μg/L (Andreozzi *et al.*, 2003)
- German rivers, <0.005–0.139 μg/L (Halling-Sørensen *et al.*, 1998)
- Effluent sedimentation tank, up to 12 μg/L (Halling-Sørensen *et al.*, 1998)
- River Elba, up to 0.024 μg/L (Wiegel *et al.*, 2004)
- STP effluent, California, USA, 0.007–0.037 μg/L (Gross *et al.*, 2004)
- Santa Ana River, California, USA, 0.013–0.151 μg/L (Gross *et al.*, 2004)
- STP effluent, Switzerland, up to 2.2 μg/L (Tauxe-Wuersch et *al.*, 2005)
- STW effluent, UK, 1,979--4.239 μg/L (Roberts & Thomas, 2006)
- River Tyne, UK, 0.144–2.370 μg/L (Roberts & Thomas, 2006)
- STP effluents, Canada, 0.079–1.885 μg/L (Metcalfe *et* al., 2003)

Ibuprofen has been detected in German drinking water at 0.003 µg/L (Webb et *al.*, 2003).

18.52 Naproxen

Naproxen significantly reduced ovulatory efficiency and progesterone (PG) production both in *vivo* and *in vitro* in human chorionic gonadotropin (hCG)-treated rabbits (Zanagnolo *et al.*, 1996). Naproxen has been detected in the environment:

- Sewage treatment plant (STP) effluent, Finland 0.017–0.057 µg/L (Lindqvist *et al.*, 2005)
- River Elbe, up to 0.032 µg/L (Wiegel et *al.*, 2004)
- STP effluent, Källby, Sweden, 0.25 µg/L (Bendz *et al.*, 2005)
- STP effluents survey, 1.12–5.22 µg/L (Andreozzi *et al.*, 2003)
- STP effluent, California, USA, 0–0.089 µg/L (Gross *et al.*, 2004)
- Santa Ana River, California, USA, 0–0.022 µg/L (Gross *et* al., 2004)
- STP effluents, Canada, 0.021–0.524 µg/L (Metcalfe et al., 2003)

18.5.3 Diclofenac

Diclofenac inhibits ovulation in the rat and rabbit (Armstrong & Grinwich, 1972; Espey, 1983; O'Grady *et* nl., 1972). Diclofenac delays implantation in the rat (Carp *et al.*, 1988). Diclofenac has been detected in the environment:

- STP effluent, France, 0.25–0.41 µg/L (Ferrari *et al.*, 2003)
- STP effluent, Greece, 0.89 µg/L (Ferrari *et al.*, 2003)
- STP effluent, Italy, 0.47–5.45 µg/L (Ferrari *et al.*, 2003)
- German rivers, 0.015–0.49 µg/L (Halling-Sørensen *et* **al.**, 1998)
- STW effluent, UK, median 0.424 µg/L (Ashton et *al.*, 2004)
- STW effluent, UK, 0.261–0.598 µg/L (Roberts & Thomas, 2006)
- STP effluent, Finland, 0.011–0.040 µg/L (Lindqvist *et al.*, 2005)
- STP effluent, Källby, Sweden, 0.12 µg/L (Bendz *et* **al.**, 2005)
- STP effluents survey, 0.68–5.45 µg/L (Andreozzi el *al.*, 2003)
- STP effluent, Switzerland, up to 1.9 µg/L (Tauxe-Wuersch et *al.*, 2005)
- River Elbe, up to 0.033 µg/L (Wiegel *et al.*, 2004)
- STP effluents, Canada, 0.005–0.359 µg/L (Metcalfe *et al.*, 2003)

Diclofenac has been detected in German drinking water at 0.006 µg/L (Webb *et al.*, 2003).

18.5.4 Indomethacin

Administration of indomethacin has been demonstrated to induce delayed follicular rupture or luteinised unruptured follicle (LUF) in previously ovulating women (Stone et *al.*, 2002). Indomethacin affects fertility: it is concluded that the antifertility effect of indomethacin at the time of implantation is exerted by reducing progesterone concentrations in plasma and uterine fluid, possibly affecting steroidogenesis, and by reducing the percentage of albumin in plasma and in uterine fluid, probably by increasing renal excretion of albumin. These effects of indomethacin provide an environment within the uterus that would not support embryo implantation and development (El Banna et *al.*, 1993). Tndomethacin been detected in the environment:

- German rivers, up to 0.121 µg/L (Halling-Sørensen *et al.*, 1998)
- STP effluents, Canada, 0.010–0.378 µg/L (Metcalfe et *al.*, 2003)

18.5.5 Conclusions on non-steroidal anti-inflammatory drugs

There is little evidence for an adverse effect on fecundity by ibuprofen or naproxen. In addilion, the removal efficiency during sewage treatment for both compounds is higher than 90%. Both diclofenac and indomethacin affect fecundity at least partly through an endocrine-disrupting mechanism and have been detected in the environment. Diclofenac has been detected in STP effluents and even in drinking water. In addition, it is used in higher amounts than indomethacin. Based on these data, diclofenac would be first and indomethacin second in possible risk.

18.6 Antipyretic drugs

18.6.1 Acetaminophen or paracetamol

Acetaminophen or paracetamol is a non-opiate, non-salicylate analgesic and antipyretic drug. It is present in more than 850 over-the-counter and prescription formulas (Prescott, 2000). In humans acetnrninophens can significantly lower basal levels of gonadotrophin and estradiol (Cramer *et al.*, 1998) and can therefore be considered as possible endocrine disrupters. Several in *vivo* animal studies suggest that acetaminophen may also alter some hormone-regulated processes in reproductive tissues, It was reported to reduce the reproductive capacity, testicular weight and spermatogenesis of mice (Reel *et al.*, 1992) and reduced estradiol-induced uterine peroxidase activity and nuclear progesterone receptor protein in immature mice (Patel & Rosengren, 2001). It has been detected in surface water monitoring studies at concentrations of up to 10 µg/L (Boxall, 2004; Daughton & Ternes, 1999; Lam *et al.*, 2004).

18.6.2 Conclusions on acetaminophen

Considering the amount of acetaminophen in use nowadays, presence in the environment and the evidence of its effects on fecundity, this compound is relevant for further investigation,

Table 18.2 The consumption of **lipid** regulators **in the Netherlands** in **defined** daily dose (DDD)

Pharmaceutical	2000	2001	2002	2003	2004
Clofibrate	89 050	83277	72237	59263	53 325
Gemfibrozil	6 202 900	6090700	5727000	5 462 000	5 058 300

18.7 Peroxisome proliferators

Exposure **to some** peroxisome proliferators leads to *toxic* effects on sex organ function, possibly by alterations of steroid hormone metabolism. This mechanism **marks** these drugs as possible endocrine disruptors. Two examples of widely used peroxisome proliferators are the lipid regulators clofibrate and gemfibrozil. The consumption of lipid regulators in the Netherlands in DDD was estimated **as** given in Table 18.2.

18.7.1 Clofibrate

Clofibrate affects hCG and progesterone concentrations (Hashimoto *et* nl., 2004). Clofibrate has a selective stimulatory effect on the hormonal action of estradiol in the mammary gland but not in the uterus (Xu *et al.*, 2001). The clinical **significance** of these **findings is** unknown; however, according to the manufacturer (Ayerst Laboratories, **New** York), clofibrate use has been associated with impotence and decreased libido **in** men. Clofibrate **has** been reported to be uterotrophic to immature female rats (Chandra *et al.*, 1982), but others could not confirm these findings (Ashby *et* al., 1997). Clofibrate has been detected in the environment:

- STP effluent, Italy, 0–0.68 µg/L (Ferrari *et al.*, 2003)
- STP effluent, Sweden, 0.46 µg/L (Ferrari et al., 2003)
- River water, **various** locations, up to 1.75 µg/L (Halling-Sørensen *et al.*, 1998)
- STP effluent, Switzerland, 0.020–0.025 µg/L (Tauxe-Wuersch *et al.*, **2005**)
- **STW** effluent, **UR**, up **to** 0.044 µg/L **(Roberts & Thomas,** 2006)
- STP effluents, Canada, 0.002–0.044 µg/L (Metcalfe *et al.*, 2003)

Clofibrate has been detected in groundwater and surface water up to concentrations 0.100 µg/L (Stalker *et al.*, 2004). Clofibrate has been detected in German drinking water 0.070 µg/L **(Webb** *et al.*, 2003) and in drinking water in the concentration of 0.025–0.100 µg/L (Stolker et *al.*, 2004).

U.7.2 Gemfibrozil

Exposure to environmental levels of gemfibrozil leads to bioconcentration **of** the drug in plasma and a reduction of plasma testosterone levels

(Mimeault *et al.*, 2005). Gemfibrozil affects hCG and progesterone concentrations (Hashimoto *et al.*, 2004). Male rats given about 17 times the average daily human dose of gemfibrozil showed inconsistent and equivocal lower rates of fertility relative to the concurrent controls (FitzGerald *et al.*, 1984). Gernfibrozil is occasionally associated with impotence and decreased libido (Bain *et al.*, 1990; Pizarro *et al.*, 1490). In *vitro* studies using mt tissues have reported that gemfibrozil and other inducers of hepatic peroxisome proliferation may alter the steroidogenic function of Leydig cells (Liu *et al.*, 1996).

Exposure to gemfibrozil results in decreased expression of enzymes that inactivate estradiol. The reported increased expression of aromatase may explain why male rats exposed to gemfibrozil have higher serum estradiol levels. These higher estradiol levels in male rats have been thought to be mechanistically linked to Leydig cell hyperplasia and adenomas (Corton *et al.*, 1997).

Gemfibrozil has been detected in the environment:

- River Elbe, up to 0.027 µg/L (Wiegel *et al.*, 2004)
- STP effluent, Källby, Sweden, 0.18 µg/L (Bendz *et al.*, 2005)
- STP effluent, California, USA, 0.015–0.065 µg/L (Gross *et al.*, 2004)
- Santa Ana River, California, USA, 0.001–0.059 µg/L (Gross *et al.*, 2004)
- STP effluents survey, 0.84–4.76 µg/L (Andreozzi *et al.*, 2003)
- STP effluents, Canada, 0.005–1.493 µg/L (Metcalfe *et al.*, 2003)

18.7.3 Conclusions on peroxisome proliferators

Both clofibrate and gemfibrozil are candidates for further study in view of their endocrine-mediated mechanism of action as well as their environmental detection.

18.8 Antihypertensive drugs

18.8.1 Methyldopa (Aldomet)

Methyldopa is a drug that is used to treat high blood pressure. It works by relaxing the blood vessels so that blood can flow more easily through the body. Methyldopa decreased sperm count, sperm motility, the number of late spermatids and the male fertility index when given to male rats at 200 and 400 mg/kg/day (3.3 and 6.7 times the maximum daily human dose when compared on the basis of body weight; 0.5 and 1 times the maximum daily human dose when compared on the basis of body surface area) (Weiss, 1991). Methyldopa appears in breast milk (Beardmore *et al.*, 2002; White *et al.*, 1985). Methyldopa interferes with sex hormone homeostasis via an increase in prolactin levels. Elevated prolactin serum concentrations inhibit gonadotropin secretion and sex steroid synthesis. Because prolactin concentrations higher than 60 µg/L are associated with anovulation, women with hyperprolactinemia typically present with menstrual irregularities such as oligomenorrhea or amenorrhea and infertility. In addition, approximately 40–70% of women with hyperprolactinemia will have galactorrhea (Arze *et al.*, 1981). Hyperprolactinemia in men, although rare, may cause decreased libido, erectile dysfunction, infertility, galactorrhe, or gynecomastia (Ou et al., 1991; Molitch, 1992). Methyldopa has been detected in the environment:

- River Lee, 17.5 µg/L (Richardson & Bowron, 1985)

18.8.2 Conclusions on methyldopa

Methyldopa is relevant for further analysis, based on its endocrine modulation, reported effects on fecundity and presence in the environment.

18.9 Anticonvulsants

The consumption of anticonvulsants in the Netherlands in DDD was estimated as given in Table 18.3.

18.9.1 Carbamazepine

Carbamazepine affects sex hormone homeostasis through increases in serum sex hormone-binding globulin (SHBG) concentrations in both men and women with epilepsy. Over time, the increase in serum SHBG levels leads to reduced bioactivity of testosterone and estradiol, which may result in reduced potency in men and menstrual disorders in some women, and thus to reduced fertility (Isojarvi *et al.*, 2005). Use of carbamazepine is associated with changes in serum sex-hormone levels and sperm abnormalities in men with epilepsy (Isojarvi *et al.*, 2004; Mikkonen *et* nl., 2004). However, Roste *et al.* (2003) could not demonstrate any significant changes in semen quality. Male rats fed carbamazepine for 30–60 days had decreased

Table 18.3 The consumption of anticonvulsants in the Netherlands in defined daily dose (DDD)

Pharmaceutical	2000	2001	2002	2003	2004
Carbamazepine	10352500	10216900	10175100	10134200	10028900
Valproic acid	10877000	11378900	12017200	12648200	13301600
Phenobarbital	3278100	3240900	3034300	2962300	2972100
Phenytoin	6288200	5893700	5550700	5338900	5175200

testicular weight, sperm cell concentration, live sperm, and percentage of progressively motile spermatozoa (Soliman *et al.*, 1999). Carbamazepine is highly persistent in the environment:

- STP effluent, Berlin, Germany, >1 µg/L (Zuehlke *et al.*, 2004)
- Several STP effluents, Italy, 0.3 µg/L (Zuccato *et al.*, 2005)
- STP effluent, Källby, Sweden, >1 µg/L (Bendz *et al.*, 2005)
- River Elbe, Germany, >1 µg/L (Wiegel *et al.*, 2004)
- STP effluent, France, 0.98–1.2 µg/L (Wiegel *et al.*, 2004)
- STP effluent, Greece, 1.03 µg/L (Wiegel et *al.*, 2004)
- STP effluent, Italy, 0.3–0.5 µg/L (Wiegel *et al.*, 2004)
- STP effluent, Sweden, 0.87 µg/L (Wiegel *et al.*, 2004)
- STP effluents survey, 0.87–1.2 µg/L (Andreozzi *et al.*, 2003)
- STP effluent, Peterborough, Canada, 0.251 µg/L (Miao & Metcalfe, 2003)
- STP effluents, Canada, 0.007–0.126 µg/L (Metcalfe et *al.*, 2003)

Carbamazepine has been detected in groundwater up to concentrations of 1.1 µg/L (Heberer, 2002; Stolker et *al.*, 2004). Carbamazepine has been detected in drinking water in the concentration of 0.030 µg/L (Heberer, 2002; Webb *et al.*, 2003) and up to 0.025 µg/L (Stolker *et al.*, 2004).

18.9.2 Valproic acid

Valproic acid medication is possibly endocrine disrupting as it may modulate serum androgen concentrations and it reduces serum FSH levels in men with epilepsy. In women, use of valproic acid appears to be associated with a frequent occurrence of reproductive endocrine disorders characterised by polycystic changes in the ovaries, high serum testosterone concentrations (hyperandrogenism) and menstrual disorders (Isojarvi *et al.*, 2005). Use of valproic acid is associated with changes in serum sex-hormone levels, sperm abnormalities and a lower testicular size/body mass index (BMI) ratio in men with epilepsy (Mikkonen *et al.*, 2004; Roste *et al.*, 2003). Valproic acid has not been detected in the environment so far.

18.9.3 Phenobarbital

Phenobarbital increases serum SHBG concentrations in both men and women with epilepsy which influences sex hormone homeostasis. Over time, the increase in serum SHBG levels leads ta reduced bioactivity of testosterone and estradiol, which may result in reduced potency in men and menstrual disorders in some women, and thus to reduced fertility (Isojarvi *et al.*, 2004). Phenobarbital inhibits the biological clock control of ovulation in hamsters (Alleva & Alleva, 1995). Phenobarbital delays ovulation and affects oocyte function in the rodent (Stoker *et al.*, 2001). Phenobarbital has not been detected in the environment *so* far.

18.9.4 Phenytoin

Phenytoin is an endocrine modulator as it increases serum SHBG concentrations in both men and women with epilepsy. Over time, the increase in serum SHBG levels leads to reduced bioactivity of testosterone and estradiol, which may result in reduced potency in men and menstrual disorders in some women, and thus to reduced fertility (Isojarvi *et al.*, 2004). Phenytoin inhibits both the first ovulation and uterine development in gonadotropin-primed immature rats (Tamura *et al.*, 2000). Phenytoin has not been detected in the environment so far.

18.9.5 Conclusions on anticonvulsants

Several anticonvulsants affect fecundity through an endocrine-disrupting mechanism and are consumed in large quantities, but only carbamazepine is highly persistent and bas been detected in STP effluents, in groundwater and even in drinking water.

18.10 Serotonin reuptake inhibitors

SSRIs are a class of antidepressants. They act within the brain to increase the amount of the neurotransmitter, serotonin (5-hydroxytryptamine or 5-HTP), in the synaptic gap by inhibiting its reuptake.

18.10.1 Fluoxetine hydrochloride (Prozac)

There was a significant increase in the incidence of sexual dysfunction (i.e. delayed orgasm or ejaculation, impotence) in humans taking fluoxetine. Sexual dysfunction was positively correlated with dose. Individuals experienced substantial improvement in sexual function when the dose was diminished or the drug was withdrawn. Men showed more incidence of sexual dysfunction than women, but women's sexual dysfunction was more intense than men's (Gregorian et al., 2002; Hu *et al.*, 2004; Montejo-Gonzalez *et al.*, 1997; Montgomery *et al.*, 2002). Fluoxetine affects sex hormone homeostasis through the elevation of prolactin levels, and a modest elevation persists during administration; however, possibly associated clinical manifestations (e.g. galactorrhea and breast enlargement) were observed (Ficicioglu *et al.*, 1995; Haddad & Wieck, 2004; Jorgensen *et al.*, 1996; Masala *et al.*, *1979;* Meltzer *et* al., 1982). Decreased ovary weight, and corpora luteal depletion and uterine atrophy were observed in females receiving fluoxetine alone (Cortes *et al.*, 1978; Fell *et al.*, 2004,2005).

