Ministerie van Verkeer en Waterstaat

Directoraat-Generaal Rijkswaterstaat

Rijksinstituut voor Kust en Zee/RIKZ

Chemical study on estrogens

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Preface

In the framework of the project "Investigating for chemicals in the future", the North Sea Directorate has put the department of Rijkswaterstaat Institute for Coastal and Marine Management (RIKZ) in charge, to start a study on unknown chemicals. The object of this project is to identify the most important contaminants, which present a threat to the North Sea and the identification of gaps in policy, management and knowledge. In the project monitoring data are evaluated and a number of "new" substances are proposed as a potential threat for the North Sea. On the 30th of June BKH Consulting Engineers has received the order to make a study on a selection of the natural and synthetic estrogens. This study will be directed on the whole track of estrogens in the environment. From production and emission to immission, waste and effects.
The project is coordinated by Mrs drs A.M.C.M. Pijnenburg of RIKZ. The project-leader of BKH is Mrs drs C.P. Groshart. The authors of the report are: Mr drs P.C. Okkerman and Mrs drs C.P. Groshart.

Summary

General
Natural estrogens are mainly formed in the ovary, during pregnancy in the placenta and in smaller quantities in the adrenal glands and testicles. Natural estrogens control the development of secondary female sex characteristics and together with the gestagens control almost all of the reproductive processes in women.
Synthetic estrogens are used as a pharmaceutical product as an oral contraceptive and hormonal replacement therapy. In the USA synthetic estrogens are also used in veterinary the induce growth of animals. The most commonly used synthetic estrogen is 17a-ethinyloestradiol. A synthetic estrogen is in principle a more stable compound in order to fullfill a wanted effect, before the substance is degraded and metabolised.

Sources and emissions
Primary production of natural estrogens occurs in female mammalian animals and in female humans.
Synthetic estrogens as 17ß-oestradiol and 17a-ethinyloestradiol are produced in the Netherlands, Germany and the USA but production volumes are largely unknown.

The total emissions of endogenous estrogens by human beings and animals in the Netherlands can be estimated at approximately 50 kilograms per day. This figure is possibly an underestimate, since neither, other livestock - such as rabbits, ducks, sheep, goats, horses, etc - nor companion animals, were included.
Emissions of ethinyl oestradiol ('the pill') are estimated at 43 grams per day. This may be an underestimate, in view of the fact that the progestagens that are also in the anticonception pill, may also be metabolised to steroid metabolites. In any case, this contribution is insignificant compared with the estimated total emissions of natural estrogens (50 kg/day). It should be noted that emissions from countries surrounding the Netherlands have not been taken into consideration.

Assuming a leaching of 3% from applied manure to surface water and an average rainfall of 60 mm/month, the concentration in surface water, will be 1.3 µg/l after application of the maximum allowed amount of manure of sows in 1 month and 0.43 µg/l in 3 months in local waters. After application of manure of cows in the maximum allowed amount in 1 month, the concentration in surface water is 0.9 µ/l and in 3 months 0.3 µg/l in local waters.

Estimated concentrations in regional waters range from 19-76 ng/l for the river Rhine and from 35-140 ng/l for the river Meuse.

Environmental characteristics and toxicity in aquatic systems
All oestradiols, oestrone and ethinyloestradiol have a low vapour pressure and a low water solubility. The log Kow varies from 3.13 for oestrone to 4.01 for both oestradiols. This indicates a moderate potential to bioaccumulate. There are no data on bioaccumulation.

Occurrence and behaviour in aquatic systems
There is no information on hydrolysis or photolysis.
There are some differing results for biodegradation in STP in Germany and Brazil. The differences are probably due to the difference in temperature. Based on the

limited data it can be concluded that oestradiols and oestrone are fairly rapidly degraded. Ethinyloestradiol seems to be more persistent. Under anaerobic conditions degradation is considerably lower.
Concentrations in the Netherlands have only been measured in one study in 1997. Highest concentrations were measured in Lobith and Eijsden (upto 5.5 ng/l). In large surface waters and marine waters, dilution occurs and concentrations are hardly detectable. Concentrations in sewerage in The Netherlands are lower than in the surrounding countries (UK, Germany)

There are very limited data on concentrations in food: milk of carying cows contains about 1 µg estrogen/l.

Toxicity
Estrogens are (de)oxidated, hydrolysed and methylised in liver and conjugated with glucuronic acid or sulfate. 17ß-oestradiol is easily oxidised to oestrone, which is further metabolised to oestriol or 2-methoxy-oestrone. Ethinyloestradiol is metabolised to hydroxy-ethinyloestradiol and further metabolised to methoxy-ethinyloestradiol or deethinylated to oestrone. Estrogens are excreted in faeces and urine. All estrogens are suspected carcinogens. Data on acute toxicity indicate that estrogens are very toxic to mammalians.

Toxicity data are scarce. Based on endocrine disrupting effects all estrogens are very toxic. Based on acute effects on survival and reproduction 17a-ethinyloestradiol is also very toxic to freshwater algae and crustaceans.
Based on chronic effects on survival and reproduction 17ß-oestradiol, oestrone and 17a-ethinyloestradiol are only slightly toxic to marine crustaceans.

There are no limit values derived for the estrogens. There is a iMPC derived for 17a-ethinylopestradiol of 1 µg/l. Concentrations in surface water in the Netherlands do not exceed this iMPC.

Policy
There is hardly any policy on estrogens in relation to the environment. 17ß-Oestradiol, oestrone and 17-ethinyloestradiol are on the OSPAR list VI. However there are no actions to this group of substances, yet.
There is, however, a ban on the use of estrogens as growth inducers in farm animals in Europe (88/146/EEC). In Canada and the USA estrogens are still used for this purpose. This ban on meat from farm animals treated with estrogens, has led to an discussion with Canada and the USA.
Furthermore there is a regulation that estrogen may not be part of cosmetic products (76/768/EEC). There are also plans for a directive on assessing the risk of environmental exposure of veterinary products and a similar directive on medicines is launched as a draft.

Conclusions and recommendations
In general data on estrogens are very scarce. Data should be produced on behaviour, distribution, production and emission.
Also, more data on acute and chronic toxicity should be produced.
Furthermore there should be a guideline on how to treat effects on the endocrine system. Especially interesting is, how these endocrine effects influence the populations.

Recommended is to conduct a study into the production volumes of estrogens and the concentrations of estrogens in groundwater, drinking water and food.

1 Introduction

1.1 Backgrounds

Natural estrogens consist of the substances 17a-Oestradiol, 17ß-Oestradiol, and Oestrone. Synthetic estrogens consist of the substances 17a-ethinyloestradiol and mestranol, which exert the same activity as natural estrogens, but are used in pharmaceuticals. Because mestranol is the degradation product of ethinyloestradiol and has a lower activity, this substance is not further discussed in this report.

The word "estrogen" refers to the class of hormones produced by the body with estrus activity. The three most important hormones of this estrogen class are oestrone, oestradiol, and oestriol. In popular writing, however, each of the specific members of the class continues to be referred to as estrogen. (Lee, 1999). The natural estrogens are produced in the body of humans, cattle, pets and other mammals. Large amounts of the natural estrogens are excreted from the body and may reach the environment through sewage waste-water and the application of manure.

Discharges of human drugs (like synthetic estrogens) and their metabolites from the production facilities, hospitals and private household effluent as well as the disposal of non-used drugs pose a load on the environment. Pharmaceuticals are suspicious environmental contaminants as they are biologically active, which obviously is a part of their nature. Furthermore pharmaceuticals are usually lipophilic in order to be able to pass membranes and often have a low biodegradability (persistent) in order to avoid the substance to be inactive before having a curing effect. This poses a potential for bioaccumulation and persistence in the environment. With a few exceptions, pharmaceuticals are not high-production volume chemicals, and the expected environmental concentrations may consequently be assumed low. To present a risk to the environment the substances should be able to cause effects at low exposure levels, i.e. preferably have a high receptor affinity (Christensen, 1998).

About the effects of estrogens on the aquatic environment little information is available. This is alarming because several estrogens are found in the aquatic environment. To get an opinion on the consequences of the occurrence of these chemicals in the aquatic environment, the underlying report is composed. This report gives an overview of the available knowledge on estrogens in regard to the aquatic environment. Important criteria for selecting these chemicals were:

- they are used and/or produced in the Netherlands;
- they are on several attention lists;
- they are expected to be persistent and bioaccumulative;
- they are expected to present a danger to the environment.

This report is produced in the framework of the project “Investigating for chemicals in the future”.

1.2 Objectives

The objectives of this study with regard to the estrogens are:
To give an analysis of the problems in the aquatic environment: a description of the load, occurrence, behaviour and effects and a analysis of the problems which

indicate how the presence of the estrogens may disturb the functioning of the different water systems by effects on sensitive organisms. Furthermore giving an overview of the national and international policy.

In this study the most recent information on estrogens has been used. It is possible that in some attention areas the essential information is not yet available. In these cases recommendations for further research will be done.
The study is broadly set up. The next aspects will be handled. In chapter 2 the chemical characteristics of the estrogens are described. In chapter 3 the production process is clarified and the use of these chemicals is described. In chapter 4 the sources of emissions, primarily to the aquatic environment, are estimated and specified. In chapter 5 and 6 the behaviour in the environment and the occurrence in the environment are described, respectively. In chapter 7 and 8 an overview is given of the toxicity data and the policy, respectively.

1.3 Limitations

In principle the study conforms itself to information that has a relation to aquatic systems. The situation around air or soil will be briefly described. Furthermore the emphasis lies on the situation in the Netherlands. In some cases the situation of the basins of Rhine, Meuse and Schelde will be commented. The information will be presented briefly. For more extensive information referred is to the concerned sources.

2 Physical chemical properties

2.1 Identification

In this study the risks are assessed of natural and artificially produced estrogens in the aquatic environment. In regard to the current environmental concern about their occurrence in the environment, behaviour and effects of the following compounds are studied:

- Oestradiols: 17a-Oestradiol and 17ß-Oestradiol
- Oestrone
- 17a-Ethinyloestradiol

Natural estrogens are mainly formed in the ovary, during pregnancy in the placenta and moreover in smaller quantities in the adrenal glands and testicles. In general they control the development of the secondary female sex characteristics and together with the gestagens they regulate almost all of the reproductive processes in women.

The natural estrogens have an (very stable) aromatic A ring and a phenolic hydroxyl group in position 3. They are transported in the blood bound to plasma globulins or albumins, metabolized in the liver and excreted in the urine or via the bile and intestines. From the intestines they can be reabsorbed again.
A woman's daily estrogen secretion is 25-100 µg depending on the menstrual cycle; this quantity can rise to 30 mg/day at the end of pregnancy. But estrogens are produced not only in humans, all the animals are estrogen-producers, too. A pregnant mare, for example, produces 100 mg/day (Turan , 1995).

Synthetic estrogens are used primarily as pharmaceuticals as oral contraceptives and for hormonal replacement therapy. The natural estrogens are orally inactive or only at higher dosage active, since they are quickly metabolized. More active and above all more stable compounds were developed by the introduction of an ethinyl-group in position 17- of the oestradiol molecule. Ethinyl oestradiol and its 3-Me-ether mestranol are the most frequently used estrogen components in contraceptives. Through the introduction of the ethinyl-group the D ring becomes extremely stabilized against oxidation. Thus, oestrone - which plays a central role in the metabolism of the natural hormones - cannot be formed here. The consequence of this increased stability is, that ethinyl oestradiol is excreted up to 80% unchanged in conjugated form (Ranney, 1977).

2.1.1 Oestradiols

17-ß-oestradiol is a natural as well as an synthesized hormone, formed from testosterone via 19-OH-testosterone.

Table 2.1:
Characteristics and physical properties of 17a-Oestradiol (Sax, 1989)

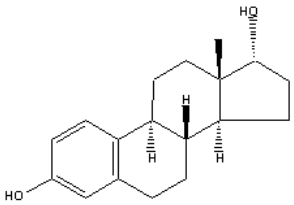
Substance	17α-Oestradiol
CAS number	57-91-0
Chemical formula	C18H24O2
Molecular mass	272.42
Physical state	-
Chemical structure	
Synonyms	3,17-dihydroxy-estratriene; 3.17-α-dihydroxyoestra-1,-3,5(10)-triene; estrat-1,3,5(10)-triene-3,17-α-diol; 1,3,5-estratriene-3,17-α-diol; estradiol-17α
Technical products	-

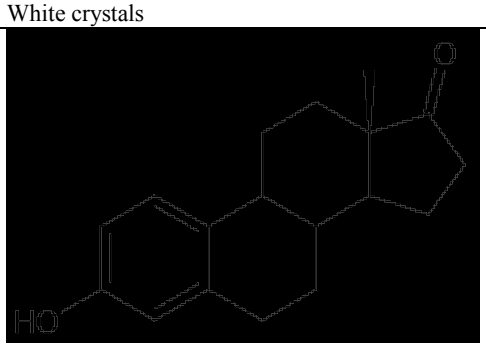
Table 2.2:
Characteristics and physical properties of 17ß-Oestradiol (Chemfinder, 2000)

Substance	17β-Oestradiol
CAS number	50-28-2
Chemical formula	C18H24O2
Molecular mass	272.39
Physical state	-
Chemical structure	
Synonyms	Beta-estradiol; estradiol; dihydrofolliculin; dihydroxyestrin; 1,3,5(10)-estratriene-3,17b-diol; 3,17-dihydroxy-delta (1,3,5-10)-estratriene; 3,17-epidihydroxyestratriene; estradiol-17beta; 17beta-estradiol; estra-1,3,5(10)-triene-3,17beta-diol;
Technical products	Alora; Climara; Climara Forte; Climaderm; Dermestril; Estrace; Estracomb TTS; Estraderm; Estraderm MX; Estraderm TTS; Estrapak 50; Estreva; Estrifam; Estring; Estrofem; Estrofem 2; Estrofem Forte; Evorel; Femtran; Fempatch; Ginedisc; GynPolar; Menorest; Oesclim; Oestradiol Berco; Progynon; Progynova; Sandrena Gel; Sisare Gel; Systen; Tradelia; Vagifem; Vivelle; Zumenon

2.1.2 Oestrone

Oestrone is a metabolite of 17ß-Oestradiol. It occurs in pregnancy urine of women & mares, in follicular liquor of many animals, in human placenta, in urine of bulls & stallions and in palm-kernel oil (HSDB, 2000).

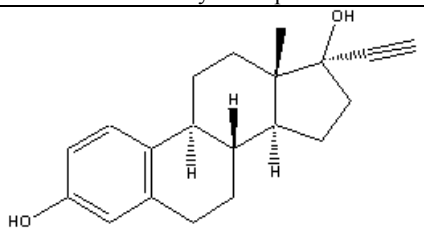
Table 2.3:
Characteristics and physical properties of oestrone (Sax, 1989; Chemfinder, 2000)

Substance	Oestrone
CAS number	53-16-7
Chemical formula	C18H22O2
Molecular mass	270.40
Physical state	White crystals
Chemical structure	
Synonyms	Folliculin; ketohydroxyestrin; 1,3,5(10)-estratrien-3-ol-17-one; Oestrone; beta-estrone; estra-1,3,5(10)-trien-17-one, 3-hydroxy-; estrol; oestrin; 3beta-hydroxyestra-1,3,5(10)-trien-17-one; 3-hydroxy-1,3,5(10)-estratriene-17-one; aquacrine; crinovaryl; cristallovar; crystogen; oisynformon; E; endofolliculina, esterone; 1,3,5(10)-estratrien-3-ol-17-one; α-1,3,5(10)-estratien-3β-ol-17-one; estrin; estrone; estrona (spanish); estrona-a; estrugenone; estrusol; femestrone injection; femidyn; folikrin; folipex; folisan; follestrine; follicular hormone; folliculine benzoate; follicunodis; follidrin; glandubolin; hestrone; hormofollin; hormovarine; 3-hydroxy-17-keto-estra-1,3,5-triene; 3-hydroxy-17-keto-oestra-1,3,5-triene; 3-hydroxy-oestra-1,3,5(10)trien-17-one; 3-hydroxy-1,3,5(10)-oestratrien-17-one; kestrone; ketodestrin; ketohydroxy-estratriene; ketohydroxyoestrin; kolpon; menagen; menformon; α-1,3,5-Oestratrien-3β-ol-17-one; 1,3,5-oestratien-3-ol-17-one; 1,3,5(10)-oestratien-3-ol-17-one; oestroform; oestroperos; ovex; ovifollin; perlatan; solliculin; theelin; thelestrin; thelykinin; thynestron; tokokin; unden; wnyestron
Technical products	-

2.1.3 Ethinyl-oestradiol

Ethinyl oestradiol is a synthetic hormone with the steroidal main structure of 17ß-oestradiol connected with an ethinyl group. Ethinyl oestradiol acts directly without further metabolic transformation. Since the ethinyl group leads to an increase of metabolic stability after oral administration of latter substance, bioavailability is higher than that of the unchanged 17ß-oestradiol. Ethinyl oestradiol is mainly applied as the estrogenic component of oral hormone contraceptives in combination with various gestagens. In modern, low dosed oral contraceptives the daily dose is 20 to 50 µg/woman during the 21 day administration/treatment period (Seibert, 1995).

Table 2.4:
Characteristics and physical properties of 17a-Ethinyloestradiol (Sax, 1989; Chemfinder, 2000)

Substance	17α-Ethinyloestradiol
CAS number	57-63-6
Chemical formula	C20H24O2
Molecular mass	296.44
Physical state	Fine white to creamy white powder
7Chemical structure	
Synonyms	Ethynyl estradiol; 17-ethynyl estradiol; 17α-ethynyl-1,3,5(10)-estratriene-3,17β-diol; estone; 19-norprega-1,3,5(10)-trien-20-yne-3,17-diol, (17 alpha)-; 19-nor-17alpha-pregna-1,3,5(10)-trien-20-yne-3,17-diol; amenoron; chee-o-genf; 3,17beta-dihydroxy-17alpha-ethynyl-1,3,5(10)-estratriene; diognat-e;diogyn-e; dyloform; EE; esteed; estigyn; estinyl; estoral (orion); estroals; estra-1,3,5(10)-triene-3,17beta-diol, 17 alpha-ethynyl-; ethidol; ethinoral; 17alpha-ethynyl-3,17-dihydroxy-delta-(sup1,3,5)-estratriene; primogyn; primogyn c (or m); progynon c; eticyclin; eticyclol; etinestrol; etinestryl; ginestrene; inestra; linoral; lynoral; menolyn; neo-eatrone; novestrol; oradiol; orestralyn; palonyl; perovex; feminone; roldiol; spanestrin; ylestrol; 17alpha-ethynyl-3-hydroxy-1,3,5(10)-estratrien-17beta-ol; Ethinylestradiol; ethinyloestradiol; 17-ethynyl-3,17-dihydroxy-1,3,5-oestratriene; 17α-ethynylestradiol; 17α-ethynylestradiol-17-β; 17α-ethynylestra-1,3,5(10)triene-3,17-β-diol; ethynyloestradiol; 17α-ethynyloestradiol; 17α-ethynyl-17-β-oestradiol; 17α ethynyloestradiol-17-β; 17-ethynyloestra-1,3,5(10)triene-3,17-β-diol; 17α-ethynyl-1,3,5-oestratriene-3,17-β-diol; 17α-ethynyl-1,3,5(10)-oestratriene-3,17-β-diol; 17α-ethynyloestra-1,3,5(10)triene-3,17-β-diol; ethinylestriol; ethinyloestradiol; 3,17β-dihydroxy-17-α-ethynyl-1,3,5(10)-oestratiene; 17α-ethinyl-3,17-dihydroxy-Δ1,3,5-estratriene; 17α-ethinyl-3,17-dihydroxy-Δ1,3,5-oestratriene; 17-ethinylestradiol; 17-ethinyl-3,17-estradiol; 17α-ethinylestradiol; 17α-ethinyl-17β-estradiol; 17α-ethinylestra-1,3,5(10)triene-3,17-β-diol; 17-ethinyl-3,17-oestradiol; ethinyl-oestranol; 17α-ethinyloestra-1,3-5(10)triene-3,17-β-diol; 17α-ethinyl-Δ(sup 1,3,5(10))oestratriene-3,17-β-diol
Technical products	Anovlar; Anovulatorio; Brevicon; Brevinor; Brevinor 21; Brevinor 28; Brevinor-1 21; Brevinor-1 28; Ciclovulan; Estrinor; Gencept; Genora; Jenest-28; Loestrin; Micronor; Milli; Minovlar; Modicon; N.E.E.; Nelova; Neocon; Nodiol; Norcept-E; Norethin; Norimin; Norinyl; Norlestrin; Orlest; Ortho 7 7 7; Ortho 1 35; Ortho-Novum; Ortho-Novum 7 7 7; Ortho-Novum 1 35; Ortho-Novum 1 50; Ovcon; Ovysmen; Ovysmen 0.5 35; Ovysmen 1 35; Synphase; Synphasic 28; Triella; Tri-Norinyl; Trinovum; Trinovum

2.2 Physico-chemical characterisation

2.2.1 Oestradiols

Chemical and physical data for oestradiols are summarised in table 2.5. All oestradiols have a very low vapour pressure and a low water solubility. Oestradiol is moderately lipophilic with a log Kow of 4.01.

Table 2.5:
Chemical and physical data of oestradiols (Sax, 1989; Chemfinder, 2000; SRC, 1996; RIWA, 1998; Yalkowsky, et al., 1986 from CIS envirofate).

Compound	17α-oestradiol	17β-oestradiol
CAS no	57-91-0	50-28-2
Commercial product	-	-
Molecular formula	C18H24O2	C18H24O2
Molecular mass	272.42	272.39
Melting point (°C)	152 (calc)	173
Decomposition Point (°C)	-	-
Vapour Pressure (Pa)	1.68E-6 (calc)	1.68E-6 (calc)
Solubility H20 (mg /1)	82 (SRC calc)	82 (SRC calc)
	-	3.6 (meas.)
Log K_{ow} (exp)	4.01	4.01
Log K_{oc} (calc)	4.2	4.2

2.2.2 Oestrone

The volatility of oestrone is very low as well as the solubility in water. Oestrone is not lipophilic with a log K_{ow} of 3.13 (SRCPhysProp Database, 2000).

Table 2.6:
Chemical and physical data of oestrone (Chemfinder, 2000; Merck, 1989; SRC PhysProp. Database, 2000)

Compound	Oestrone
CAS no	53-16-7
Molecular formula	C18H22O2
Molecular mass	270.40
Melting point (°C)	254.5 - 260.2
Boiling point (°C)	154
Decomposition Point (°C)	-
Vapour Pressure (10^{-6} Pa)	$1.42E^{-7}$ mm Hg at 25°C estimated
Henry's law constant	$3.8E^{-10}$ atm-m^3/mole at 25°C estimated
Solubility H_20 (25°C; mg/l)	30
	147 (calc. SRC)
Well soluble in (25°C; g/kg)	Alcohol
	Benzene
	Ether
	Chloroform
Log K_{ow} (exp.)	3.13

2.2.3 Ethinyloestradiol

17a-Ethinyloestradiol is a white to creamy white powder with a very low solubility in water and a low vapour pressure (see table 2.7). Ethinyl oestradiol is low to moderate lipophilic with a low Kow of 3.67.

Table 2.7:
Chemical and physical properties of 17a-Ethinyloes-tradiol (Sax, 1989; Chemfinder, 2000; Christensen, 1998)

Compound	17α-Ethinyloestradiol
CAS no	57-63-6
Molecular formula	C20H24O2
Molecular mass	296.44
Melting point (°C)	142-146
Boiling point (°C)	411
Decomposition Point (°C)	-
Vapour Pressure (Pa)	3.6^{E}-7 (exp. SRC)
Solubility H_20 (mg/l)	4.75
	116 (calc. SRC)
Log K_{ow}	3.67 (exp. SRC)

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3 PRODUCTION AND USE

3.1 Uses

Humans and livestock produce estrogens naturally, which is considered to be the most important source of estrogens. Additionally estrogenic pharmaceutical products are used in both human and veterinary medicine. Estrogen is taken by millions of women each day, as oral contraceptive and hormone replacement therapy in deficiency states. These include primary amenorrhea and delayed onset of puberty as well as management of the menopausal syndrome (Kristensen, 1995 in Christensen, 1998). In the United States, conjugated estrogens have historically been most commonly used as menopausal drugs (Hardman & Limbird, 1996 in Christensen, 1998).
Likewise, In the USA, millions of feedlot cattle are administered estrogens for growth enhancement purposes, which is prohibited in the European Union.

