Pharmaceuticals


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 a food transportation medium 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 serotonin reuptake inhibitors (SSRIs) (fluoxetine hydrochloride, fluvoxamine maleate and sertraline), beta-blockers (propranolol, metoprolol and atenolol), progestins (ethynodiol diacetate, norethindrone and levonorgestrel) and estrogens (17α-ethynylestradiol), antibiotics (sulfamethoxazole-trimethoprim combination, tetracycline, doxycycline, minocycline and erythromycin). The key factors which affected inclusion of lists 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 fecun-
dity 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 prioritises 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 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 anovulatory infertility (Baird, 1979). They may be present with amenorrhea (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 (Yarkoui et al., 1978) and oestrogens (Furuhjelm et al., 1980). Amenorrhoea is also associated with high-dose corticosteroids (Turkington & MacIndoe, 1972), danazol (Dmowski, 1984; Judd 1976; 1992); this can be drug-induced. 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 (Yarkoui et al., 1978) and oestrogens (Furuhjelm et al., 1980). Amenorrhoea is also associated with high-dose corticosteroids (Turkington & MacIndoe, 1972), danazol (Dmowski, 1984; Judd 1976; 1992); this can be drug-induced.

18.2.2 Sexual dysfunction
Sexual function may be divided into three categories reflecting the sexual response cycle: (1) 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).

18.1.3 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 (De la et al., 2003; Dusing, 2005; Klomer, 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 & Procacci, 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) (Hedlund & Andersson, 1985) and prazosin (Melman et al., 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 et 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 (Morrisette 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 (Fovaeus 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. Thoridazine 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, 1991). 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 link 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
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 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 leakage 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 measures 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 the 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 local 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 luteinised 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 al., 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)

<table>
<thead>
<tr>
<th>Pharmaceutical,</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibuprofen</td>
<td>29093500</td>
<td>30426100</td>
<td>31550800</td>
<td>31926100</td>
<td>24283400</td>
</tr>
<tr>
<td>Naproxen</td>
<td>36432400</td>
<td>36715000</td>
<td>35531500</td>
<td>33585600</td>
<td>29445000</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>4404800</td>
<td>3934600</td>
<td>3574500</td>
<td>3316100</td>
<td>2890100</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>54018300</td>
<td>53731200</td>
<td>52343500</td>
<td>51529900</td>
<td>48853700</td>
</tr>
</tbody>
</table>

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 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, but 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)
- STP 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)
- STP 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)

18.53 Diclofenac
Diclofenac inhibits ovulation in the rat and rabbit (Armstrong & Grinwich, 1972; Espey, 1983; O’Grady et al., 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 et al., 2003)
- STP effluent, Switzerland: up to 1.9 μg/L (Tauxe-Wierson 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).

18.54 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). Indomethacin 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 addition, 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 acetaminophens 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)

<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clofibrate</td>
<td>89050</td>
<td>83277</td>
<td>72237</td>
<td>59263</td>
<td>53325</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>6202900</td>
<td>6090700</td>
<td>5727000</td>
<td>5462000</td>
<td>5058300</td>
</tr>
</tbody>
</table>

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 al., 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., 1997), 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 (Metcalf 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 (Stolk 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
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). Gemfibrozil is occasionally associated with impotence and decreased libido (Bain et al., 1990; Pizarro et al., 1990). 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 con-
centrations higher than 60 $\mu$g/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, galactorrhea, or gynecomastia (Ou et al., 1991; Molitch, 1992). Methyldopa has been detected in the environment:

- River Lee, 17.5 $\mu$g/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 al., 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](image)

<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>10352500</td>
<td>10216900</td>
<td><strong>10175100</strong></td>
<td>10134200</td>
<td>10028900</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>10877000</td>
<td>11378900</td>
<td>12017200</td>
<td>12648200</td>
<td>13301600</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>3278100</td>
<td>3240900</td>
<td>3034300</td>
<td>2962300</td>
<td>2972100</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>6288200</td>
<td>5893700</td>
<td><strong>5550700</strong></td>
<td>5338900</td>
<td><strong>5175200</strong></td>
</tr>
</tbody>
</table>