In rat reproduction studies, there is an increase in stillborn pups, a decrease in pup weight and an increase in pup deaths following maternal exposure to fluoxetine during gestation and during both gestation and lactation (Nulman & Koren, 1996; Stanford & Patton, 1993). The effect of fluoxetine on labour and delivery in humans is unknown. Fluoxetine crosses the placenta; therefore, there is a possibility that Ruoxetine may have **adverse** effects on the newborn (Gentile, 2005; Heikkine et ***al.,*** 2002; Hendrick *et al.*, 2003; Morisson *et al.*, 2005; **Pohland** ***et*** *al.*, 1989). In humans, the relatively slow elimination of fluoxetine (elimination half-life of 1–3 days after acute administration and 4-6 days after chronic administration) and its active metabolite, norfluoxetine (elimination half-life of 4-6 days after acute and chronic administration), leads to significant accumulation of these active species in chronic use and delayed attainment of **steady** state, even when a fixed dose is used. After 30 days of dosing at 40 mg/day, **plasma** concentrations of fluoxetine in the range of 91–302 ng/ml and norfluoxetine in the range of 72–258 ng/ml have been observed. Plasma concentrations of fluoxetine were higher than those predicted by single-dose studies, because fluoxetine's metabolism is not proportional to dose. Norfluoxetine, however, appears to have linear pharmacokinetics. Its mean terminal half-life after **a** single dose **was** 8.6 days and after multiple dosing **was** 9.3 days. Steady state levels after prolonged dosing are similar **to** levels seen at 4–5 **weeks.** The Iong elimination half-lives of fluoxetine and norfluoxetine ensure that, even when dosing is stopped, active drug substance will persist in the body for weeks (primarily **depending** on individual characteristics, previous dosing regimen, and length of previous therapy at discontinuation). This is of potential consequence when drug discontinuation is required or **when** drugs are prescribed that might interact with fluoxetine and norfluoxetine following the discontinuation of fluoxetine (Johnson ***et*** *al.*, 2005; Wilens *et al.*, 2002; Young & Ashton, 1996).

Fluoxetine and its metabolite norfluoxetine were detected at levels greater **than** 0.1 ng/g **in** all tissues examined **from** fish residing **in** a municipal effluent-dominated stream in North **Texas, USA (Brooks et** *al.*, 2005). Fluoxetine was detected in most STP effluents and **some** surface water samples in the Iower Great Lakes (Lake Ontario and Lake Erie), at sites near the two STPs for the city of Windsor (ON, Canada), and at sites in Hamilton Harbour (ON, Canada) (Metcalfe ***et*** *al.*, **2003). According** to a BBC report (BBC, 2004) traces of the fluoxetine can be found in the **drinking** water according to the UK Environment Agency.

18.10.2 Fluvoxamine maleate (Luvox)

Similar to fluoxetine, fluvoxamine also cause **a** significant increase in the incidence of **sexual** dysfunction (i.e. delayed **orgasm** or ejaculation, impotence) in humans. Sexual dysfunction **was** positively correlated with dose. Individuals experienced substantial improvement in sexual function **when** the dose was diminished or the drug was withdrawn. Men showed more incidence of sexual dysfunction than women, but women's sexual dysfunction was more intense than men's (Dorevitch & Davis, 1994; Gregorian el ***al.,*** **2002;** Montejo-Gonzalez ***et*** *al.*, 1997; Montgomery *et al.*, 2002). In another study in humans, the incidence of sexual dysfunction during fluvoxamine therapy in healthy volunteers is 35% (Nafziger et *al.*, 1999). Fluvoxamine has an elimination half-life of 15 hours in patients with normal hepatic function. In patients with cirrhosis and the elderly, there may be as much as a 40–50% reduction in clearance and dosing should be adjusted accordingly.

18.10.3 Sertraline (Zoloft)

A decrease in fertility was seen in one of two rat studies at a dose of 80 mg/kg (four times the maximum recommended human dose (MRHD) on a mg/m^2 basis) (Davies and Klowe 1998). When female rats received sertraline during the last third of gestation and throughout lactation, there was an increase in the number of stillborn pups and in the number of pups dying during the first four days after birth. Pup body weights were also decreased during the first four days after birth. These effects occurred at a dose of 20 mg/kg (1 times (i.e. the same as) the MRHD on a mg/m^2 basis). The no effect dose for rat pup mortality was 10 mg/kg (half the MRHD on a mg/m^2 basis). The decrease in pup survival was shown to be due to *in utero* exposure to sertraline. The clinical significance of these effects is unknown. There are no adequate and well-controlled studies in pregnant women. Sertraline and its metabolite desmethylsertraline were detected at levels greater than 0.1 ng/g in all tissues examined from fish residing in a municipal effluent-dominated stream in North Texas, USA (Brooks *et al.*, 2005).

18.10.4 Conclusions on serotonin reuptake inhibitors

In previous studies fluoxetine hydrochloride showed clear effects on fecundity. Although environmental levels of fluoxetine hydrochloride may be low, the mere fact that its half-life is very long (days instead of hours, as it is for most pharmaceuticals) means that it can persist in the body for weeks and accumulate due to prolonged exposure. Fluvoxamine's half-life is also relatively long and clearly has an effect on fecundity but no data on environmental levels are available. Data on sertraline also shows possible effect on fecundity but there is no data on environmental levels in the European setting. For fluoxetine, an endocrine mechanism of fecundity effects has been indicated. For both other compounds the situation is less clear, but in view of their common primary mechanism of action further study of this class of compounds is warranted.

18.12 Beta-blockers

The consumption of beta blockers in the Netherlands in DDD was estimated as given in Table 18.4.

Table 18.4 The consumption of beta blockers in the Netherlands in defined daily dose (DDD)

Pharmaceutical	2000	2001	2002	2003	2004
Propranolol	9300300	9092100	8967400	9025200	9030500
Metoprolol	80386700	86849600	92654400	98935200	108001000
Atenolol	57244000	60216300	62832400	66683700	70251900

18.11.1 Propranolol

Propranolol has been identified as having endocrine-disrupting potential as it affects both total and free testosterone (Rosen *et al.*, 1988; el Sayed et al., 1998). Propranolol induces a significant decrease in percent of progressive motility of sperm, a significant increase in sperm head and tail abnormalities, and histopathological alterations in testis, epididymis and seminal vesicles (el Sayed *et al.*, 1998). Studies on the binding of propranolol in rat Leydig cell cultures suggest that propranolol is capable of inhibiting testosterone synthesis in the testis (Tinajero *et al.*, 1993). Propranolol acts in *vitro* as a spermicide for human sperm at a concentration of about 2 x 10^{-3} M (Zipper et *al.*, 1982). Propranolol has been detected in the environment:

- STP effluent, Källby, Sweden, 0.03 µg/L (Bendz et *al.*, 2005)
- STP effluents survey, 0.01–0.09 µg/L (Andreozzi et *al.*, 2003)
- STW effluent, UK, 0.195–0.373 µg/L (Roberts & Thomas, 2006)
- STW effluent, UK, median 0.076 µg/L (Ashton *et al.*, 2004)

18.11.2 Metoprolol

Metoprolol affects both total and free testosterone levels (Rosen et *al.*, 1988; el Sayed *et al.*, 1998). Metoprolol induces a significant decrease in percent of progressive motility of sperm, a significant increase in sperm head and tail abnormalities, and histopathological alterations in testis epididymis and seminal vesicles (el Sayed et *al.*, 1998). Metoprolol has been detected in surface water up to concentrations of 0.100 µg/L (Stolker et *al.*, 2004). Metoprolol has been detected in the environment:

- STP effluent, Källby, Sweden, 0.19 µg/L (Bendz *et al.*, 2005)
- STP effluents survey, 0.08–0.39 µg/L (Andreozzi *et al.*, 2003)
- River Saale, >0.100 µg/L (Wiegel *et al.*, 2004)

18.11.3 Atenolol

Atenolol affects both total and free testosterone levels (Rosen *et al.*, 1988; el Sayed *et al.*, 1998). Atenolol induces a significant decrease in percent of progressive motility of sperm, a significant increase in sperm head and tall abnormalities, and histopathological alterations in testis, epididymis and seminal vesicles (el Sayed *et al.*, 1998). Atenolol causes a significant reduction in testosterone release by rat Leydig cells (Fogari *et al.*, 2002; Khan et *al.*, 2004). Atenolol has been detected in the environment:

- STP effluent, Källby, Sweden, 0.14 µg/L (Bendz *et al.*, 2005)
- Several STP effluents, Italy, 0.466 µg/L (Zuccato et *al.*, 2005)

18.11.4 Conclusions on beta-blockers

All three beta-blockers are endocrine modulators as they affect testosterone levels and spermatogenesis. However, metoprolol and atenolol are consumed in far higher quantities than propranolol. In addition, all three compounds have been detected in the environment.

18.12 Steroid contraceptives

General

Table 18.5 is a summary of the steroids currently used as contraceptives. Table 18.6 is a summary of the combined oral contraceptive preparations available on the market.

Pharmacological effects of progestins in oral contraceptives

A number of pharmacological effects contribute to the contraceptive effects of progestins. These include inhibiting ovulation by suppressing the function

Table 18.5 Contraceptive progestins

Class compound	Name	Relative progestational activity (arbitrary units)	Relative androgenic activity (arbitrary units)
19 Nor-testosterone progestins			
Estranes	Norethindrone	1	1
	Norethindrone acetate	1.2	1.6
	Ethynodiol diacetate	1.4	0.6
Gonanes	Levonorgestrel	5.3	8.3
	Norgestrel	2.6	4.2
	Norgestimate	1.3	1.9
	Desogestrel	9	3.4
	Gestodene	12.6	8.6
Pregnane progestins			
	Megestrol acetate	0.4	0
	Medroxyprogesterone acetate	0.3	0

Table 18.6 **Available** combination oral contraceptives

	Name	Progestin (mg)	Type of estrogen (mcg)
50 mcg estrogen	Ogestrel/Ovral	Norgestrel (0.5)	EE (50)
	Necon/Nelova/Norethin/Norinyl/Ortho-Novum 7/50	Norethindrone (1.0)	Mestranol (50)
	Ovcon 50	Norethindrone (1.0)	EE (50)
	Norlestrin 1/50	Norethindrone acetate (1.0)	EE (50)
	Demulen 50/Zovia 1/50	Ethynodiol diacetate (1.0)	EE (50)
<50 mcg estrogen plus monophasic	Lo-Ovral/Low-Ogestrel	Norgestrel (0.3)	EE (30)
	Ovcon 35	Norethindrone (0.4)	EE (35)
	Desogen/Ortho-cept	Desogestrel (0.15)	EE (30)
	Levlen/Levora/Nordette	Levonorgestrel (0.15)	EE (30)
	Ortho-Cyclen	Norgestimate (0.25)	EE (35)
	Necon/Nelova/Norinyl/Norethrin/Ortho-Novum 1/35	Norethindrone (1.0)	EE (35)
	Mircette	Desogestrel (0.15)	EE (20)
	Brevicon/Modicon/Necon/Nelova 0.5/35	Norethindrone (0.5)	EE (35)
	Loestrin 1.5130	Norethindrone acetate (1.5)	EE (30)
	Alesse/Levlite	Levonorgestrel (0.1)	EE (20)
	Locstrin 1/20	Norethindrone acetate (1.0)	EE (20)
	Demulen/Zovia 1/35	Ethynodiol diacetate (1.0)	EE (35)
<50 mcg **estrogen plus multiphasic**	**Ortho-Novum** 7/7/7	Norethindrone (0.5, 0.75, 1.0)	EE (35, 35, 35)
	Tri-Levlen/Triphasil/Trivora	Levonorgestrel (0.05, 0.075, 0.125)	EE (30, 40, 30)
	Jenest	Norethindrone (0.5, 1.0)	EE (35, 35)
	Necon/Nelova/Ortho-Novum 10/11	Norethindrone (0.5, 1.0)	EE (35, 35)
	Ortho Tri-Cyclen	Norgestimate (0.18, 0.215, 0.250)	EE (35, 35, 35)
	Tri-Norinyl	Norethindrone (0.5, 1.0, 0.5)	EE (35, 35)
	Estrostep	Norethindrone acetate (1.0, 1.0, 1.0)	EE (20, 30, 35)

of the hypothalamic–pituitary-ovarian (HPO) axis; modifying the subsequent pituitary surge of LH and FSH; slowing transport of the ovum through the Fallopian tubes, which limits the time available for fertilisation; thickening cervical mucus, which impedes sperm transit; and inhibiting the activation of spermatic enzymes required for ovum penetration (capacitation). Thus, the primary mechanism of oral contraceptives defines these compounds as endocrine disruptors.

Family tree of contraceptive progestins

Synthetic progestins used in oral contraceptives can be classified as those that are structurally related to progesterone or testosterone, Progestins structurally related to progesterone include progesterone itself and medroxyprogesterone acetate compounds that have 21 carbons. Progestins structurally related to testosterone are structural derivatives of testosterone and are not synthesised from testosterone. Removal of the methyl group from the testosterone molecule produces norethindrone, a compound with high progestational activity, high oral activity, and almost no androgenicity. Adding an additional methyl group forms an ethyl group and produces the compound norgestrel, which has even greater progestational activity than norethindrone. Norgestrel is synthesised chemically into dextro-norgestrel, an inactive form, and levonorgestrel, the active form. Another classification of progestins uses the terms gonane or estrane and is based on the number of carbons: gonanes have 17 carbons, and estranes have 18 carbons. A family tree of contraceptive progestins is presented in Table 18.7.

Biologically active forms of progestins

When assessing a contraceptive progestin, several factors need to be considered. The first consideratian is whether the progestin is in active form or needs to be converted. Some progestins are prodrugs that must be converted to biologically active forms. The next is the progestin's affinity for human tissues, including inhibition of ovulation and binding **affinity** to human receptors. The third consideration is the pharmacokinetic profile, including half-life and bioavailability. The clinical relevance of animal data compared with human data should also be assessed. Five estrane progestins are in commercial use. Three of these – norethindrone acetate, ethynodiol

Table 18.7 Family tree **of** contraceptive progestins

Gonanes (levonorgestrel family}	Estranes (norethindrone family)
Levonorgestrel	Norethindrone
Desogestrel	Norethindrone acetate
Norgestimate	Ethynodiol acetate
Festodene	Lynestrenol

diacetate, and lynestrenol – are prodrugs. Before these three can exert progestational activity, they must undergo biochemical conversion to norethindrone, their biologically active form (Stanczyk & Roy, 1990). Levonorgestrel and gestodene ate gonane progestins that are active in their current forms. Desogestrel and norgestimate are prodrugs that must undergo biochemical conversion in the liver. Desogestrel is transformed to 3-keto-desogestrel, which is its only active form, whereas norgestimate is converted to levonorgestrel and levonorgestrel-3-oxime, which are its active forms (Stanczyk, 1997).

Bioavailability of progestins

The extent to which a contraceptive progestin enters the circulation without undergoing hepatic metabolism determines its bioavailability. There is a great deal of interindividual variability in the bioavailability of contraceptive progestins. The range goes from gestodene (>90%) and levonorgestrel (~90%) to the metabolites produced by norgestimate (<25%). Norethindrone and 3-keto-desogestrel (active form of desogestrel) are in the intermediate range at approximately 64% and 62%, respectively (Back et *al.*, 1918, 1981; Orme *et al.*, 1991; Stanczyk & Roy, 1990).

Serum half-lives

Serum half-lives of contraceptive progestins are not absolute values, but change depending on whether women receive progestin only or oral contraceptives which additionally contains an estrogenic compound. Levonorgestrel has been shown to have the longest half-life of 15 hours. Both 3-keto-desogestrel (the active form of desogestrel) and gestodene have half-lives of 12 hours, and the half-life of norethindrone is 7 hours (Fotherby & Caldwell, 1994). All progestins were given in combination with ethinyl estradiol (30–35 µg).

Plasma levels

Plasma levels of norethindrone (1000 µg dose) and levonorgestrel (150 µg dose) after a single oral dose indicate that a considerably higher level of norethindrone (about 14 ng/mL) occurs within the first hour as compared with levonorgestrel (about 2 ng/mL). However, levels of norethindrone fall precipitously to undetectable levels – below 1 ng/mL at 24 hours compared to levonorgestrel, which is still detectable at 48 hours (Stanczyk, 1994).

Relative binding affinities for human uterine progesterone receptor

In vitro studies of uterine progesterone receptor binding of progestins give a range of relative binding affinities (RBAs), depending on the species studied, various study parameters and compounds used for comparison. Compounds such as levonorgestrel (LNG) have a very high affinity for the human uterine progesterone receptor, as does 3-keto-desogestrel (3-

keto-DSG), levonorgestrel-17-acetate (LNG-17-acetate) and gestodene (GSD). Two prodrugs, desogestrel (DSG) and norgestimate (NGM), do not bind to the human uterine progesterone receptor. Among the norgestimate metabolites, levonorgestrel-3-oxime (LNG-3-oxime) has a very low RBA for human uterine progestin receptors, even though serum levels may be high. LNG-17-acetate, however, has substantial progestational activity, but is barely detectable in serum following administration of norgestimate (Juchem *et* al., 1993).

Dose/ovulation inhibition dependence

In studies looking at various progestins combined with 30–35 µg ethinylestradiol (EE), the progestin dose needed for ovulation inhibition varied widely from high doses for norethindrone (approximately 400 µg per day) and norgestimate (200 µg per day), to levonorgestrel and desogestrel (60 µg per day), to smaller doses for gestodene (approximately 30 µg per day) (Teichmann, 1996).