3.1.1 Natural estrogens

17ß-Oestradiol is the most important naturally occurring estrogenic hormone and a key intermediate in industrial synthesis of other estrogens and of various hormonal 19-norsteroids - progestagens and anabolics. Oestradiol or its esters are the active ingredients in dozens of medical preparations and an even larger number of steroid drugs include the various 19-norsteroids, synthesized from oestradiol (Pivnitsky, 1998).
Steroids are biosynthesised from cholesterol by certains enzymes in the sex and adrenal glands. The specific action of the specific hormones may be changed by reversible conversions of these hormones in the body (RIWA, 2000).

It is secreted by the ovaries in normal cycling in adult females and by the placenta in pregnant females. It is essential for the growth and normal maintenance of the uterine lining, for the development of the accessory and secondary female sex characters, and for support of pregnancy (Prosser, 1973). It is used in human medicine for the treatment of symptoms of the climacteric, particularly for vasomotor and psychological disturbances (IARC V.21,1979). It is also used for local treatment of atrophic vaginitis, for the chemotherapy of advanced prostatic carcinoma, and for the prevention of postpartum breast engorgement. 17ß-Oestradiol is also used in the treatment of primary amenorrhea, delayed onset of puberty, and chemotherapy of breast neoplasms in postmenopausal women. It is believed to be a component of hormones derived from pregnant mares'urine used in cosmetic skin preparations. 17ß-Oestradiol is used in veterinary medicine for estrogenic hormone therapy, as well as in food-producing animals as a growth promoter (IARC V.21, 1979).

3.1.2 Synthetic estrogens

Oral contraceptives have been used since the early 1960s and are now used by about 90 million women worldwide. 'The pill' is given as a combination of an estrogen and a progestogen or as sequential therapy. Since the 1970s, progestogen-only pills have been available. Continuous development of the formulas and the development of new progestogens have allowed for lower dosages with fewer acute side-effects, while offering effective, convenient contraception.

The estrogen component of combined oral contraceptives is either ethinyloestradiol or mestranol, and the progestogens used are cyproterone acetate, desogestrel, ethynodiol diacetate, gestodene, levonorgestrel, lynoestrenol, megestrol, norethisterone, norethisterone acetate, norethynodrel, norgestimate and norgestrel. Currently, the most commonly used estrogen is ethinyloestradiol, and commonly used progestogens are levonorgestrel and norethisterone.

Large differences exist in the worldwide use of oral contraceptives. These products were already being used extensively in the 1960s in northern Europe (e.g. the Netherlands, Sweden and the United Kingdom) and the United States. Extensive use of oral contraceptives by adolescents was documented in Sweden and the United Kingdom as early as 1964. Very little use of oral contraceptives is reported in Japan, the countries of the former Soviet Union and most developing countries. Contraceptive use also differs in relation to religion, ethnicity, educational level, use before or after marriage and use before or after first pregnancy.

The type of oral contraceptives prescribed differs between countries, and both the type of oral contraceptive and the doses of estrogens and progestogens have changed between and within countries over time.

Oral contraceptives may be used for emergency post-coital contraception, and the components of oral contraceptives are used to treat peri- and post-menopausal symptoms and a number of other conditions.

3.1.3 Veterinary use

In the USA feed livestock receives growth enhancing hormones as estrogen, progesterone, and androgens. For example, oestradiol benzoate in conjunction with trenbolone acetate (testosterone derivative) is used for increased weight gain and feed efficiency. Oestradiol benzoate is metabolized in vivo to 17ß-oestradiol, oestrone, and related conjugates (Syntex Animal Health, 1995).

3.2 Major producers

The major known producers are given in Table 3.1.

Table 3.1:
Major producers of 17ß-oestradiol (E1) and 17ß-ethinyloestradiol (EE1) (RIWA, 1998, HSDB, 2000)

Substance	Country	Producer	Location
E1, EE1	The Netherlands	Diosynth	Oss
E1, EE1	Germany	Schering	-
	USA	Warner-Lambert Co Parke Davis Div.	Morris Plains NJ Holland, MI

The 1998 Chemical Buyers Directory lists two USA suppliers of oestradiol, and Chemcyclopedia 98 names three suppliers (Tilton, 1997; Rodnan, 1997). In 1983, the USA imported in total 20 kg of 17ß-oestradiol (USITCa, 1984). Firms from the USA also imported 70 kg of the 3-benzoate form of oestradiol in 1983, while in 1976 this was 172 kg and in 1975 3 kg (IARC V.21, 1979). Commercial production of 17ß-oestradiol was reported as early as 1939 in the USA by the U.S. Tariff Commission (IARC S.4, 1982).

World production of oestradiol and oestrone totals many hundred kilograms per year (Pivnitsky, 1998).

With a few exceptions, pharmaceuticals like 17a-ethinyloestradiol, are not high-production volume chemicals, and the expected environmental concentrations may consequently be assumed low. To present a risk to the environment the substances should be able to cause effects at low exposure levels, i.e. preferably have a high receptor affinity (Christensen, 1998).

Based on defined daily doses (DDD) 17ß-oestradiol and estrogens used in oral contraceptives are the most used sex hormones. Approx. 22 million DDD 17ß-oestradiol have been used in Denmark in 1996. This equals approx. 45 kg/year as one DDD is 2 mg (WHO, 1995 in Christensen, 1998). In 1995 DDD was 24.3 million/year which equals 49 kg/year applied (Halling-Sorensen, 1998).
The estrogen used in contraceptives is mainly 17a-ethinyloestradiol (EE2), but also to a smaller extent its 3-methyl ether mestranol (Juul, 1997 in Christensen, 1998). However mestranol has no estrogenic action itself, but is metabolized to EE2 in the body (Bolt, 1974; Hardman & Limbird, 1996 in Christensen, 1998). In Denmark 3.64 kg EE2 is calculated to be used in Denmark in 1996. Oral contraceptives contain 20-35 µg/pill (Hardman & Limbird, 1996; Juul, 1997 in Christensen, 1998).

3.3 Production processes

3.3.1 Synthesis of 17ß-Oestradiol and oestrone

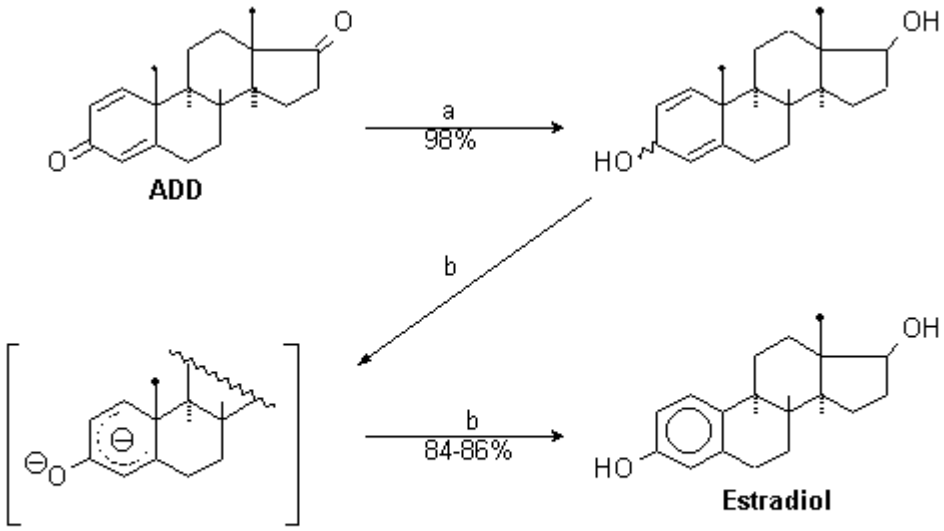
17ß-Oestradiol is a naturally occurring steroid hormone produced endogenously by all mammalian species. The production rate in humans ranges between 6 µg/24 hr in prepubescent boys and 945 µg/24 hr in normal adult cycling females.

At present, industrial synthesis of oestradiol or oestrone makes use of androsta-1,4-diene-3,17-dione (ADD) as a starting material. ADD is available from microbiological side chain degradation of sterols - sitosterol and cholesterol. Two methods for synthesis are known and find large-scale application for the chemical conversion of ADD into estrogens. The first method is high-temperature pyrolysis of ADD producing oestrone. The second method is the Dryden method, which consists in the treatment of 17-protected ADD with sodium metal at an elevated temperature with subsequent deprotection. Oestradiol is obtained by a hydride reduction of oestrone.

A modification of the second method includes 17-ethyleneketalization of ADD (the first step) and A-ring aromatization by Dryden reaction followed by ketal hydrolysis (one-pot procedure, the second step). The primary product is oestrone, produced with a total yield of 72% (from ADD).

Another method consists of the reduction of carbonyl groups of ADD (the first step) and triple deprotonation of the resulting diol by a strong base, followed by the spontaneous conversion of trianion formed into oestradiol (the second step). This procedure results in a yield of 83-85%.

A new method has been developed for the ring A aromatization of androsta-1,4-diene-3,17-dione (ADD). The method consists in the reduction of ADD into the corresponding dienediol, by means of sodium bis-(2-methoxyethoxy)aluminium hydride (Red-Al) and the subsequent double C,O-deprotonation of the latter in ring A by n-butyllithium. This deprotonation results in a spontaneous expulsion of 19-methyl group and formation of oestradiol in a total yield of 83-85% compared to 70-75% in other methods (see Figure 3.1) (Pivnitsky, 1998).



Reagents and conditions: a) $NaAlH_2(OCH_2CH_2OCH_3)_2$, PhMe, 50-60 ^{0}C, 1 h; b) *n*-BuLi (5 eq.), PhMe, 100 ^{0}C, 11 h.

Figure 3.1:
Synthesis of Oestradiol

Except for the above mentioned methods, oestrone can also be isolated from mexican yam (Osol et al., 1975), moghat roots or date palm pollen grains (Merck (Budavari), 1989) and from pregnant human urine, or can be synthesised from ergosterol (Lewis, 1993).

3.3.2 Synthesis of ethinyloestradiol

The concentration of ethinyloestradiol in the contraceptive pill ranges from 20 to 50 µg, with 35 µg most commonly prescribed. The regimen consists of taking the "pill" for 21 consecutive days, followed by 7 days without intake, prior to the next 21-day cycle (Arcand-Hoy et al., 1997).

The synthesis of ethinyloestradiol is largely unknown.
The production of medicines is a highly complex procedure, usually consisting of a large number of steps and covering a considerable period of time. Most medicinal products consist only partly of the active substances: the active compounds. In case of the pill, the active substances are certain hormones. Small quantities of these substances are usually sufficient to achieve the desired effect (AKZO, 1997).

The contraceptive pill Marvelon contains only 0.18 mg of active substances. The rest consists almost entirely of fillers and, to a small extent, ingredients which protect the active substances. The pharmacologically active substances of Marvelon are desogestrel (a progestagenic hormone) and ethinyloestradiol. Desogestrel is prepared from diosgenin into the final hormone (AKZO, 1997).

After each step in the production process, a different intermediate product is obtained, which has to be dried and analyzed to determine whether it meets the criteria set. The processing takes place in small and large reaction vessels depending on the quantities involved. The pharmacologically active substances are made in various ways:

1. Chemically by chemical reaction from raw materials;
2. Biochemically by physical processing of biological materials and by processes involving micro-organisms (biotechnology).

Diosynth produces end products (hormones in powder form) which are raw materials for Organon. The contraceptive pill Marvelon is produced in production runs of 7.5 million tablets with 0.002% of pharmacologically active substances. 99.998% comprises auxiliary substances such as potatostarch, lubricants, antioxidants, lactose, etc. (AKZO, 1997).

In Stumpf, et al. (1996) the total prescribed estrogens and contraceptives in Germany are given as 531 million doses of estrogen and 21 million doses of contraceptives in 1990. In 1993 this were 945 million doses estrogens and 263 million doses of contraceptives.

3.3.3 Wastewater treatment

Once the natural or synthetic estrogens are excreted in form of their conjugated metabolites, they will be found in wastewater. Laboratory experiments with optimized microorganism cultures and added nutrients (Tabak and Bunch, 1970) have shown that it takes several weeks, before estrogens are disappeared from the system (Table 3.2). The most stable molecule in this system was ethinyl oestradiol.

Table 3.2:

Loss of natural and synthetic estrogens in activated sludge (in %)

Substance	Week 1	Week 2	Week 3	Week 4
Estriol	81	89	97	100
Oestradiol	90	96	100	100
Oestrone	94	98	100	100
Ethinyloestradiol	73	82	90	95

From: Tabak and Bunch (1970)

Another study (Norpoth et al., 1973) found that ethinyl oestradiol and mestranol remained in activated sludge fully unchanged over 5 days (see Table 3.3). Table 3.3 also shows that the synthetic gestagens, which are chemically much less stable than the natural estrogens, are much faster decomposed than ethinyl oestradiol. Presumably, the stability of the natural hormones might be somewhere in between of them. Since by the latter experiment, activated sludge and wastewater taken from a sewage treatment plant, without added nutrients were used, its biological circumstances were closer to reality than that of the previous one.

Table 3.3 Stability of synthetic estrogens and gestagens in an activated sludge model (in %) Norpoth et al. (1973)

Substance	16 h	24 h	48 h	72 h	96 h	120 h
Norethisterone acetate	23	-	-	-	-	-
Chlormadinone acetate	-	40	-	-	-	-
Norgestrel	-	30	8	-	-	-
Lynesrenol	-	58	42	10	-	-
Megestrol acetat	-	-	39	19	-	-
Medroxy progesteron acetate	-	-	30	22	8	-
Mestranol	-	100	100	100	100	100
Ethinyl oestradiol	-	100	100	100	100	100

In another test after 14 days, 50% of the oestrogens were mineralised (Schweinfurth, 1996 in RIWA, 2000).
There are no studies available that could reveal the fate of the conjugated estrogen metabolites in wastewater. From a chemical point of view these conjugates are fairly stable. At the beginning of the estrogen metabolite research hot acid hydrolysis was used to release the free estrogens from their conjugates, a procedure, during which a considerable part of the free estrogens became immediately decomposed. However, the estrogen conjugates can carefully be cleaved by enzymatic hydrolysis as well. The enzymes, that can mildly cleave the conjugates are quite often isolated from microorganisms, which might be present in wastewater as well. But it is not determined yet, how active these enzymes under these circumstances are.

3.4 Demands and developments

In Germany the annual prescription of 17a-ethinyloestradiol amount to approximately 50 kg (Ternes, et al., 1999b).

3.5 Conclusions and recommendations

Primary production of natural estrogens occurs in female mammalian animals and in female humans. Synthetic estrogens as 17ß-oestradiol and 17a-ethinyloestradiol are produced in the Netherlands, Germany and the USA but production volumes are largely unknown. Based on presciptions amounts of 50 kg/year are estimated.

Recommended is to conduct a study into the production volumes of estrogens.

3.6 References

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4 Emissions to aquatic environment

The natural sex hormones that are produced endogenously by humans and other mammals are, in part, excreted in urine and in faeces. This applies also to the synthetic hormones in the oral contraceptive pill.

The emissions into the environment are in this study confined to humans, cattle, pigs, horses and chickens. Of these categories pregnant women, pregnant animals and egg-laying chickens excrete the largest quantities of sex hormones (Dutch Health Council, 1999). Other sources such as industry are considered of minor importance and negligible. In the majority of mammals, excretion occurs primarily via the urine. In ruminants such as cattle and sheep, on the other hand, excretion takes place principally via the faeces (Velle, 1976). Only the most important excretion routes are studied.

4.1 Emission routes

Natural estrogens
The fate of the hormones that are emitted into the environment is dependent on the starting point of the emission. Thus, for example, hormones that are excreted by humans mainly find their way into water via wastewater treatment plants (STPs). However, during heavy rainfall, the sewage treatment system in the Netherlands have an overflow which discharges directly into the surface water. Consequently, in the case of an overflow, degradation by bacteria in the STPs does not take place.

In the case of livestock, the route via which the hormones find their way into the environment also determines the fate of the substance. Pregnant cows spend a large part of the year at pasture during which excreted natural hormones find their way directly on to the land via the faeces, and subsequently, by a process of leaching, into the surface water. In contrast to pregnant cows, pregnant sows are virtually always in a stable. The emission into the environment will therefore in this case depend on the degradation by bacteria during manure storage. The manure from the sows is regularly spread on to the land between spring and October. Poultry litter is mainly used in arable farming and partly used in horticulture in the Netherlands and other countries (in some cases in a dried and pelletted form). However, the exportation of pelletted poultry litter is limited due to environmental regulations abroad.

The manner in which the manure is applied to the land can also determine the fate of the hormones. Livestock manure is currently for the most part injected or ploughed into the ground. The natural hormones end up several centimetres below the ground and are then protected from the sunlight so photodegradation does not occur (Dutch Health Council, 1999).

Synthetic estrogens
Discharges of human drugs and their metabolites from the production facilities, hospitals and private household effluent as well as the disposal of nonused drugs pose a load on the environment. The entry route to the environment will consequently be via the sewer systems. Veterinary drugs may enter the environment more directly than does human drugs, for instance via growth

promoters used in fish farming or field application of manure from animals treated with drugs (Christensen, 1998).

After having an internal curing effect somewhere in the human body, a medical substance will be excreted through urine or faeces as a mixture of metabolites, as unchanged substances or conjugated with an inactivating substituent attached to the molecule.

A synthetic estrogen can have three fates:
- ultimately mineralised to carbon dioxide and water
- the substance is lipophilic and not readily biodegradable: partly retained in sludge
- the substance is metabolized to a more hydrophilic form of the parent lipophilic substance but still persistent (Halling-Sorensen et al., 1998).

4.2 Emission of natural hormones of human and animal origin

The emission of estrogenes by cattle, pigs, chicken and horses is estimated. Next to these animals there are also 1.4 million sheep (of which 700,000 lambs) and 150,000 goats, of which 86.000 milk goats (CBS, 1999 in RIWA, 2000). However the addition of these animals will be relatively small compared to the emissions by cattle, pigs, chicken and pregnant horses (RIWA, 2000).

4.2.1 Cattle

The gestation period of cows normally varies between 277 and 286 days. The production and excretion of estrogens begins to increase markedly after approximately 110 days of the gestation period. The major compounds excreted, are 17a-oestradiol, 17ß-oestradiol and oestrone in unconjugated form (56%, 32% and 11%, respectively) (Hoffman, 1997). According to Hoffman, the total estrogen concentration in the faeces at the end of pregnancy is, on average, 0.5 mg estrogenl/kg faeces (Hoffmann, 1997). After only 28 weeks, Desaulniers already found average total estrogen concentrations of around 1 mg estrogen/kg faeces (Desaulniers, 1989). Between day 115 and parturition, the average excretion of estrogens is 83 µg/kg faeces (Hoffmann, 1997). This estimate appears to be on the low side compared with other data. For example, others found average concentrations of 187 µg (17a-oestradiol) and 947 µg (total estrogens)/kg faeces between 20 and 28 weeks (no measurements were taken after 28 weeks) (Bamberg, 1984, Desaulniers, 1989 in Dutch Health Council, 1999). In the study of Desaulniers (1989) the concentrations estrogens in faeces of pregnant musk oxes were much higher: 231-365 µg estrogen/ kg faeces. The concentration at the end of the pregnancy rised to 6,300-17,000 µg estrogens/kg faeces (Desaulniers, et al., 1989 in RIWA, 2000). In another study the concentration of 17a-oestradiol was always higher than 22 µg/kg faeces and rised to over 100 µg/kg faeces at the end of the pregnancy. The concentration of a-oestradiol in faeces was 10 times higher than the amounts of oestrone and 17ß-oestradiol (Mostl, et al., 1984 in RIWA, 2000).
The average total estrogen excretion in faeces per day varies from 1.8 mg/day (Mostl, etal, 1984) to 15 mg/day (Desaulniers, etal, 1989).

Although it is probably an underestimation the Dutch Health Council calculated the emissions in the Netherlands, based on the data produced by Hoffmann for pregnant cows to be 1.5 kg/day (Dutch Health Council, 1999).

RIWA, 2000 also calculated the excretion of estrogens in faeces and urine for the Netherlands, based on pregnant, non-pregnant cows and calves.

RIWA (2000; Blok, 2000, pers comm.) furthermore uses the excretion of estrogens in urine of pregnant cows: total excretion of 9696 mg in urine during pregnancy with an average urine production of 60 l/day from Hoffman, et al. (1997). Based on this the excretion in urine is 36 mg/day. This means the excretion through urine accounts for 95% of the total excretion. The total excretion amounts to 37.3 mg/day. Using the 37.3 mg/day value of Hoffman (et al., 1997) the total excretion by 777,730 pregnant cows in the Netherlands (based on RIWA, et al., 2000) is 29 kg per day.

The excretion by non-pregnant cows varies from 26 µg/kg faeces (Mostl, et al., 1984 in RIWA, 2000) to 34 µg/kg faeces (Desaulniers, et al., 1989 in RIWA, 2000). Using an average value of 30 µg/kg faeces and a total of 1,025,724 non-pregnant cows in 1999, the total excretion by all non-pregnant cows is about 1.1 kg estrogens per day (RIWA, 2000).

The excretion by young cattle, based on Terplan, etal, 1990 (in RIWA, 2000) is summarised in Table 4.1.

Table 4.1:
Excretion by young cattle (Terplan, etal, 1990 in RIWA, 2000)

Substance	Excretion in urine (in µg/l)	Excretion in faeces (in µg/kg)
17β-oestradiol	0.5	2
α-Oestrone	16	12
Testosterone	21	7

Based on a faeces production of 12.5 kg/day and a urine production of 25 l/day, the total excretion of 1,701,688 calves in 1999 is around 1 kg estrogens/day and 1 kg testosterone/ day (RIWA, 2000).

The total excretion by cattle based on RIWA (2000) and the Dutch Health Council (1999) is summarised in Table 4.2.
The Dutch Health Council calculated a total excretion of 540 kg/year and RIWA (2000) of 11,351 kg/year. The difference between these calculations can be explained primarily by the considerable excretion through urine of cattle, only accounted for by RIWA (2000) and the different numbers of cattle.

Table 4.2:
Overview of the production of estrogens in the Netherlands by cattle

Group of cattle	Faeces production in kg/animal/day	Conc. of estrogens in faeces in µg/kg faeces	Excretion in faeces (in g/day)[2]	Excretion in urine (in g/day)	Total excretion (kg/day)	Total excretion per year (in kg)	Reference
Young cattle ≥ 1y (female)[4] 508,000	12.5	51.5[1]	202	-	0.2	73.7	Dutch Health Council, 1999
Dairy cows and pregnant cows[4] 1,600,000	25	51.5[1]	1,277	-	1.3	466	Dutch Health Council, 1999
Total	-	-	1,479	-	1.5	540	Dutch Health Council, 1999
Pregnant cows 777,730	25	54[3]	1,011	28,000	29	10,585	RIWA, 2000
Non-pregnant cows 1,025,724	25	30[5]	769	378	1.1	401.5	RIWA, 2000
Young cattle 1,701,688	12.5	14	298	701[6]	1	365	RIWA, 2000
Total	-	-	2,078	29,079	31.1	11,351	RIWA, 2000

1. The concentration of 83 µg/kg faeces applies to the period from 115 days to parturition. Spread out over the entire pregnancy, this gives 51.5 µg/kg faeces.
2. On average, cows calve for the first time at 2.2 years of age and are slaughtered at 4.6 years. Given a gestation period of 270 days, this means that they are pregnant for 62% of their lifespan. This percentage has been used to calculate the total oestrogen production.
3. Based on the average excretion during pregnancy: 1.3 mg/day. This is calculated from the total excretion of 361.25 mg in faeces during pregnancy with an average faeces production of 25 kg/day and 54 µg/kg estrogen in faeces.
4. Cows over 1 year of age and dairy cows, are the two groups of cattle which are pregnant for a significant amount of time.
5. Using an average value of 30 µg/kg faeces with a faeces production of 25 kg/day and that 33% of excretion occurs through urine (Erb, etal, 1977 in RIWA, 2000) the daily excretion of non-pregnant cows is 750 µg/day in faeces and 369 µg/day in urine.
6. Based on an urine production of 25 l/day and a concentration of 16.5 µg/l in urine.

