Fate and Behaviour of Steroid Oestrogens in Rivers: A Scoping Study

R&D Technical Report P161

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Research Contractor:

Institute of Hydrology

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The document provides background and experimental information on the fate of steroid oestrogens in the aquatic environment. This information should be used to assess the risk of these substances being bioavailable contaminants in receiving waters and to identify further work to complete data/information gaps and underpin relevant control measures. The report is to be used by Environment Agency staff and others managing discharges to controlled waters.

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EXECUTIVE SUMMARY

Previous research has shown that effluents from sewage treatment works contain chemicals that have oestrogenic activity. The compounds that contribute most to the effect have been identified as the natural oestrogens oestrone and 17β-oestradiol and the synthetic hormone ethinyl-oestradiol. In order to help to understand the implications of these discharges to rivers the Environment Agency commissioned a scoping study on the fate and behaviour of these three oestrogens in rivers.

The research has covered three main areas: (1) A review of existing published physico-chemical data relevant to the fate and behaviour of steroids in rivers: (2) Laboratory experiments to determine values of some of these parameters for which data were missing and were appropriate for UK rivers: (3) A modelling study to estimate the potential distribution of these chemicals in illustrative UK river environments downstream of an effluent discharge. The literature review revealed that there were very limited data available on the distribution of the three oestrogens in rivers. Most data have been obtained from various stages of the effluent treatment process through to the point of discharge. Those data that are available indicated values at around the ng/l level. Higher reported values, particularly from early research, have been questioned. Other studies have tried to estimate likely river concentrations based on natural excretion and medical prescription levels. These studies generally predicted concentrations within the ranges of those observed.

Data on physico-chemical properties that might influence concentrations in rivers were very scarce. Some basic data on solubility, and octanol-water partition coefficient were published, but sorption distribution coefficients were not available. Degradation rates had been estimated for the hormone oestrogens in activated sludge and drinking water, but not for river waters. However, some microbial degradation mechanisms for 17β-oestradiol and oestrone had been proposed.

The laboratory studies reported here used material collected from the Rivers Thames, Aire, Calder, and the estuaries of the Tyne and Tees, and concentrated on sorption and degradation experiments. The experiments included, for 17β-oestradiol: Sorption to laboratory equipment, sorption-desorption kinetics, the measurement of sorption distribution coefficients for bed and suspended sediments and mineralisation of the phenol A ring. For all three oestrogens, anaerobic and aerobic biodegradation in the water column.

In the experimental system used, the majority of sorption to the bed-sediments occurred within 24 hours but small quantities were still being sorbed after 5 days. Desorption released less compound than was sorbed and gave distribution coefficients (K_d) 2-3 times higher than on sorption. Values of K_d (l/kg) for sorption of 17β-oestradiol to the bed-sediments were estimated to be 23-46 for the Thames, 43-67 for the Aire, 34-56 for the Calder, 20-34 for the Tees estuary and 54 for the Tyne estuary. There was a positive correlation with organic carbon content and with decreasing particle size. However, K_{oc} values, calculated showed a wide variation (610-2,650, (l/kg)).

K_d values for the sorption of 17β-oestradiol to suspended sediment were estimated as 1690, 3364 and 106 for the Rivers Aire, Calder and Thames respectively. For all rivers these values exceeded those for the bed-sediments. For the Aire and the Calder this represented a 100 fold increase on the K_d of the bed sediment. Assuming that the organic carbon content is primarily responsible for the extent of sorption, this implies a 5 fold increase in sorption efficiency of the organic carbon in the suspended sediment over the bed-sediments. For the River Thames the change is less dramatic. This may be attributable to the nature of the suspended sediment which for the Thames was predominantly live algae and for the Aire and Calder was composed of decaying organic aggregates.

Under aerobic conditions 17β-oestradiol was degraded at high concentrations, in river waters with half-lives of <3 days for the Aire and Calder, 4 days for the Thames, 6 days for the Tees and 27 days for the Tyne estuaries. 17β-oestradiol was shown to be converted to oestrone, which was then further degraded at a similar rate. Ethinyl-oestradiol was shown to be much more persistent with a half-live of 46 days compared to 4 days for 17β-oestradiol under the same experimental conditions. Under anaerobic conditions, degradation of 17β-oestradiol in river water was still relatively rapid in samples from the Aire and Calder, although the oestrone so formed was much more persistent. Both compounds showed greater persistence in the other river samples. Ethinyl-oestradiol showed no degradation under anaerobic conditions over 46 days in samples from the Thames. Variability between replicates was much greater under anaerobic conditions and it was not possible to derive sensible half-lives.
Microbial cleavage of the steroid ring system was demonstrated by release of radiolabelled CO_2 from the phenolic ring of 17β-oestradiol (position 4).

The EXAMS model has been set up for river systems based on the Rivers Thames, Aire and Calder for the three oestrogens. The model has been used to estimate likely environmental concentrations in the water column, and bed-sediments using the best available data on the physico-chemical properties of the steroid oestrogens in these systems. However, the concentrations predicted were based on average loadings of oestrogens and may not reflect in absolute terms those concentrations that might be observed in water samples from these rivers.

Despite their affinity suspended sediments, the vast majority of the steroid oestrogens within the water column were predicted to be in the dissolved phase. Concentrations under average conditions were predicted to vary between 0.21 and 0.37 ng/l for 17β-oestradiol, 0.27 and 0.44 ng/l for oestrone and 0.024 and 0.038 ng/l for ethinyl-oestradiol. Under low-flow conditions of the river, predicted concentrations increased by a factor of between 4 and 10 times the average concentrations at the point of discharge. Degradation processes were shown to be unimportant under average conditions but more significant under summer low flow conditions. River bed-sediments were shown to account for between 13% and 92% of the chemical loads entering the system. This was controlled by the value of the distribution coefficient for the chemical/sediment system.

A series of recommendations have been made to take forward research in the area of the fate and behaviour of steroid oestrogens in river systems. These cover four areas, (1) laboratory studies to include measurements of sorption for oestrone and ethinyl-oestradiol and degradation rates on suspended and bed-sediments and how speciation might effect oestrogenicity, (2) selected monitoring to confirm model predictions, (3) further dynamic modelling based on specifically

collected data and (4) development of quality standards for oestrogens in river that will ensure protection of stream fauna.

KEY WORDS

Oestrogens, 17β-oestradiol, oestrone, ethinyl-oestradiol, fate, behaviour, rivers, modelling, degradation, sorption, distribution coefficient.

1. INTRODUCTION

1.1 Background: Concern about Environmental Oestrogens

The question as to whether chemicals in the environment can influence the hormonal system of humans or wild animals was discussed in the 70's as a consequence of the increasing use of synthetic steroids as oral contraceptives and in animal production. The conclusion then was that there was no need for action (Rathner and Sonnenborn 1979). However, since then there have been numerous observations of endocrine disruption in fish and other water animals especially close to sewage works. For example unusually high incidences of hermaphrodite fish were observed (Sumpter and Jobling, 1995). These observations led to the desire to identify which substances were causing these effects. While in special cases of trade effluents the cause could be tentatively put down, for example to alkylphenols from wool scouring mills (Harries *et al.,* 1997), effluents from sewage treatment works, receiving mainly domestic sewage, could also show oestrogenic effects (Sumpter and Jobling, 1995).

1.2 Excretion of Steroid Oestrogens by Humans and Other Mammals

Natural oestrogens are formed from testosterone, the main androgen (male sex hormone), in the ovary, during pregnancy in the placenta and in smaller quantities in the adrenal glands and testicles. The main oestrogens are 17β-oestradiol, oestrone and oestriol. They control the development of the secondary female sex characteristics and female behaviour and are together with the gestagens responsible for regulating almost all of the reproductive process in women. Figure 1.1 shows some metabolic pathways of 17β-oestradiol in the liver. All metabolites still contain the steroid ring system with the aromatic A-ring by which the oestrogens differ from other steroids. Before they are excreted the oestrogens are conjugated to oestrogen-sulphate-esters or oestrogen-glucuronides or double and mixed conjugates (Turan, 1996), which renders them virtually inactive as hormones, but also makes them much more polar and therefore much more water soluble than the free compound. The normal daily oestrogen secretion of women is 24-100 µg depending on the menstrual cycle and can rise to 30 mg towards the end of pregnancy (Turan, 1996).

The natural hormones are orally inactive or active only at very high doses, because they are rapidly metabolised. Blocking the oxidation to oestrone by introducing a ethinyl group in position 17α of 17β-oestradiol leads to much more stable products which remain in the body long enough to be used as oral contraceptives (Turan, 1996). The consequence of this increased stability is that the synthetic steroid ethinyl-oestradiol is excreted up to 80% unchanged in conjugated form (Ranney 1977, quoted from Turan, 1996). Figure 1.2 gives an overview of the metabolism of ethinyl-oestradiol.

Because the natural as well as the synthetic steroids are excreted in an inactivated form, an effect on rivers receiving these steroids through sewage treatment works effluents, would seem unlikely if the conjugates stayed intact. From a chemical point of view the conjugates are quite stable. At the beginning of the oestrogen metabolite research, quite drastic measures such as hot acid hydrolysis were used to release the steroids from their conjugates, a procedure

during which a considerable part of the free oestrogens became immediately decomposed. There is, however, a gentler way of cleaving the conjugates by enzymatic hydrolysis. These enzymes are often isolated from micro-organisms which might also be present in wastewater (Turan, 1996). This poses a problem for the environment. If enzymes capable of releasing the oestrogens from their conjugates are active in sewage treatment works, relatively large quantities of active oestrogens may be released during treatment. Oestrogens were indeed found in the unconjugated form in treated sewage (Desbrow *et al.* 1996), indicating that the conjugates got split in the sewer or during treatment. In a recent paper Ternes *et al.* (1999b) showed that bacteria present in activated sludge can readily split glucuronides.

In principle the same excretion mechanisms as for humans apply to farm animals leading to locally increased oestrogen concentrations where large animal stocks are kept (compare values by Shore *et al.*, 1993 in table 2.1).

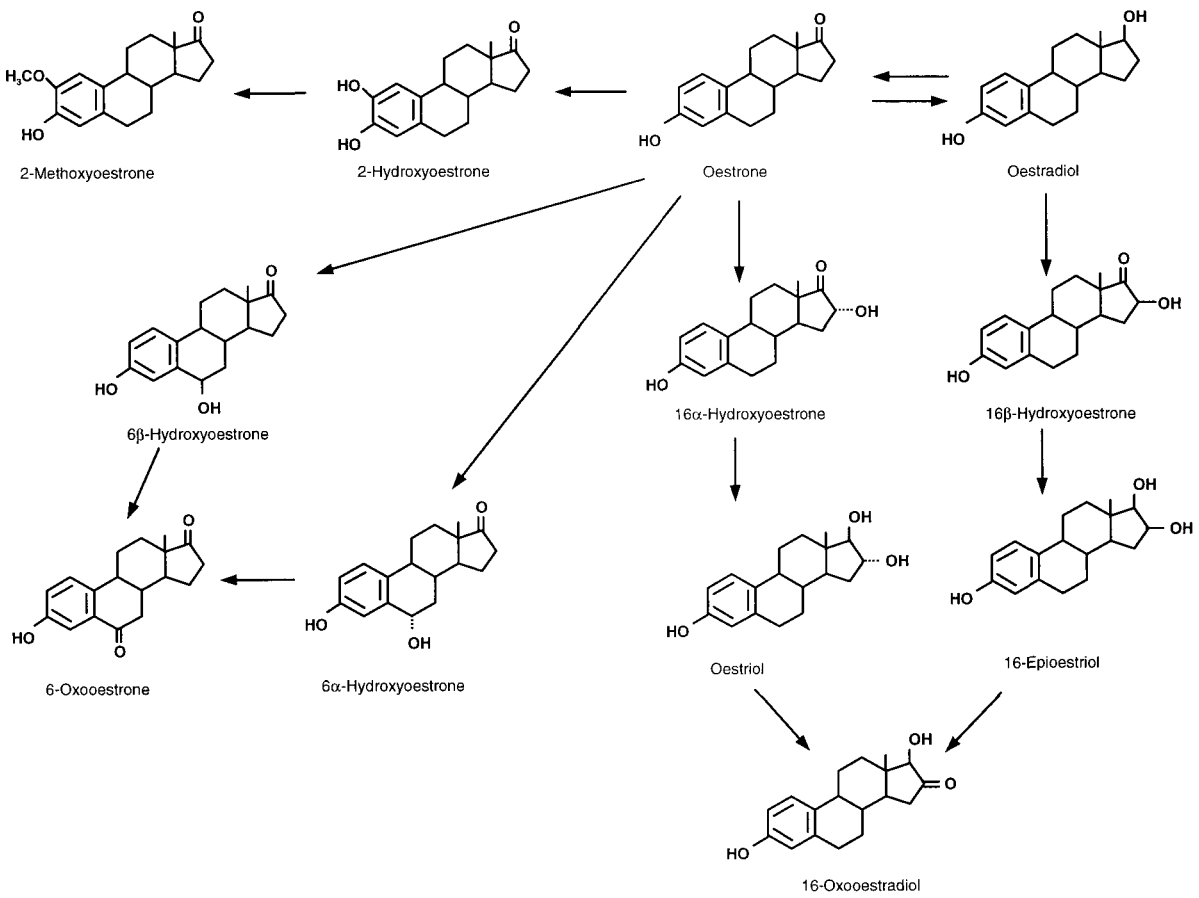


Figure 1.1 Metabolism of 17β-oestradiol in humans (examples, from Turan, 1996)

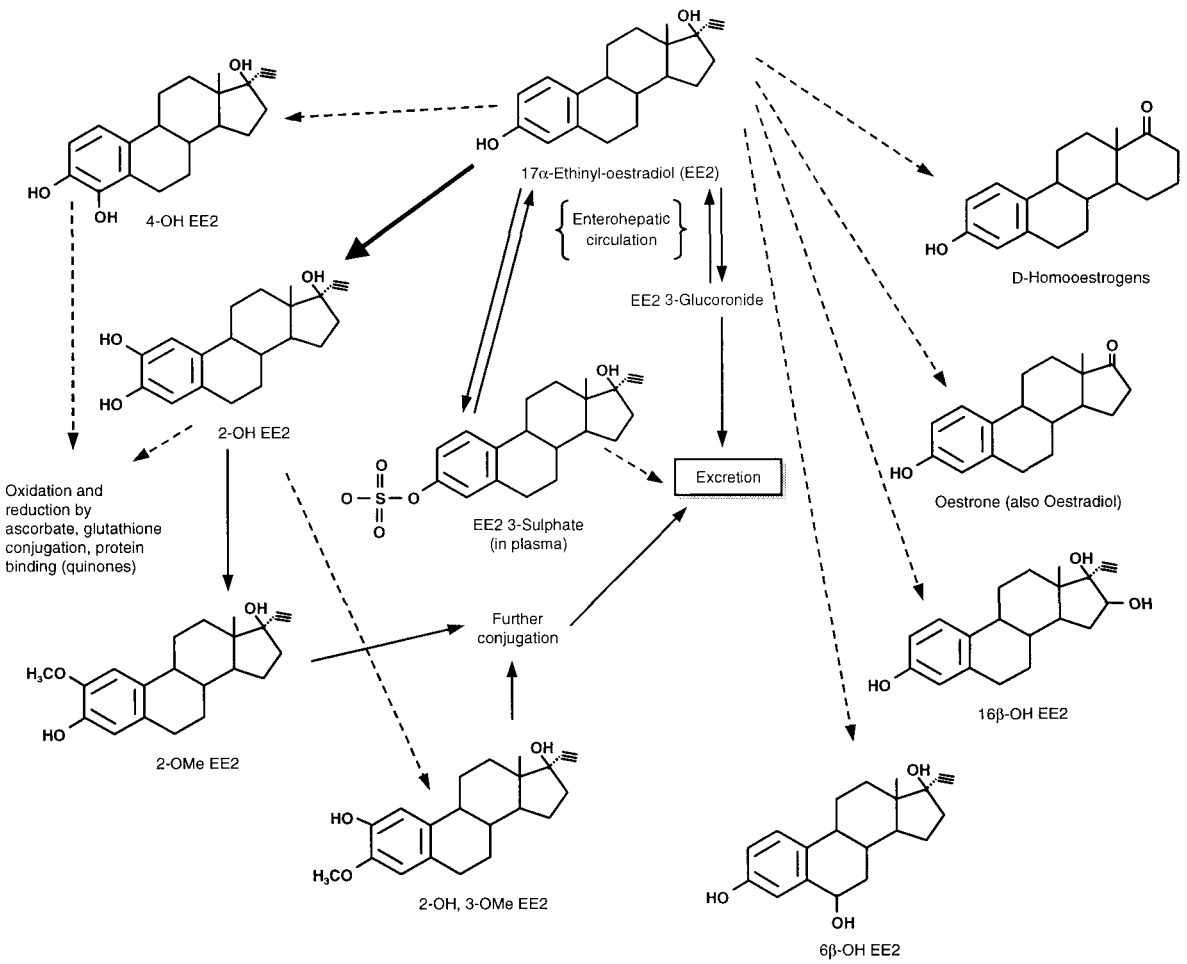


Figure 1.2 Metabolism of ethinyl-oestradiol in humans (from Guerngerich, 1990)

1.3 Effects of Steroid Oestrogens on Fish

Fish regulate their reproductive system by the same hormones as mammals. It is therefore not surprising that they are affected by sex hormones in the water. One effect often used as a biomarker for oestrogenic effects is the production of vitellogenin by male fish. Vitellogenin is an egg-yolk protein precursor normally only produced by adult females. In many polluted stretches of rivers vitellogenin production by males and juvenile females has been observed as well as increased vitellogenin levels in females (Purdom *et al.*, 1994, Sumpter and Jobling, 1995, Harries *et al.*, 1997). In more extreme cases there have also been increased incidences of hermaphroditism in fish. For a male fish the production of egg yolk protein represents a waste of energy, which can be a cause of stress. Among the chemicals suspected of causing these effects are the natural and synthetic steroids. Steroids are found in the water in much lower concentrations than other pollutants, but it is the nature of hormones that they are especially effective at very low concentrations (Patlak, 1996). For example Purdom *et al.* (1994) showed that at 10°C vitellogenin synthesis was induced by 10 ng/l ethinyl-oestradiol in male rainbow trout while at 16.5°C as little as 0.5 ng/l was effective. This means that the steroid oestrogen concentrations found in sewage treatment works effluents and rivers

(compare table 2.1) are frequently high enough to cause some effects and that concentrations close to or below the detection limit even of advanced methods may affect fish.

