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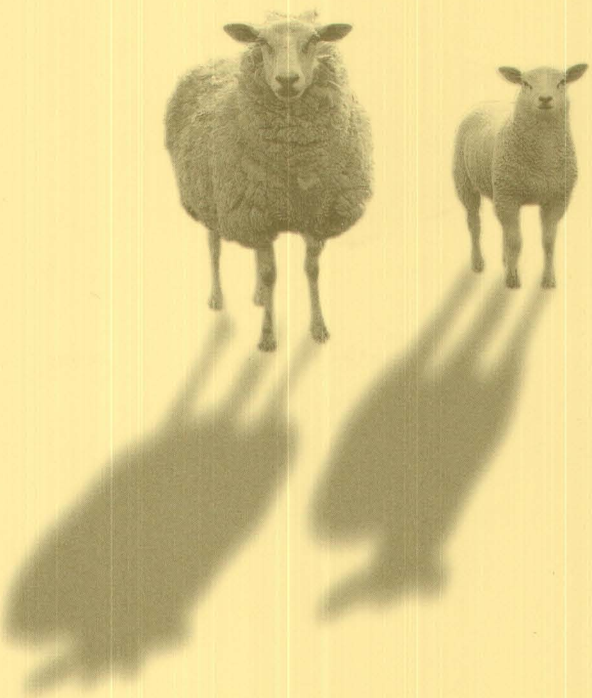
December 2000

Source and environmental fate of natural oestrogens



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ABSTRACT

This report contains an evaluation of the literature for the estimation of environmental emissions of natural and synthetic oestrogens. The natural oestrogens emitted by the human population are present in urine and faeces and enter the sewer. In sewage treatment plants they are partially degraded and then diluted in the surface water. For about 20 times a year on average, during occasional stormwater overflow, raw sewage is discharged temporarily into the surface water.

For the Dutch population the total excretion to the sewer of natural steroid hormones is estimated at 3.2 kg/day. For the synthetic contraceptive ethinyloestradiol the estimated emission is 43 g/d. After biological purification with an efficacy of 95 respectively 50% the natural hormones will have a concentration of 50 ng/l and the diethyl oestradiol 6.5 ng/l. At an average dilution factor 20 for the daily sewage flow of the European population to the daily run-off in rivers, the expected concentration of ethinyloestradiol is 0.3 ng/l and of natural hormones about 2.5 µg/l. These predicted concentrations are rather close to the measured concentrations.

Besides ethinyloestradiol several progestogens are used in modern contraceptives. Very little is known about degradation and the effect of metabolites. Based on a "worst case" approach it is estimated that the human emission of steroids could be increased by 40% by their progestogen metabolites.

The emission of hormones by domestic animals in manure is normally collected in storage basins and after use as fertiliser, a small fraction is leaching and enters the drains and ditches. The total Dutch animal stock produces about 46 kg oestrogens/day. Assuming that after fertilising 3% leaches to the drains and that the manure of a year is spread during a period of three months, the predicted concentration in polder ditches is between 75 and 150 ng/l. The highest concentration can be expected if the yearly produced manure is spread at once over the land at the highest permitted rate. This is the case with pig manure on land used for corn. If the manure of cows is spread over grass land in regular intervals around the summer season, the predicted concentration may be about 40 ng/l.

A similar level is calculated for the entire catchment area of Rhine and Meuse with the emission from the given amount of cattle in the catchment area. The yearly production of manure spread in three months will give a concentration of 76 ng/l in Rhine and 140 ng/l in Meuse. With a more regular distribution the concentrations may be around 40 ng/l. Assuming that in river water some 95% degradation may occur, the predicted level could be 2 ng/l.

The total emission of natural hormones by cattle is much higher than that by the human population. However, the predicted concentration in surface water is uncertain, mainly because the degradation in soil and surface water is unknown.

Measured concentrations of natural hormones in Dutch surface water show a very variable concentration. The present data are possibly not representative for the loading by land application of manure. The sampling points and the sampling times are not chosen in order to validate the highest predicted levels. The highest measured values for the sum of 17α-, 17β-oestradiol and oestrone are around 12 ng/l. In many cases, however, the value is below the detection limit.

It can be concluded that the predicted concentrations on the basis of estimated emission, degradation, leaching and dilution are in fairly good agreement with the highest of the few available data. The measurements in surface water, however, are still indicative and not based on adequate monitoring at those places where the highest concentrations are expected. Too little data are known about the environmental fate and behaviour of hormones in manure after land application. Representative measurements for concentrations in liquid manure and polder ditches are absent.

Based on the highest observed level of the ethinyloestradiol in river water and drinking water, it can be concluded that the risk for human health as a consequence of drinking water is negligible. Even with a concentration of 5 ng/l ethinyloestradiol, which is the highest observed level in Dutch surface water, the daily intake would be a factor 3500 below the effective dose for contraceptives. Based on experience from producers a dosis of a factor 200 below the effective data gives no secondary effects.

It can be expected that in small surface waters near the outfall of treatment plants there will be effects on fish. This is based on an effective concentration for vitellogen induction in male fish of 0.9 ng/l for ethinyloestradiol and 17β-oestradiol and the highest measured concentrations in effluents of Dutch sewage treatment plants (8 and 12 ng/l respectively). This is in agreement with field observations of effects in UK.

For 17α-oestradiol or oestrone a 100-200 times higher effective dose is reported. A content of about 150 ng of oestrone/l can be expected in ditches around agricultural land intensively fertilised with manure, and subsequent heavy rainfall.

The environmental risk to fish in the neighbourhood of emission sources seems rather high. It is not yet clear what the relative effectiveness is of the most common chemical form oestrone during shorter periods of exposure. Moreover, it is needed to validate the predicted concentrations in ditches by adequate measurements.

1 INTRODUCTION

1.1 Reason for this investigation

In recent years great interest arose in hormone-disturbing substances, viz. approximately fifty synthetic substances suspected of possibly disturbing the hormonal system of vertebrates. It concerns various organohalogens (dioxins, PCB's), pesticides and degradation products of detergents (nonyl-phenol).
These substances have been associated with observed sexual defects of animals in nature and with the decline of human sperm count during the past thirty years. The cause/consequence ratio, however, is highly controversial (Wright, 1996). The possible effect of these xeno-oestrogens cannot be assessed without considering a possible effect by exposure to natural hormones. The high human and animal density in the Netherlands causes relatively high emissions of natural hormones into water. After degradation and dilution the concentration of these substances in water is low. However, since a very low concentration of hormones may already form a risk, effective environmental concentrations cannot be excluded beforehand. For example, a concentration of 0.9 ng per litre of ethinyloestradiol in water may already influence the hormonal system in fish, by for instance inducing the formation of female proteins which are connected to the formation of eggs (vitellogen) in male fish (Purdom *et al.,* 1994).

1.2 Objective

To assess the risk for human and animal health caused by the emission of natural hormones, this study includes an estimation of the environmental emissions by the most frequently excreted hormones.
Since the structures of the various hormones and their metabolites only differ slightly, the emission of the entire group of related substances has been determined, wherever possible. In this report the following subjects are described:

1. the contribution of oestrogen emissions by humans;
2. the contribution of oestrogen emissions by domestic animals;
3. the degradation and distribution of oestrogens in the environment;
4. an estimation of the oestrogens concentrations in surface water and groundwater;
5. a risk assessment for fish in surface water and a risk assessment for humans via the consumption of drinking water.

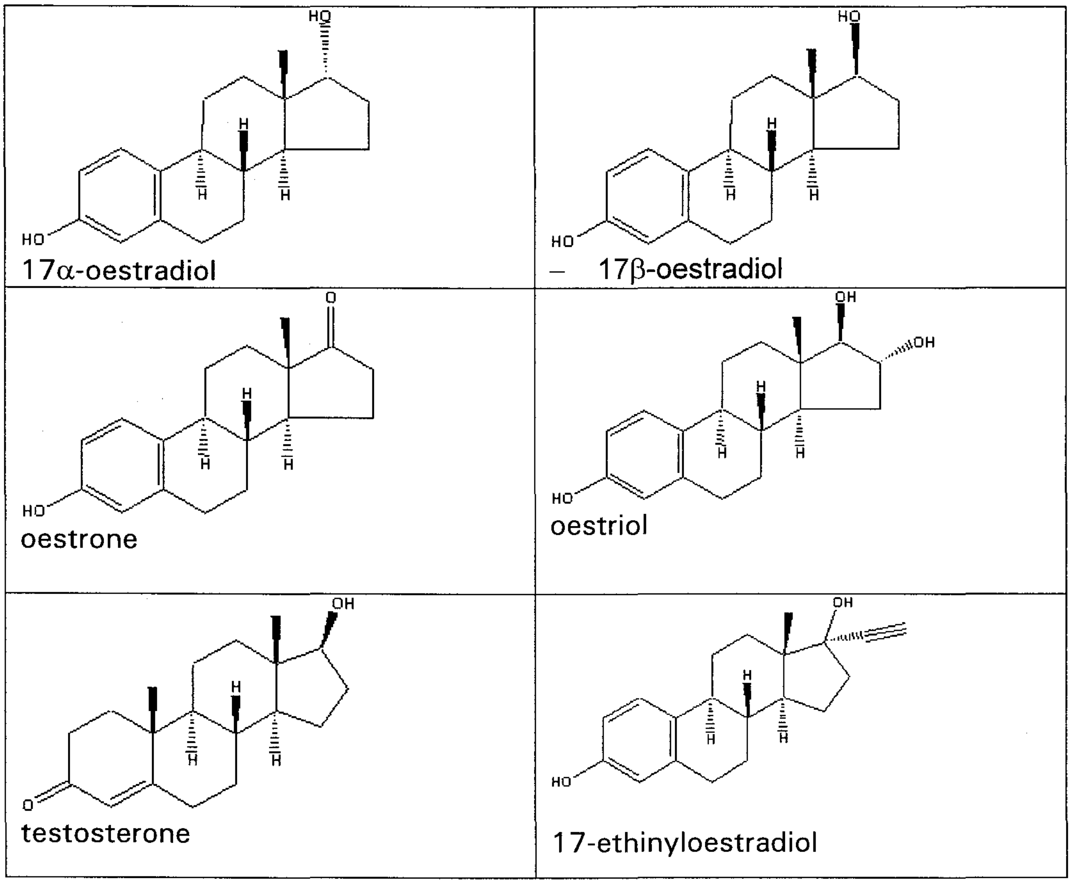
2. HORMONES

2.1 General

Oestrogens control the balance between sex-related processes in the body. Their chemical composition comprises a steroid skeleton and a number of substituted groups. The nature of these substitutes is responsible for the specific oestrogenic activity of these substances. The specific activity to target organs may change by the occurrence of various reversible hormonal conversions in the body.