Effect of oral contraceptives on sex hormone binding globulin/testosterone

The results are presented (Van der Vange *et al.*, 1990) of a study comparing seven oral contraceptives with regard to their effect on SHBG, total testosterone (total T), and free testosterone (free T). The oral contraceptives all contained EE (30–40 µg per dose) but different types and doses of progestin. In this study, the increases in SHBG were extremely variable, and total T varied to a lesser degree. (One oral contraceptive, CPA 2000 CPA µg/EE 35 µg, actually caused total T to increase.) Despite these variations, all the oral contraceptives reduced free T to a similar degree. A decrease in free T is considered the most important factor when evaluating the effect of oral contraceptives on acne and other androgenic conditions.

Oral contraceptive effects on androgens

The effects of two 20 µg EE oral contraceptives, LNG 100 µg and norethindrone acetate (NETA) 1000 µg, were compared (Thorneycroft *et al.*, 1999) on androgen levels and acne lesion counts. Patients were evaluated at baseline and during cycle 3 (days 17 to 21) for androgen and SHBG levels, acne lesion count and weight. Results demonstrated that, among the 41 evaluable women at the end of the study, there were statistically significant reductions in all measured androgen levels. At the end of three cycles, both 20 µg EE formulations decreased androgens and increased SHBG from baseline, although the oral contraceptive with NETA increased the mean SHBG more than the oral contraceptive with LNG. Compared with the formulation consisting of EE and LNG, the formulation consisting of EE and NETA was associated with two times greater relative increase in SHBG. At the same time, the formulations had equivalent decreases in bioavailable testosterone.

Oral contraceptive changes in biochemical markers of androgenicity

Changes in biochemical markers of androgenicity were studied in 58 young women (>14 years old) randomised to placebo (n = 29) or a low-dose oral contraceptive, EE 20 μg/LNG 100 μg (*a* = 29). Mean percentage changes from baseline were determined at the end of cycles 4 and 6. Statistically significant ($P < 0.05$) reductions were noted in 3α-androstanediol glucuronide (3a-diol G), as well as marked reductions in the treatment group in androstenedione (A), androsterone glucuronide (AG), dihydrotestosterone (DHT), total testosterone (TT) and dehydroepiandrosterone sulphate (DHEAS), although the reductions were not statistically significant. Statistically significant reductions in the oral contraceptive group were observed for A, AG and 3α-diol G vs. increases with placebo. The oral contraceptive significantly decreased androgen levels in ovarian (A, TT) and peripheral (3α-diol G) compartments as compared to placebo (Stanczyk *et al.*, 2000).

Adverse effects of progestins

Adverse effects of progestins are reviewed for individual compounds below.

18.12.1 Ethynodiol diacetate

Following oral administration of ethynodiol diacetate plus mestranol to mice, increased incidences of pituitary tumours were observed in animals of each sex. Ethynodiol diacetate plus ethinyloestradiol was tested for carcinogenicity by oral administration to mice and rats. In mice, it induced increased incidences of pituitary tumours in animals of each sex and of malignant tumours of connective tissues of the uterus In rats, malignant mammary tumours were produced in animals of each sex (*IARC Monographs*, 1979a).

18.12.2 Norethindrone, norethindrone acetate

Aneuploidy was observed in oocytes of mice treated with high doses of norethindrone acetate. In a test for dominant lethal mutations in which female mice were exposed orally to norethindrone acetate, no increase was seen in one strain of mice, and a second strain showed an increase only when females were mated within two weeks of treatment, However, the compound did not induce aneuploidy or chromosomal aberrations in cultured human lymphocytes. Neither norethindrone nor its acetate was mutagenic to bacteria (IARC *Monographs*, suppl. 1987).

Norethindrone and its acetate were tested by oral administration in mice and rats, and by subcutaneous implantation in mice. In mice, norethindrone and its acetate increased the incidence of benign liver-cell tumours in males; norethindrone increased the incidence of pituitary tumours in females and produced granulosa-cell tumours in the ovaries of females. Norethindrone increased the incidence of benign liver-cell tumours and benign and malignant mammary tumours in male rats (*IARC Monograph,* 1979b). Rats fed 3-4 mg/kg body weight (bw) per day norethindrone acetate (about 100 times the daily human dose) for two years had an increased incidence of neoplastic nodules of the Liver; an increase in the incidence of uterine polyps was seen in females (Schardein, 1980). In rats given weekly intramuscular injections for 104 weeks of norethindrone enanthate at doses of 10, 30 and 100 mg/kg bw (20, 60 and 200 times the daily human contraceptive dose), there was a dose-related increase in pituitary gland tumours in males, whereas in females no effect on pituitary glands was observed with the lowest dose and a reduction in pituitary tumours was observed with the highest dose. Benign mammary tumours were observed in males at all doses, but there was little effect in females; the incidence of malignant mammary tumours was greatly increased in both males and females given the two higher dose levels and was dose-related. A dose-related increase in the incidence of liver tumours was also seen in animals of each sex (El Etreby & Neumann, 1980).

18.12.3 Levonorgestrel

The combination of T plus LNG suppressed sperm production much more than T alone (Bebb *et al.*, 1996). Some 67% of the T plus LNG group achieved azoospermia (33% for T alone group). Severe oligospermia developed in 94% of the T plus LNG group compared with the 61% T alone group. T plus LNG also suppressed sperm production more rapidly than T alone. Time to azoospermia was 9.9 ± 1.0 vs. 15.3 ± 1.9 weeks in the T plus LNG and T alone groups, respectively (mean ± SEM; $P < 0.05$).

Consumption of Levonorgestrel

The consumption of defined daily doses of estrogens with progestogens in fixed proportions in the Netherlands in 2000–2004 is estimated as given in Table 18.8. The table shows that the use of Levonorgestrel in contraceptives in Netherlands is higher than the use of other progestogens. This combined with longer half-life implies a possibility of higher environmental concentrations.

18.12.4 Conclusions on progestins

From the above-compiled data on progestins, Levonorgestrel seem to be the most relevant compound for further analysis, since in experimental studies it showed significant effects on fecundity. Though there are no reported results on presence in the environment, we believe that this is mainly due to lack of attempts for detection of this compound in the environment. We must take inta account that the amount of Levanorgestrel

Table 18.8 The consumption of defined daily doses (DDD) of estrogens with progestogens in fixed proportions in the Netherlands in 2000–2004

Pharmaceutical	2000	2001	2002	2003	2004
Estrogen with lynestrenol (Ministat ®)	17 925 900	15 678 100	14 017 600	13 563 700	914 490
Estrogen with norethisteron (Neocon ®)	5 501 200	5 458 900	5 424 400	5 471 800	596 630
Estrogen with levonorgestrel (Microgynon ®)	254 781 700	275 320 400	298 341 600	318 354 500	82 111 800
Estrogen with desogestrel (Marvelon ®)	139 903 000	125 862 600	104 173 300	94 577 100	5 761 400
Estrogen with gestodeen (Meliane ®)	59 303 500	59 994 700	51 045 000	47 089 300	5 214 500
Estrogen with norgestimaat (Cilest ®)	5 327 500	5 057 000	4 440 000	4 118 900	345 290
Estrogen with drospirenon (Yasmin ®)		5 072 400	9 349 400	9 809 000	3 216 800
Estrogen with norelgestromine (Evra ®)				109 740	71 101
Total	482 742 800	492 444 100	486 791 300	493 094 040	98 232 011

used is high, which suggests a high likelihood for presence in the environment, especially wastewater treatment plants, and that the half-life is relatively long, which suggests a possibility for bioaccumulation.

18.12.5 Ethynylestradiol

The synthetic analogue of 17β-estradiol, 17α-ethynylestradiol (EE2), is a potent xenoestrogen and the most widely used estrogenic component of modern oral contraceptive preparations in the world. Around 95–98% of plasma EE2 is bound, virtually all to albumin (Akpoviroro et *al.*, 1981). The affinity to the hormone-binding proteins in serum, including SHBG, albumin and α-fetoprotein strongly influence the *in vivo* estrogenicity of a compound. Only the unbound fraction, less than 5% of the total, is considered biologically active (Orme *et al.*, 1983). It is estimated that about 98% of the endogenous 17β-estradiol is bound to binding proteins, especially SHBG, resulting in only a small percentage available to the cells (Ben Rafael *et* al., 1986).

The major site of metabolism for EE2 is the liver, with the two major metabolic pathways being 2-hydroxylation and 16β-hydroxylation. **These** pathways result in a number of metabolites, which are then conjugated with glucuronide and/or sulphate, and are considered to be biologically inactive (IARC Monographs, 1979a). However, a major portion of EE2 is conjugated directly with glucuronic acid and excreted in the urine.

In contrast to the metabolites of natural estrogens, *a* significant proportion of the metabolites of EE2 are excreted by the faecal route. In radiolabelled studies, the ratio of faecal/urine radioactivity has been reported to he. about 4:6, and the total recovery of radioactivity from both sources is about 80%. One study reported that about 30% is excreted in the faeces, of which one-third is excreted as the unchanged form (which may be a result of deconjugation in the colon). The remainder is excreted in the urine mainly as the EE2 glucuronide conjugate, Other glucuronide (and to a lesser extent sulphate) conjugates include: 2-hydroxyoestradiol; 2-methoxyethynyloestradiol and 3-methoxy-2-hydroxy-ethynylestradiol. It has been reported that only 1% of unchanged EE is excreted in the urine, although a higher value of 16% has also been reported. De-ethynylated estrogens (e.g. estrone, 17β-estradiol and estriol) only account for 1–2% of the dose in women (Orme *et al.*, 1983).

Effects of 17α-ethynylestradiol related to **fecundity**

EE2 is used as an oral contraceptive in humans and as such its effects on fecundity in humans is well known. The effects of EE2 on animals has been investigated in several studies and some results are given below. These studies may give some indication of what the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) in humans might be.

The relative estrogenic potency (REP) of EE2 compared with 17β-estradiol and estimated using *in vitro* assays has been stated to be between 0.5 and 5.71 (Tanaka *et al.* (2001), REP_{EE2} = 0.5; Korner *et al.* (2001), REP_{EE2} = 0.91; Gutendorf and Westendorf (2001), REP_{EE2} = 1.25, 1.25 and 5.71).

The effects of exposure to EE2 upon the reproductive success of a marine fish was investigated recently (Robinson *et al.*, 2003). Sand goby (*Pomatoschistus minutus*) were exposed for seven months to EE2 or a sewage effluent containing known xeno-estrogens (alkylphenol polyethoxylates) and bred using within treatment crosses. Nominal exposure concentrations were 6 ng/L EE2, **0.3** or 0.03% v/v sewage effluent. **At** the end of the breeding trials, expression of hepatic zona radiata protein (Zrp) and vitellogenin (Vtg) mRNA were determined. Exposure to 6 ng/L **EE2** induced Zrp and Vtg mRNA expression in male and female sand goby, impaired male maturation and reproductive bebaviour, reduced female fecundity and reduced egg fertility. As a consequence, fertile egg production of the EE2-exposed population was reduced by 90%. Exposure to sewage effluent (0.3% v/v) increased adult mortality and female Zrp and Vtg mRNA expression, but did not induce male vitellogenesis. Exposure to EE2 and 0.3% v/v sewage effluent impaired development of the male urogenital papilla. Fish exposed to 0.03% v/v sewage effluent produced more fertile eggs than those exposed to 0.3% effluent, or those receiving no effluent.

In another study (Berg *et al.*, 1999) two synthetic estrogens, diethylstilbestrol (DES) and EE2, were injected into the yolks of embryonated eggs, At a dose as low as 2 ng EE2/g egg, all male embryos became feminised, containing ovary-like tissue in the left testis. The extent of feminisation of the testes was determined by measuring the relative area of the ovary-like component. Persistent Mullerian ducts oviducts in male embryos, and malformations of the Mullerian ducts in females occurred at 2 ng EE/g egg and higher doses. DES was approximately one-third to one-tenth as potent as EE2. The morphological changes studied were dose-dependent, indicating that they are useful as test end points for estrogenic activity. Feminisation of the left testis in males proved to be the most sensitive end point

Papoulias *et* al. (1999) evaluated the effects of a model environmental estrogen, EE2, on the Japanese medaka (*Oryzias latipes*, a freshwater fish) using a nano-injection exposure. Gonad histopathology indicated that a single injection of 0.5–2.5 ng EE2/egg can cause phenotypic sex-reversal of genetic males to females. Sex-reversed males had female-typical duct development and the secondary sex characteristics were generally consistent with phenotype. No instances of gonadal intersexes were observed. EE2 also appeared to reduce growth but not condition (weight-at-length) and exposed genetic females appeared to have a higher incidence of atretic follicles relative to controls. The results suggested that EE2 may influence sexual differentiation and development.

Scholz and Gutzeit (2000) exposed Freshly hatched Japanese medaka (*Oryzias* latipes) for two months to nominal EE2 concentrations of 0, 1, 10 or 100 ng/L under semi-static conditions. The exposure period was followed by a six week recovery period in order to detect long-lasting effects on sexual differentiation. Sex ratio, gonadal growth, spawning, fecundity, histology as well as ovarian gene expression of aromatase was monitored. Growth was unaffected in all treatment groups. At 100 ng/L, all genetically male rnedaka were sex reversed and had developed an ovary. At lower test concentrations, no alteration of testicular structure was detected (including testis–ova or ovarian-like structures) and male fertility appeared to be unchanged. In genetic females, significantly reduced ovarian weight was observed at 10 and 100 ng/L as well as a significantly decreased egg production rate. There was a 80% reduction in egg production at 10 ng/L and complete inhibition occurred at the highest test concentration, likely to be caused by the absence of males. Aromatase, which is normally only expressed in ovaries, was also detectable in testis of genetic males exposed to 10 ng/L.

A full life-cycle study with fathead minnow (*Pimephales promelas*) revealed a variety of effects on survival, growth, gross development, gonad development, sex determination and reproductive maturity (Lange *et al.*, 2001). Newly fertilised embryos (<24 h old) were exposed to nominal concentrations of 0.2, 1, 4, 16 and 64 ng/L in flow-through conditions at 25 ± 1 °C for 305 days (four days pre-hatch and 301 days post-hatch). Exposure concentrations were confirmed by radioimmunoassay analysis and ranged from 58 to 84% with mean measured values ≥70%. Hatching success of embryos was not significantly different from controls at any exposure concentration (NOEC >64 ng/L). Larval growth was reduced at 16 ng/L and a NOEC of 4 ng/L identified at day 28. In addition, juvenile fish growth was reduced when sampled at days 28 and 56 and NOEC and LOEC values of 1 and 4 ng/L were reported, respectively.

Gross morphological changes were seen in fish at test concentrations of 16 and 64 ng/L. No males (with appropriate secondary sexual characteristics and territorial behaviour) were seen after 172 days post-hatch at a concentration of 4 ng/L or above. Histology of exposed fish at 56 days post-hatch revealed a female : male sex ratio of 84:5 (with ova–testes in 11% of fish) at a concentration of 4.0 ng/L. No significant effects were seen at lower test concentrations. After 172 days post-hatch, no testicular tissue was observed in any fish exposed to 4 ng/L. Thus the NOEC and LOEC values based on gonad histology were 1 and 4 ng/L, respectively.

There are several other studies reported in the open literature where similar results to the above are reported (Metcalfe *et al.*, 2001; Nash *et al.*, 2004; Wenzel *et al.*, 2001; Zillioux *et al.*, 2001).

Production volume and use of 17α-ethylnylestradiol

EE2 can be used in human medicine to treat various gynaecological disorders and post-menopausal breast cancer. However, its largest use is in oral contraceptives, when it is usually administered in combination with a synthetic progestin. Its concentration in the contraceptive pill ranges from 20 to 50 µg, with 35 µg most commonly prescribed (Archand-Hoy *et al.*, 1998).

An annual use of 0.029 tonnes of EE2 has been estimated in the UK (Webb, 2000). By comparison, it has been estimated that 0.088 tonnes of oral contraceptives (EE2 and mestranol) are used annually in the US (Archand-Hoy *et al.*, 1998).

Persistence of 17α-ethylnylestradiol in the environment

Synthetic estrogens (EE2 and mestranol) are more resistant to microbial degradation than natural steroids (estradiol, estrone, estriol). The data on the physicochemical properties of EE2 and its environmental fate (Table 18.9) indicate that the compound is relatively persistent in the aquatic environment. It is likely that adsorption of EE2 to soil is a major removal process (log K_{oc} = 3.8).

It has been reported that EE2 is highly stable and is not sufficiently eliminated during biological treatment of the wastewater (Ternes et al.,

Table 18.9 Physicochemical properties and environmental fate data

Physicochemical property	Value
Physical state at ambient temperature	Solid
Water solubility	4.7–19 mg/L[1]
Octanol–water partition coefficient (log K_{ow})	3.62–4.7
Organic carbon water partition coefficient (log K_{oc})	3.8 (4.5)
Type of degradation	
Aquatic–abiotic	Sorption is the major removal process with photolysis being of lower importance and volatilisation being negligible
Aquatic–biotic	A number of laboratory studies have indicated that EE2 is relatively persistent
Terrestrial	No data are available on the persistence of EE2 in soil though it is likely that adsorption to soil is a major removal process

1999). Several studies have examined the persistence of EE2 in rivers (Jurgens *et al.*, 2002), activated sewage and wastewater effluent, After the primary treatment of the sewage, the mean percent of remaining EE2 is equal to 75%. After the secondary treatment, approximately 65% remaining EE2 was detected (Tabak *et al.*, 1981). These values correspond to the study of EE2 removal rates in French STPs (mean removal rate – 40%, Cargouet *et al.*, 2004). In this study, it was estimated that EE2 accounted for 35–50% of the estimated estrogenic activity in rivers. Close persistency values were observed by Kuch and Ballschmiter, 2000 (43%). The low removal rate of EE2 can be explained by its slow microbial degradation during the treatment process (Tabak *et al.*, 1981; Ternes *et al.*, 1999). In addition, EE2 concentrations in the STPs can be increased by the partial conversion of other drugs into this molecule (Kuhnz *et al.*, 1997).

