MELiSSA

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Preliminary requirements to test hormones and pharmaceutical drugs countermeasures

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Notation

AOP	Advanced Oxidation Process
E1	Estrone
E2	17β-estradiol
E3	Estriol
EDC	Endocrine Disrupting Compound
EE2	17α-ethynylestradiol
GAC	Granular Activated Carbon
HRT	Hydraulic Retention Time
MBR	Membrane Bioreactor
MF	Microfiltration
NF	Nanofiltration
PAC	Powdered Activated Carbon
PPCP	Pharmaceutical, Personal Care Product
RO	Reverse Osmosis
SRT	Sludge Retention Time
STP	Sewage Treatment Plant
UF	Ultrafiltration

1. Introduction

Any biologically based Life Support System (LSS) will sooner or later face recalcitrant compounds and the accumulation of (xenobiotic) compounds. In MELiSSA's case (Micro-Ecological Life Support System Alternative), a closed loop LSS, the factors of accumulation and incomplete conversion are magnified as no external manipulations are allowed. BELISSIMA envisages studying the fate of micro-nutrients, the potential accumulation of recalcitrant compounds or xenobiotics, and the influence of those accumulations or depletions on the microbiota -and consequently on the process performances- present in a small scale MELiSSA loop. In the MELiSSA loop, the first compartment is an anaerobic reactor operating under VFA-producing conditions. Apart from non-absolute degradation conditions (cf. MAP study "A total and biosafe liquefaction compartment for MELISSA, AO-99-LSS-015), accumulation through non-degradation and ab/adsorption is to be expected.
On a terrestrial level domestic wastewater treatment plants encounter various pharmaceuticals, such as endocrine disrupting synthetic hormones, β-blockers… that prove to be difficult to remove using conventional technologies. As several of these compounds will most likely also be used in space, their recalcitrant nature needs to be assessed in the context of the MELiSSA cycle. Apart from anthropogenic substances, the organisms in the MELiSSA cycle itself will produce troublesome compounds. Little is known about these substances, such as bacterial toxins, signalling compounds, phytohormones, … and their fate in closed loop systems.

In the following paragraphs the compounds that deserve further attention will be pinpointed and the possible processes with their respective sample points and drawbacks will be mentioned. In addition, the possible countermeasures against the prevalence and accumulation of these compounds in the closed loop LSS will be discussed.

2. Hormones

2.1. General

Steroid hormones are a group of biologically active compounds that are synthesized from cholesterol and have in common a cyclopentanoperhydrophenanthrene ring. Natural steroids are secreted by the adrenal cortex, testis, ovary and placenta in human and animal, and include glucocorticoids, mineralocorticoids and sex steroids. Sex steroids are generally divided into three functional groups: estrogens, androgens and progestagens. All the steroid hormones exert their action by passing through the plasma membrane and binding to intracellular receptors. In addition, there are some synthetic steroids such as 17α-ethynylestradiol (EE2) and mesantrol (MeEE2) used as contraceptives.

The hormones or endocrine active compounds, which will be evaluated within this project are the human estrogens and androgens and the synthetic hormone EE2, the most common and persistent hormone present in the contraceptive pill. These groups of hormones are most likely to occur in urine and faeces, and belong to the feed in compartment I.
The most important representative of the estrogen group is 17β-estradiol (E2). In addition, there are less potent estrogens, such as estrone (E1) and estriol (E3) (Figure 1). For the androgens, testosterone and its derivate dihydrotestosterone are the most important forms. Less potent androgens are androstenedione and dihydro-epiandrosterone (Figure 1).

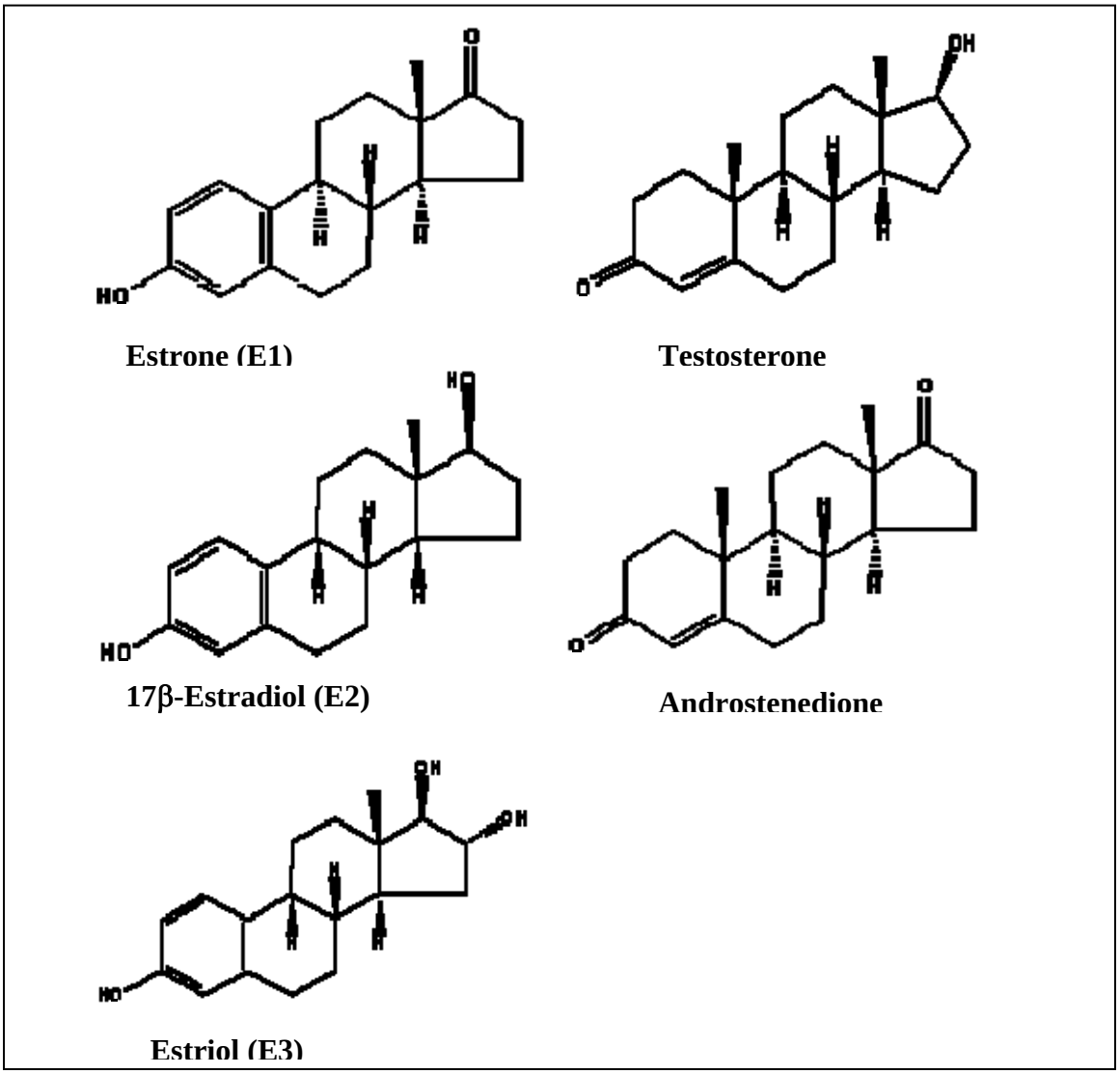


Figure 1. Chemical configuration of the most important estrogenic (left) and androgenic (right) hormones.

Steroid hormones are hydrophobic substances and thus difficult to be excreted by kidneys. Therefore, steroid hormones are metabolized in the human liver (eg. oxidation, hydrolysis, methylation, …) before they are conjugated with glucuronic acid, phosphates or sulphates. This conversion makes them more soluble and facilitates the excretion through urine and faeces. The conjugated forms (estrogenically inactive) are cleaved to free estrogens through microbial processes before or during sewage treatment (Panter et al., 1999; Ternes et al., 1999; Johnson & Williams, 2004). The majority of excreted steroids in the faeces are in the unconjugated form (Johnson & Williams, 2004). The important role of intestinal metabolism of estrogens is experimentally shown. Conjugated estrogens, both sulfate and glucuronide, excreted from the bile are largely deconjugated by the natural intestinal flora prior to excretion. Significant deconjugation of sulfate forms occurs in the gut, indicating the presence of steroid desulfating bacteria. Strictly anaerobic desulfating bacterial strains have been isolated from human feces, which were capable of cleaving estrone-3-sulphate and estradiol-3-sulphate.
Except for the parent compounds, metabolites or conjugated compounds can occur in urine, and may appear during degradation processes in compartment I. All these compounds from human origin are likely to occur as input to the BELISSIMA loop.

2.2. Occurrence of hormones in terrestrial environments

Please find enclosed in appendix 1 and 2 tables showing terrestrial data on hormone occurrence

2.3. Expected concentrations of hormones in space environment

2.3.1.Androgens

With regard to levels of excretion of androgen hormones, few data are available. Dittmer (1961) reported total androgen levels up to 100 µg/mL in daily urinary excretion by male. These numbers are rather high compared to a more recent paper where basal levels of urinary excretion of male hormones, including several metabolic forms, range from 0.028 µg/mL for testosterone up to 1.9 µg/mL for androsterone (Wang et al., 2005). The latter data, which were obtained by GC analysis, are likely more accurate. This range can be taken into account to assess the content of androgens which will be put into compartment I, as soon as is decided upon the amount of urine to be put into the feed.

2.3.2.Natural estrogens

According to Williams and Stancel (1996), the total daily excretion rate of natural estrogens ranges from 10 to 100 µg for woman, 5 – 10 µg for women after the menopause and 2 – 25 µg for men. Average excretion values from a study amongst female inhabitants of a Roman condominium were 32, 14 and 106 µg/day of conjugated E1, E2 and E3, respectively (D'Ascenzo et al., 2003). According to Adlercreutz et al. (1986) women excrete in urine about 7 µg E1, 2.4 µg E2 and 4.6 µg E3 in unconjugated

form daily. Approximately 0.4 µg E2, 1.25 µg E3 and 0.5 µg E1 is eliminated in faeces per day (Adlercreutz et al.; 1994). Fotsis et al. (1980) reported a daily excretion in urine of unconjugated forms as 3.0 µg E2, 8.0 µg E1 and 4.8 µg E3. Witters et al. (2002) made a summary of available literature data (minimum-maximum values) on human excretion of estrogens. Based on a mean composition of crew in space consisting of 3 man and 3 women, an average range of daily excretion of estrogens in urine per population head will be between 10 and 140 µg total estrogens (Table 1Table 1). The latter does not consider the use of anticonceptive pill. This estimated range can be taken into account to assess the content of estrogens which will be put into compartment I, as soon as is decided upon the amount of urine to be put into the feed.

Table 1. **Estimates of excretion of natural estrogens in human urine in space, based on proposed composition of crew (ESA) and numbers from Witters et al. (2006)**

Population group	Daily estrogen excretion (µg/day)		Crew in space (# persons)	Range daily excretion (µg/day)	
	Min.	Max.		Min.	Max.
Children (0-15 yrs)	nd	20	0		
Male (15-64 yrs)	7	85	3	21	340
Female (15-64 yrs)	16.3	250	3	48.9	750
Pregnant female	6859	30000	0		
Eldery (> 64 yrs)	nd	28	0		
Total crew			6	69.9	1090
Population head (average)				11.7	181.7

2.3.3.Synthetic estrogens

Except for the natural hormones, the synthetic hormone EE2, the most common and persistent hormone present in the contraceptive pill, is studied. The daily dose of women using the contraceptive pill is calculated to be approximately 35 µg taken during 21 days of a 28 day period (Katzung, 1995). Up to 80 % of the EE2 digested is excreted as unmetabolized conjugates (Ranney, 1977; Maggs et al., 1983). Of the daily dose, 22 – 50 % of EE2 is excreted in urine of which about 64 % is conjugated and approximately 30 % is excreted in faeces (Reed et al., 1972). The oral bioavailability of EE2 is about 42 % due to an extensive first-pass metabolism in the intestinal wall and liver (Weber et al., 1996). More than 30 % of EE2 is sulphated, which accounts for approximately 60 % of the first-pass metabolism (Back et al., 1979, 1982). Only 1 – 2 % of the administered EE2 has been found to be de-ethynylated and transformed to E1, E2 or E3 (Ranney, 1977). The contribution of EE2 to the total amount of excreted estrogens is only about 1 % but this compound is considerably more persistent in sewage treatment plants compared to the natural hormones (Ternes et al., 1999a, b).
These estimated ranges can be taken into account to assess the content of estrogens which will be put into compartment I, as soon as is decided upon the amount of urine to be put into the feed.

2.4. Biological degradation of hormones

2.4.1.Aerobic degradation of hormones

2.4.1.1. *Aerobic biodegradation of androgens*

Several species of bacteria, including *Nocardia restrictus* (Gram-positive) and *Comamonas testosteroni* (Gram-negative) (formerly *Pseudomonas testosteroni*), are known for the ability to utilize testosterone and various other steroids as sole carbon and energy sources. The mechanism by which testosterone is degraded in *N. restrictus* and *C. testosteroni* was eagerly studied, and the main intermediate compounds in the degradation pathway of these bacteria, especially *N. restrictus*, were determined in the 1960s (Coulter et al., 1968; Gibson et al., 1966; Sih et al., 1966). Complete assimilation of steroids is achieved through a complex pathway involving many enzymatic steps of oxidation responsible for the breakdown of the steroid nucleus. The catabolic enzymes for steroid metabolism in C. *testosteroni* are not constitutively expressed but are induced by their respective substrates such as testosterone and progesterone.
Esperanza et al. (2004) studied the removal of testosterone and androstenedione in two pilot-scale municipal wastewater treatment plants. It was shown that these androgens were completely removed from the aqueous phase. Layton et al. (2000) showed that testosterone is capable of being mineralized by biosolids from four different municipal wastewater treatment plants. Lorenzen et al. (2005) concluded that testosterone is rapidly and thoroughly biodegraded in agricultural soils under a range of conditions typical of a temperate growing season and thus is unlikely to pose a long-term risk to adjacent aquatic environments. Testosterone was shown to be easily biodegraded in activated sludge wastewater treatment processes (Pauwels et al., 2006, in preparation). Mansell et al. (2004) showed that the dominating removal mechanism for testosterone during soil-aquifer treatment is adsorption to the porous media matrix and that additional attenuation to below detection limit occurred in the presence of bioactivity. This additional removal occurred regardless of dominating redox conditions (aerobic vs. anoxic) or the type of organic carbon matrix present (hydrophobic acids, hydrophilic carbon vs. colloidal carbon). Svenson and Allard (2004) reported that the removal of androgenicity in treatment plants with biological treatment was efficient (96 – 99 % removal). Apparently the androgenic compounds were more easily converted into inactive forms as compared to the estrogens tested in the same effluents. There was no difference in performance regarding removal of androgens between different types of secondary treatment. Both activated sludge and solid supported microorganism treatments seemed equally effective. Efficient removal of androgens by biological treatment of sewage effluents was also reported from the UK (Kirk et al., 2002).