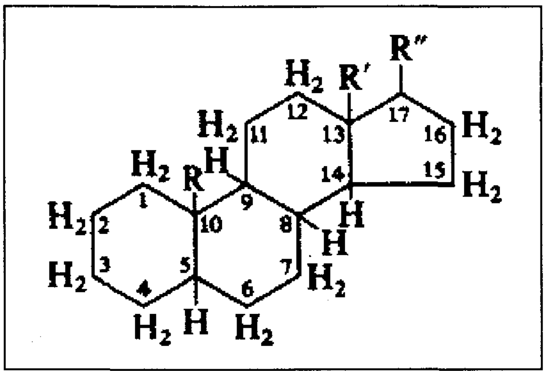
Biosynthesis of hormones takes place from cholesterol via the conversion by certain enzymes in sex glands, the adrenal gland and some other organs. Fig. 1 shows the most common active forms and groups (Stumpf *et al.*, 1996).

Figure 1: Most potent oestrogens



The most potent hormone in vertebrates is 17β-oestradiol. Oestrone and oestriol are excreted as well, however, their oestrogenic activity is lower. The oestrogenic activity of hormones is described as potency compared to DES (diethylstilbestrol). For oestradiol this potency is 1/4 of DES and for oestrone 1/10 of DES (Korenman, 1969 in Shore *et al.,* 1988).

Figure 2: The numbering of C-atoms in the steroid skeleton



2.2 Structure

Natural oestrogens are based on a steroid skeleton, consisting of 17 C-atoms in three C-6 rings and one C-5 ring. Figure 2 shows the numbering of C-atoms in the steroid skeleton. A diol bears two hydroxyl groups at position 3 and 17. At these positions a dion has two oxy groups. Progesterone has an acetyl group on position 17. By the addition of only one hydroxyl group on position 11 corticosterone is derived from progesterone. Moreover, cortisol has an additional hydroxy group on position 17.

The female hormone oestradiol differs from the male hormone testosterone only by the binding of a hydroxyl group instead of an oxy group at position 3. Oestriol only differs from oestradiol by an additional hydroxyl group at position 16. Testosterone and oestrone only differ by the exchange of the hydroxyl group and the oxy group at position 3 and 17.

Dihydrotestosterone, important for the expression of the male sexual characteristics, has only one additional hydrogen at position 5 compared to testosterone.

2.3 Degradation and excretion of oestrogens in the human body

Oestrogens (de)oxidise, hydrolyse and methylate in the liver before conjugating with glucuronic acid or sulphate, which increases the water solubility. Subsequently, they are excreted through urine and faeces. The excretion of mammals and humans consists of, i.a., 17β-oestradiol, 17α-oestradiol and oestrone. 17β-Oestradiol readily oxidises into oestrone, which can be converted into oestriol via 16α-hydroxy oestrone. Moreover, oestrogens can be excreted in various forms, like 17β-hydroxy oestrone, 16-ketoestrone or 16-epioestriol. 17β-oestradiol is mainly present as 17β-oestradiol-3-glucurone, oestrone-3-sulphate and oestrol; oestrol is mainly present as oestriol-16-glucurone (Ternes *et al.*, 1999).

3. EMISSIONS BY THE HUMAN POPULATION

3.1 Introduction

Excretion by females, besides 17α-oestradiol and oestrone, contains mainly 17β-oestradiol. These hormones are present in urine and faeces, in urine often in a conjugated form. The ratios found between conjugated and unconjugated substances vary strongly. Subsequently, the conjugated substances are hydrolysed in the sewage. Consequently, in this report only the excretion of the total amount of oestrogens is mentioned.

Since the excretion differs for each group, a distinction has been made for different ages and groups.

Table 1: Oestrogen emissions by the human population, per group (Aherne & Briggs, 1989)

Group	Excretion (μg/day)	Estimated average excretion (μg/day)
Children	1 - 40	20
Men	40 - 130	85
Women (not pregnant)	50 - 450	250
Women (after menopause)	5 - 50	28

According to Forth *et al.*, 1992 (in Stumpf *et al.*, 1996) the female excretion of oestrogens amounts to 25-100 μg/day, depending on the stage in the menstrual cycle.

3.2 Excretion by pregnant females

During pregnancy the hormone level strongly increases and consequently the excretion of oestrogen in urine and faeces. Halling-Sorensen *et al.* (1998) and Shore *et al.* (1993a) state that the emission by females at the end of a pregnancy may amount to 2,500 μg/day. According to Adlercreutz & Martin (1976) the excretion of oestrogens at the end of a pregnancy may amount up to 30,000 μg/day (Health Council, 1999). Turan (1996) mentions an emission of 28,000 μg/day.

Allowing for an exponentially increasing excretion from 450 to 30,000 μg/day within a 9-months period, the average excretion in this study is assumed to be 10,000 μg/day during the entire period.

The expected demographic Dutch populational constitution, according to data by CBS (1996) in 2000, is shown in Table 2.

Table 2: Constitution of the Dutch population in 2000 (CBS, 1996)

0-14 years:	16%
14-19 years:	8%
19-65 years:	63%
65 years and older:	13%

The number of births is estimated to be 200,000/yr. This figure is based on 150,000 pregnant females (or 0.9% of the total population) and a pregnancy period of 9 months.

Table 3 shows the average oestrogen excretion of the total Dutch population.

Table 3: Average oestrogen excretion by the Dutch population

Group	Percentage of Dutch population	Oestrogen excretion per day	Proportional contribution per group
Youth until 14:	16% *	20 µg/d	≈ 3.2 µg/d
Youth 14 – 19:	8% *	100 µg/d	≈ 8 µg/d
Male adult:	32% *	85 µg/d	≈ 27.2 µg/d
Female adult:	31% *	250 µg/d	≈ 77.5 µg/d
Female, pregnant:	0,9% *	10000 µg/d	≈ 90 µg/d
Elderly:	13% *	28 µg/d	≈ 3.6 µg/d
Average oestrogen excretion per person in the Netherlands			≈ 209.5 µg/d

These data show that the average oestrogen emission to the sewer in the Netherlands by 16 million inhabitants amounts to 3 kg/day. The calculation proves that the oestrogen excretion by pregnant women makes up half of the total amount. This indicates that the assumed average excretion over a period of 9 months is of great influence.

For comparison, Rathner & Sonnenborn (1979) have calculated an average excretion by the municipal population of 200 µg of hormones/inhabitant/day (equal to 3.2 kg/day for the Netherlands).

At a discharge of 200 l of water per person per day into wastewater treatment plants the concentration of natural oestrogens in raw sewage is calculated to be approximately 1 µg/l.

Johnson & Williams (1999) estimated the excretion for a number of cities in the UK. They calculated an oestradiol concentration of 14-24 ng/l in the influent. This concentration strongly differs from the concentration mentioned above, since Johnson & Williams assume a much lower value for the daily excretion by men and non-pregnant women.

3.3 Excretion by the use of contraceptives

3.3.1 Ethinyloestradiol in the contraceptive pill

The contraceptive pill contains several synthetic hormones, the most common and persistent one being ethinyloestradiol. Estimations by the Health Council (1999) state the daily dose of α-ethinyloestradiol by women using the contraceptive pill to be 35 μg.

Urine excretion contains approximately 35% of this ethinyloestradiol, whereas faeces contain approximately 30% (Reed *et al.*, 1972). Kulkarni & Goldzieher (1970) have estimated the amount of excreted ethinyloestradiol to range from 35 to 54%. 80% of the amount of α-ethinyloestradiol is excreted in an unaltered form (Turan, 1996), the form of the remaining 20% is unknown.

In order to determine the total amount of excreted ethinyloestradiol, the excretion per female is set at 70% of the total intake (0.7x35 g/day). Approximately 24.4% of the Dutch population consists of women between the age of 16 and 50 (CBS, 1996). According to CBS 45% of this group uses contraceptive pills. The number of Dutch women using contraceptive pills thus is 11% of 16 million inhabitants = 1.76 million.

Based on these figures, the total excretion of ethinyloestradiol in the Netherlands amounts to $0.7 \times 35 \times 1.76 \times 10^6 = 43$ g/day.

Based on 16 million inhabitants using 200 l water per day it can be assumed that the ethinyloestradiol concentration in the influent in water treatment plants amounts to 13.4 ng/l.

3.3.2 Synthetic progestogens in the contraceptive pill

The present contraceptive pill increasingly consists of a combination of ethinyloestradiol and a variable amount of synthetic progestogens. The progestogen dose is 10-20 times higher than the ethinyloestradiol concentration. There are approximately 10 different progestogens, including levonorgestrel, desogestrel, gestogens, norgestrel, norethisterone, lynoestranol, and medroxy progestenone. The progestogens are modifications of various groups of the steroid skeleton, such as 13-ethyl- and 17-α-pregnyl (C CH) substitution and various other groups.

Generally, progestogens are metabolised in the body and their behaviour in the environment is less persistent than that of ethinyloestradiol. Too little data are available for a good mass balance. Therefore the following is assumed for a worst-case scenario:
- a combination pill is used by half of the users (0.5 x 1.76 million users);
- the average dose of progestogen amounts to ten times the ethinyloestradiol dose (350 μg/day);

- excretion and behaviour in water treatment plants leads to a conversion into steroid metabolites for 20%.

Based on these assumptions, it can be concluded that the concentration of these progestogens in the effluent of water treatment plants is:

$0.5 \times 1.76 \times 10^6 \times 350\ \mu g/day \times 0.2 = 61.6 \times 10^6\ \mu g/day \approx 62\ g/day.$

This calculation indicates that the contribution of progestogens is low compared to a contribution of approximately 0.16 kg by natural oestrogens. This is described in Chapter 6.

However, no data are available on the further environmental behaviour of these metabolites nor on the vitellogenic induction in fish and the total environmental emission of steroids.

4. EMISSIONS BY DOMESTIC ANIMALS

4.1 Introduction

This chapter contains an assessment of the total oestrogen emission by domestic animals. To that end the oestrogen excretions by cattle, pigs, chicken and horses are estimated. Besides these animals there are: 1.4 million sheep, 40,000 of which being lambs and 150,000 goats, 86,000 of which being milk goats (CBS, 1999). The contribution of sheep and goats will be relatively low in comparison with the millions of cattle, pigs and chicken and the high oestrogen excretion of pregnant horses. Consequently the oestrogen excretions by sheep and goats have not been included in this investigation.