Additionally, reports from laboratory biodegradation studies (Desbrow et *al.*, 1998) indicated that EE2 was highly stable and persistent in activated sludge, with no detectable degradation occurring after 120 h of treatment. The solubility of EE2 in pure water and sewage treatment water was reported *to* be 4.2 and 4.7 mg/L, respectively, which was three-fold less soluble than natural steroidal estrogens. This Pact is believed to contribute to the increased resistance of EE2 to biodegradation as compared with natural steroidal estrogens.

According to Ying *et al.*, 2002, EE2 was principally persistent under selected aerobic conditions. Camparatively, 70–80% of added estradiol (E2) was mineralised to CO_2 within 24 h by biosolids from wastewater treatment plants, whereas the mineralisation of EE2 was 25–75-fold less. EE2 was also reported to be degraded completely within six days by nitrifying activated sludge and resulted in the formation of hydrophilic compounds.

EE2 was found to be microbially degraded (Colucci and Topp, 2001). According to the studies, the dissipation half-life of EE2 ranged from 7.7 days at 4°C to 3 days at 30°C.

Exposure routes

There are several exposure routes that may lead to contamination of food or water with EE2. If we neglect routes 3 and 4 (*see* Fig. 18.1) related to effects which would exist just in the vicinity of the production plant and should be negligible if the necessary precautions are in operation at the plant, then the most likely exposure route would be route 1 in Fig. 18.1. After the human usage part of the EE2 would either be discarded and end up in landfills (route 5) or would end up in sewage through human excreta (route 6) and from there would enter an STP.

EE2 can be further transported from a landfill as effluent through the landfill effluent treatment system, and from these into the sanitary sewage or STP (route 8), or could be released into surface waters or land (route 13), depending on the level of treatment applied to it. Landfill leachate can percolate the containment system and pollute soil and groundwater (route 15); however, this exposure route should not represent a significant threat to the environment in a well-designed and maintained landfill and therefore will not be considered further.

The sewage sludge from a STP, among other options like incineration for example, may be disposed into a landfill, route 11, or could be used in agriculture, route 16. The EU Directive which regulates the use of sewage sludge in agriculture is 86/278/EEC. However, this Directive does not mention endocrine disrupters such as EE2 or E2, and therefore tests for such EDs are not required.

From the agricultural fields the endocrine-disrupting chemicals (EDCs) may be transported to surface waters, soil and groundwater by leaching, dissipation and sun-off, route (18). If EE2 is transported to surface waters it may end up in the food chain by bioacumulation in fish or as water for domestic use, and if it reaches groundwater it may further be used as tap water for human consumption. Once sewage sludge is applied to agricultural fields, EE2 may end up in plants through plant uptake. However, Directive 86/278/EEC instructs that sludge must not be applied to soil in which fruit and vegetable crops are growing or grown, or less than 10 months before fruit and vegetable crops are to be harvested. Grazing animals must not be allowed access to grassland or forage land less than three weeks after the application of sludge.

Unless direct measurements show otherwise, the risks from EE2 being present in the air due to evaporation from landfills, STPs and agricultural fields where sewage sludge is applied, will be considered to be negligible.

Evidence for presence of 17α-ethylnylestradiol in the environment

Several studies were examining the presence of EE2 in STPs in raw sewage as well as effluent. In a study by Stumpf *et al.* (1996) EE2 was detected in all 20 STPs investigated above the quantification level of 1 ng/L and in 15 effluents >10 ng/L. The median concentration of EE2 was 17 ng/L and the maximum 62 ng/L. Similar results have been, found in the UK where concentrations in effluents were up to 7 ng/L EE2 (Aheme and Briggs, 1989; Desbrow et *al.*, 1998).

Belfroid *et al.* (1999) reported that EE2 was detected at one occasion in three and two STP effluent samples in Netherlands, respectively. The data also showed that concentrations of all hormones were higher in domestic effluents than in industrial effluents.

Ternes *et al.* (1999) reported that in the raw sewage of the Brazilian STP of Penha/Rio dc Janeiro, the natural estrogens 17β-estradiol and estrone were detected with average concentrations of 0.021 and 0.040 μg/L, respectively. In the German municipal STP close to FrankfurtrMain the raw sewage was contaminated by E2 and estrone with average concentrations of 0.015 and 0.027 μg/L, respectively. The evaluated removal rates were much lower than those obtained in the Brazilian STP. For instance, the loads of estrone and EE2 were not appreciably reduced while passing through the German STP. Considering the standard deviation no elimination rate could be evaluated. The differences between the absolute removal rates of the German and Brazilian STP might be caused by the low temperatures in the German sampling period with –2 °C on average compared to above 20°C in Rio *de* Janeiro. E2 and 16α-hydroxyestrone were eliminated with a higher efficiency than EE2 and estrone. In German STPs median values could be evaluated for EE2 in the range of 1 ng/L (detection limit). In comparison, the concentrations of EE2 were higher in Canadian effluents compared with those determined in the German STP effluents (median: 9 ng/L).

In a study performed by Larsson *et al.* (1999), the effluent from STP in Sweden was analysed. The results revealed significant levels of estrogenic substances in sewage effluent water (4.5 ng/L for EE2). The steroids were mainly present in unconjugated form. Since humans primarily excrete both natural estrogens and EE2 as conjugates (Ranney, 1977), these results suggest that deconjugation (activation) occurs within the sewage system, and/or that the conjugates are more rapidly degraded. The ratio between EE2 and natural estrogens in the water is higher than the theoretical ratio based an human secretion rates of natural and synthetic estrogens (von Rathner and Sonneborn, 1979), indicating a faster degradation of the natural estrogens. Larsson *et al.* (1999) reported that the bile of fish caged downstream of the STP contained estrogenic substances at concentrations 10^4–10^6 times higher than water levels. The estimated EE2 concentration in the creek was 1.5 ng/L during the experiment, taking into account the flow rate through the STP and the dilution in the creek, while the EE2 concentration in bile from caged juvenile rainbow trout exposed to diluted sewage effluent water for four weeks showed concentrations of EE2 of approximately 1 µg/g bile. This shows that exposure to different environmental estrogens results in accumulation of prominent amounts of these substances.

A recent study by Cargouet *et al.* (2004) showed that the concentration of EE2 in STP influents in Paris area has a mean value ranging from 4.9 to 7.1 ng/L, which represents 11–15% of the total detected steroids.

All these results show that EE2 is present in the raw sewage as well as effluent of STPs irrespective of the country were the tests are performed. Once released in the surface waters/rivers the effluent is diluted and depending on the extent of dilution EE2 may be detectable or not.

In the UK, immunoassay detection revealed the presence of EE2 in rivers in concentrations below 5 ng/L in September 1982 and 2–15 ng/L in August 1987 (Aherne and Briggs, 1989). In Germany, in the Ruhr district, EE2 has been detected in surface water in concentrations between <1 and 4 ng/L (Stumpf *et al.*, 1996).

Caged fish held downstream of most STW produced vitellogenin, indicating the presence of estrogenic substances (Harries et *al.*, 1996, 1997; Purdom *et al.*, 1994). The nature of the inducer(s) was, however, not clearly elucidated.

Hohenblum *et al.* (2004) monitored surface waters in Austria for EE2 and some other compounds, and found EE2 in four samples with maximum concentration of 0.33 ng/L.

Vogel *et al.* (2003) studied continuous infiltration experiments over a period of two years and run-off experiments in order to investigate the behaviour of EDCs in agricultural soils after sewage sludge application. In infiltration experiments transport of EE2 towards lower soil layers was observed. They did not detect considerable EE2 concentrations in the leachate, leading them to the conclusion that adsorption to the soil matrix and/or biodegradation prevent in some cases a direct EE2 transport to groundwater. However, since the experimental conditions were very specific (groundwater table >90 cm below ground surface, high soil organic matter) infiltration of EE2 to the groundwater under certain conditions cannot be ruled out. Recent studies have shown that disposal of animal manure to agricultural land could lead to movement of estrogenic steroids into surface and groundwater (Peterson et *al.*, 2001). Peterson et *al.* (2001) measured 17β-estradiol concentrations ranging from 6 to 66 ng/L in mantled karst aquifers in northwest Arkansas. The observed 17β-estradiol concentration trends imitated the changes in stage over the recharge event. The contamination was associated with poultry litter and cattle manure waste applied on the area.

Hohenblum *et al.* (2004) detected EE2 in one sample of 112 tested, though 17β-estradiol was detected in about half the samples. This study supports the findings of Vogel *et al.* (2003) that the adsorption to the soil matrix and/or biodegradation prevent a direct EE2 transport to groundwater. The maximum concentration of E2 found was 0.79 ng/L while EE2 was 094 ng/L.

18.12.6 Conclusions on 17α-ethylnylestradiol

There is awareness of the importance of investigating the presence of EE2 in the environment, and many of the previous studies included EE2 as the main representative of the synthetic steroids

As can be seen from the above studies, evidence exists of the presence of EE2 in the environment. EE2 was found basically in every medium where an attempt was made for its detection, i.e. raw sewage, STP effluent, rivers and even groundwater. We are not aware of any attempt being made to detect EE2 in landfills, landfill effluent or agricultural fields where sewage sludge is applied. The detection of EE2 is difficult since its concentrations in the environment are usually on the detection limit, but this certainly does not mean that the risk from EE2 is negligible. EE2 can cause changes in animals in very low concentrations. Chronic exposure under laboratory conditions, including studies of chronic exposure over two complete generations, to as little as 1 ng/L EE2 (below the limits of chemical detection for most effluents) was sufficient to sex reverse male zebrafish and 1.5 ng/L stimulated vitellogenesis in juvenile fish (Om *et al.*, 2003; Hahlbeck *et* nl., 2004). Bioaccumulation, as shown by Larsson *et al.* (1999), can increase the EE2 concentrations by several orders of magnitude. One should also not forget that EE2 concentrations in the STPs can be increased by the partial conversion of other drugs into this molecule (Kuhnz *et al.*, 1997). Finally, several studies have shown that EE2 is more persistent in the environment than the natural estrogens. All the above facts make EE2 a compound of major interest for further study.

18.13 Antibiotics

The consumption of antibiotics in the Netherlands in DDD is estimated as given in Table 18.10.

18.13.1 Sulfamethoxazole–trimethoprim combination

Trimethoprim is a folic acid antagonist. As such, it can cause abnormal embryo development in experimental animals (Helm *et al.*, 1976). A role of trimethoprim therapy in human birth defects has not been established. Treatment with a sulfamethoxazole/trimethoprim combination causes a drop in sperm concentration between 7 and 88%. Possible mechanisms for this effect is folate deprivation of spermatogenic cells through the inhibitory action of trimethoprim on dihydrafolate reductase (Murdia *et al.*, 1978). A decrease in sperm concentration and total number of sperm has been

Table 18.10 The consumption of antibiotics in the Netherlands in defined daily dose (DDD)

Pharmaceutical	2000	2001	2002	2003	2004
Trimethoprim	1592200	1575300	1530200	1554100	1503100
Sulfamethoxazole/ trimethoprim	2403900	2284800	2206300	2141900	2070700
Doxycycline	12059200	11825300	11382000	10813900	10507400
Tetracycline	1239660	1086542	945461	887715	870980
Minocycline	1355100	1412200	1372700	1373900	1504500
Erythromycin	602910	609710	5470220	489480	478670

reported after treatment of rams with sulfamethoxazole–trimethoprim combination (Tanyildizi & Bozkurt, 2003).

Treatment with a sulfamethoxazole–trimethoprim combination leads to a significant impairment of spermatogenesis (Crotty *et al.*, 1995). In *vitro* analysis of human sperm function by Hargreaves *et al.* (1998) has shown that the combination with trimethoprim increases the sensitivity of spermatozoa to the drug approximately 10-fold. Trimethoprim and sulfamethoxazole are highly persistent (Bendz *et al.*, 2005). Sulfamethoxazole has been detected in the environment:

- STP effluent Källby, Sweden, 0.02 μg/L (Bendz *et al.*, 2005)
- STP effluents survey, 0.05–0.09 μg/L (Andreozzi *et* al., 2003)
- STW effluent, UK, median <0.050 μg/L (Ashton *et al.*, 2004)
- River Elbe, up to 0.070 μg/L (Wiegel *et* al., 2004)
- Several STP effluents, Italy, 0.13 μg/L (Zuccato *et al.*, 2005)
- River water, about 1.0 μg/L (Halling-Sørenson *et al.*, 1998)
- STP effluents Wisconsin, USA, 0.05–0.37 μg/L (Karthikeyan & Meyer, 2006)
- STP effluent, Germany, median 0.40 μg/L (Hirsch et *al.*, 1999)
- Surface water, Germany, median 0.03 μg/L (Hirsch *et al., 1999)*
- STP effluent, Canada, median 0.243 μg/L (Miao *et al.*, 2004)
- STP effluents, Sweden, 0.135–0.304 μg/L (Lindberg et *al.*, 2005)

Trimethoprim has been detected in the environment:

- STP effluent Källby, Sweden, 0.04 μg/L (Bendz *et al.*, 2005)
- STP effluents survey, 0.04–0.13 μg/L (Andreozzi *et al.*, 2003)
- STW effluent, UK, median 0.070 μg/L (Ashton et *al.*, 2004)
- STW effluent, UK, 0.218–0.322 μg/L (Roberts & Thomas, 2006)
- River Tyne, UK, 0.004–0.019 μg/L (Roberts & Thomas, 2006)
- River Elbe, up to 0.040 μg/L (Wiegel *et al.*, 2004)
- STP effluents, Wisconsin, USA, 0.05–0.55 μg/L (Karthikeyan & Meyer, 2006)
- STP effluent, Germany, median 0.32 μg/L (Hirsch *et al.*, 1999)

- STP effluents, Canada, 0.009–0.194 μg/L (Metcalfe *et al.*, 2003)
- STP effluents, Sweden, 0.066–1.34 μg/L (Lindberg *et al.*, 2005)

18.132 Tetracycline

Tetracycline appears to be relatively non-toxic to spermatogenesis (Kushniruk, 1976; Timmermans, 1974). It has significant effects on sperm movement. Effects have been seen at concentrations as low as 2.5 mg/ml, well within those achieved following therapeutic doses of the antibiotic (Hargreaves *et al.*, 1998). Tetracyclines are rapidly metabolised and moreover form relatively stable complexes with metal cations (Miao *et al.*, 2004). Another source classifies tetracycline as non-degradable (Halling-Sørenson et *ah*, 1998). Nevertheless, it has been detected in STP effluents. Tetracycline has been detected in the environment:

- STP effluents, Wisconsin, USA, 0.05–0.37 μg/L (Karthikeyan & Meyer, 2006)
- STP effluent, Canada, median 0.151 μg/L (Miaa *et al.*, 2004)
- River water, about 1 μg/L (Halling-Sørenson *et al.*, 1998)

18.13.3 Doxycycline

Doxycycline decreases hyperactivation of cryopreserved human sperm (King *et al.*, 2997). Doxycycline has been detected in the environment:

- STP effluents, Canada, 0.04 μg/L (Miao *et al.*, 2004)
- STP effluents, Wisconsin, USA, 0.05 μg/L (Karthikeyan & Meyer, 2006)
- STP effluents, Sweden, up to 915 ng/L (Lindberg *et al.*, 2005)
- Sweden sewage sludge: some samples had 1.5 mg/kg dry weight (Lindberg *et al.*, 20053

18.13.4 Minocycline

Minocycline has been shown to be toxic to sperm (Schlegel *et al.*, 1991). Minocycline may interfere with oral contraception, causing breakthrough bleeding (De Groot *et al.*, 1990). No data were available on persistence and environmental fate.

18.13.5 Erythromycin

Erythromycin had significant effects on rapid movement of sperm at concentrations >100 μg/ml (Hargreaves *et al.*, 1998). Erythromycin application in pregnancy is associated with an increase in cardiac malformations in infants (Kallen & Olausson, 2003; Kallen *et al.*, 2005). It may inhibit hepatic degradation of carbamazepine and theophylline (Blagg & Gleckman, 1981;

Mitch, 1989). It has a prolonged stability with a half-life of over one year (Zuccato *et* ai., 2005) and has been detected in the environment:

- STP effluents, Italy, 47 ng/L (Zuccato *et al.*, 2005)
- STP effluents, Canada, 80 ng/L (Miao et *al.*, 2004)
- STP effluents, Wisconsin USA 20 ng/L (Karthikeyan & Meyer, 2006)
  River water, around 1 µg/L (Halling-Sørenson et *al.*, 1998)
- STP effluents, Germany, median level 2.5 µg/L(Hirsch *et* ah, 1999)
- Surface waters, Germany, median level 150 ng/L (Hirsch *et al.*, 1999)
- STP effluent, UK, up to 290 ng/l (Roberts & Thomas, 2006)
- River water, UK, up to 70 ng/L (Roberts & Thomas, 2006)
- River water, Germany, up to 70 ng/L (Wiegel et *al.*, 2004)
- STW effluent, UK, mean 109 ng/L (Ashton *et al.*, 2004)

18.13.6 Conclusions on antibiotics

The sulfamethoxazole–trimethoprim combination appears to be the most important pharmaceutical in this group in view of mechanism of action, persistence and environmental exposure. Second in this group is erythromycin, for which the mechanism of action is less clear but the effects are relevant for fertility. In addition, this compound is stable in the environment and has been detected through many environmental studies. These compounds share significant effects on spermatogenesis and sperm function, which warrant their inclusion in this compilation, although the mechanism of action is less clear. Further study is needed as to the possible causation of these effects through endocrine mechanisms. Although doxycycline has the highest usage pattern, limited information on effects on fertility and on persistence precludes conclusions on the priority of studying this compound.

18.14 Risk assessment

According to the European Chemicals Bureau guidance (European Chemicals Bureau, 2003) the assessment of compaunds is based on four main components: hazard identification, dose–response assessment, exposure assessment and risk characterization. A detailed description of these components is given below.