2.4.1.2. *Aerobic biodegradation of natural and synthetic estrogens*

Cleavage of estrogen conjugates

Estrogen conjugates are cleaved into their active forms, as found in batch experiments using activated sludge (Ternes et al., 1999a). The initial transformation of E2-conjugates to an estrogenically active product occurs more rapidly than degradative loss (Panter et al., 1999). Deconjugation of glucuronide conjugates is expected to already take place in sewer systems, while cleavage of the sulphuric conjugates, (which need arylsulphatase for cleavage), will only happen in sewage treatment plants (STPs) as this demands more specialistic micro-organisms (Baronti et al., 2000). This is confirmed with measurements at the STP entrance, where free estrogens and sulphated estrogens were the dominant species (D' Ascenzo et al., 2003). Also in lab scale experiments with wastewater and the addition of both types of conjugates, it took approximately 3 days for the sulphate conjugates, against 7 h for the glucuronide conjugates to reach half the initial concentration (D'Ascenzo et al., 2003).

Aerobic degradation of estrogens

In aerobic batch experiments it was shown that after a period of 1 – 3 h, more than 95 % of E2 was oxidized to E1 (Ternes et al., 1999a). In the same experimental set up, EE2 appeared to be stable. Also Norpoth et al. (1973) found no degradation of EE2 in activated sludge after an incubation time of five days. The findings for the conversion of E2 to E1 were confirmed in experiments with river water samples, in which E2 was converted into E1 and mineralized according first order kinetics (Jürgens et al., 2002).

E1 can be mineralized by cleavage initiating the A-ring (Layton et al., 2000) or initiated at C-17 of ring D (Lee & Liu, 2002). In the first case, the postulated mechanism is ring cleavage by hydroxylation at C-4, followed by an oxidative fission between C-4 and C-5 by a dioxygenase, from there on it can be converted into either pyridine carbolic acid, where no CO_2 is formed, or to 3aα-H-4α-[3'-propanoicacid]-5β-[2-ketopropyl]-7aα-methyl-1-1inanone or 3aα-H-4α-[3'-propanoicacid]-5β-[4'-but-3-enoicacid]-7aβ-methyl-1-1indanone, where CO_2 is formed (Coombe et al., 1966). However, D-ring cleavage is more likely since lactone has been identified as a metabolite (Lee and Liu, 2002). Eventually, estrogens will be mineralized, as after 25 days, 24 – 45 % of radio labeled ^{14}C E2 has been converted to CO_2 by micro-organisms from river water (Jürgens et al., 2002) and 70 – 80 % was converted into CO_2 by sludge from municipal STPs after 24 h (Layton et al., 2000). Also EE2 can be mineralized as after 24 h 40 % of ^{14}C-EE2 was converted into CO_2 (Layton et al., 2000).

All the k-values obtained from literature are summarized in De Mes et al. (2005). In this review, an attempt has been made to standardize k-values for the applied dry matter content in different batch tests and therefore expressed in 1/g SS/day. The general trend in the conversion rates is that the conversion of E2 to E1 is rapid, in some cases even a few minutes, and that EE2 is sometimes not converted at all, or in a far slower rate, with halflifes of 6 h up to 5 days.

Natural estrogens are thought to be biodegraded via a pathway where bacteria can use the conversion for growth, whereas EE2 is thought to be biodegraded by co-metabolism, in which an organic compound is modified but not utilized for growth (Vader et al., 2000). Nitrifying sludge is held responsible for the conversion of EE2 by the use of the enzyme ammonium monooxygenase, which inserts oxygen into C-H bonds (Vader et al., 2000). The nitrifying activated sludge converted EE2 to more hydrophilic metabolites almost completely in about six days, while sludge with a very low nitrifying capacity did not convert EE2 (Vader et al., 2000). Using N-allylthiourea (ATU), a chemical that inhibits the nitrification by blocking the ammonium monooxygenase enzyme, resulted in slower conversion of EE2, while the conversion rates of E1 and E2 remained the same. If ATU is applied on a pure culture of nitrifying bacteria the conversion is completely blocked, while in activated sludge it was only slowed down, suggesting that in activated sludge also other bacteria are able to convert EE2 (Shi et al., 2004a).

Influence of initial concentration

Another remarkable trend is that the conversion appears to be a lot faster when the initial concentration of the estrogens is lower. This can either indicate an inhibition of the estrogens on the sludge or it can be due to another unknown phenomenon. Inhibition by EE2 has been confirmed in a biological oxygen demand (BOD) test with activated sludge at 28 °C in the dark, and with addition of 60 mg/L E2 or EE2. E2 is biodegraded, but addition of EE2 led to a lower BOD than the blank (Kozak et al., 2001). The latter was confirmed in a toxicity test with nitrifying sludge, a sensitive group of microorganisms towards toxicants, which shows toxic effects for concentrations above 10 mg EE2/L (Kozak et al., 2001). This trend of higher conversion rates at lower concentrations has also been found in a river water sample, when the conversion rate for E2 was slightly higher spiking with 0.1 µg/L compared to 100 µg/L, while oxygen depletion was not the case (Jürgens et al., 2002). Also Ternes et al. (1999a) observed faster degradation at 1 µg/L of E2 compared to 1 mg/L. Another example was found by Shi et al. (2004b), finding faster degradation at 0.2 µg/L compared to 0.2 mg/L.

Influence of temperature

The degradation rate is depending on the temperature. In the temperature range from 5 to 10 °C, the k_d value is 4.2 day^{-1} for E2 and 0.14 day^{-1} for EE2, while in the range of 20 to 25 °C, the k_d values are 6.0 day^{-1} and 0.29 day^{-1} (Jürgens et al., 2002).

Influence of adaptation

Adaptation of the microorganisms is of importance as sludge from a STP was able to remove 84 % of ^{14}C-E2 and 85 % of ^{14}C-E1, against less than 4 % by industrial sludge unexposed to estrogens (Layton et al., 2000). The industrial sludge might consist of a different bacteria population that is not capable of converting E1 and E2. Mineralisation by STP sludge of ^{14}C-EE2 was 25–75-fold less; only 40 % was converted in 24 h (Layton et al., 2000). It is not clear whether this can be explained by the presence or absence of nitrifying bacteria, or that other bacteria are capable of the conversion of estrogens.

Influence of sludge retention time (SRT)

The type of sludge can also be important as shown in tests with both activated sludge and sludge from a membrane bioreactor (MBR) (Joss et al., 2004). MBR sludge showed a 2-3-fold faster conversion, which was explained by the longer SRT of MBR sludge. The smaller floc size of MBR sludge results in a higher specific surface area, enhancing transfer in the floc. The SRT seems to be of most importance as shown in research comparing the degradation of EE2 in a conventional system with a very high SRT of 52 – 237 days, with a MBR. No significant differences in removal were found (Clara et al., 2004). Lyko et al. (2005) reviewed several papers which compared the elimination efficiency of estrogenic trace contaminants in MBR and CAS (conventional activated sludge) systems resulting in the generation of Table 2.

Table 2. Elimination efficiencies of MBRs and CAS process (after Lyko et al., 2005)

Compound	MBR rejection (%)	CAS rejection (%)	Reference
E1	96.3	91.2	Hegemann et al. (2002)
E2	100	91.0	Hegemann et al. (2002)
EE2	92.4	100	Hegemann et al. (2002)
E1	93.8-99.7	87.8-97.5	Zühlke and Dünnbier (2003)
E2	95.7-98.5	94-97.5	Zühlke and Dünnbier (2003)
EE2	81.9-93.6	59.4-81.5	Zühlke and Dünnbier (2003)
E2-eq	75	58	Holbrook et al. (2002)

Influence of hydraulic retention time (HRT)

Longer HRTs give higher removal efficiencies of E1, E2 and EE2 as illustrated by STPs in the UK, in which removal is significantly better at a HRT of around 13 h compared to 2-5 h (Kirk et al., 2002). This is confirmed by Svenson et al. (2003), reporting removal below detection limit for the Klävlinge plant with a HRT of 20 h and the Ekebyverket plant including a wetland with a HRT of 7 days. Approximately 99 % removal was achieved in the Vimmerby plant with a HRT of 12 h, which was longer than the 2-8 h applied in most other plants in this research, only removing about 58 – 94 %. Cargouët et al. (2004) found better removal for E1 (58 %) and E2 (60 %) in the plants Evry and Valenton with an HRT of 10 – 14 h compared to a plant in Achères with a HRT of 2 – 3 h in which a removal of 44 % for E1 and 49 % for E2 was established. In the plant containing three biofilters including nitrification and denitrification in Colombes with an HRT of 2.5 – 4 h, 55 % of E1 and 43 % of E2 were removed. In all the four plants removal of EE2 was approximately 40 %.

The influence of increased SRT is illustrated by a STP in Wiesbaden which has been upgraded from a BOD removal plant to a nutrient removal plant, with substantial higher SRTs, increasing from < 4 days to 11 – 13 days. Batch experiments with sludge from the old plant did not show any reduction of EE2 (Ternes et al., 1999a), while at the increased SRT a reduction of around 90 % is established in the full scale plant, which can indicate the growth of microorganisms capable of degrading EE2 (Andersen et al., 2003). So below a certain SRT, degradation of EE2 will not occur.

Isolation of estrogen degrading strains

There have been a few attempts to isolate a microorganism that can specifically convert estrogens. The fungus *Fusarium proliferatum*, has been isolated from a cowshed sample and is capable of converting EE2 (Shi et al., 2002). The fungus was able to remove 97 % of EE2 at an initial concentration of 25 mg/L in 15 days at 30 °C and gave a k_d value of 0.6 day^{-1} at an optimum pH of 7.2 (Shi et al., 2002). This resembles a half-life of 1.2 day, which is remarkably faster than measured in activated sludge. The role that fungi can play is degradation by production of enzymes, as was shown in a test with direct addition of the enzyme laccase to a solution of E1 and EE2. In three days around 40 % of E1 and 75 % of EE2 disappeared (Tanaka et al., 2000). Fungi might also be responsible for the conversion of EE2 in STPs, since they can also be present in activated sludge.

From an activated sludge plant, a gram-negative bacterium, possibly from the genus *Novosphingobium*, was isolated and was capable of degrading E2 and E1, but not EE2 (Fujii et al., 2002). The culture was able to degrade 60 % of E2 in 14 days and 40 % of E1 in 20 days. The degradation of E2 was not enhanced by the addition of yeast extract or glucose. Among 20 white-rot fungal strains have been screened for the removal capacity of a variety of (xeno)estrogens, including E1 and E2 (Fujita et al., 2002). Removal was not established in seven of the tested strains for either E1 or E2 or both, in other strains the removal varied from 5.5 % to over 99.9 %.

Yoshimoto et al. (2004) isolated four strains of *Rhodococcus* from an activated sludge plant which completely and rapidly degraded 100 mg/L of E1, E2, E3 and EE2.

Sorption on sludge

Estrogens are hydrophobic organic compounds of low volatility, with log K_{ow} values of 3.43 for E1, 3.94 for E2 and 4.15 for EE2 (Lai et al., 2000). As an indication, compounds with a log K_{ow} below 2.5 exhibit a low sorption potential, between 2.5 and 4.0 a medium sorption potential and higher than 4.0 a high sorption potential (Rogers, 1996). Due to their physico-chemical properties, steroid estrogens should be adsorbed onto sludge.

In a recent Danish study (DEPA, 2004) on removal processes in activated sludge, the results indicated that at common sludge densities in Danish STPs about 35 – 45 % of E1, 55 – 65 % of E2 and EE2 can be expected to be sorbed to the sludge. In a test with activated sludge in a concentration of 2 – 5 g/L, only 20 % of labelled EE2 remained in the aqueous phase after one hour, when 20 % mineralization was observed, concluding that 60 % can be bound to the sludge (Layton et al., 2000).

However, Andersen et al. (2003) carried out a mass balance of estrogens in a German municipal STP and they concluded that only 5 % of the estrogens are sorbed onto digested sewage sludge. They also stated that E1 and E2 show slow sorption kinetics and no equilibrium between the sorbed and dissolved estrogens is established.

2.4.2.Anaerobic biodegradation of hormones

2.4.2.1. *Anaerobic biodegradation of androgens*

To the best of our knowledge, the anaerobic degradation pathways have not been elucidated up to now.