1.4 Scope of this Report

This report presents data used to make a preliminary assessment of the fate and behaviour of 17β-oestradiol, oestrone and ethinyl-oestradiol in rivers. The work comprised 4 parts, firstly a literature review of current knowledge of the occurrence and properties of the compounds in surface waters (section 2). Secondly, additional work in the laboratory to provide data on important physico-chemical properties of, in particular, 17β-oestradiol and one or both of oestrone and ethinyl-oestradiol, for selected UK rivers (sections 3 and 4). Thirdly, to use the data from the literature and new laboratory measurements to make initial estimates of the distribution of the steroid oestrogens in typical UK river environments (section 5). Finally, to provide recommendations as to how monitoring and research into the fate and behaviour of 17β-oestradiol, oestrone and ethinyl-oestradiol should be taken forward (section 7).

2. LITERATURE REVIEW

2.1 Search for the Cause of Endocrine Disruption in Aquatic Fauna

Several studies have observed oestrogenic effects in sewage treatment works effluents (e.g. Sumpter and Jobling 1995, Purdom *et al.*, 1994, Harries *et al.*, 1997). This led to the conclusion that the substance(s) responsible must be common to most of them and therefore be domestic rather than industrial in origin.

A large number of chemicals have been shown in laboratory experiments to possess endocrine disrupting properties. These include such different compounds as:

1) natural oestrogens: 17β-oestradiol, oestriol, oestrone and others
2) synthetic oestrogens (used as contraceptives and in hormone replacement therapy) mainly ethinyl-oestradiol and mestranol...

   The natural and synthetic oestrogens are all steroids, while the following groups have a molecular structure that resembles the steroid ring system to a greater or lesser degree, but it is not yet possible to predict oestrogenicity by the molecular structure alone.

3) Phyto-oestrogens (plant-oestrogens), present for example in soy-products
4) Chemicals which are produced for a different purpose:
   - Alkylphenols (degradation products of alkylphenol polyethoxylates (APEOs), used in industrial surfactants)
   - DDT
   - pesticides: e.g. lindane (g-hexachlorocyclohexane, or g-HCH), toxaphene, dieldrine, endosulphane (Pesticide news 39, March 1996 lists 40 different pesticides causing endocrine disruption)
   - pthalates
   - PCBs,
   - Bisphenol A

Many of these chemicals are known to be present in at least some effluents but often at much lower concentrations than that required to explain the observed effects on fish.

Desbrow *et al.* (1996) developed a method to isolate the oestrogenic fraction of an undiluted sewage treatment works effluent and identify the substance(s) responsible without prejudging where the oestrogenic activity might be found in the complex mixture. Only one oestrogenic fraction was found in all samples and 17β-oestradiol, oestrone and in some cases ethinyl-oestradiol could be identified in that fraction. The steroids were found in their unbound form. Thus it they could show that the oestrogenic effects observed in the undiluted sewage treatment works-effluents were due to these steroids.

2.2 Concentrations of Oestrogens in the Environment

Oestrogens entering a river from a sewage works outlet will be subject to a number of processes (e.g. dilution, sorption to bed-sediments and suspended sediments, degradation) which might be expected ,eventually, to reduce the concentration in the aqueous phase. It is plausible that oestrogens may enter rivers via routes other than from sewage treatment work outlets. Hydrophobic compounds such as steroid oestrogens can sorb to sludge solids (Shore *et al.*, 1993). These compounds may survive anaerobic digestion and then be disposed to land with the sludge. Subsequent runoff may then carry the compounds to a water course. Alternatively, steroid oestrogens excreted by farm animals, such as cattle and chickens may also escape from soil or farm yards to water courses (Shore *et al.*, 1993).

The data for environmental concentrations especially of natural oestrogens is relatively scarce, because the concentrations, although high enough to cause effects on fish at least under some circumstances (temperature, long term-exposure, early life stages...), are often close to or below the detection limit even of advanced methods, and the focus of research has so far been mainly on xeno-oestrogens, for example alkylphenols (e.g. Jobling and Sumpter, 1993).

A summary of reported concentrations of the natural oestrogens 17β-oestradiol and oestrone and the synthetic 17β-oestradiol derivate ethinyl-oestradiol is given in table 2.1.

Values for "effective oestradiol binding equivalent concentrations" by Montagnani (1996) are also included. It has to be mentioned though, that these are not the same as actual steroid concentrations because a great number of non-steroidal chemicals can bind to the oestrogen receptor (see Chapter 2.1 for examples). So these figures could be due to completely different substances than the others, e.g. pesticides, which may explain the discrepancy to Shore (1993) as far as the influence of rainfall is concerned.

Table 2.1 Summary of oestrogen concentrations in the environment

sample	compound	results [ng/l]	method	remarks	reference
STW effluents (8 samples) treatment: activated sludge	ethinyl-oestradiol	<1- 7	immunoassay	all samples in SE England Aug 1987. The results have been questioned by Harries *et al.*, 1995	Aherne and Briggs 1989
Rivers (13 samples)	as above	2-15	as above	as above	
Impounding reservoirs	as above	1-3	as above	as above	
Potable water	as above	<1-4	as above	as above	
Effluents of 3 domestic STWs in the Netherlands on two occasions each	ethinyl-oestradiol (EE2) oestradiol (E2) oestrone (E1)	EE2: <0.2-7.5 E2: <0.6- 12 (median 0.9) E1: <0.4-47	GC/MS/MS after extraction and fractionation in HPLC	on one of the occasions oestrogen glucuronide conjugates were also determined, but could only be found for oestrone (0-2.7 ng/l)	Belfroid *et al.* 1999
Effluents of 2 industrial STWs in the Netherlands on two occasions each	as above	EE2: <0.2-2.6 E2: <0.4-1.8 E1: <0.1-11 (median 0.4) E1 in one STW on both occasions detectable, E2, EE2 only on one occasion	as above	on one of the occasions oestrogen glucuronide conjugates were also determined, but were found to be below detection limit	
surface waters of 11 estuarine and freshwater locations in the Netherlands	as above	EE2: <0.1 - 4.3 E2: <0.3 - 5.5 E1: <0.1 - 3.4 (median 0.3)	as above	oestrogen glucuronides could not be found in two of the three samples investigated. In one location very low concentrations (<1ng) were found	

Summary of oestrogen concentrations in the environment, continued

sample	compound	results [ng/l]	method	remarks	reference
7 STW-effluents in England on three occasions each	ethinyl-oestradiol (EE2) oestradiol (E2) oestrone (E1)	E1 and E2 always detectable E2: 2.7-48 (mean 11) E1: 1.4-76 (mean 16) EE2 only in two STW effluents on all dates and in one on one date detectable. max: 4.3	1. HPLC fractionation 2. Test of oestrogenicity of the fractions 3. concentration of the active fraction (solid phase extraction) 4. Ion-trap GC/MS	Steroids are the only biologically active substances identified in the 7 STW effluents Hormones were present in the biologically active **unbound** form No oestrogenicity detectable in extracts of suspended solids	Desbrow et. al. 1996
Bavarian a) surface waters b) sewage effluent	ethinyl-oestradiol	a) <0.2 b) 0.3-0.5	not given by Römbke *et al.*	free compound	Kalbfus 1995, quoted from Römbke *et al.*, 1996
Drinking water in various towns in Saxony, (Germany) and Opole (Poland)	ethinyl-oestradiol	0.11-34.2 (average 8, mainly due to high values in Dresden)	ELISA for the free compound	some of the results have been confirmed by GC/MS (personal note from Jörg Oehlmann)	Lebietzka, 1996
Mineral water	ethinyl-oestradiol	3.08	ELISA for the free compound	as above	
River water of Neiße and Mandau in Saxony and the Czech Republic	ethinyl-oestradiol	0.6 - 18.8 average 7.4	ELISA for the free compound	as above	
Zittau STW	ethinyl-oestradiol	influent 6.8 activated sludge 57 effluent 8.0	ELISA for the free compound	higher values during treatment because of conjugate cleavage	

Summary of oestrogen concentrations in the environment, continued

sample	compound	results [ng/l]	method	remarks	reference
Influent or primary treated (mechanical treatment) effluent	ethinyl-oestradiol (EE2) oestradiol (E2) oestrone (E1)	EE2: <5 E2: 6-15 E1: 26-29	GC/MS after solid phase extraction	Further samples that had been preserved (1% formaldehyde) and stored before analysis are not included here, because this seemed to alter the results. Higher values are found for oestriol (Influent: 53-250 ng/l, effluent: <10-33). Removal rates can be calculated for E1: 66-87 %	Lee and Peart, 1998
Effluent of Canadian sewage works	as above	EE2: <5 E2: <5 E1: 2-14	as above		
5 STW effluents SE England	effective oestradiol binding equivalent concentrations	3.88 5.34 13.05 5.06 0.24	radio receptor assay	Substances that bind to E2-receptor. These are not necessarily steroids. Exceedingly high levels during period of heavy rainfall.	Montagnani 1996
River water prior to STW discharge	as above	5.31	as above	as above	
Netherlands drinking water	ethinyl-oestradiol	0.06	Mass Spectrometry	Freudenthal *et al.*, 1975	quoted from Rathner et al., 1979
Netherlands river water	ethinyl-oestradiol	0.3	Mass Spectrometry	as above	

Summary of oestrogen concentrations in the environment, continued

sample	compound	results [ng/l]	method	remarks	reference
56 springs and wells for drinking water supply in South Germany	oestradiol	0-0.9 average: 0.2	radioimmunologic	probably only free compound	Rurainski *et al.*, 1977
as above	ethinyl-oestradiol	0-22.5 average: 2	EE2-radio receptor ligand assay	probably only free compound	
as above	summary of steroids including esters. Calculated as ethinyl-oestradiol	0-104 average: 41	fluorometric: method of Ittrich	including conjugates results probably too high because of unspecific fluorescence	
Tel Aviv area a) raw sewage b) anaerobic tank c) activated sludge d) effluent e) slow sand filter (3 months)	oestrogen	a) 48-141 b) 7-39 c) 19-64 d) 7-50 e) 2	radio-immunoassay for oestradiol (?), with 25% cross reaction with oestrone Total oestrogen concentration may be much higher (Turan 1996)	The values go apparently up and down during treatment. Maybe due to cleavage of conjugates which are not included in the figures (?) Highest values at the end of exceptionally dry period.	Shore *et. al.* 1993
Effluent from small sewage treatment units on farms	as above	summer: 343 winter: 153	as above	before dry period	
Municipal effluent	as above	summer: 116 winter: 39	as above	before dry period	
a) wells in N. Israel b) lake Kinneret (shore, 1991) c) drinking water from lake (500m offshore)	as above	a) 3 b) 4-23 c) <2-22	as above	-	

Summary of oestrogen concentrations in the environment, continued

sample	compound	results [ng/l]	method	remarks	reference
Effluents of 20 German sewage works	ethinyl-oestradiol (EE2) oestradiol (E2) oestrone (E1)	EE2 detectable in all effluents (max. 62 , median 17) E2 only in 8 (max. 20) E1 in none	GC/ion-trap-MS after solid phase extraction at pH 3. Detection limit 1 ng/l	**are conjugates cleaved at pH 3 ?** Values may include an unknown impurity not distinguishable from EE2 with this method. (Ternes *et al.* 1999a)	Stumpf *et al.*, 1996
Rivers in Germany 10 sites	as above	only EE2 detectable 0-4			
Drinking water	as above	< det. limit			
Concentrations in raw and treated sewage (USA)	oestradiol	ca. 50 % loss during treatment effluent: 0-20 average 10	TLC and Gas-Liquid - Chromatography after hydrolysis and liquid/ liquid extraction	**results include the conjugates** loss for primary treatment only: around 40 %	Tabak *et. al.* 1981
	oestrone	50 to 60% loss during treatment effluent: 0-40 average: 20	as above	**results include the conjugates** loss for primary treatment only: around 40 %	
	ethinyl-oestradiol	30 to 40% loss during treatment effluent: 250-1,780 average: 810	as above	**results include the conjugates** loss for primary treatment only: 20-30 %	

Summary of oestrogen concentrations in the environment, continued

sample	compound	results [ng/l]	method	remarks	reference
Concentrations in 16 German STW effluents	oestrone	<1 – 70 Median: 9 90-percentile: 22	GC/MS/MS after solid phase extraction at pH 3. Detection limit 1 ng/l	The method is based on Stumpf *et al.* but improved to distinguish better between the hormones and other impurities	Ternes *et al.* 1999a
	oestradiol	<1- 3 Median: n.d. 90-percentile: 2			
	ethinyl-oestradiol	<1- 15 Median: 1 90-percentile: 4			
Concentrations in 10 Canadian STW effluents	oestrone	<1 – 48 Median: 3 90-percentile: 10	as above	as above	
	oestradiol	<1- 64 Median: 6 90-percentile: 14			
	ethinyl-oestradiol	<1- 42 Median: 9 90-percentile: 29			
Concentrations in 15 German rivers and streams	oestrone oestradiol ethinyl-oestradiol	only oestrone in three rivers detectable: 0.7-1.6; all others <0.5	GC/MS/MS after solid phase extraction at pH 3. Detection limit 0.5 ng/l	as above	
River Lee (UK)	ethinyl-oestradiol	traces (<1)	GC/MS	qualitative survey of organic substances in the River Lee	Waggot 1980

Note on table 2.1:

- It is not always clear whether or not the quoted figures include the conjugated steroids, which are not biologically active but can be cleaved enzymatically to reveal the original active compound.
- The values of Tabak *et al.* (1981) are much higher than the other quoted values, presumably because with their method the conjugates are chemically cleaved at the beginning of the procedure and therefore included in the figures and the values are for total extractable oestrogens, whereas in the other values compounds sorbed to suspended solids are probably not included.
- Cross reactions with the matrix may have distorted some results especially where detection methods other than GC/MS were used.

In addition to measurements of environmental concentrations, some workers have tried to estimate likely concentrations. (table 2.2). Although these estimates often represent worst case scenarios, it is clear from the above summary, that at least in some stretches of some rivers, steroid concentrations in the water could be high enough to account for oestrogenic effects on fish, which have sometimes been observed at concentrations as low as 0.5 ng/l ethinyl-oestradiol (Purdom *et al.*, 1994).

Table 2.2 Estimates for concentrations of natural and synthetic hormones. Calculated on basis of prescriptions and natural excretion

sample	compound	estimates	reference
American sewage influent (no metabolism)	ethinyl-oestradiol	2.16	Arcand-Hoy *et al.* (1998)
American sewage influent (no metabolism)	conjugated oestrogens from HRT	41.5	Arcand-Hoy *et al.* (1998)
American sewage influent (no metabolism)	oestradiol as growth enhancing hormone in animal production	14.2	Arcand-Hoy *et al.* (1998)
Berlin sewage	ethinyl-oestradiol	15 ng/l	Rathner & Sonnenborn (1979)
Berlin sewage	natural oestrogens	800 ng/l	Rathner & Sonnenborn (1979)
English surface water	ethinyl-oestradiol	3 ng/l	Richardson & Bowron (1985), quoted from Römbke *et al.* (1996)
River Main, Germany	ethinyl-oestradiol	0.7-2.5 ng/l	Kalbfus (1995), quoted from Römbke *et al.* (1996)
River Main, Germany	oestradiol	1.2 ng/l	Kalbfus (1995), quoted from Römbke *et al.* (1996)
German surface water	ethinyl-oestradiol	0.2 ng/l	Römbke *et al.* (1996)
German surface water	oestradiol	10 ng/l	Römbke *et al.* (1996)
German surface water	oestriol	5 ng/l	Römbke *et al.* (1996)

2.3 Removal and Degradation of Oestrogens

A summary of previous research on the degradation and removal of oestradiol, oestrone and ethinyl-oestradiol is given in table 2.3. The comparison of concentrations before and after treatment in sewage treatment works, mentioned in table 2.1 (Tabak *et al.*,1981, Shore, 1993 and Lebietzka, 1996), could also be included in this summary. No data was available for degradation rates in the natural environment.