Additionally, risk assessment can be extended and supported with the help of (quantitative) structure–activity relationships (QSARs), which assess compounds from the point of view of their structural properties. Moreover, ii specific restrictions on data collected so far are met, QSARs may be useful in reducing the number of animal tests.

In the frame of the current study, the hazard identification step includes selection of compounds which bear the intrinsic potential to disrupt the human endocrine system and cause fertility and fecundity problems in target populations. The key factors, which influenced the selection, were identified as follows (Luijten *et al.*, 2005):

- Potential and subsequent evidence of adverse effects related to fertility and fecundity.
- Production volume.
- Presence in the environment (STP effluent, rivers, groundwaters, soil, etc.).
- Persistence in the environment,
- Ability to reach the target populations through relevant exposure pathways.

18.14.1 Dose–response assessment

The objective of the dose–response assessment is to analyse results of in *vivo/in vitro* studies for subsequent estimation of so-called threshold doses/concentrations, which do not produce adverse effects on species/cells being tested. In the risk characterisation step these results are extrapolated using uncertainty factors to obtain safe dosesiconcentrations of the compound for target populations.

There are several approaches for estimation of such threshold values, which depend on the acceptance by regulatory agencies, data available and subpopulations of interest:

- no (low) observable adverse effect level (N(L)OAEL);
- benchmark dose (BMD);
- probabilistic analysis (PA).

Each approach has inherent advantages and disadvantages. Firstly, among currently accepted approaches BMD is more accurate in estimation of 'safe' doses. Secondly, the BMD approach can be extended by applying probabilistic analysis, which is based on resampling and combines ranges of plausible dose estimates together with their uncertainties. Additionally, by applying distributions of uncertainty factors instead of point estimates, it is possible to estimate risks for different subpopulations (general/sensitive). Finally, it is possible to apply these approaches in a consecutive manner.

No (low) observable effect level

The N(L)OAEL represents the highest experimental dose for which no adverse effects have been documented (Crump *et al.*, 1995). This approach is currently recognised and accepted by all regulatory agencies both in EU and USA. The principal procedure for calculation of N(L)OAEL is NOSTASOT dose (Crump *et al.*, 1995).

Although simple and straightforward, the N(L)OAEL bas many limitations, such as:

- the selection of a 'safe' dose is limited **to** the set **of** experimental doses;
- N(L)OAEL **varies with** the number of **species** being tested;
- the slope of the dose–response plays tittle role **in** determining N(L)OAEL;
- if no **'safe'** dose was determined, a **new** set **of** studies **should** be carried **out,** which is both time and resource consuming.

In case of failure to determine a N(L)OAEL from **the** initial studies, another option is to use LOAEL (lowest observable adverse **effect** level) and **additionally** introducing another uncertainty **factor (usually** 10).

Benchmark dose

The BMD (Crump, 1984) is the statistical lower **confidence** limit **for a** dose that produces a predetermined change in response rate of an adverse effect (benchmark response, BMR) compared with background. Unlike N(L)OAEL, BMD takes into account the whole dose–response information **by fitting** the mathematical model to dose–response data. **Therefore,** slopes are **taken** into account, which decreases the **uncertainty of** tho resulting **'safe'** doses.

The sequence of steps for determination of BMD is the following:

1. Fit **a mathematical** model **to** the **data (Crump,** 1984): using maximum likelihood procedures, the predefined model (polynomial, Weibull, etc.) is being fitted to the set of dose–response pairs.
2. Definition of BMR: define the **change** in **response rate, specific** to given study (typical **values** are 1%, *5%* and 10%) and with the help of **fitted** model determine the corresponding dose (this dose is the point estimate which is **the** basis of **confidence** limits calculation) (Crurnp *et al.*, 1995).
3. Determination **of** BMD (Cox & Lindley 1974): BMD is defined to be the lower confidence limit of the dose obtained on **step** 2. **In most** cases, 95% lower limit is sufficient.

The BMD is currently accepted by US Environmental Protection **Agency** and is increasingly recognised as **the more accurate** approach **than the** NOAEL.

Probabilistic analysis

PA (Slob & Pieters, 1998) represents 'sale' dose in terms of distribution, thus combining the range of plausible **values** together with their uncertainties. The basic idea is to replace the point **estimate** obtained by, for example, the **BMD approach by the** set **of values,** generated according to some predefined distribution model **(usually** log-normal). Therefore, **the first** steps **are** similar to BMD **approach. For generation** of **the** set of values some resampling technique can be used (Monte Carlo or Latin hypercube) (Vose, 2000). In the risk characterisation step, the resulting distribution can be combined with distributions of uncertainty factors to obtain uncertainty distribution of 'safe' human dose.

18.14.2 Exposure assessment

The objective of exposure assessment is to quantify the doses of the compounds, identified in hazard identification step, which are taken by target populations. The results of exposure assessment are then compared with dose–response assessment results in the risk characterisation step.

The core of exposure assessment includes identification of relevant pathways of exposure (Luijten *et al.*, 2005) and estimation of total chemical intake with respect to these pathways. Estimation of total chemical intake is based on both chemical concentrations in food and consumption patterns. The exposure assessment methods can be subdivided into three classes:

- screening tools;
- tools based on specific data;
- confirmatory methods.

Tools which are based on specific data are especially useful in risk characterisation step for comparison with results of dose–response analyses. There are three currently applied techniques to combine chemical concentration and consumption patterns (Kroes *et* al., 2002):

- *Point estimates:* assume single (best guess) estimates for both concentration and consumption.
- *Simple distributions:* a method that employs distributions of consumption variables but uses a fixed value for the concentration. The results are more informative than those of the point estimates because they take account of the variability that exists in food consumption patterns.
- *Probabilistic analysis:* variables are described in terms of distributions to characterise their variabilities and/or uncertainties. The method takes account of all the possible values that each variable could take and weights each possible outcome by the probability of its occurrence.

Provided that data are adequate and models are selected properly, probabilistic assessment should provide the most realistic estimates of exposure.

When data are adequate, it is preferable to apply simple distributions and probabilistic analysis for exposure assessment because they both take into account the probabilistic nature of consumption patterns. The comparison of the results, obtained with the help of these two techniques, will determine the most suitable approach for risk characterisation. Finally, since PA replaces point estimates with distributions, this provides additional data for comparison with results of dose–response assessment (also represented by distributions).

18.14.3 Risk characterisation

The final stage of risk assessment process combines results obtained from previous steps, The main objective is to compare results of dose–response assessment and exposure assessment in order to identify the strategy for eliminating/reducing the risk.

Estimation of reference dose

Reference dose (RfD) (Environmental Protection Agency, 1993) is the dose of the chemical, which is regarded to be safe for target population. RfD is the result of extrapolation of 'safe' doses estimated for species being tested during dose–response assessment. In order to extrapolate doses to sensitive humans uncertainty factors are applied:

$$\mathrm{RfD} = \frac{\mathrm{N(L)OAEL}}{\mathrm{UF}_1 \times \mathrm{UF}_2 \times \ldots \times \mathrm{UF}_n} \tag{18.1}$$

where UF_i corresponds to *i*th uncertainty factor. The most widely applied uncertainty factors include (Crump *et al.*, 1995):

- *H* (interhuman): describes variation in sensitivity in target population (default 10).
- *A* (animal to man): accounts for the uncertainty in extrapolating animal data to humans (default 10).
- *S* (subchronic to chronic): accounts for the uncertainty in extrapolating from subchronic to chronic NOAELs (default 10).

The value of the denominator in equation. (18.1) should not exceed 10^4.

More advanced approaches, such as probabilistic analysis, assume distributions of uncertainty factors instead of point estimates, This gives more flexible results provided there are enough data for determination of such distributions (but default lag-normal distributions are considered to be plausible) (Vermeire *et al.*, 20013.

Comparison of dose–response data and exposure data

The final stage of risk characterisation is to compare dose–response and exposure data and draw a conclusion on further actions. The comparison is based on evaluation of the margin of safety (MOS) (European Chemicals Bureau, 2003). The possible outcomes of the comparison and therefore of the whole risk assessment process are the following:

- Need for further information and/or testing.
- At present no need for further information and/or testing and no need for risk reduction measures.
- Need for limiting the risk.

W.14.4 Quantitative structure–activity relationships

QSARs are estimation methods developed and used for prediction of specific effects/properties of chemicals which are basecl on the structure of the substance. QSAR models have been created for a range of end points, including several toxicological and ecotoxicological end points and physicochemical/fate parameters (European Chemicals Bureau, 2003).

In case of exposure assessment, in the absence of experimental data, e.g. if it is not possible to obtain reliable measured data, specific physicochemical/fate parameters may be derived by applying **QSARs.** For the risk characterisation ***step,*** if the comparison of exposure and dose–response steps is inconclusive, QSARs may serve as a supporting tool in taking decisions. Additionally, **QSARs** may also be used to optimise the testing strategies.

It should be noted that estimates resulting from **QSARs** cannot be the only basis of risk assessment for a given compound, since **QSARs** are an estimation method and therefore there is a certain probability that the estimate is poor. Instead, **QSARs** should be seen as a complementary tool, which evaluated together with dose–response and exposure assessment can provide a more complete understanding of the characteristics of the substance. Furthermore, the result of QSARs should be evaluated for consistency in the light of available **experimental** data and validated estimates from other end points.

The development of a QSAR is based on the assumption that chemical substances which reach and interact with a target site by the same mechanism do so because of their similar chemical properties. Since different mechanisms of interaction usually will depend on different properties, **QSARs** must be generally developed for each mode of action. Some QSARs axe developed using quantitative data in order to predict a quantitative parameter. There arc two types of predictive methods:

- formalised methods;
- expert judgement.

Forrnalised methods are methods which can be subjected to validation, e.g. applied by one assessor and are both reproducible and transparent to other assessors. They are basecl on mathematical computations and/or fixed rules. Critical evaluation of the models should be carried out, including the evaluation of the appropriateness and validity of the descriptor variables, the evaluation of the form of the models and the methods used to construcl the models. These models should be applied critically acknowledging the limitations of the model, such as which compounds are within the domain of the model. Consequently, the specific information concerning the model which is used should be made available to the other **assessors** in order to ensure transparency and reproducibility (European Chemicals Bureau, 2003).

Methods based on expert judgement rely en the expert's experience and intuition. They are generally non-quantitative methods based on structural similarity and/or analogues. These methods should be used with caution, as they rely on the judgement of the individual assessor and may not be reproducible by the others.

For a QSAR to be used for the risk assessment process, it is necessary that the end point estimated is compatible with an end point used in the risk assessment. If such compatibility exists, then the QSAR can be used for such purposes as:

- assisting in data evaluation;
- contributing to the decision-making process on whether further testing is necessary to clarify an end point of concern and, if further testing is needed, to optimise the testing strategies, where appropriate;
- establishing parameters (descriptors) which are necessary to conduct the exposure and/or effects assessment;
- identifying effects which may be of potential concern on which test data are not available.

Validated QSARs are not currently available for human health-related toxicity end points. Instead, expert judgement is used in the light of data on close structural analogy and/or the presence of 'structural alerts' (i.e. fragments associated with affects) in the substance. However, recently the techniques have been developed aiming to incorporate advanced classification schemes in order to categorise compounds on the basis of numerical representation of their chemical structure (Asikainen *et al.*, 2006).

18.15 Conclusions

As part of the Food & Fecundity EC FP6 project, a prioritisation list has been created of pharmaceutical compounds with the potential to enter the environment and the human food chain and with suspected or proven ability to affect human fecundity through an endocrine mechanism of action. The list is based on an extensive literature search while considering the following criteria:

1. Do the available data indicate existence of an endocrine mechanism of action with an effect on fecundity?
2. Is the production volume sufficiently large to cause concern?
3. Has the PP been detected in food and/or environment?
4. Is the PP sufficiently persistent in the environment?

The chapter also discusses the endocrine and alternative mechanisms by which the drugs can affect human fecundity in men and women. An overview of possible pathways by which pharmaceutical products can enter the environment and human food chain are discussed, identifying water as the major media for transport and dispersion of pharmaceutical products in the environment and therelore providing potential lo also enter the human food chain. The currently available data are too limited to allow for definitive conclusions about human risks due to environmental exposures to endocrine-active drug residues. On the other hand, this study shows that such exposures are actually occurring, although seemingly below thresholds for human concern. However, given trends of increasing production and use of the drugs involved, both environmental and human exposure levels are likely to increase as well. When estimating the related actual risks for human health, additional consideration should be given to the fact that exposures to a range of pharmaceutical residues are likely to occur simultaneously, increasing the chance for combined exposures above thresholds of significant endocrine effects. The increasing amount of emerging new data also illustrates the enhanced awareness about hazards and anticipated possible risks of endocrine-disrupting pharmaceutical residues for human fecundity.

It is concluded Lhat for a series of pharmaceuticals, current data on production, use pattern and environmental fate warrant further study on possible human exposure and health risks. The risk assessment paradigm is reviewed and will be applied to forthcoming data on concentrations of the selected compounds in drinking water and foodstuffs. These analyses will enable informed conclusions about current risks of human exposure to pharmaceutical residues via the food chain.

18.16 References

AHERNE GW and BRIGGS a (1989) The relevance of the presence of certain synthetic steroids in the aquatic environment. *J Pharm Pharmacol* 41, 735–736.

AKPOVIRORO JO, MANGALAM M, JENKINS N and FOTHERBY K (1981) Binding of the contraceptive steroids medroxyprogesterone acetate and ethynyloestradiolin blood of various species. *J. Steroid Biochem.*, 14,493498.

ALLEVA JJ and ALLEVA FR (1995) Synergistic inhibition by benzodiazepine and barbiturate drugs of the clock control of ovulation in hamsters. *Chronobiol. Int.*, 12, 1–7.

ANDERSEN OP, HANSEN P and MADSEN H (1981) Hyperprohctinernic amenorrhea induced by metoclopamid (PrimperanR). *Acta Obstet. Gynecol. Scand.*, 60, 341–342.

ANDREOZZI R, RAFFAELE M and NICKLAS P (2003) Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment. *Chemosphere*, 50, 1319–1330.

ARCHAND-HOY LD, NIMROD AC and BENSON WH (1998) Endocrine-modulating substances in the environment: estrogenic effects of pharmaceutical products. *Int. J. Toxicol.* 17,139–158.

ARMSTRONG DT and GRINWICH DL (1972) Blockade of spontaneous and LH-induced ovulation in rats by indomethacin, an inhibitor of prostaglandin biosynthesis. I. *Prostaglandins*, 1, 21–28.

ARZE RS, RAMOS JM, RASHID HU and KERR DN (1981) Amenorrhoea, galactorrhoea, and hyperprolactinaemia induced by methyldopa. *Br. Med. J. (Clin. Res. Ed)*, 283, 194.

ASRBY J, LEFEVRE PA, ODUM J, TINWELL H, KENNEDY SJ, BERESFORD N and SUMPTER JP (1997) Failure to confirm estrogenic activity for benzoic acid and clofibrate: implications for lists of endocrine-disrupting agents. *Regul. Toxicol. Pharmacol.*, **26,** 96–101.

ASHTON D, HILTON M and THOMAS KV (2004) Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom. *Sci. Total. Environ.*, **333,** 167-184.

ASIKAINEN A, KOLEHMAINEN M, RUUSKANEN J and TUPPURAINEN K (2006) Structure-based classification of active and inactive estrogenic compounds by decision tree, LVQ and kNN methods. *Chemosphere*, **62**(4), 658–673.

BACK DJ, BRECKENRIDGE AM, CRAWFORD FE, *et al.* (1978) Kinetics of norethindrone in women. II. Single-dose kinetics. *Clin. Pharmacol. Ther.*, **24,** 448–453.

BACK DJ, BATES M, BRECKENRIDGE JM, et *al.* (1981) The pharmacokinetics of levonorgestrel and ethinylestradiol in women – studies with Ovran and Ovranette. *Contraception*, 23,229–239.

BAIN SC, LEMON M and JONES AF (1990) Gemfibrozil-induced impotence. *Lancet*, 336, 1389.

BAIRD DT (1979) Endocrinology of female infertility. *Br. Med. Bull.*, **35,** 193–198.

BANCROFT J, TENNENT G, LOUCAS K and CASS J (1974) The control of deviant sexual behaviour by drugs. I. Behavioural changes following oestrogens and anti-androgens. *Br. J.* Psychiatry, **125,** 310–315.

BARNES TR, BAMBER RW and WATSON JP (1979) Psychotropic drugs and sexual behaviour. *Br. J. Hosp. Med.*, **21,** 594–600.

BATHEN J (1978) Propranolol erectile dysfunction relieved. *Ann. Intern. Med.*, **88,** 716–717.

BBC (2004) Prozac 'found in drinking water' (http:llnewabbc.co.uW1/h"l/health/ 3545684.stm).

BEARDMORE KS, MORRIS JM and GALLERY ED (2002) Excretion of antihypertensive medication into human breast milk: a systematic review. *Hypertens.* Pregnancy, **21,** 85–95.

BEBB RA, ANAWALT BD, CHRISTENSEN RB, PAUWEN CA, BREMNER WJ and MATSUMOTO AM (1996) Combined administration of levonorgestrel and testosterone induces more rapid and effective suppression of spermatogenesis than testosterone alone: a promising male contraceptive approach. *J. Clin. Endocrinol. Metab.*, **81**(2), 757–762.

BEELEY L (1984) Drug-induced sexual dysfunction and infertility. Adverse *Drug React Acute Poisoning Rev.*, **3,** 23–42.

BELFROID AC, VAN DER HORST A, VETHAAK AD, SCHÄFER AJ, RIJS GB, WEGENER J and COFINO WP (1999) Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands. *Sci. Total Environ.*, **225**(1–2), 101–108.