4.2 Oestrogen emission by cattle

The excretion of cows mainly consists of 17β-oestradiol, 17α-oestradiol and oestrone. Several investigations have been carried out into the concentrations of these hormones in faeces and urine. Strikingly, the measured concentrations strongly vary. In order to determine the oestrogen emission, the manure production of (milk) cows is set at 25 kg/day and the urine excretion is set at 60 l/day (pers. comm. IKC). Cattle is classified in: pregnant cows, milk cows and heifers. The numbers of animals in the Netherlands (CBS, 1999) are shown in Table 4.

Table 4: The numbers of cattle in the Netherlands

Group of cattle		Number in the Netherlands (CBS, 1999)
Pregnant cows (1)	(d+e)	777,730
Non-pregnant cows (2)	(a+b+c-d)	1,025,724
Heifers (3)	(f+g)	1,701,688
Heifers for milk production	CBS (f)	1,373,637
Heifers for meat production	CBS (g)	328,051
Milk cows and calves, total	CBS (a)	1,588,489
Store cattle / pasture cattle	CBS (b)	61,245
Older female store cattle	CBS (c)	10,278
Yearlings for milk production	CBS (d)	634,288
Yearlings for meat production	CBS (e)	143,442

(1) The number of pregnant cows in the Netherlands was calculated as being the sum of yearlings for milk cows and store cattle.

(2) The number of adult non-pregnant cows in the Netherlands was calculated as being the sum of milk cows and calves (total) plus store cattle / pasture cattle plus older female store cattle minus yearlings for milk production. Cows for meat production are not included since, in general, these are no adult animals.

(3) Heifers were calculated as being heifers for milk production and meat production.

4.2.1 Oestrogen emission by pregnant cows

The gestation period of cows is approximately 280 days. During this period their oestrogen excretion is higher. The period in between gestations for milk cows is approximately 185 days.

A study into the oestrogen excretion of 20 cows (Desaulniers *et al.*, 1989) shows the course of the total amount of emitted oestrogens. In the first 12 weeks the average excretion amounted to 34.6 µg/kg of manure. From week 14 on the oestrogen excretion increased. From week 20 till the end of the gestation period the average oestrogen excretion amounted to 947 µg/kg of manure. Based on these data the calculated oestrogen excretion during gestation amounted to 583 µg/kg of manure.

Remarkably the oestrogen concentration in manure of four pregnant musk-oxen was much higher. The same study mentions an oestrogen concentration for these animals of 231-365 µg/kg of manure after 20 weeks. This concentration increased at the end of the gestation period to 6,300-17,000 µg oestrogen/kg of manure.

An other study (Möstl *et al.*, 1984) into oestrogens in the manure of 39 pregnant cows and 21 non-pregnant cows showed an 17α-oestradiol concentration in manure during the first gestation weeks which was equal to that of non-pregnant cows. From week 14 on the concentration of α-oestradiol in manure increased and remained < 22 µg/kg of manure. Subsequently this concentration increased to well over 100 µg/kg at the end of the gestation period. The concentration of α-oestradiol in manure proved to be ten times higher than the concentrations of oestrone and 17β-oestradiol.

A third study (Hoffman *et al.*, 1997) into the oestrogen concentration in manure of pregnant cows showed somewhat lower values. In Table 5 the values, derived from a diagram, are given. The values are the sum of conjugated and unconjugated oestrogen.

Table 5: Total oestrogen concentration in cow manure during different gestation periods

Number of days Since fertilisation	Oestrogen excretion calculated as: Period (days)*manure excretion (kg/day)* oestrogen excretion (µg/kg)	Total oestrogen excretion per gestation period (µg)
0 – 100	100 * 25 * 0	0
100 – 150	50 * 25 * 25	31,250
150 – 230	80 * 25 * 50	100,000
230 – 250	20 * 25 * 110	55,000
250 – 270	20 * 25 * 350	175,000
Total oestrogen excretion in manure during gestation		361,250
Mean oestrogen excretion in manure during gestation (mg/day)		1.3

This investigation included the oestrogen concentrations in urine. A strong increase was found in the concentration of sulfoconjugated oestrone and sulfoconjugated 17α-oestradiol, starting at 150 days after fertilisation. In table 6 the total excretion in urine is summarised.

Table 6: Oestrogen excretion in urine by cows in different gestation periods *(Amounts are given in ng/mosmol. At a mean osmotic value of 1030 mosmol/l, 1 ng/mosmol corresponds to 1 mg/l. The average urine production of pregnant cows amounts to approximately 60 l/day)*

Number of days Since fertilisation	Oestrogen excretion calculated as: Period (days) * urine excretion (l/day) * oestrogen excretion (mg/l)	Total oestrogen excretion per gestation period (mg)
0 – 100	100 * 60 * 0	0
100-150	50 * 60 * 0,4	1,200
150-230	80 * 60 * 0,6	2,880
230-250	20 * 60 * 1,4	2,016
250-270	20 * 60 * 3	3,600
Total oestrogen excretion in urine during gestation		9,696
Mean oestrogen excretion in urine during gestation (mg/day)		36

This study was based on a gestation period of 270 days. The sum of the average conjugated and unconjugated oestrogen concentration in faeces and urine during the entire gestation period amounts to 37.3 mg/day.

Table 7 shows an overview of the results of the investigations mentioned.

Table 7: Mean measured values of total oestrogens in faeces and urine of pregnant cows

Source	Oestrogen concentration in manure	Oestrogens in manure (at a production of 25 kg manure/day)	Oestrogens in urine	Total oestrogen excretion per day
Desaulniers *et al.*, 1989	582 μg/kg manure	15 mg/day	-	> 15 mg/day
Hoffman *et al.*, 1997	54 μg/kg manure	1.3 mg/day	36 mg/day	≈ 37.3 mg / day
Möstl *et al.*, (1984)	22–110 μg/kg manure (average 70)	1.8 mg/day	-	> 1.8 mg / day

The measured oestrogen concentrations in faeces and urine vary strongly, possibly caused by the different measuring methods and to what extent these measurements include the conjugated oestrogen. Besides unconjugated oestrogens, Hoffman *et al.* (1997) also measured various forms of conjugated oestrogens, both in faeces and in urine. For that reason this study is based on the values calculated by Hoffman *et al.* (1997). An emission of 37 mg of oestrogens/day by pregnant cows means that the total oestrogen excretion of 777,730 pregnant cows in the Netherlands amounts to 29/day.

4.2.2 Oestrogen emission by non-pregnant cows

Desaulniers *et al.* (1989) determined the amount of hormones in faeces of four non-pregnant cows. The average oestrogen excretion in manure amounted to 34 µg/kg. Möstl *et al.* (1984) found a concentration of 26 µg/kg in manure. The calculation of oestrogen excretion by non-pregnant cows is based on 30 µg of oestrogens/kg of manure at a production of 25 kg manure/day.
Assuming an oestrogen excretion of 33% in urine (Erb *et al.*, 1977), the daily excretion of non-pregnant cows will amount to 750 µg/day in manure and 369 µg/day in urine. The total daily oestrogen excretion amounts to 1.1 mg. Based on 1,025,724 non-pregnant cows (see table 4) the total oestrogen excretion by all non-pregnant cows will amount to approximately 1.1 kg/day.
For this category no data are available on oestrogens in urine. Should the oestrogen concentration in urine from pregnant cows not be 33% of the total excretion (according to Erb *et al.*, 1977), but 95% (like in the measurements by Hoffman *et al.*, 1977), the total excretion will be 15 kg, rather than 1.1 kg.

Oestrogen emission by heifers

A study into the oestrogen concentrations in urine of 19 weeks old bull calves showed the following concentrations (Terplan *et al.*, 1990):

17β-oestradiol:	0.5	µg/l
α-oestradiol:	16	µg/l
testosterone:	21	µg/l

In faeces the following values were measured:

17β-oestradiol:	2	µg/kg
α-oestradiol:	12	µg/kg
testosterone:	7	µg/kg

Assuming a daily production by calves of 12.5 kg of manure and 25 l of urine, the total daily oestrogen emission of 1,701,688 calves (see table 4) amounts to approximately 1 kg and the testosterone emission amounts to approximately 1 kg. The total steroid hormone emission by heifers amounts to approximately 2 kg/day.

4.3 Oestrogen emission by pigs

Several investigations have been carried out into the oestrogen concentration in urine of pregnant pigs. The objectives of these investigations were pregnancy diagnosis of pigs, and whether progeny size could be determined based on the oestrogen concentration in urine. Oestrogen measurements in pig manure are unknown. Therefore, in order to determine the total oestrogen emission by pigs and breeding sows, it is assumed that the oestrogen emissions in manure and urine are equal.

The average amount of slurry (faeces and urine) produced by store sows is 3.5 kg/day, roughly divided into 1.3 kg of manure and 2.2 l of urine per day. Breeding pigs without piglets produce 6 kg of slurry on an average (1.5 kg of manure and 4.5 l of urine). Breeding pigs with piglets produce approximately 16 kg/day of slurry (4 kg of manure and 12 l of urine) (pers. comm. Imag-DLO Institute for environmental and agricultural techniques, Wageningen).

4.3.1 Oestrogen emission by breeding sows

It is assumed that breeding sows are pregnant around the year. The gestation period is approximately 115 days.

During two gestation phases the level of oestrogen concentration is increased, which is during the phase 22-31 days after fertilisation and during 80 days after fertilisation until birth. Urine contains conjugated oestrogens, particularly as oestrone sulphate. The concentrations of other oestrogens during gestation are insignificant (Velle, 1960). In the phase 20-30 days after fertilisation the average oestrogen concentration amounts to approximately 500 μg/l (Atkinson & Williamson, 1986).

Another study into oestrogen concentrations in urine proved that the measured concentrations strongly depends on the amount of HCL used for extraction. Using 6 vol% of HCL, the mean oestrogen value found for pregnant sows was 46 μg/l and 1.6 μg/l for non-pregnant sows. Using 15 vol% of HCL the values found were approximately 4 times higher (161 μg/l for pregnant sows and 7 μg/l for non-pregnant sows). With the latter method also oestradiol was measured, calculated as 17β-oestradiol having a value of 5.6 μg/l for pregnant sows and 5.8 μg/l for non-pregnant sows (Velle, 1960).

In order to calculate the oestrogen excretion by breeding sows, it is assumed that the excretion during day 21 till day 32 amounts to 161 μg/l. During the second peak phase, from day 80 till day 115 (35 days of the gestation period) the oestrogen concentration is 10-20 times higher than during the first peak phase (Edgerton *et al.*, 1971; Reaside, 1963).