The values by Stumpf *et al.* (1996) would suggest a faster degradation rate for ethinyl-oestradiol than for 17β-oestradiol, which is a contradiction to the findings of other authors.

This value however, was based on just one plant where concentrations were monitored over 5 days. Oestrogens were determined in the filtered samples, therefore removal by sorption to the sludge also contributed to the difference between influent and effluent. The input concentrations of 17β-oestradiol were already much lower than of ethinyl-oestradiol. The authors suggest a difference in human metabolism as the cause for this, but an initial faster degradation of 17β-oestradiol taking place already in the sewer could also be an explanation.

Table 2.3 Removal and degradation of oestrogens

substance	Experiment set-up	results	remarks/method	reference
ethinyl-oestradiol (EE2)	shaking batch system with activated sludge (diluted to 0.7 g/l dry weight) and additional carbon source, EE2-conc.: 50 ng/l -1 mg/l	55-80% degradation in 8 weeks, depending on origin of sludge Initial conc. had little influence on degradation rates	ELISA for free compound after extraction from water or solids activated sludge from: •pharmaceutical, •municipal, and •brewery- sewage treatment works.	Lebietzka, 1996
ethinyl-oestradiol	activated sludge model conc. not given	no degradation in 5 days	TLC (fluorometric) concentration sorbed on sludge below detection	Norpoth *et al.*, 1973
oestradiol	degradation in drinking water conc. not given	after 10 days 61.1% remaining unaltered	radio - TLC	Rurainski *et al.*, 1977
ethinyl-oestradiol	degradation in drinking water conc. not given	after 10 days 77.6% remaining unaltered		
oestradiol including conjugates	elimination in a STW	75%	difference between influent and effluent. GC-MS of filtered sample	Stumpf *et al.*, 1996
ethinyl-oestradiol including conjugates	elimination in a STW	89%		

Removal and degradation of oestrogens, continued

substance	Experiment set-up	results	remarks/method	reference
oestrone (E1) oestradiol (E2) ethinyl-oestradiol (EE2)	activated sludge addition of trace minerals shaking at 25°C steroids (20mg/l) dispersed in media	Loss after one week: E1: 94% E2: 90% EE2: 73%	spectrophotometric analysis of chloroform extracts. Expts. use 20 mg/l (above solubility !)	Tabak & Bunch 1970
Oestrone (E1) Oestradiol (E2) Ethinyloestradiol (EE2)	elimination in STW (Penha/Rio de Janeiro, Brazil) average temperature >20°C	a) Activated sludge treatment E1: 83% E2: 99.9% EE2: 78% b) Trickling filter: E1: 67% E2: 92% EE2: 64%	GC/MS/MS of filtered sample	Ternes *et al.*, 1999a
Oestrone (E1) Oestradiol (E2) Ethinyloestradiol (EE2)	elimination in STW (Frankfurt/Main, Germany) during winter: average temp. -2°C	E1: no reduction E2: 64% EE2: no reduction	GC/MS/MS of filtered sample. For E1 and E2 highest values after preliminary clarification. (conjugate cleavage?)	
Oestradiol-glucuronide	shaking batch system with activated sludge (1:10 diluted) conc. 1 mg/l and 1 µg/l	most E2 released within one day at 1mg/l and within 5 min at 1µg/l. No lag-phase observed for glucuronidase	GC/MS/MS of filtered sample.	Ternes *et al.*, 1999b

Removal and degradation of oestrogens, continued

substance	Experiment set-up	results	remarks/method	reference
Oestrone (E1) Oestradiol (E2) Ethinyloestradiol (EE2)	shaking batch system with activated sludge (1:10 diluted) conc. 1 mg/l and 1 µg/l	1 mg/l: after 1-3 hrs 95% of E2 is converted to E1, which then disappeared within 3 days. Half lives: E2 < 1hr, E1 approx. 1 day. Much faster at 1µg/l: both gone after 5 hrs EE2: much more stable, loss of about 20% over 2 days	GC/MS/MS of filtered sample.	Ternes *et al.*, 1999b

2.4 Fate and Behaviour in Rivers

The release of oestrogenic chemicals into the environment does not necessarily mean that these substances will remain available to aquatic organisms. Harries *et al.* (1997) assessed the oestrogenic activity of five UK rivers in vivo by exposing caged male trout to primary sewage treatment works effluents and to river waters at several distances downstream of the discharge and measuring the blood vitellogenin levels after three weeks. They found that the oestrogenic activity of two of the assessed sewage works was dissipated within a few meters of the discharge point and one very small sewage treatment works showed no oestrogenic activity at all, whereas at the other two sewage treatment works the oestrogenic activity could be detected in the river several kilometres downstream. Therefore, the oestrogenic effects were reduced after discharge, probably due to dilution, degradation and sorption of the oestrogenically active substance(s). Clearly, before the environmental significance of oestrogenic substances can be estimated, knowledge of the fate and behaviour of these substances once they are discharged into the rivers has to be improved.

3. MATERIALS AND METHODS

3.1 Collection of River Water and Sediment Samples

The river water, suspended sediment, and bed-sediment samples were collected from a number of different river environments:

Two highly polluted urban and industrialised rivers:

- The River Aire, 8 km downstream of Leeds (National Grid Reference (NGR) SE 379 288) on 6 Sep. 1996, 18. Dec. 1996 and 18. Jul. 1997.
- The River Calder, 16 km downstream of Wakefield. (NGR SE 405 207), on 16. Jan. 1997 and 18. Jul. 1997

A relatively rural stretch of a river:

- The River Thames near Wallingford (NGR SU 614 903) on 5. Dec. 1996, 15. Apr. 1997, 27. Jun. 1997 (sediment and water) and 4. Jun. 1997, 21. Jul. 1997, 9. Sep. 1997 and 13. Oct. 1997 (water only): Sediments were collected from different sites within a few meters of each other: inside a boathouse, from a nearby slipway and from the river bottom mid-channel.

Two urban/industrial estuaries:

- The estuary of the Tees: at Seal Sands (NGR NZ 535 265) and Bran Sands (NGR NZ 555 265) on 31. Jul. 1997.

- The estuary of the R. Tyne: at Hebburn (NGR NZ 325 658) on 1. Aug. 1997.

Water samples were collected in 1 l glass bottles from 5 cm below the river surface and stored at 4°C prior to use.

The maximum storage period for the water samples was 1 month at this temperature. Bed-sediments were collected from 1-2 m from the river bank by skimming the top 5 cm with 1 l bottles. For the collection of the bed-sediments from the estuaries a specially designed mechanical grab was used. Prior to sorption experiments with the bed-sediments, the samples were air-dried, passed through a 2 mm sieve and stored at room temperature.

3.2 Methods

3.2.1 Characteristics of the Water Samples

Dissolved Organic Carbon (DOC)

DOC in river water was measured using a TOCsin II Aqueous Carbon Analyser (Phase Separations Ltd.). The 0.2µm-filtered sample was acidified and purged of CO_2 and then pumped through a heated capillary inlet tube and forced into an oxidation furnace, where the DOC was converted to CO_2. The carbon dioxide formed was then mixed with hydrogen over a nickel catalyst to form methane. The methane, evolved from the original organic carbon, was measured using a flame ionisation detector.

Suspended Sediment Load

The suspended sediment load was determined by weight by passing 250 ml river water through a pre-weighed 0.2µm filter and reweighing the dried filter.

3.2.2 Characteristics of Sediments

Particle Size of Bed-sediments

The particle size of the < 900 µm fraction was assessed using a laser granulometer (Coulter LS 130). This gives the percentage of the different fractions as percent of total volume, not mass.

Clay Mineralogy of Bed-sediments

The clay mineralogy was determined using an x-ray diffraction system. The results given are semi-quantitative and refer to the groups, not the individual minerals.

Total Organic Carbon of Bed-sediments

Total organic carbon was assessed for the air dried and sieved sediments using the method of Gaudette *et al.* (1974), which was specially devised for sediment organic carbon measurements. This method uses exothermic heating and oxidation with potassium dichromate and concentrated sulphuric acid and the titration of excess dichromate with ferrous ammonium sulphate solution. For each bed-sediment, three replicate samples were measured.

3.2.3 Development of Techniques for Handling Steroid Oestrogens: Sorption of 17β-Oestradiol to Laboratory Equipment

To assess the suitability of different laboratory containers for experiments with 17β-oestradiol the sorption of the compound to different laboratory materials was tested. 10 ml purified water with 5 µg/l radiolabelled 17β-oestradiol (4-^{14}C-17β-oestradiol, 0.74 MBq m/l, 99% purity, DuPont NEN) was added to containers of either glass, polytetrafluoroethene (PTFE), polycarbonate or polypropylene (three replicates each). The containers were incubated at room temperature (20 +/- 2°C) and sampled after 24 and 48 h. Sampling was done by drawing 0.7 ml into a syringe which already contained 0.7 ml methanol. This was then added to a plastic scintillation vial, mixed with 5 ml scintillant and counted for 5 minutes. At 48 h the remaining liquid in each container was poured into three pre-weighed scintillation vials and counted again. The containers were then rinsed three times with cold water and absorbed 17β-oestradiol determined by extracting them overnight on a shaker at room temperature with 5 ml methanol. The methanol from the extraction was then poured into two scintillation vials and counted as above.

3.2.4 Determination of the Octanol/Water partition coefficient (K_{ow})

The octanol/water partition coefficient (K_{ow}) of 17β-oestradiol was determined using a carbon-14 labelled analogue (4-^{14}C-oestradiol, Du Pont NEC-127, 7.4 MBq/mg, stock solution 0.1 g/l in ethanol). 10 ml of 10 µg/l 17β-oestradiol in purified water and 0 µl, 50 µl, 200 µl octanol (duplicates) were placed in improvised separation funnels (chromatography mini-prep columns, BioRad, with the filter at the bottom removed). These were placed on a shaker for one hour during which they were removed every 20 minutes for an additional rigorous mixing on a whirly mixer. The samples were then centrifuged (10 minutes, 675*g) to assist phase separation. The aqueous phase of each funnel was sampled in triplicate by removing 1-2 ml into pre-weighed scintillation vials and determining the exact amount by weight. 5 ml scintillant (Ultima Gold, Canberra Packard) was added and the vials placed in a liquid scintillation counter (Beckmann LS 6500 instrument) to be counted for 5 minutes. The counts were compared to those of the original solution. The loss of 17β-oestradiol without addition of octanol was used to estimate the relative loss through handling.
The concentration in octanol was calculated as the difference between the original concentration and the remaining concentration in water after taking into account any sorption to the plastic. K_{ow} was calculated as the ratio of mass per volume in octanol and water.

3.2.5 Sorption of 17β-Oestradiol to Bed-Sediments

Sorption and Desorption Kinetics

The sorption kinetics were investigated for bed-sediment samples collected from the R. Aire (6. Sep. 1996) and R. Thames (27. Jun. 1997). 5 g of the airdried sediment (Thames) were

added to 15 ml of river water from the same origin (0.2 µm filtered to remove all suspended sediments) in 45 ml PTFE screw-top centrifuge tubes (Nalgene). 75 µl ^{14}C-17β-oestradiol was added to the water/sediment samples from a freshly made up stock solution (1 mg/l) to give a concentration of 5 µg/l. The same amount of 17β-oestradiol was added to centrifuge tubes containing 15 ml purified water to act as standard. The centrifuge tubes were laid diagonally (so the liquid did not touch the polypropylene lids) on an orbital shaker and shaken at 90 rpm at room temperature. Using a sacrificial sampling technique, the replicates were removed at intervals of 5 min, 30 min, 4 h, 24 h and 48 h. Following centrifugation at 4749 g for 15 min, each tube was sampled 3 times by removing 0.7 ml of the supernatant into 0.7 ml methanol with a syringe. This was then added to a further syringe connected to a 0.45 µm PTFE-filter. The sample was filtered directly into a scintillation vial, mixed with 5 ml scintillant and counted for 5 minutes. The amount of radioactivity still present in the aqueous phase was compared with the sediment-free pure water controls and the amount sorbed calculated by difference. The supernatant was then replaced by fresh filtered river water from the same site and the samples returned to the shaker to be analysed after 0.5 h and 24 h of desorption.

To clarify that the delayed sorption observed in the first experiment was not due to insufficient mixing of the sample, only 1 g sediment (Thames 27.6.97 and Aire 6.9.96) was used in a second experiment of the same kind. The smaller size of the forming pellet should ensure that the transport mechanisms to the particles were sufficient. Because in the previous experiment equilibrium had not yet been reached after 2 days a longer timescale was used: The samples were analysed after 1 d, 2 d and 6 d of sorption. During the sorption times all the samples were kept anaerobic (2.5 l anaerobic jar with AnaeroGen pack, Oxoid) and dark to slow down degradation. Desorption was for 30 min and 24 h aerobic as above.

Influence of Storage and Oxygen Status on Sorption Potential of Bed-Sediments

The influence of air-dried compared to wet storage on 17β-oestradiol sorption potential, was tested with R. Thames bed-sediment (27. June 1997). A subsample was air-dried and prepared in the normal way whilst the remaining sediment was stored wet and cool (4°C). 1 g dry sediment or the equivalent of the wet sediment (1.55 g) were added to water from the same site (total amount of water 15 ml) and spiked with 17β-oestradiol to give a final concentration of 5 µg/l. The samples were incubated by shaking for 24 h at room temperature before centrifugation and measurement of the aqueous phase concentrations.

To study the influence of anaerobic versus aerobic conditions on sediment sorption, samples were taken from the R. Thames (27. June 1997) and R. Aire (18. Dec. 1996). Subsamples of 3 g of the air-dried and sieved samples were mixed with 15 ml of filtered river water. Those samples destined for anaerobic incubation were placed with loose caps in a 2.5 l anaerobic jar with an AnaeroGen gas pack, for 24 h prior to the addition of 17β-oestradiol. Similarly those for anaerobic incubation were left to equilibrate for 24 h before the addition of 17β-oestradiol. Following this period, the tubes were opened and radiolabelled 17β-oestradiol added to give final concentrations of 2.5, 5, and 10 µg/l. A fresh AnaeroGen pack was added to the samples for anaerobic incubation and all the samples were allowed to equilibrate for 21

h on the orbital shaker. The aqueous concentrations were then measured and K_d determined as described in the following paragraph.

Establishing Sorption Distribution Coefficients for 17β-Oestradiol and Bed-Sediments

Any laboratory method chosen to measure distribution coefficients for an organic compound will be a compromise. Long incubation periods may be necessary to achieve equilibrium, however, biodegradation will often present this from being achieved by removing the sorbate from the system. Sterilising the sediments would prevent this, however, the sterilising process may completely alter the nature of the sorbent surface.

For the comparison of different bed-sediments 1 to 5 g of the airdried sediment (depending on the expected K_d from the known TOC) and 15 ml 0.2 µl filtered water of the respective site were used. The protocol followed that used in the kinetic experiments with the exception that incubation was for 20 h only, and carried out under ambient aerobic conditions. Three replicates at three 17β-oestradiol concentrations (2.5 µg/l, 5 µg/l and 10 µg/l) were used to establish an isotherm. It is reasonable to expect that an isotherm derived from these concentrations would be relevant for the lower natural concentrations of a few ng/l, since the sorbent surfaces would still be in great excess with respect to the sorbate, and therefore the observed concentrations would be in the linear range of the Langmuir-isotherm. The slope of a best fit line through the data for solid phase versus aqueous phase concentration yielded the distribution coefficient.
For some of the samples a second distribution coefficient (desorption) was measured after 20 h of desorption as described in the previous section.

The loss of radioactivity in the aqueous phase can be attributed to sorption not degradation because although in some samples oestradiol is completely converted to oestrone within 3 days through enzymatic oxidation (compare section 4.5.1) this can be seen as merely a different form of the molecule with similar physico-chemical and toxicological properties. An example for the properties is the solubility in double distilled water which is 12.4 mg/l for oestrone and 13.0 mg/l for 17β-oestradiol.