4.2.2 Pigs

The normal gestation period for pigs is between 110 and 120 days. Excretion of estrogens occurs principally via the urine, and takes the form of oestrone sulphate. Estrogen production and excretion rise sharply during the first 27 days of pregnancy to values of 1.6 mg oestrone sulphate/litre urine (Atkinson, 1987 in Dutch Health Council, 1999). Between 20 and 30 days of pregnancy the average concentration of estrogens in urine is about 500 µg/l (Atkinson & Williamson, 1987 in RIWA, 2000). After 27 days a sharp fall of oestrone levels occurs to values slightly above the level prior to pregnancy. Between around day 50 and parturition, the production and excretion rise considerably once again, reaching concentrations of 5 mg per litre urine (Raeside, 1963 in Dutch Health Council, 1999).
It is difficult to estimate the emissions due to the poor correspondence between the quantities given in the Atkinson and Reaside studies. Thus Atkinson only gives the concentrations during the first 30 days. During the same period of pregnancy, Reaside found concentrations of 0.2 mg/l, expressed in terms of total estrogens. It

is possible that the values given by Reaside, which date back to 1963, are structurally too low on account of the analytical techniques that were used at that time. It would appear both from the Reaside study and from that of Edgerton (Edgerton, 1971) that the concentration of estrogens in the urine at the end of the pregnancy is higher than the maximum from the first 30 days by a factor of between 10 and 20. Application of these factors to the Atkinson's data would give concentrations considerably in excess of 5 mg/l at the end of the pregnancy. Based on an estimate from the curves presented by Reaside and Atkinson, an average concentration of estrogens in the urine throughout the pregnancy of 0.5 to 1 mg/l is derived. From this estimate the total emissions of estrogens by sows in the Netherlands is produced (Table 4.4) (Dutch Health Council, 1999).

From another study it appeared that the measured concentrations in urine strongly depended on the amount of HCL used during extraction. With 6 vol% HCL the average concentration of estrogens in urine was 1.6 µg/l with non-pregnant sows. With 15 vol% HCL the concentration of estrogens in urine was 7 µg estrogen/l for non-pregnant sows and 161 µg estrogen/l for pregnant sows with 5.8 µg/l 17ß-oestradiol for non-pregnant sows and 5.6 µg 17ß-oestradiol/l for pregnant sows (Velle, 1960 in RIWA, 2000).

RIWA (2000) calculated the excretion of estrogens by pregnant and non-pregnant sows using the 161 µg estrogen/l as the concentration of estrogen in urine. The calculation is summarised in Table 4.3.

Table 4.3:
Excretion of estrogen by pregnant and non-pregnant sows

Group of pigs	Period of pregnancy in days (total 115 days)	Excretion in µg/l	Excretion during period of pregnancy (in mg)	Excretion in urine and faeces (mg/day)	Total excretion In kg/year	Based on
Pregnant sows 6,774,084	10 (21-32)	161[a]	7.2			Velle, 1960 in RIWA, 2000
	70	20[a]	6.3			Estimated, slightly above concentration before pregnancy (RIWA, 2000)
	35 (80-115)	2415[a]	380.4			10-20 times higher hormone concentration than 21-32 days (Edgerton, etal, 1971 and Reaside, 1963 in RIWA, 2000)
	Total		393.9	6.8[c,d]	3,869	RIWA, 2000
Non-pregnant sows 6,774,084	-	7.2[b]		0.0317[c]	73	RIWA, 2000
Total					3942	RIWA, 2000

- a. assuming an excretion of 4.5 l urine per day
- b. assuming an excretion of 2.2 l urine per day
- c. assuming that 50% of the estrogens is excreted in urine and 50% in faeces.
- d. assuming 6 kg faeces and urine/day/sow this gives an average excretion of 1.13 mg/kg

Table 4.4:

Emission of estrogens by sows in the Netherlands (Dutch Health Council, 1999)

Population	Number of animals (*1000)	Urine production (litres/animal/ day)	Conc. of estrogens in urine (in mg/l)	Total estrogen excretion through urine (in kg/day)	Total estrogen excretion (in kg/year)
Sows	1610	5	0.5-1	4-8	1460-1920

The Dutch Health Council calculates the estrogen excretion by sows to be 1460-1920 kg/year and RIWA (2000) calculates the estrogen excretion by pigs to be 3942 kg/year. However, the RIWA (2000) calculation, uses considerable higher numbers of pigs, which are probably more accurate. Therefore the emission will be about 4000 kg/year.

4.2.3 Chickens

Besides estrogens, chickens also excrete fairly large quantities of testosterone. Table 4.5 gives the concentrations of both hormones in litter from chicks, layers and cocks (Shore, 1993).

Table 4.5:
Concentrations of testosterone and estrogen hormones in poultry litter from chicks, layers and cocks (Shore, 93).

	testosterone (µg/kg litter)	estrogens (µg/kg litter)
chicks (f)	133	65
chicks (m)	133	14
Layers	254	533
Cocks	670	93

Estrogens are primarily excreted in the form of 17a-oestradiol, oestrone and 17ß–oestradiol (Bishop, 1991). The total quantities of hormones that are excreted are based only on concentrations in poultry litter for layers (see Table 4.6). Layers account for the greater part of the poultry population in the Netherlands and, excrete by far the largest quantities per bird (see Table 4.5).

Table 4.6:
Estimate of the emission of estrogens via poultry litter (Dutch Health Council, 1999).

Population	Layers
No. of birds (*1000)	41,000
Litter production (g/bird)	50
Concentration of estrogens in litter (ng/l)	533
Total excretion of estrogens (g/day)	1090
Total excretion of estrogens (kg/year)	398.8

In RIWA (2000) the emission of estrogen by chicken is also calculated. Assumed is that no degradation of the concentration estrogens occurs during storage, although in another study the concentration was diminished with 34% after 30 hours (at 25°C in a wet form) but remained the same after 24 hours (at 100°C in a dry form) (Shore & Shemesh, 1992 in RIWA, 2000).

In Table 4.7 the used concentrations are given for the different groups of chicken.

Table 4.7:
Excretion of estrogen in chickens manure (RIWA, 2000)

Excretion in chicken layer		Estrogens/chicken (µg/kg dw)	Estrogens/chicken (µg/kg manure)1	Estrogens/chicken (µg/year)	Total estrogen excretion (kg/year)
Chicks 53 million	Male 26.5 million	14	702	7	0.1855
	Female 26.5 million	65	3402	34	0.901
Layer hens 42.5 million		533	2823	70503	300
Cocks		93	493	12253	-

1. assuming 50% liquid
2. assuming faeces production of 10 kg/year
3. assuming faeces production of 25 kg/year

4.2.4 Horses

Turan (1996 in RIWA, 2000) says that pregnant horses excrete 100 mg estrogen per day, although this is probably only during the last period of the pregnancy. Using this amount of excretion, the excretion by pregnant horses is 2.1 kg per day assuming 23,388 pregnant horses and a pregnancy period of 11 months (RIWA, 2000).

4.2.5 Total animal emission through excretion

In Table 4.8 the total estimated yearly emission through animals is given.

Table 4.8:
Overview of total estimated emission of estrogens via animals.

Category of animals	Total estimated emission/year In kg/year	Total estimated excretion in kg/day	Reference
Cows	539.7	1.5	Dutch Health Council, 1999
Pigs	1460-1920	4 - 5.3	Dutch Health Council, 1999
Chicken	398.8	1.1	Dutch Health Council, 1999
Total	2398.5 – 2858.5	6.6 – 7.9	Dutch Health Council, 1999
Pregnant cows	10,585	29	RIWA, 2000
Non-pregnant cows	401.5	1.1	RIWA, 2000
Young cattle	730	2	RIWA, 2000
Pregnant sows	3,869	10.6	RIWA, 2000
Non-pregnant sows	73	0.2	RIWA, 2000
Chicken-layer hens	292	0.8	RIWA, 2000
Horses	766.5	2.1	RIWA, 2000
Total	16,863	46.2	RIWA, 2000

The total emission of animals calculated by the Dutch Health Council amounts to 2.4 to 2.8 tonnes/year. RIWA (2000) calculated a total emission of approx. 17 tonnes/year. Because the estimate of RIWA uses more accurate numbers of animals and also takes account of the excretion of non-pregnant animals, this estimate of emission is considered to be the most accurate. However it still should be considered that other animals (natural animals and pets) also excrete estrogens through urine and faeces, which may indicate that the estimate for emission still may be an underestimate.

4.2.6 Humans

Endogenously produced estrogens

Women excrete 17a-oestradiol, oestrone and 17ß-oestradiol. The excretion of these hormones occurs partly by urine and partly by faeces, in urine mostly in the conjugated form. The ratios of conjugated and non-conjugated steroids vary largely. In sewage water the conjugated steroids are again hydrolysed (RIWA, 2000).

During the final period of pregnancy, women excrete approximately 30 mg estrogens per day via the urine (Adlercreutz, 1976, Fotsis, 1987). This consists mainly of conjugated 17ß-oestradiol, oestriol and oestrone. The excretion during pregnancy varies from 450 to 30000 µg/l. Based on an estimated average concentration of 10 mg estrogens per day throughout the entire pregnancy and approximately 180,000 pregnant women (in 1998), the total excretion is approximately 2 kilograms per day and 657 kg/year, assuming as the same number of pregnant women over the entire year. The number of pregnant women in 1998 has been equated with the number of births in that year (Dutch Health Council, 1999).
RIWA (2000) also calculates the endogenous produced estrogens by humans. However, RIWA, also takes the excretion of non-pregnant humans into account and calculates a total excretion of 3.35 kg/day and 1223 kg/year (see Table 4.10).

Excretion of estrogens differs between men and women and differs with age. In Table 4.9 an overview is given of the excretion of estrogens by humans per group (Aherne & Briggs, 1989 in RIWA, 2000).

Table 4.9:
Excretion of estrogens by humans per group (from RIWA, 2000).

Group	Excretion (µg/day)	Estimated average excretion (µg/day)	Reference
Children	1-40	20	Aherne & Briggs, 1989
Males	40-130	85	Aherne & Briggs, 1989
Females (not pregnant)	50-450	250	Aherne & Briggs, 1989
Females (not pregnant)	25-100[1]	62	Forth, et al., 1992 in Stumpf et al., 1996
Females (after menopause)	5-50	28	Aherne & Briggs, 1989
Pregnant females (end of pregnancy)	2500	-	Halling-Sorensen, et al., 1998 Shore, et al., 1993a
Pregnant females (end of pregnancy)	30000	-	Adlercreutz, et al., 1976
Pregnant females (end of pregnancy)	28000	-	Turan, et al., 1996

1 dependent on the menstruation cycle

Table 4.10:
Average excretion of estrogens in the Netherlands (RIWA, 2000)

Group (age in years)	Division of the Dutch population (in %)	Excretion of estrogen (µg/day)	Proportional addition to the excretion (µg/day)
Youth, age up to 14	16	20	3.2
Youth, 14 to 19	8	100	8
Adult male (19-65)	32	85	27.2
Adult female (19-65)	31	250	77.5
Pregnant female	0.9[1]	10000	90
Elderly (>65)	13	28	3.6
Average excretion of estrogens (per person)	-	-	209.5[3]
Average excretion of estrogens (total population)[2]	-	-	3.35 kg/day
Average excretion of estrogens (total population) per year	-	-	1223 kg/year

1 Based on 200,000 births per year from 150,000 pregnant women (=0.9% of the population) and a pregnancy of 9 months;
2 16 million people;
3 Rathner & Sonnenborn (1979) estimated an average excretion of 200 µg/estrogens per inhabitant per day.

In another study the following yearly excretion is mentioned.
The excretion of estrogens from fertile, pregnant and menopausal women and from pregnant mares, the following estimated excretion of estrogens can be calculated: fertile women 55 kg/year; pregnant women: 560 kg/year; menopausal women: 7 kg/year and pregnant mares: 540 kg/year. When it is assumed that the therapeutically administered E2 is metabolized in the same manner as endogenous E2, it can be concluded that the extra estrogen load due to 45 kg E2 used therapeutically contributes less than 5% compared with the natural excretion (Christensen, 1998).
In prepubescent boys the endogenous E2 production amounts to 6 µg/day (Farber, & Arcos, 1983; CVMP, 1997a) and in adult men to 45-48 µg/day (Farber & Arcos, 1983; Griffin, 1996 in Christensen, 1998) in the testis(10-15%) and in peripheral tissue (85-90%). The calculated intake is maximum 85 ng/day (Christensen, 1998).

The oral contraceptive pill

The active substance in the oral contraceptive pill (mainly ethinyl oestradiol) is excreted via urine and faeces. A study revealed that following administration of labelled ethinyl oestradiol, 22 to 50% found its way into the urine and 30% into the faeces (Reed, 1972). In faeces primarily the unconjugated form of the ethinyloestradiol has been found. In urine, 16% of the total dose was also found in unconjugated form (Dutch Health Council, 1999).
Other studies estimate the excretion of ethinyloestradiol between 35 and 54% (Kulkarni & Goldzieher, 1970 in RIWA, 2000). Of the total estimated amount a-ethinyloestradiol 80% is excreted in unchanged form (Turan, 1996 in RIWA, 2000).

In 1998 there are estimated to be around 1.4 million oral contraceptive pill users in the Netherlands (Statistics Netherlands). The average daily dose of ethinyloestradiol via 'the pill' is estimated to be 35 µg and the total excretion of ethinyloestradiol in

the Netherlands therefore at 50 grams per day. This is a maximum value, since it is based on total excretion (100%) of (unmetabolised) ethinyloestradiol (Dutch Health Council, 1999).

RIWA, 2000 also calculated the excretion of ethinyloestradiol in the Netherlands. RIWA estimated the excretion of ethinyloestradiol to be 70% of the total intake (0.7 * 35 µg/day). Around 24.4% of the Dutch population exists of women between 16 and 50 year of which 45% uses the contraceptive pill (=11% of 16 million inhabitants= 1.76 million). Based on this, the total excretion of ethinyloestradiol in the Netherlands is calculated to be (0.7 * 35* 1.76 * 10^6) 43 g/day (RIWA, 2000).

Because RIWA (2000) estimated an excretion of 70% of the total intake and the Dutch Health Council 100%, the estimate of RIWA is more accurate.

It should, however, be stressed that nowadays increasing use is made of a combination of ethinyloestradiol with a variable amount of synthetic progestagenes for the conception pill. The dose of prostagene is around 10 to 20 times higher than the concentration of ethinyloestradiol. The are about 10 different progestagenes used: levonorgesterole, desogestrole, gestodene, norgestrole, norethisterone, lynestrenone and medroxy progesterone. The progesterones are also modifications of the steroid skelet of estrogens and may be metabolised to steroidmetabolites (RIWA, 2000).
This could mean that the emission of estrogen through the use of anticonception pills, may be much higher.

4.3 Leaching of estrogens to surface water

The leaching of 17ß-oestradiol from chicken layer on grassland in Israel has been examined by Nichols et al. (1998 in RIWA, 2000). When applying 5000 kg chicken manure/ha with a concentration of 903.9 µg 17ß-oestradiol/kg dw assuming an moisture content of 24.5%, at a rainfall of 50 mm in 1 hour the maximum concentration would be 6.8 µg 17ß-oestradiol/l assuming all estrogen is solved in the water. The actual measured concentration in the water flowing off at the ditch is averaged at 3.5 µg/l, which is 51% of the maximum. However the ditch in this experiment was at 3 m distance. When the distance was 21m, only 3% leeched to the surface water (Nichols et al. 1998 in RIWA, 2000). However the study differs from the Dutch situation because the rain was very intensive, there was a slope of 3% and a dry soil.
In another experiment 56% of the oestradiol and 59% of the estrone were strongly bound to the soil and could only be extracted using organic solvents (Shore etal, 1993a in RIWA, 2000).

RIWA (2000) calculated the leeching of estrogens to the surface water in two scenario's: for regional waters and local waters.

Local waters
In 2000 the maximum amount of manure allowed on grasslands equals 85 kg P/ha/year. Manure of sows consist 3.6 kg P/1000 kg manure which indicates a maximum of 23,600 kg manure of pigs may be applicated on 1 ha grassland. With an average concentration of 1.13 mg estrogens/kg manure, an average rainfall of 60 mm/month and an application period of 1 month, the concentration in the rain water is 44 µg estrogens/l. Assuming that 3% leaches from soil, the concentration in local surface water will be 1.3 µg/l (RIWA, 2000). When the application period is 3 months (this means 180 mm rain) the concentration will be 0.43 µg/l.

Manure of pregnant cows consist 1.8 kg P/1000 kg manure which indicates a maximum of 47,000 kg manure of pigs may be applicated on 1 ha grassland. With an average concentration of 0.4 mg estrogens/kg manure, an average rainfall of 60 mm/month and an application period of 1 month, the concentration in the rain water is 31.3 µg estrogens/l. Assuming that the leeching is 3%, the concentration in local surface water will be 0.9 µg/l (RIWA, 2000). When the application period is 3 months (this means 180 mm rain) the concentration will be 0.3 µg/l.

Regional waters
For regional waters the emission to the basins of the rivers Rhine and Meuse is estimated based on the live stock in the area. Assuming that 3% of estrogens in manure reach the surface water and based on the number of live stock in Germany and the Netherlands, the concentration using a daily-averaged scenario is 19 ng/l for the river Rhine. For the 3 month-scenario the concentration will be the 4-fold of the daily-averaged scenario: 76 ng/l. For the river Rhine the emission is primarily caused by cattle (90%) (RIWA, 2000).
Assuming that 3% of estrogens in manure reach the surface water and based on the number of live stock Belgium and the Netherlands, the concentration using a daily-averaged scenario is 35 ng/l for the river Meuse. For the 3 month-scenario the concentration will be the 4-fold of the daily-averaged scenario: 140 ng/l. For the river Meuse the emission by cattle is about 70% (RIWA, 2000).

Sewage water
The estimated content of natural estrogens in unpurified sewage water (influent) is 1 µg/l based on a total discharge of 200 l per person per day to the sewage treatment plant (RIWA, 2000).
The estimated content of ethinyloestradiol in unpurified sewage water (influent) is 13.4 ng/l based on a total discharge of 200 l per person per day to the sewage treatment plant (RIWA, 2000).

Drinking water
In Christensen (1998) the concentrations of EE2 in drinking water are estimated at 0.3 and 1.2 ng/l for the regional and local scenario, resp. Intake through drinking water in local scenario is 6.32 10^{-7} mg EE2 x kg bw wt x d ~ 44 ng/day or 85 ng/day (if not biodegradable).

4.4 Conclusion

The total emissions of endogenous estrogens by human beings and animals in the Netherlands can be estimated at approximately 50 kilograms per day. This figure is possibly an underestimate, since neither, other livestock - such as rabbits, ducks, sheep, goats, horses, etc - nor companion animals, were included.
Emissions of ethinyl oestradiol ('the pill') are estimated at 43 grams per day. This may be an underestimate, in view of the fact that the progestagens that are also in the anticonception pill, may also be metabolised to steroid metabolites. In any case, this contribution is insignificant compared with the estimated total emissions of natural estrogens (50 kg/day). It should be noted that emissions from countries surrounding the Netherlands have not been taken into consideration.

Assuming a leaching of 3% from applied manure to surface water and an average rainfall of 60 mm/month, the concentration in surface water, will be 1.3 µg/l after application of the maximum allowed amount of manure of sows in 1 month and 0.43 µg/l in 3 months in local waters. Application of manure of cows in the maximum allowed amount in 1 month, the concentration in surface water is 0.9 µ/l and in 3 months 0.3 µg/l in local waters.

Estimated concentration in regional waters range from 19-76 ng/l for the river Rhine and from 35-140 ng/l for the river Meuse.

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5 Behaviour in the aquatic environment

5.1 Introduction

The behaviour of organic micropollutants in the aquatic environment is determined by the properties of the compound (solubility, hydrophobicity, volatility,) and by the characteristics of the water system of concern (residence time of the water, sedimentation area, organic matter content, etcetera). These compound and system specific properties determine to what extent a compound will accumulate in organisms.

5.2 Solubility and volatilisation

The water solubility of a compound is a good indication of the extent to which this compound can be transported with water. In general poorly soluble compounds have a high affinity for silt particles in a water system. This is the reason that the compound will settle together with the sediment and suspended particles and thereby the transport along with the water stream will be slowed down. Poorly soluble compounds can also accumulate in organisms more easily. Solubility and vapour pressure further determine together whether a compound will evaporate out of water. The volatility of a compound is characterised by its Henry constant. In Table 5.1 the values for water solubility, vapour pressure and Henry Coefficient are given (based on Chapter 2.2).

Table 5.1:
Solubility and volatisation of oestrogens

	17α-oestradiol	17β-oestradiol	Oestrone	17α-Ethinyloestradiol
Solubility in mg/l	82 calc.	3.6	30	4.75
Solubility In mol/m3	0.300	0.013	0.110	0.016
Vapour pressure In Pa	$1.68\ 10^{-6}$ (calc.)	$1.68\ 10^{-6}$ (calc.)	$1.42\ 10^{-7}$ (calc.)	$3.6\ 10^{-7}$ (exp.)
Henry Coefficient In atm-m3/mole	$5.6\ 10^{-6}$ (calc.)	$7.7\ 10^{-9}$ (calc.)	$7.7\ 10^{-9}$ (calc.)	$4.44\ 10^{-10}$ (calc.)

The volatilisation of estrogens from surface water to air may be estimated by the Henry's Law constant. This is calculated by dividing the vapour pressure by the solubility. The calculated values of the Henry's Law constant suggests that volatilisation would be insignificant from all bodies of water (Lyman et al., 1982). Due to its relatively low vapour pressure and its tendency to adsorb to soil, estrogens are not expected to volatilise significantly from wet or dry soil surfaces.

Estrogens are not volatile and are therefore relatively short lived in the atmosphere. Therefore, estrogens are unlikely to enter the atmosphere in large amounts. As the lifetime of the oestradiols and oestrone in the atmosphere is relatively short they are unlikely to be transported a long distance from its point of emission.

5.3 Sorption

The extent of sorption of a compound strongly depends on the compound's hydrophobicity and the availability of organic matter in soil, sediment or suspended particles. The hydrophobicity of a compound is characterised by its octanol water partition coefficient (K_{ow}). To what extent the compound will adsorb onto soil, sediment or suspended solids further depends on the organic matter e.g. organic carbon content of these media. The specific affinity of a compound can be directly related to organic carbon content by means of the K_{oc} value. For various media, the organic carbon content is known. Furthermore, detailed measurements have been performed on adsorption of organic compounds onto these media. According to the TGD for risk assessment, partition coefficients of hydrophobic chemicals in organic carbon / water systems (K_{oc}) can be derived from the following equation:

$\log K_{oc} = 0.81 * \log K_{ow} + 0.10 \quad \text{for } 1.0 < \log K_{ow} < 7.5$

With a standard fractional organic carbon content of soil, sediment and suspended sediment taken as 2, 5 and 10% respectively, specific adsorption constants (Kp) for soil, sediment and suspended sediment can be calculated directly from the K_{oc} or K_{ow} value.

For the distribution coefficients of oestradiol between water and various sediments, Jurgens detected values of 20-67 l/kg (Jurgens, 1999) (see Table 5.2). For (labelled) oestradiol and oestrone, respectively, Shore (1993) detected 56% and 59% binding to the soil (removable only with organic solvents). Testosterone, however, proved extremely easy to leach out. According to Nichols (1997), oestradiol is mobile in the soil judging by the concentrations that have been found in ditches.

It appears from the above findings that estrogens do not bind strongly to soil or sediments. The Nichols study may possibly relate in part to oestradiol in conjugated form. The conjugated form is considerably more soluble in water than the 'free' form, and it might therefore be expected to be extremely mobile.