![Image](image)
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 (Zuehkle 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 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). 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 has 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 Fluoxetine 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 long 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 lower 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 et al., 2002; Montejo-Gonzalez et al., 1997; Montgomery et al., 2002).
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/m² 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/m² 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 desmethyIsertraline 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)

<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propranolol</td>
<td>9300300</td>
<td>9092100</td>
<td>8967400</td>
<td>9025200</td>
<td>9030500</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>80386700</td>
<td>86849600</td>
<td>92654400</td>
<td>98935200</td>
<td>108001000</td>
</tr>
<tr>
<td>Atenolol</td>
<td>57244000</td>
<td>60216300</td>
<td>62832400</td>
<td>66683700</td>
<td>70251900</td>
</tr>
</tbody>
</table>

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 $2 \times 10^{-3}$ 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 tail 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

<table>
<thead>
<tr>
<th>Class compound</th>
<th>Name</th>
<th>Relative progestational activity (arbitrary units)</th>
<th>Relative androgenic activity (arbitrary units)</th>
</tr>
</thead>
</table>
| **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                                           |
| **Pregnan progestins**
| Megestrol acetate | 0.4                            | 0                                                |
| Medroxyprogesterone acetate | 0.3                        | 0                                                |
**Table 18.6 Available combination oral contraceptives**

<table>
<thead>
<tr>
<th>Name</th>
<th>Progestin (mg)</th>
<th>Type of estrogen (mcg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>50 mcg estrogen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ogestrel/Ovral/</td>
<td>Norgestrel (0.5)</td>
<td>EE (50)</td>
</tr>
<tr>
<td>Necon/Nelovia/</td>
<td>Norethindrone (1.0)</td>
<td>Mestranol (50)</td>
</tr>
<tr>
<td>Norethin/Norinyl/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ortho-Novum 7/50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovcon 50</td>
<td>Norethindrone (1.0)</td>
<td>EE (50)</td>
</tr>
<tr>
<td>Norlestrin 1/50</td>
<td>Norethindrone acetate (1.0)</td>
<td>EE (50)</td>
</tr>
<tr>
<td>Demulen 50/Zovia 1/50</td>
<td>Ethynodiol diacetate (1.0)</td>
<td>EE (50)</td>
</tr>
<tr>
<td><strong>&lt;50 mcg estrogen plus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>monophasic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lo-Ovral/</td>
<td>Norgestrel (0.3)</td>
<td>EE (30)</td>
</tr>
<tr>
<td>Low-Ogestrel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovcon 35</td>
<td>Norethindrone (0.4)</td>
<td>EE (35)</td>
</tr>
<tr>
<td>Desogen/Ortho-cept</td>
<td>Desogestrel (0.15)</td>
<td>EE (30)</td>
</tr>
<tr>
<td>Levlen/Levora/</td>
<td>Levonorgestrel (0.15)</td>
<td>EE (30)</td>
</tr>
<tr>
<td>Nordette</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ortho-Cyclen</td>
<td>Norgestimate (0.25)</td>
<td>EE (35)</td>
</tr>
<tr>
<td>Necon/Nelova/</td>
<td>Norethindrone (1.0)</td>
<td>EE (35)</td>
</tr>
<tr>
<td>Norinyl/Norethin/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ortho-Novum 1/35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mircette</td>
<td>Desogestrel (0.15)</td>
<td>EE (20)</td>
</tr>
<tr>
<td>Brevicon/Modicon/</td>
<td>Norethindrone (0.5)</td>
<td>EE (35)</td>
</tr>
<tr>
<td>Necon/Nelova 0.5/35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loestrin 1.5/30</td>
<td>Norethindrone acetate (1.5)</td>
<td>EE (30)</td>
</tr>
<tr>
<td>Alesse/Levrette</td>
<td>Levonorgestrel (0.1)</td>
<td>EE (20)</td>
</tr>
<tr>
<td>Locstrin 1/20</td>
<td>Norethindrone acetate (1.0)</td>
<td>EE (20)</td>
</tr>
<tr>
<td>Demulen/Zovia 1/35</td>
<td>Ethynodiol diacetate (1.0)</td>
<td>EE (35)</td>
</tr>
<tr>
<td><strong>&lt;50 mcg estrogen plus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>multiphasic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ortho-Novum 7/7/7/</td>
<td>Norethindrone (0.5, 0.75, 1.0)</td>
<td>EE (35, 35, 35)</td>
</tr>
<tr>
<td>Tri-Levlen/Triphasil/</td>
<td>Levonorgestrel (0.05, 0.075, 0.125)</td>
<td>EE (30, 40, 30)</td>
</tr>
<tr>
<td>Trivora</td>
<td>Norethindrone (0.5, 1.0)</td>
<td>EE (35, 35)</td>
</tr>
<tr>
<td>Jenest</td>
<td>Norethindrone (0.5, 1.0)</td>
<td>EE (35, 35)</td>
</tr>
<tr>
<td>Necon/Nelova/</td>
<td>Norgestimate (0.18, 0.215, 0.250)</td>
<td>EE (35, 35, 35)</td>
</tr>
<tr>
<td>Ortho-Novum 10/11</td>
<td>Norethindrone (0.5, 1.0, 0.5)</td>
<td>EE (35, 35)</td>
</tr>
<tr>
<td>Ortho Tri-Cyclen</td>
<td>Norethindrone acetate (1.0, 1.0, 1.0)</td>
<td>EE (20, 30, 35)</td>
</tr>
</tbody>
</table>
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 consideration 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>
<thead>
<tr>
<th>Table 18.7 Family tree of contraceptive progestins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonanes (levonorgestrel family)</td>
</tr>
<tr>
<td>Levonorgestrel</td>
</tr>
<tr>
<td>Desogestrel</td>
</tr>
<tr>
<td>Norgestimate</td>
</tr>
<tr>
<td>Festodene</td>
</tr>
<tr>
<td>Estranes (norethindrone family)</td>
</tr>
<tr>
<td>Norethindrone</td>
</tr>
<tr>
<td>Norethindrone acetate</td>
</tr>
<tr>
<td>Ethynodiol acetate</td>
</tr>
<tr>
<td>Lystrenol</td>
</tr>
</tbody>
</table>
diacetate, and lynestrenol – are prodrugs. Before these three can exert progestational activity, they must undergo biochemical conversion to nor-ethindrone, their biologically active form (Stanczyk & Roy, 1990). Levo-
norgestrel and gestodene are 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 3Q–35μg ethinyl-estradiol (EE), the progestin dose needed for ovulation inhibition varied widely from high doses for norethindrone (approximately 400μg per day) and norgestimate (200μg per day), to levonorgestrel and desogestrel (60μg per day), to smaller doses for gestodene (approximately 30μg 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 (30–40μg 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 20μg EE oral contraceptives, LNG 100μg and norethindrone acetate (NETA) 1000μg, 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 20μg 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 (3α-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 into account that the amount of Levonorgestrel...
Table 18.8 The consumption of defined daily doses (DDD) of estrogens with progestogens in fixed proportions in the Netherlands in 2000–2004