2.4.2.2. *Anaerobic biodegradation of natural and synthetic estrogens*

Little research has been done on the fate of estrogens under anaerobic conditions. Work by Holbrook et al. (2002) is in this respect interesting. They evaluated the mass balance of estrogen activity (by use of yeast estrogen receptor test) in liquid and solid phases of pilot and full-scale waste water treatment facilities. They demonstrated that 5 to 10 % of the estrogenic activity of the influent became associated with the biosolids, and between 26 (aerobic) to 43% (anaerobic) appeared in the treated liquid effluent. Except for the estrogenic activity which is not biodegradable and will appear in effluents, the digestion process of biosolids appeared to be a significant sink for estrogenic compounds (Holbrook et al., 2002). Similar observations were made by Braga et al. (2005). In a primary STP, removal of estrogens was mainly due to sorption to the solids. In an advanced STP with activated sludge reactors (anoxic & aerobic zones) 85 to 96% of natural estrogens were removed, while EE2, the synthetic hormone appeared resistant to biological treatment and undetectable levels were explained as the result of sorption.
Bed sediment was used to examine the potential for E2 to be degraded anaerobically at 20 °C, and was fairly rapidly converted to E1, almost completely after an incubation of 2 days (Jürgens et al., 2002). In batch experiments with activated sludge supernatant under anaerobic conditions (purged with N_2), after 7 days 50 % of the spiked amount of E2 (initial concentration of 200 µg/L) was converted into E1 (Lee and Liu, 2002). No further degradation of E1 was observed, so E1 may accumulate as a by-product. Autoclaved samples were used as sterile controls. Under strict anaerobic conditions, E1 is expected to convert into E2, rather than E2 is converted to E1. This pathway was shown by Joss et al. (2004) who also showed the subsequent removal of E2 under anaerobic conditions without nitrate. So somehow under anaerobic conditions, there are still electron acceptors available, like Fe^{3+} and various organic oxidative compounds, responsible for the conversion.
Carballa et al. (2005) investigated the removal of E1, E2 and EE2 during mesophilic and thermophilic anaerobic digestion of sewage sludge. It was stated that after sludge adaptation removal percentages were 65 – 95 % and 40 – 90 % for E1 + E2 respectively EE2. Joss et al. (2004) indicated that the degradation of natural estrogens (E1 and E2) takes place under all redox conditions (aerobic, anoxic and anaerobic), but at significantly different rates. For E1, an increase by a factor 3 – 5 was observed in the transitions from anaerobic to anoxic (nitrate available but no molecular oxygen) and from anoxic to aerobic (O_2 available in solution). The reduction of E1 and E2 and the subsequent removal of E2 could be shown to take place under anaerobic conditions without nitrate.

Moreover in a recent Danish study (DEPA, 2004) the degradation of E1, E2 and EE2 was studied under aerobic and anaerobic conditions in a simulated activated sludge system. It is concluded that under anaerobic conditions, the degradation rates for E1 and EE2 were considerably (10 – 20 times) lower than under aerobic conditions while the degradation of E2 was not significantly different.

Ying et al. (2003) evaluated the sorption and degradation of 5 endocrine disrupting compounds (EDCs), including E2 and EE2, in sediment and groundwater from an aquifer. The estrogens did show medium affinity for the aquifer material (sorption coefficients K_f of 24.2 for EE2 and 90.9 for E2). In anaerobic conditions, no degradation was observed for EE2 while E2 degraded very slowly within 70 days in native groundwater (Ying et al., 2003). In Ying et al. (2005), it was shown that E2 was biotransformed to E1 under both aerobic and anaerobic conditions.

A few other papers on anaerobic conditions indicate that steroid compounds will remain almost unchanged. EE2 tested under anaerobic conditions in river water samples showed no degradation over 46 days (Jürgens et al., 1999). No degradation of the three estrogens was found by Pakert et al. (2003) in batch tests with sludge from an anaerobic sludge digester. Similarly Ivashechkin et al. (2004) indicate that endocrine disrupting chemicals sorb to sludge and that no degradation of these compounds is expected during anaerobic digestion.

Matsui et al. (2000) observed that the E2 concentrations and estrogen activity of the dewatering liquid from the sludge treatment were even more than twice as high as the inflow to the plant. Several reasons explain this fact. First, conjugated compounds originating from primary sludge are expected to be cleaved in the digester; second, the dissolution of particles due to the digestion process may release estrogens by desorption; and third, the E1 to E2 reverse reaction could be shown to take place in an anaerobic environment.

Johnson and Williams (2004) reported that strictly anaerobic desulphating strains are capable of cleaving E1-3-sulphate and E2-3-sulphate, thus increasing their concentrations. In contrast, Clara et al. (2004) and Kreuzinger et al. (2004a) indicated that the anaerobic digestion stabilisation accelerates the breakdown of natural estrogens.

Sorption on sludge

The information on the adsorption on anaerobic sludge is scarce. Pakert et al. (2003) found in batch tests with anaerobic sludge with a TSS content of 30 g/L, 75 % of E2, 85 % of E1 and 90 % of EE2 was adsorbed. Kunst et al. (2002) reported values adsorbed to anaerobic sludge during sludge treatment of 3 – 115 µg/kg TS for E2 and 3 – 330 µg/kg TS for E1. EE2 was not detected.

Anoxic biodegradation

Under anoxic conditions the conversion rates lay in between those under anaerobic and aerobic conditions. For example the degradation of EE2 was 11 h under anaerobic conditions, 2.8 h under aerobic and 5.6 h under anoxic conditions (Joss et al., 2004).

Andersen et al. (2003) indicated that the natural estrogens are degraded mainly in the denitrifying tank (anoxic conditions).

2.5. Other technologies for the removal of hormones (chemical, physical)

2.5.1.Coagulation/Flocculation

Metal salts (aluminium sulfate, ferric chloride) and softening chemical (calcium oxide, sodium carbonate) are commonly added to destabilize particles present in water or to precipitate new particles (coagulation), aggregate particles (flocculation), and improve settling characteristics of particles (clarification). As has been observed at full-scale treatment plants, coagulation did not have any enhancing effect on the removal of estrogens, which is also tested in batch tests by the addition of ferric chloride (5 – 50 mg/L) to a 15 ng/L E1 solution at different pH values (5 – 11.4) leading to no removal of E1 (Ong et al., 2001; Chang et al., 2004). Also Schäfer and Waite (2002) showed that the addition of ferric chloride does not change the E1 concentration. Westerhoff et al. (2005) showed that aluminium sulfate (4.7 – 6.3 mg Al^{3+}/L) and ferric chloride (9.8 – 13.1 mg Fe^{3+}/L) coagulants or chemical lime softening removed less than 20 % of E1, E2, E3, EE2, progesterone, androstenedione and testosterone at initial concentrations of about 100 ng/L. Also adsorption by iron phosphate precipitates would be unlikely to sorb large quantities of steroid estrogens (Johnson et al., 2000). Recently, Bodzek et al. (2006) showed that the efficiency of the coagulation process was not good enough to remove E1, E2, E3 and EE2 completely from water.
Snyder et al. (2002) reported elimination of E2 around 43 % during the coagulation process. However, no reduction was observed for EE2. Kobuke et al. (2002) observed that estrogenic activity was somewhat eliminated by the coagulation-flocculation process (around 50 %), although not complete. This can be due to the fact that most of the components responsible for the occurrence of estrogenic activity are low molecular organic compounds, which are not removed by this process.

2.5.2.Chlorination process

Chlorine is a strong oxidant used primarily as a disinfectant in drinking water treatment. Several studies (Hu et al., 2003 and Moriyama et al., 2004) showed that βE2 and EE2, respectively, reacted rapidly with HOCl and are completely removed (Table 3). However, several chlorinated by-products were formed. Moreover, it has been reported that some of the chlorinated products have carcinogenicity and/or mutagenicity (Moriyama et al., 2004). Thus, it is important to identify the products from the reaction of EDCs with available chlorine and their estrogenic activities associated (Hu et al., 2003; Moriyama et al., 2004). Indeed, Hu et al. (2003) could determine mainly the formation of 4-chloro-E2, 2,4-dichloro-E2, and 2,4-dichloro-E1, and other non-identified compounds.

Hu et al. (2003) concluded that the products in aqueous chlorinated βE2 solution elicited estrogenic activity. Moreover, Moriyama et al. (2004) confirmed the formation of two products in highly chlorinated solutions after 60 min. (4-chloro-EE2, 1–6 mol%; 2,4-dichloro-EE2, 3– 25 mol%). The estrogenic activities of 4-chloro-EE2 were similar to those of the parent EE2.
Contrary to these reports, Liu et al. (2005) showed that 15 min. of chlorination resulted in a dramatic decrease of the estrogenic activity of E2 and EE2.
In theory, the estrogenic activity of endocrine disrupters should be removed with a sufficient reaction time, if the structures are transformed or sufficiently degraded to lose their bioactive sites. This process was confirmed by the results from Lee et al. (2004), in which the estrogenic activity of E2 was completely removed after 24 h chlorination. However, such a long disinfection period is not practical in wastewater treatment plants.

Table 3. Removal of estrogens by chlorination processes

Compound	Concentration	Removal (%)	Reaction time	Added dose	Reference
βE2	50 µg/L[a]	100	10 min.	1.46 mg/L of sodium hypochlorite	Hu et al. (2003)
βE2	1 mg/L[a]	100[c]	15 min.	7 mg/L of sodium hypochlorite	Liu et al. (2005)
βE2	10^{-7} M[a]	100[b]	24 h	1.5 mg/L of chlorine	Lee et al. (2004)
EE2	0.2 mmol/L[a]	100	5 min.	1 mmol/L of chlorine	Moriyama et al. (2004)
EE2	1 mg/L[a]	100[c]	15 min.	7 mg/L of sodium hypochlorite	Liu et al. (2005)

[a] Synthetic water
[b] Complete removal of estrogenic activity
[c] Dramatically decrease of estrogenic activity

2.5.3.Ozonation and Advanced Oxidation Processes (AOPs) with ozone

During ozonation, two strong oxidants can lead to the transformation of organic compounds: molecular O_3 and hydroxyl radicals ($HO^{\cdot}$) (Hoigne and Bader, 1983a, 1983b). O_3 is a selective electrophile that reacts with amines, phenols and double bonds in aliphatic compounds, while $HO^{\cdot}$ reacts less selectively with organic compounds (von Gunten et al., 2003). Due to the selective nature of ozone, micropollutant transformation may require the use of AOPs, such as O_3/H_2O_2, O_3/UV or H_2O_2/UV.
Ternes et al. (2003), Nakagawa et al. (2002), and Kosaka et al. [132] could remove considerably various estrogens during ozonation treatment.
Applying 10 – 15 mg/L ozone with a contact time of 18 min., it is possible to remove E1 in a concentration of 0.015 µg/L to below the detection limit from STP effluent from an activated sludge plant treating municipal wastewater in Germany (Ternes et al., 2003). Nakagawa et al. (2002) showed a removal percentage of 95 % for the removal of 9.7 – 28 ng/L of E1 and 3.0 – 21 ng/L of βE2 from wastewater from a secondary treatment using an ozone concentration of 5 mg/L and a reaction time of 10 min.

E2 is highly reactive towards ozone because of the two reactive hydroxyl groups (Kosaka et al., 2000). During a treatment with O_3/H_2O_2, ozone is more selective than $HO^{\cdot}$, and since E2 is a highly reactive target, it will be removed quite easily even in the presence of radical scavenging compounds such as humic acid (Kosaka et al., 2000).
It was shown by Liu et al. (2005) that 5 mg/L of ozone and a contact time of 15 min. resulted in 90 % removal of E2 and 100 % removal of EE2 both with an initial concentration of 1 mg/L. The ozonation of E2 still retained a certain degree of estrogenic activity but it was shown that this was caused by residual E2 and not by byproducts. Ozonation of EE2 was shown to reduce the estrogenic activity of EE2.
Furthermore, the ozonation products formed are currently unknown (Ternes et al., 2003). However, hydroxylated estrogens should lose their affinity for the estrogen receptor to greatly reduce the known estrogenic activities of wastewater, but this assumption has not been proved (Ternes et al., 2003). Moreover, Huber et al. (2003) concluded that modifications caused by ozonation or AOPs should be sufficient to eliminate the estrogenic effects of EE2. However, the reactions with ozone and OH radicals during an ozonation process will not result in the complete mineralization of EE2.
Huber et al. (2003) determined, in bench-scale experiments, the rate constants of EE2 for ozonation ($k_{O3} = 7 \times 10^9\ M^{-1}\ s^{-1}$) and AOP ($k_{OH} = 9.8 \times 10^9\ M^{-1}\ s^{-1}$). However, EDCs co-exist with other organic and inorganic compounds, whose concentrations are relatively high in environmental water. The reaction of $HO^{\cdot}$ is less selective, and thus the generated $HO^{\cdot}$ is ineffectively consumed by the coexisting compounds. It is assumed that EDCs removal efficiencies are dependent on the initial concentrations of EDCs, co-existing compounds and their reactivities toward ozone and $HO^{\cdot}$.
In a study comparing sand filtration, ozone/hydrogen peroxide (AOP), microfiltration and reverse osmosis for the removal of estrogenicity from municipal wastewater, only AOP and reverse osmosis were able to remove total estrogenic activity for over 97 % while in other options, the removal was insufficient (Shishida et al., 2000).

2.5.4.Photolysis reactions and photocatalytic degradation

UV lamps are used widely for microbial disinfection of water and wastewater. In several cases, they also have been used for treatment of micropollutants. Because several ECDs and Pharmaceutical, Personal Care Products (PPCPs) have chromophores that lead to absorption of light at UV wavelengths, many may be amenable to transformation during UV treatment. Photolysis reactions have been extensively studied for estrogens removal from aqueous environment (Liu et al., 2004 and Segmuller et al., 2000). Liu and Liu (2004) examined the UV-light and UV–Vis-light (high-pressure mercury lamp) direct photolysis of two estrogens, βE2 and E1, in aqueous solution at high concentrations, 3.0 – 20 mg/L. The two estrogens undergo fast direct photolysis under irradiation with an UV disinfection lamp, and a high-pressure mercury lamp can also induce the photolysis of E1. The photolysis of both the estrogens causes the breakage and oxidation of benzene rings to produce compounds containing carbonyl groups. Photodegradation of EE2 in the solid state was observed but products were not identified (Segmuller et al., 2000).