3.2.6 Establishing Sorption Distribution Coefficients for 17β-Oestradiol to Suspended Sediments

Because of the difficulty in measuring sorption to the low natural concentrations of suspended sediments, the experiments had to be preceded by a concentration step. The concentration step was done on site for the Thames (4.6.97) sample using a high capacity pump feeding water from 5 cm below the surface to a continuos flow centrifuge (Brownbill, *et al.,* 1992) to give a concentration factor of around 40. For R. Aire and R. Calder (18.7.97) samples the concentration step was undertaken in the laboratory. A BECKMANN J2-21 centrifuge was used to spin three 250 ml centrifuge bottles which were filled with water from each of the two sites and centrifuged 1 h at 14 000 rpm. Most of the supernatant was then discarded and the

remaining settled sediments resuspended in a smaller volume (55 ml). This concentrated sediment was kept suspended in a bottle by stirring with a magnetic flea. 5 ml was then added to each replicate PTFE centrifuge tubes. Radiolabelled 17β-oestradiol was added to give concentrations of 2.5, 5 and 10 µg/l. The tubes were positioned on the shaker and rotated at room temperature at 120 rpm for 20 h. The tubes were then centrifuged at 4749 g for 30 min before samples being taken from the aqueous phase using the technique described previously and assessed for radioactivity. The concentration of 17β-oestradiol bound to the solid phase was calculated by difference.

3.2.7 Degradation of 17β-Oestradiol, Oestrone and Ethinyl-Oestradiol

To measure the degradation, relatively high concentrations (200-400 µg/l) were used in the microcosm experiments. Whilst this concentration is many times higher than that which would normally be encountered in rivers, it does establish whether the indigenous river microorganisms are competent to degrade these compounds. The temperature of 20°C, used in the experiments, does occur in summer for about a month in the R. Thames and for periods in rivers in the Yorkshire area (Lewis *et al.*, 1997).

Analytical Technique for Measuring Non-radioactive Steroids

The degradation experiments were carried out with non-radioactive chemicals.

The samples, which contained 50% methanol to inhibit microbial action and prevent sorption to the filter, were 0.45µm filtered (Nalgene PTFE-filters) into 2 ml HPLC vials with teflon/rubber septa and stored for up to one month at 4°C prior to analysis by HPLC. Samples were taken into the HPLC via a 150 µl loop. A C18 column 2 x 250 mm (COLUMBUS) with a CH_3CN : H_2O, 42:58 eluent and a UV absorption detector was used. The concentration was determined from the absorption at 220 nm giving a detection limit of 25 µg/l in the 50% methanol samples i.e. a detection limit of 50 µg/l in the original samples. The absorption at 230 nm was also measured, the ratio of the values for 220 nm and 230 nm indicating whether an observed peak was likely to be the expected steroid or another compound eluting at the same time.

Aerobic and Anaerobic Biodegradation of 17β-Oestradiol in Different River Waters

River water samples from the R. Thames (21 July 1997), R. Aire (18 July 1997), R. Calder (18 July 1997), Tees estuary (31 July 1997) and Tyne estuary (1 August 1997) were collected and stored at 4°C for up to 40 days prior to the start of the experiment. On arrival at the laboratory the pH, DOC and suspended sediment content of the water was tested. For each river water sample, 50 ml quantities were added to six PTFE conical flasks. River water controls were sterilised by autoclaving. Using a methanol 17β-oestradiol stock (concentration 6.74 g/l) 3.71 µl was added to each flask to achieve a final concentration of 500 µg/l 17β-oestradiol. Half of the flasks were placed in anaerobic jars, ensuring that their tops were not screwed down, where anaerobic conditions were generated by the addition of

AnaeroGen packs (Oxoid). Indicator test strips (Anaerotest, Merck) were added to these jars to confirm the removal of O_2 from the headspace. All the samples (aerobic and anaerobic) were incubated at 20°C. The flasks were sampled at the start of the experiment and after 3, 6, 10, 14, 20, 23, 28, 35, 42 and 49 d. This was achieved by withdrawing 700 µl aliquots into a syringe containing 700 µl methanol before filtering through a 0.45 µm PTFE filter (Nalgene) into 2 ml HPLC vials with teflon/rubber septa. Sampling the anaerobic flasks meant opening them to the air for approximately 20 minutes before regeneration of anaerobiosis with another AnaeroGen pack. Samples were stored for up to 14 days at 4°C prior to analysis by HPLC.

Degradation of Ethinyl-Oestradiol compared to 17β-Oestradiol

Thames water (13. Oct. 1997) was spiked with either 300 µg/l 17β-oestradiol or the same amount ethinyl-oestradiol. Triplicate samples and sterile controls were incubated under aerobic or anaerobic conditions as described in the previous paragraph and sampled at the start of the experiment and after 2, 4, 7, 11, 15, 24, 31, 39 and 46 days, following the same protocol as above.

Mineralisation of 17β-Oestradiol Phenol Ring A

In contrast to the previous degradation experiments in which the loss of the parent molecule over time was measured, in this experiment the evolution of $^{14}CO_2$ from the phenol ring of 17β-oestradiol (A) was monitored. To compare with the previous degradation experiment the same concentration of 17β-oestradiol was selected (500 µg/l, of which 10 µg/l was radio-labelled).

Triplicate samples of 40 ml of R. Aire (18.7.97), R. Calder (18.7.97) and R. Thames (1.9.97) water were added to 125 ml glass conical flasks (Quickfit). To measure the evolution of $^{14}CO_2$ an air pump feeds air through a soda lime CO_2 scrub and a drechsel flask to moisturise the air. Via a manifold the air is passed over the conical flasks containing the sample and through a 5 ml granular activated charcoal cartridge (to capture volatilised 17β-oestradiol or by-products) and bubbled into a drechsel flask containing 50 mM NaOH to trap any evolved $^{14}CO_2$. All the flasks were connected via neoprene tubing. The conical flasks containing the samples and attendant apparatus were maintained within a 20°C incubator. The air pump was switched on every 3 days to purge the headspace of the conical flasks. The air flow was regulated to approx. 0.5 l/min distributed between the 12 flasks for 3 hours giving about 120 total air changes in the headspace of the conicals. 1 ml was then withdrawn from the NaOH solution and mixed with scintillant cocktail prior to counting in the scintillation counter (each sample counted twice for 20 min). Autoclaved samples were treated in the same way and used as sterile controls.
The apparatus used is shown in figure 3.1.

At the end of the experiment a mass balance was undertaken for the radiolabel. The remaining radioactivity in the batches was measured by sampling 1 ml into the scintillation vials. The remaining liquid (37-38 ml) was then passed under gravity through solid phase

extraction cartridges (Variant) to concentrate the parent and hydrophobic by-products containing the aromatic ring.

The radioactivity that passed through the column in form of more hydrophilic by-products was also measured. Any compounds sorbed to the neoprene tubing or the emptied charcoal tube were measured by extracting the tubes with methanol.

To measure the radioactive carbon trapped on the charcoal, the apparatus above was modified by replacing the incubator by a furnace in which the charcoal was placed for 1 h at 850°C. As there was only room for one sample at a time, the air flow was reduced to 0.1 l/min. The apparatus is shown in figure 3.2.

Low concentration biodegradation tests

To enable the study of the degradation potential for 17β-oestradiol at lower concentrations, 1 l volumes from the R. Thames were dispensed into 1 l screw top PTFE containers. 3 containers were then sterilised by autoclaving for 30 min. From a 15 mg/l 17β-oestradiol stock in methanol, 20 µl was added to the controls and 3 non-sterile samples to give a final concentration of 0.3 µg/l. The containers were then incubated with their tops loose at 20°C. Sampling was achieved by decanting 200 ml from the containers into 250 ml glass bottles. These were extracted the same day by passing through pre-conditioned C18, 3 cc cartridges (Isolute, IST Ltd, UK) and eluting in 1 ml of methanol prior to HPLC analysis. Spike tests on this extraction technique gave 82-96 % recovery. From the same R. Thames sample, 100 ml volumes were added to 250 ml Quickfit conical flasks. Four replicate flasks were autoclaved (121^{0}C for 30 min) as sterile controls and four left un-sterilised. From the 1 mg/l ^{14}C-oestradiol stock, 10 µl was added to each flask to give a final concentration of 0.1 µg/l (100 ng/l). Mineralisation of the compound was measured using the same apparatus and conditions as described previously.

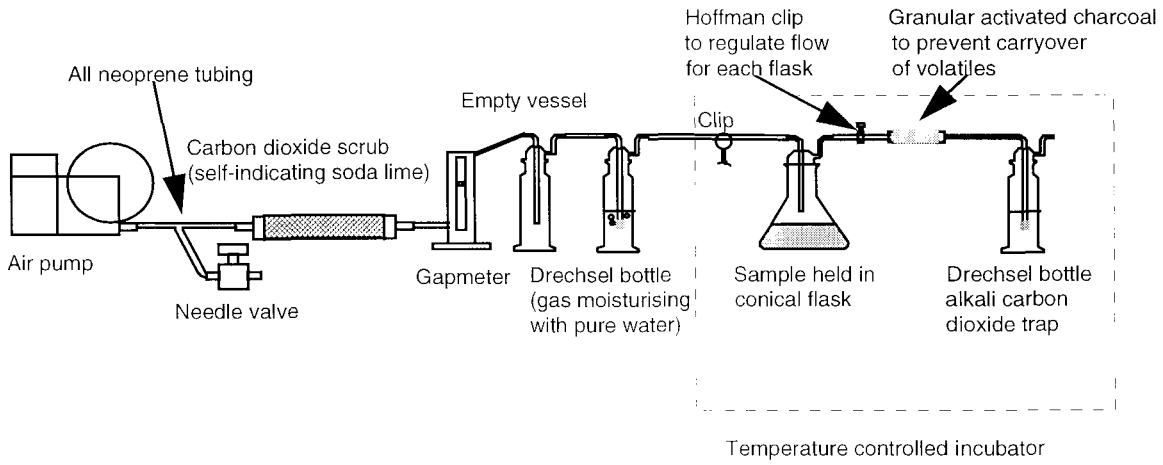


Figure 3.1 Schematic diagram of apparatus for 17β-oestradiol mineralisation

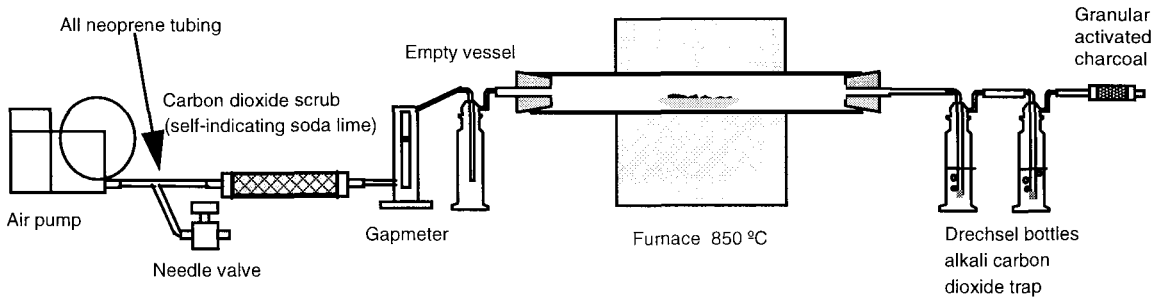


Figure 3.2 Schematic diagram of apparatus to release radiolabel from charcoal

4. RESULTS AND DISCUSSION

4.1 Development of Methods: Sorption of 17β-Oestradiol to Laboratory Equipment

It is important when studying the behaviour of organic chemicals, particularly those with hydrophobic properties, to consider sorption to laboratory equipment. In previous experiments with another hydrophobic chemical (octylphenol) it was found that the presence of 50% methanol reliably prevented sorption to all tested materials (Johnson *et al.* 1998). Therefore, all experiments were sampled by withdrawing the sample into a syringe which already contained the same amount of methanol.

While this treatment was suitable for the sampling procedure as such, it was also neccessary to find containers in which aqueous oestrogen solutions can be stored without much loss. In this experiment the sorption of 17β-oestradiol to different materials was monitored over a two day period. Measuring the remaining 17β-oestradiol in solution after one and two days gave evidence of sorption to the plastic materials, but the variability in the results was relatively high, due to sampling errors. The most conclusive results were obtained by extracting the sorbed fraction from the containers with methanol. The results shown in table 4.1 demonstrate that small quantities of 17β-oestradiol adsorb to glass or plastic containers over a 2 d period. Glass and PTFE proved to be the containers, which adsorbed the least 17β-oestradiol, so these were selected for the fate and behaviour experiments. Since oestrone and ethinyl-oestradiol have similar properties to oestradiol, these containers would also be expected to be suitable for handling these steroids.

Table 4.1 Oestradiol sorbed to laboratory equipment, means of three replicates (± refers to 2*standard deviation =95 % confidence interval)

Material	Glass conicals Quickfit, 125 ml	PTFE tubes Nalgene, 45 ml	Polycarbonate tubes Sterilin, 30 ml	Polypropylene tubes Simport, 50 ml	Polypropylene tubes Corning, 50 ml
sorption after 2 days	0.25 % (±0.14)	0.40 % (±0.52)	1.03 % (±0.22)	1.92 % (±0.08)	4.10 % (±1.08)

4.2 Octanol/Water Partition Coefficient, K_{ow}

The K_{ow} of a molecule is viewed as an index of its hydrophobicity. This property is seen as a reliable indicator of a molecule's potential to sorb to organic materials. K_{ow} has been used to estimate the likely sorption to organic carbon in sediments (Karickhoff, 1981, Di Toro, 1991). The method used gave a log K_{ow} of 3.1 ± 0.1. The oestradiol solution used still contained a small proportion of ethanol, which may have influenced the partition, but as the ethanol concentration was only 0.01% it is expected that the influence was negligible.

It is interesting to note that the K_{ow} of oestradiol is a factor of 10 below the xenobiotic

oestrogens nonyl- and octylphenol (Ahel and Giger, 1993). Therefore, it would be predicted that 17β-oestradiol would have a lower potential to sorb to sediments in rivers than the oestrogenic alkylphenols. The results shown in table 4.2 show indeed a lower sorption capacity of 17β-oestradiol compared to 4-tert-octylphenol (log K_{ow} = 4.12 ± 0.10, Ahel and Giger, 1993)
Another value for the hydrophobicity of 17β-oestradiol was given by Schweinfurth and Länge (1996) with Log P_{ow} for 17β-oestradiol of 4.0. However it is not clear from the report what the units are or the methods used.

4.3 Sorption of 17β-Oestradiol to Bed-Sediments

The basic assumption of the sorption experiments is that ^{14}C-17β-oestradiol not detected in the aqueous phase must be associated with the solid phase. This assumes that the 17β-oestradiol has not been lost through degradation. However, the degradation experiments show that most of the 17β-oestradiol may be transformed to oestrone with the Aire and Calder samples (less with the other samples) within three days. In the conversion of 17β-oestradiol to oestrone, H_2 is removed, but the steroid ring system is not affected, so no loss of the radiolabel would occur. 17β-Oestradiol and oestrone have similar chemical properties and oestrogenic effects (Tabak, 1981, Fishman and Martucci, 1980). It can be argued that the transformation of a proportion of 17β-oestradiol to oestrone would not significantly affect the interpretation of the results.
The degradation rates in the presence of bed-sediments however, have not been determined due to technical problems, but as the measured distribution coefficients are in the expected range (see chapter 4.3.2), it is unlikely that large amounts of radiolabel have been lost as CO_2 or volatile by-products during the course of the experiments.

4.3.1 Influence of Storage and Oxygen Status

As a practical method to compare bed-sediment sorption properties, samples were pre-dried and stored before use. The sediment samples were then re-wetted for the sorption experiments, which were carried out under ambient aerobic conditions. However, this procedure may in some way affect the chemistry of the sediments and thus their ability to sorb the test chemical. Thus, the results gained from sediments, which have been previously dried, may differ from the potential sorption under natural conditions. The influence of the air-drying preparation compared to maintaining the sediments in the wet form was tested with R. Thames bed-sediment (27. June 1997). The storage conditions had no significant influence on the sample, with wet stored and dry stored sediment giving K_d values of 25 (SD 2.7) and 20 l/kg (SD 2.9) respectively.
Comparing aerobic with anaerobic conditions on bed-sediment sorption gave K_d values of 18 and 24 l/kg respectively for the R. Thames (27. Jun. 1997) and 40 and 47 l/kg for the R. Aire (18. Dec. 1996) samples. When all of the data points which made up the respective curves were compared using a t-test these differences were not found to be significant at α=0.1.

4.3.2 Sorption and Desorption Kinetics

Sorption

Figures 4.1 and 4.2 show the oestradiol concentration in the water column and on the bed-sediment over 44 hours with 5 g bed sediment or 6 days with 1g bed sediment respectively (individual values and mean of three replicates). Although some caution may be required in the interpretation of the results, for the reasons discussed above, the sorption kinetics follow an expected pattern. An initial rapid sorption over the first 24 h is followed by a slow increase of sorbed oestradiol over the following 5 d. There is some evidence that sorption is initially more rapid with the Aire bed-sediment sample, perhaps due to its higher TOC (2.4 % compared to 1.1 %), than with the Thames.