BEN RAFAEL Z, MASTROIANNI L, JR, MELONI F, LEE MS and FLICKINGER GL (1986) Total estradiol, free estradiol, sex hormone-binding globulin, and the fraction of estradiol bound to sex hormone-binding globulin in human follicular fluid. *J. Clin. Endocrinol. Metab.*, **63,** 1106–1111.

BENDZ D, PAXEUS NA, GINN TR and LOGE FJ (2005) Occurrence and fate of pharmaceutically active compounds in the environment, a case study: Hoje River in Sweden. *J. Hazard. Mater.*, **122,** 195–204.

BERG C, HALLDIN K, FRIDOLFSSON A-K, BRANDT I and BRUNSTRO B (1999) The avian egg as a test system for endocrine disrupters: effects of diethylstilbestrol and ethynylestradiol on sex organ development. *The Science of the Total Environment*, **233,** 57-66.

BIRON P (1979) Diminished libido with cimetidine therapy. Can. *Med. Assoc. J.*, **121,** 404–405.

BLAGG NA and GLECKMAN RA (1981) Erythromycin. new indications and toxicities. *Postgrad. Med.*, **69**(3), 59-68.

BOYD AS (1988) Varicoceles and male infertility. *Am. Fam. Physician*, **37**, 252–258.
BOXALL AB (2004) The environmental side effects of medication. *EMBO Rep.*, 5, 1110–1116.
BROOKS BW, CHAMBLISS CK, STANLEY JK, RAMIREZ A, BANKS KE, JOHNSON RD and LEWIS RJ (2005) Determination of select antidepressants in fish from an effluent-dominated stream. *Environ. Toxicol. Chem.*, **24**, 464–469.
BUVAT J (2003) Androgen therapy with dehydroepiandrosterone. *World J. Urol.*, **21**, 346–355.
CAMERON ST, CRITCHLEY HO, THONG KJ, BUCKLEY CH, WILLIAMS AR and BAIRD DT (1996) Effects of daily low dose mifepristone on endometrial maturation and proliferation. *Hum. Reprod.*, **11**, 2518–2526.
CARGOUET M, PERDIZ D, MOUATASSIM-SOUALI A, TAMISIER-KAROLAK S and LEVI Y (2004) Assessment of river contamination by estrogenic compounds in Paris area (France). *Science of the Total Environment*, **324**, 55–66.
CARP HJ, FEIN A and NEBEL L (1988) Effect of diclofenac on implantation and embryonic development in the rat. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, **28**, 273–277.
CAVALIER M (1995) Sexually speaking... the dance. *Alaska Med.*, 37,113.
CHANDRA D, THULASIMANY M and BISWAS AR (1982) Uterotropic effect of clofibrate and phenylbutazone in immature female rats. *Indian J. Physiol. Pharmacol.*, **26**, 147–151.
CHANG SW, FINE R, SIEGEL D, CHESNEY M, BLACK D and HULLEY SB (1991) The impact of diuretic therapy on reported sexual function. *Arch. Intern. Med.*, **151**, 2402–2408.
CHOWDHURY AR (1987) Effect of pharmacological agents on male reproduction. *Adv. Contracept. Deliv. Syst.*, 3,347–352.
CLAYTON DO and SHEN WW (1998) Psychotropic drug-induced sexual function disorders: diagnosis, incidence and management. *Drug Saf.*, 19, 299–312.
COHEN AJ and BARTLIK B (1998) Ginkgo biloba for antidepressant-induced sexual dysfunction. *J. Sex Marital Ther.*, **24**, 139–143.
COLUCCI MS and TOPP E (2001) Persistence of estrogenic hormones in agricultural soils: II. 17a-Ethynylestradiol. *J. Environ. Qual.*, **30**, 2077–2080.
COMPTON MT and MILLER AH (2001) Priapism associated with conventional and atypical antipsychotic medications: a review. *J. Clin. Psychiatry*, **62**, 362–366.
CORTES A, MERINO G and CONTRERAS E (1978) Effect of fluoxetine (Lilly II0I40) on serotonin, noradrenaline and acetylcholine in isolated organs. *Cell. Mol. Biol. Incl. Cyto. Enzymol.*, 23,179–182.
CORTON JC, BOCOS C, MORENO ES, MERRITT A, CATTLEY RC and GUSTAFSSON JA (1997) Peroxisome proliferators alter the expression of estrogen-metabolizing enzymes. *Biochimie*, **79**, 151–162.
COX DR and LINDLEY DV (1974) *Theoretical Statistics*. Chapman & Hall, London.
CRAMER DW, LIBERMAN RF, HORNSTEIN MD, MCSHANE P, POWERS D, LI EY and BARBIERI R (1998) Basal hormone levels in women who use acetaminophen for menstrual pain. *Fertil. Steril.*, **70**, 371–373.
CROTTY KL, MAY R, KULVICKI A, KUMAR D and NEAL DE, JR (1995) The effect of antimicrobial therapy on testicular aspirate flow cytometry. *J. Urol.*, **153**, 835–838.
CRUMP K (1984) A new method for determining allowable daily intakes *Fundamental Appl. Toxicol.*, **4**, 854–871.
CRUMP K, ALLEN B and FAUSTMAN E (3995) *The Use of Benchmark Dose Approach in Health Risk Assessment.* EPA/630/R-94/007.
CUTLER GB JR, SAUER MA and LORIAUX DL (1939) SC 25152: a potent mineralocorticoid antagonist with decreased antiandrogenic activity relative to spironolactone. *J. Pharmacol Exp. Ther.*, 209,144–146.

DAIL WG, MCGUEFEE L, MINORSKY N and LITTLE S (1987) Responses of smooth muscle strips from penile erectile tissue to drugs and transmural nerve stimulation. *J. Auton. Pharmacol.*, **7**, 287–293.

DAUGHTON CG and TERNES TA (1999) Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environ. Health Perspect.*, **107**, Suppl 6, 907–938.

DAVIES TS and KLOWE WM (1998) Preclinical toxicological evaluation of sertraline hydrochloride. Drug *Chem Toxicol.*, **21**(4), 521–537.

DE GROOT AC DE, ESHUIS H and STRICKER BH (1990) In effectiviteit van orale anticonceptic tijdens gebruik van minocycline (in Dutch). *Ned Tijdschr Geneeskd.* **134**(25), 1227–1229.

DELLA CA, PFIFFNER D, MEIER B and HESS OM (2003) Sexual activity in hypertensive men. *J. Hum. Hypertens.*, **17**, 515–521.

DESBROW C, ROUTLEDGE EJ, BRIGHTY FC, SUMPTER JP and WALDOCK M (1998) Identification of estrogenic chemicals in STW effluent. 1. Chemical fractionation and *in vitro* biological screening. *Environ. Sci. Technol.*, **32**(11), 1549–1558.

DMOWSKI WP (1988) Danazol-induced pseudomenopause in the management of endometriosis. *Clin. Obstet. Gynecol.*, **31**, 829–839.

DOREVITCH A and DAVIS H (1994) Fluvoxamine-associated sexual dysfunction. *Ann. Pharmacother.*, 28, 872–874.

DUSING R (2005) Sexual dysfunction in male patients with hypertension: influence of antihypertensive drugs. *Drugs* **65**, 773–786.

EL BANNA AA, AL GAHTANI SJ and SEDRANI SH (1993) Effect of indomethacin administered during the normal time of implantation on the progesterone and protein composition of plasma and uterine flushings of rabbits. *J. Reprod. Fertil.*, **97**, 277–285.

EL ETREBY MF and NEUMANN F (1980) Influence of sex steroids and steroid antagonists on hormone-dependent tumors in experimental animals. In: Iacobelli S, King RJB, Lindner HR and Lippman ME, eds, *Hormones and Cancer*, New York, Raven Press, pp. 321–336.

EL SAYED MG, EL SAYED MT, ELAZAB ABD ES, HAFEIZ MA, EL KOMY AA and HASSAN E (1998) Effects of some beta-adrenergic blockers on male fertility parameters in rats. *Dtsch. Tierärztl. Wochenschr.*, **105**, 10–12.

ENVIRONMENTAL PROTECTION AGENCY (1993) Reference Dose (RfD): Description and Use in Health Risk Assessments. US EPA http://www.epa.gov/iris/rfd.htm

ESPEY LL (1983) Comparison of the effect of nonsteroidal and steroidal antiinflammatory agents on prostaglandin production during ovulation in the rabbit. *Prostaglandins*, **26**, 71–78.

ESPEY LL, STEIN VI and DUMITRESCU J (1982) Survey of antiinflammatory agents and related drugs as inhibitors of ovulation in the rabbit. *Fertil. Steril.*, **38**, 238–247.

EUROPEAN CHEMICALS BUREAU (2003) European Commission Technical Guidance Document in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation No. 1488/94 on Risk Assessment for Existing Substances, Office for Official Publications of the European Communities: Luxembourg, 1996 (and as revised, 2003).

FALKSON CI, FALKSON G and FALKSON HC (1991) Long term follow-up of patients with advanced prostatic cancer treated with nasal buserelin. *Ann. Oncol.*, 2, 303–304.

FELL MJ, MARSHALL KM, WILLIAMS J and NEILL JC (2004) Effects of the atypical antipsychotic olanzapine on reproductive function and weight gain in female rats. *J. Psychopharmacol.*, **18**, 149–155.

FELL MJ, GIBSON R, MCDERMOTT E, SISODIA G, MARSHALL KM and NEILL JC (2005) Investigation into the effects of the novel antipsychotic ziprasidone on weight gain and reproductive function in female rats. *Behav. Brain Res.*, **160**, 338–343.

FENT K, WESTON AA and CAMINADA D (2006) Ecotoxicology of human pharmaceuticals. *Aquatic Toxicol.*, **76**, 122–159.

FERRARI B, PAXEUS N, LO GR, POLLIO A and GARRIC J (2003) Ecotoxicological impact of pharmaceuticals found in treated wastewaters: study of carbamazepine, clofibric acid, and diclofenac. *Ecotoxicol. Environ. Saf.*, **55**, 359–370.

FERNANDEZ PR, KESSLER AG and RAWLINS RG (1991) Modern approaches to the treatment of human infertility through assisted reproduction. *P. R. Health Sci. J.*, **10**, 75–51.

FICICIOGLU C, TEKIN HI, ARIOGLU PF and OKAR I (1995) A murine model of adenomyosis: the effects of hyperprolactinemia induced by fluoxetine hydrochloride, a selective serotonin reuptake inhibitor, on adenomyosis induction in Wistar albino rats. *Acta Eur. Fertil.*, **26**, 75–79.

FITZGERALD JE, PETRERE JA and DE LA IGLESIA FA (1987) Experimental studies on reproduction with the lipid-regulating agent gemfibrozil. *Fundam. Appl. Toxicol.*, **8**, 454–464.

FOGARI R, PRETI P, DEROSA G, MARASI G, ZOPPI A, RINALDI A and MUGELLINI A (2002) Effect of antihypertensive treatment with valsartan or atenolol on sexual activity and plasma testosterone in hypertensive men. *Eur. J. Clin. Pharmacol.*, **58**, 177–180.

FOTHERBY K and CALDWELL ADS (1994) New progestogens in oral contraception. *Contraception*, **49**, 1–31.

FRANCES A and KAPLAN HS (1982) Patient on hypertensive drug has increasing erectile problems. *Hosp. Community Psychiatry*, **33**, 431–432.

FRAUNFELDER FT and MEYER SM (1985) Sexual dysfunction secondary to topical ophthalmic timolol. *JAMA*, **253**, 3092–3093.

FOVAEUS M, ANDERSSON KE and HEDLUND H (1987) Effects of some calcium channel blockers on isolated human penile erectile tissues. *J. Urol.*, **138**, 1267–1272.

FURUHJELM M, RYDNER T and CARLSTROM K (1980) Hyperprolactinemia in cases of infertility and amenorrhea. *Acta. Obstet. Gynecol. Scand.*, **59**, 137–141.

GENTILE S (2005) The safety of newer antidepressants in pregnancy and breastfeeding. *Drug Saf.*, 28, 137–152.

GIULIANO F and ALLARD J (2001) Dopamine and sexual function. *Int. J. Impot. Res.*, **13** Suppl 3, S18–S28.

GNOTH C, GODEHARDT E, FRANK-HERRMANN P, FRIOL K, TIGGES J and FREUNDL G (2005) Definition and prevalence of subfertility and infertility. *Hum. Reprod.*, 20, 1144–1147.

GODO G (1984) Hyperprolactinaemia and female infertility. *Acta. Med. Hung.*, **41**, 185–193.

GONZALEZ-VILLAPANDO C, SZABO M and FROHMAN LA (1980) Central nervous system-mediated stimulation of prolactin secretion by cimetidine, a histamine H2-receptor antagonist: impaired responsiveness in patients with prolactin-secreting tumors and idiopathic hyperprolactinemia. *J. Clin. Endocrinol. Metab.*, **51**, 1417–1424.

GREGORIAN RS, GOLDEN KA, BAHCE A, GOODMAN C, KWONG WJ and KHAN ZM (2002) Antidepressant-induced sexual dysfunction. *Ann. Pharmacother.*, 36, 1577–1589.

GROSS B, MONTGOMERY-BROWN J, NAUMANN A and REINHARD M (2004) Occurrence and fate of pharmaceuticals and alkylphenol ethoxylate metabolites in an effluent-dominated river and wetland. *Environ. Toxicol. Chem.*, **23**, 2074–2083.

GUPTA S, MASAND P, ASHTON AK and BERRY SL (1999) Phenelzine-induced sexual dysfunction treated with sildenafil. *J. Sex Marital Ther.*, **25**, 131–135.

GUTENDORF B and WESTENDORF J (2001) Comparison of an array of *in vitro* assays for the assessment of the estrogenic potential of natural and synthetic estrogens, phytoestrogens and xenoestrogens. *Toxicology*, **166**, 79–89.

HGDDAD PM and WIECK A (2004) Antipsychotic-induced hyperprolactinaemia: mechanisms, clinical features and management. *Drugs*, **64**, 2291–2314.

HAFEZ ES and HAFEZ SD (2005) Erectile dysfunction: anatomical parameters, etiology, diagnosis, and therapy. Arch. *Androl.*, 51,1531.

HAHLBECK E, KATSIADAKI I, MAYER I, ADOLFSSON-ERICI M, JAMESJ and BENGTSSON BE (2004) The juvenile three-spined stickleback (*Gasterosteus aculeatus* L.) as a model organism for endocrine disruption 11–kidney hypertrophy, vitellogenin and spiggin induction. *Aquat. Toxicol.*, **70**(4), 311–326.

HALLING-SØRENSEN B, NORS NIELSEN S, LANZKY PF, INGERSLEV F, HOLTEN LÜTZHØFT HC and JØRGENSEN SE (1998) Occurrence, fate and effects of pharmaceutical substances in the environment – a review. *Chemosphere*, 36,357–393.

HARGREAVES CA, ROGERS S, HILLS F, RAHMAN F, HOWELL RJ and HOMA ST (1998) Effects of co-trimoxazole, erythromycin, amoxycillin, tetracycline and chloroquine on sperm function in *vitro. Hum. Reprod.*, 13,1878–1886.

HARRIES JE, SHEAHAN DA, JOBLING S, MATTHIESSEN P, NEALL P, ROUTLEDGE EJ, RYCROFT R, SUMPTER JP and TYLOR T (1996) A survey of estrogenic activity in United Kingdom inland water. *Environ. Toxicol. Chem.*, **15**, 1993–2002.

HARRIES JE, SHEAHAN DA, JOBLING S, MATTHIESSEN P, NEALL P, SUMPTER JP, TYLOR T and ZAMAN N (1997) Estrogenic activity in five United Kingdom rivers detected by vitellogenesis in caged male trout. *Environ. Toxicol. Chem.*, **16**, 534–542.

HASHIMOTO F, OGUCHI Y, MORITA M, MATSUOKA K, TAKEDA S, KIMURA M and HAYASHI H (2004) PPARalpha agonists clofibrate and gemfibrozil inhibit cell growth, down-regulate hCG and up-regulate progesterone secretions in immortalized human trophoblast cells. *Biochem. Pharmacol.*, 68,313–321.

HEBERER T (2002) Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicol.* Lett, 131, 5–17.

HEDLUND H and ANDERSSON KE (1985) Comparison of the responses to drugs acting on adrenoreceptors and muscarinic receptors in human isolated corpus cavernosum and cavernous artery. *J. Auton. Pharmacol.*, **5**, 81–88.

HEDLUND H and ANDERSSON KE (1989) Effects of prazosin in men with symptoms of bladder neck obstruction and a non-hyperplastic prostate. *Scand. J. Urol. Nephrol.*, 23,251–254.

HEIKKINE T, EKBLAD U and LAINE K (2002) Transplacental transfer of citalopram, fluoxetine and their primary demethylated metabolites in isolated perfused human placenta, *Brit. J. Obst. Gynecol.*, **109**, 1003–1008.

HELM F, KRETZSCHMAR R, LEUSCHNER F and NEUMANN W (1976) [Investigations on the effect of the combination sulfamoxole/trimethoprim on fertility and fetal development in rats and rabbits (author's tranls)]. *Arzneimittelforschung.*, *26*, 643–651.

HENDRICK V, STOWE ZN, ALTSHULER LL, HWANG S, LEE E and HAYNES D (2003) Placental passage of antidepressant medications. *Am. J. Psychiatry*, **160**, **993–996**.

HIRSCH R, TERNES T, HABERER K and KRATZ KL (1999) Occurrence of antibiotics in the aquatic environment. *Sci. Total Environ.*, **225**, 109–118.

HOHENBLUM P, GANS O, MOCHE W, SCHARF S and LORBEER G (2004) Monitoring of selected estrogenic hormones and industrial. chemicals in groundwaters and surface waters in Austria. *Sci.* Total *Environ.*, 333,185–193.

HOLMES B and SORKIN EM (1986) Indoramin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in hypertension and related vascular, cardiovascular and airway diseases. Drugs, **31**, 467–499.