During the remaining days (70) the oestrogen excretion is somewhat higher than before the gestation period (for this calculation assumed to be 20 μg/l). Based on a gestation period of 115 days and 4.5 l of urine per day, the average oestrogen excretion in urine is 3.4 mg/day during the gestation period. Assuming the excretion in manure to be equally high, this results in a total oestrogen emission of 6.8 mg/day. Based on 1,553,977 breeding sows in the Netherlands (CBS, 1999), this gives a total oestrogen contribution by breeding sows of 10.6 kg/day. In table 8 the emission data for breeding sows are given.

Table 8: Oestrogen excretion by pregnant breeding sows

Breeding sow	Excretion (μg/l)	Excreted amount of urine/day	Excreted amount of oestrogens/period in gestation
10 days	161	4.5 litre	7.2 mg
70 days	20	4.5 litre	6.3 mg
35 days	*2415	4.5 litre	380.4 mg
Oestrogen excretion in urine during gestation (115 days):			393.9 mg
Oestrogen excretion in urine/day:			3.4 mg
Oestrogen excretion in manure/day (assumption):			3.4 mg
Oestrogen excretion manure + urine/day during gestation:			6.8 mg
Total oestrogen excretion breeding sows (1.553.977):			10.6 kg/day
Mean oestrogen excretion per breeding sow in μg/kg of slurry (manure + urine)			1.13 mg/kg

4.3.2 Oestrogen emission by store sows

Velle (1960) determined the oestrogen emission of non-pregnant pigs to be 7.2 μg/l of urine. The average urine excretion by store sows amounts to 2.2 l/day. Also in this case it is assumed that the excreted amount of oestrogen in manure is equal to that in urine. This results in an oestrogen excretion per store pig of 31.7 μg/day. Based on 6,774,084 store sows in the Netherlands (CBS, 1999), this gives an oestrogen contribution by store sows of 0.2 kg/day.

4.3.3 Conclusion oestrogen emission by cattle and pigs

The oestrogen contribution by breeding sows is considerably higher (10.6 kg/day) than the contribution by store sows (0.2 kg/day). The total oestrogen emission by pigs in the Netherlands amounts to approximately 10.8 kg/day. It should be noted that these data are based on investigations carried out in 1960. Present techniques make use of GC-MS after enrichment of the sample through C18-columns, whereas the older method is based on hydrolysis with boiling HCL and extraction with n-hexane, followed by gas chromatography with a recovery of only 50%.

4.4 Oestrogen emission by chicken

Like other types of manure, chicken manure is stored some time before applying it as fertiliser on the land. The oestrogen concentration in chicken manure (laying hens) decreased by 34% after having been stored for 30 hrs at 25°C in a wet form. The oestrogen concentration in dry manure remained constant after 24 hrs of storage at 100°C. Another study into oestrogens showed that the oestrogen concentration in chicken manure has not decreased after 6 months of storage (Shore & Shemesh, 1992 in Shore *et al.*, 1993a).

Shore *et al.* (1993b) determined the amounts of testosterone and oestrogens in dry chicken manure. The dry content of this chicken manure amounts to 530 g/kg of manure (IKC, 1993). Chicken produce up to 25 kg/year, while the production of chicks amounts to 10 kg. Table 9 shows the oestrogen and testosterone concentrations.

Table 9: Hormone concentrations in chicken manure (Shore *et al.*, 1993b)

Hormone excretion in manure	Testosterone (µg/kg d.s.)	Oestrogens (µg/kg d.s.)	Testosterone (µg/kg manure)	Oestrogens (µg/kg manure)	Testosterone (µg/ year)	Oestrogens (µg/year)
Male chicks	133	14	70	7	700	70
Female chicks	133	65	70	34	700	340
Laying hens	254	533	135	282	3375	7050
Cocks	670	93	355	49	8875	1225

An investigation into the oestrogen concentration in chicken manure by Mather & Common (1969), quoted by Shore *et al.* (1988) stated that the excreted urine of non-laying hens contains 0.27 µg of oestrone and 1.4 µg of oestradiol. This emission by urine is negligible compared to the emission by manure.

The total amount of laying hens in the Netherlands is 42.5 million (CBS, 1999). The daily hormone contribution is 42.5 million x 7.05 mg / 365 = 0.8 kg of oestrogens and 0.4 kg of testosterone. This results in a total amount of steroid hormones of 1.2 kg/day.

In the Netherlands there are 53 million broilers. Assuming half of them being male and half of them being female, the total oestrogen contribution of broilers amounts to 29 g/day and the testosterone contribution amounts to 100 g/day. The contribution by cocks is negligible because of the limited number of cocks.

4.5 Oestrogen emission by horses

Turan (1996) has stated that the oestrogens excretion by pregnant mares is 100 mg/day. This seems to be high compared to the excretion by cows whose size is equal to that of horses (max 37 mg/day). Probably this high emission concerns only the last part of the gestation period. Assuming the emission would be 100 mg/day, this results in an emission by pregnant mares of 2.1 kg of oestrogen/day at a number of foal births of 23,388 and a gestation period of 11 months.

4.6 Comparison of oestrogen emissions by various domestic animals

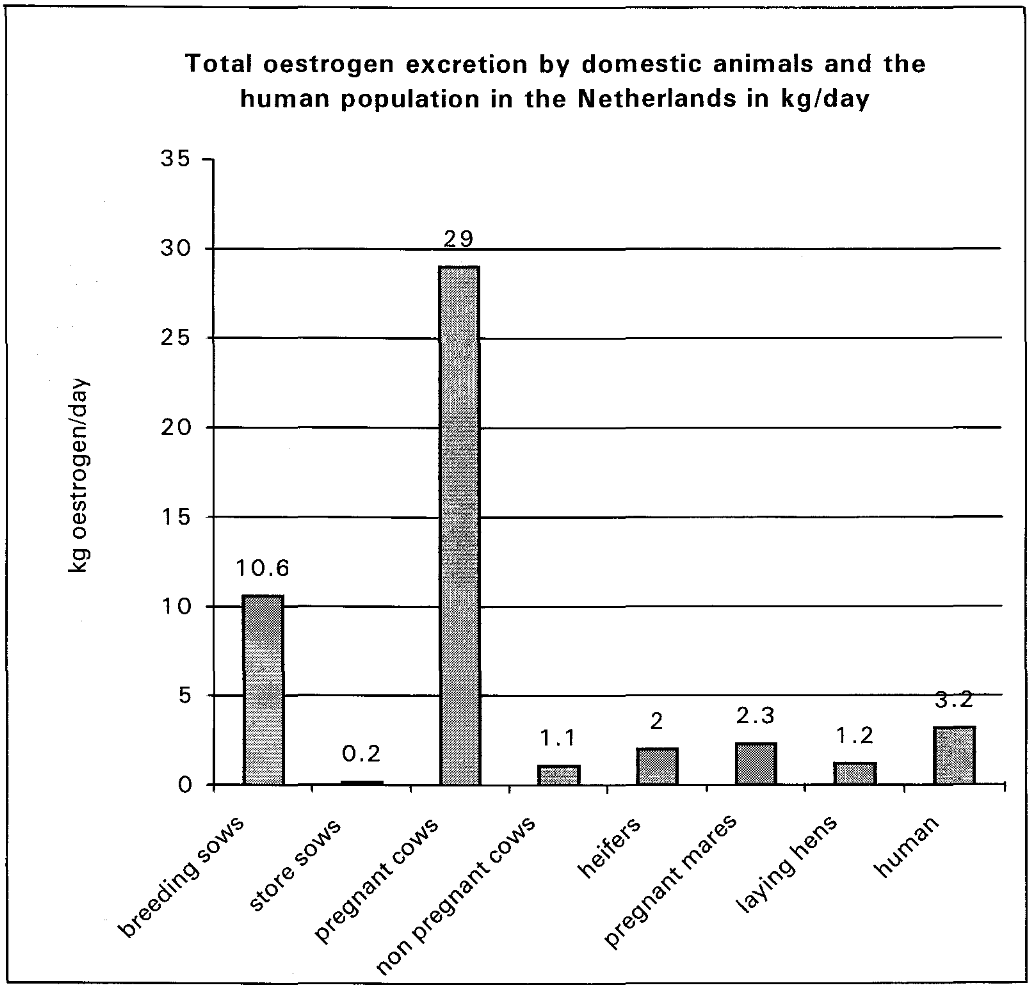
Table 10 and Fig. 3 show the relative contribution of the various domestic animals to the environmental emissions.

Table 10: Oestrogen excretion by humans and domestic animals in the Netherlands (kg/day)

GROUP	Oestrogen excretion in kg/day
Breeding sows	10.6
Store sows	0.2
Pregnant cows	29
Non-pregnant cows	1.1 (15)*
Heifers	2
Laying hens	1.2
Mares	2.1
Total, livestock	46.2 (60)*
Total, humans	3.2

*depending on an assumed contribution in urine

Figure 3: Oestrogen excretion by humans and domestic animals in the Netherlands



5. OESTROGEN LEACHING TO SURFACE WATER

5.1 Degree of oestrogen leaching

Nichols *et al.* (1998) have investigated the leaching of 17β-oestradiol from chicken manure on pasture in Israel. During the experiment 5,000 kg of chicken manure per ha was applied to the pasture at a concentration of 903.9 μg of 17β-oestradiol/kg dry content. The liquid content of this manure was 24.5%. Assuming the oestrogen would dissolve completely, it can be calculated that the concentration in water is 6.8 μg/l at a rainfall of 50 mm during 1 hr. The average measured concentration in leached water on ditch sides, after applying 50 mm of rain for one hour, amounted to 3.5 μg/l or 51% of the applied oestrogens.

A degree of leaching of 51% is comparable to the results of another experiment in which radioactively labelled steroids were applied to heavy soil, which was subsequently irrigated for a few months. The experiment showed that 56% of the oestradiol and 59% of the oestrone had adhered to the soil and could only be extracted by organic solvents (Shore *et al.*, 1993a).

The experiments by Nichols *et al.* (1998) showed that leaching strongly depends on the length of the grass field. Leaching ranged between 51% for an average length of 3 meter and 3% for an average length of 21 meter. This may be caused by infiltration of 17β-oestradiol to the soil. Conservative conclusions from this study may be that, for land having polder ditches at a distance of 100 meter, 3% of the applied 17β-oestradiol enters the surface water through rainwater.