These optimised batch sorption experiments show the behaviour of 17β-oestradiol and its attachment to sorption sites, but do not reflect the natural river conditions where contact between free chemical and the bed-sediments would be fleeting.
For the distribution coefficient experiments a 20 h incubation, whilst not being long enough to allow the establishment of a true equilibrium, was chosen as being suitable for comparative studies between the bed-sediments. During this time 80-90% of the sorption would be expected.

Desorption

Replacing the aqueous phase with fresh (17β-oestradiol-free) water and then allowing further mixing resulted in a small proportion of the originally sorbed compound returning into solution. Thus, the experiment continued for a further 24 h under ambient aerobic conditions. The new distribution coefficients were invariably higher than the first ones, which indicates either a greater proportion of the 17β-oestradiol remaining on the solid phase, or 17β-oestradiol being eliminated by degradation. As discussed above the degradation results with water from these sites imply that the latter explanation is unlikely, but it is not clear how the presence of bed sediment influences degradation rates.
The amount of 17β-oestradiol being desorbed decreased slightly with longer sorption times. This apparent increase in sorption strength or hysteresis effect has often been noted with organics and natural sediments or soil aggregates (Kan *et al.* 1994, Pignatello and Xing, 1996).
Table A1-A3 in the appendix show the sorption and desorption distribution coefficients (K_d, kg/l) over time for both experiments. The correlations between the corresponding K_ds in both experiments indicate that the different experimental conditions had no significant influence on the sorption kinetics. Therefore, the slow sorption was probably not due to insufficient transport mechanisms to the sediment aggregates, which would have been improved by the use of a much smaller amount of sediment in the second experiment.

4.3.3 Distribution Coefficients (20 h) for 17β-Oestradiol and the Bed-Sediments

The curves drawn to calculate distribution coefficients (K_d) for 17β-oestradiol between the river water (suspended sediment-free) and bed-sediments were derived from the assumption that a linear isotherm exists at these low 17β-oestradiol concentrations (2.5-10 µg/l, which is well below the solubility of 13 mg/l, Tabak, 1981). Environmental concentrations would be likely to be even lower, in the range of a few ng/l (see table 2.1). Assuming a linear isotherm, K_d values derived at these concentrations should be valid for lower concentrations as well.

Table 4.2 shows sorption and desorption K_d for a number of bed-sediments in relation to their properties. By way of comparison, the sorption K_d of the more hydrophobic xenobiotic oestrogen, octylphenol (OP) for the same sediments have been included, where available (from Johnson *et al.*, 1998).

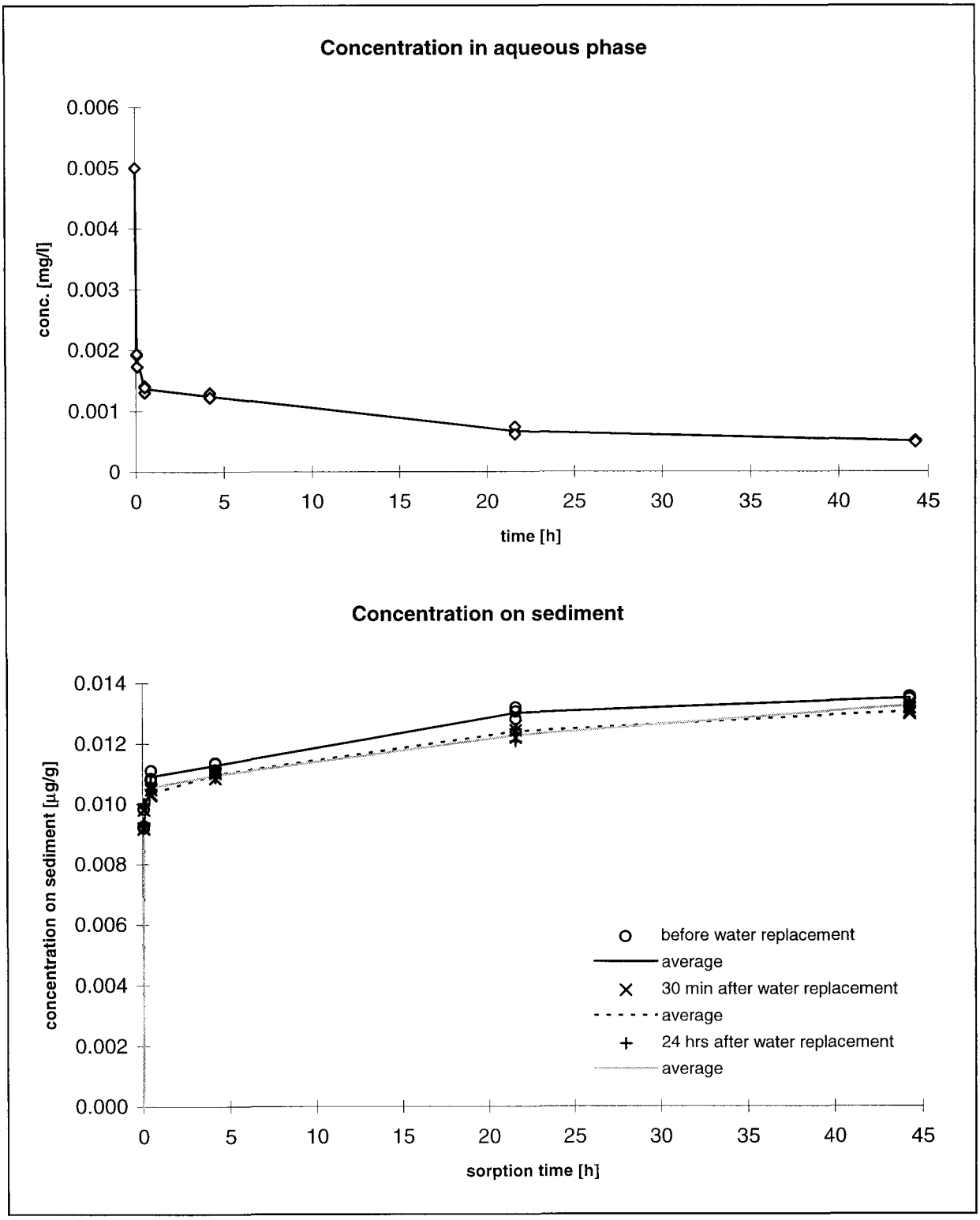


Figure 4.1 Sorption and desorption kinetics for Thames (Jun.1997) bed-sediments

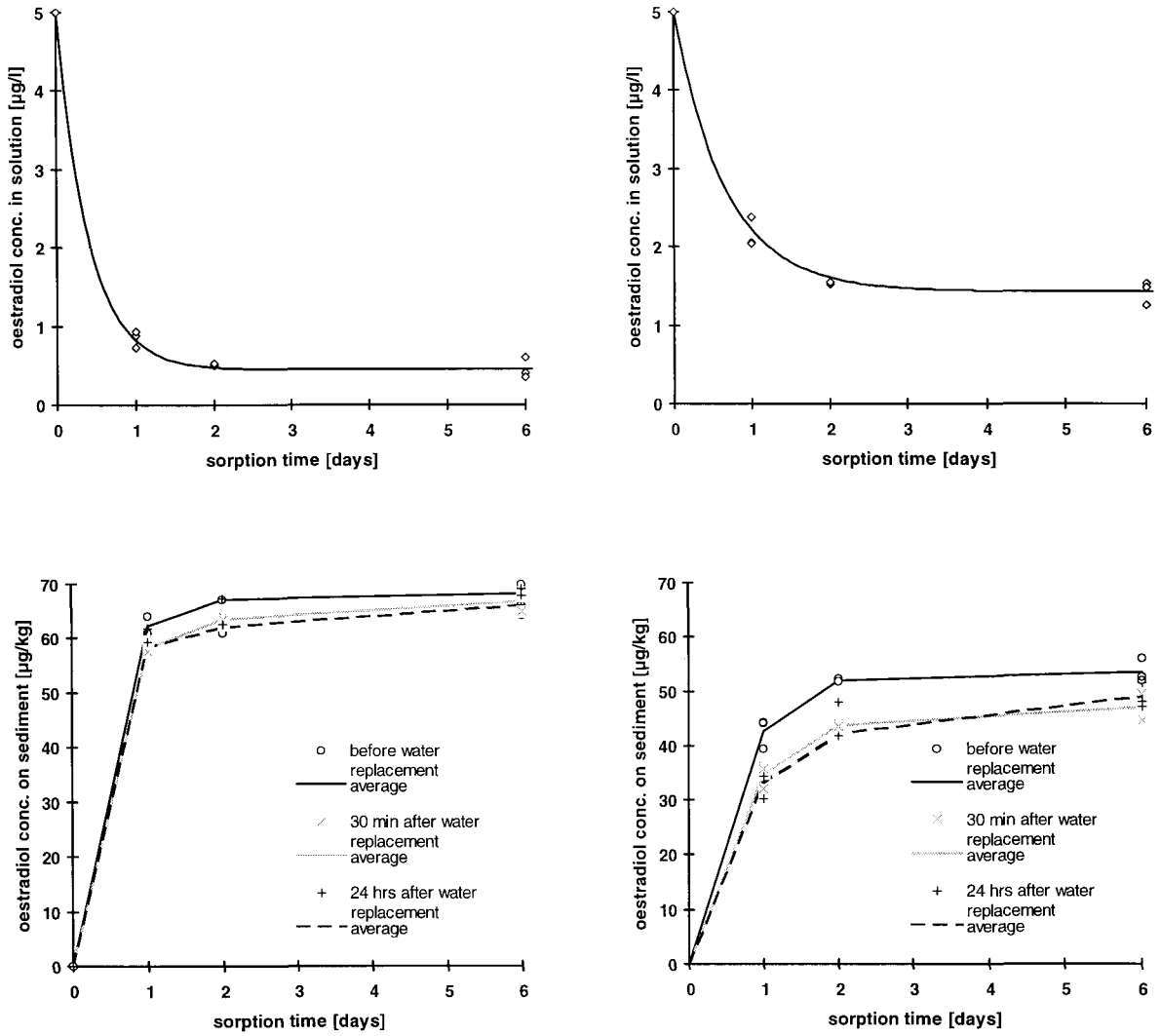


Figure 4.2 Sorption and desorption kinetics for Thames and Aire bed-sediments (6 days anaerobic equilibration)

Table 4.2 Distribution coefficients for 17β-oestradiol and the bed-sediments in relation to their properties

sample / characteristics	Aire 6.Sep96	Aire 18.Dec96	Aire 18.Jul97	Calder 16.Jan97	Calder 18.Jul97	Thames mid-channel 5.Dec96	Thames in boathouse 15.Apr97	Thames slipway 27.Jun97	Tees Bran Sands 31.Jul97	Tees Seal Sands 31.Jul97	Tyne 1.Aug97
% clay	9.2	7.4	6.97	6.6	6.82	5.5	4.8	6.0	6.2	7.7	9.4
% silt	72.9	62.8	66.3	55	49.8	38	43	41.4	42.6	52.2	76.5
% sand	17.9	29.8	26.7	38.4	43.4	56	51.8	52.6	51.1	40.1	14.1
% Kaolinite	50	20	40	25	60	10	10	40	60	55	55
% Illite	30	65	50	65	30	10	10	15	35	40	40
% Smectite	20	15	10	10	10	80	80	45	5	5	5
% TOC	2.42	7.02	10.04	5.71	3.33	1.76	2.88	1.08	0.92	2.0	3.74
Kd 1 sorption [l/kg]	**64****	**43**	**67**	**56**	**34**	**12.6**	**46**	**20**	**20**	**34**	**54**
r^2	0.953	0.958	0.992	0.971	0.949	0.928	0.992	0.924	0.980	0.986	0.978
SD	5.3	3.4	2.3	3.7	3.0	1.3	1.5	2.2	1.1	1.5	3.3
Kd 2 desorption [l/kg]	132	----	125	----	84	----	----	57	38	83	135
r^2	0.990	----	0.987	----	0.983	----	----	0.997	0.995	0.999	0.993
SD	4.8	----	5.3	----	4.2	----	----	1.2	1.1	1.0	4.2
Kd 2/Kd 1	2.1	----	1.9	----	2.5	----	----	2.9	1.9	2.4	2.5
Koc [l/kg]	2,645	612	667	980	1,032	715	1,599	1,852	2,188	1,717	1,439

Sorption coefficients of octylphenol for the same sediments

Kd	259	707	----	582	----	61	114	56	----	----	----
Koc [l/kg]	10,373	10,071	----	10,193	----	3,467	3,958	5,185	----	----	----

** has been stored wet for 8 months before drying (possible change)

The sorption coefficients for the different samples span from 12.6 for the very sandy sample of the Thames to 67 in the Aire. Using the relationship between K_{ow} and K_{oc}, described by Di Toro *et al.* (1991), a K_{oc} value of around 1,200 might be expected for 17β-oestradiol. The table shows values between 612-2,645 l/kg, which is within the uncertainty range of a factor of 2 or 3 mentioned by Di Toro.

The relationship between 17β-oestradiol and the organic carbon of the sediments seems much less clear than that with the more hydrophobic OP. With OP, consistently higher K_{oc} values were obtained with the Aire and Calder samples than with those obtained with the Thames. No such pattern was observed with the less hydrophobic 17β-oestradiol. Perhaps 17β-oestradiol is attracted to a wider spectrum of sorbent surfaces.

A correlation can be shown between both particle size and TOC with sorption capacity of the bed-sediments (figure 4.3), the bed-sediments containing higher proportions of small particles or larger TOC's giving higher K_d values. Although the r^2 values were rather poorer than those obtained in similar experiments with the more hydrophobic octylphenol (Johnson *et al.*, 1998).

Where a second distribution coefficient (desorption) has been determined it was always 2-3 times greater than the original sorption distribution coefficient. This hysteresis effect, whereby 17β-oestradiol is more difficult to desorb than predicted by the original K_d value, was also observed in the kinetics experiment described above (figures 4.1 and 4.2)

The results of the desorption can be described as follows:

- Replacing the aqueous phase with fresh (17β-oestradiol-free) water and then allowing further mixing resulted in a small proportion of the originally sorbed compound returning into solution. Thus, the experiment continued for a further 24 h (68 h in total);
- The new distribution coefficients were invariably higher than the first ones, which indicates either a greater proportion of the 17β-oestradiol remaining on the solid phase, or 17β-oestradiol being eliminated by degradation;
- The degradation results with these samples imply that the latter explanation is unlikely;
- The amount of 17β-oestradiol being desorbed decreased with longer sorption times; and
- The apparent increase in sorption strength or hysteresis effect has often been noted with organics and natural sediments or soil aggregates (Kan *et al.*, 1994, Pignatello and Xing, 1996). Table A1-A3 in the appendix show the sorption and desorption distribution coefficients (K_d) over time for both experiments.