HOLZBEIERLEIN JM, CASTLE EP and THRASHER JB (2003) Complications of androgen-deprivation therapy far prostate cancer. *Clin.* Prostate Cancer, **2**, 147–152.

HOLZBEIERLEIN JM, MCLAUGHLIN MD and THRASHER JB (2004) Complications of androgen deprivation therapy for prostate cancer. *Curr. Opin. Urol.*, **14**, 177–183.

HU XH, BULL SA, HUNKELER EM, MING E, LEE JY, FIREMAN B and MARKSON LE (2004) Incidence and duration of side effects and those rated as bothersome with selective serotonin reuptake inhibitor treatment for depression: patient report versus physician estimate. *J. Clin. Psychiatry*, **65**, 959–965.

HUMMER M, KEMMLER G, KURZ M, KURZTHALER I, OBERBAUER H and FLEISCHHACKER WW (1999) Sexual disturbances during clozapine and haloperidol treatment for schizophrenia. Am. *J. Psychiatry*, **156**, 631–633.

IARC Monographs (1979a) **21**, 233–255, 257–278, 387–395.

IARC Monographs (1979b) **21**, 441–460.

IARC Monographs (1987) Suppl. 6: 427429.

ISOJARVI JI, LOFGREN E, JUNTUNEN KS, PAKARINEN AJ, PAIVANSALO M, RAUTAKORPI I and TUOMIVAARA L (2004) Effect of epilepsy and antiepileptic drugs on male reproductive health. Neurology, 62,247–253.

ISOJARVI JI, TAUBOLL E and HERZOG AG (2005) Effect of antiepileptic drugs on reproductive endocrine function in individuals with epilepsy. CNS *Drugs*, **19**, 207–223.

JOHNSON DJ, SANDERSON H, BRAIN RA, WILSON CJ, BESTARI KJ and SOLOMON KR (2005) Exposure assessment and microcosm fate of selected selective serotonin reuptake inhibitors. *Regul. Toxicol. Pharmacol.*, **42**, 313–323.

JORGENSEN H, KNIGGE U, KJAER A and WARBERG J (1996) Interactions of histaminergic and serotonergic neurons in the hypothalamic regulation of prolactin and ACTH secretion. *Neuroendocrinology*, 64,329–336.

JOSEPH JC and SCHUNA AA (1990) Management of hypertension in the diabetic patient. *Clin.* Pharm, **9**, 864–873.

JUCHEM M, POLLOW K, ELGER W, HOFFMANN G and MÖBUS V (1993) Receptor binding of norgestimate – a new orally active synthetic progestational compound. *Contraception*, **47,283–294.**

JUDD SJ, LAZARUS L and COMPTON P (1976) Hyperprolactinaemia: its physiology and investigation. *Med. J. Aust.*, **2**, 23–25.

JURGENS HD, HOLTHAUS KIE, JOHNSON AC, SMITH JJL, HETHERIDGE M and WILLIAMS RJ (2002) The potential for estradiol and ethinylestradiol degradation in English rivers. *Environ. Toxicol. Chem.*, **21**(3), 480–488.

KHAN UA, ASLAM M and SAEED SA (2004) Effect of beta adrenergic antagonist on the production of testosterone by rat's Leydig cells. *J. Ayub. Med. Coll. Abbottabad.*, **16**, 26–28.

KALLEN EAJ and OLAUSSON PO (2003) Maternal drug use in early pregnancy and infant cardiovascular defect. *Reprod. Toxicol.*, *17,255–261.*

KALLEN BAJ, OLAUSSON PO and DANIELSSON BR (2005) Is erythromycin teratogenic in humans? *Reprod. Toxicol.*, 20,209–214.

KANALY KA and BERMAN JR (2002) Sexual side effects of SSRI medications: potential treatment strategies for SSRI-induced female sexual dysfunction. *Curr. Womens Health* **Rep.**, *2,409416.*

KATZ IM (1986) Sexual dysfunction and ocular timolol. *JAMA*, **255**, 37–38.

KARTHIKEYAN KG and MEYER MT (2006) Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. *Sci. Total Environ.*, **361**(1–3), 196–207.

KEENE LC and DAVIES PH (1999) Drug-related erectile dysfunction. *Adverse Drug React. Toxicol. Rev.*, **18**, 5–24.

KELLY DL and CONLEY RR (2006) A randomized double-blind 12-week study of quetiapine, risperidone or fluphenazine on sexual functioning in people with schizophrenia. *Psychoneuroendocrinology*, **31**(3), 340–346.

KHER R and KALLA NR (1996) Antifertility effects of an LHRH agonist in male mice. *Contraception*, **53**, 299–306.

KILLICK S and ELSTEIN M (1987) Pharmacologic production of luteinized unruptured follicles by prostaglandin synthetase inhibitors. *Fertil. Steril.*, **47**, 773–777.

KING K, CHAN PJ, PATTON WC and KING A (1997) Antibiotics: effecton cryopreserved-thawed human sperm motility *in vitro*. *Fertil. Steril.*, **67**(6), 1146–1151.

KLEIN TA, RICHMOND JA and MISHELL DR, JR (1976) Pelvic tuberculosis. *Obstet. Gynecol.*, **48**, 99–104.

KLONER RA (2003) Erectile dysfunction in the cardiac patient. *Curr. Urol. Rep.*, **4**, 466–471.

KNOBIL E (1988a) The neuroendocrine control of ovulation. *Hum. Reprod.*, **3**, 469–472.

KNOBIL E (1988b) The hypothalamic gonadotrophic hormone releasing hormone (GnRH) pulse generator in the rhesus monkey and its neuroendocrine control. *Hum. Reprod.*, **3**, 29–31.

KNOBIL E (1990) The GnRH pulse generator. Am. *J. Obstet. Gynecol.*, **163**, 1721–1727.

KOLETTIS PN (2003) Evaluation of the subfertile man. *Am. Fam. Physician*, **67**, 2165–2172.

KORNER W, SPENGLER P, BOLZ U, SCHULLER W, HANF V and METZGER JW (2001) Substances with estrogenic activity in effluents of sewage treatment plants in southwestern Germany. 2. Biological analysis. *Environ. Chem. Toxicol.*, *20*, 2142.

KROES R, MULLER D, LAMBE J, LOEWIK MRW, VAN KLAVEREN J, KLEINER J, MASSEY R, MAYER S, URIETA I, VERGER P and VISCONTI A (2002) Assessment of intake from the diet. *Food Chem. Toxicol.*, **40**, 327–385.

KUSHNIRUK II (1976) [Effect of several antibacterial preparations on the concentration of nucleic acids in spermatogenic epithelial cells]. *Tsitol. Genet.*, **10**, 342–344.

KUCH HM and BALLSCHMITER K (2000) Determination of endogenous and exogenous estrogens in effluents from sewage treatment plants at the ng/L-level. *Fresenius J. Anal. Chem.*, **366**, 392–395.

KUHNZ W, HEUNER A, HUMPEL M, SEIFERT W and MICHAELIS K (1997) *In vivo* conversion of norethisterone and norethisterone acetate to ethinyl etradiol in postmenopausal women. *Contraception*, 56, 379–385.

LABBATE LA, CROFT HA and OLESHANSKY MA (2003) Antidepressant-related erectile dysfunction: management via avoidance, switching antidepressants, antidotes, and adaptation. *J. Clin. Psychiatry*, **64** Suppl 10, 11–19.

LAM MW, YOUNG CJ, BRAIN RA, JOHNSON DJ, HANSON MA, WILSON CJ, RICHARDS SM, SOLOMON KR and MABURY SA (2004) Aquatic persistence of eight pharmaceuticals in a microcosm study. *Environ. Toxicol. Chem.*, 23, 1431–1440.

LÄNGE R, HUTCHINSON TH, CROUDACE CP, SIEGMUND F, SCHWEINFURTH H, HAMPE P, PANTER GH and SUMPTER JP (2001) Effects of the synthetic estrogen 17α-ethinylestradiol on the life-cycle of the fathead minnow (*Pimephales promelas*). *Environ. Toxicol. Chem.*, **20**, 1216–1227.

LARSSON DGJ, ADOLFSSON-ERICI M, PARKKONEN J, PETTERSSON M, BERG AH, OLSSON P-E and FORLIN L (1999) Ethinyloestradiol – an undesired fish contraceptive? Aquatic *Toxicol.*, **45**, 91–97.

LESKO LM, STOTLAND NL and SEGRAVES RT (1982) Three cases of female anorgasmia associated with MAOIs. *Am. J. Psychiatry*, **139**, 1353–1354.

LINDBERG RH, WENNBERG P, JOHANSSON MI, TYSKLIND M and ANDERSSON BA (2005) Screening of human antibiotic substances and determination of weekly mass flows in five sewage treatment plants in Sweden. *Environ. Sci. Technol.*, 39, 3421–3429.

LINDQVIST N, TUHKANEN T and KRONBERG L (2005) Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters *Water Res.*, **39**, 2219–2228.

LIU RC, HAHN C and HURTT ME (1996) The direct effect of hepatic peroxisome proliferators on rat Leydig cell function *in vitro*. *Fundam. Appl. Toxicol.*, **30**, 102–108.

LUIJTEN M, TOMENKO V, POPOV V and PIERSMA AH (2005) A scenariospecific prioritization List of chemicals bearing a high potential of affecting human fecundity D1.1. Contract No. FOOD-CT-2004-513953.

MANTERO F and LUCARELLI G (2000) Aldosterone antagonists in hypertension and heart failure. *Ann. Endocrinol (Paris)*, **61**, 52–60.

MARIK J and HULKA J (1978) Luteinized unruptured follicle syndrome: a subtle cause of infertility. *Fertil. Steril.*, **29**, 270–274.

MARTI B (1991) Health effects of recreational running in women. Some epidemiological and preventive aspects. *Sports Med.*, **11**, 20–51.

MASALA A, DELITALA G, DEVILLA L, ALAGNA S and ROVASIO PP (1979) Enhancement of insulin-induced prolactin secretion by fluoxetine in man. *J. Clin. Endocrinol. Metab.*, **49**, 350–352.

MATUSZKIEWICZ-ROWINSKA J, SKORZEWSKA K, RADOWICKI S, SOKALSKI A, PRZEDLACKI J, NIEMCZYK S, WLODARCZYK D, PUKA J and SWITALSKI M (1999) The benefits of hormone replacement therapy in pre-menopausal women with oestrogen deficiency on haemodialysis. *Nephrol. Dial. Transplant*, **14**, 1238–1243.

MELMAN A, FERSEL J and WEINSTEIN P (1984) Further studies on the effect of chronic alpha-methyldopa administration upon the central nervous system and sexual function in male rats, *J. Urol.*, **132**, 804–808.

MELTZER HY, FANG VS, TRICOU BJ and ROBERTSON A (3982) Effect of antidepressants on neuroendocrine axis in humans. *Adv. Biochem Psychopharmacol.*, **32**, 303–316.

MESTON CM (2004) A randomized, placebo-controlled, crossover study of ephedrine for SSRI-induced female sexual dysfunction. *J. Sax Marital Ther.*, **30**, 57–68.

MESTON CM, GORZALKA BB and WRIGHT JM (1997) Inhibition of subjective and physiological sexual arousal in women by clonidine. *Psychosom. Med.*, **59**, 399–407.

METCALFE CD, METCALFE TL, KIPARISSIS Y, KOENIG BG, KHAN C, HUGHES RJ, CROLEY TR, RAYMOND EM and POTTER T (2001) Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by *in vivo* assays with Japanese medaka (*Oryzias latipes*). *Environ. Toxicol. Chem.*, **20**, 297–308.

METCALFE CD, MIAO XS, KOENIG BG and STRUGER J (2003) Distribution of acidic and neutral drugs in surface waters near sewage treatment plants in the Lower Great Lakes, Canada. *Environ. Toxicol. Chem.*, **22**, 2881–2889.

MIAO XS and METCALFE CD (2005) Determination of carbamazepine and its metabolites in aqueous samples using liquid chromatography-electrospray tandem mass spectrometry. *Anal. Chem.*, 75, 3731–3738.

MIAO XS, BISHAY F, CHEN M and METCALFE CD (2004) Occurrence of antimicrobials in the final effluents of wastewater treatment plants in Canada. *Environ. Sci. Technol.*, **38**, 3533–3541.

MIKKONEN K, TAPANAINEN P, PAKARINEN AJ, PAIVANSALO M, ISOJARVI JI and VAINIONPAA LK (2004) Serum androgen levels and testicular structure during pubertal maturation in male subjects with epilepsy. *Epilepsia*, **45**, 769–776.

MIMEAULT C, WOODHOUSE AJ, MIAO XS, METCALFE CD, MOON TW and TRUDEAU VL (2005) The human lipid regulator, gemfibrozil bioconcentrates and reduces testosterone in the goldfish, *Carassius auratus*. *Aquat. Toxicol.*, **73**, 44–54.

MITCH RA (1989) Carbamazepine toxicity precipitated by intravenous erythromycin. *DICP*, **23**(11), 878–879.

MOLITCH ME (1992) Pathologic hyperprolactinemia. *Endocrinol. Metab. Clin. North Am.*, 21,877–901.

MONTEJO-GONZALEZ AL, LLORCA G, IZQUIERDO JA, LEDESMA A, BOUSONO M, CALCEDO A, CARRASCO JL, CIUDAD J, DANIEL E, DE LA GJ, DERECHO J, FRANCO M, GOMEZ MJ, MACIAS JA, MARTIN T, PEREZ V, SANCHEZ JM, SANCHEZ S and VICENS E (1997) SSRI-induced sexual dysfunction: fluoxetine, paroxetine, sertraline, and fluvoxamine in a prospective, multicenter, and descriptive clinical study of 344 patients. *J. Sex Marital Ther.*, **23**, 176–194.

MONTGOMERY SA, BALDWIN as and RILEY A (2002) Antidepressant medications: a review of the evidence for drug-induced sexual dysfunction. *J. Affect. Disord.*, **69**, 119–140.

MORRISSETTE DL, SKINNER MH, HOFFMAN BB, LEVINE RE and DAVIDSON JM (1993) Effects of antihypertensive drugs atenolol and nifedipine on sexual function in older men: a placebo-controlled, crossover study *Arch. Sex Behav.*, **22**, 99–109.

MORRISON JL, RIGGS KW and RURAK DW (2005) Fluaxetine during pregnancy: impact on fetal development. *Reprod. Fertil. Dev.*, **17**, 641–650.

MOSS HB (1983) More cases of anorgasmia after MAOI treatment. *Am. J. Psychiatry*, **140**,266.

MOSS HB and PROCCI WR (1982) Sexual dysfunction associated with oral antihypertensive medication: a critical survey of the literature. *Gen. Hosp. Psychiatry*, **4**, 121–129.

MULLER SC, EL DAMANHOURY H, RUTH J and LUE TF (1991) Hypertension and impotence. *Eur. Urol.*, **19**, 29–34.

MURDIA A, MATHUR V, KOTHARI LK and SINGH KP (I 978) Sulpha-trimethoprim combinations and male fertility. *Lancer*, 2,375–376.

NAFZIGER AN, BERTINO JS, JR, GOSS-BLEY AI and KASHUBA AD (1999) Incidence of sexual dysfunction in healthy volunteers on fluvoxamine therapy. *J. Clin. Psychiatry*, **60**, 187–190.

NASH JP, KIME DE, VAN DER VEN LT, WESTER PW, BRION F, MAACK G, STAHLSCHMIDT-ALLNER P and TYLER CR (2004) Long-term exposure to environmental concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish. *Environ. Health Perspect.*, **112**(17), 1725–1733.

NEWMAN RJ and SALERNO HR (1974) Letter: Sexual dysfunction due to methyldopa. *Br. Med. J.*, 4,106.

NULMAN I and KOREN G (1996) The safety of fluoxetine during pregnancy and lactation. *Teratology*, **53**, 304–308.

OEHNINGER S and ALEXANDER NJ (1991) Male infertility: the focus shifts to sperm manipulation. *Curr. Opin. Obstet. Gyaecol.*, **3**, 182–190.

O'GRADY JP, CALDWELL BV, AULETTA FJ and SPEROFF L (1972) The effects of an inhibitor of prostaglandin synthesis (indomethacin) on ovulation, pregnancy, and pseudopregnancy in the rabbit. Prostaglandins, **1**, 97–106.

OLIVE DL., LINDHEIM SR and PRITTS EA (2003) Endometriosis and infertility: what do we do for each stage? *Curr. Womens Health Rep.*, **3**, 389–394.

ORME MLE, BACK DJ and BRECKENBRIDGE AM (1983) Clinical pharmacokinetics of oral contraceptives. *Clin. Pharmacokinetics*, **8**, 95–136.

ORME M, BACK DJ, WARD S, *et al.* (1991) The pharnacokinetics of ethynylestradiol in the presence and absence of gestodene and desogestrel. *Contraception*, 43, 305–316.

ORN S, HOLBECH H, MADSEN TH, NORRGREN L and PETERSEN GI (2003) Gonad development and vitellogenin production in zebrafish (*Danio rerio*) exposed to ethinylestradiol and methyltestosterone. *Aquat. Toxicol.*, **65**(4), 397–411.

OU YC, HWANG n, YANG CR, CHANG CL, CHANG CH, WU HC and HWANG YF (1991) Hormonal screening in impotent patients. *J. Formos. Med. Assoc.*, **90**, 560–564.

PALL M, FRIDEN BE and BRANNSTROM M (2001) Induction of delayed follicular rupture in the human by the selective COX-2 inhibitor rofecoxib: a randomized double-blind study. *Hum. Reprod.*, 16,1323–1328.

PAPOULIAS DM, NOLTIE DB and TILLITT DE (1999) An *in vivo* model fish system to test chemical effects on sexual differentiation and development: exposure to ethinyl estradiol. *Aquatic Toxicol.*, 48, 37–50.