The photosensitized degradation of EE2 in solution gave a hydroperoxide derivative (Segmuller et al., 2000).
Photocatalysis, as the name suggests, involves light and a catalyst to bring about a chemical reaction. Titanium dioxide photocatalysis is an alternative technique for the breakdown of organic pollutants in water and air. In titanium dioxide photocatalysis for water purification the pollutants are usually organic compounds and, therefore, the overall process can be summarized by the following equation.

$$\text{Organic pollutant} + O_2 \xrightarrow[h\nu,\ \lambda \le 400\ nm]{TiO_2} CO_2 + H_2O$$

Ohko et al. (2002) investigated E2 degradation by TiO_2 photocatalysis using TiO_2 (1 g/L) in suspension. After 30 min. reaction time 99 % of the initial E2 concentration (10^{-6} M) was removed. It was confirmed that E2 in water is completely mineralized as a result of the photocatalytic reactions. It was concluded in this study that the phenol moiety of the βE2 molecule should be the starting point of the photocatalytic oxidation. In addition, since the intermediate products do not have a phenol ring, Ohko et al. (2002) presumed that their estrogenic activities are negligible.
Coleman et al. (2000 and 2005) investigated the photolytic and photocatalytic degradation of estrogens in water using immobilized TiO_2. The reactions carried out in a batch reactor with TiO_2 immobilized on Ti alloy (Coleman et al., 2000) resulted in 98 % removal of E2 after 3.5 h reaction time and initial concentrations of 0.05 – 3 µmol/L. A quartz coil reactor coated internally with titanium dioxide (Degussa P-25) was shown effective for the removal of E2, E3 and EE2 in water at initial concentrations of 3 µM (Coleman et al., 2005). The results showed that photocatalysis and photolysis are capable of degrading all three oestrogens in water. It was shown that photocatalysis is much more effective than photolysis alone and all reactions follow pseudo first order kinetics. 17α-ethynyloestradiol degrades the fastest for both photocatalysis and photolysis followed by E2 and E3. This was attributed to the triple bond of the ethynyl group which absorbs UV light more easily. It was also shown that the relation ship between initial concentration (0.1 – 3 µM) and rate is linear for both photocatalysis and photolysis of E2 in water. Photocatalysis degrades E2 at twice the rate of photolysis.

2.5.5.Membrane filtration

Membrane filtration processes include microfiltration (MF, macropores > 50 nm), ultrafiltration (UF, mesopores 2 – 50 nm), nanofiltration (NF, micropores < 2 nm), reverse osmosis (RO, dense < 2 nm), dialysis, and electrodialysis (ED). NF distinguishes itself from RO as it only retains multivalent ions, so it has an economic advantage when the retention of monovalent ions is not required (Schäfer et al., 2003).
The most important way to remove estrogens with membrane filtration is by retention on the membrane or by adsorption to organic particulates, since membrane pores are still larger than the radius of for example E1, which is 0.84 nm, while the average pore radius

for a 1000 Da membrane is 0.94 nm (Schäfer et al., 2002b). The adsorption capacity of the membranes for hormones could be affected by membrane types, pH, affinity of hormones to water, as well as the presence of other organics (Chang et al., 2002b). E1 retention is higher in the presence of organics (Schäfer et al., 2002b; Schäfer and Waite, 2002), since the compound is attached to the organic, which is retained by the membrane.
A number of commercially available NF and RO membranes have been investigated for the retention of E1 dissolved in carbonate buffer (Schäfer et al., 2003). In general the retention at an initial concentration of 100 ng/L was very good, 95 – 99 % with the exception of one, which was 80 %. For the membrane types used, both size exclusion and adsorptive effects are responsible for maintaining high retention of E1. Adsorptive effects appear to be particularly important for retention by NF membranes exhibiting relatively low ion retentions. These adsorptive effects may be driven by hydrogen bonding between E1 and the membranes. Deprotonation leads to a significant decrease in retention, possibly as a result of a critical role of the hydroxyl-group or as a result of strong electrostatic repulsive forces (Schäfer et al., 2003). The amount of sorption of E1 was researched for different types of membranes, different pH values, ionic strength and competition by other organics (Chang et al., 2002b). It was concluded that E1 has a higher affinity for hydrophobic membranes. There was not much difference between an ionic strength of 0.02 and 0.2 M and the pH only has an influence above pH 11, as the molecules become charged, lowering the affinity for the membrane, since they are both negatively charged. There was competition with other organic materials, since E1 removal in a buffer solution showed higher removal compared to E1 removal in surface water and secondary effluent, although the removal was not influenced dramatically. The retention on the membrane decreases with the increase in the surface concentration and a breakthrough will occur when the surface concentration reaches the equilibrium value for the corresponding feed concentration (Chang et al., 2002a, b).
Even with MF or UF, pore sizes are too big and the main removal mechanism will be adsorption to the membrane, which is low at neutral pH and decreased at pH higher than 10.5 (Schäfer and Waite, 2002).
Kimura et al. (2004) investigated the removal of E2 by two types of RO membranes. It was shown that at an initial E2 concentration of 100 µg/L, the polyamide membrane removed 83 % of E2 while the cellulose acetate membrane only removed 29 % of E2.
Nghiem et al. (2004) investigated the removal of the natural hormones E1, E3, progesterone and testosterone by NF at initial concentrations of 100 ng/L. The results indicated that adsorption of hormones to the membrane polymer is the dominant removal mechanism in the early stages of filtration. Because the adsorptive capacity of the membrane is limited, the final retention stabilizes when the adsorption of hormones has reached equilibrium. At this later filtration stage, the overall hormone retention is lower than that expected based solely on the size exclusion mechanism. This behavior is attributed to partitioning and subsequent diffusion of hormone molecules in the membrane polymeric phase, which ultimately results in a lower retention. Hormone diffusion in the membrane polymeric matrix most likely depends on the size of the

hormone molecule, hydrogen bonding of hormones to membrane functional groups, and hydrophobic interactions of the hormone with the membrane polymeric matrix.

One of the possible solutions to enhancing removal of endocrine compounds from secondary effluent is to combine low-pressure membrane processes such as UF or MF with other physicochemical separation methods in so-called **hybrid membrane processes**. In such systems, MF or UF membranes can be a positive barrier for various particulates including clays, metal oxides, algae, bacteria, and parasites, while adsorption to added particulates (activated carbon) or hydrolysable coagulants could be effective for removal of dissolved organic compounds.
Microfiltration was compared to a microfiltration Powdered Activated Carbon (PAC) hybrid system for the removal of E1 from a buffer solution (12.2 – 13.8 ng E1/L) and a secondary effluent (14.8 – 15.4 ng E1/L) (Ong et al., 2001; Chang et al., 2004). For the buffer solution it was shown that the retention of E1 to the MF membrane was less than 5 % before PAC addition. For a PAC dosage lower than 20 mg/L, the removal rate was a strong function of PAC dosage. For PAC concentrations of 20 mg/L or higher, about 91 % removal was achieved in the first hour and maximum removal (96 %) was reached in 3 h. A lower E1 removal was achieved with the secondary effluent over the period of the tests, suggesting that the presence of other organics not only impacted the removal degree but also the removal rate (Chang et al., 2004).

2.5.6.Activated carbon

PAC has an adsorption capacity between 2 – 62 ng/mg for E1 applied at concentrations of 3.6 – 65 ng/L (Ong et al., 2001). The adsorption of E1 is linear in a buffer solution, whereas using surface water and STP effluent it is not due to a preloading with other organics adsorbing to PAC as well. In a buffer solution with a concentration of 100 ng E1/L, a concentration of 5 mg/L PAC was removing more than 80 % of E1, and at 20 mg/L more than 95 %, whereas for surface water containing E1 100 ng/L 80 % removal was only achieved at a PAC concentration of 50 mg/L and for STP effluent it was not achieved at this concentration. As a post-treatment system emphasizing on the removal of estrogens, the use of PAC may not be suitable, as a lot of PAC will be needed to achieve a sufficient removal. Chang et al. (2004) reported adsorption capacities in carbonate buffer solution ranging from about 1.0 to 17 ng/mg and in secondary effluent from 0.3 to 5.7 ng/mg for equilibrium dissolved estrone concentrations of 1.3 – 17.4 ng/L.
Westerhoff et al. (2005) investigated the removal of estrogens and androgens from several spiked surface waters by adding different concentrations of PAC. The average estrogen removal percentages with a PAC concentration of 5 mg/L and a contact time of 4 h were 76 % for E1, 84 % for E2, 60 % for E3 and 77 % for EE2 for initial concentrations between 50 and 170 ng/L. The average progesterone removal percentage was 86 % (5 mg PAC/L, 4 h contact time) at an initial concentration of 50 ng/L. The average androgen removal percentages with a PAC concentration of 5 mg/L and a contact time of 4 h were 79 % for testosterone and 80 % for androstenedione for initial concentrations of 60 and 70 ng/L respectively. The removal percentages were increased

by increasing the PAC concentration. At a low dosage of 1 mg/L, removal of steroids ranged between 40 and 75 %, while dosages of 20 mg PAC/L effectively removed > 91 % of the steroids. It was also shown that the removal of βE2 was nearly independent for initial concentrations between 6.8 ng/L and 1360 ng/L (Westerhoff et al., 2005).
Fuerhacker et al. (2001) concluded that the adsorption of E2 to Granular Activated Carbon (GAC) is insufficient as at equilibrium, only 49 – 81 % of the E2 in the 1 – 100 ng/L range is adsorbed in deionised water.

2.5.7.Treatment with manganese oxide

De Rudder et al. (2004) explored the use of manganese oxide (MnO_2) as an oxidative removal substrate. MnO_2 is a well-known solid phase oxidant, and its surface redox reactions with xenobiotic organic chemicals have been extensively studied. In natural waters, the main manganese source is Mn(II). It has been proposed that the oxidation of Mn(II) in humic-rich environments is a possible mechanism by which bacteria can utilize the large biologically recalcitrant pools of carbon contained in humic substances. After being oxidized, the manganese precipitates around cells or accumulates on slime layers or sheaths (Corstjens et al., 1992). This precipitated manganese is postulated by the latter authors to abiotically oxidize humic and fulvic acids releasing low molecular organic compounds such as pyruvate, acetone, formaldehyde, and acetaldehyde. The latter are then bioavailable for the Mn-oxidizing organisms. Although historically most research regarding Mn(II)-oxidizing bacteria has focused on *Bacillus* species, *Leptothrix discophora* and *Pseudomonas putida* (Francis et al., 2001), new microorganisms have recently been shown to oxidize manganese, indicating that microbiological Mn oxidation is widespread in nature (Tebo et al., 2000).
De Rudder et al. (2004) obtained an EE2 removal of 81.7% when synthetic wastewater containing 15 µg EE2/L was treated using manganese oxide (MnO_2) (reaction time of 1.12 h). Moreover, since the MnO_2 reactor was not yet saturated after 40 days of treatment, they concluded that EE2 was not only adsorbed to the MnO_2 granules, but most probably also degraded into others compounds. Thus, the self-regenerating cycle of MnO_2 seems possible. This can make this treatment cost-effective, because the matrix does not have to be replaced (De Rudder et al., 2004). However, De Rudder et al. (2004) did not identify the EE2 metabolites and neither their estrogenic activity.

2.5.8.Electrolysis

Electrolysis of the effluent can be considered. This approach has been tested successfully for MBR effluent with EE2 as a model compound at LabMET at power consumptions of 0.06 to 0.6 kWh/m³ water treated. The drawback of this technology is the formation of chlorinated by-products which tend to be more recalcitrant than the parent compound. Another drawback is the pollution of the electrodes by oppositely charged particles. This technology is only feasible if the water is particle free. This is no problem because the membranes of compartment I remove the particles from the water.

2.5.9.Hydrothermal oxidation

Another promising technology is the hydrothermal oxidation at high temperatures and high pressures. This technology yields total liquefaction of all materials under certain conditions with a concomitant complete sterilization, given the reaction conditions. This technology seems very promising. However, regarding the removal or degradation of xenobiotic compounds, no data are available.

2.5.10. Bioaugmentation by slow-release tubes

For biological technologies, dedicated microorganisms can be selected to perform the tasks at hand. The dedicated microorganisms will have to function at low cell densities whilst being resilient to washout. Firstly, the Slow Release Seeding of dedicated bacteria, in which dedicated bacteria are primed and confined in slow release capsules is of interest. The microorganisms contained in the slow release capsules procreate inside the tubes, and slowly seed viable, optimally degrading bacteria into the system of concern. This technology has recently been developed for use in a bioreactor for the continuous removal of 3-chloroaniline, and has proven very useful (Boon et al., 2002). Because of slow microbial adaptation and growth, there is seldom sufficient metabolic capacity to protect reactors from these xenobiotics. Lab cultured inocula, which can transform the xenobiotica very efficiently in pure cultures, are however usually of little effectiveness once they are inoculated into an established microbial community.

3. Pharmaceuticals

3.6. General

- Antibiotics

There are several classes of antibiotics, which can be subdivided according to their molecular structure into:
- Aminoglycosides: ex. Gentamicin, Tobramycin, Amikacin
- β-Lactams: ex. penicillins, cephalosporins, carbapenems, monobactams
- Glycopeptides: ex. Vancomycin
- Lincosamides: ex. Clindamycin
- Macrolides: ex. Erythromycin
- Oxazolidinones: ex. Linezolid
- Quinolones and Fluoroquinolones: ex. Ciprofloxacin
- Sulfonamides: ex. Sulfamethoxazole
- Streptogramins
- Tetracyclines: ex. Tetracycline

In this Technical Note, the group of Fluoroquinolones (Ciprofloxacin) and Sulfonamides (Sulfamethoxazole) were studied. The reason for their selection is explained in TN80.12.

- Analgesics + Anti-inflammatory drugs

There are several drugs that suppress inflammation in a manner similar to steroids, but without their side effects, referred to as non-steroid anti-inflammatory drugs (NSAIDS). Many of these pharmaceuticals also have analgesic (pain killing) and/or antipyretic (fever reducing) activities. There are many different types of NSAIDS available over the counter, such as Ibuprofen, and also under prescription, such as Naproxen and Diclofenac. Also aspirin is included in this study as analgesic and anti-inflammatory agent.

- β-blockers

Examples of β-blockers are Metoprolol, Propanolol, Timolol, Betaxolol, Bisoprolol, Carazolol.

- Anti-depressants

The most common form of anti-depressants is a group called benzodiazepines, which includes Temazepam and Diazepam (most commonly known as Valium).

3.7. Occurrence of selected pharmaceuticals in terrestrial environments

Please review documents in Attachments 1 and 2.