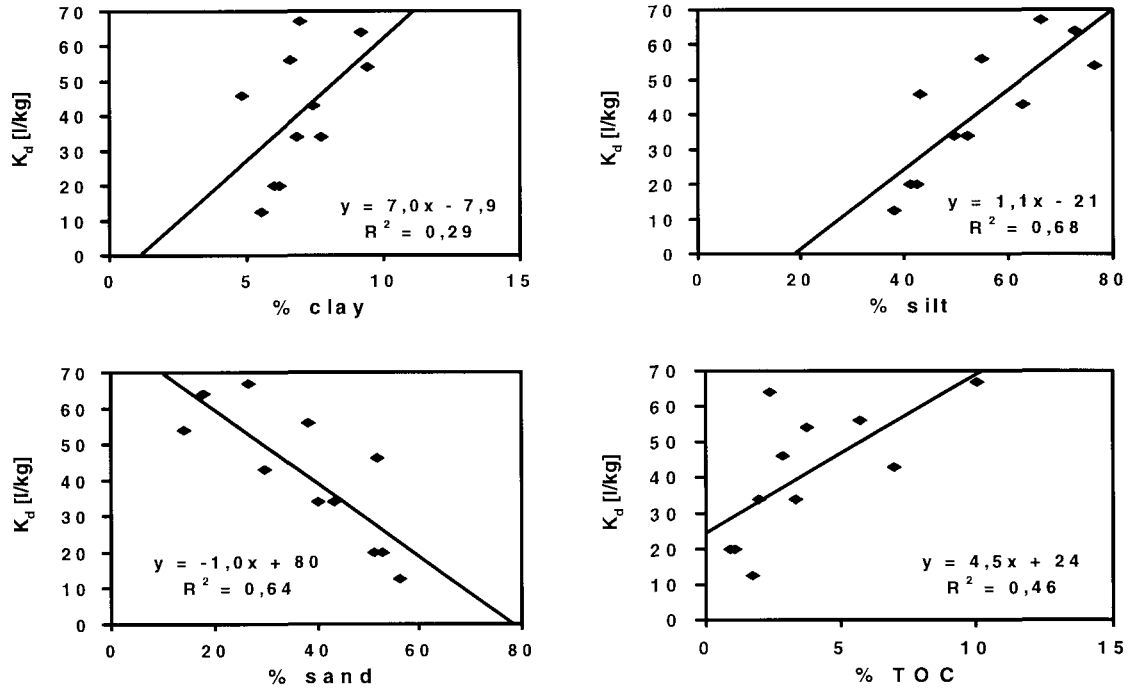


Figure 4.3 Correlations between bed-sediment properties and their K_d values

4.4 Sorption of 17β-Oestradiol to Suspended Sediments

The occasions when river water and hence suspended sediments were sampled from all of the rivers coincided with low to medium flow periods. Thus, the suspended sediments do not represent those that might be found during storm/high flow periods. For the rivers Aire and Calder, K_d values of 1690 and 3364 respectively were obtained for their suspended sediments (table 4.3), which is a hundred fold greater than for the bed-sediments and also higher than the K_{oc} of the respective bed-sediments (table 4.2). For the amount of suspended sediment present this translates to 7-10% 17β-oestradiol being sorbed to the solid phase. Assuming that the suspended sediments were 100% particulate organic matter, and the organic matter comprises 58% organic carbon (Hope *et al.*, 1994) then the K_{oc} can be estimated as 2,900 for the Calder and 5,800 for the Aire (both 18 July 1997). Therefore, the organic carbon of the suspended sediments was about 5 times more effective at sorbing 17β-oestradiol than that of the bed-sediments. . Since the suspended sediments are likely to be less than 100% organic matter this is likely to be an underestimate. Observations using a light microscope suggested that these suspended sediments consisted mainly of organic aggregates (little evidence of algae or clay minerals). Pinder *et al.* (1997) reported consistently low concentrations of chlorophyll a throughout the season in the Aire and Calder rivers. They suggested that low algal productivity in these rivers was related to industrial effluents colouring the water and hence impairing light penetration. Given that the R. Aire sample was collected 8 km downstream from Knostrap sewage treatment works (STW) (2.1×10^5 m^3/d entering 15.1×10^5 m^3/d annual mean river flow), and the Calder sample 16.4 km downstream from Calderdale STW (4.4×10^4 m^3/d entering 15.4×10^5 m^3/d annual mean river flow), much of the particulate organic matter may have originated from these sources. Alternatively, the organic inputs from the STW could have stimulated the river bacteria to such an extent that they formed aggregates.

Microscopic observations of the suspended sediment collected from the Thames in June 1997 revealed the material to consist mainly of green algae. The K_d for the suspended sediment of this sample was calculated as 106, which translates to only 0.25% of 17β-oestradiol being removed from the aqueous phase by sorption to the suspended sediment. Assuming that the algae were also 58% organic carbon a K_{oc} of 183 can be calculated, which is an order of magnitude lower than that of the corresponding bed-sediments. Koelmans *et al.* (1995) noted that live algae absorbed markedly less of a hydrophobic organic compound than algae, which had been allowed to decay. Table 4.3 summarises the findings for the suspended sediments.

Table 4.3 Sorption of 17β-oestradiol to suspended sediments

Sample	suspended sed. [mg/l]	Kd	SE	r^2	amount sorbed to susp. sed. (calculated)
Thames, 04. Jun 1997	20	106	19	0.81	0.25%
Aire, 18. Jul 1997	45	1690	120	0.97	7%
Calder, 18. Jul 1997	32	3364	221	0.97	10%

4.5 Degradation of Steroids in River Waters

4.5.1 Aerobic and Anaerobic Degradation of 17β-Oestradiol

Difficulties were experienced with the initial degradation experiments, which included sediments, due to imperfect sterilisation of the controls and the difficulty of detecting 17β-oestradiol in the presence of bed-sediment. Sterilisation was successful when controls were autoclaved twice at 121°C and the steroids were added directly from the methanol stock, rather than from an (0.2 µm-filtered) aqueous stock solution. Throughout the 49 d incubation (at 20°C) no decrease in 17β-oestradiol concentration was observed with the sterile controls (figure 4.4). The most rapid transformation of 17β-oestradiol occurred with the Aire and Calder river water samples, with half-lives less than 3 days. The Thames gave a half-life of 4 d, the Tees 6 d and the Tyne 27 d. Thus, the poorest degradation rates were observed in the estuary river water samples. Perhaps the freshwater/sea water interface with its higher salt content inhibited microbial activity. Conductivity tests on the river water confirmed the high salt content of these estuarine samples compared to the other rivers (data not shown). The sterile controls suggested oestradiol was still available in the estuarine samples, in as far as concentrations did not change during the experiment, despite filtering. (The fact that towards the end of the experiment the concentrations in the sterile controls started to go down is due to the samples becoming unsterile through contamination while sampling)

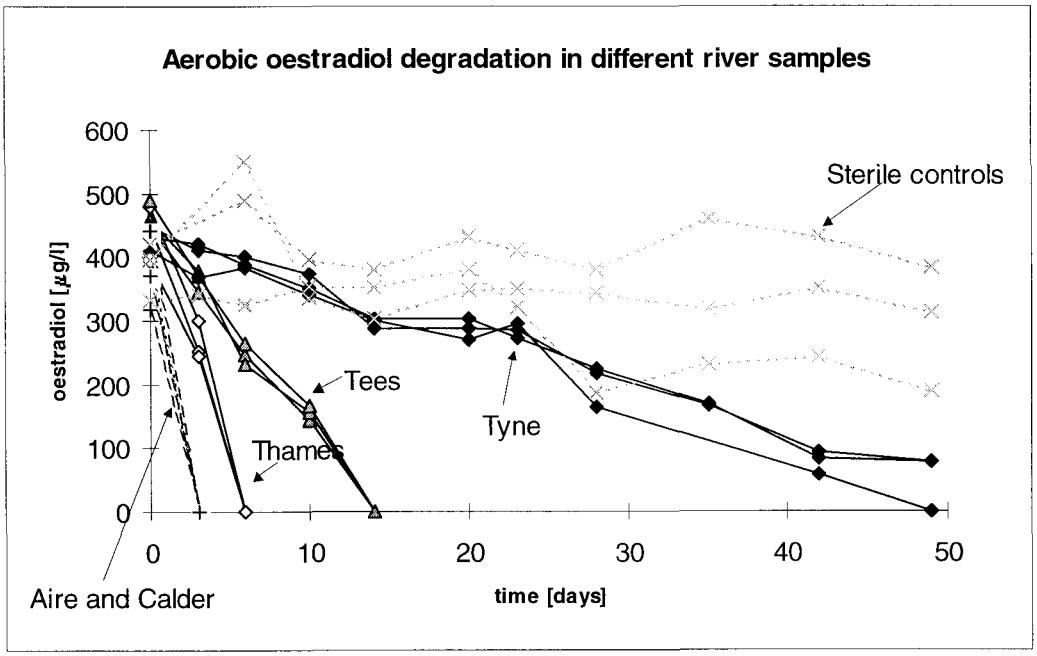


Figure 4.4 Aerobic degradation of 17β-oestradiol in different river waters

Examination of the HPLC traces showed that the first step of 17β-oestradiol degradation is transformation into oestrone (another oestrogen). The observed 17β-oestradiol and oestrone concentrations in relation to the initial concentration of 17β-oestradiol are shown in figure 4.6. After the 17β-oestradiol was converted to oestrone, oestrone was also degraded, but the subsequent by-products have not been identified. Thus, assuming the breakdown products of oestrone are not oestrogenic, the removal of oestrogenicity of 17β-oestradiol in rivers might be predicted to take 6 days for the R. Aire, 10 days for the Rivers Calder and Thames, 14 days for the Tees estuary, and longer than 49 days for the Tyne estuary. A possible pathway for aerobic conditions as it has been suggested by Coombe *et al.* (1966) is shown in figure 4.5. It is important to emphasise these values might correspond to ideal summer temperatures, but that the compounds might be twice as persistent at winter temperatures. It is not known whether degradation would be as rapid with the low natural concentrations of 17β-oestradiol, but the preliminary results shown in section 4.5.3 indicate that although the initial conversion to oestrone may happen at similar rates when lower concentration are applied, complete breakdown of the product is not as rapid as with the high concentrations applied here.

Bearing in mind the existence of anaerobic zones within the bed-sediments and their porewaters it is important to examine the anaerobic degradation potential of 17β-oestradiol. Ideally such an experiment would be carried out with the bed-sediments, however this presented technical difficulties due to other unknown compounds emanating from the sediments masking oestradiol on the HPLC. Therefore, the anaerobic incubation contained river water only. The results can be seen in figures 4.7 and 4.8.

The anaerobic test system was imperfect in that it permitted the transient entry of air during sampling. Clearly, anaerobic conditions imposed a break on oestradiol degradation. It could be argued that the degradation that was observed only occurred during periods when air was introduced for an hour or so during sampling. Thus, over the total incubation period of 1176 h, for perhaps 9 sampling periods of 1 h air was present. Given the half-lives measured under aerobic conditions, this would seem inadequate to account for the amount of oestradiol transformation which was actually measured. As observed with the aerobic experiment, transformation of 17β-oestradiol was rapid with the Aire and Calder river water. However, this transformation appeared partly reversible and the oestrone which was produced was much more persistent under anaerobic than aerobic conditions, with 20-60% of the 17β-oestradiol present as oestrone after 49 d incubation. With the Thames 60% of the 17β-oestradiol was still present as oestrone after 49 d, whilst with the Tyne and Tees estuaries almost 100% of the 17β-oestradiol could still be identified as the original compound or as oestrone. Thus, 17β-oestradiol can be seen to be more persistent under anaerobic conditions, the extent of this increased persistence in bed-sediments has yet to be tested.

4.5.2 Comparison of Degradation Rates for 17β-Oestradiol and Ethinyl-Oestradiol

A comparison between the aerobic degradation potential of the natural hormone 17β-oestradiol and its synthetic derivate ethinyl-oestradiol was carried out in river water collected from the R. Thames 13. Oct. 1997 (figure 4.9). The results confirm previous findings (Tabak and Bunch 1970, Tabak *et al.* 1981, Rurainsky, 1977), that ethinyl-oestradiol is more stable than the natural steroids. While 17β-oestradiol showed a half-life of 4 days, as in the previous experiment, and was no longer detectable after one week, a reduction of the original ethynil-oestradiol by 50% was only reached at the end of the experiment after 46 days. Thus the degradation of ethinyl-oestradiol under these conditions takes about 10 times as long as that of 17β-oestradiol. Under anaerobic conditions the degradation of 17β-oestradiol was slowed down as in the previous experiments and no change could be observed for ethinyl-oestradiol over the whole 46 day incubation period (data not shown).
Because of this greater stability, synthetic steroid hormones may play an important ecotoxicological role although they are released in much smaller quantities than the natural steroids.

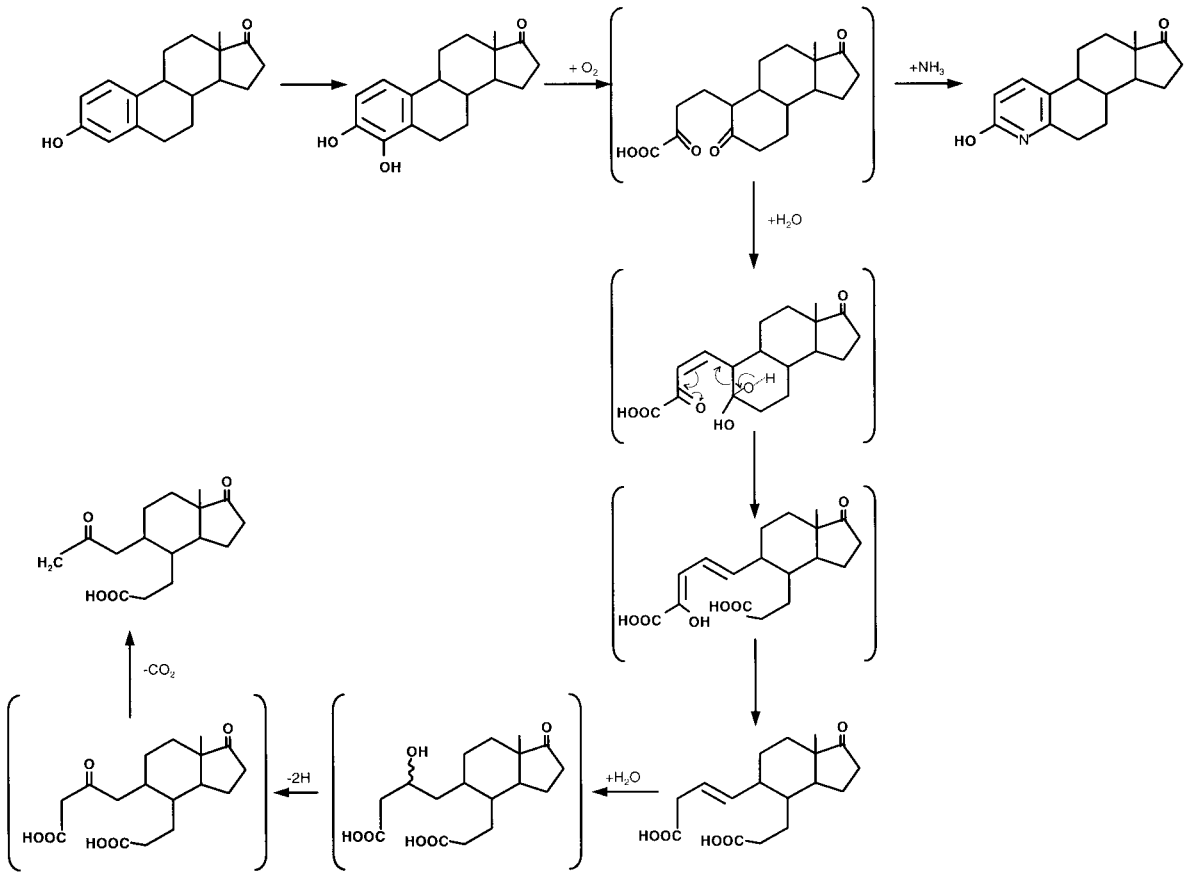


Figure 4.5 Abridged tentative scheme for the metabolism of oestrone (Coombe, 1966)