M. Boerjan (DLO Lelystad) is preparing an investigation in order to measure the degree of leaching under Dutch circumstances. The results of the investigation by Nichols *et al.* (1998) differ from the Dutch situation since the slope of the surface amounted to 3% and the surface had been rained upon very intensively during a short period (the amount of rain falling in one month under normal circumstances was applied within one hour in this experiment). Moreover, unlike in Israel, the soil in the Netherlands is often saturated. If the degree of leaching were to be measured over a period of several months, allowances should be made for the conversion into oestrone and the presence of oestrone, whether or not conjugated, in the applied fertiliser.

5.2 Calculation of the degree of oestrogen leaching to surface water

Two methods have been applied for an estimation of the oestrogen concentration in surface water. The first method is based on a small-scale area with a polder ditch around an area, which is intensively fertilised with manure. The second method is based on a total Rhine or Meuse catchment area and the livestock in that area.

Calculation of concentrations in polder ditches

The calculation of the oestrogen concentration in drain water is based on both the calculated oestrogen concentration in slurry of breeding sows and pregnant cows, and on a highest permitted rate of manure application. The calculation is based on 1 ha of land with a polder ditch in which the total amount of rainwater is collected.

Around the year breeding sows are stabled. The slurry is collected and can be spread in certain periods (February through September). In practice spreading takes place in early spring (February through April), before crops are growing.

Investigations into chicken manure proved that the oestrogen and testosterone concentrations are almost unchanged after six months of storage (Shore *et al.*, 1992 in Shore *et al.*, 1993a). Therefore this calculation also proceeds from an unchanged oestrogen concentration in slurry of breeding sows and cows. In 2000 the highest permitted amount of fertiliser spread on pasture is 85 kg of phosphate/hectare/year (pers. comm. Bureau Hefflingen). Slurry produced by breeding sows contains 3.6 kg of phosphate per 1000 kg of manure. This implies that the highest permitted amount of spread fertiliser on one ha of land is 23,600 kg. Slurry of breeding sows contains approximately 1.13 mg of oestrogen/kg of manure (see table 8). Hence, the amount of oestrogens applied to the land is 1.13 x 23,600 mg per hectare. Assuming an average rainfall of 60 mm/month, the oestrogen concentration in rainwater amounts to 44 μg/l if the highest permitted amount of fertiliser is spread within one month and the degree of leaching is spread over one month.

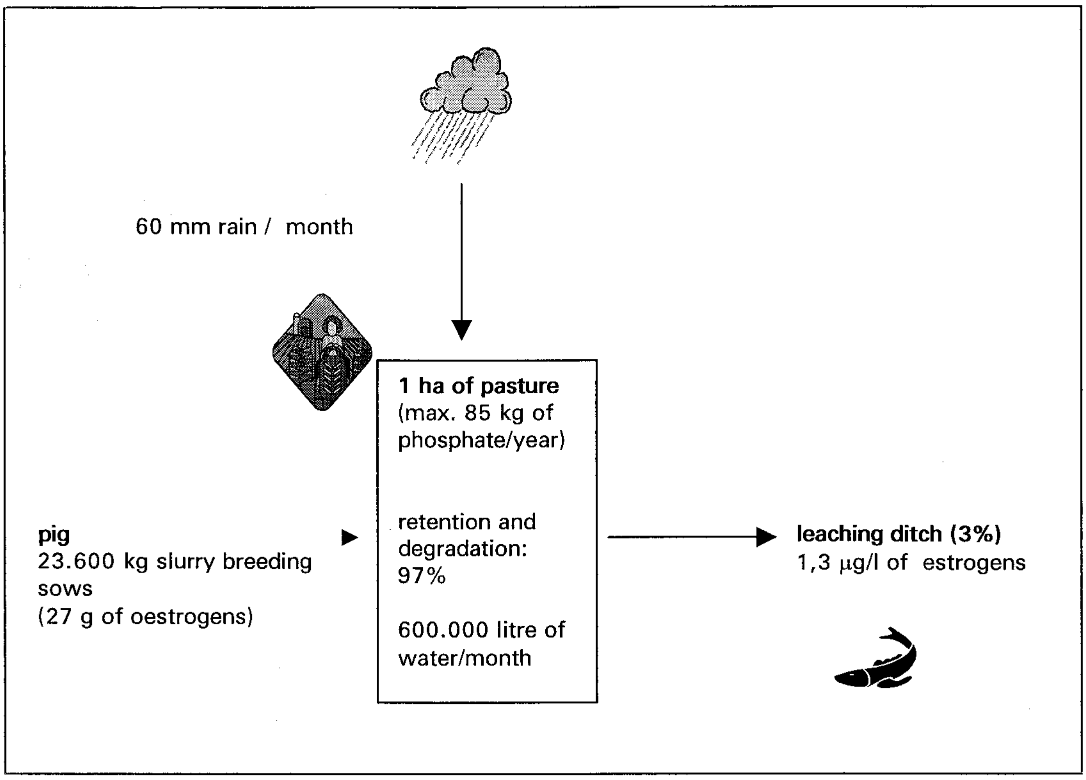
Based on a degree of leaching of approximately 3% of oestradiol, oestrone and sulfo-conjugated compounds, the concentration in a ditch is 1.3 μg/l.
It should be noted that:
- 3% leaching was not measured under Dutch circumstances in the period February-April;
- on a larger scale the period of spreading and hence the period of leaching may be 3 months, implying that calculations carried out with 3 times less the amount of manure and 3 times more the amount of water. This results in an oestrogen concentration of 0.14 μg/l.

Comparable calculations can be made for slurry from pregnant cows using the following basic assumptions:
- 1000 kg of slurry from pregnant cows contains 1.8 kg of phosphate (IKC, 1993);
- highest permitted rate of spreading fertiliser of 85 kg phosphate/ hectare in 2000; this amount is spread in one month (= 47,000 kg of slurry/-hectare);
- the oestrogen concentration in slurry is at least 0.4 mg/kg (37 mg in 60 l of urine and 25 kg of manure);
- this causes an oestrogen application on the land of 19 g;
- 60 mm of rainfall in one month (600 m^3/ha);

- leaching amounts to 3%, resulting in a concentration in surface water of 0.9 µg/l at a rate of spreading and leaching of 1 month, and 0.1 µg/l at a rate of three months.

There is hardly any difference between calculations for manure of breeding sows and milk cows. The result of the calculations for the 3-months scenario would range between 150 and 100 ng/l.

Figure 4: Oestrogen leaching caused by spreading slurry from breeding sows to ditches



5.3 Leaching per catchment area

For the catchment area of the rivers Meuse and Rhine the oestrogen concentration has been estimated. This estimation was based on the number of domestic animals in the provinces of the catchment areas. A classification has been made according to breeding sows, store sows, milk cows, heifers, laying hens and breeding cows.

The following aspect should be noted with regard to the calculation:
- horses have been omitted because of both unavailability of information on the number of horses within the catchment area and because of the absence of representative measurements on the oestrogen emissions;
- data are absent for the river Rhine with regard to domestic animals in France and Luxembourg and two German provinces;
- data on laying hens in Germany are based on the total amount of poultry. No differentiation has been made between laying hens and other poultry. It has been assumed that this just concerns laying hens;

- the oestrogen excretion by breeding cows has been equated with the excretion by pregnant cows;
- the basic assumption is that 3% of the emitted oestrogen from manure and urine eventually enters the surface water;
- in a daily average scenario the mean emission per day (after 97% retention and degradation) is divided across the mean river flow;
- in a three months′ scenario the manure is assumed to be spread for a period of only three months of the year, which indicates a quadruple concentration compared to the daily average scenario.

Details of this calculation are given in the spreadsheets in Appendices 1 and 2.

The calculation shows a concentration of 19 ng/l in the river Rhine and 35 ng/l for the Meuse at a daily average scenario. For a three months' scenario this means 76 and 140 ng/l, respectively.

In the river Rhine the emission has been caused by milk cows for 90%. If the manure were to be spread regularly within 6 months, the concentration might become approximately 40 ng/l.

In the Meuse the emission by milk cows amounts to approximately 70%. If this manure were to be spread regularly within 6 months and the pig manure only for 3 months, the concentration might become 50-90 ng/l.

6. MEASURED HORMONE CONCENTRATIONS IN WATER

6.1 General

During the past years some investigations have been carried out into the oestrogen effects on fish and into the oestrogen concentration in wastewater, surface water and drinking water.

The various studies show that male fish in the effluent of purification plants produce vitellogen. In the Southern UK the effect of effluent on a wild population of flounders (*Platichtys flesus*) has been investigated. This study proved that of the total number of fish exposed to effluent of a main purification plant, 60% of the male fish produced vitellogen, whereas in a sparsely populated area this amounted to only 20%. Moreover, up to 53% of the fish in the former location showed testis defects, compared to 0% in the latter location. Also the liver of the former population was enlarged which is an indication of an increased intoxication activity (Lye *et al.*, 1997). This study shows the presence of several hormone disturbing substances in the effluent. Its is unknown to what extent this is caused by natural oestrogens.

In the effluent of municipal wastewater sometimes a hydrolysis step has been performed prior to oestrogen measurements, in order to convert conjugated oestrogens to unconjugated oestrogens for a total-oestrogen measurement. In contrast with oestrogen in faeces and urine (see paragraph 4.2.2), oestrogens proved to be present in the effluent mainly in an unconjugated form. The purification step already hydrolyses the conjugated oestrogens (Wegener *et al.*, 1999). Hence, performing a hydrolysis step prior to measurements is of no importance to the results of the oestrogen measurements in the effluent.

6.2 Concentrations in wastewater

Ethinyloestradiol

Several investigations have been carried out into oestrogens in unpurified wastewater (Influent) (Belfoid *et al.*, 1999, Wegener *et al.*, 1999, Kalbfus 1997, Schweinfurth *et al.* 1996, Stumpf *et al*, 1996, Kalbfus 1995; Shore *et al.*, 1993a, Aherne & Briggs, 1989; Rathner & Sonneborn *et al*, 1979; Wilson, 1978; Freudental *et al,* 1975).

The highest found ethinyloestradiol concentration in purified wastewater (effluent) in the Netherlands is 8 ng/l (Belfoid *et al.*, 1999). The highest found concentration in effluent amounted to 62 ng/l (Stumpf *et al.*, 1996).
The median concentration in this investigation was 17 ng/l. The values found in the other investigations were much lower, ranging from below the detection limit of 0.5 ng/l to max. 8 ng/l. In 13 water purification plants in Germany the detected ethinyloestradiol concentrations were not above the detection limit of 1 ng/l (Wegener *et al.*, 1999).