3.8. Expected concentrations of pharmaceuticals in space environment

In the following paragraphs, excretion percentages of pharmaceuticals are given. These estimated ranges can be taken into account to assess the content of pharmaceuticals which will be put into compartment I, as soon as is decided upon the amount of urine to be put into the feed.

3.8.1.Antibiotics

Urine concentrations of Sulfamethoxazole can be considerably higher than the concentrations in the blood. The average percentage of the dose recovered in urine from 0 to 72 hours after a single oral dose is 84.5 % for total sulfonamide. 30 % of the total sulfonamide is excreted as free sulfamethoxazole, with the remaining as N4-acetylated metabolite.

The proportion of the relative amount of metabolites to the total amount of drug excreted in urine increased from 29.7 % after intravenous administration to 42.7 % after oral dosing of Ciprofloxacin, indicating a first-pass effect of the liver.

The structure of both compounds is shown in Figure 2.

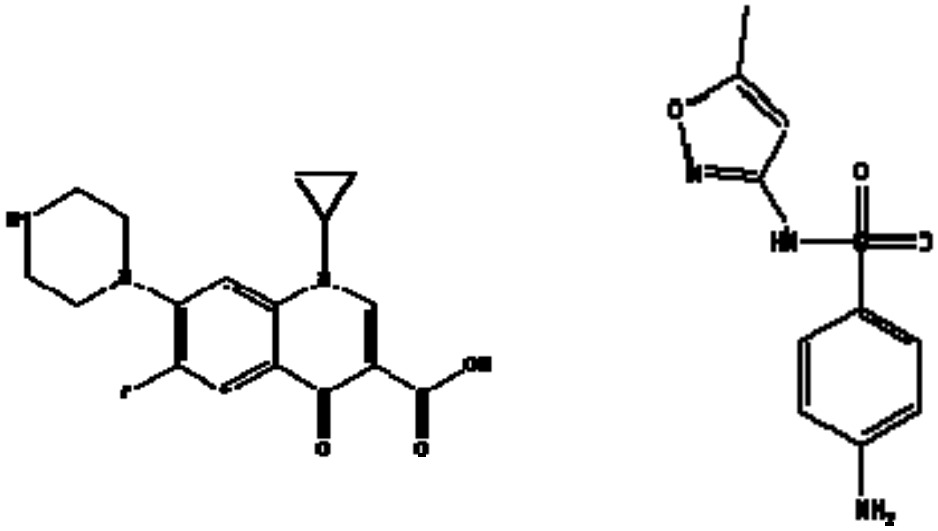
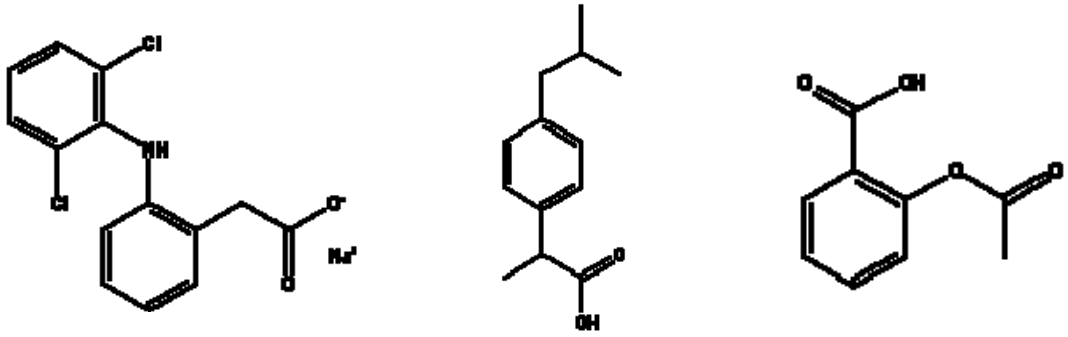


Figure 2. Chemical structure of Ciprofloxacine (left) and Sulfamethoxazole (right)

3.8.2.Analgesics + Anti-inflammatory drugs

Ternes et al. (1998) reported that 15 % of Diclofenac is excreted in urine as unchanged drug and < 1 % as glucuronides. For Ibuprofen the amount of unchanged drug excreted in urine varies between 1 and 8 %, whereas the percentage of glucuronides is 14 %.
The major urinary metabolites of Aspirin include salicyluronic acid, salicyl-O-glucuronide, salicyl ester glucuronide and free salicylic acid.
The structures for all compounds are given in Figure 3.



Diclofenac **Ibuprofen** **Aspirin**

Figure 3. Chemical structure of some important NSAIDS

3.8.3.β-blockers

According to Ternes et al. (1998) < 1 % of Propanolol and 3 – 10 % of Metoprolol is excreted as unchanged drug in urine.

3.8.4.Anti-depressants

The anti-depressant Diazepam is usually not detected in sewage. It is thought to be completely metabolized in the human body and is excreted as oxazepam and demethil-diazepam (Suárez et al., 2005).

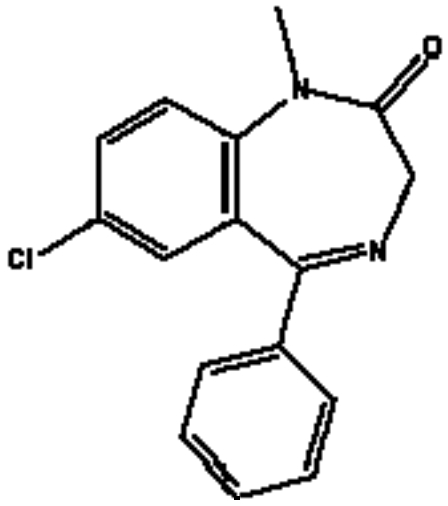


Figure 4. Chemical structure of Diazepam

3.9. Biological degradation of pharmaceuticals

3.9.1.Aerobic degradation of pharmaceuticals

3.9.1.1. *Antibiotics*

Generally, biological treatment processes have been shown to be ineffective in the removal of antibiotics. For example, Ingerslev and Halling-Sorensen (2000) found that 12 different sulfonamides were not readily biodegradable in activated sludge. Khan and Ongerth (2004) reported Sulfamethoxazole removal percentages of 5 – 27 %, whereas Kreuzinger et al. (2004a) reported a removal range in STPs of 33 – 91 %. Also Perez et al. (2005) reported that sulfonamides were characterized by a general biodegradability in the primary and secondary treatment. Drillia et al. (2005) studied the aerobic degradation of Sulfamethoxazole in an aerobic sequencing batch reactor inoculated with non-adapted activated sludge. It was found that Sulfamethoxazole was eliminated even when the feed concentration was as high as 383 mg/L. Since the inoculum used for the SBR start-up was not acclimated to the pharmaceutical, the microorganisms responsible for the biodegradation of Sulfamethoxazole must be common bacteria; species present in the activated sludge process. Sulfamethoxazole served as a carbon and /or nitrogen source for the bacteria and it seemed that the enzymatic mechanism responsible for the Sulfamethoxazole degradation was not activated as long as there was readily degradable

carbon source available in excess of ammonium. In case there is a depletion of easily biodegradable matter, Sulfamethoxazole degradation is more likely to take place.
Al-Ahmad et al. (1999) and Kümmerer et al. (2000) reported that Ciprofloxacin was not biodegradable in the Closed Bottle Test. The behaviour of fluoroquinolone antibacterial agents (Ciprofloxacin) during mechanical-biological wastewater treatment was studied by Golet et al. (2003). It was shown that wastewater treatment resulted in a reduction of the fluoroquinolone mass flow of 88 – 92 %, mainly due to sorption on sewage sludge. These results suggest sewage sludge as the main reservoir of fluoroquinolone residues. Wetzstein et al. (1999) studied degradation of Ciprofloxacin by Basidiomycetes by monitoring $^{14}CO_2$ formation from [^{14}C]-Ciprofloxacin in liquid cultures. Sixteen species inhabiting wood, soil, humus, or animal dung produced up to 35 % $^{14}CO_2$ during 8 weeks of incubation.
Also Vieira da Silva (2005) made a literature review and discusses the extended group of several antibiotics.

3.9.1.2. *Analgesics + Anti-inflammatory drugs*

Ternes et al. (1998) reported a maximal Acetylsalicylic acid removal percentage of 81 % in a German STP.
The Diclofenac removal percentage has been reported by several auhors (Table 4).
Ibuprofen is mainly excreted as two metabolites: hydroxyl (IBP-OH) and carboxyl (IBP-CX). It is widely reported in literature (Weigel et al., 2004; Ternes et al., 2001; Buser et al., 1999) that while IBP and IBP-CX were almost quantitatively eliminated (> 95 %) during biological treatment, IBP-OH was hardly affected (less than 20 %) and thus is the dominant compound in STP effluents and rivers. In literature, the removal efficiencies of Ibuprofen range from 0 to 100 %, depending on the type of treatment used and the SRT of the plant (Table 4). Kanda et al. (2003) reported that the removal of Ibuprofen is higher at plants using activated sludge treatment or an oxidation ditch compared to biological filters or reed beds; again related to the SRT.
In literature, the removal efficiencies of Naproxen range from 15 – 93 % (Table 4).

Table 4. Removal efficiencies of the major anti-inflammatory drugs according to literature

Compound	Removal efficiency	Reference
Diclofenac	69 %	Ternes et al., 1998
	0 %	Tauxe-Wuersch et al., 2005
	0 – 69 %	Kreuzinger et al., 2004a
	17 %	Heberer et al., 2002
	53 – 74	Strenn et al., 2004
	23 ± 30 %	Quintana et al., 2005
	9 – 60 %	Lindqvist et al., 2005
	71 %	Roberts and Thomas, 2005
	75 %	Andreozzi et al., 2003
	50 %	Buser et al., 1998
	5 – 9 %	Stumpf et al., 1999
	7 – 31 %	Khan and Ongerth, 2004
	< 20 – 80 %	Clara et al., 2005
	< 10 – 80 %	Paxeus, 2004
Ibuprofen	60 – 70 %	Carballa et al., 2004
	90 %	Ternes et al., 1998
	22 – 75 %	Stumpf et al., 1999
	96 – 99 %	Buser et al., 1999
	14 – 100 %	Kanda et al., 2003
	(), 4 – 52 %	Khan and Ongerth, 2004
	< 20 – 99 %	Clara et al., 2005
	0 – 99 %	Kreuzinger et al., 2004a
	52 – 99 %	Paxeus et al., 2004
	> 90 %	Strenn et al., 2004
	> 90 %	Fahlenkamp et al., 2004
	> 90 %	Metcalfe et al., 2003
	97 ± 4 %	Quintana et al., 2005
	98 %	Roberts and Thomas, 2005
	53 – 79 %	Tauxe-Wuersch et al., 2005
	(), 78 – 100 %	Lindqvist et al., 2005
Naproxen	40 – 55 %	Carballa et al., 2004
	66 %	Ternes et al., 1998
	15 – 78 %	Stumpf et al., 1999
	3 – 58 %	Khan and Ongerth, 2004
	42 – 93 %	Paxeus, 2004
	40 – 100 %	Metcalfe et al., 2003
	100 %	Thomas and Foster, 2004
	71 ± 18 %	Quintana et al., 2005
	55 – 98 %	Lindqvist et al., 2005

3.9.1.3. *β-blockers*

Both Propanolol and Metoprolol show a high degree of persistence in the aquatic environment, although they exhibit a high metabolic rate in humans (Bendz et al., 2005). Also Roberts and Thomas (2005) reported that Propanolol was not removed during sewage treatment. The treatment of wastewaters by activated sludge usually did not result in any practical removal (< 10%) of Metoprolol and Atenolol (Andreozzi et al., 2003; Paxeus et al., 2004). However, Ternes et al. (1998) reported 83 % removal of Metoprolol and 96 % removal of Propanolol in a German municipal STP.

3.9.1.4. *Anti-depressants*

Suárez et al. (2005) observed Diazepam removal rates below 10 % during a nitrifying-denitrifying process in an activated sludge system with an initial concentration of 20 ppb. Kreuzinger et al. (2004a) reported Diazepam removal percentages up to 25 % in a wastewater treatment plant with a SRT of 24 days. Kreuzinger et al. (2004b) reported that Diazepam hardly showed any removal during wastewater treatment and remained stable during post treatment steps as well as in the groundwater. Beausse et al. (2004) reported removal percentages below 50 % during aerobic wastewater treatment. Van der Hoeven (2004) reported a maximal Diazepam removal percentage of 93 % in a STP.

3.9.2.Anaerobic biodegradation of selected pharmaceuticals

3.9.2.1. *Antibiotics*

Fountoulakis et al. (2004) studied the effect of Sulfamethoxazole on mesophilic methanogenesis at concentrations ranging from 0 up to 400 mg/L. The results showed that Sulfamethoxazole did not affect methanogenesis even at high concentrations. Carballa et al. (2005) reported a very high removal of Sulfamethoxazole by degradation in both the mesophilic (> 95 %) and thermophilic (85 – 95 %) range, independently of the HRT.

3.9.2.2. *Analgesics +Anti-inflammatory drugs*

Fountoulakis et al. (2004) studied the effect of Diclofenac on mesophilic methanogenesis at concentrations ranging from 0 up to 400 mg/L and they tried to relate the final effect with the tendency of the compounds to sorb on the anaerobic biomass. The results were that Diclofenac caused severe inhibition at high concentrations (200 – 400 mg/L), moderate inhibition at a concentration of 100 mg/L and no inhibition at all at 10 and 50 mg/L. They found a direct correlation between the level of the pharmaceuticals inhibition and the affinity to sorb on the anaerobic sludge. But it should be pointed out that at the concentrations levels usually prevailing in STPs, no significant impact of any pharmaceutical is anticipated. Carballa et al. (2005) could not obtain clear results for the anaerobic digestion of sewage sludge spiked with initial Diclofenac concentrations of 10 µg/L. In some cases the removal could not be quantified due to the high deviation of the data. In those cases where it was possible, the efficiencies ranged between 25 and 75 %.
Carballa et al. (2005) reported a medium elimination of Ibuprofen in both mesophilic (30 – 60 %) and thermophilic (40 – 55 %) digestion of sewage sludge with initial concentrations of 20 µg/L.
Carballa et al. (2005) showed very high removal of Naproxen (initial concentration = 20 µg/L) by degradation in both the mesophilic (80 – 85 %) and thermophilic (80 – 95 %) range, independently of the HRT.