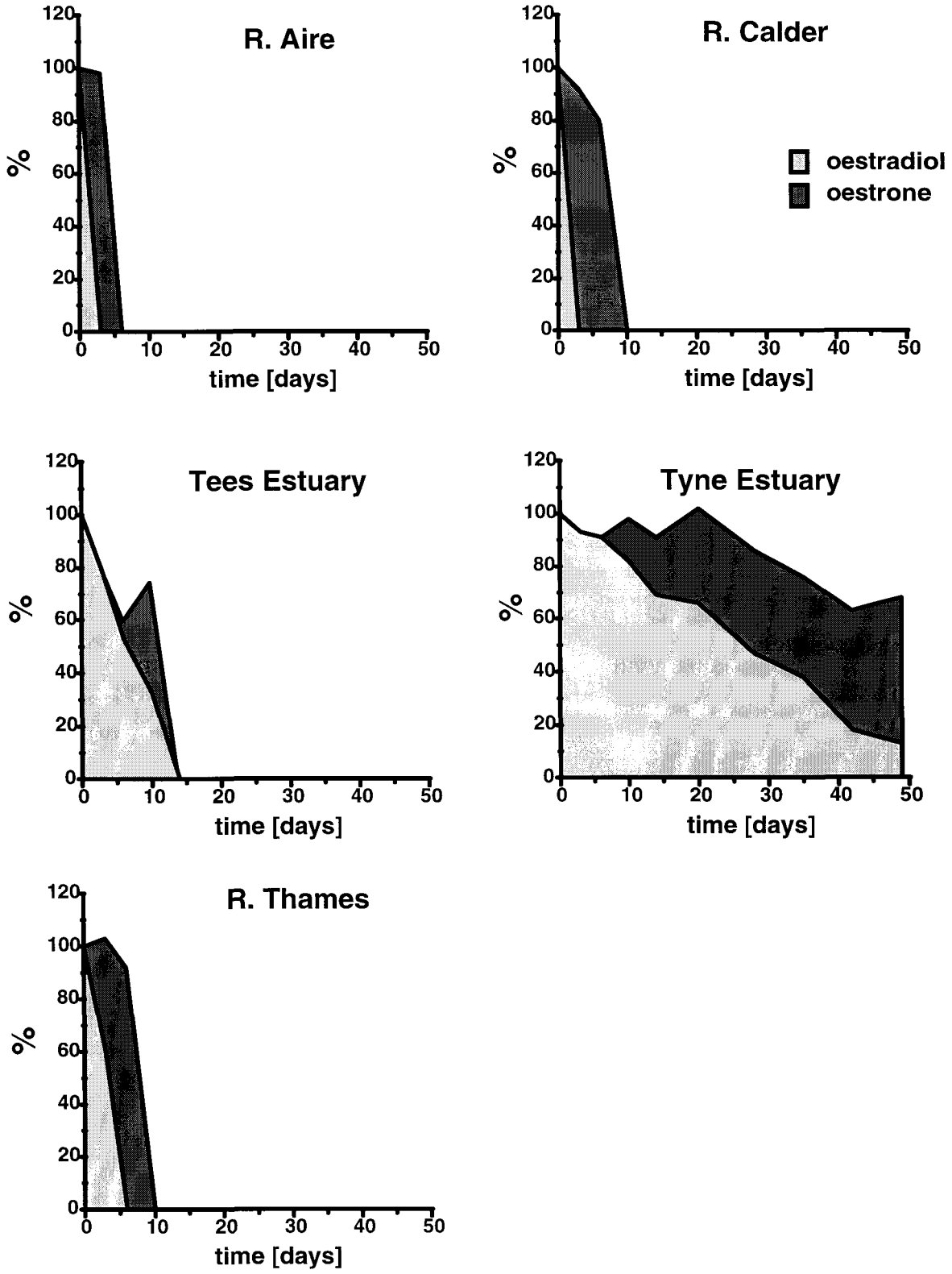


Figure 4.6 17β-Oestradiol conversion to oestrone under aerobic conditions

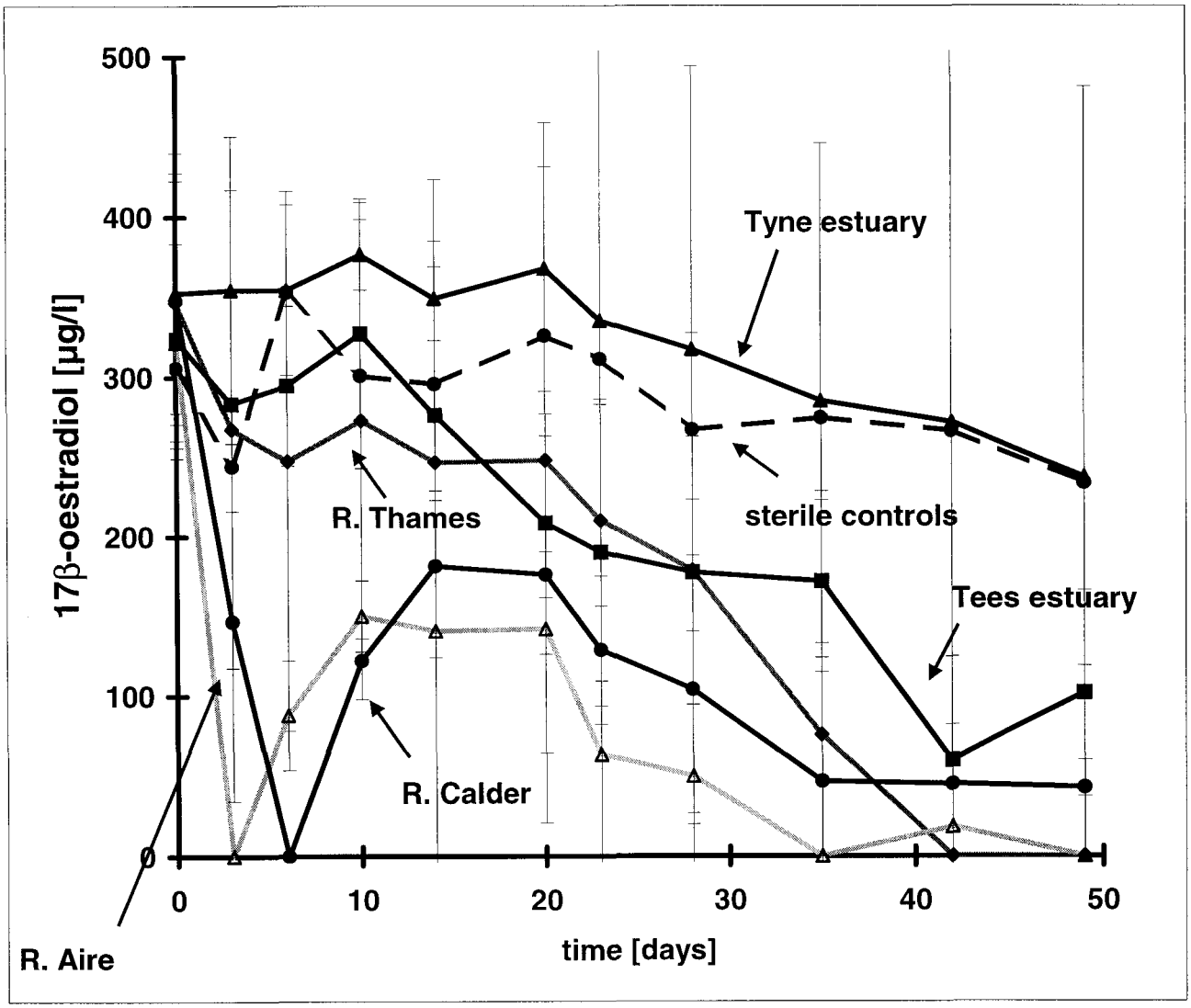


Figure 4.7 Anaerobic degradation of 17β-oestradiol in different river waters (means and 2* SD)

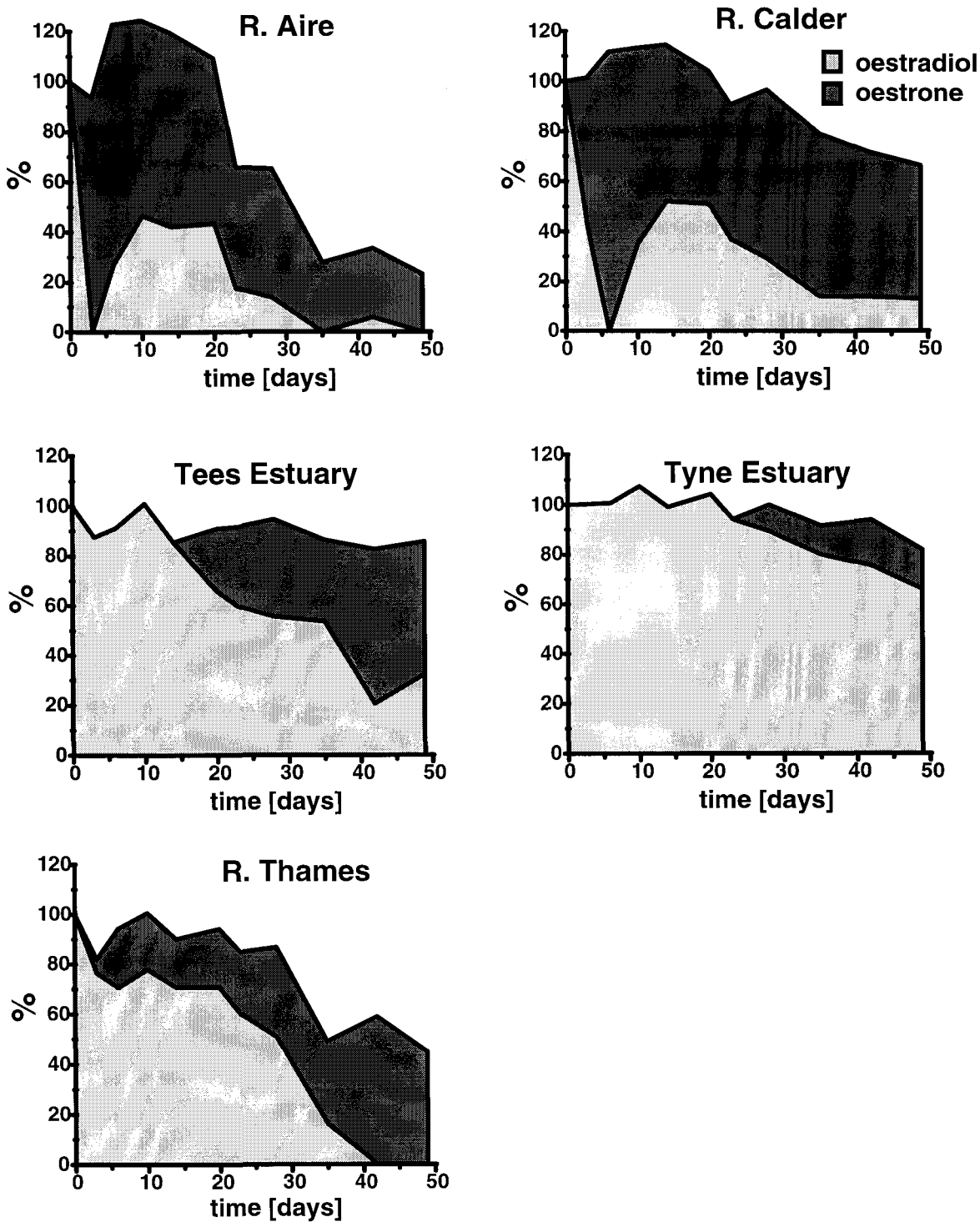
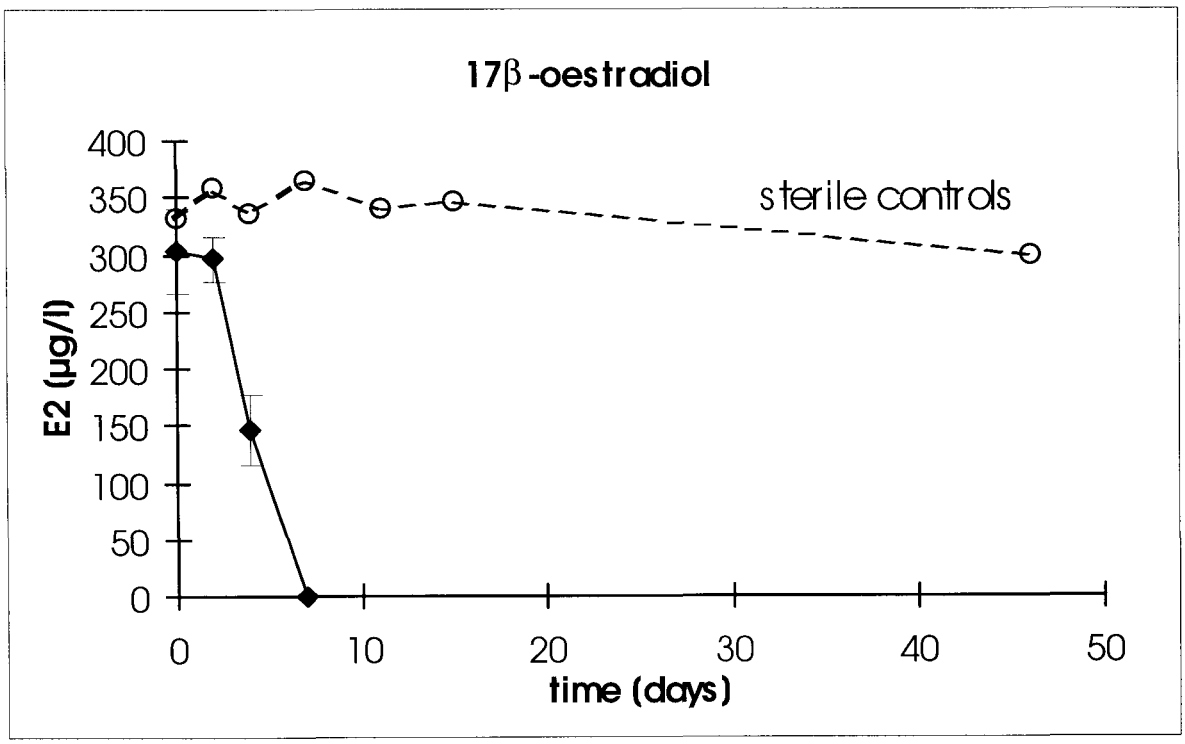


Figure 4.8 17β-Oestradiol conversion to oestrone under anaerobic conditions



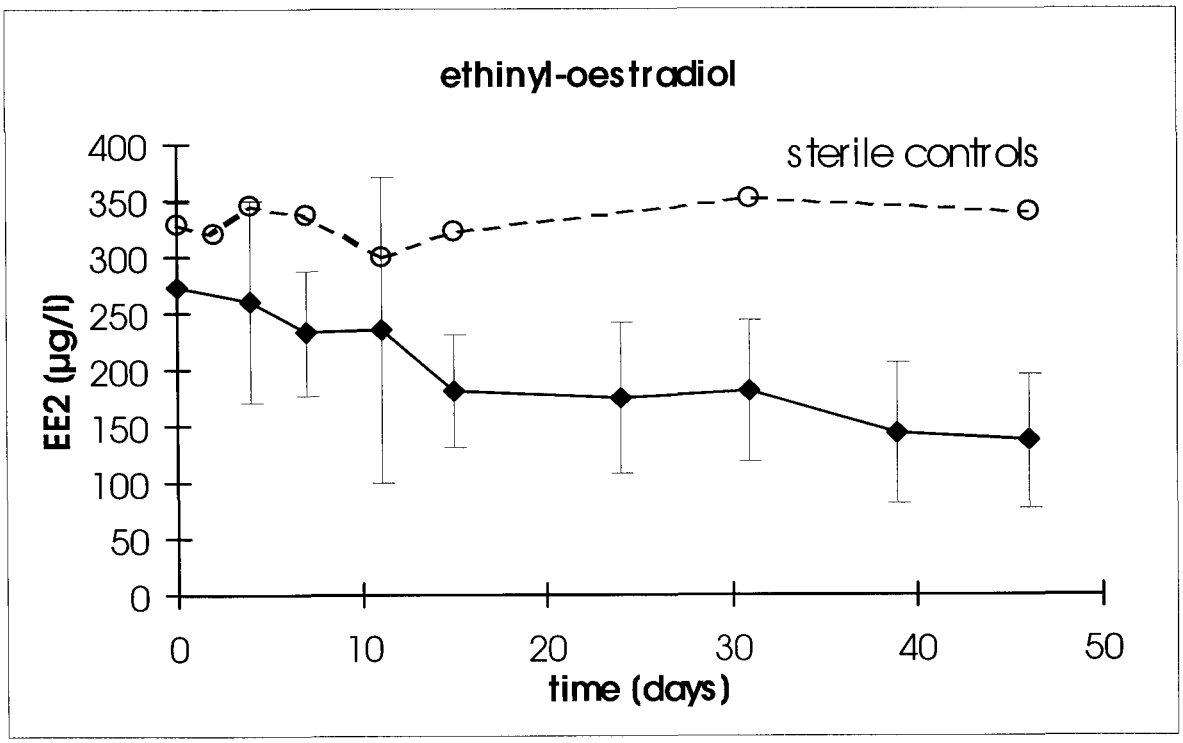


Figure 4.9 **Comparison of aerobic degradation of 17β-oestradiol and ethinyl-oestradiol in Thames river water (means and 2* SD, the sterile controls are shown without their standard deviations))**

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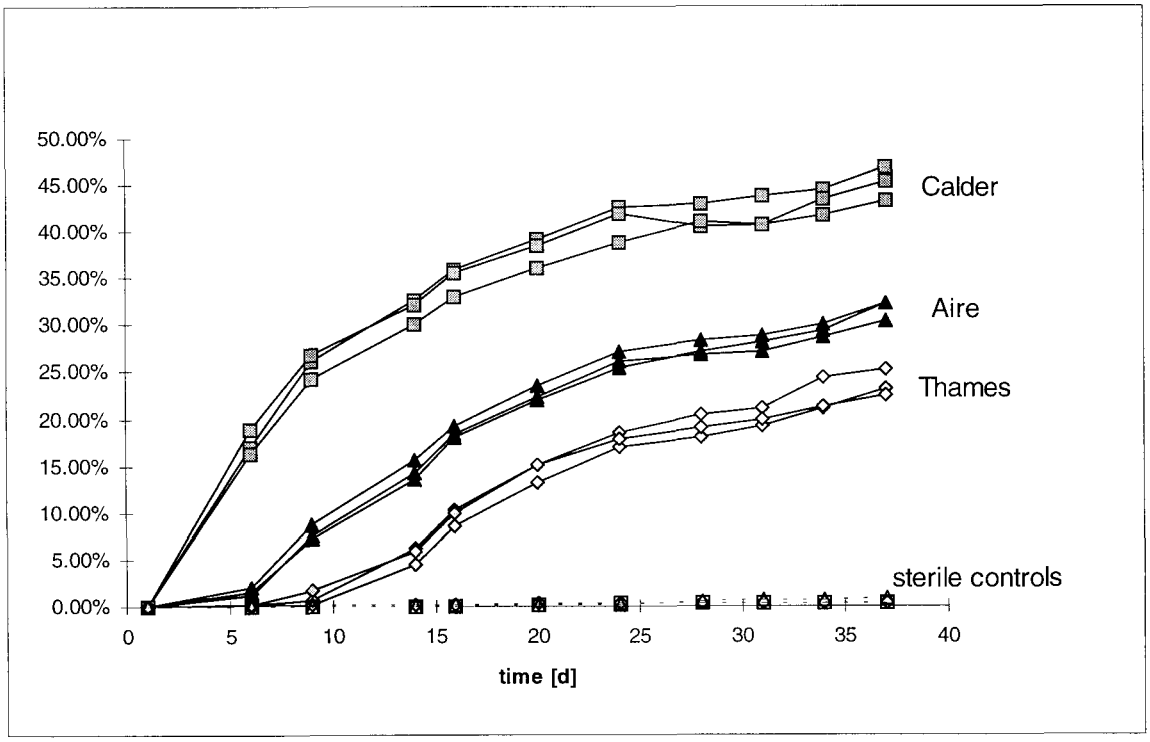

Figure 4.11 Mineralisation of 17β-oestradiol in river waters (aerobic)

Table 4.4 Mass balance for the radiolabel after 37 days, percent of original amount added

	Steriles	Thames	Calder	Aire
amount mineralised	0.5%	23.7%	45.2%	31.6%
left in parent	98.2%	53.1%	28.3%	46.4%
hydrophilic fraction in parent	*1.3%*	*23.0%*	*10.0%*	*14.6%*
sorbed to conicals	0.3%	0.4%	0.1%	0.3%
sorbed to charcoal	0.3%	1.9%	2.0%	0.4%
sorbed to tubing	0.5%	2.1%	1.3%	0.6%
total recovery	99.8%	81.1%	76.9%	79.3%
missing volatiles	0.2%	18.9%	23.1%	20.7%

4.5.4 Low concentration biodegradation experiments

In an effort to examine whether 17β-oestradiol would actually be degraded at more realistic concentrations, 0.3 µg/l 17β-oestradiol was added to samples of R. Thames water. Whilst no change was seen in the sterile controls (table 4.5), 17β-oestradiol was degraded to below detection limits within 3 d. Whilst 230 ng/l is still high, compared to literature values for likely river water concentrations, it would have represented about 0.02% of the total organic carbon available to the bacteria (3.4 mg/l DOC). On the basis of this experiment, it would seem that low 17β-oestradiol concentrations will not be degraded more slowly then the rates previously seen at 200-300 µg/l level, indeed it may even be more rapid.