PATEL R and ROSENGREN RJ (2001) Acetaminophen elicits anti-estrogenic but slot estrogenic responses in the immature mouse. *Toxicol. Lett.*, 122, 89–96.

PENTLAND 8, ANDERSON DA and CRITCHLEY JA (1981) Failure of ejaculation with indoramin. *Br. Med. J. (Clin. Res. Ed.]*, 282, 1433–1434.

PETERSON EW, DAVIS RK and ORNDORFF HA (2001) 17β-Estradiol as an indicator of animal waste contaminntion in mantled karst aquifers. *J. Environ. Qual.*, 29, 826–834.

PIERCE JR, JR (1983) Cimetidine-associated depression and loss of libido in a woman. Am. *J. Med. Sci.*, 286, 31–34.

PIZARRO S, BARGAY J and D'AGOSTO P (1990) Gemfibrozil-induced impotence. *Lancet*, 336,1135.

POHL R (1983) Anorgasmin caused by MAOIs. *Am. J. Psychiatry*, 140,510.

POHLAND RC, BYRD TIC, HAMILTON M and KOONS JR (1989) Placental transfer and fetal distribution of fluoxetine in the rat. *Toxicol. Appl. Pharmacol.*, 98, 198–205.

PRESCOTT LP (2000) Paracetamol: past, present, and future. *Am. J. Ther.*, 7, 143–147.

PURDOM CE, HARDIMAN PA, BYE VJ, ENO NC, TYLER CR and SUMPTER J (2994) Estrogenic effects of effluents from sewage treatment works. *Chem. Ecol.*, 8, 275–285.

RANNEY RE (1977) Comparative metabolism of 17α-ethynyl steroids used in oral contraceptives. *J. Toxicol. Environ. Health*, 3, 139–166.

RATHNER M VON and SONNEBORN M (1979) Biologishe wirksame Oestrogene in Trink- und Abwasser. *Forum Staedte.* Hygiene, 30,4549.

REEL JR, LAWTON AD and LAMB JC (1992) Reproductive toxicity evaluation of acetaminophen in Swiss CD-1 mice using a continuous breeding protocol. *Fundam. Appl. Toxicol.*, 18, 233–239.

RICHARDSON ML and BOWRON JM (1985) The fate of pharmaceutical chemicals in the aquatic environment. *J. Pharm. Pharmacol.*, 37, 1–12.

ROBERTS PH and THOMAS W (2006) The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. *Sci. Total Environ.*, 356(1–3), 143–153.

ROBINSON CD, BROWN E, CRAFT JA, DAVIES IM, MOFFAT CF, PIRIE D, ROBERTSON F, STAGG RM and STRUTHERS S (2003) Effects of sewage effluent and ethynyl oestradiol upon molecular markers of oestrogenic exposure, maturation and reproductive success in the sand goby (*Pomatoschistus minutus*, Pallas). *Aquatic* Toxicol., 62, 119–134.

ROSEN RC and MARIN H (2003) Prevalence of antidepressant-associated erectile dysfunction. *J. Clin. Psychiatry*, 64 Suppl 10, 510.

ROSEN RC, KOSTIS JB and JEKELIS AW (1988) Beta-blocker effects on sexual function in normal males. *Arch.* Sex *Behav.*, 17,241–255.

ROSEN RC, LANE RM and MENZA M (1999) Effects of SSRIs on sexual function: a critical review. *J. Clin. Psychopharmacol*, 19, 67–85.

ROSSI AG, SOARES JM, JR, MOTTA EL, SIMOES MJ, OLIVEIRA-FILHO RM, HAIDAR MA, RODRIGUES DL and BARACAT EC (2002) Metoclopramide-induced hyperprolactinemia affects mouse endometrial morphology. *Gynecol. Obstet.* Invest., 54,185–190.

ROSTE LS, TAUBOLL E, HAUGEN TB, RJORNENAK T, SAETRE ER and GJERSTAD L (2003) Alterations in semen parameters in men with epilepsy treated with valproate or carbamazepine monotherapy. *Eur. J. Neurol.*, 10,501–506.

RUDKIN L, TAYLOR MJ and HAWTON K (2004) Strategies for managing sexual dysfunction induced by antidepressant medication. *Cochrane Database Syst. Rev.*, CD003382.

SCHARDEIN JL (1980) Studies on the components of an oral contraceptive agent in albino rats II. Progestogenic component and comparison of effects of the components and the combined agent. *J. Toxicol. Environ. Health*, **6**, 895–906.

SCHLEGEL PN, CHANG TS and MARSHALL FF (1991) Antibiotics: potential hazards to male fertility. *Fertil. Steril.*, **55**(2), 235–242.

SCHOLZ S and GUTZEIT HO (2000) 17-a-ethinylestradiol affects reproduction, sexual differentiation and aromatase gene expression of the medaka (*Oryzias* latipes). *Aquatic Toxicol.*, **50**, 363–373.

SEGRAVES RT (1988) Sexual side-effects of psychiatric drugs. *Int. J. Psychiatry Med.*, **18**, 243–252.

SHEN WW and MALLYA AR (1983) MAOI-induced inhibited Female orgasm, *Am. J. Psychiatry*, **140**, 1275.

SHEN WW and PARK S (1982) Thioridazine-induced inhibition of female orgasm. *Psychiatr. J. Univ. Ott.*, 7,249–251.

SHEN WW and SATA LS (1983) Inhibited female orgasm resulting from psychotropic drugs. A clinical review. *J. Reprod. Med.*, **28**, 497–499.

SHEN WW and SATA LS (1990) Inhibited female orgasm resulting from psychotropic drugs. A five-year, updated, clinical review. *J. Reprod. Med.*, **35**, 11–14.

SIDI AA, CAMERON JS, DUFFY LM and LANGE PH (1986) Intracavernous drug-induced erections in the management of male erectile dysfunction: experience with 100 patients. *J. Urol.*, 135,704–706.

SILVESTRI A, GALETTA P, CERQUETANI E, MARAZZI G, PATRIZI R, FINI M and ROSANO GM (2003) Report of erectile dysfunction after therapy with beta-blockers is related to patient knowledge of side effects and is reversed by placebo. *Eur. Heart J.*, **24**, 1928–1932.

SLOB W and PIETERS MN (1998) A probabilistic approach for deriving acceptable human intake Limits and human health risks from toxicological studies: general framework. *Risk Analysis*, 18,787–798.

SMALDONE M, SUKKARIEH T, REDA A and KHAN A (2004) Epilepsy and erectile dysfunction: a review. *Seizure*, **13**, 453–459.

SMITH PJ and TALBERT st (1986) Sexual dysfunction with antihypertensive and antipsychotic agents. *Clin. Pharm.*,5,373–384.

SOLIMAN GA and ABLA AM (1999) Effects of antiepileptic drugs carbamazepine and sodium valproate on fertility of male rats. *Dtsch. Tierarztl. Wochenschr.*, **106**, 110–113.

SPARWASSER C, SCHMELZ HU, DRESCHER P, ECKERT R and MADSEN PO (1998) Role of intracellular Ca2+ stores in smooth muscle of human penile erectile tissue. *Urol. Res.*, **26**, 189–193.

STANCZYK FZ (1994) Structure–function relationships, potency, and pharmacokinetics of progestogens. In: Lobo RA, ed. *Treatment of the Postmenopausal Woman: Basic and Clinical Aspects*. New York: Raven Press; 69–89.

STANCZYK FZ (1997) Pharmacokinetics of the new progestins and influence of gestodene and desogestrel on ethinylestradiol metabolism. *Contraception*, **55**, 273–282.

STANCZYK FZ and ROY S (1990) Metabolism of levonorgestrel, norethindrone, and structurally related contraceptive steroids *Contraception*, **42**, 67–96.

STANCZYK FZ, LEYDEN J, KEMPERS S, *et al.* (2000) The effect of an oral contraceptive containing 20 µg ethinyl estradiol and 100 µg levonorgestrel (EE 20/LNG 100) on ovarian, adrenal, and peripheral androgens *Gynecol Endocrinol.*, **14**(suppl), 137. Abstract R.T59.

STANFORD MS and PATTON JH (1993) In utero exposure to fluoxetine HCl increases hematoma frequency at birth. *Pharmacol. Biochem Behav.*, **45**, 959–962.

STOKER TE, GOLDMAN JM and COOPER RL (2001) Delayed ovulation and pregnancy outcome: effect of environmental toxicants on the neuroendocrine control of the ovary (1). *Environ. Toxicol. Pharmacol.*, **9**, 117–129.

STOLKER AA, NIESING W, HOGENDOORN EA, VERSTEEGH JF, FUCHS R and BRINKMAN UA (2004) Liquid chromatography with triple-quadrupole or quadrupole-time of flight mass spectrometry for screening and confirmation of residues of pharmaceuticals in water. *Anal. Bioanal. Chem.*, **378**, 955–963.

STONE S, KHAMASHTA MA and NELSON-PIERCY C (2002) Nonsteroidal anti-inflammatory drugs and reversible female infertility: is there a link? *Drug Safety*, **25**, 545–551.

STUMPF M, TERNES TA, HABERER K and BAUMANN W (1996) Nachweis von naturlichen und synthetischen Ostrogenen in Klaranlagen und Fliessgewassern. *Vom Wasser* (in German), **87**, 251–261.

TABAK H, BLOOMHUFF RN and BUNCH RL (1981) Steroid hormones as water pullutants II, *Dev. Ind. Microbiol.*, **22**, 497–519.

TADOKORO N, VOLLENHOVEN B, CLARK S, BAKER G, KOVACS G, BURGER H and HEALY D (1997) Cumulative pregnancy rates in couples with anovulatory infertility compared with unexplained infertility in an ovulation induction programme. *Hum. Reprod.*, **12**, 1939–1944.

TAMURA K, ABE Y and KOGO H (2000) Phenytoin inhibits both the first ovulation and uterine development in gonadotropin-primed immature rats. *Eur. J. Pharmacol.*, **398**, 317–322.

TANAKA H, YAKOU Y, TAKAHASHI A, HIGASHITAMI K and KOMORI M (2001) Comparison between estrogenicities estimated from DNA recombinant yeast assay and from chemical analyses of endocrine disruptors during sewage treatment. *Water Sci. Technol.*, **43/2**, 125–132.

TANYILDIZI S and BOZKURT T (2003) The effects of lincomycin–spectinomycin and sulfamethoxazole–trimethoprim on hyaluronidase activities and sperm characteristics of rams. *J. Vet. Med. Sci.*, **65**(7), 775–880.

TAUXE-WUERSCH A, DE ALENCASTRO LF, GRANDJEAN D and TARRADELLAS J (2005) Occurrence of several acidic drugs in sewage treatment plants in Switzerland and risk assessment. *Water Res.*, **39**, 1761–1772.

TEICHMANN AT (1996) *Empfängnisverhütung: eine vergleichende Übersicht aller Methoden, Risiken und Indikationen*. Stuttgart, Germany: Georg Thieme Verlag; 23–31.

TERNES TA, STUMPF M, MUELLER J, HABERER K, WILKEN RD and SERVOS M (1999) Behavior and occurrence of estrogens in municipal sewage treatment plants – I. Investigations in Germany, Canada and Brazil. *Sci. Total Environ.*, **225**, 81–90.

THORNEYCROFT I, STANCZYK F, BRADSHAW K, *et al.* (1999) Effect of low-dose oral contraceptives on androgenic markers and acne. *Contraception*, **60**, 255–262.

TIMMERMANS L (1974) Influence of antibiotics on spermatogenesis. *J. Urol.*, **112**, 348–349.

TINAJERO JC, FABBRI A and DUFAU ML (1993) Serotonergic inhibition of rat Leydig cell function by propranolol. *Endocrinology*, **133**, 257–264.

TURKINGTON RW and MACINDOE JH (1972) Hyperprolactinemia in sarcoidosis. *Ann. Intern. Med.*, **76**, 545–549.

UHLER ML, HSU JW, FISHER SG and ZINAMAN MJ (2001) The effect of nonsteroidal anti-inflammatory drugs on ovulation: a prospective, randomized clinical trial. *Fertil. Steril.*, **76**, 957–961.

VANGE N VAN DER, BLANKENSTEIN MA, KLOOSTERBOER HJ, *et al.* (1990) Effects of several low-dose combined oral contraceptives on sex hormone binding globulin, corticosteroid binding globulin, total and free testosterone. *Contraception*, **41**, 345–352.

VERMEIRE T, PIETERS M, RENNEN M and BOS P (2001) Probabilistic assessment factors for human health risk assessment. RIVM report 601516005, TNO report V3489.
VOGEL D, GEHRING M, TENNHARDT L, WELTIN D and BILITEWSKI B (2003) Mobility and fate of endocrine disrupting compounds (EDCs) in soil after application of sewage sludge to agricultural land. In: Pullammanappallil, Pr., McComb, A., Diaz, L. F., Bidlingmaier, W. (eds.): *Proc. 4th Int. Conference ORBIT Ass. on Biol. Process. Organics: Adv. Sustain. Soc.*, April 30–May 2, 2003, Perth, Australia, vol. **1**, 241–250.
VOSE D (2000) *Risk Analysis: A Quantitative Guide.* John Wiley & Sons, Chichester.
WAGHMARAE D (1972) Infertility. *East Afr. Med. J.*, **49**, 698–702.
WARDLE PG, MITCHELL JD, MCLAUGHLIN EA, RAY BD, MCDERMOTT A and HULL MG (1985) Endometriosis and ovulatory disorder: reduced fertilisation in vitro compared with tubal and unexplained infertility. *Lancet*, **2**, 236–239.
WARNEKE L (1994) Phenelzine and sexuality. *J. Clin. Psychiatry*, **55**, 216–217.
WEBB S (2000) Risk assessment for pharmaceuticals. In: *Proceedings of International Seminar on Pharmaceuticals in the Environment.* 9 March 2000, Brussels, Technological Institute.
WEBB S, TERNES T, GIBERT M and OLEJNICZAK K (2003) Indirect human exposure to pharmaceuticals via drinking water. *Toxicol. Lett.*, **142**, 157–167.
WEBSTER J (1979) Male sexual dysfunction and cimetidine. *Br. Med. J.*, **1**, 889.
WEINER RI (1996) Cellular basis of the GnRH pulse generator. *Nippon Sanka Fujinka Gakkai Zasshi*, **48**, 573–577.
WEISS RJ (1991) Effects of antihypertensive agents on sexual function. *Am. Fam. Physician.*, **44**, 2075–2082.
WENZEL A, SCHÄFERS C, VOLLMER G, MICHNA H and DIEL P (2001) *Research Efforts Towards the Development and Validation of a Test Method for the Identification of Endocrine Disrupting Chemicals.* Final Report Contract B6-7920/98/000015.
WHITE WB, ANDREOLI JW and COHN RD (1985) Alpha-methyldopa disposition in mothers with hypertension and in their breast-fed infants. *Clin. Pharmacol. Ther.*, **37**, 387–390.
WIEGEL S, AULINGER A, BROCKMEYER R, HARMS H, LOFFLER J, REINCKE H, SCHMIDT R, STACHEL B, VON TUMPLING W and WANKE A (2004) Pharmaceuticals in the river Elbe and its tributaries. *Chemosphere*, **57**, 107–126.
WILENS TE, COHEN L, BIEDERMAN J, ABRAMS A, NEFT D, FAIRD N and SINHA V (2002) Fluoxetine pharmacokinetics in pediatric patients. *J. Clin. Psychopharmacol.*, **22**, 568–575.
WIRSHING DA, PIERRE JM, MARDER SR, SAUNDERS CS and WIRSHING WC (2002) Sexual side effects of novel antipsychotic medications. *Schizophr Res.*, **56**, 25–30.
XU S, ZHU BT, CAI MX and CONNEY AH (2001) Stimulatory effect of clofibrate on the action of estradiol in the mammary gland but not in the uterus of rats. *J. Pharmacol. Exp. Ther.*, **297**, 50–56.
YARKONI S, SPITZ IM, BEN DAVID M and POLISHUK WZ (1978) Failure of bromocriptine to suppress prolactin in majeptil-induced hyperprolactinemia. *Acta. Eur. Fertil.*, **9**, 185–188.
YOUNG AH and ASHTON CH (1996) Pharmacokinetics of fluoxetine. *Trends Pharmacol. Sci.*, **17**, 400.
YING G-G, KOOKANA RS and RU X-J (2002) Occurrence and fate of hormone steroids in the environment. *Environment Int.*, **28**, 545–551.
ZANAGNOLO V, DHARMARAJAN AM, ENDO K and WALLACH EE (1996) Effects of acetylsalicylic acid (aspirin) and naproxen sodium (naproxen) on ovulation, prostaglandin, and progesterone production in the rabbit. *Fertil. Steril.*, **65**, 1036–1043.
ZAWAR MP, DESHPANDE NM, GADGIL PA and MAHANTA AA (2003) Histopathological study of endometrium in infertility. *Indian J. Pathol. Microbiol.*, **46**, 630–633.

ZILLIOUX EJ, JOHNSON IC, KIPARISSIS Y, METCALFE CD, WHEAT JV, WARD SG and LIU H (2001) The sheepshead minnow as an *in vivo* model for endocrine disruption in marine teleosts: a partial life-cycle test with 17-alpha-ethynylestradiol. *Environ. Toxicol. Chem.*, **20**, 1968–1978.

ZIPPER J, BRUZZONE ME, ANGELO S, MUNOZ V and WHEELER RG (1982) Effect of topically applied adrenergic blockers on fertility. *Int. J. Fertil.*, **27**, 242–245.

ZUCCATO E, CASTIGLIONI S and FANELLI R (2005) Identification of the pharmaceuticals for human use contaminating the Italian aquatic environment. *J. Hazard. Mater.*, **122**, 205–209.

ZUEHLKE S, DUENNBIER U and HEBERER T (2004) Determination of polar drug residues in sewage and surface water applying liquid chromatography–tandem mass spectrometry. *Anal. Chem.*, **76**, 6548–6554.