Based on current knowledge of the fate of these hormones, it is not possible, to estimate concentrations in the surface water from emission data. It should be emphasized that natural hormones emitted by livestock do not, in general, find their way into STPs. Moreover, there are indications that hormones in the environment are not degraded very rapidly and may be mobile in the soil. It is therefore likely that concentrations in the surface water in areas of intensive livestock production may be considerably higher than in the vicinity of urban areas. Nevertheless in chapter 4.3 concentrations in local and regional waters have been estimated.

Table 5.2:
Sorption coefficients

Substance	17α-oestradiol	17β-oestradiol	Oestrone	17α-Ethinyloestradiol
Log Kow	4.01	4.01	3.13	3.67
Log Koc l/kg	20-67	20-67	-	-

5.4 Transformations in freshwater and marine environments

5.4.1 Hydrolysis and photolysis

No information on the hydrolysis of estrogens in water are reported.

Photolysis is the transformation of a chemical by direct absorption of radiant energy into a new chemical or chemicals different from the precursor.
The reaction of estrogens with hydroxyl radicals in the atmosphere are estimated by the AOPWIN program with a half-life for the reaction of hydroxyl radicals in the atmosphere of 1.043 h, 0.085 d and 382 d for the oestradiols, oestrone and 17a-ethinyloestradiol, respectively. Estrogen released to the atmosphere is therefore likely to be degraded by reaction with hydroxyl radicals except for 17a-ethinyloestradiol.

5.5 Biodegradation and mineralisation

5.5.1 Natural estrogens

Ultimately, it is the degradation of natural hormones and the mobility of these substances, which determine their concentration in the surface water. Degradation occurs primarily via bacteria. Mobility is dependent, among other things, on the physical/chemical properties of the substances and on the binding characteristics of the substrates in the different environmental compartments.

The degradation rate of hormones has been measured in various matrices and environmental compartments. In bacterial cultures, for example, Tabak detected a percentage degradation of 70 - 94% for oestradiol, oestriol and oestrone after one week (Tabak, 1970). Broadly comparable degradation rates have been found in STPs. Stumpf identified a decrease of 75% for oestradiol and 89% for ethinyl oestradiol after five days (Stumpf, 1996). In a modern STP in Israel, lower degradation rates of between 20 and 88% have been found for estrogens after five days (Shore, 1993).

Ternes et al., 1999a studied concentrations of estrogens in STP effluents in Germany, Brazil and Canada in 1997. It was observed that in STP discharges mainly the natural estrogens oestrone, 17ß-oestradiol, 16a-hydroxyestrone as well as the synthetically altered contraceptive 17a-ethinyloestradiol could be measured in the lower ng/l range. Mestranol was only present in two samples and 17ß-oestradiol-17-valerate could not be detected at all.

In a Brazilian municipal STP the observed removal rates ranged from 64% for 17a-ethinyl oestradiol in effluent of a 'biological filter' to above 99.9% for 17ß-oestradiol in the effluent of the aeration tank. Oestrone and 17a-ethinyloestradiol were comparably eliminated up to 83% and 78%, respectively after 6 days (See Table 5.3). In a German municipal STP the loads of oestrone and 17a-ethinyloestradiol were not appreciably reduced. Considering the standard deviation no elimination rate could be evaluated. However, 16a-hydroestrone and 17ß-oestradiol were removed with reductions in concentrations of 68% and about 64%, respectively (Ternes et al., 1999). The authors indicate that a major factor causing the differences between the Brazilian and German STP might be the difference in temperature with 2°C on average in Germany compared to above 20°C in Brazil. It should be researched in greater detail, whether temperature, microbial activity or rain events cause the differences in removal.

Table 5.3:
STP loads, concentrations in influent and effluent and removal % in three STPs in Brazil, Germany and Canada (Ternes et al., 1999)

Location	Substance	load (g/day)	concentration raw sewage in influent (ng/l	Median (maximum) concentration in effluent (ng/l	Removal (%)
Brazil	17β-oestradiol	2.5	21	0.02	99.9
	Oestrone	5	40	7	83
	17α-ethinyloestradiol	0.6	5	1	78
Germany	17β-oestradiol	0.5	15	2 (3)	64
	Oestrone	1	27	9 (70)	-
	17α-ethinyloestradiol	0.05	1.5	1 (15)	-
Canada	17β-oestradiol	-	A	6 (64)	-
	Oestrone	-	A	3 (10)	-
	17α-ethinyloestradiol	-	A	9 (29)	-

· A: concentrations in the same range as concentrations in influent of German STP

In view of the fact that degradation mainly occurs via the bacterial route, it seems likely that the degradation rate in surface water is lower than in bacterial cultures or STPs. According to as yet unpublished results, under aerobic conditions oestradiol in river water is converted into oestrone within the space of a few days (Jurgens, 1999). The further degradation of oestrone would likewise take a few days. James also detected a conversion of oestradiol to oestrone in river water (James, 1998). In that study it took 20 days for oestradiol and oestrone and 17-ethinyl oestradiol to be substantially degraded. Under anaerobic conditions in sediment, the conversion is considerably slower (Jurgens, 1999). Moreover, it appeared that the conversion of oestradiol to oestrone was reversible. Under both sets of conditions, ethinyl oestradiol was considerably more persistent than oestradiol (Jurgens, 1999).

In poultry litter, degradation of estrogens and testosterone also appears to be virtually absent after several months. Even industrial processing of poultry litter, or beating to 100°C for 24 hours, did not appear to influence the hormone concentration to any significant extent (Shore, 1993). Nichols investigated the runoff into ditches of estrogens from poultry litter that has been applied to pasture (Nichols, 1997). This leaching process was still taking place after the pasture had been irrigated for a week.

Although the literature is limited, degradation of hormones in the environment appears to take several days under the most favourable circumstances. Under less favourable circumstances, however - e.g. deficiency of oxygen and micro-organisms - the degradation will be considerably slower. In a substrate such as poultry litter, there is - as was stated above - only minimal degradation even after several months. It is not known to what extent this also applies to pig and cattle manure.

The half-life of 17ß-Oestradiol in drinking water is 10 days (39% degradation) (Rurainski, 1977 in Verschueren, 1983).

5.5.2 Synthetic estrogens

17a-Ethinyloestradiol (EE2) cannot be assumed to biodegrade significantly. Norpoth et al., 1973 (in Christensen, 1998) report from a study where 100% of the EE2 test substance remained after a 5-day aerobic sludge experiment. Tabak and Bunch, 1970 report that EE2 was less biodegradable than estrone, estradiol, and estriol in a laboratory where the degradation was investigated in sludge

supplied with nutrient and an optimum content of microorganisms. Five percent of EE2 remained after 2 weeks. Conclusion: inherently or not biodegradable (in Christensen, 1998).

Degradation of ethinyloestradiol by nitrifying activated sludge (Nitrosomonas europaea). The sludge was fed with only mineral salts medium containing ammonium as the sole energy source. The oxidation rate was 50 mg NH_4/g DW/h. This activated sludge was also able to degrade ethinyloestradiol at a maximum rate of 1 µg/g DW/h; complete degradation within 6 days. Using sludge with an insignificant nitrifyling capacity of 1 mg NH_4/g DW/h no degradation of ethinyloestradiol was seen. Oxidation of ethinyloestradiol by nitrifying sludge resulted in the formation of hydrophilic compounds, which probably have lower pharmacological activity than ethinyloestradiol. Ethinyloestradiol degradation is mediated by ammonium monooxygenase activity.
At low temperatures reduced nitrification is a well-known phenomenon. The higher growth rate of nitrifying bacteria in summer are due to increased temperatures. The ability to degrade ethinyloestradiol is correlated with a temperature-dependent microbial processes such as nitrification.
Nitrifying bacteria occur in biological wastewater treatment systems but also abundantly in natural ecosystems. Ethinyloestradiol degradation by nitrifying microoroganisms is a cometabolic process.
There are remarkable seasonal changes in ethinyloestradiol concentrations in effluents (Desbrow et al., 1998 en Belfroid et al., 1999 in Vader et al., 2000).

5.6 Bioconcentration

Bioconcentration is the process in which micro contaminants are taken up by organisms. The contaminant is concentrated to higher internal concentrations in case it is not metabolised by the organism as compared to the case that it is metabolised. Bioconcentration is considered to be a partition process between water and organisms and comparable with for example sorption and octanol-water partitioning. Bioconcentration of compounds in aquatic organisms can occur through uptake of compounds directly from the water (bioaccumulation) or through food (biomagnification). Bioaccumulation can be measured in different ways. Organisms can be exposed to water with contaminants until equilibrium is attained (internal contents do not increase anymore). The bioconcentration factor (BCF) can be calculated from the ratio between the content in organisms and water: BCF = Corganism / C water. The BCF can be expressed on lipid basis as well as on fresh weight basis.

There are no data on the BCF of estrogens. Based on the log Kow values, estrogens are expected to moderately bioaccumulate.

5.7 Prediction of the environmental distribution of estrogens

Transport in air
Due to the low vapour pressures and low Henry coefficient of the estrogens, estrogens are not expected to volatise to air. When in air the natural estrogens are rapidly removed by photo-oxidation with OH radicals. Ethinyloestradiol is not susceptible to photo-oxidation.

Transport in soil
Due to moderate log Kow, the estrogens are expected to sorb to soil. Due to low water solubility, estrogens are not likely to be transported through ground water.

Transport in water
Due to low water solubility and moderate log Kow, the estrogens are expected to moderately sorb to suspended particles in water.

5.8 Distribution in water systems

The behaviour of a substance and its distribution in the environment is primarily governed by properties such as solubility in water, volatility and biodegradability. To what extent these properties are favoured or hindered, depends further on the environmental conditions. The influence of environmental circumstances on the substance's behaviour, can be simulated through modelling of the relevant mass transfer processes. Two modelling programmes, EPIWIN from the Syracuse Research Corporation and EUSES from the EU, have been used to compute the volatilisation from surface water and the substance distribution over air, water and sewage sludge during wastewater treatment.

With the EPIWIN estimation program it is possible to estimate environmental properties from the compound's chemical structure (chemical bond estimation method). Computed values are subsequently used to calculate basic emission distributions between water, air and soil, or the (a)biotic degradation in water and atmosphere. For compounds without experimental data for relevant environmental properties, this estimation method can provide a first indication of the required properties and behaviour in the environment. However, calibrations computations for compounds with reliable data show that sometimes computed values for K_{ow}, K_{oc} and Henry's Law coefficient significantly differ from experimental values and that accordingly, emission distributions will deviate substantially from results obtained with experimental data.
For the selected compounds, the environmental behaviour in lakes, rivers and wastewater treatment plants was quantified. Furthermore, a theoretical half-life value was computed for atmospheric photolysis in order to obtain an indication of the persistence of compound after it is released into the atmosphere. Basic conditions for volatilisation from river and lake are given in table 5.4.

Table 5.4: Basic parameters for volatilisation (EPIWIN)

	River	Lake
Water depth (m)	1	1
Wind velocity (m/s)	3	0.5
Water current velocity (m/s)	1	0.05

For computation of the emission distribution during wastewater treatment, an activated sludge plant was chosen with a regular set-up consisting of primary sedimentation, aeration and secondary sedimentation. Under these conditions, the biodegradability of the selected compounds was assumed to be negligible.

The modelling results are summarised in table 5.5. From the volatilisation results for river and lake, it shows that compounds, with very low Henry's Law coefficients such as $3.8\ 10^{-10}$ and $7.94\ 10^{-12}$ estrogens are not removed from surface waters.

Table 5.5:
Results of EPIWIN and EUSES estimation programmes for environmental distribution of selected brominated compounds

Property	17α-oestradiol	17β-oestradiol	Oestrone	17α-Ethinyloestradiol
Log Kow (measured)	4.01	4.01	3.13	3.67
Log Koc	-	-	-	-
H (calculated) (atm m^3/mole)	$3.64\ 10^{-11}$	$3.64\ 10^{-11}$	$3.8\ 10^{-10}$	$7.94\ 10^{-12}$
Volatilisation half-life from river	117300 y	117300 y	433.5 y	21720 y
Volatilisation half-life from lake	852900 y	852900 y	3153 y	158000 y
Half-life for reaction with hydroxyl radicals[a]	1.043 h	1.043 h	0.085 d	382 d

· H: Henry's law constant.
[a]: Calculated from OH reaction rate constant estimated by the method of Atkinson and assuming a OH radical concentration of 1.5×10^6 molecules/cm^3 and 12 hours sunlight/day

5.9 Conclusions and recommendations

With respect to the environmental properties and behaviour in the aquatic systems the following conclusions can be drawn for the selected compounds:

1. Estrogens appear not to bind strongly to soil or sediment.

2. There are no data on bioaccumulation, but based on their log Kow, estrogens are expected to have a moderate bioaccumulating potential.

3. There is no information on hydrolysis or photolysis. The oestradiols and oestrone are photolysed rapidly in air in contradiction with ethinyloestradiol.

4. There are some differing results for biodegradation in STP in Germany and Brazil. The differences are probably due to the difference in temperature. Based on the limited data it can be concluded that oestradiols and oestrone are fairly rapidly degraded. Ethinyloestradiol seems to be more persistent. Under anaerobic conditions degradation is considerably lower.

5. Additional data on bioaccumulation, biodegradation, hydrolysis, photolysis and mobility in soil should be generated.

5.10 References

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6 Occurrence in the aquatic environment

6.1 Analytical techniques

Techniques for trace analysis of steroids in water have been developed by Belfroid et al. (1999), Stumpf et al. (1996), and Schlett and Pfeifer (1996).

In the study of Belfroid et al. (1999) samples were collected in glass sampling bottles with a Teflon stopper. All bottles were rinsed with HPLC water and dried for 10 hours at 250°C and pre-treated with a dimethyl dichlorosilane (DMDCS) solution. Hereafter they are rinsed successively with toluene and methanol (for residue analysis).

Pre-treatment with DMDCS is meant to fill in so-called active sites in the glass that may bind hormones.

In Table 6.1 the analysis methods are summarised.

Table 6.1:
Analytical methods for hormones

Sample	Extraction and clean-up	Separation and detection	Detection limit	Reference
surface water influent effluent	Extracted with a SDB-XC disk; eluted with methanol, concentrated and dissolved in purified hexane Extraction hexane/HPLC/SIL A	GC MS/MS	3.1-19 ng/l	Belfroid et al., 1999
surface water influent effluent	Acidified sample; concentrated with SPE using Lichrolut, eluted with acetone/ silica gel chromato-graphy with hexane/acetone as the eluent	GC MS	1 ng/l	Stumpf, et al., 1996
surface water influent effluent	Derivatization with MSTFA/TMIS/DTE	GC MS	1 ng/l	Schlett and Pfeifer, 1996
Sewerage	Extraction hexane/HPLC/SIL A	GC MS/MS	15-124 ng/l	Belfroid et al., 1999
Sewage effluents	Derivatized with pentachloropropionic acid anhydride	GC/MS	5 ng/l E1, E2 10 ng/l E3	Lee, et al., 1998
Sewage effluents	Solid-phase extraction (SPE) of a 20 l sample, liquid chromatographic fractionation and ion trap GC/MS analysis	GC/MS	5 ng/l E1, E2 10 ng/l EE2	Desbrow et al., 1997 in Lee, et al., 1998
Influent residue	Freeze-dried, ASE technique with dichloromethane /acetone (50/50)	GC MS/MS		Belfroid et al., 1999
Suspended solids sediment	Extract freeze-dried powdered sample solvent dichloromethane/acetone (50/50) ASE extraction procedure	GC MS/MS		Belfroid et al., 1999
Fish	Freeze-dried, ASE technique with dichloromethane /acetone (50/50)	GC MS/MS		Belfroid et al., 1999

E1= oestrone, E2= 17ß-oestradiol, E3=oestriol, EE2= 17a-ethinyloestradiol

6.1.1 Watery matrices

Estrogens
In the method described by Belfroid sampling water was filtered immediately in the laboratory over 0.45 µm and 1.2 µm glass filters and extracted with a SDB-XC disk. Substances on the disk were eluted with methanol, concentrated and dissolved in purified hexane (using a combination of C18 and NH_2 columns). The extract was dissolved in HPLC eluens (methanol/water 65/35) and purified using a High Liquid Performance Chromatography (HPLC). The hormone containing fraction was collected and condense-dried. Extracts were silylated with SIL A reagents, washed in water and dried over sodiumsulphate. Detection was performed with GC-MS/MS.

Typical recoveries of 17a-oestradiol, 17ß-oestradiol, oestrone and 17a-ethinyloestradiol, using established methods are in the range of 88%, 88%, 98% and 96%, respectively (SD 8-14). Detection limits in surfaces waters, effluent and influent and in sewage sludge are given in Table 6.2 (Belfroid et al., 1999). The detection limit is strongly influenced by contamination of the samples. Normally 1 litre or a few grams of sludge are sufficient for detection. It appeared that 10 days storage at 4°C had no significant effect on the estrogen concentration in the sample.
Influents were also analysed for hormones in the residue after filtration. The filters including the remaining matrix were freeze-dried and shaken with DCM for 30 minutes. The sample was concentrated and treated and analysed as described above (Belfroid, et al., 1999).

Table 6.2:
Detection limits of estrogens in the different compartments.

Substance	Surface water (ng/l)	Effluent (ng/l)	Influent (ng/l)	Sewage sludge (ng/g dry weight)
17α-oestradiol	0.1	0.1-1.3	0.1-1.3	0.2-2.1
17β-oestradiol	0.3-0.6	0.4-0.6	<1	0.2-2.1
Oestrone	0.1-0.3	0.1-0.4	<1	2.1-11
17α-ethinyloestradiol	0.1-0.3	0.2-1.8	0.2-1.4	1.4-17

Stumpf et al. (1996) reported a method suitable for detection of natural and synthetic estrogen in matrices containing high organic contents (such as secondary effluents). Samples of 1 liter were filtered using 0.45 µm glass fiber filters than acidified to pH 3. Analytes are concentrated using SPE with a mixture of Lichrolut-EN and Lichrolut C18 (Merck). The extracted materials were eluted with acetone and cleaned up using silica gel chromatography with hexane/acetone as the eluent. The analytes were silylated and then analysed using GC/MS. Recovery rates at 10 ng/l were 76 to 97%. The detection limit was 1 ng/l for oestrone, oestradiol, estriol, mestranol and 17a-ethinyloestradiol. Schlett and Pfeifer (1996) reported a method for the detection of steroidal hormones that is similar to that of Stumpf et al., 1996. The sample is not acidified prior to SPE, and cleanup of the extract is not necessary. For derivatization a 1,000:2:2 mixture of MSTFA/TMIS/DTE is used.

Estrogen glucuronids
Hormone glucuronids are analysed by treating the extract with the enzyme ß-glucuronidase, which causes the present hormone glucuronids to be hydrolysed to the original hormones. By analysing extracts treated with the enzyme and extracts not treated with the enzyme, the content of hormone glucuronids can be derived. After elution of the extraction disk, the methanol is concentrated and the residue solved in 5 ml 0.2 M sodiumacetate. To this, 50 µl ß-glucuronidase (type H2, B-

glucuronidase activity 100,000 E/ml, sulfatase activity 5,000 E/ml) is added, after which hydrolyses occurs during the night at 37°C. After hydrolyses, the buffer is extracted with a combination of C18 and NH_2-columns of Baker (preactivated with ethylacetate and water). Elution is done with 3 x 1 ml ethylacetate buffer. The eluate is then concentrated. Further clean-up with HPLC was done as described above. The recovery of the procedure was 105 and 116% (Belfroid et al., 1999).

6.1.2 Non-watery matrices

Hormone analysis in suspended solids, sediment, sewage and activated sludge and/or biota may use an accelerated solvent extractor (ASE) with dichloromethane/acetone (50/50) as solvent. Before extraction the samples are freeze-dried. The ASE technique extracts samples for 20 minutes at high pressure (200 psi) and 100°C using the solvent. The further procedure for treatment and analysis is the same as described above (Belfroid et al., 1999).

6.1.3 Immunological methods

Immunological methods of analysis have been widely used for monitoring concentrations in water, mainly because of their relative simplicity of use, requiring little or no extractive operations, their application to large batch analyses, and especially their sensitivity (Caddy, 1984). They do, however, suffer from disadvantages in that they do not always possess the specificity required for distinguishing metabolites from the parent drug (Paxton and Donald, 1980). In 1978, determination of hormones was developed by applying radioimmunoassay (RIA) techniques.

The use of the alternative non-radioactive immunological procedure, an enzyme multiplied immunoassay technique (EMIT®), is based on competitive protein binding using an enzyme as a label and an antibody as a specific binding protein. The enzyme activity is related to the amount of drug in the sample and is measured spectrophotometrically. Although this assay is very easy to use and requires no radiochemical facilities, it is not used very often for determination as the sensitivity is relatively low. The lower limit of sensitivity of the assay was 0.8 mg/ml with a coefficient of variation for the assay of <5% (Siegel et al., 1990). One has to be careful to use citric acid in combination with the EMIT® assay because the enzyme glucose-6-phosphate dehydrogenase (G6PD) used in the EMIT® assay for ethosuximide and phenytoin (Paton and Logan, 1986) is inhibited 38% by citric acid (8 mmol/l).

Another direct immunoassay, which is not subject to the disadvantages associated with the use of radioisotopes in RIA is described by De Boever et al. (1990). They developed a chemiluminescence immunoassay (CIA) using isoluminol for the detection of oestradiol.

6.1.4 Human body fluids

A variety of methods exist for the determination of ethinyl oestradiol in plasma. A competitive protein binding assay was described by Verma, et al. , 1975 based on the competitive protein binding assay for oestradiol originally described by Corker and Exley, (1970). However, most methods for ethinyloestradiol involve the use of a radioimmuno- assay following the description of a radioligand binding assay for oestradiol by Korenman (Korenman et al., 1969a). Rao et al. (1974) compared 3 antisera for ethinyloestradiol; 2 were 7-linked and the other was 6-linked. Some cross-reaction was seen (particularly to 6-hydroxyethinyloestradiol), varying between 25% and 75%, and the best antibody appeared to be the 7-linked antiserum. Initial results reported from radioimmunoassay data suggested that peak plasma concentrations of ethinyloestradiol after a 50 µg oral dose were in

the range of 400 to 1000 pg/ml (de la Pena et al., 1975; Elstein et al., 1976). Pasqualini et al. (1975) described a radioimmunoassay for ethinyloestradiol that could detect 65 pg/ml, but with later studies it became clear that peak concentrations of ethinyloestradiol after a 50 ug dose are rarely higher than 200 pg/ml.
Radioimmunoassays have also been described by Akpoviroro & Fotherby (1980), Back et al. (1979a), Cortés-Gallegos et al. (1979), de la Pena et al. (1975), Dyas et al. (198l), Elstein et al. (1976), Hümpel et al. (1979), Kaufman et al. (1981), Kundu (1974), Kundu et al. (1977), Morvay et al. (1980), Nilsson and Nygren (1978), Stanczyk et al. (1980), and Warren and Fotherby (1974b). In general, most of these immunoassays are reliable but it is usually necessary to extract the plasma samples initially or submit them to chromatography. Combined oral contraceptive steroid preparations containing norethisterone and ethinyloestradiol often contain 1 mg of norethisterone - at least a 20 times higher dose than that for ethinyloestradiol. A quoted cross-reaction of less than 1 % for an ethinyloestradiol antiserum with norethisterone will then assume significant proportions in vivo. The radioimmuno-assay described by Nilsson and Nygren (1978) is known to be unsatisfactory if norethisterone is taken at the same time as ethinyloestradiol. Methods of prior clean-up of plasma samples before submitting them to radioimmunoassay include column chromatography (Stanczyk et al., 1980), use of a nonspecific antiestrogen antiserum (Dyas et al., 198l), or extraction with organic solvents (Back et al., 1979a). The sensitivity of most of these radioimmunoassays is in the range of 5 to 20 pg/ml. A rapid radioimmunoassay has been described by Morvay et al. (1980).
Other methods of assay of ethinyloestradiol include a further competitive protein binding assay using the rabbit uterine cytosol preparations (Warren and Fotherby, 1973), and an enzyme-mediated inimunoassay (Turkes et al., 1981) that can detect 2 pg/ml of ethinyloestradiol. Mass spectrometric assays have also been developed. Tetsuo et al. (1980) have described an assay that can measure 5 pg/ml of ethinyloestradiol in urine, while the method described by Siekmann et al. (1978) can only detect 25 pg/ml - a result that compares unfavourably with many of the radioimmunoassay methods. Fotherby et al. (198la) have used isotope dilution mass spectrometry to measure ethinyloestradiol and have used the method to examine the specificity of their radioimmunoassay. There was a good correlation between the two assay systems, suggesting that the radioimmunoassay was relatively specific for ethinyloestradiol. These mass spectrometric assays are probably best utilised in checking on the radioimmunoassay methods from time to time (from Orme, 1983).