The maximum values found are of the same order of magnitude as the estimated concentration in the influent (13.4 ng/l, § 6.3). This confirms the poor degradation of ethinyloestradiol.

Natural hormones

Belfroid *et al.* (1999) measured the oestrogen concentration in the effluent of a number of Dutch purification plants. The highest observed concentrations are 47 ng of oestrone/l, 5 ng of 17α-oestradiol/l and 12 ng of 17β-oestradiol/l. This gives a total amount of oestrogens of max. 64 ng/l. During the period 1994-1996 MAF and Brunel University (James *et al.*, 1997) found oestrone concentrations in the effluent ranging from 1.4-76 ng/l and 17β-oestradiol concentrations ranging from 2.7-48 ng/l.

Desbrow *et al.* (1998), cited by Johnson & Williams (1999), found 17β-oestradiol concentrations in various effluents ranging from 2.7-12 ng/l.

Wegener *et al.* (1999) detected max 80 ng of oestrone/l and max 42 ng of oestriol/l in effluent. In 8 out of 13 effluent samples oestrone concentrations were not present higher than 1 ng/l (detection limit), and in 11 effluent samples oestriol concentrations were not present higher than 1 ng/l (detection limit).

After extremely dry periods the oestrogen concentration found in Israel in municipal influent ranged from 48 to 141 ng/l and in rural areas from 135 to 350 ng/l. After treatment with activated sludge oestrogen concentrations had decreased to 24-48 ng/l. The testosterone concentration found in influent of an municipal purification plant ranged from 208 to 320 ng/l, while after purification with activated sludge the concentration was lower by a factor of 2-4 (Shore *et al.*, 1993a).

Tables 11a and 11b summarise the concentrations found in wastewater before and after purification.

Table 11a: Oestrogen concentrations in influent of municipal wastewater

Substance	Ng/l	Remark	Location	Source
Oestrogens (total)	350	max. measured values, after extreme drought	Israel, rural wastewater (influent)	Shore *et al.*, 1993a
Oestrogens (total)	141	max. measured values, after extreme drought	Israel, municipal wastewater (influent)	Shore *et al.*, 1993a

Table 11b: Oestrogen concentrations in effluent of municipal wastewater

Substance	ng/l	Remark	Location	Source
Ethinyloestradiol	62	max. measured value	Germany	Stumpf *et al.* 1996
Ethinyloestradiol	8	max. in the Netherlands	Netherlands	Belfroid *et al.* 1999
Oestrone	1.4-76	max. measured values	United Kingdom	MAF en Brunel University (1996)
Oestrone	80	max. measured value	Germany	Wegener *et al.*, 1999
Oestrone	47	max. in the Netherlands	Netherlands	Belfroid *et al.* 1999
17β-oestradiol	2.7-48	max. measured values under normal circumstances	United Kingdom	MAF en Brunel University (1994-1996)
17β-oestradiol	2.7-12	min. en max. measured values	United Kingdom	Desbrow *et al.* 1998 in Johnson & Williams (1999)
17α-oestradiol	5	maximum value	Netherlands	Belfroid *et al.* 1999
17β-oestradiol	12	Maximum value	Netherlands	Belfroid *et al.* 1999
Oestriol	42	Maximum value	Germany	Wegener *et al.*, 1999

6.3 Purification efficacy

Several investigations have been carried out into the oestrogen degradation in waster water purification plants. It is not always clear to what extent the oestrogen decrease has been the result of either adsorption or degradation. Besides, not every study has considered the formation of the various metabolites.

6.3.1 Activated sludge

The most recent study into the oestrogen degradation in water purification plants has been performed in Germany (Ternes *et al.*, 1999). In this study the effects of activated sludge treatment on the oestrogen concentrations in water have been measured, performed at both the purification plant and in various batch experiments.

Batch experiments 17β-oestradiol and 16α-hydroxy oestrone

The 17β-oestradiol concentration immediately decreased after contact with sludge. After 1-3 hrs the 17β-oestradiol concentration had decreased by 95%. Initially this was oxidised into oestrone. After 5 hrs 17β-oestradiol and oestrone concentrations had been reduced to concentrations, which were below the detection limit. Other metabolites, such as 16α-hydroxy oestrone, have not been detected.

Batch experiments with 17β-oestradiol glucuronides

Starting from a 17β-oestradiol glucuronides concentration of 1.65 μg/l, 70% of the concentration had been oxidised into oestrone after 15 minutes. After 28 hrs only 3% was recovered as 17β-oestradiol. Starting from a concentration of 1.6 ng 17β-oestradiol glucuronides/l, only 50% had been converted into oestrone after 5 minutes, while neither 17β-oestradiol nor oestrone was detected after 24 hrs.

Batch experiments with 17α-ethinyloestradiol

Experiments with 17α-ethinyloestradiol did not show a decrease in concentration after treatment with activated sludge for 48 hrs. This is in agreement with other studies, which proves that 17α-ethinyloestradiol is more persistent than natural oestrogen.

Glucuronidase activity

Since activated sludge contains many micro organisms, with their enzymes glucuronidase and sulfatase, it can be assumed that these bacteria dissociate the glucuronide and sulphate groups from the oestrogens (Ternes *et al.*, 1999). This hypothesis is confirmed by the fact that in effluent mainly unconjugated oestrogens are found such as 17β-oestradiol, oestrone, 16α-hydroxy oestrone and 17α-ethinyloestradiol.

A study lasting 6.7 hrs into the glucuronidase activity showed that approximately 19% of the 4-methylumbelliferyl-β-D-glucuronide (MUG) had been dissociated This proves that micro organisms in activated sludge are able to dissociate glucuronides. In table 12 the results of batch experiments are summarised.

Table 12: Removal efficiency in batch experiments with activated sludge

Oestrogen	residence time of activated sludge	Removal efficiency	Details
17β-oestradiol	5 hrs	100%	
Oestrone	5 hrs	100%	
17β-oestradiol glucuronides (1,65μg/l)	15 min.	70%	Converted into oestrone
17β-oestradiol glucuronides (1,6 ng/l)	24 hrs	97%	3% present as 17β-oestradiol
Glucuronidase-activity with MUG	6.7 hrs	19%	19% dissociated

(Ternes *et al.*, 1999)

Also Wegener *et al.* (1999) have performed batch experiments, during which doses of 1-100 μg/l have been added to the effluent. This clearly shows that 17β-oestradiol, like glucuronides, degrade with a half life of one day and that oestrone is formed as intermediate. Half life for oestrone is approximately 4 days. It turned out that ethinyloestradiol and mestranol in a period of 10-14 days had hardly been degraded.

Conclusion of batch experiments

It is assumed that the relatively high oestrone concentration in effluents and rivers is caused by the relatively higher oestrone stability, the dissociation of glucuronide conjugates of oestrone and 17β-oestradiol, and the oxidation of 17β-oestradiol into oestrone. In the batch experiments 17β-oestradiol and its metabolite 16α-hydroxy oestrone were readily degraded. Prior to oxidation and dimethylation the glucurone conjugates are dissociated by bacteria.

Measurements at purification plants

Recently the removal efficiency of oestrogen has been measured in various types of purification plants in Brazil (Rio de Janeiro) and Germany (Frankfurt) (Ternes *et al.*, 1999). The results are summarised in table 13.

Table 13: Oestrogen removal efficiency in purification plants

Oestrogen	Influent concentration (ng/l)	Method	Removal efficiency (6 days)
17β-oestradiol (Brazil)	21	aeration tank	99.9%
17β-oestradiol (Germany)	15	activated sludge	64%
Oestrone (Brazil)	40	aeration tank	83%
Oestrone (Germany)	27	activated sludge	0%
16α-hydroxy oestrone (Germany)	Unknown	activated sludge	68%
17-ethinyloestradiol (Brazil)	Unknown	biological filter	64%
17-ethinyloestradiol (Brazil)	Unknown	activated sludge	78%
17α-ethinyloestradiol (Germany)	Unknown	activated sludge	0%

(Ternes *et al.*, 1999)

The differences in removal efficiencies between sewage purification plants in Brazil and Germany are possibly caused by the microbiological activities under different weather conditions. During the measurements the temperature in Brazil was 20°C, whereas in Germany this was 2°C.

Laboratory experiments

In a laboratory experiment with subcultures (Tabak *et al.*, 1970) natural oestrogens were completely degraded within 3 to 4 weeks, while ethinyloestradiol was degraded for 95% within 4 weeks. Table 14 shows the summarised results.

Table 14: Removal efficiency of activated sludge in laboratory experiments (%)

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

Tabak *et al.*, (1970)

Another experiment showed that 50% of the oestrogen had mineralised after 14 days (Schweinfurt, 1996 in James *et al.*, 1997). A similar test with ethinyloestradiol showed that only 3% of the oestrogens had mineralised after 4 weeks, whereas another study demonstrated no reduction at all of the ethinyloestradiol concentration in activated sludge after 5 days (Norpoth, 1973).

Vader *et al.*, (2000) studied the degradation of ethinyl estradiol (EE) in different types of activated sludge. In normal activated sludge from a municipal plant with a minimal nitrifying capacity no degradation was observed. In a special cultured sludge with relatively high nitrifying capacity primary degradation to hydroxylated derivatives was observed. It was explained as a co-metabolic reaction of the enzyme mono-oxygenase. The rate of hydroxylation can be correlated with the rate of ammonia oxidation in order to extrapolate this phenomenon to the situation in a normal sewage treatment plant with nitrification.
The ratio of EE hydroxylation to ammonia oxidation that was found is 1:50.000. In a normal municipal plant the average amount of EE per population equivalent is 2.7 μg/d (43 g/d divided by 16 million). The average load of ammonia per inhabitant is about 13.5 g/d. So, the ratio of EE to ammonia is 1:5000.000. This would mean that the normal capacity of a treatment plant with complete nitrification would be 100 times in access of the required capacity for the hydroxylation of ethinyl estradiol.

6.3.2 Sand filtration

Shore *et al.* (1993) have investigated wastewater in Israel. These investigations proved that oestrogen concentrations had been reduced by 64-90% after three months of sand filtration. However, the amount of absorbed oestrogens other than indicated is not mentioned. The result of degradation may thus be lower. Besides, in the sampling method only the oestrone and - oestradiol concentrations have been investigated. These measurements prove that the oestrogen concentration in agricultural wastewater is approximately three times higher than the concentration in municipal wastewater. This might be caused by the oestrogen contribution from domestic animals. Table 15 shows the summarised results of sand filtration experiments.