3.9.2.3. *β-blockers*

No data were found on the anaerobic degradation of β-blockers. Fountoulakis et al. (2004) studied the effect of Propanolol on mesophilic methanogenesis at concentrations ranging from 0 up to 400 mg/L. It was shown that 50 mg/L of Propanolol caused a significant inhibition of the methanogenesis step. It should be pointed out that these concentrations are much higher than the ones expected in a spatial environment.

3.9.2.4. *Anti-depressant*

Carballa et al. (2005) reported Diazepam removal percentages between 20 and 60 % after sludge adaptation during mesophilic and thermophilic anaerobic digestion of sewage sludge with initial spiked concentrations of 20 μg/L.

3.10. Other technologies for the removal of selected pharmaceuticals (chemical, physical)

3.10.1.Coagulation/Flocculation and Flotation

Literature information about the removal of pharmaceuticals by physico-chemical processes is scarce. When some data is available, it is related to either a post-treatment or to drinking water treatment, and they are normally combined with other technologies, such as activated carbon or filtration (Ternes et al., 2002; Boyd et al., 2003; Stackelberg et al., 2004). Results can not be compared since the type and content of solids and organic matter in the raw waters of drinking water facilities differs considerably from municipal wastewaters.

Boyd et al. (2003) studied the fate of some pharmaceuticals during drinking water facilities with different treatment technologies in Louisiana and Ontario, and they reported that conventional drinking water processes (coagulation-flocculation/sedimentation step with PAC addition) do not remove Naproxen. Adams et al. (2002) reported no significant removal of selected antibiotics with aluminium or ferric salt coagulation. Similarly, Ternes et al. (2002) reported no significant elimination of selected pharmaceuticals, Carbamazepine (13 %) and Diclofenac (4%), using ferric chloride coagulation in lab-scale experiments (≈ 20 mg/L) and investigations in waterworks (6 – 13 mg Fe^{3+}/L).

Stackelberg et al. (2004) reported little or no removal of Ibuprofen and Sulfamethoxazole during conventional drinking water treatment, which includes coagulation-flocculation/sedimentation with PAC addition and filtration. He stated that sorption efficiencies depend on competition with other organic compounds; therefore, the adsorption capacity for pharmaceuticals in a facility that processes raw water that contains substantial amounts of many naturally occurring and anthropogenic organic compounds is expected to be smaller than that in laboratory and pilot-scale experiments in which fresh activated carbon and deionized water were used.

Also Westerhoff et al. (2005) reported low removal percentages (< 20 %) for Sulfamethoxazole, Naproxen, Ibuprofen, Diclofenac and other pharmaceuticals after a chemical treatment with aluminium or iron chloride.
Carballa et al. (2005) investigated the removal of pharmaceuticals present in sewage by coagulation/flocculation and flotation. During the coagulation/flocculation assays, the removal percentage for Diclofenac was 50 – 70 %, the concentration of Diazepam and Naproxen were reduced by 20 – 25 %. Ibuprofen could not be removed by means of coagulation/flocculation. During the flotation assay, the removal parameters were as follows: 40 – 50 % for Diazepam, 20-45 % for Diclofenac, 10 – 25 % for Ibuprofen, 10 – 30 % for Naproxen.

3.10.2.Chlorination process

A study by Boyd et al. (2003) indicated that chlorination may be an effective treatment for reducing the concentration of Naproxen that was observed in Mississippi River and Detroit River waters. Adams et al. (2002) showed reduction of seven spiked (50 µg/L) antibiotics in distilled water and Missouri River water by chlorination. The HPLC/UV chromatograms from this study show that oxidation by-products are being formed from chlorination. However, the possible formation of chlorinated by-products (and their relative toxicity) were not investigated. Westerhoff et al. (2005) reported removal percentages of 95 % for Diclofenac, 95 % for Naproxen, 90 – 98 % for Sulfamethoxazole, 75 % for Diazepam and 30 – 75 % for Ibuprofen during chlorination.

Chlorine dioxide (ClO_2) is an oxidant used for the disinfection of relatively high quality water, such as groundwater or treated surface water. Chemically, ClO_2 is a stable free radical that reacts with other water matrix components and micropollutants mainly through a one electron transfer reaction. ClO_2 is a highly selective oxidant with respect to specific functional groups of organic compounds like phenolic moieties or tertiary amino groups. The reactivity of these moieties is governed by speciation, because the reactivity of the phenoxide ion and the neutral form of the amine is many orders of magnitude higher than the reactivity of the neutral phenol and the protonated amine. Many pharmaceuticals exhibit phenolic moieties and/or amino groups in their structure. Huber et al. (2005b) showed that macrolide and sulfonamide antibiotics as well as estrogens and phenazones are readily oxidized by ClO_2. However, many of the investigated compounds did not react at an appreciable rate with ClO_2. Therefore, it can be concluded that ClO_2 applied in water treatment only acts as a partial barrier for pharmaceuticals. ClO_2 appears slightly more powerful than chlorine for the oxidation of pharmaceuticals. For a more comprehensive comparison of these oxidants, additional knowledge about the formation of oxidation products and their pharmacological or biological effects would be necessary.

3.10.3.Ozonation and Advanced Oxidation Processes (AOPs) with ozone

Ternes et al. (2002) showed 97 % removal of Diclofenac at an ozone dose of 0.5 mg/L and an initial spiked concentration of 1 µg/L. Ternes et al. (2003) assessed the removal of pharmaceuticals from municipal wastewater using a pilot ozonation and UV-disinfection plant receiving effluent from a German STP. In the original STP effluent, 5 antibiotics (0.34–0.63 μg L−1), 5 beta-blockers (0.18–1.7 μg L−1), 4 antiphlogistics (0.10–1.3 μg L−1) were detected. By applying 10–15 mg/L ozone (contact time 18 min.), all the pharmaceuticals investigated were no longer detected. Also Vogna et al. (2004) showed that ozonation is effective in inducing Diclofenac degradation, ensuring a complete conversion of the chlorine into chloride and a mineralization degree of 32 % after 90 min. treatment. Also H_2O_2/UV was found to be effective ensuring 39 % mineralization after 90 min. Westerhoff et al. (2005) evaluated the removal efficiency of several pharmaceuticals during ozonation. Compounds which were removed over 80 % include Sulfamethoxazole, Diclofenac, Ibuprofen, Naproxen, Diazepam. Huber et al. (2003) concluded that ozonation and other AOPs are promising processes for an excellent removal of pharmaceuticals in drinking water. Huber et al. (2005a) reported more than 90 – 99 % removal of macrolide and sulfonamide antibiotics Diclofenac and Naproxen for ozone doses >= 2 mg L-1. Also Adams et al. (2002) reported that ozonation reactions with sulfonamides (antibiotics) were rapid. Even with very low bulk ozone concentrations (below levels typically employed in water treatment plants), ozone was found to be highly effective at achieving pharmaceutical oxidation to levels below detection limits. Boyd et al. (2003) showed that ozonation is an effective treatment method for reducing the concentration of Naproxen. It should be noted that very little is known about the formation of by-products during ozone degradation of organics. Zwiener and Frimmel (2000) investigated three environmentally relevant substances (Diclofenac, Ibuprofen and Clofibric acid). From these substances only Diclofenac was sufficiently degraded by using the selective oxidant ozone alone at a concentration generally applied in drinking water treatment. The application of AOP (e.g., O_3/H_2O_2) improved the degradation efficiency of all investigated pharmaceuticals significantly. However, the degradation efficiency of an AOP is limited by the radical scavenging capacity of the matrix of the treated water. This limitation can be overcome by increasing the oxidant concentration. It has also to be taken into account that the ozone consumption by organic matter (DOC) is of fundamental importance. For instance, for a sufficient degradation of the pharmaceuticals (> 90%) the ozone concentration has to be equal to the DOC value. In drinking water treatment higher oxidant concentrations and the combination application of ozone and hydrogen peroxide are recommended for a close to quantitative degradation of pharmaceuticals. A sound assessment of the efficiency of the process and of its physiological relevance needs further information on the main degradation products and on the pathway of their formation.

3.10.4.Photolysis reactions and photocatalytic degradation

Several pharmaceutical compounds have been shown to degrade due to the action of sunlight (Boreen et al., 2003). The most extensively studied of these compounds is the analgesic/anti-inflammatory drug Diclofenac, which has been shown to degrade in the aquatic environment due to ultraviolet (UV) light.

Andreozzi et al. (2003) carried out a monitoring survey of STP effluents in Italy, France, Greece, and Sweden and found more than 20 individual pharmaceuticals. The photodegradation of six compounds (Carbamazepine, Diclofenac, Clofibric acid, Ofloxacin, Sulfamethoxazole and Propranolol) was tested. Carbamazepine and Clofibric acid were found to have the longest halflives (of the order of 100 days at the most northerly areas sampled), whereas Sulfamethoxazole, Diclofenac, Ofloxacin, and Propranolol were found to undergo faster degradation with half-lives of 2.4, 5.0, 10.6, and 16.8 days, respectively. For almost all the studied compounds, except Propranolol, the presence of nitrate ions in aqueous solutions resulted in a reduction of the measured half life. This effect may be ascribed to the formation of HO radicals due to photolysis of nitrate. The authors point out that besides pharmaceutical residues, other species targeted by OH radicals, such as naturally occurring organic constituents, are present in rivers and lakes. For this reason, the effect caused by nitrate on the degradation rates of the pharmaceuticals found in this study should be interpreted only as a tendency if no other organic molecules but the substrate are present in the test solution. A more complex situation arose when humic acids were added to the solutions containing the pharmaceuticals. Humic acids are known to exert two opposite effects on the rate of photodegradation of organic molecules in water. Due to their capability to absorb UV radiation in a broad range of wavelengths, they can reduce the available energy for the organic molecules present in the solution, thus acting as an inner filter (thus decreasing photodegradation). At the same time, the molecules of humic acids submitted to UV irradiation are promoted to a transient, excited state, in which they may react with oxygen in the solution, forming reactive species as singlet oxygen, or react directly with other organic species, thus promoting their phototransformation. The overall effect of humic acids on the phototransformation rate of an organic substance will therefore depend on the balance between these two opposite contributions. In the study, humic acids were found to act as inner filters toward Carbamazepine and Diclofenac, but as photosensitizers toward Sulfamethoxazole, Clofibric acid, Oflaxocin, and Propranolol.

Buser et al. (1998) established that up to 90% of Diclofenac entering a Swiss lake was degraded with a half-life of less than 1 h−1. Incubation of lake water, fortified with Diclofenac, exhibited no reduction in the dark, suggesting minimal chemical and biological degradation. However, when the fortified water was exposed to sunlight, rapid degradation was observed that indicated that this was the result of photodegradation. The use of sewage lagoons may therefore increase the removal of light sensitive compounds as demonstrated by Kreuzinger et al. (2004b) who showed that removal rates of Diclofenac were only 14% with just activated sludge treatment, while after further polishing in a sewage lagoon concentrations decreased to below the limits of detection.

Adsorption and biodegradation were ruled out as the cause of the decrease, as there was no developed/active sludge flock in the lagoon, leaving photodegradation as the most likely cause.
However, the extent of photo-induced degradation of pharmaceuticals can vary significantly for different pharmaceuticals, and it strongly depends on the aqueous constituents (such as humic and fulvic acids) present in solution. In addition, light levels within STPs are likely to be much lower than in the environment (effectively zero), due to the higher solids content. Indeed, Koutsouba et al. (2003) found Diclofenac to be widespread in Greek domestic sewage effluent, with concentrations in effluent ranging from 10 to 365 ng/L. Given the inherent photosensitivity of this compound, its presence in sewage effluent would seem to indicate that photodegradation is highly unlikely to take place within STPs where light penetration is minimal at best.
Andreozzi et al. (2004) also investigated the removal of pharmaceuticals by means of a TiO_2 photocatalytic system (membrane immobilized catalyst). The removal percentages obtained were 87 ± 9 % for Diclofenac (initial concentration: 3.46 mM, reaction time: 144 h), 65 ± 4 % for Diclofenac (initial concentration: 0.670 mM, reaction time: 158 h), 85 ± 4 % for Naproxen (initial concentration: 9.98 mM, reaction time: 141 h).

3.10.5.Membrane filtration

Kimura et al. (2003) investigated the rejection of organic micropollutants by polyamide NF/RO membranes in bench-scale filtration experiments. Experimental results clearly showed that negatively charged compounds (Diclofenac, Salicylic acid) could be rejected to a great extent (i.e., > 90 %) regardless of physico-chemical properties of the tested compounds. In contrast, rejection of non-charged compounds was found to be influenced mainly by the size of the compounds. It was found that the concentration range of solutes might influence the rejection efficiency of a membrane. In this study, experiments at a low concentration range were found to show lower rejection efficiency. In Kimura et al. (2004) the rejection of neutral uncharged pharmaceuticals by RO membranes was investigated in bench-scale crossflow experiments. With a polyamide membrane 70 % rejection of Sulfamethoxazole could be achieved, while with a cellulose acetate membrane 82 % of Sulfamethoxazole was rejected.

3.10.6.Activated carbon

Ternes et al. (2002) showed that filtration with GAC was very effective in removing pharmaceuticals. Even in relatively high concentrations, the pharmaceuticals could be almost completely removed at specific throughputs over 70 m³/kg. Adams et al. (2002) reported 25 – 50 % removal of antibiotics from Missouri River water in batch experiments with a PAC dosage of 5 mg/L, and > 90 % removal for a PAC dosage of 50 mg/L. Adams et al. (2002) and Boyd et al. (2003) noted that for the Louisiana drinking water treatment plant, routine addition of 2 mg/L of PAC, which is used for the removal of natural organic matter in Mississippi River water, does not appear effective in reducing low-level concentrations of Naproxen. Westerhoff et al. (2005) investigated the removal

of several pharmaceuticals by addition of 5 mg PAC/L and a contact time of 4 h. The following removal percentages were obtained: 16 % for Ibuprofen, 36 % for Sulfamethoxazole, 39 % for Diclofenac, 52 % for Naproxen and 67 % for Diazepam. At a higher PAC concentration (20 mg/L), 92 % of Diclofenac and 80 % of Ibuprofen were removed.