6.2 Concentrations in freshwater systems

Concentrations of hormones in the Dutch environment have to date only been measured in one study (Belfroid, 1999). This relates to concentrations in large rivers, estuaries and in the influent and effluent from STPs and industrial purification plants. Table 6.3 provides an overview of the results obtained for urban areas in 1997.

In both influent and effluent from STPs and industrial purification plants, the researchers only found hormones in unconjugated form. Deconjugation of substances by bacteria in purification plants is a known phenomenon and even serves as a check of the functioning of the purification plant. This deconjugation also evidently takes place before the hormones find their way into the STP. The concentrations are in the order of nanograms per litre. The emission of hormones by humans represents only a fraction of the amounts excreted by livestock.

Highest concentrations were measured in Lobith and Eysden (upto 5.5 ng/l). In surface waters such as open waters and estuarines, dilution occurs (Delfzijl, Westerschelde, Oosterschelde and Haringvlietsluizen). At these locations hormones are absent or hardly detectable.

Comparison of the concentrations measured in The Netherlands with concentrations found in other countries showed that concentrations of the hormones in surrounding countries (Germany and UK) in sewerage water and effluents of STPs were higher than in the Netherlands. However, comparison of the data is difficult as long as there are differences in detection techniques and no information on the sampling location and conditions is available.

In Germany 17a-ethinyloestradiol was detected in all 20 effluents of STPs at concentrations beyond 1 ng/l and in 15 effluents in concentrations even beyond 10 ng/l, with a highest concentration of 21 ng/l (Stumpf, 1996). In another German study 17a-ethinyloestradiol concentrations in a unknown number of STP effluents were in the range of 0.3-0.5 ng/l and 0.2 ng/l in surface water (Kalbfus, 1995). In the UK, hormone concentrations in STP effluents ranged between 1-50 ng/l oestrone, 2-50 ng/l 17ß-oestradiol and upto 7 ng/l 17a-ethinyloestradiol (Desbrow, 1997; Aherne, 1989).
The concentration 17a-ethinyloestradiol in river water is 2 – 1.5 ng/l, 1-3 ng/l in reservoir water and <5 ng/l in drinking water (Aherne & Briggs, 1995).

Table 6.3:
Levels of estrogens in water in different freshwater systems in the Netherlands (Belfroid et al., 1999).

Location	Comments	17β-oestradiol (ng/l)		17α-oestradiol (ng/l)		oestron (ng/l)		17α-ethinyloestradiol (ng/l)	
		Date one	Date two	Date one	Date two	Date one	Date two	Date one	Date two
Rhine, Lobith, 1997	Two dates 27/8, 11/11	<0.6	1.0+2.8[2]	<0.1	1.4 + 3.0[2]	1.7 + 2.9[2]		<0.3	<19[2]
Meuse, Eysden, 1997	3 dates:26/8; 5/11, 9/12	<0.8; 5.5	0.6+ 0.7[3]	<0.3; 1.1	<0.1 <0.1[3]	<2.7; 2.5	3.4+5.3[3]	<0.4; <0.2	<0.2+<0.2[3]
Nieuwe waterweg, Maassluis, 1997	Two dates: 20/8, 3/11	0.6	<0.4[2]	0.2	<0.1[2]	0.6	<0.1+1[2]	0.3	<0.2[2]
Haringvlietsluizen, 1997	one date 5/11	<0.4[2]		<0.1[2]		<0.1[2]		<0.2[2]	
House hold sewerage water, Oostergemeente, 1997	Two dates: 8/10, 18/12	16	18+<0.25[4]	2.0	1.2+<0.25[4]	56	89+<1.3[4]	3.1	<0.2+<2.1[4]
House hold sewerage sludge, Oostergemeente, 97	One date: 8/10	4.3	ng/g ds	<0.4		<2.1		<3.4	
STP influent, Amsterdam-Westerpoort, 1997	Two dates: 15/10, 1/12	9.5	48+<0.3[4]	1.3	9+<0.3[4]	87	140+<1.4[4]	1.3	9.7+<2.3[4]
STP effluent, Amsterdam-Westerpoort, 1997	Two dates: 15/10, 1/12	<0.6	12.0	<0.1	5.0	2.1	47	0.3	7.5
STP sludge (primary and excess) Amsterdam-Westerpoort, 1997	One date: 1/12	<1.4	ng/g ds	<1.4		<7.2		<12	
STP influent, Kralingseveer, 1997	Two dates: 1/10, 3/12	n.a[5]		<1.3	8	18	8.8	<1.4	8.8
STP effluent, Kralingseveer, 1997	Two dates: 1/10, 3/12	n.a[5]		<1.7	1.2	<0.4	6.3	<1.8	<0.2
STP sludge (primary and excess) Kralingseveer, 97	One date: 3/12	<2	ng/g ds	<2		<10		<16	
STP influent, Eindhoven, 1997	Two dates: 30/9, 4/12	11.0[1]	14	1.8[1,2]	1.1	(8.5, 11)[1,2]	42	(<1.4, 9.2)[1,2]	<0.2
STP effluent, Eindhoven, 1997	Two dates: 30/9, 4/12	n.a[5]		<1.3	<0.1	2.7	15	<1.4	<0.2
STP sludge (primary and excess) Eindhoven,1997	One date: 4/12	<2.1	ng/g ds	<2.1		<11		<17	
ASTP influent, location A, 1997	Two dates: 14/10, 2/12	8	25+<0.3[5]	<0.5	8+<0.3[5]	22	34+<1.3[5]	4.4	7.7+<2.0[5]
ASTP effluent, location A, 1997	Two dates: 14/10, 2/12	<0.6	1.8	<0.5	2.1	11	0.7	<1.8	2.6
ASTP sludge (primary and excess),location A,1997	One date: 2/12	<0.2	ng/g ds	<0.2		<0.9		<1.4	
ASTP influent, location B, 1997	Two dates: 16/10,16/12	1.6	n.a.[5]	0.3	n.a.[5]	3.4	n.a.[5]	<0.3	n.a.[5]
ASTP effluent, location B, 1997	Two dates: 16/10,16/12	<0.7	<0.4	<0.1	<0.1	<0.4	<0.1	<0.3	<0.2
Industrial wastewater, sewerage, location C,1997	Two dates: 9/10,10/12	3.3	1	<0.5	1	12	92	2	7.6
Industrial wastewater, sludge, location C, 1997	One date: 10/12	0.2	ng/g ds	<0.2		3.1		<1.6	
Industrial wastewater, sewerage, location D,1997	Two dates: 2/10,9/12	n.a.[5]	7.7	n.a.[5]	<0.1	n.a.[5]	43	n.a.[5]	<0.2
Industrial wastewater, sludge, location D, 1997	One date: 9/12	<1.1	ng/g ds	<1.1		<5.4		<8.6	

1: samples were treated with ß-glucuronidase
2: replicate measurements
3: data in last row concerns not filtered samples added directly to the disk
4: concentration in filtrate (dissolved in watery fraction) + concentration in residue (solid matrics)
5: not analysed due to wrong fractionation

Oestrone was the only estrogen detected in 3 of 15 German rivers and streams. Concentrations ranged between 0.7 and 16 ng/l (detection limit 0.5 ng/l) (Ternes et al., 1999a).

Nichols et al. (1997) analysed 17ß-oestradiol in poultry litter, runoff water of a pasture applied with poultry litter and municipal water. Environmental concentrations in poultry litter (water extractable fraction) were found to be 133 µg/kg. The observed concentrations in municipal water were 0.03 µg/kg and in runoff 0.3 to 1.6 µg/kg. The detection limit was found to be 0.02 µg/l.

Oestrone was the only estrogen detected in 3 of 15 German rivers and streams. Concentrations ranged between 0.7 and 16 ng/l (detection limit 0.5 ng/l) (Ternes et al., 1999a).

The predominant presence of oestrone in STP effluents and rivers is presumably a result of the relative high stability of oestrone within the STP, the cleavage of glucuronide conjugates from both oestrone and 17ß-estradiol and the oxidation of 17ß-oestradiol to oestrone (Ternes et al., 1999b).

Shore et al. (1993) measured concentrations of 0.2 to 0.5 nmol estrogen/l in raw sewage water in Tel Aviv in Israel.

6.2.1 Sediment

Hormones are mainly present in the watery fraction of the sewerage water. Only a limited percentage is accumulated in the solid matrix (Belfroid et al., 1999).

6.2.2 Organisms

No data are available on concentrations oestradiols, oestrone nor ethinyloestradiol.

6.2.3 Food

Milk of carying cows contains about 1 µg/l (Shore et al., 1993). Even after giving birth high concentrations of estrogen are found. These estrogens are partly synthesized in the udders itself and are partly derived from the maternal blood. This does not mean that neonates that are fed with cow-milk receive a high dose of estrogens through their food because during production of these milk products the estrogens are most likely to removed. Mothermilk contains neglectable amounts of estrogens (Mennes, et al., 1996).

6.2.4 Groundwater

For none of the compounds considered, measurement data were available with regard to the presence in groundwater. In the Netherlands livestock manure is currently for the most part injected or ploughed into the ground and end up several centimetres below the ground. As a result of this natural hormones are protected from the sunlight, so that photodegradation is absent. This may result in accumulation in the subsoil and may cause contamination of the groundwater.

6.2.5 Rainwater

For none of the considered compounds measurement data were available with respect to the presence in rainwater.

6.2.6 Drinking water

In Christensen, 1998 the following measurements in drinkingwater are given:
In the Netherlands: 0.6 ng/l (Rathner & Sonnenborn, 1979 in Christensen, 1998).
In Germany (wells in south and southeast): 0.83 - 6.4 ng/l (average) and 0 - 22.5 ng/l (individuel) (Rurainski et al., 1977 in Christensen, 1998). Furthermore in the

Bavarian area <0.2 ng/l (d.l.) and in a wastewater treatment plant effluent: 0.3-0.5 ng/l (Kalbfus, 1997 in Christensen, 1998).
In Israel during drought: 12-20 ng/l (Shore, et al., 1993 in Christensen, 1998).
In Great Britain <5 ng/l (d.l.) (Aherne et al., 1985 in Christensen, 1998) and <1-4 ng/l in potable water (Aherne & Briggs, 1989 in Christensen, 1998).

6.3 Measurements in marine systems

6.3.1 Surface water

Data presented in table 6.4 indicate that dilution occurs. At all seawater locations hormone concentrations were near or below detection limit. The highest measured concentration measured was 0.6 ng oestrone /l.

6.3.2 Sediment and organisms

For none of the considered compounds measurement data were available with respect to the presence in sediment or in organisms.

6.4 Occurrence in human tissues

Ethinyloestradiol has been found in the human endometrium in concentrations of 3.5 µg/g wet weight tissue. There is some evidence that concentrations of the drug in the endometrium are higher in women under the age of 40 than in women over that age (Cortes-Gallegos et al., 1979).
Ethinyloestradiol does not appear to be excreted in breast milk to any significant extent. Nilsson et al. (1978) found there was insufficient ethinyloestradiol in human milk to be detected and calculated that the daily dose to an infant consuming 600 ml of milk per day would be 10 ng [about 0.02% of dose to mother (50 µg)]. Similar figures have been found by Wijmenga and Van der Molen (1969) who gave ^{14}C-mestranol to lactating women. (Uit Orme, 1983)

6.5 Conclusions

For the considered compounds the following conclusions can be made with respect to their occurrence in the environmental systems discussed:

Concentrations in the Netherlands have only been measured in one study in 1997. Highest concentrations were measured in Lobith and Eijsden (upto 5.5 ng/l). In large surface waters and marine waters, dilution occurs and concentrations are hardly detectable. Concentrations in sewerage in The Netherlands are lower than in the surrounding countries (UK, Germany)

There are very limited data on concentrations in food: milk of carying cows contains about 1 µg estrogen/l.

Recommended is to conduct a study into concentrations in groundwater, drinking water and food.

Table 6.4:
Levels of estrogens in marine water in different water systems in the Netherlands (Belfroid et al., 1999).

Location	Comments	17β-oestradiol (ng/l)		17α-oestradiol (ng/l)		oestron (ng/l)		17α-ethinyloestradiol (ng/l)	
		Date one	Date two	Date one	Date two	Date one	Date two	Date one	Date two
Nieuwe waterweg, Beneluxtunnel, 1997	One date: 2/10	<0.3		<0.1		0.3		<0.1	
Noordzeekanaal, Ijmuiden, 1997	One date 1/10	<0.3		<0.1		0.5		<0.1	
Westerschelde – Hansweert, 1997	One date 23/9	<0.3		<0.1		<0.2		<0.1	
Westerschelde – Terneuzen, 1997	One date 24/9	<0.3		<0.1		<0.2		<0.1	
Kanaal Gent-Terneuzen, 1997	One date 24/9	0.3		<0.1		0.6		<0.1	
Oosterschelde – Oesterput 1997	One date 13/10	<0.3		<0.1		<0.2		<0.1	

1: samples were treated with ß-glucuronidase
2: replicate measurements
3: data in last row concerns not filtered samples added directly to the disk
4: concentration in filtrate (dissolved in watery fraction) + concentration in residue (solid matrics)
5: not analysed due to wrong fractionation

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7 Toxity in the aquatic environment

7.1 Mechanism of toxicity

Steroids are important in the development of female sex characteristics in humans and in regulating (either naturally or unnaturally by the pill) the reproductive cycle. These chemicals are common to all vertebrate animals, so it is conceivable that their effects could be transferable via environmental pathways to other species (UK, 1998). Steroids are generally excreted from humans in an inactive form but are found in treated sewage effluents in an activated form (UK, 1998).

The effects of estrogens depend on dose, time and probably the duration of exposure. Estrogens also act at several levels in the reproductive system, i.e. they influence specific neuronal areas in the brain, they modulate gonadotropin secretion from the pituitary gland, and they directly affect the reproductive organs. Estrogens probably exert most or all of their effects through a specific receptor and such receptors are present in the brain, pituitary, gonads, and accessory sex organs at one or another time during fetal, prepubertal, or adult life (Toppari, 1996). However the precise location and temporal expression of estrogen receptors during differentiation and development of the testis and the male reproductive tract are poorly described.

Disturbance of hormonal regulation of organogenese, oocytosis and spermatogeneses can be caused in many ways: at the level of hormonal signal transduction between pituitary, hypophyse and gonades several toxicological deregulation mechanisms, are possible (RIKZ, 1996).

The direct estrogenic substances may act in at least 3 different ways. At first they may bind to the estrogen receptor, in which case the mechanism of action is identical to 17ß-oestradiol. The second mechanism is based on stimulation of the endogenous production of 17ß-oestradiol. A third mechanism could be the diminishing of 17ß-oestradiol degradation. Furthermore there are substances that may bind to the estrogen receptor but do not cause an estrogenic effect in organisms. These substances are anti-estrogenic, because they prevent the binding of endogenous oestradiol and thereby cause endocrine disruption. There are also substances that influence the hormone regulation in an indirect way, e.g. because they influence the metabolism or the excretion of 17ß-oestradiol (RIKZ, 1996).

7.1.1 Metabolism

Estrogens are metabolized rapidly in the testes, e.g., by specific sulfotransferases, after which they cannot bind to their receptor (Toppari, 1996).

By the Environment Agency in the Uk three steroid hormones were identified in seven STW effluents: 17ß-oestradiol, oestrone and ethinyloestradiol. The source of the natural steroids was believed to be human and the presence of free (unconjugated) hormone indicated a biotransformation from the excreted bound (conjugated) form in the sewer and/or during treatment (UK, 1998).

Differences between 17ß-oestradiol and ethinyloestradiol lie mainly in the oral bioavailability, where ethinyloestradiol is much more potent due to the first-pass hepatic metabolism. Ethinyloestradiol is used therapeutically as having the same

mode of action as 17ß-oestradiol. Ethinyloestradiol has approximately the same affinity to the estrogen receptor as 17ß-oestradiol (Bergink, et al., 1983, Kaspar & Witzel, 1985; Cheskis, 1997 in Christensen, 1998) but at the same time ethinyloestradiol is believed to be slightly more efficacious than 17ß-oestradiol once at the receptor (Cheskis, 1997 in Christensen, 1998).

a) Oestradiols and oestrone

Estrogens are (de)oxidated, hydrolysed and methylised in the liver before they conjugate with glucuronic acid or sulfate, which increases the water solubility. Then they are excreted by urine and faeces. Mammals and humans excrete 17ß-oestradiol, 17a-oestradiol and oestrone. 17ß-oestradiol is easily oxidised to oestrone, which can be metabolised by 16a-hydroxyestrone to oestriol. Furthermore estrogens may be excreted in several other forms: 17ß-hydroxyestrone, 16-ketoestrone or 16-epioestriol. 17ß-oestradiol is primarily found as 17ß-oestradiol-3-glucuron, estrone-3-sulfate and oestriol. Oestriol is primarily found as oestriol-16-glucuron (Ternes, et al., 1999 in RIWA, 2000).

17ß-oestradiol is administered orally. After intake, an extensive first-pass hepatic metabolism to the less potent estrogens as estrone and estriol takes place with the latter being the major urinary metabolite. A variety of sulfate and glucuronide phase II metabolites are also excreted along with a minor part of nontransformed 17ß-oestradiol (Hardman & Limbird, 1996 in Christensen, 1998).

In dogs the major metabolite of oestrone in plasma was its glucuronide accompanied by a small amt of estradiol-17ß-3-glucuronide, oestrone sulfate and oestradiol-17ß-glucuronide. After an intraperitoneal injection of oestrone into female Wistar rats, 2-hydroxyoestrone glucuronide was the major urinary metabolite (HSDB, 2000).

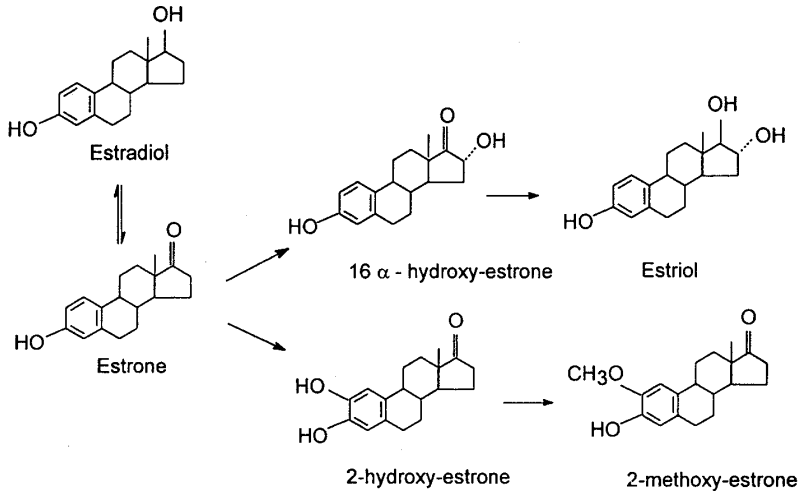

Figure 7.1: Metabolism of oestradiol

b) Ethinyloestradiol

The studies of Purdom, et al. (1994) and Sheanhan, et al. (1994) confirm that the synthetic hormone ethinyloestradiol is partly responsible for effects on fish in the environment. In sludge from sewage sludge treatment plants the hydroxymetabolite of ethinyloestradiol is activated to an estrogenic active form.

The hydroxymetabolite of ethinyloestradiol is the excretion product of women that use ethinyloestradiol as anticonception. This metabolite itself is not estrogenic and does not bind to the estrogen receptor (RIKZ, 1996).

Most medical substances are metabolised to phase I or phase II metabolites. Phase I reactions usually consist of oxidation, reduction or hydrolysis. Phase II reactions involve conjugation, which normally results in inactive compounds.
The phase II metabolites, which the liver often transforms medical substances into, will be hydrolysed in nature. The conjugated metabolites are often more hydrophobic than the phase II conjugated substances, which enables them to bioaccumulate (Halling-Sorensen, 1998).

The metabolism of ethinyloestradiol is rather complex and involves both phase I and II metabolism. The primary phase I route has been shown to be 2-hydroxylation (Bolt, 1974, 1979; Bolt et al., 1974 in Christensen, 1998). However, far from all ethinyloestradiol is phase I metabolized (Maggs, et al., 1983 in Christensen, 1998). Before excretion, ethinyloestradiol and its phase I metabolites are conjugated to a high degree to water-soluble compounds (Helton, et al., 1976; Maggs, et al., 1983; Park & Maggs, 1986 in Christensen, 1998). The rate of excretion of free ethinyloestradiol is thus very low. However, it has been shown that the conjugates may undergo bacterial hydrolysis (Kulkarni & Goldzicher, 1970 in Christensen, 1998). It has been speculated by other authors that free ethinyloestradiol may be reformed by bacterial hydrolysis in the sewer system, in waste water treatment plants, or in nature (Kalbfus, 1997; Rurainski, et al., 1977 in Christensen, 1998).

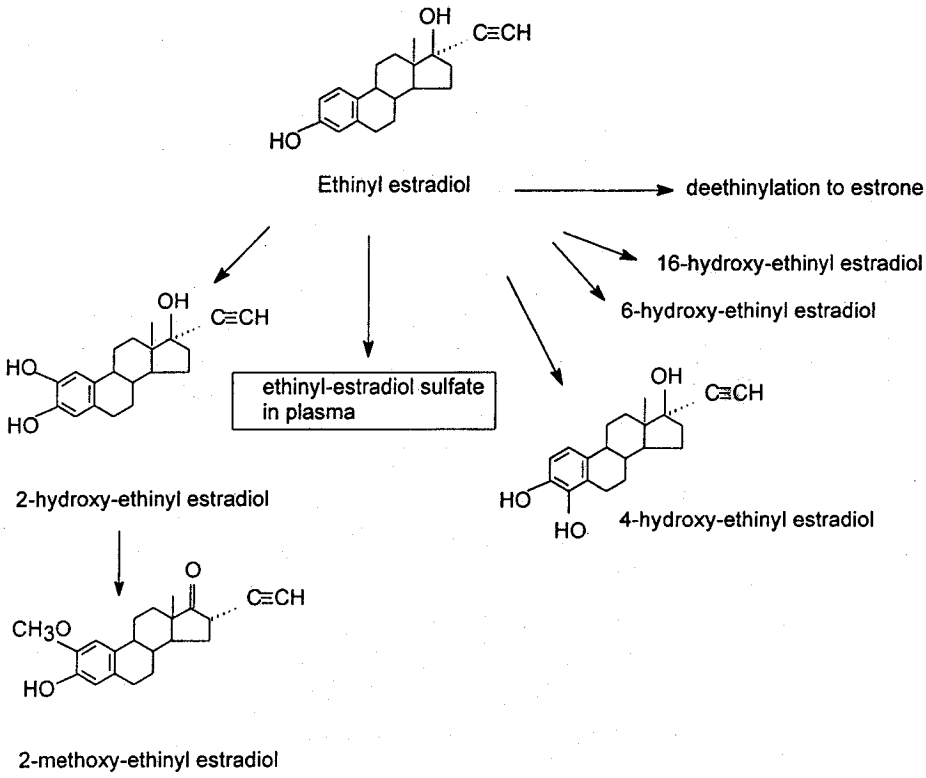

Fig 7.2:
Metabolism of ethinyloestradiol

Figure 7.2 illustrates the various routes of metabolism of ethinyloestradiol.