Table 15: Oestrogen degradation after sand filtration (Shore *et al.*, 1993)

	Oestrone + β-oestradiol concentration (ng/l)
Wastewater, agricultural	135 – 350
Wastewater, Tel Aviv	54 – 135
Purified water	6,5 – 50
After sand filtration (3 months)	2.7

6.3.3 Purification with other methods

Experiments with oestradiol and ethinyloestradiol demonstrated that these components react well with chlorine and ozone and that they completely disappear after treatment with powdered activated carbon (PAC). Application of these techniques for the drinking water production resulted in a removal of more than 95%. Coagulation and filtration were not effective, although it should be noted that this experiment was carried out with water containing a low concentration of organic substance (Hutchinson *et al.*, 1997).

6.4 Concentrations in surface water

Ethinyloestradiol

In rivers in the south-east of the UK an ethinyloestradiol concentration has been found of 2-15 ng/l (Aherne & Briggs, 1989), being the highest concentration found in surface water. Freudental *et al.* (1975) had found an ethinyloestradiol concentration in Dutch waters ranging from 0.06 to 0.3 ng/l.

Other investigations in Germany and the United Kingdom mention much lower concentrations ranging from some tenths of nanogrammes per litre to some nanogrammes per litre (Wegener *et al.*, 1999; Stumpf *et al.*, 1996; Schweinfurth *et al.*, 1996; Kalbfus, 1995; Wilson 1978).

Natural hormones

The highest observed natural hormone concentration in Dutch surface water amounts to 6 ng/l (Belfroid *et al.*, 1999). This value has been measured in autumn. It should be noted that most measurements (75%) showed concentration levels below the detection limit (0.1-2.4 ng/l) for the various oestrogens.

In the same investigation the values have been itemised to the different hormones. The highest observed 17β-oestradiol concentration in Dutch water amounted to 5.5 ng/l, whilst for oestrone this was 5.3 ng/l and for 17α-oestradiol 1.1 ng/l.

For the sum of 17β- and 17α-oestradiol, 17α-ethinyloestradiol and oestrone the values in the Meuse, near Eijsden, are 3.7, 9.1 and 4.0 ng/l, respectively and in The Rhine, near Lobith, the values are 2.0, 5.3 and 13.0 ng/l, respectively.

Recently, investigations have been carried out into oestrogen concentrations in surface water in Germany as well. In three out of 15 rivers oestrone concentrations were observed ranging from 0.7 to 1.6 ng/l. In the remaining rivers oestrogens were not detected (detection limit 0.5 ng/l) (Ternes *et al.*, 1999).

Another study in Germany showed that all concentrations in surface water were below the detection limit of 1 ng/l (Stumpf *et al.*, 1996). Wegener *et al.* (1999) found up to 4 ng/l of oestrone in a lake near Cologne. The concentrations in The Rhine were below 1 ng/l, except for one sample.
The results are summarised in Table 16.

Table 16: Oestrogen concentrations found in surface water

Substance	ng/l	Location	Source
Ethinyloestradiol	2-15	England	Aherne & Briggs, 1989
Total oestrogens	6	The Netherlands	Belfroid *et al.* 1999
17α-oestradiol	1.1	The Netherlands	Belfroid *et al.* 1999
17β-oestradiol	5.5	The Netherlands	Belfroid *et al.* 1999
Oestrone	5.3	The Netherlands	Belfroid *et al.* 1999

For polder ditches of an agricultural parcel a concentration has been estimated of 100-150 ng/l, for a three months' scenario. For the catchment areas of The Rhine and Meuse the estimated values for a three months' scenario are 70 and 120 ng/l, respectively, and for a daily average scenario 19-35 ng/l.

These values are clearly above the observed values of max. 4-9 ng/l. It should be considered that the sampling locations at Lobith and Eijsden are not representative for the emissions of the livestock in Brabant and Gelderland. Moreover, measurements have been performed in August, November and December. This period is not representative for the manure spreading period.

Finally, it should be noted that in the estimation no allowances have been made for degradation in river water. Recent measurements by the Institute of Hydrology, at the request of the Environmental Agency in the UK (Jurgens *et al.*, 1999), show that removal by adsorption to sludge in rivers hardly occurs and the greater part of the steroid hormones remains in solution. Half life of 17β-oestradiol and oestrone in the rivers Thames, Aire and Calder is approximately 3-4 days and in the estuaries of the rivers Tyne and Tees this is 6-27 days. Half life of ethinyloestradiol was approximately 10 times longer.

6.4.1 Drinking water

Ethinyloestradiol concentrations, observed in drinking water, ranged from 1 to 4 ng/l (Aherne & Briggs, 1989). Measurements in Berlin did not indicate ethinyloestradiol values above the detection limit of 0.02 ng/l (Rathner &

Sonneborn, 1979). In the Netherlands the detected concentration in purified water ranged from 0.3 to 0.06 ng/l (Freudental *et al.*, 1975). Rurainski (1977) found an ethinyloestradiol concentration in Germany ranging from 0.85 to 2.89 ng/l. For natural hormones the concentration in groundwater ranged from 0.12 ng/l in sources at a depth of 100 m to 0.3 ng/l at the surface. The results are summarised in table 17.

Table 17: Oestrogen concentrations in drinking water

Substance	ng/l	Remark	location	Source
Ethinyloestradiol	1-4	Highest observed level	England	Aherne & Briggs, 1989
Ethinyloestradiol	<0.02	below detection limit	Berlin	Rathner & Sonneborn, 1979
Ethinyloestradiol	0.06	Highest observed level in the Netherlands	Netherlands	Freudental *et al.*, 1975
Ethinyloestradiol	0.85-2.89		Germany	Rurainski, 1977
Natural oestrogens	0.12-0.3		Germany	Rurainski, 1977

Conclusion

The results show that, of the various chemical forms of oestrogens, mainly ethinyloestradiol and oestrone concentration levels are highest both in effluents of sewage purification plants and in surface water. Ethinyloestradiol has been detected in drinking water as well. This confirms that ethinyloestradiol is more persistent than natural oestrogens. The relatively high oestrone concentrations can be explained by the conversion of 17β-oestradiol into oestrone, which is more persistent than the other metabolites. Then again, oestrone is more readily degradable than ethinyloestradiol.

7. RISK ASSESSMENT

7.1 Risk to human population and mammals by exposure to oestrogens

The highest observed concentrations in main rivers are < 5 ng/l. For drinking water production from surface water by means of bank filtration or sand filtration it can be assumed that further degradation of approximately 80% will occur. Therefore, natural oestrogen concentrations in drinking water of < 1 ng/l can be expected. At a consumption of 2 l/day per person this results in an intake of < 2 ng/day.

In order to establish the possible effects to the human population, a comparison was made of the intake by dairy products with the intake of ethinyloestradiol as contraceptive.

The effective dose of ethinyloestradiol in the contraceptive pill amounts to 35 µg/day, which is 17,500 times more than the possible oestrogen intake by drinking water. Moreover, in drinking water mainly oestrone as a natural oestrogen derivative will be present. At oral intake this substance is far less effective than ethinyloestradiol (Ranney, 1977 in Turan 1996). Even the highest observed ethinyloestradiol concentration of 4 ng/l results in a dose well below the effective dose by a factor of 500.

Based on the target limit, set by pharmaceutical industry to protect co-workers exposed to substances containing active components of the contraceptive pill, it has been concluded that a dose of a hundredth below the active dose will give no secondary effects (Derikx-van de Ven *et al.*, 1997).

Intake of natural oestrogens also takes place by the consumption of milk and meat. In table 18 the oestrogen concentrations found in cow's milk are given (Erb *et al.*, 1977). According to Aherne & Briggs (1989) the oestrogen concentration in a consumed quantity of meat of 100 g/day is approximately 3 ng. At a milk consumption of 0,5 l/day the dose per day would be approximately 100 ng, still being 350 times less than the effective ethinyloestradiol dose. Since this is mainly 17α-oestradiol, no effects are expected.

If cattle were to drink ditch water, in which the effluent of a purification plant is discharged, ethinyloestradiol might be taken in. At a consumption of 60 l/day, containing 50 ng of ethinyloestradiol/l (undiluted effluent), the daily dose amounts to 3 µg. This worst case dose is still 10 times below the effective dose for humans and approximately 100 times below the effective dose per kg body weight.

Table 18: Oestrogen concentrations in cow's milk

Hormone	Concentration in cow's milk (ng/l)
Oestrone	28
17β-oestradiol	13
17α-oestradiol	160
Total oestrogens	201

7.2 Risks to the ecosystem

Emission calculations show that in sensitive areas close to the source and in periods of elevated emission levels, the oestrogen concentration in surface water might rise to approximately 100-150 ng/l. Main component probably being oestrone. In river parts further downstream concentrations of up to 5 ng/l have been found and in many rivers concentrations stay below the detection limit (0.5 ng/l).
The observed ethinyloestradiol concentrations vary from 8 ng/l in effluents of purification plants to < 0.1 ng/l in surface water. At places where the effluents are poorly diluted, the No Observed Effect Concentration (NOEC) for vitellogen induction may be exceeded.

Few data are available on the specific efficacy of oestrone to fish. For ethinyloestradiol a NOEC for vitellogenin induction is known of 0.9 ng/l (Purdum *et al.*, 1994). Normally a specific activity of a steroid compound is related to the activity of the reference substance DES towards the oestrogen receptor system. If these ratios are also valid for the vittelogenin induction in fish, the NOEC for oestrone and vitellogenin induction might be 2.5 ng/l.

According to these calculations the environmental risk, expressed in a ratio between predicted concentration and effect limit, varies between well above 1 and well below 1. Especially during manure spreading periods effects could be expected in polder ditches and draining waters. Effects can also be expected when purified or unpurified municipal wastewater is discharged, depending on dilution, in particular when the effluent dilution is less than a factor 20, and in areas for storm water overflow.

Benson *et al.*, (2000) have not detected vitellogen induction until 100 ng/l of 17β-oestradiol (21-days catfish). Moreover, their investigations showed that by injection the effective doses of oestrone and oestriol are 100-150 times higher than the effective dose for 17β-oestradiol. This might indicate that oestrone has no effect until 10 μg/l, and also that no effective concentrations occur in Dutch surface water.

8. CONCLUSIONS AND RECOMMENDATIONS

8.1 Conclusions

The following conclusions can be drawn from this study:

Conclusions with regard to the oestrogen excretion by the human population up to the concentrations in drinking water.