3.10.7.Treatment with manganese oxide

Zhang et al. (2005) reported that fluoroquinolones are highly susceptible to manganese oxide-facilitated oxidation. Reaction of fluoroquinolones with manganese oxides yielded various N-dealkylated, hydroxylated and possibly coupling oxidation products. No other references on the use of manganese oxide for the removal of pharmaceuticals were found.

3.10.8.Electrolysis

The electrochemical oxidation of drug residues in water was investigated by Weichgrebe et al. (2004). It was shown that the electrochemical oxidation is a sufficiently effective method for destroying drug residues like Acetylsalicylic acid, Tetracycline and Gentamicine in water. Similar degradation results are achieved with a C-anode (modified by manganese oxides) and a Pt anode.

3.10.9.Hydrothermal oxidation

Regarding the removal or degradation of pharmaceuticals by means of hydrothermal oxidation, no data are available.

4. Avoidance and pretreatment as countermeasures

Any biologically based Life Support System (LSS) will sooner or later face recalcitrant compounds and the accumulation of (xenobiotic) compounds. In MELiSSA's case, a closed loop LSS, the factors of accumulation and incomplete conversion are magnified as no external manipulations are allowed. Therefore a first measure that should be taken to decrease the potential accumulation of recalcitrant compounds or xenobiotics, is prevention of intake and in case of intake minimization of dose. In this respect the general biodegradability and biological activity of each xenobiotic compound should be taken into account.

An additional countermeasure is the possible isolation of the feaces and urine fractions of those crew members that are exposed to an antibiotic treatment or other pharmaceutics. A pretreatment of the urine and faeces fractions could be foreseen here. A highly destructive technique, like (hydro)thermal destruction could be considered for those streams.

5. Conclusions

Table 5. Synthesis of the different removal techniques with respective efficiencies or drawbacks for the considered test hormones and pharmaceutical drugs (*)

Technology	Compounds					
	Hormones		Pharmaceuticals			
	Androgens	Estrogens	Antibiotics	Analgesics	β-blockers	Antidepressiva
Aerobic biodegradation	96-99%	E1: 91-99% E2: 60-98% EE2: 0-100 %	Sulfamethoxazole 5-91% Ciprofloxacine: 88-92%	Aspirin: 81% Diclofenac: 0-80% Ibuprofen: 0-100% Naproxen: 15-93%	Metoprolol: 0-83% Propanolol: 0-96%	Diazepam: 10- 93%
Anaerobic biodegradation	x	degradation considerable slower than aerobic	Sulfamethoxazole 85-95%	Diclofenac: 25-75% Ibuprofen: 30-60% Naproxen: 80-95%	No data found	Diazepam: 20-60%
Coagulation/ Flocculation	Less than 20% removed, E2 max. 43%, EE2 0%		No removal	Diclofenac: 4-70% Ibuprofen: 0-25% Naproxen: 0-30%	No data found	Diazepam: 40-50%
Chlorination process	Complete removal, but harmful chlorinated side products		Sulfamethoxazole 90-98%	Diclofenac: 95% Ibuprofen: 30-75% Naproxen: 95%	No data found	Diazepam: 75%
Ozonation and AOPS	> 97% estrogenicity removal (E1, E2, EE2)		90-99% removal for sulfonamide antibiotics	Diclofenac:32-97% Ibuprofen: >80% Naproxen: >80%	No detection after ozon	Diazepam: >80%
Photolysis and photocatalysis	Complete degradation estrogens (E2, E3, EE2), non identified products		Sulfamethoxazole $t_{1/2}$ = 2.4 d	Diclofenac: 14-90% $t_{1/2}$ = 5.0 d	Propanolol: $t_{1/2}$ = 16.8 d	No data found
Membrane filtration	95-99% E1, 29-83% E2		Sulfamethoxazole 70-82%	Aspirin: >90% Diclofenac: >90%	No data found	No data found
Activated Carbon	76-95% E1, 49-84% E2, 77% EE2, androgen 79%		Sulfamethoxazole : 36%	Ibuprofen: 16-80% Naproxen: 0% Diclofenac: 39-92% Naproxen: 52%	No data found	Diazepam: 67%
Manganese oxide treatment	82% EE2		Highly susceptible for oxidation	No data found	No data found	No data found
Electrolysis	Succesful removal but chlorinated side products		Effective for tetracycline and gentamicine	Effective for aspirin	No data found	No data found
Hydrothermal oxidation	Promising but no data for hormones available		No data found	No data found	No data found	No data found
Bioaugmentation by SRT	Promising but no data for hormones available		No data found	No data found	No data found	No data found

(*) Please note that the removal efficiencies stated in Table 5 were obtained in specific conditions. The consistency and compatibility with MELiSSA technologies and conditions should be checked on a case by case basis before final technology selection can be done.

6. Impact on design

6.11. Experimental Set-up

In MELiSSA, the first compartment's (C1) task - the anaerobic degradation of plant and human waste streams and the reuse of its bioconverted constituents through VFA, mineral and water recuperation- is intended to be as complete as possible. The main focus for detoxification and elimination of hazardous exogenous and endogenous compounds in a MELiSSA-like closed loop system must therefore lie in the optimised performance of C1. This approach not only stresses the importance of C1's performance, but also ensures the subsequent compartments will be safeguarded from exposure to disruptive compounds. In order to assist MELiSSA's C1, supporting technologies can be suggested to tackle the most recalcitrant of these disruptive compounds and avoid their accumulation by treating either the complete effluent of C1 at the C1 to C2 interface, or creating a recycling - stream that is being subjected to the additional technologies which is then subsequently re-introduced into C1, as shown in Figure 5.

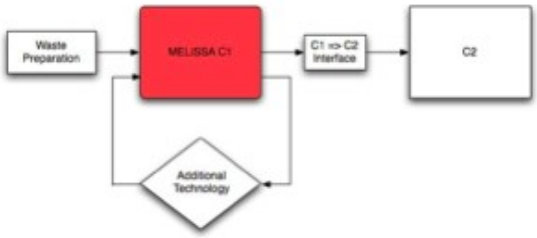


Figure 5: Location of countermeasures - C1

In case endo- or exogeneous compounds are introduced in Compartments *other than in C1*, technologies should also be in place to act locally.

BELISSIMA envisages an extensive characterisation of C1 in the early stages of its running, and as the project continues, the other compartments will be characterised. The fate of artificially introduced chemical problem-compounds according to a yet-to-be completed test plan will provide valuable insight in the way:

- The component behaves in the compartment, on its biology and on the subsequent compartments
- The performance of the compartment
- The bioconversions, accumulation issues, analytical issues allow sufficiently detailed study
- The fate of the chemical as it travels through MELiSSA/BELISSIMA

The choice of additional technologies of worth to MELiSSA and BELISSIMA will to a large extent be guided by the preliminary test results. A certain worst-case- approach will be held as the model compounds chosen in BELISSIMA will not reflect all eventualities but a representative cross-section of terrestrially relevant compounds in use in Space.
As becomes clear from overlooking the plethora of potentially hazardous compounds that might accumulate in- or disturb MELiSSA, and from the multitude of biological and physico-chemical technologies in use for the elimination of these compounds in terrestrial applications, some general points of attention come to mind:

For biological systems:

- No biological system solely will be sufficiently and rapidly adaptable to degrade occurring compound(s). Given the low concentrations at which compounds may be present, the fact that components will not be constantly present, biological systems will not always include the necessary enzymes to degrade all compounds
- Biological systems will not give sufficient assurance that micropollutants were eliminated to non physiologically active concentrations, given the analytical bias that will occur in the measurements, given the large numbers of potential by-products, ...
- In case the recalcitrant chemical was not previously recognised as being troublesome or is not routinely screened for, its removal cannot be verified
- Augmentation of C1 using dedicated organisms seems a valid technology for constitutively present components such as female hormones. Bio-Augmentation of other compounds is problematic given the demand for axenic running of all the compartments, except CI.
- Biological systems require COD and micronutrients, and thus compete with the actual MELiSSA / BELISSIMA compartments

For physico-chemical systems:

- Several of these technologies require the input of additional chemicals such as chlorine, ozone, hydrogen peroxide.
- Efficient though they are, in most cases the by-products are not known and they rarely show absolute removal of the harmful compound.
- Several of these technologies (e.g. advanced oxidation processes, hydrothermolysis) mineralise substantial fractions of the COD. This is not strictly a problem, but for the sizing of the compartments, a very relevant factor.
- Membrane technologies act as an efficient means to prevent transfer of disruptive components, though concentrate the contaminant. A subsequent technology is required to take care of the concentrate.
- Some technologies (hydrothermolysis and similar technologies) have a large power consumption and generate heat.

Requirements

Testing the countermeasure technologies should be done using BELISSIMA effluents to closely approximate MELiSSA conditions. In case too large amounts of BELISSIMA effluents are required to test the countermeasure technologies, ESA and VITO may decide to use a synthetic effluent designed to closely resemble BELISSIMA effluents. For this reason, detailed characterization of the BELISSIMA effluents is required under nominal operating conditions, specifying COD, BOD, fibre content (when relevant), nitrogen species, phosphorus species, major minerals, … Along the same line, detailed characterization of the effluent from spike-ing experiments is required.

6.12. Influence of countermeasures and impact on design

The exact design of the countermeasures depends largely on the findings of the BELISSIMA study. It is therefore not possible to give an accurate account for the countermeasure impact on design. Secondly, direct implication of the countermeasure technologies is not planned in this first stage of BELISSIMA.
However, given the above mentioned points of attention with relation to possible countermeasure technologies, and given that the countermeasures would be installed on BELISSIMA, we would advise to have:

- A adaptable cooling system to provide cooling for the effluents of physico-chemically treated effluents
- A means to introduce augmentation-strands into the compartments (axenically for those compartments where this is relevant). Ideally, a sluice is installed that allows axenic access.
- Fittings and valves suitable to connect external modules (pumps, piping, ...).

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Appendix 1 & 2

Table 11
Concentrations of pharmaceuticals in municipal German STP effluents; results from 1996 to 1998 [36,51,52,58]

Analyte	LOQ (µg/l)	Number STPs	$n >$ LOQ	Median (µg/l)	90-percentile (µg/l)	Maximum (µg/l)
Lipid regulator						
Bezafibrate	0.25	49	48	2.2	3.4	4.6
Gemfibrozil	0.050	49	39	0.40	0.84	1.5
Clofibric acid (metabolite)	0.050	49	47	0.36	0.72	1.6
Fenofibric acid (metabolite)	0.050	49	41	0.38	0.68	1.2
Antiphlogistics						
Diclofenac	0.050	49	49	0.81	1.6	2.1
Ibuprofen	0.050	49	42	0.37	1.2	3.4
Indomethacin	0.050	49	49	0.27	0.40	0.60
Naproxen	0.050	10	10	0.30	0.42	0.52
Ketoprofen	0.050	49	37	0.20	0.25	0.38
Phenazon	0.10	30	28	0.16	0.30	0.41
ASA	0.10	49	22	0.22	0.32	1.5
Salicylic acid (metabolite)	0.050	36	9	< LOQ	0.063	0.14
Betablocker						
Metoprolol	0.025	29	29	0.73	1.3	2.2
Propranolol	0.025	29	28	0.17	0.23	0.29
Betaxolol	0.025	29	17	0.057	0.10	0.19
Bisoprolol	0.025	29	17	0.057	0.13	0.37
β_2-Sympathomimetics						
Terbutalin	0.050	29	11	< LOQ	0.087	0.12
Salbutamol	0.050	29	10	< LOQ	0.072	0.17
Psychiatric drug						
Diazepam	0.030	20	8	< LOQ	0.03	0.04
Antiepileptic						
Carbamazepine	0.050	30	30	2.1	3.7	6.3
Antibiotics						
Clarithromycin	0.020	8	8	0.14	0.24	0.26
Roxithromycin	0.020	10	10	0.68	0.80	1.00
Chloramphenicol	0.020	10	1	< LOQ	< LOQ	0.56
Sulfamethoxazol	0.020	10	10	0.40	0.90	2.00
Trimethoprim	0.020	10	9	0.32	0.62	0.66
Dehydrato-erythromycin (metabolite)	0.020	10	10	2.50	5.10	6.00
X-ray contrast media						
Iopamidol	0.010	25	21	0.66	8.0	15
Iopromide	0.010	24	23	0.75	4.4	11
Diatrizoate	0.010	25	22	0.08	1.5	8.7
Iomeprol	0.010	12	10	0.37	2.8	3.8
Estrogens						
Estrone	0.001	38	20	0.001	0.021	0.070
17β-Estradiol	0.001	38	13	< LOQ	0.002	0.003
17β-Estradiol-17-valerate	0.004	38	0	< LOQ	< LOQ	< LOQ
17α-Ethinylestradiol	0.001	38	9	< LOQ	0.001	0.015
16α-Hydroxyestrone	0.001	15	11	0.001	0.004	0.005

LOQ: limit of quantification. STP: sewage treatment plant effluents (identical with the number of investigated STPs).

tion generally exceeded 75%. The determined S.D. (1σ, n=3) are always below 15% at a spiking level of 50 ng/l. Even in the raw influent and the final effluent from municipal STPs the mean recoveries of estrogens were mostly above 70%.