The metabolism of ethinyloestradiol in humans starts with the introduction of the 17a ethinyl group into the oestradiol molecule, which renders the compound to be relatively resistent to metabolism by 16a-hydroxylase, which in the case of oestradiol leads to the formation of 16a-hydroxy-oestrone and oestriol. M. Williams et al. (1 975) identified 4 de-ethynylated estrogens in the urine of women given 3H-ethinyloestradiol (oestrone, oestradiol-170, oestriol, and 2-methoxyoestradiol- 1 70), but J. Williams et al. (1975) found only 1 to 2% of the dose was de-ethynylated in women.

Ethinyloestradiol is chiefly metabolised by aromatic hydroxylation, as is also the case for natural estrogens. The main hydroxylated derivatives are ring A metabolites of which 2-hydroxy-ethinyloestradiol is probably the most important (Abdel-Aziz and Williams, 1970; J. Williams et al., 1975; M. Williams et al., 1975 in Orme, et al., 1983).

The catechol metabolite (2-hydroxy-ethinyloestradiol) may be converted to 2-methyoxy-ethinyloestradiol and both may be excreted as sulphate and glucuronide metabolites (Helton et al., 1976; J. Williams et al., 1975; M. Williams et al., 1975). Ethinyloestradiol also undergoes hydroxylation at the 4 position and to a small extent at the 6 and 16 positions, resulting in 6a- and 160-hydroxy-ethinyloestradiol (Bolt et al., 1974b; M. Williams et al., 1975). These hydroxylated metabolites are also subject to conjugation chiefly with glucuronide (Helton et al., 1976).
Perhaps the most unusual metabolic route for ethinyloestradiol is D-homoannulation. This metabolic step involves oxidation at the ethynyl triple bond followed by rearrangement (ring D enlargement) and oxidative elimination of co-situated carbon atom. This metabolic route is uncommon in humans (Abdel-Aziz and Williams, 1970) although the metabolite has been detected (M. Williams et al., 1975 in Orme, et al., 1983).

Ethinyloestradiol concentrations in plasma are greatly exceeded by the concentration of ethinyloestradiol sulphate (Akpoviroro and Fotherby, 1980; Back et al., 1980f; Bird and Clark, 1973; Warren and Fotherby, 1974b). The ratio of ethinyloestradiol to ethinyloestradiol sulphate in plasma may vary from 11:6 to 1:22 (in Orme, et al., 1983)

Both ethinyloestradiol and levonorgestrel are more than 90% bound in plasma (Akpoviroro et al., 1981; Jenkins et al., 1980) and at least for ethinyloestradiol the protein binding is similar in most animal species. However, ethinyloestradiol does not bind to SHBG (Akpoviroro et al., 1981). Ethinyloestradiol sulphate, which is present in higher concentrations in plasma than ethinyloestradiol itself, is also bound to albumin and is not displaced from its binding sites by ethinyloestradiol. Using equilibrium dialysis techniques, the binding of ethinyloestradiol in plasma is 97 to 98%, while levonorgestrel is 93 to 95% and norethisterone 79 to 80% bound (Back et al., 1982b; Orme, 1982 in Orme, et al. 1983).

7.2 Toxic effects in the aquatic environment

7.2.1 General

This paragraph decribes the data on toxicity retrieved from the literature.
Toxic effects for species in the aquatic environment are distinguished into acute and chronic effects. Furthermore a distinction is made between (pelagic) water organisms and ((epi) bentic) sediment organisms. It is not possible to base this distinction on the larger taxonomic groups. Within every group there are representatives of a typical bentic and a typical pelagic way of living. Even within

one species there can be a shift of one compartment to the other during the development from larvae to adult. The placing of a taxonomic group under pelagic or bentic organisms is therefore arbitrar.

In this report the available toxicity data are presented per group of species. The retrieved toxicity data are not evaluated except for the lowest values used for deriving the iMPC. However, when available, the test methodology used is reflected, which gives an indication about the quality of the data.
The level of toxicity of the synthetic estrogens is classified according to the classification system in Annex 2.1.

7.2.2 Toxic effects in freshwater aquatic environment

a) General

In this report the Daphnids, are incorporated with the pelagic freshwater environment. The other freshwater crustaceans and insect larvae are incorporated with the bentical environment. Algae, bacteria, protozoa and fish are incorporated with the pelagic environment. Data from tests with sediment with several organisms are also incorporated with the bentical environment.
The decision whether a test is acute or chronic depends on the generation time of the specific species (group) and in principal chronic tests should enclose more than 1 generation. In this report the toxicity tests on insects, crustaceans and molluscs with a testing time of 96 h and less, are regarded as acute. The other tests are regarded as chronic. For algae, bacteria and protozoa the EC_{50} values at 96 h and less are regarded as acute and the NOEC values at 96 h as chronic. For fish the data are regarded per test. Tests on early life stages (ELS) are regarded as chronic. In table 7.2 to 7.4 all retrieved acute and chronic toxicity data of the estrogens for freshwater organisms are presented. Table 7.1 gives an overview of the level of toxicity. In this table the data from crustaceans of pelagic and bentic environments are combined.

Table 7.1:
Overview of the toxicity data on estrogens in the freshwater environment classified according to the classification system in Annex 2.1
0 = very slightly toxic * = slightly toxic ** = moderately toxic *** = very toxic.

Chemical	CAS no	Algae		Crustaceans		Fish		Amphibian		Reptilian	
17α-Oestradiol	57-91-0	-	-	-	-	-	-	-	-	-	-
17β-Oestradiol	50-28-2	-	-	-	-	***[a]	*** [a]	*** [a]	*** [a]	-	-
Oestrone	53-16-7	-	-	-	-	-	*** [a]	*** [b]	-	-	-
17α-Ethinyloestradiol	57-63-6	***	-	***	-	*** [c]	*** [d]	-	-	-	-

a. Vitellogenin induction
b. Binding affinity to ER
c. Morphologcal and histology effects
d. Biochemical effects

b) 17α-Oestradiol

There are no data on the ecotoxicity of 17a-oestradiol.

c) 17β-Oestradiol

The tables 7.2a and b show the toxicity data of 17ß-Oestradiol for water and sediment organisms. All data refer to endocrine effects such as induction of vitellogenin and disturbance of sex-ratio. There are only data on fish, amphibians and reptilians. Based on these data 17ß-oestradiol is very toxic. There are no toxicity data available on sediment organisms. Effect concentrations are far below the limit of water solubility of 82 mg/l.

The conjugated forms of oestradiol also exhibit an estrogenic activity in fish. Oestradiol glucuronide has 10% of the activity of oestradiol, and oestrone sulphate, 100% (Peterson, 1993 in Dutch Health Council, 1999).

Table 7.2.a:
Retrieved acute effect concentration (LC_{50} and/or EC_{50}) (µg/l) of 17ß-Oestradiol for groups of species from the freshwater aquatic environment

Class	Substance	Conc. (µg/l)	Testing time	Effect type	Organism	Method	Literature (source)
Waterorganisms							
Fish		100 pM (~27.2 ng/l)	96 h	Induced vitellogenin-mRNA	Salmo gairdneri Hepatocytes		Islinger, et al., 1999
Fish		1 nmol (~0.272 µg) 1 µmol (~0.272 mg)	1x injection, 10 days later mating	NOEC increased embryo and larval mortality LOEC increased embryo and larval mort., sex-reversal	Danio rerio	28°C, other effects: karyorrhexis and karyolysis in kidney, bile stasis, atresia in ovaries, limited failure of testicular spermatogenesis	Olsson, et al., 1999
Fish	E2	4	48 h	LOEC sex-reversal (primarily into females or dev. Of testis-ova)	Oryzias latipes	25°C,	Hartley, et al., 1998
Fish	Estradiol benzoate	100 µg/day	Injected 3 consecutive days	Induction of vitellogenin	Heteropneustes fossilis	Level of vitello-genin in males << in females	Sundararajet al., 1981
Fish		2 mg/kg bw	Injected intraperitoneal	Maximum response of induction of EP (vitellogenin)	Ictalurus punctatus Males	18-22 °C, fed daily; solved in propylene glycol, dose-dependent	Bradley, et al., 1989
Amphibian	-	10^{-9} M (~0.272 µg/l)	36 h incubation of hepatocytes	LOEC increased vitellogenin-mRNA	Xenopus laevis male hepatocytes	Saturation reached at 10^{-6} M, Dose-dependent	Kloas, et al., 1999
Amphibian		$59*10^{-9}$ M (~16.7 µg/l)		IC50 Binding affinity ER	Xenopus laevis Liver cells		Lutz, et al., 1999
Reptilian		5 ng/egg 400 pg/egg (~40 ng/kg)	Dose pipetted directly onto the eggshell	ED50 Sex-reversal increased: female fraction 14.4% sex reversal of animals	Trachemys scripta elegans	28.6°C, eggs placed in incubators; (5 ng/egg as 1.7 ng/egg exogenous oestradiol and 3.3 ng/egg endogenous oestradiol)	Sheehan, et al., 1999
Reptilian		1µg/kg bw	Injected intra-musculary	Increased vitellogenin level	Chrysemys picta, males	In sesame oil Dose-dependent	Ho, et al., 1985
Reptilian	E2	0.1 µg	Topical application onto eggshell	LOEC sex-ratio (50% females)	Trachemys scripta	26°C: male producing temp.) (dose-dependent)	Crews, et al., 1991
Reptilian	Estradiol benzoate	0.1 µg 1 µg	Injection+ topical application onto eggshell	NOEC sex-ratio (% females) LOEC	Trachemys scripta	26°C (male producing temp.), dose-dependent relation	Crews, et al., 1991
Reptilian		1 mg/kg	Injected daily for 7 d and on alternate days until 21 d	Induction of vitellogenin production	Trachemys scripta	Injection, 1 concentration, dissolved in corn oil	Palmer, et al., 1995
Reptilian		1 µg/5 µl 10 µg/5µl	Applied to eggshell surface at 5 or 11 d after oviposition	NOEC alteration of hatchling sex-ratio, decreased male hatchlings LOEC alteration of hatchling sex-ratio, higher mortality	Eublepharis macularius	Dose-dependent	Tousignant, et al., 1994
Reptilian	E2	5 µg	Topical application onto eggshell	EC sex-ratio (100% females)	Trachemys scripta	26°C (male producing temp.) single dose	Crews, et al., 1995
Reptilian		0.25 mg/kg bw 1mg/kg bw	Single injection	Increased plasma vitelogenin level (dose-related)	Chrysemys picta females Chrysemys picta males		Ho, et al., 1981

Table 7.2.b:
Retrieved chronic effect concentration (NOEC) (µg/l) of 17ß-Oestradiol or groups of species from the freshwater aquatic environment

Class	Substance	Conc. (µg/l)	Testing time	Effect type	Organism	Method	Literature (source)
Waterorganisms							
Fish	>99% pure	0.001 m 0.01 m	21 d	NOEC LOEC Increase in vitellogenin production (dose-related)	Oncorhynchus mykiss Males	Continous flow-through of borehole water	Routledge, et al., 1998
Fish	>99% pure	0.01 m 0.1 m	21 d	NOEC Increase in vitellogenin production (dose-related) LOEC	Rutilus rutilus Males	Continous flow-through of borehole water	Routledge, et al., 1998
Fish	>99% pure E1 and E2	0.025 E1, 0.025 E2, m	21 d	LOEC Increase in vitellogenin production	Oncorhynchus mykiss Males	Continous flow-through of borehole water	Routledge, et al., 1998
Fish	-	0.032 0.1	21 d	NOEC vitellogenin induction and inhibition of testicular growth (dose-related) LOEC	Pimephales promelas	Continous flow-through	Panter, et al., 1998
Fish	E2	0.1 n	10 d	LOEC Vitellogenin induction	Leuciscus idus	Flow through	Allner, et al., 1999
Fish	>99% pure	>0.1 m	21 d	NOEC Increase in vitellogenin production	Rutilus rutilus Females	Continous flow-through of borehole water	Routledge, et al., 1998
Fish	E2	10	4 w	EC100 sexual displays	Poecilia reticulata	25°C, flow-through	Bayley, et al., 1999
Fish		10	90 d	Total reversal of sexes	Cyprinus carpio males	Intermittant flow through, 1 conc. tested, pos. Control	Gimeno et al., 1996 (RIKZ,1996)
Fish		<500	4 – 24 d related to hatching	LOEC mortality (dose-dependent)	Salmo gairdneri	11-12°C, tap water, dosed 2h every 3rd day, in total 10 x.	Kristfalusi, et al., 1998
Fish	E2	1 µg/day	Injected daily for 20 days	Increased vitellogenin levels, no effect on ovarian weight	Heteropneustes fossilis, females, hypophysectomiz.	Dose-dependent increase	Sundararaj, et al., 1981
Fish	E2	1 µg	3 spaced injections on day 0, 28 and 48	Increased intensity of response after successive exposures	Heteropneustes fossilis, males and hypophysectomiz. females,	Level of vitellogenin males <<females after each injection	Sundararaj, et al., 1981
Fish	E2	0.05 mg/kg in diet	7 d	Increased vitellogenin	Salmo gairdneri	Vitellogenin increase in females > in males	Carlson, et al., 1999
Fish		0.5 mg/kg bw/week	25 d, ip	Increase in plasma vitellogenin; reduction in GSI; severe effects on histological testicular structure	Zoarces viviparus	Given in half doses twice a week	Christiansen, et al., 1998
Fish		0.5 mg/kg food	Feed, 1 x day, 6d/week	EC100 Inhibition of gonadal development, marked testicular regression	Salmo gairdneri	10-19°C	Billard, et al., 1981
Fish		5 mg/kg diet 12.5mg/kg diet	Oral daily approx. 7-10 w	41% reversal in sex differentiation of males to females 100% sex-reversal	Oryzias latipes	Sex-reversed females were mostly fertile, dosed daily from hatching until 12 mm stage.	Yamamoto, et al., 1963
Class	Substance	Conc. (µg/l)	Testing time	Effect type	Organism	Method	Literature (source)

Fish		30 mg/kg feed	76 d	Cumulative mortality approached 50%, smaller, enlargded liver, spleen and kidneys, gall bladder distended, intestine dilated with fluid	Salmo gairdneri	12-13°C	Herman, et al., 1988
Amphibian		$1.84*10^{-10}$ M (~50 ng/l) $1.84*10^{-9}$ M (~500ng/l)	8 d, in 1 ml cultures in 5 µg/l propylene glycol	NOEC Induction of vitellogenin synthesis LOEC Induction of vitellogenin synthesis	Xenopus laevis Liver cultures	Daily renewal, $1.84*10^{-9}$ M is Similar to normal estrogen levels in plasma of female vertebrates	Wangh, et al., 1975
Amphibian	-	10^{-8} M (~2.72 µg/l)	12 w	LOEC increased number of female fenotypes	Xenopus laevis	Tap water, 22-23°C, renewal 3 x week	Kloas, et al., 1999
Amphibian		1 mg/kg	Ip daily:7d monitored on day 14	Induction of vitellogenin production	Xenopus laevis	Injection, 1 conc. tested, dissolved in corn oil	Palmer, et al., 1995
Reptilian		5 µg/kg bw 500 µg/kg bw	Multiple injection at o, 1, 2, 3, 4, 6, 7, 8 d	NOEC LOEC Increased plasma vitelogenin level (slow but linear increase; dose-dependent)	Chrysemys picta males		Ho, et al., 1981
Reptilian		300 µg/kg bw	3 weekly injections with increasing dose	Increase of calcemia following a total injection of 1.7-2.5 µg	Lacerta vivipara, ovariectomized		Gavaud, 1986
Reptilian		400 µg/kg bw	Multiple injection at 0, 4, 8, 12, 16, 18, 20d	Increased plasma vitelogenin level (linear increase)	Chrysemys picta females	Vitellogenin level only increased after day 6	Ho, et al., 1981

n=nominal, m=measured

d) Oestrone

The tables 7.3a and b show the toxicity data of oestrone for water and sediment organisms. All data refer to endocrine effects such as induction of vitellogenin and disturbance of sex-ratio. There are only data on fish, amphibians and reptilians. Based on these data oestrone is very toxic. There are no toxicity data available on sediment organisms. Effect concentrations are far below the limit of water solubility of 30 mg/l.

It is known that oestrone can potentiate the action of oestradiol in fish. This raises the question of whether this also occurs in connection with combined exposure to oestrone and xenobiotic substances, which also possess an estrogenic action. If this is the case then the risks of these substances for fish could be considerably greater (Dutch Health Council, 1999).

Table 7.3.a:
Retrieved acute effect concentration (µg/l) of oestrone for groups of species from the freshwater aquatic environment

Class	Substance	Conc.	Testing time	Effect type	Organism	Method	Literature (source)
Waterorganisms							
Fish	Pure powder E1	0.5 mg	Sub-scutaneous between the scales	EC Enlargment of urinogenital papillae in males and females	Oryzias latipes, males and females		Yamamoto, et al., 1955
Fish	E1	12 mg/kg diet	feed	LOEC Sex-reversal (14% males reversed to females)	Oryzias latipes	Majority of the reversed males are fertile	Yamamoto, 1958
Fish	E1	25 mg/kg diet	feed	EC Sex-reversal (76% males reversed to females)	Oryzias latipes	Majority of reversed males fertile	Yamamoto, 1958
Fish	E1	50 mg/kg diet	feed	EC100 Sex-reversal (100% males reversed to females)	Oryzias latipes	Majority of the reversed males are fertile	Yamamoto, 1958
Amphibian	E1	$59*10^{-9}$ M (~15.9 µ g/l)		IC50 Binding affinity to ER	Xenopus laevis Liver cells		Lutz, et al., 1999
Reptilian	E1	5 µg/egg	Topical applicatio n onto eggshell	EC sex-ratio (100% females)	Trachemys scripta	26°C (male producing temp.) single dose	Crews, et al., 1995

Table 7.3.b:
Retrieved chronic effect concentration (NOEC) (µg/l) of oestrone for groups of species from the freshwater aquatic environment

Class	Substance	Conc. (µg/l)[b]	Testing time	Effect type	Organism	Method	Literature (source)
Water organisms							
Fish		0.0099 0.0318 0.0099-0.9927	21 d	NOEC LOEC vitellogenin induction and inhibition of testicular growth LOEC Change in testes morphology	Pimephales promelas	Continous flow-through, dose-related effect [a]	Panter, et al., 1998
Fish	>99% pure	0.025 0.050	21 d	NOEC Increase in vitellogenin production (dose-related) LOEC	Onchorhynchus mykiss, Males	Continous flow-through of borehole water, conc. measured	Routledge, et al., 1998
Fish	E1	0.066 m	7 d	LOEC Vitellogenin induction	Leuciscus idus	Flowthrough 15°C	Allner, et al., 1999
Fish	E1	1 µg/day	Injected daily for 20 days	Increased vitellogenin levels (dose-dependent), no effect on ovarian weight	Heteropneustes fossilis, females, hypophysec-tomized		Sundararaj, et al., 1981

· a the positive control (100 ng oestradiol/l) proved more potent at inhibiting testicular growth than the corresponding level of oestrone (99.3 ng/l).
b. m=measured; n- nominal.

e) 17α-Ethinyloestradiol

The tables 7.4a and b show the toxicity data of 17a-ethinyloestradiol for water and sediment organisms. Almost all data refer to endocrine effects such as induction of vitellogenin and disturbance of sex-ratio. There are however some acute EC50/LC50's on algae and crustaceans. Based on these data 17a-ethinyloestradiol is very toxic. There are no toxicity data available on sediment organisms. Effect concentrations are below the limit of water solubility of 4.75 mg/l.

The synthetic mestranol, which is metabolised to ethinyloestradiol by ortho-methylisation in the mammal liver, is just as active in fish as ethinyloestradiol (RIKZ, 1996).
At an intraperitoneal dose of 0.66 mg/kg ethinyloestradiol the vitellogenin content was 50,000 times the background value in fish. For mestranol this was about the same. 17ß-Oestradiol at 0.6 mg/kg gave a 25000 times higher vitellogenin content than the background value in fish (RIKZ, 1996).

Table 7.4.a:
Retrieved acute effect concentration (mg/l) of 17a ethinyloestradiol for groups of species from the freshwater aquatic environment

Class	Concentration (mg/l)	Testing time	Effect type	Organism	Method	Literature (source)
Waterorganisms						
Algae	0.84	-	EC50	Algae		Kopf, 1995 (in Halling-Sorensen, 1998
Crustaceans	0.105	-	EC50 Reproduction	Daphnia		Kopf, 1995 (in Halling-Sorensen, 1998
Crustaceans	5.7	Acute	LC50	Daphnia		Kopf, 1995 (in Halling-Sorensen, 1998
Fish	0.7-0.9 ng/l <0.1-0.9 ng/l	4 d 4 d	Changed biochemical effects Changed morphology and histology	Oncorhynchus mykiss	Flow through	Sheahan, et al., 1994 (AQUIRE)
Fish	10^{-9} M (~0.3 mg/l)	96 h	Maximum inducible vitellogenin-mRNA expression rate	Salmo gairdneri hepatocytes	EC50 value is at least as less as that of 17β-oestradiol	Islinger, et al., 1999

Table 7.4.b:
Retrieved chronic effect concentration (NOEC) (ng/l) of 17a-thinyloestradiol for groups of species from the freshwater aquatic environment

Class	Concentration (ng/l)	Testing time	Effect type	Organism	Method	Literature (source)
Waterorganisms						
Fish	<0.1 0.1-0.9	196 d	NOEC Biochemical effects on plasma LOEC Biochemical effects on plasma morphology	Oncorhynchus mykiss	Flow Through	Sheahan, et al., 1994 (AQUIRE)
Fish	0.5	10 d	LOEC Increase in plasma vitellogenin	Salmo gairdneri Males	Flow Through	Purdom, et al., 1994 (UBA, 1995)
Fish	1 4 >64	300 d	NOEC egg laying and number of egg LOEC Growth retardation in developing fish, affected maturation and reproduction NOEC Vitellogenin concentration	Pimephales promelas	Lifecycle test	Laenge, et al., 1997 (SETAC abstracts 1997)
Fish	0.05 ng/egg 0.5 ng/egg	7d - >2 months	NOEC Sex-reversal LOEC Sex-reversal	Oryzias latipes Males	Flow through. Life cycle	Papoulas, et al., 2000
Fish	2.5 ng/egg	7d - >2 months	NOEC Survival to adult, length and weight	Oryzias latipes	Flow through, Life cycle	Papoulas, et al., 2000
Fish	0.66 mg/kg	7 d	Increased biochemical effects and increased morphological changes in liver	Ictalurus punctatus	Injection	Nimrod, et al., 1996 (AQUIRE)
Avian	2 ng/g egg	12 d injection	EC100 sex-reversal: all male embryos became feminized with ovary-like tissue in left testis (dose-dependent)	Coturnix japonica	Incubated at 37.5°C and 60% humidity, injected into yolks of embryonated eggs	Berg, et al., 1999

f) Overview of the toxicity data for fresh water organisms

In table 7.5 and 7.6 an overview is given of the lowest retrieved acute and chronic effect concentrations. The concentrations are reflected in **µg/l.**

Table 7.5:
Overview of the lowest retrieved acute effect concentrations (L(E)C_{50}) (in µg/l) of the estrogens in the freshwater environment

Substances	Algae	Crustaceans	Fish	Amphibian	Reptilian
17α-Oestradiol	-	-	-	-	-
17β-Oestradiol	-	-	0.0272[a]	0.272 [a]	5 ng/egg [b]
Oestrone	-	-	12 mg/kg [c]	15.9 [d]	5 µg [e]
17α-Ethinyloestradiol	840	105	<0.0001-0.0009 [f]	-	-

a.LOEC Vitellogenin induction, b. ED50 sex-reversal, c.LOEC sex-reversal, d.Binding affinity to ER (IC50), e.sex-reversal 100%, f. LOEC Morphologcal and histology effects

Table 7.6:
Overview of the lowest retrieved chronic effect concentrations (NOEC) (in µg/l) of the estrogens in the freshwater environment

Substances	Algae	Crustaceans	Fish	Amphibian	Reptilian/ Avian
17α-Oestradiol	-	-	-	-	-
17β-Oestradiol	-	-	0.001 [a]	0.05 [a]	5 µg/kg bw [a]
Oestrone	-	-	0.0099 [a]	-	-
17α-Ethinyloestradiol	-	-	<0.0001 [b]	-	-

a. NOEC vitellogenin induction
b. NOEC biochemical effects

Tables 7.5 and 7.6 show that most data are available on fish and that most available ecotoxicity data on estrogens are on endocrine effects.

g) Effects observed in the environment

No data on estrogens are retrieved on effects observed in the environment.