<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen with lynestrenol (Ministat®)</td>
<td>17925900</td>
<td>15678100</td>
<td>14017600</td>
<td>13563700</td>
<td>9144900</td>
</tr>
<tr>
<td>Estrogen with norethisteron (Necon®)</td>
<td>5501200</td>
<td>5458900</td>
<td>5424400</td>
<td>5471800</td>
<td>596630</td>
</tr>
<tr>
<td>Estrogen with levonorgestrel (Microgynon®)</td>
<td>254781700</td>
<td>275320400</td>
<td>298341600</td>
<td>318354500</td>
<td>82111800</td>
</tr>
<tr>
<td>Estrogen with desogestrel (Marvelon®)</td>
<td>139903000</td>
<td>125862600</td>
<td>104173300</td>
<td>94577100</td>
<td>5761400</td>
</tr>
<tr>
<td>Estrogen with gestodene (Meliane®)</td>
<td>59303500</td>
<td>59994700</td>
<td>51045000</td>
<td>47089300</td>
<td>5214500</td>
</tr>
<tr>
<td>Estrogen with norgestimaat (Cilest®)</td>
<td>5327500</td>
<td>5057000</td>
<td>4440000</td>
<td>4118900</td>
<td>345290</td>
</tr>
<tr>
<td>Estrogen with drospirenon (Yasmin®)</td>
<td>5072400</td>
<td>9349400</td>
<td>9809000</td>
<td>3216800</td>
<td></td>
</tr>
<tr>
<td>Estrogen with norelgestromine (Evra®)</td>
<td></td>
<td></td>
<td></td>
<td>109740</td>
<td>71101</td>
</tr>
<tr>
<td>Total</td>
<td>482742800</td>
<td>492444100</td>
<td>486791300</td>
<td>493094040</td>
<td>98232011</td>
</tr>
</tbody>
</table>