- For the Dutch population the daily excretion of natural hormones is approximately 3.2 kg, half of this being caused by pregnant women. Calculated concentrations in the influent of waster water purification plants amounted to approximately 1,000 ng/l.
- Measurements in purification plants, using activated sludge, show that removal efficiency for natural hormones amounts to 70-100%.
- Assuming a purification efficiency for natural hormones of 95%, this could result in a concentration in the effluent of approximately 50 ng/l. A limited number of measurements showed a highest concentration of 64 ng/l. The highest observed oestrone concentration was 47 ng/l.
- For the Dutch population the total excretion of ethinyloestradiol (coming from the contraceptive pill) is estimated at 43 g/day.
- All experiments proved 17α-ethinyloestradiol to be more persistent than natural hormones. In batch experiments no conversion took place after 48 hrs. In purification plants degradation ranged from 0-78% (6 days), depending on the installation. Only laboratory experiments showed higher degradation rates of 73% after one week and 95% after four weeks.
- The expected ethinyloestradiol concentration in influents of purification plants is 13 ng/l based on calculation of the total number of contraceptive users. Assuming a purification efficiency for this substance of 50%, the expected concentration in effluent is 6.5 ng/l. The highest concentration detected in effluent was 8 ng/l.
- The sewage flow of the European population can be diluted by a factor 20 in the run-off in rivers. Therefore, the expected average ethinyloestradiol concentration in surface water is approximately 0.4 ng/l.
- In most experiments ethinyloestradiol concentrations in surface water amounted to some nanogrammes per litre. The highest concentrations of 2-15 ng/l have been observed in the UK.
- In only one investigation natural oestrogens in drinking water have been measured. The highest observed concentration was 0.3 ng/l. In a number of investigations ethinyloestradiol has been measured. Here the highest observed concentration was 4 ng/l.
- Oestrogen concentrations found in drinking water cannot result in an effective dose.
- Highest observed ethinyloestradiol concentrations in effluent from purification plants and in surface water may result in exceeding the NOEC for vitellogen induction.

Conclusions with regard to emission by domestic animals up to the concentrations in surface water.

- The average oestrogen emission by pregnant cows amounts to 37 mg/day, whilst for non-pregnant cows this is approximately 1 mg/day. The average oestrogen emission by breeding sows amounts to 7 mg/day and the excretion by store sows is minimal (31 µg/day). Also the emission by laying hens is minimal, however, since there are 45 million laying hens in the Netherlands, the entire oestrogen contribution per day results in approximately 1 kg/day. At the end of the gestation period the oestrogen emission by mares is assumed to be approximately 100 mg/day. However, no data are available to confirm this high value.
- The total oestrogen emission by Dutch livestock is dominated by breeding sows and pregnant cows (10.6 and 22 kg/day, respectively). The total emission by the Dutch livestock is approximately 10 times higher than the excretion by the human population.
- Should the highest permitted amount of slurry from breeding sows or milk cows be spread on the land, it can be calculated that the concentration in ditches, during a fertilising period of three months, may rise to 150 ng/l.
- Based on the size of livestock in the catchment area of Rhine or Meuse, it can be calculated that the increase in concentration might amount to 75 and 140 ng/l, respectively, during a fertilising period of three months. Regular spreading of manure over the summer period (6 months) could result in concentrations in the Rhine and Meuse of approximately 40 and 90 ng/l, respectively.
- Degradation of natural oestrogens in surface water has not been established unambiguously. Results of measurements vary between 0% and 100% in the course of approximately 5 days.
- Highest observed oestrone and β-oestradiol concentrations in surface water amounted to 5.3 and 5.5 ng/l, respectively.
- Differences between calculated and measured values may be explained by the fact that measurements have been performed in autumn and near Lobith and Eijsden. The highest leaching of oestrogens from manure of domestic animals is assumed to take place in spring, when manure is being spread on the land.
- Based on a threshold value of 2.5 ng/l for vitellogen induction by oestrone, similar effects are very likely to occur in agricultural areas and in surface water to which effluent is discharged by water purification plants.
- If oestrone has no effect until 10 µg/l, there would be no effects at all in the Netherlands.

8.2 Recommendations

As a result of knowledge hiatuses, established in this study, the following is recommended:

- measure oestrogen concentrations in various types of slurry;
- measure oestrogen concentrations in small waters shortly after spreading manure of breeding sows or milk cows on the land and after leaching by rain. In this way insight can be gained into the actual oestrogen leaching from manure;
- for a more representative idea of oestrogen concentrations in the rivers Rhine and Meuse frequent measurements on various locations should be performed during one year;
- investigations into vitellogen induction by oestrone in male fish;
- investigations into the degradation of natural oestrogens in Dutch surface water;
- investigations into progestogen emissions and degradation, and into vitellogen induction by progestogens and their metabolites.

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APPENDIX 1

Calculation of the natural oestrogen concentration in the Meuse emitted by domestic animals in the Netherlands and Belgium

Calculation of natural oestrogen concentration in the Meuse emitted by domestic animals in the Netherlands and Belgium

REGIOS MEUSE	**breeding sows**	**store pigs**	**milk cows**	**non-pregnant cows**	**heifers (calves < 1jr)**	**pregnant mares**	**layers**	**breeding cows**
Total the Netherlands (Limburg and Noord-Brabant)	921607	3670713	305712	169683	132381	2353	20894170	?
Total Belgium, except Meuse and Ardennes	85800	429000	237600	261400	323300	?	5205400	104400
Total Meuse catchment area (Belgium and the Netherlands)	1007407	4099713	543312	431083	455681	2353	26099570	104400
Oestrogen excretion (mg/animal/day)	6.8	0.03	37.3	1.1	0.59	100	0.019	37.3
Oestrogen excretion (mg/group of animals/day)	6850368	122991	20265538	474191	268852		495891.8	3894120
Total excretion Meuse catchment area (g/day)	32372							
average flow rate Meuse catchment area 1974-1998 (m3/s) near Keizersveer	320							
average flow rate Meuse catchment area 1946-1998 (l/d)	2.80E+10							
Oestrogen concentration in Meuse (100% leaching, 0% degradation) ng / l	1156							
Oestrogen concentration in Meuse (3% leaching) ng / l	**35**							
Source: CBS 1999								

APPENDIX 2

Calculation of the natural oestrogen concentration in the Rhine, emitted by domestic animals in the Netherlands and Germany

Calculation of natural oestrogen concentration in the Rhine, emitted by domestic animals in the Netherlands and Germany								
REGIOS RHINE	breeding sows	store pigs	milk cows	non-pregnant cows	heifers (calves < 1jr)	pregnant mares	layers	breeding cows
THE NETHERLANDS								
DRENTHE	34601	149629	94205	24909	41151		1538970	
FLEVOLAND	4674	32574	27040	3582	10855		566700	
FRIESLAND	16693	53953	268594	24773	215985		996880	
GELDERLAND	289422	1368220	259405	90274	108006		12063780	
NOORD-HOLLAND	4419	17859	81466	18159	30742		175080	
OVERIJSSEL	192272	938347	256142	78892	104909		2655760	
UTRECHT	38370	215055	90180	15455	30121		12063780	
ZUID-HOLLAND	22701	145686	108650	19980	34833		480490	
TOTAL THE NETHERLANDS	603152	2921323	1185682	276024	576602	4483	30541440	
LUXEMBOURG								
LUXEMBOURG	?	?	?	?	?	?	?	?
GERMANY								
ARNSBERG	44700	662500	73600	19400	79900	?	869200	14900
DARMSTADT	16100	174800	43500	11300	47100	?	889600	7800
DUESSELDORF	62900	550800	91900	13500	90600	?	1204300	15500
FREIBURG	20200	217400	97400	21100	94200	?	628300	14400
GIESSEN	20700	204300	50800	13200	51200	?	458100	9400
KARLSRUHE	15200	153900	36900	7500	41500	?	612600	4700
KOBLENZ	15700	177000	49200	23700	54000	?	673900	11400
KOELN	11100	101300	100600	17500	70900	?	842200	17500
MEURTHE-ET-MOSELLE	?	?	?	?	?	?	?	?
MITTELFRANKEN	59400	501600	149600	9900	148700	?	832600	22800
MOSELLE	?	?	?	?	?	?	?	?
MUENSTER	277000	3260100	177600	14200	270300	?	3277100	14600

Calculation of natural oestrogen concentration in the Rhine, emitted by domestic animals in the Netherlands and Germany								
REGIOS RHINE	breeding sows	store pigs	milk cows	non-pregnant cows	heifers (calves<1jr)	pregnant mares	layers	breeding cows
OBERFRANKEN	31100	282200	118700	7400	93300	?	400500	18800
RHEINHESSEN-PFALZ	9000	106100	20200	8400	24300	?	921700	3800
SAARLAND	2600	25500	17300	7700	19000	?	193800	3400
STUTTGART	178400	688600	143000	17500	147100	?	1836700	19700
TRIER	15900	103600	79100	19100	60400	?	242100	17300
TUEBINGEN	92500	564700	213000	14900	157200	?	1661900	31100
UNTERFRANKEN	51700	401800	49900	8100	64200	?	540300	7800
TOTAL GERMANY	924200	8176200	1512300	234400	1513900	?	16084900	234900
FRANCE								
LOW-RHINE	?	?	?	?	?	?	?	?
HIGH-RHINE	?	?	?	?	?	?	?	?
VOSGES	?	?	?	?	?	?	?	?
Total France	?	?	?	?	?	?	?	?
Total Rhine catchment area (the Netherlands and Germany)	1527352	11097523	2697982	510424	2090502	4483	46626340	269800
Oestrogen excretion (mg/animal/day)	6.8	0.03	37.3	1.1	0.59	100	0.019	37.3
Oestrogen excretion (mg/group of animals/day)	10385994	332926	100000000	561466	1233396		885900.46	10063540
Total excretion Rhine catchment area (g/day)	123463							
average flow Rhine catchment area 1946-1998 (m3/s)	2222							
average flow Rhine catchment area 1946-1998 (l/d)	1.90E+11							

Calculation of natural oestrogen concentration in the Rhine, emitted by domestic animals in the Netherlands and Germany		
oestrogen concentration in Rhine, 100% leaching and 0% degradation (ng/l)	650	
Oestrogen concentration in Rhine (3% leaching) ng / l	**19**	

Remarks:

- Excretion by horses has not been included because a) the excretion in manure is unreliable and b) insufficient data on the number of horses in catchment area.
- It is assumed that the oestrogen excretion by breeding cows equals that of pregnant cows.
- In Germany no distinction has been made into layers and other chicken. It is assumed that all chicken are layers.

Source CBS: 1999

Colophon

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