7.2.3 Comparing exposure concentrations to environmental criteria

Until now no environmental criteria are set for estrogens. Therefore a comparison with exposure concentrations can not be made.

7.2.4 Toxic effects in marine aquatic environment

a) General

In table 7.8 to 7.10 the retrieved acute and chronic toxicity data of the estrogens for marine organisms are reproduced. In this report the toxicity tests on crustaceans and molluscs with a testing time of 96 h and less, are regarded as acute. The other tests are regarded as chronic. For algae the EC_{50} values at 96 h are regarded as acute and the NOEC values at 96 h as chronic. For fish the data are evaluated per test. Tests on early life stages (ELS) are regarded as chronic. No distinction is made between water and sediment organisms.

Table 7.7 gives an overview of the level of toxicity.

Table 7.7:
Overview of the toxicity data on estrogens in the marine environment classified according to the classification system in Annex 2.1.
0 = very slightly toxic * = slightly toxic ** = moderately toxic *** = very toxic.

Chemical	CAS no	Algae		Molluscs		Crustaceans		Fish	
		Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic
17α-Oestradiol	57-91-0	-	-	-	-	-	-	-	-
17β-Oestradiol	50-28-2	-	-	-	-	*** [a]	*	-	-
Oestrone	53-16-7	-	-	-	-	-	*	-	-
17α-Ethinyloestradiol	57-63-6	-	-	-	-	-	*	-	-

a. LOEC inhibition of settlement

No data are retrieved on 17a-oestradiol. The data on the other substances are described in the next paragraphs.

b) 17α-Oestradiol

There are no data on the ecotoxicity of estrogens to marine organisms.

c) 17β-Oestradiol

The table 7.8a and b show the toxicity data of 17ß-oestradiol for marine organisms.
Almost all data refer to endocrine effects such as disturbance of sex-ratio. Furthermore there are effects on settlement of cocks. There are only data on crustaceans and fish. There are however some chronic data on survival and reproduction. Based on these data 17ß-oestradiol is slightly toxic. The effect concentrations are far below the limit of water solubility of 82 mg/l.

Table 7.8.a:
Retrieved acute effect concentration (LC_{50} and/or EC_{50}) (µg/l) of 17ß-Oestradiol for groups of species from the marine aquatic environment

Class	Concentration (µg/l)	Testing time	Effect type	Organism	Method	Literature (source)
Crustaceans	0.1	24h	LOEC Inhibition of settlement (trend: less inhibition at higher conc.)	Balanus amphitrite	25-28°C, seawater, oestradiol used as positive control	Billinghurst, et al., 1998
Crustaceans	1	48-96h	LOEC Inhibition of settlement	Balanus amphitrite	25-28°C, seawater, oestradiol used as positive control	Billinghurst, et al., 1998
Crustaceans	1	From egg hatching until cypris stage	EC100 Increased cypris major protein (CMP, related to barnacle vitellin)	Balanus amphitrite, nauplius stage larvae	22°C, seawater,	Billinghurst, et al., 2000

Table 7.8.b:
Retrieved chronic effect concentration (NOEC) (µg/l) of 17ß-Oestradiol for groups of species from the marine aquatic environment

Class	Concentration (µg/l)	Testing time	Effect type	Organism	Method	Literature (source)
Crustaceans	23	10 d	LOEC stimulating effect on relative egg production rate	Acartia tonsa	Art. seawater, fed daily, renewal, oestradiol as positive control, >98% pure	Andersen, et al., 1999
Crustaceans	>=100	10 d	NOEC Survival	Tisbe battagliai	Renewal 3 x pw Life cycle test	Hutchinson, et al., 1999
Crustaceans	>=100	21 d	NOEC Survival	Tisbe battagliai	Renewal 3 x pw Life cycle test	Hutchinson, et al., 1999
Crustaceans	>=100	21 d	NOEC Reproduction	Tisbe battagliai	Renewal 3 x pw Life cycle test	Hutchinson, et al., 1999
Crustaceans	0.01 mg/kg bw	5 w, injected	NOEC Moult cycle duration, stimulation of ovarian development, increase of mean ovary weight relative to body weight	Penaeus esculentus	Art. seawater, 29-31‰, 26-27°C, flow through	Koskela, et al., 1992
Fish	>30 mg/kg feed	100 d	NOEC Mortality LOEC Decreased weight 100% sex-reversal (all females) after 21d	Salmo salar		Herman, et al., 1991
Fish	>30 mg/kg feed	100 d	NOEC Mortality, sex reversal after 16 m LOEC Decreased weight, enlarged liver	Salvelinus namaycush		Herman, et al., 1991

d) Oestrone

The table 7.9 shows the toxicity data of oestrone for marine organisms. There are no data on acute toxicity to marine organisms and only limited data on chronic toxicity (1 study).
There are only data on crustaceans. Based on these data oestrone is slightly toxic. Effect concentrations are far below the limit of water solubility of 30 mg/l.

Table 7.9:
Retrieved chronic effect concentration (NOEC) (µg/l) of oestrone for groups of species from the marine aquatic environment

Class	Concentration (µg/l)	Testing time	Effect type	Organism	Method	Literature (source)
Crustaceans	>=100	10 d	NOEC Survival	Tisbe battagliai	Renewal 3 x pw Life cycle test	Hutchinson, et al., 1999
Crustaceans	>=100	21 d	NOEC Survival	Tisbe battagliai	Renewal 3 x pw Life cycle test	Hutchinson, et al., 1999
Crustaceans	>=100	21 d	NOEC Repro-duction	Tisbe battagliai	Renewal 3 x pw Life cycle test	Hutchinson, et al., 1999

e) 17α-Ethinyloestradiol

The tables 7.10 shows the toxicity data of 17a-ethinyloestradiol for marine organisms.
There are no data on acute toxicity to marine organisms and only limited data on chronic toxicity (1 study).
There are only data on crustaceans. Based on these data 17a-ethinyloestradiol is slightly toxic. Effect concentrations are far below the limit of water solubility of 4.75 mg/l.

Table 7.10:
Retrieved chronic effect concentration (NOEC) (µg/l) of 17a-Ethinyloestradiol for groups of species from the marine aquatic environment

Class	Concentration (µg/l)	Testing time	Effect type	Organism	Method	Literature (source)
Crustaceans	>=100	21 d	NOEC Reproduction	Tisbe battagliai	Renewal 3 x pw Life cycle test	Hutchinson, et al., 1999
Crustaceans	>=100	10 d	NOEC Survival	Tisbe battagliai	Renewal 3 x pw Life cycle test	Hutchinson, et al., 1999
Crustaceans	>=100	21 d	NOEC Survival	Tisbe battagliai	Renewal 3 x pw Life cycle test	Hutchinson, et al., 1999

f) Overview of toxicity data for marine organisms.

In the Tables 7.11 and 7.12 an overview is given of the lowest retrieved acute and chronic effect concentrations. The concentrations are reflected in **µg/l.**

Table 7.11:
Overview of the lowest retrieved acute effect concentrations ($L(E)C_{50}$) (in µg/l) of the estrogens in the marine environment.

Substances	Algae	Molluscs	Crustaceans	Fish
17α-Oestradiol	-	-	-	-
17β-Oestradiol	-	-	0.1 [a]	-
Oestrone	-	-	-	-
17α-Ethinyloestradiol	-	-	-	-

a. LOEC Inhibition of settlement

Table 7.12:
Overview of the lowest retrieved chronic effect concentrations (NOEC) (in µg/l) of the estrogens in the marine environment.

Substances	Algae	Molluscs	Crustaceans	Fish
17α-Oestradiol	-	-	-	-
17β-Oestradiol	-	-	=100	>30 mg/kg feed
Oestrone	-	-	=100	-
17α-Ethinyloestradiol	-	-	=100	-

From the Tables 7.11 and 7.12 it is clear that toxicity data on the estrogens are scarce. From the available data it seems that these substances are slightly to very toxic to marine organisms.

7.3 Standards and derivation of iMPCs

In MilBoWa (1999) harmonized standards are derived for several environmental compartments for a number of chemicals. The purpose of MilBoWa (1999) is to create a system of limit- and target values for soil and surface water.
A limit value is a quality level that minimally should be achieved or maintained. A target value is a quality level at which no adverse effects are to be expected. The limit value is based upon the "maximal permissible concentration" (MPC), the target value on the "negligible concentration level" (NC). At time it could be possible that different MPCs were operative for the same substance because there were also MPCs derived in the framework of the admission of plant protection products and biocides. In 1999 (Kalf, et al., 1999) the procedure for the derivation of MPCs for admission policy of plant protection products and biocides and the setting of environmental quality standards are harmonised.
As a starting-point it is formulated that a MPC is comparable to the concentration at which at least 95% of the species in the ecosystem will be protected (method of Van Straalen and Denneman (1989), modified to the model of Aldenberg and Slob (1991; 1993). There is also formulated that the negligible risk level is comparable to 1% of the MPC.

For the estrogens there are no standards derived yet.

For the derivation of MPCs directly from ecotoxicological endpoints two different methods are used: the refined effect assessment method and the preliminary effect assessment method. Because long term chronic data are preferred above short term acute data the aim is to apply the refined effect assessment method. However application of this method is based on data availability: at least four NOEC values are needed for four different taxonomic groups of organisms. If these data are not available the preliminary effect assessment method is applied. In this case in principle the TGD is applied. In figure 7.3 the direct method for MPC derivation is presented.

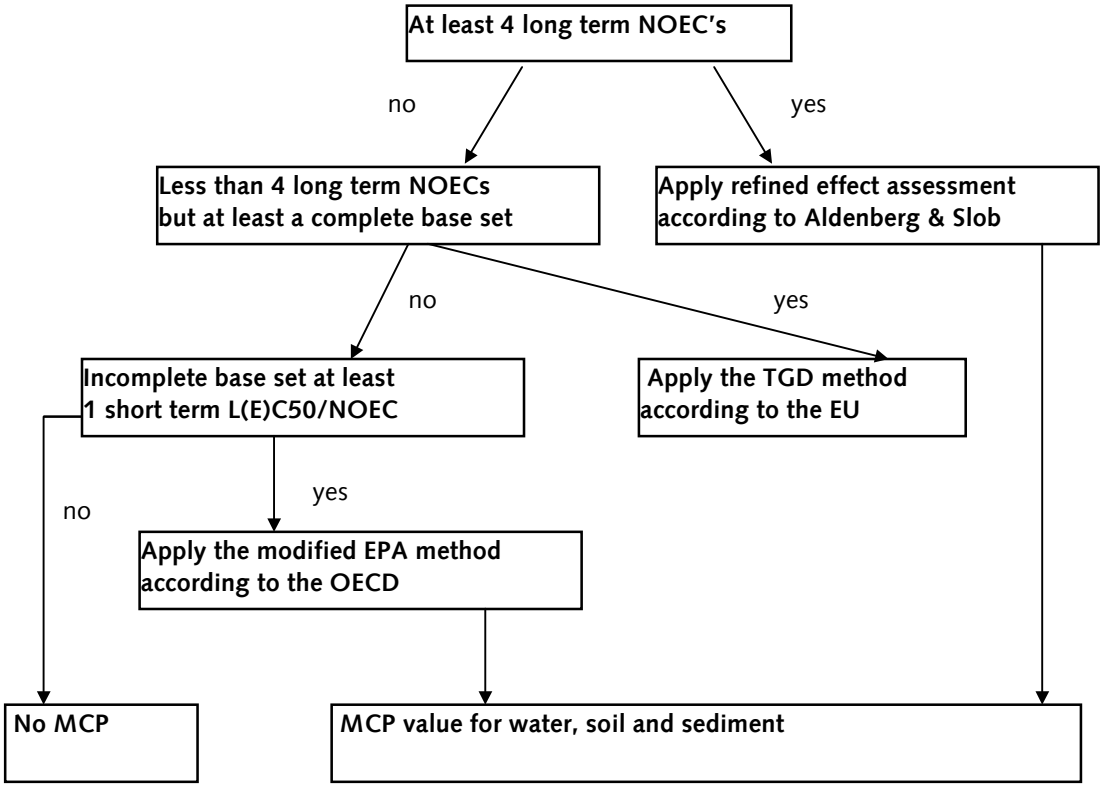

Fig 7.3:
Scheme for the derivation of the MPC: direct method

The aim of the environmental quality standards is that the MPC is set at a level that protects all species in an ecosystem. However, in order to be able to use extrapolation methods like the one of Aldenberg & Slob, a 95% protection level is chosen as a sort of cut-off value.
The 95% protection level can be defined for an individual substance if there are NOEC values for at least four different groups of species (e.g. fish, mollusc, crustacean and algae) available. The method of Aldenberg & Slob assumes that the NOECs used for estimating distribution, fit the log-logistic distribution.
If there are not enough data to applicate the method of Aldenberg & Slob, the preliminary effect assessment method is used. In principle the assessment factors of the ECB (1996), laid down in the Technical Guidance Documents (TGD), are used. The application of the TGD assessment factors is presented in Table 7.13.

Table 7.13:
Assessment factors for aquatic toxicity data following EU/TDG (ECB, 1996) according to EUSES (EC, 1996)

Available valid data	Assessment factor to be applied to the lowest L(E)C50 or long-term NOEC
At least one short-term L(E)C50 from each of 3 trophic levels of the base-set (fish, Daphnia and algae)	1000[a]
One long-term NOEC (either fish or Daphnia)	100[b]
Two long-term NOECs from species representing two trophic levels (fish and/or Daphnia and/or algae)	100[b] 50[c]
Long-term NOECs from at least 3 species (normally fish, Daphnia and algae) representing three trophic levels	50[c] 10[d]
Field data or model ecosystems	Reviewed on a case by case basis

- Base set= 3 L(E)C50 values from acute aquatic toxicity tests, carried out with 3 organisms each representing a different trophic level (algae, Daphnia and fish).
- NOEC should be from long term test and L(E)C50 form short test.

a. Except for substances with intermittent release under no circumstances should a factor lower than 100 be used in deriving a iMPCwater from short-term toxicity data.
b. An assessment factor of 100 applies to a single long-term NOEC (fish or Daphnia) if this NOEC was generated for the trophic level showing the lowest L(E)C50 in short-term tests.
   An assessment factor of 100 applies also to the lowest of two long-term NOECs covering two trophic levels when such NOECs have not been generated from that showing the lowest L(E)C50 of short-term tests.
c. An assessment factor of 50 applies to the lowest of two NOECs covering two trophic levels when such NOECs have been generated covering that level showing the lowest L(E)C50 in the short-term tests. It also applies to the lowest of 3 NOECs covering three trophic levels when such NOECs have not been generated from that level showing the lowest L(E)C50 in the short-term tests.
d. An assessment factor of 10 will normally only be applied when long-term toxicity NOECs are available from at least three species across three trophic levels (e.g. fish, Daphnia, and algae or a non-standard organism instead of a standard organism). The PNECwater should be calculated from the lowest available NOEC. This is only sufficient, however, if the species tested can be considered to represent one of the more sensitive groups. This is particularly important of the substance does not have the potential to bioaccumulate. If it is not possible to make this judgement, then an assessment factor of 50 should be applied to take into account any interspecies variation in sensitivity.
e. For compounds with a high log Kow no short term toxicity may be found. Also, even in long term tests this may be the case or steady state may still not have been reached. For tests with fish for non-polar narcosis the latter can be substantiated by the use of long-term QSARs. It can be considered to use a higher assessment factor in such cases where steady state seems not to have been reached.

f. For substances for which no toxicity is observed in short term tests a long term test has to be carried out if the log Kow > 3 (or BCF > 100) and if PEClocal/regional is > 1/100th of the water solubility. The NOEC from this test can then be used with an assessment factor of 100. If in addition another NOEC from an algae test of the base set is determined, an assessment factor of 50 is applied.

There are two exceptions to the use of the TGD method:
1. Only when long term NOECs on three trophic levels are available, a comparison with data from the (complete) base set is no longer demanded.
2. It is inferred that for more hydrophobic compounds, short term toxicity data may not be representative, since the time span of an acute test may be too short to reach a toxic internal level. In those cases, base set completeness is not demanded and an assessment factor of 100 may be applied to a chronic test, which should not be an alga test if this is the only chronic test available.

If the base set is incomplete, the TGD method cannot be applied, abitrar safety factors are used (the modified EPA-method (OECD, 1992)): a factor 10 and/or 1000 wille be applied to the NOEC and/or L(E)C50, respectively, to derive the MPC. It should be stressed here that this exception may only be used if the TGD can not be applied.
In Table 7.8 the safety factors of the modified EPA method, dependent on the number of available toxicity data, are presented.

The calculated MTR in this report will be defined as "indicative MPC" (iMPC).
In contradiction to the limit and target values the derived iMPCs have only a technical status and no political value. They are not legally set and may change as soon as more toxicity data become available and/or an MTR is derived by the INS-project.

Table 7.14:
Safety factors for the derivation of iMPCs in surface water (modified EPA method).

Available toxicity data	Safety factor
Lowest acute L(E)C_{50} or QSAR estimation for acute toxicity	1000
Lowest acute L(E)C_{50} or QSAR estimation for acute toxicity for at least algae, crustaceans and fish	100
Lowest NOEC or QSAR estimation for chronic toxicity	10*
Lowest NOEC or QSAR estimation for chronic toxicity for at least algae, crustaceans and fish	10

* this value will be compared with the value based on acute L(E)C_{50} values. The lowest value will be selected.

Based on the retrieved toxicity data the iMPCs are derived using the procedure described by Kalf (1999). For the derivation of the iMPCs the fresh and salt water toxicity data are combined just as the data concerning pelagic and bentic organisms (based on the assumption that bentic organisms are exposed through the same medium as the pelagic organisms). Biomagnification is not included in this calculation. The toxicity data used for the derivation of the iMPCs are reflected in Annex 2.2. The iMPCs are in table 7.15.

The iMPCs for sediment are calculated using the equilibrium partition (EP) method (see Slooff, 1992 Beek, 1993).

MPC sed = MPC water x Kp

The equilibrium partition coefficient is calculated with the K_{oc} using the following formula:

$Kp = K_{oc} \times foc\ (l.kg^{-1})$

In the calculation the standard soil is assumed to contain 5% organic carbon.

Table 7.15:
iMPCs for surface water (µg/l) and sediment (µg/kg dry soil).

Substance	Surface water (µg/l)		Sediment (µg/kg)	
	IMPC	Method	IMPC	Method
17α-Oestradiol	-	-	-	-
17β-Oestradiol	-	-	-	-
Oestrone	-	-	-	-
17α-Ethinyloestradiol	1	EPA 100	-	-

There can only be derived an iMPC for ethinyloestradiol for surface water, as there are not enough data on the toxicity of the other estrogens to derive an iMPC. Because there is no log Koc available, the iMPC for sediment cannot be calculated. As no limit values are derived for the estrogens there is no comparation possible with the iMPCs. However a comparison with the concentrations in the environment can be made. In Table 7.16 the concentrations in water and wastewater from chapter 6.2 and 6.3 are summarized. Data are available on all estrogens. Only the concentrations of ethinyloestradiol can be compared with the iMPC. The concentrations of ethinyloestradiol in surface water in the Netherlands range from <0.2 to 19 ng/l in fresh water and are <0.1 ng/l in marine water. In wastewater and the sludge the concentrations vary from <0.3 to 9.7 ng/l. This means that in the Netherlands the concentrations in freshwater, marine water, wastewater and sludge do not exceed iMPC.

Table 7.16:
Concentration ranges in fresh and marine surface water, wastewater and sludge in ng/l on several locations based on data from chapter 6.2 and 6.3

Location	Fresh water/ Marine	17α-Oestradiol In ng/l	17β-Oestradiol In ng/l	Oestrone In ng/l	17α-Ethinyloestradiol In ng/l
Netherlands 1997	**Fresh** Surface water[a]	<0.1-3.0	<0.4-5.5	<0.1-5.3	<0.2-19
Netherlands 1997	Wastewater and sludge[a]	<0.1-9	<0.2-2.5	<0.4-140	<0.3-9.7
Netherlands 1997	**Marine** Surface water[b]	<0.1	<0.3-0.3	<0.2-0.6	<0.1

a. based on table 6.3
b. b. based on table 6.4

7.4 Human toxicity

a) General effects

The estrogens are listed as endocrine disrupters (Colborn, 1993) and also have effects on thyroid hormones.
Oestradiol is the most potent estrogenic hormone in all vertebrates. The binding specificity of estrogen receptors for oestradiol is virtually identical in highly diverse species - for example, the trout and the human being. In other words: in vertebrates, the system of hormonal and accompanying receptors has undergone little change in the course of evolution. Because of the extremely high binding specificity of natural (and also synthetic) hormones, exposure to low concentrations results in effects on reproduction in all species. Synthetic hormones, such as those in the oral contraceptive pill, are specifically intended to prevent reproduction at low concentrations.

By their very nature, estrogens will be present in ecosystems, at extremely low concentrations as a result of the excretion of these hormones by vertebrates. Such concentrations are not expected to have any effects on animals. Due to the large numbers of human beings and livestock in the Netherlands and the surrounding countries, the extent of the emission of natural hormones can no longer be regarded as natural.

Synthetic estrogens as ethinyloestradiol and e.g. diethylstilboestrol (DES) are more resistant to degradation and removal thereby increasing their potency over natural forms. These substances are very potent and even at very low concentrations exert estrogenic activity for long periods. Compared to 17ß-Oestradiol the synthetic forms are somewhat less potent in vitro than in vivo because it is the protection from degradation in vivo that enhances their activity. Implications for inadvertent exposure are uncertain. The organism may be able better to metabolise and eliminate natural hormones. Effects might be expected where there is continual exposure to the hormone, or when removal mechanims are not in developed (juvenile) stages (UK, 1998).

b) Oestradiols

In Table 7.17 the acute toxicity of 17a-oestradiol is given. The values indicate that 17a-oestradiol is very toxic to mammalians. There are no mammalian toxicity data on 17ß-oestradiol available.

Table 7. 17 The acute toxicity of 17a-Oestradiol

Dose/Effect	Mode/Species	Concentration	Source
ETA, teratogen	Imp/Guineapig	3 mg/kg	Sax, 1989
-	Dni:other / human	100 mg/l	Sax, 1989

ETA= Equivocal tumorigenic agent
DNI= DNA inhibition
Imp= Implant

The substance is carcinogenic to animals (IARC V6, 1974 IARC V21, 1979 IARC S4, 1982 IARC S7, 1987). It produces various tumours after implantation, s.c. and oral implantation in animals. The Dutch Expert Committee on Occupation Standards has evaluated all available information and concluded that the evidence for activity in short-term tests is inadequate. The compound is well investigated, but the evidence of a carcinogenic effect is insufficient to classify this compound as probably carcinogenic to humans. This compound is classified as suspect carcinogen (DECOS, 1995).

c) Oestrone

In Table 7.18 the acute toxicity of oestrone is given. The values indicate that oestrone is very toxic to mammalians.