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 (Akpowiro 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 be 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-methoxy-ethynylestradiol 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<sub>EE2</sub> = 0.5; Korner et al. (2001), REP<sub>EE2</sub> = 0.91; Gutendorf and Westendorf (2001), REP<sub>EE2</sub> = 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 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 behaviour, 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 medaka 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 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.,
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 (Metcalf et al., 2001; Nash et al., 2004; Wenzel et al., 2001; Zillioux et al., 2001).

**Production volume and use of 17α-ethynylestradiol**

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-ethynylestradiol 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.,
Physicochemical properties and environmental fate data

<table>
<thead>
<tr>
<th>Physical state at ambient temperature</th>
<th>Solid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water solubility</td>
<td>4.7–19 mg/L,¹</td>
</tr>
<tr>
<td>Octanol–water partition coefficient (log $K_{ow}$)</td>
<td>3.62–4.7</td>
</tr>
<tr>
<td>Organic carbon water partition coefficient (log $K_{ow}$)</td>
<td>3.8 (4.5)</td>
</tr>
</tbody>
</table>

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.

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 fact 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. Comparatively, 70–80% of added estradiol (E2) was mineralised to CO₂ 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 EDCs 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 bioaccumulation 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-ethynylestradiol 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 concentrations 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 Netheners, 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 de 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 Frankfurt/Main the raw sewage was contaminated by EE2 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 on 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 – 10^5 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, 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., 1994, 1996; Purdom et al., 1997). 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 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, 2003). 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 0.94 ng/L.

18.12.6 Conclusions on 17α-ethynylestradiol
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 al., 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 (Kuhn 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 dihydrololate 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)

<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethoprim</td>
<td>1592200</td>
<td>1575300</td>
<td>1530200</td>
<td>1554100</td>
<td>1503100</td>
</tr>
<tr>
<td>Sulfamethoxazole-trimethoprim</td>
<td>2403900</td>
<td>2284800</td>
<td>2206300</td>
<td>2141900</td>
<td>2070700</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>12059200</td>
<td>11825300</td>
<td>11382000</td>
<td>10813900</td>
<td>10507400</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1239660</td>
<td>1086542</td>
<td>945461</td>
<td>887715</td>
<td>870980</td>
</tr>
<tr>
<td>Minocycline</td>
<td>1355100</td>
<td>1412200</td>
<td>1372700</td>
<td>1373900</td>
<td>1504500</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>602910</td>
<td>609710</td>
<td>547020</td>
<td>489480</td>
<td>478670</td>
</tr>
</tbody>
</table>

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)
Endocrine-disrupting chemicals in food

- 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)

18.13.2 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 al., 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 (Miao 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., 1997). 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., 2005)

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 al., 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-Sørensen et al., 1998)
- **STP effluents, Germany**, median level 2.5 μg/L (Hirsch et al., 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 compounds 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, if 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 doses/concentrations 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 NOST-TASOT dose (Crump et al., 1995).

Although simple and straightforward, the N(L)OAEL has 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 little 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 the 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) (Crump 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,
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 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:

\[
RfD = \frac{N(L)OAE L}{U F_1 \times U F_2 \times \ldots \times U F_n}
\]  \hspace{1cm} (18.1)

where \( U F_i \) 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 log-normal distributions are considered to be plausible) (Vermeire et al., 2003).

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 based 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 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 are developed using quantitative data in order to predict quantitative parameters. There are two types of predictive methods:

- **Formalised methods;**
  expert judgement.

Formalised 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 based 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 construct 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 on 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 therefore 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 that 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

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: impli-


Endocrine-disrupting chemicals in food


CUTLER GB, SAWER 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.


Ecotoxicology

Endocrine-disrupting chemicals in food


Effect of drugs on reproductive endocrine function in individuals with epilepsy. *CNS Drugs*, 19, 207–223.