The improved confirmation using GC/MS/MS is essential for the detection of 17α-ethinylestradiol since an unknown compound exhibited exactly the same retention time [36]. Both EI spectra showed the m/z values of 440 (molecular weight

Table 12
Concentrations of pharmaceuticals in German rivers and streams; results from 1996 to 1998 [36,51,52,58]

Analyte	LOQ (µg/l)	Number STPs	$n >$ LOQ	Median (µg/l)	90-percentile (µg/l)	Maximum (µg/l)
Lipid regulator						
Bezafibrate	0.025	43/22	39	0.35	1.2	3.1
Gemfibrozil	0.010	43/22	28	0.052	0.19	0.51
Clofibric acid (metabolite)	0.010	43/22	35	0.066	0.21	0.55
Fenofibric acid (metabolite)	0.010	43/22	26	0.045	0.17	0.28
Antiphlogistics						
Diclofenac	0.010	43/22	43	0.15	0.80	1.20
Ibuprofen	0.010	43/22	35	0.07	0.28	0.53
Indometacin	0.010	43/22	35	0.04	0.17	0.20
Naproxen	0.010	20/20	20	0.07	0.15	0.39
Ketoprofen	0.010	43/22	5	< LOQ	0.12	0.12
Phenazon	0.020	26/20	21	0.024	0.15	0.95
ASA	0.020	43/22	17	< LOQ	0.16	0.34
Salicylic acid (metabolite)	0.010	35/19	24	0.025	0.13	4.1
Betablocker						
Metoprolol	0.010	45/23	38	0.045	1.2	2.2
Propranolol	0.010	45/23	26	0.012	0.44	0.59
Betaxolol	0.010	45/23	1	< LOQ	< LOQ	0.028
Bisoprolol	0.010	45/23	19	< LOQ	0.19	2.9
β_2-Sympathomimetics						
Terbutalin	0.010	45/23	0	< LOQ	< LOQ	< LOQ
Salbutamol	0.010	45/23	2	< LOQ	< LOQ	0.035
Psychiatric drug						
Diazepam	0.030	30/20	0	< LOQ	< LOQ	< LOQ
Antiepileptic						
Carbamazepine	0.030	26/20	24	0.25	0.82	1.1
Antibiotics						
Clarithromycin	0.020	33/22	7	< LOQ	0.15	0.26
Roxithromycin	0.020	52/40	23	< LOQ	0.20	0.56
Chloramphenicol	0.020	52/40	4	< LOQ	< LOQ	0.06
Sulfamethoxazol	0.020	52/40	26	0.03	0.14	0.48
Trimethoprim	0.020	52/40	10	< LOQ	0.09	0.20
Dehydrato-erythromycin (metabolite)	0.020	52/40	31	0.15	0.63	1.7
X-ray contrast media						
Iopamidol	0.010	25/25	24	0.49	1.6	2.8
Iopromide	0.010	25/25	22	0.10	0.55	0.91
Diatrizoate	0.010	25/25	23	0.23	6.4	ca. 100
Iomeprol	0.010	12/12	12	0.10	0.47	0.89
Estrogens						
Estrone	0.0005	15/15	3	< LOQ	0.001	0.0016
17β-Estradiol	0.0005	15/15	0	< LOQ	< LOQ	< LOQ
17β-Estradiol-17-valerate	0.002	15/15	0	< LOQ	< LOQ	< LOQ
17α-Ethinylestradiol	0.0005	15/15	0	< LOQ	< LOQ	< LOQ
16α-Hydroxyestrone	0.0005	15/15	0	< LOQ	< LOQ	< LOQ

LOQ: limit of quantification.

(MW) of silylated 17α-ethinylestradiol) and 425 (MW 'minus' CH_3), however, with a different ratio. Using MS/MS detection of the target ion m/z 425 a confirmation with regard to identification and quantification of 17α-ethinylestradiol can be carried out. Due to the fact that the MS/MS spectra of the contraceptive and the unknown impurity are different, a precise quantitation is possible using the product ions 193 m/z and 231 m/z of the precursor ion 425 m/z. Using single MS detection the probability of determining excessive concentrations cannot be excluded. There is a distinct possi-

Table 6. Actual concentrations of E1, E2 and EE2, measured in influents and effluents of STPs

References	Country	Location	Method of treatment	Detection method	Estrone E1 (ng/l)			17β-Estradiol E2 (ng/l)			17α-Ethynylestradiol EE2 (ng/l)		
					Influent	Effluent	% removal	Influent	Effluent	% removal	Influent	Effluent	% removal
Baronti et al. (2000)	Italy	Cobis	Activated sludge	LC-ESI-MS-MS	71 (35)	9.62 (5)	86 (10)	16.1 (7)	1.48 (1.02)	91(8)	3.93 (5.14)	0.64 (0.31)	84 (23)
		Fregene			67 (18)	4.06 (1.5)	94 (3)	9.2 (5)	0.92 (0.74)	90(10)	3.39 (2.35)	0.68 (0.68)	80 (24)
		Ostia			50.6 (13)	44.62 (25)	12 (54)	14.68 (7)	2.44 (1.19)	83(11)	2.48 (1.76)	0.79 (0.32)	68 (26)
		Roma Sud			35.2 (10)	30.34 (16)	14 (52)	8.6 (2.3)	1.89 (0.94)	78(12)	2.95 (2.08)	0.66 (0.37)	78 (20)
		Roma Est			50.4 (14)	7.66 (2.6)	85 (7)	9.3 (2)	0.75 (0.08)	92(2)	2.28 (1.57)	0.44 (0.20)	81 (16)
		Roma Nord			36.8 (8)	13.88 (15)	62 (41)	11.46 (3)	0.98 (0.55)	91 (5)	2.95 (2.33)	0.48 (0.10)	84 (13)
Belfroid et al. (1999a)	Netherlands	STP A'dam-Westpoort (Oct)	Aeration tank	GC/MS/MS *Italic:after addition of enzyme for unconjugation*	87; *200*	2.1;*2.1*	99	9.5; *10*	<0.6; <0.6	94	1.3; *1.5*	<0.3; 0.5	67
		STP A'dam-Westpoort (Dec)			140	47	66	48	12	75	9.7	7.5	23
		STP Kralingsveer (Dec)	Carousel		100	6.3	94	31	0.7	98	8.8	<0.2	98
		STP Eindhoven (Oct)	Aeration tank		*10.3* (1)	2.7; *5.4*	48	*11*	n.a.		*9.2*	<1.4; <1.4	84
		STP Eindhoven (Dec)			42	15	64	14	1.1	92	<0.2		
Vethaak et al. (2002)		Several plants	Activated sludge	GC/MS/MS	20–130	<0.3–11		17–150	<0.8	70	<0.3–5.9	<0.3–2.6	
Bruchet et al. (2002)	France	STP Esniere sur Oise	Activated sludge	GC/MS	20	8	60	10	3		2.4	1.4	42

Table 6. Continued

References	Country	Location	Method of treatment	Detection method	Estrone E1 (ng/l)			17β-Estradiol E2 (ng/l)			17α-Ethynylestradiol EE2 (ng/l)		
					Influent	Effluent	% removal	Influent	Effluent	% removal	Influent	Effluent	% removal
Tabak et al. (1981)	USA	Batavia	Trickling Filter	TLC and GLC after hydrolysis and liquid/ liquid extraction	20 (20)	10 (7)	50 (61)	10	<10		1070 (810)	670 (590)	37 (73)
		Fairfield	Activated sludge		10 (7)	10	0 (70)	10	<10		890 (630)	530 (430)	40 (64)
		Lebanon			20 (14)	10	50 (35)	10	<10		880 (520)	540 (370)	39 (56)
		Milford	Trickling filter		40 (20)	20 (14)	50 (43)	10 (7)	10		1330 (610)	910 (630)	32 (57)
		New Richmond	Contact stabilisation		40 (20)	20 (7)	50 (31)	20 (7)	10	50 (18)	1290 (710)	920 (580)	29 (60)
		Bethel	Trickling filter		30 (20)	20 (14)	33 (64)	10 (7)	10		1000 (640)	660 (470)	34 (63)
		Loveland			30 (20)	10 (7)	67 (32)	10 (7)	10		990 (520)	630 (400)	36 (52)
		Little Miami	Primary		50 (30)	30 (14)	40 (46)	20 (7)	10 (7)	50 (39)	1770 (710)	1320 (640)	25 (47)
		Bromely	Contact stabilization		40 (14)	10 (14)	75 (36)	10 (7)	10		1480 (640)	1040 (530)	30 (47)
		Muddy Creek	Primary		50 (20)	30 (14)	40 (37)	20 (7)	10	50 (18)	1590 (750)	1160 (660)	27 (54)
		Hamilton	Activated sludge		20 (14)	10 (7)	50 (50)	10	<10		1270 (400)	780 (320)	39 (32)
		Glendale	Trickling Filter		30 (20)	20 (7)	33 (50)	10 (7)	10		1000 (690)	600 (490)	40 (64)
Adler (2001)	Germany	Southern and middle Germany	Five STPs	GCMS, Unconjugated After adding enzymes	<0.5–20, 2 med. 2–16, 3 med.	<0.1–18, 0.3 med. <0.1–57, 0.4 med.	87	<0.5–4, 2 med. 1–22, 3 med.	<0.05–0.6, 0.2 med. 0.2–2, 0.9 med.	70	1–14, 12 med. 1–45, 26 med.	<0.05–0.6,0.2 med. <0.1–2, 0.4 med.	98

		Seven STPs	GCMS, Unconjugated	2–25, 6 med.	<0.05–130, 3 med.	14	1–9, 1 med.	<0.05–4, 0.7 med.	30	1–9 4 med.	<0.1–4, 0.5 med.	71		
			After adding enzymes	8–25, 14 med.	<0.1–170, 12 med.		2–19, 3 med.	0.2–6, 3 med.		5–15, 7 med.	<0.1–40, 2 med.			
Andersen et al. (2003)	Germany	Wiesbaden	(1) primary clarifier	GCMS	65.7	74.9	−14	15.8	10.9	31	8.2	5.2	37	
			(2) denitrification-1		74.9	37.3	50	10.9	10.3	6	5.2	1.5	71	
			(3) denitrification-2		37.3	2.8	92	10.3	<1	90	1.5	1.2	20	
			(4) nitrification		2.8	<1	64	<1	1.8	−80	1.2	<1	>20	
			(5) secondary clarifier		<1	<1		1.8	<1		<1	<1		
			Total removal				>98			>94			>88	
			(2) denitrification-1	GCMS; on sludge(ng/g)	10.1			2.7			1.9			
			(3) denitrification-2		6.9			2.3			2.2			
			(4) nitrification		5.6			2.2			<1.5			
			(6) digested sludge (solid)		25.2			5.1			<1.5			
			(6) digested sludge(liquid)		67.1 (ng/l)			5.4 (ng/l)			<1 (ng/l)			
Schullerer et al. (2002)	Karlsruhe		(1) activated sludge with N and P removal	GC/MS	130	26	80	32	3.2	90	55	9.0	84	
			(2) trickling filter		26	11	58	3.2	2.7	16	9.0	7.0	22	
							92			92			87	
	Ebingen		(1) primary clarifier		120	65	46	35	17	51	20	n.d.	>91	
			(2) activated sludge with N and P removal		65	2.1	97	17	6.0	65	n.d.	2.7		
			(3) activated carbon		2.1	n.d.	>99	6.0	n.d.	>97	2.7	1.8	33	
													91	
	Lautlingen		(1) primary clarifier		49	34	31	31	29	6	59	55	7	
			(2) activated sludge with N and P removal		34	3.3	90	29	1.3	96	55	3.7	93	
			(3) activated carbon		3.3	n.d.	>98	1.3	n.d.	>97	3.7	2.2	41	
													96	

med. = median value, n.a. = not available.
SD in brackets if available.
Standard deviations for the removal are calculated according propagation of uncertainty (Rubinson 2000).

Cargouët et al. (2004)		Evry	Activated sludge +(de) nitrification	GC/MS	17.6 (0.5)	7.2 (0.8)	59 (5)	11.1 (1.7)	4.5 (1.4)	59 (14)	5.4 (0.6)	3.1 (0.6)	43 (13)
		Valenton			15.2 (1.8)	6.5 (1.2)	57 (9)	17.4 (1.7)	7.2 (0.8)	59 (6)	7.1 (0.9)	4.4 (1.2)	38 (19)
		Colombes	Upflow Biolfilters		9.6 (1.5)	4.3 (0.6)	55 (9)	11.6 (0.6)	6.6 (1.4)	43 (12)	4.9 (1.0)	2.7 (0.8)	45 (20)
		Achères	Activated sludge		11.2 (2.3)	6.2 (0.8)	45 (13)	17.1 (0.6)	8.6 (0.9)	50 (6)	6.8 (1.4)	4.5 (0.8)	34 (18)
Lee and Peart (1998)	Canada	Burlington (Dec)	Activated sludge	GC/MS	26 (1)	6 (0.5)	77 (2)	7 (0.6)	<5	>29 (6)	n.a.		
		Burlington (Jan)			53 (4)	8 (1)	85 (2)	14 (2)		>64 (5)			
		Dundas			69 (1)	9 (1)	87 (1)	7 (0.7)		>29 (7)			
		Edmonton			109 (5)	72 (2)	34 (4)	<5		n.a.			
		Guelph (Dec)			66.5 (12)	17 (1)	74 (5)	<5					
		Guelph (Jan)			41 (4)	14 (1)	66	15 (2)		67 (4)			
Nasu et al. (2001)	Japan	27 plants autumn	Activated sludge	ELISA	n.a.			0.03–0.090 0.042med.	n.d.–0.043 0.013 med.	69			
		Winter						0.02–0.094 0.047 med.	0.0003–0.03 0.014 med.	70			
Lebietzka (1996), p. 257, quoted in Jürgens et al. (1999)	Germany	Zittau STW	Activated sludge	EE2 ELISA	n.a.						6.8	8.0	–18
Petrovic et al. (2002)	Spain	Calaf	n.a.	HPLC-LCMS	<2.5–115	<2.5–8.1	n.a.	11.0–30.4	<5.0	n.a.	<5.0		n.a.
		Igualada			<2.5–4.6	<2.5–2.7		<5.0	<5.0–7.6				
		Piera			<2.5–13.1	<2.5		<5.0	<5.0				
		Manresa			<2.5–56.5	<2.5–7.2		<5.0–14.5	<5.0				
Shore et al. (1993)	Israel	Tel Aviv	(1) anaerobic tank	E2 Radio-immunoassay	48–141	7–39	22–64	n.a.					
			(2) activated sludge		7–39	6–50							