Table 7.18:
The acute toxicity of Oestrone

Dose/Effect	Mode/Species	Concentration	Source
-	Oral / dnd-rat	870 nmol/kg	Sax, 1989
-	Ovr/ cyt-hamster	50 µmol/l	Sax, 1989
TDL0 teratogen	Scu / rat	10 µg/kg	Sax, 1989
TDL0 reproduction	Oral / rat	100 µg/kg	Sax, 1989
TDL0 eta/teratogen	Imp / guinea pig	640 µg/kg	Sax, 1989
TDL0 eta	Par / mouse	1200 µg/kg (W-I)	Sax, 1989
TDL0 reproduction	Imp/ man 60 d male	1586 µg/kg	Sax, 1989
TDL0 eta	Imp / guineapig	1800 µg/kg	Sax, 1989
TDL0 eta/teratogen	Imp / guineapig	2 mg/kg	Sax, 1989
-	Ipr / cyt-rat	10 mg/kg	Sax, 1989
TDL0 neoplastic effects	Oral / mouse	11 mg/kg (68w-C)	Sax, 1989
TDL0 eta	Imp / rat	16 mg/kg	Sax, 1989
TDL0 reproduction	Scu / mouse	28 mg/kg	Sax, 1989
TDL0 eta/teratogen	Scu / guineapig	40 mg/kg (18W-I)	Sax, 1989
TDL0 eta	Scu / mouse	48 mg/kg (24W-I)	Sax, 1989
TDL0 eta	Imp / mouse	48 mg/kg	Sax, 1989
TDL0 eta	Imp / rat	80 mg/kg	Sax, 1989
TDL0 neoplastic effects	Scu / mouse	108 mg/kg (90w-I)	Sax, 1989
TDL0 eta	Imp / hamster	320 mg/kg (59W-C)	Sax, 1989
TDL0 eta	Imp / hamster	640 mg/kg (38W-I)	Sax, 1989

eta= Equivocal tumorigenic agent; imp= implant ; scu= subscutaneous;ipr= intraperitoneal;cyt=cytogenic analysis;dnd=dna damage; par= parenteral

The Dutch Expert Commitee on Occupational Standards has evaluated this compound. They conclude that there is inadequate evidence for carcinogenicity of oestrone in humans (IARC S4, 1982, DECOS, 1995). The compound is carcinogenic to animals. It produces malignant tumours in various organs after skin painting, implantation, injection and oral administrations. The evidence of carcinogenic effect is insufficient to classify this compound as probably carcinogenic to humans. This compound is classified as a suspect carcinogen (DECOS, 1995).

d) Ethinyloestradiol

In Table 7.19 the acute toxicity of ethinyloestradiol is given. The values indicate that ethinyloestradiol is very toxic to mammalians.

Table 7.19:
The acute toxicity of 17a-ethinyloestradiol

Dose/Effect	Mode/Species	Concentration	Source
-	Dni-human:lym	50 µmol/l	Sax, 1989
TDL0 carcinogen	Oral rat	6 µg/kg (2y-C)	HSDB, 2000
TDL0 reproduction	Women	20 µg/kg (4 d preg)	HSDB, 2000
TDL0 reproduction	Rat	55 µg/kg (11 d preg)	HSDB, 2000
TDL0 reproduction	Women	160 µg/kg (57 w preg)	HSDB, 2000
TDL0 reproduction	Oral / mouse	200 µg/kg	Sax, 1989
TDL0 reproduction	Rat	250 µg/kg (1-4 d preg)	HSDB, 2000
TDL0 reproduction	Women	500 µg/kg (5d preg)	HSDB, 2000
TDL0 reproduction	Women	500 µg/kg (1-5d preg)	HSDB, 2000
TDL0 reproduction	Rat	560 µg/kg 14 d male)	HSDB, 2000
TDL0 reproduction	Rat	860 µg/kg (1 d preg)	HSDB, 2000
TDL0 reproduction	Scu / rat	1 mg/kg	Sax, 1989
TDL0 reproduction	Rat	3 mg/kg (9-20 d preg)	HSDB, 2000
TDL0 carcinogen	Imp rat	5 mg/kg	HSDB, 2000
TDL0 reproduction	Rat	4500 µg/kg (1-9 d preg)	HSDB, 2000
TDL0 glandular effects	Oral women	21 mg/kg	HSDB, 2000
TD carcinogen	Oral rat	245 mg/kg (35w-C)	HSDB, 2000
TDL0 carcinogen	Imp. Hamster	621 mg/kg	HSDB, 2000
LD50 glandular effects	Oral mouse	1737 mg/kg	HSDB, 2000
LD50 glandular effects	Oral rat	2952 mg/kg	HSDB, 2000

imp= implant ; scu= subscutaneous; dni= DNA inhibition; lym=lymphocytes

The Dutch Expert Committee on Occupational standards has evaluated all available information on mutagenicity, genotoxicity and human data and concludes that there is insufficient evidence that ethinyl-oestradiol is carcinogenic to humans. The compound is carcinogenic to animals. It produces malignant tumours in various organs after oral administration in animals. The mutagenic activity is short-term tests is inadequate. This compound is well investigated, but evidence of a carcinogenic effect is insufficient to classify this compound as probably carcinogenic to humans. This compound is classified as a suspect carcinogen (DECOS, 1995).

e) Mestranol.

The Dutch Expert Committee on Occupational standards has evaluated all available information on mutagenicity, genotoxicity and human data and concludes that there is insufficient evidence that Mestranol is carcinogenic to human. The compound is carcinogenic to animals. It produces malignant tumours in various

organs after oral administration. The mutagenic activity is short-term tests is limited. This compound is well investigated, but evidence of a carcinogenic effect is insufficient to classify this compound as probably carcinogenic to humans. This compound is classified as a suspect carcinogen (DECOS, 1995).

f) Exposure routes in the aquatic environment.

Contamination of the aquatic environment (surface water and sediment) can pose a threat to public health. The hazards can be caused by direct and/or indirect contact with the contaminants. In principle, uptake of contaminants by humans can take place by ingestion (oral), dermal contact (via the skin) and inhalation (via the lungs).
In case of the natural estrogens, these substances are already in the body of mammalians (endogenous production). However additional estrogens may disturb the internal hormone balance. Exposure of synthetic estrogens occurs primarily through oral intake.

g) Human health risk assessment evaluation with exposure to sediment

BKH (1991) has conducted a study into the human health risks of recreants potentially exposured to contaminants in sediment. Because children are seen as the most vulnerable group, recreating children are used as a starting point for the derivation of a human-toxicologic based advisory value (HTBA-value) for contaminations in sediment.
Above this value adverse health effects may be expected. In Annex 2.3 the human health assessment evaluation-method is described. The HTBA-values are based on ADI-values (Acceptable Daily Intake) from the literature and the K_{ow}-values. There are no ADI-values for the natural and synthetic estrogens. Because there are no ADI-values available, the HTBA-value could not be derived.

7.5 Conclusions and recommendations

7.5.1 Conclusions

Mechanism of toxicity
Estrogen substances act through binding on the estrogen receptor, stimulation of endogenous production of 17ß-oestradiol and dimishing of 17ß-oestradiol degradation.

Metabolism

Estrogens are (de)oxidated, hydrolysed and methylised in liver and conjugate with glucuronic acid or sulfate. 17ß-oestradiol is easily oxidised to oestrone, which is further metabolised to oestriol or 2-methoxy-oestrone. Ethinyloestradiol is metabolised to hydroxy-ethinyloestradiol and further metabolised to methoxy-ethinyloestradiol or deethinylated to oestrone. Estrogens are excreted in faeces and urine.

Toxicity in freshwater environment

Toxicity data are scarce. Based on endocrine disrupting effects all estrogens are very toxic. Based on acute effects on survival and reproduction 17a-ethinyloestradiol is also very toxic to algae and crustaceans.

Toxicity in saltwater environment

Toxicity data are scarce. Based on chronic effects on survival and reproduction 17ß-oestradiol, oestrone and 17a-ethinyloestradiol are only slightly toxic to crustaceans.

Limit values and indicative MPCs

There are no limit values derived for estrogens. There is a iMPC derived for 17a-ethinylopestradiol of 1 µg/l. Concentrations in surface water in the Netherlands do not exceed this iMPC. The HTBA-value could not be derived.

Humane toxicity

All estrogens are human suspected carcinogens. Data on acute toxicity indicate that estrogens are very toxic to mammalians.

7.5.2 Recommendations

More data on acute and chronic toxicity should be produced. Furthermore there should be a guideline on how to treat effects on the endocrine system. Especially interesting is, how these endocrine effects influence the populations.

7.6 References

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8 Policy overview

8.1 National environmental policy

8.1.1 Netherlands

In the National Environmental Policy Plan (NMP, 1989) and the more recently published National Environmental Policy Plan-3 (NMP-3, 1997) the general environmental policy is described.
In the year 2010 the environmental targets and target values must be reached. Concerning the reverse of the risks caused by high concentrations of chemicals, specific policy targets have been set in the National Environmental Policy Plan of 1989. These targets imply the aim to not exceed the Maximum Permissible Concentrations (MPCs) and the Negligible Concentrations (NCs) in 2010, by means of prevention and reconstruction. These values are guidelines but not legally binding. When the environmental quality standards are set, other aspects, such as political and technical feasability, are also taken into account. Target values are either set at the NC or at the background value. The derivation of MPCs and NCs is explained in chapter 7.3. For the natural and synthetic estrogens no specific quality standards, MPCs or NCs have been set.
In the report on integral standardisation on substances (INS, 1997) environmental quality standards have been derived.
The current water policy is reflected in the Fourth Note on Watermanagement (1997). In this note the targets and headlines of the policy for the national water management are given.

8.1.2 Other country specific policy

8.2 European Commission

The following regulations are effectuated in the European union: Estrogen must not form part of the composition of cosmetic products. Member states should prohibit the marketing of cosmetic products containing the substance (Council Directive Relating To Cosmetic Products (76/768/EEC)) (Halling-Sorensen, 1998).

The use in livestock farming of 17ß-oestradiol and related derivatives having a hormonal action is prohibited. Administering for therapeutic purposes is autorized only by a veterinarian in the form of an injection. An animal, which has been treated may not be slaughtered before expiry of a fixed period (Council Directive 88/146/EEC) (Halling-Sorensen, 1998).

January 1998 EU has taken into operation a directive describing the technical guideline for assessing the risk of the environmental exposure of veterinary medical substances (AEU Note for guidance EM/CAMP/055/96-FINAL).
A similar directive have been launched as a draft manuscript covering a risk assessment procedure of the exposure of medical substances for human treatment (III/5504/94 EU Draft guideline).
The proposed directive prescribe that a risk assessment should be part of the approval procedure of new medical substances (Cwiertniewicz, 1994). The US Food and Drug Administration have already had for several years issued its Environmental Assessment Technical Handbook for FDA-required environmental Assessment (FDA, 1985; FDA, 1987). But it seems that this legislation is not of high

concern, since only very few new medical substances in recent years, to the authors knowledge, have been subjected to a complete risk assessment where a battery of appropriate ecotoxicological tests were included.
The Food and Drug Administration (FDA) issued the final rule requiring compliance for human pharmaceuticals under the National Environmental Policy Act (NEPA) in 1985. In March 1987, FDA prepared and issued its Environmental Assessment Technical Handbook for FDA-required environmental Assessment (Cwiertniewicz, 1994; FDA, 1985; FDA, 1987). Finally in 1995, FDA issued its guidance for industry for the submission of an environmental assessment in human drug application and supplements.
In Europe the legislation on the area has first been initiated in the beginning of the 90s and it distinguishes between medical substances which do not contain (or consists of) genetically modified organisms (GMOs) and medical substances which do contain (or consist of) genetically modifed organisms (GMO). These two groups are further divided into veterinary medicinal products and human medicinal products. All new EU policy making will include an environmental dimension. This means that all new draft EU laws will be assessed for potential environmental impact. Directive 81/852/EEC (as amended since 1 April 1993) is known as the technical directive concerning veterinary medicinal products. It outlines in sections on ecotoxicity the basic requirements of conducting an environmental risk assessment. A note for guidance (EMEA/CVMP/055/96-FINAL) issued in 1997 gives a detailed technical guideline for assessing the risk of veterinary medical substances.
The technical directive for human medicinal products (EU directive 75/318/EEC) does not include any reference to ecotoxicity or ecotoxicology and there are apparently no plans about doing this. In this way it differs from its veterinary counterpart (EU directive 81/852/EEC). A detailed technical draft guideline III/5504/94 is issued in 1994 indicated that the same approach applicable for veterinary also would apply for human medicinal substances. This technical guideline has not yet been finalised (Halling-Sorensen, 1998).

8.2.1 Current development

There is a dispute between the U.S. and the European Union on the import of meat treated with growth enhancers. In Europe the use of these products in livestock is prohibited. However, a panel of the World Trade Organization has decided that the European Union's ban on hormone-treated beef is illegal (Andrews, 1997).

These enhancers consist of the naturally occurring hormones progesterone, testosterone and oestradiol, and the synthetic hormones zeranol, trenbolone acetate and melengestrol acetate. Whereas, approximately 90 percent of the fed cattle in the U.S. are implanted with growth enhancers.

The EU Scientific Committee of veterinary measures relating to Public Health (SCVPH) proposes to ban on the use of 17ß-oestradiol and its steroid derivatives in farm animals definitely. As there will be an establishment of a new system on meat hormones, the Commission considers the embargo to conform to the World Trade Organisation rules and so hopes that Canada and the USA will accept this (Eur-op news, 2000).

8.3 International policy

8.3.1 OSPAR

The estrogens oestradiol, oestrone, DES and ethinyloestradiol are on the OSPAR-list in group VI. There are no further actions to this group of substances, yet.

8.4 Policy on emissions

8.4.1 Exposure limits

Establishing an ADI and an ARL for a hormone that is produced endogenously at variable level in human beings was considered unnecessary. Residues resulting from the use of this substance as a growth promotor in accordance with good animal husbandry practice are unlikely to pose a hazard to human health (WHO, 1988).

8.4.2 Emission limits

There is no national legislation in the European Commission that inspect estrogens in the atmospheric emissions, fluid discharges and waste.

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Annex 1: Abbreviation list

ADD	Androsta-1,4-diene-3,17-dione
ADI	Acceptable daily intake
AOPWIN	Aerobic oxidation programme
APME	Association of Plastics Manufacturers in Europe
ASE	Accelerated solvent extractor
ASE	Accelerated solvent extractor
ASTP	Activated sludge treatment plant
BADGE	Bisphenol A diglycidyl ether
BCF	Bioconcentration factor
BOD	Biological Oxygen Demand
BPA	Bisphenol A
BUA	Umwelt Bundes Amt
CAS	Chemical Abstract Services
CD	Compact discs
CIA	Chemiluminescence immuno assay
COD	Chemical Oxygen Demand
CYT	Cytogenic analysis
DES	Diethylstilboestrol
DGD	Decision guidance document
DGXI	Directorate general 11
DIFFCHEM	Working group of OSPAR on Diffuse sources of chemicals
DMDCS	Dimethyl dichloro silane
DMDCS	Dimethyl dichloro silane
DND	DNA damage
DNI	DNA inhibition
DOC	Dissolved Oxygen Content
E&E	Electrical and electronic
E1	Oestrone
E2	17β-oestradiol
E3	Oestriol
EC	European Commission
EC10	Effect concentration with 10% effect
EC100	Effect concentration with 100% effect
EC50	Effect concentration with 50% effect
ECD	Electron capture detector
ECNI	Electron capture negative ionisation
ED50	Effect dose with 50% effect
EE1	17α-Ethinyloestradiol
EHC	Environmental Health Criteria
ELS	Early life stage
EMIT	Enzyme multiplied immuno assay technique
EP	Equilibrium partition coefficient
EPA	Environmental Protection Agency
EpR	Epoxy resin
ER	Estrogen receptor
ER	Estrogen receptor
ETA	Equivocal tumorigenic agent
EU	European Union
FAO	Food and agriculture organisation of the UN
Foc	Fraction organic matter in soil
G6PD	Glucose-6-phosphate dehydrogenase
GC	Gas chromatography
GC/MS	Gas chromatography/ Mass Spectrometry
GLP	Good laboratory practice
GSI	Gonadosomatic index
HCl	Chloric acid
HPLC	High performance liquid chromatography
HRGC	High resolution GC

HRMS	High resolution MS
HTBA	Human toxicologic based advisory value
IC50	Inhibition concentration
IMP	Implant
iMPC	Indicative Maximum Permissible Concentration
IMTR	Indicative MTR
IP	Intraperitoneal
IPCS	International Program for Chemical Safety (a WHO program)
IPR	Intraperitoneal
ISO	International Standards Organisation
IUCLID	International uniform chemical information database
KEMI	Swedish national chemicals inspectorate
Koc	Organic carbon content
Kom	Organic matter content
Kow	Octanol water partitioning coefficient
Kp	Equilibrium partition coefficient
LC100	Effect concentration with 100% mortality
LC50	Effect concentration with 50% mortality
LD50	Effect dosis with 50% effect
LOEC	Lowest observed effect concentration
MLD	Mild effects
MLSS	Mixed liqour suspended solids
MPC	Maximum Permissible Concentrations
MS	Mass spectrography
MTR	Maximal tolerable risk
NAA	Neutron activation analysis
NC	Negligible concentration
NEO	Neoplastic effects
NMP	National Environmental Policy Plan
NOEC	No observed effect concentration
NR	Negligible risk
OECD	Organisation for Economic Cooperation and Development
OSPAR	Convention for the Protection of the Marine Environment of the North-East Atlanti
OVR	Ovary
Par	Parenteral
PC	Polycarbonates
PEC	Predicted effluent concentration
PIC	Prior informed consent
PNEC	Predicted no effect concentration
POP	Persistent organic pollutants
Preg	Pregnant
PVC	Polyvinyl chloride
QSAR	Quantitative structure analysis r
Red-Al	Sodium bis-(2-methoxyethoxy) aluminium
REP	Reproduction
REP	Reproduction
RIA	Radio immuno assay
RIVM	National institute for human health and environment
SCAS	Semi-continuous activated sludge
SCU	Subcutaneous
SD	Standard deviation
SEV	Severe effects
SHBG	Sex hormone binding globulin
SIM	Selection ion monitoring
SMLs	Specific Migration Limits
STWS	Sewage treatment works
STP	Sludge treatment plant
TDI	Tolerable daily intake
TDL0	Lowest toxic dose with no effect
TER	Teratogen
TGD	Technical Guidance Document
ThCO2	Theoretical CO2 production
ThOD	Theoretical oxygen demand
TOC	Total Organic Carbon

UN	United Nations
UN-ECE	United Nations Environmental Commission for Europe
UNEP	United Nations Environment Programme
UPE	Unsaturated polyesters
VIC	Voluntary Industry Commitment
VROM	Ministry of spatial planning and environment
WHO	World Health Organisation
WTP	Wastewater treatment plant
WWTP	Wastewater treatment plant

Annex 2: Background information on aquatic toxicity

Annex 2.1: Used classification systems for aquatic toxicity

Toxicity to algae (96-h, EC_{50}), crustaceans (48-h, LC_{50}) and fish (96-h, LC_{50}):

Class $E(L)C_{50}$ (mg/l)

very toxic		< 1	
moderately toxic	1	-	10
slightly toxic	10	-	100
very slightly toxic		> 100	

Toxicity to aquatic organisms: chronic tests:

Class NOEC (mg/l)

very toxic		< 0.01	
moderately toxic	0.01	-	0.1
slightly toxic	0.1	-	1
very slightly toxic		> 1	

Toxicity to birds: acute oral LD_{50} (mg/kg body weight)

Class LD_{50} (mg/kg)

very toxic		< 5	
moderately toxic	5	-	50
slightly toxic	50	-	500
very slightly toxic		> 500	

Annex 2.2: Overview of the toxicity data per group of organisms (freshwater and marine) used for the derivation of iMTRs in surface water

Table 1:
Acute toxicity data (mg/l)[a]

Substance	Taxonomic group	L(E)C50 (mg/l)	Safety factor
17α-Ethinyloestradiol	Algae	0.84	-
	Crustaceans	0.105	-

a. QSAR calculations using the equations given for non-polar narcosis in Appendix II of Chapter 4 in the Technical Guidance Document have not been used because of the high log Kow which makes the QSAR unreliable.

Table 2:
Chronic toxicity data (mg/l)[a]

Substance	Taxonomic group	NOEC (mg/l)	Safety factor
17β-oestradiol	Crustaceans	>0.1[b]	-
Oestrone	Crustaceans	>0.1 [b]	-
17α-Ethinyloestradiol	Crustaceans	>0.1	100/EPA

a. QSAR calculations using the equations given for non-polar narcosis in Appendix II of Chapter 4 in the Technical Guidance Document have not been used because of the high log Kow which makes the QSAR unreliable.
b. Not enough data

Annex 2.3: Human health risk assessment evaluation method

Human health risk may occur by direct contact of recreating people with contaminants in the aquatic environment (water, sediment). Playing children (1.5 - 4.5 years old with a body weight of 14 kg) are seen as the most vulnerable groups based on age-bound factors. The relevant exposure routes are through oral intake and dermal contact. The intake (in mg/day) can be calculated for the separate exposure routes for 1 day of playing at the waterside in a worst-case scenario (for a detailed description see BKH, 1991):

Oral intake through sediment

The oral intake through sediment is:

$I_{o,sed} = S_1 * 10^{-6} *$ level B

in which:

$I_{o,sed}$	daily oral intake of contaminants via sediment (mg/kg bw)
S_1	sediment intake in mg dw/day (1020 mg dw/day)
10^{-6}	conversion factor for units in the given dimensions
B	level of contamination in soil material in mg/kg dw

Oral intake through suspended matter

The oral intake through suspended matter is:

$I_{o,susp} = I * S_2 * 2 * 10^{-9} *$ level B

$I_{o,susp}$	daily oral intake of contaminants via suspended matter (mg/kg bw)
I	intake surface water (50 ml/day)
S_2	concentration in suspended matter (300 mg dw/l)
2	correction factor for higher concentrations in suspended matter
10^{-9}	conversion factor for units in the given dimensions
B	level of contamination in soil material in mg/kg dw

Oral intake through surface water

The oral intake through surface water is:

$I_{o,wat} = W_i * 10^{-3} * (10^{0.21}/f_{oc}*K_{ow}) *$ level B

$I_{o,wat}$	daily oral intake of contaminants via water (mg/kg bw)
W_i	intake of surface water (50 ml/day)
10^{-3}	conversion factor for units in the given dimensions
f_{oc}	organic carbon fraction in sediment (0.05)
K_{ow}	partition coefficient octanol/water
B	level of contamination in soil material in mg/kg dw

Dermal contact with sediment

The dermal contact with sediment is:

$I_{d,sed} = O_{skin} * B_{b,skin} * A * M * 10^{-6} *$ level B

$I_{d,sed}$	daily dermal intake of contaminants via sediment (mg/kg bw)
O_{skin}	surface of skin exposed (2800 cm^2)
$B_{b,\ skin}$	area of skin covered with sediment parts (0.5 mg dw/cm^2)
A	absorption coefficient (0.12/day)
M	matrix effect: the effect of the binding of contaminants to soil particles on body intake (0.15)
10^{-9}	conversion factors for units in the given dimensions
B	level of contamination in soil material in mg/kg dw

Dermal contact with suspended matter

Dermal contact with suspended matter is negligible compared to dermal contact with sediment.

Dermal contact with water

Dermal contact with water is:

$I_{d,wat} = O_{skin} * t * A'' * B_{w,skin} * 10^{-9} * C_w$

$I_{d,wat}$	daily dermal intake of contaminants via water (mg/kg bw)
O_{skin}	exposed skin surface (4560 cm^2)
t	exposure time (3 hours/day)
A''	absorption coefficient (0.01/hour)
$B_{w,\ skin}$	area of skin covered with water (0.5 µg/cm^2)
10^{-9}	conversion factors for units in the given dimensions
C_w	concentration in water; this is:

$Cw = 2*(10^{0.21})/(f_{oc} * K_{ow}) * 10^3 *$ level B

with:

f_{oc}	organic carbon fraction in sediment (0.05)
K_{ow}	partition coefficient octanol/water
10^3	conversion factor for units in the given dimensions
B	level of contamination in soil material in mg/kg dw

For the calculation of the yearly-averaged daily intake, the daily intake should be multiplied with a factor 30/365; the number of playing days at the water side is estimated at 30 per year.
For the calculation of concentrations of individual contaminants in sediment, the yearly-averaged daily total intake through the above-mentioned exposures routes are compared to a human health guidance value, at which there is a maximal tolerable risk (MTR, ADI). This means the ADI should be multiplied by 14 kg body weight and 0.05 (MTR) to derive the maximum intake per day for a child of 14 kg. In this way HTBA-values may be derived.

In the report of BKH (1991) the level at which there is a maximal tolerable risk (MTR) is linked to the intervention-value-level, an environmental quality level that is established in view of direct measures and at which there is a "serious risk for human health". For the derivation of the HTBA-values for sediment, above which there is a "serious risk", the total contribution of exposure to sediment is set at a maximum of 5% of the MTR. Using this percentage, it is expected that other sources as well as the contribution of other substances, which comparable effects, are sufficiently encountered for.