Table 4.5 Concentration of 17β-oestradiol [µg/l] in spiked R. Thames water samples (mean of three observations plus standard deviations)

Treatment	t = 0	SD	3 d	SD	5 d	SD
Sterile	0.28	0.05	0.26	0.017	0.28	0.06
Non-sterile	0.23	0.04	<0.15	NA*	<0.15	NA*

*NA not applicable

The same R. Thames water sample was spiked with radiolabelled oestradiol to a concentration of 0.1 µg/l and mineralisation of the 'A' ring monitored by radiolabelled CO_2 evolution. There was close to zero radioactivity evolved from the sterile treatments (fig. 4.12), the small quantity (1.6%) found after 100 d almost certainly reflected volatilisation of the parent. Over the 100 d incubation, 20% mineralisation was observed with the non-sterile treatment (fig. 4.12). At the end of the experiment 80-94% of the radioactivity could be accounted for, the remainder, presumably, converted into more volatile products. Unlike the transformation of the parent molecule, the complete mineralisation process has taken longer than observed previously with a concentration over 1000 times greater (fig. 4.11). With the previous R. Thames sample collected on 1,9,97 and spiked with 500 µg/l 20% was mineralised after 40 d. When the 29,5,98 sample was spiked with 0.1 µg/l, only 10% had been mineralised after 40 d.

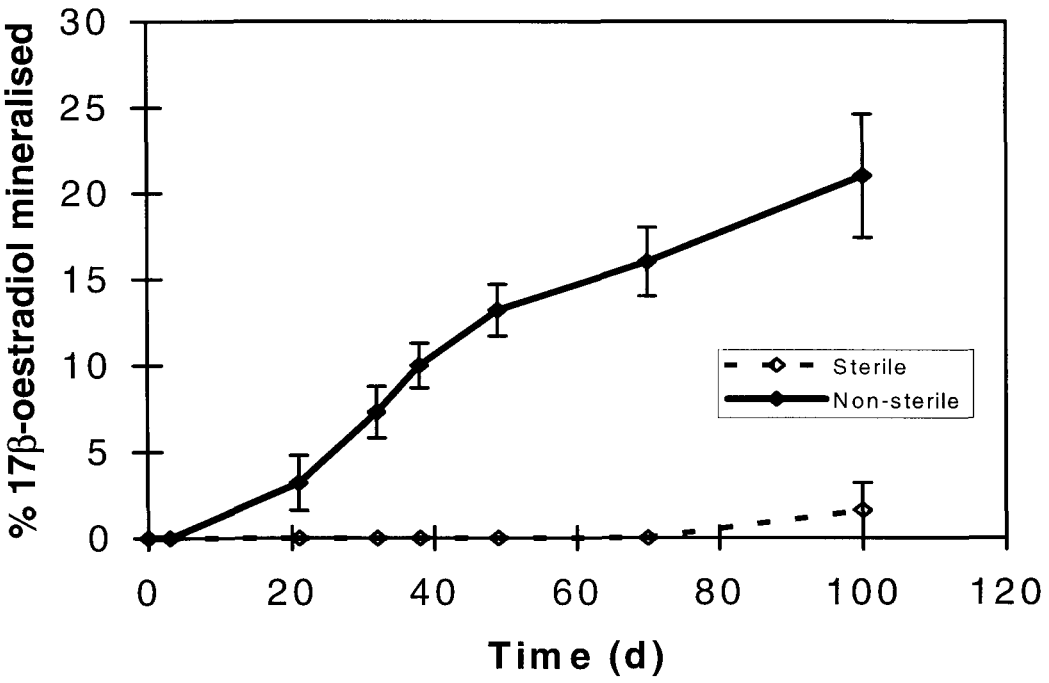

Figure 4.12 Mineralisation of 0.1 µg/l oestradiol by water collected from the R. Thames

5. ENVIRONMENTAL FATE AND EXPOSURE: PRELIMINARY MODELLING STUDIES

5.1 Introduction

The previous sections have identified values of physico-chemical properties for the steroid oestrogens, 17β-oestradiol, oestrone and ethinyl-oestradiol. These have been determined by literature review and by laboratory experiments using materials collected from particular English rivers and estuaries. The aim of this section is to use these data to give a preliminary assessment of how these compounds are likely to become distributed in river systems. This has been achieved by applying the Exposure Assessment Modelling System (EXAMS) version 2.96 (Burns, 1996) to environmental systems based on those rivers from which material was taken and used in the laboratory experiments. In this way, long term, steady state environmental concentrations of these compounds have been predicted for rivers that are similar to the Rivers Thames, Aire and Calder, using model parameters that were measured using material from these rivers and assuming a certain input loading. The modelled environments are presented as only being based on these rivers because the geometry and reach structure is much more simplified than might be used in a full scale model of these rivers. However, the channel dimensions, flow rates and other physical parameters used were taken from observations of these rivers. A similar modelling exercise for estuaries was out with the resources available for this scoping study.

5.2 The EXAMS Model

The EXAMS model was developed by the United States Environmental Protection Agency as an aid in making hazard assessments solely from laboratory descriptions of the chemistry of new, toxic organic compounds (Burns, 1982). The model gives estimates of the exposure, fate and persistence of a chemical in a given environment. Exposure is the concentration to which an organism in any of the defined environmental compartments will be exposed. Fate is an estimate of the amounts of the compound that is either transformed (by whatever process) or transported through the environmental system. Persistence is the rate at which the compound will be removed from the environment if the input loading were to cease. In this study the model was used to assess the fate and exposure levels of 17β-oestradiol, oestrone and ethinyl-oestradiol given a steady input loading.

5.2.1 The Model Environment

Three model environments were used in this study based on the Rivers Aire, Calder and Thames. In each case the river was modelled as a series of ten, identical 1 km reaches with each reach comprising a water column compartment above a bed-sediment compartment (figure 5.1). The physical dimensions of each compartment for the three rivers is given in table 5.1. These dimensions were used in all of the model runs. The depths of the Rivers Aire and Calder were

halved for assessments made under summer flow conditions. The River Thames on the other hand, is regulated for boat traffic and its depth was kept constant.

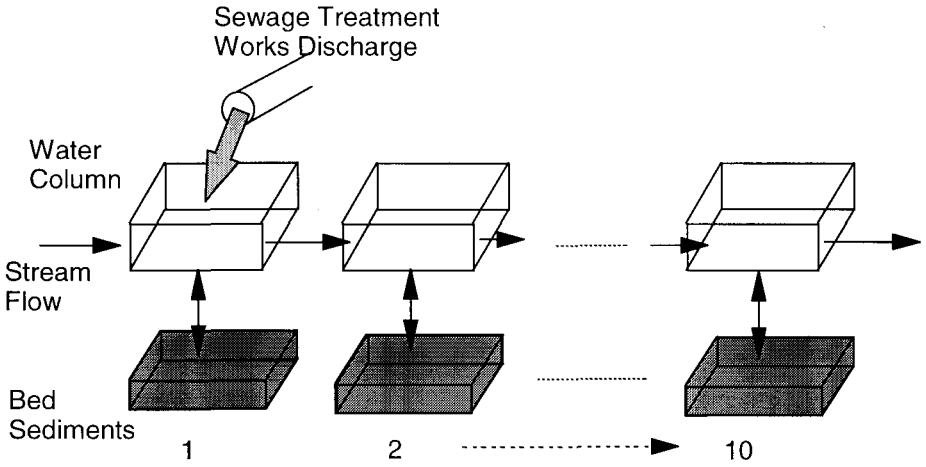

Figure 5.1 Model structure used for the simulation of the Rivers Thames, Aire and Calder with EXAMS

Table 5.1 Main compartment dimensions of the river environments used in the EXAMS model

	Thames	Aire	Calder
Water Column			
Depth (m)	2.0(2.0)	1.0(0.5)	1.0(0.5)
(low flow value)			
Width (m)	30	20	20
Length (m)	1000	1000	1000
Channel Cross Section	Rectangular	Rectangular	Rectangular
Bed-sediments			
Depth (m)	0.1	0.1	0.1
Width (m)	30	20	20
Length (m)	1000	1000	1000

The water column compartment was assumed to comprise sub-compartments representing stream biota and suspended sediments. A chemical entering the water column will become distributed between the water, suspended sediments and biota depending on its physical properties (see section 5.2.2). The suspended sediment concentrations used were estimated for the Rivers Aire and Calder based on observed values taken from the Land Ocean Interaction Study (LOIS) data base and an arbitrary value was used for the River Thames. The amount of biota in the water column was also unknown for all three values and again an arbitrary value was assigned. The values used are given in table 5.2.

Within EXAMS the bed-sediments are considered to be a porous medium comprising a solid phase within which water and biota is held. A chemical that enters into the bed-sediments will become distributed between the solid, water and biota, again according to its physical properties (see section 5.2.2.3). The sizes of these three sub-compartments for the three rivers were estimated because no measured data were readily available (Table 5.2).

5.2.2 Processes Modelled

EXAMS allows consideration of the effect on a chemical pollutant of a wide range of physical and chemical processes: advection, dispersion, sorption, degradation and volatilisation. All of these have been included to a greater or lesser extent within this study.

Table 5.2 Suspended solids and stream biota concentrations and bed-sediment properties for the three EXAMS river environments modelled.

	Thames	Aire	Calder
Suspended Solids (mg/l)	50.0	25.0	15.0
Stream Biota (mg/l dry weight)	10.0	10.0	20.0
Bed-sediments Water Content (%)	135	135	135
Bulk Density of Bed-sediments (g/cm^3)	1.2	1.2	1.2
Benthic Biota (g/m^2 dry weight)	0.0	0.0	0.0

Advection

Advected flows are calculated simply by conservation of mass. No change in volume of a compartment is allowed and therefore the inputs to a compartment must equal the outputs. The aim of this modelling study was to investigate possible environmental concentrations of oestrogens under steady state conditions. The steady flow through the system was specified by giving the flow rate into the first water column compartment. Flows in subsequent compartments also took this value because no inputs or abstracts were included in the river environments.

Advection of bed-sediments was not included in this model because no data were available on mass loads transported under different flow regimes. This was thought reasonable as there was unlikely to be large bed-sediment movement under the average and low flow conditions that were considered in this study.

Dispersion

Longitudinal dispersion effects with respect to water and contaminant transport will not be important under the steady flow conditions used in this modelling study, therefore they have not been included.

Capture of organic chemicals by bed-sediments can occur by a number of mechanisms including; direct sorption onto sediment surfaces, entrainment at the interface by sediment

dwelling animals and scouring of sediments followed by their subsequent re-settlement. Although some of these processes are continuous some are intermittent and therefore within EXAMS, the dispersion equation is used as an efficient summary of the macro-scale effects of these micro-scale processes. The exchange of environmental volume, F (m^3/h) is calculated from

$$F = D\frac{XSA}{CL},$$

where D is the vertical dispersion coefficient (m^2/h), XSA is the cross sectional area between the sediment and the water column (m) and CL is the characteristic length of the dispersion pathway (m). This is then modified to account for the exchange of both water and solids. The value of XSA is defined in the physical description of the river environment, the characteristic length can be calculated as distance between the mid points of the two compartments and a value of D was taken from typical river values (Burns *et al*, 1982). The values used in this study are given in table 5.3.

It should be noted that this method accounts for chemical exchange occurring on average between the bed-sediments and the water column within a single river reach. The model system has not been constructed to consider transport of suspended sediments from upstream reaches and their subsequent settlement on the river bed. This is quite acceptable under the steady state conditions represented in this study because there should be no net loss/generation of suspended sediments. This is because as the processes generating suspended sediments should be in equilibrium with those encouraging re-settlement on the river bed. Mass movement of bed-sediments into the water column will only occur under high flows and a quiescent period will be required for their subsequent return to the bed. This process representation requires a dynamic modelling approach and could be considered in future work.

Table 5.3 Values of the dispersion coefficients, cross-sectional area and characteristic lengths used to determine water column/bed-sediment interactions for the three EXAMS river environments modelled.

	Thames	Aire	Calder
Dispersion Coefficient (m^2/h)	10^{-4}	10^{-4}	10^{-4}
Cross Sectional Area (m^2)	$3x10^4$	$2x10^4$	$2x10^4$
Characteristic Length (m) (summer conditions)	1.05(1.05)	0.55(0.3)	0.55(0.3)

Sorption

Suspended and Bed-sediments

In both the water column and the bed-sediments an organic compound can become distributed between the solid and dissolved phases. The distribution coefficient K_d, for a given chemical is defined as the steady state ratio of the solid phase concentration to the dissolved phase. EXAMS assumes a linear sorption isotherm, that is the value of K_d remains constant over a range of dissolved and sorbed concentrations. For neutral organic compounds it has been shown that the organic carbon content of the solid phase is the most important factor in determining the value of K_d (Karickhoff, 1981). The value of K_d normalised to the organic carbon content, known as K_{oc}, of the solid phase is relatively invariant for a given chemical. EXAMS uses this value to compute values of the K_d for an organic compound with respect to both the bed-sediment and the suspended sediments.

In this study the values of K_{oc} for 17β-oestradiol and the values of organic carbon content of the solid phases in each of the three river systems were chosen to give the K_ds measured in the laboratory (table 5.4). No data were available for either oestrone or ethinyl-oestradiol. In the case of oestrone its chemical structure is so close to that of 17β-oestradiol it is reasonable to assume that it would show a similar K_d for each of the river systems modelled. For ethinyl-oestradiol the K_{oc} value was calculated from the octanol/water partition coefficient (K_{ow}) using the equation, $K_{oc} = 0.41K_{ow}$ (Karickhoff, 1981). The values of organic carbon content used to obtain the correct value of K_d for 17β-oestradiol were then used to calculate a value of K_d for ethinyl-oestradiol for each of the three river environments (Table 5.4).

Biota

Uptake of organic chemicals by both stream and benthic organisms is usually represented through a bioconcentration factor (BCF). The BCF is defined as the ratio of the concentration of chemical in the tissue of the animal to that in the surrounding water and is therefore similar to the distribution coefficient for sediments. In the model environments described here, biota were only considered in the water column. Measured values of BCF were not available for the oestrogens being studied, but it has been shown that there is a good relationship between the BCF and the octanol/water partition coefficient, K_{ow}. Veith (1980) and Oliver and Niimi (1983) gave the following regression equations for estimating BCF:

$$\log BCF = 0.76 \log K_{ow} - 0.23$$

$$\log BCF = -0.869 + 0.997 \log K_{ow}$$

These equations were used to give a range of possible values for BCF to be used in the EXAMS model (5.4).

Table 5.4 Values of the distribution coefficients used for 17β-oestradiol, oestrone and ethinyl-oestradiol for each of the three river environments modelled.

Chemical	Distribution Coefficient	Thames	Aire	Calder
17β-Oestradiol and Oestrone	Suspended Sediments	106	1690	3364
	Bed-sediments	20	67	34
	BCF, water column	---------- 631-1259 ----------		
Ethinyl-oestradiol	Suspended Sediments	370	5910	11790
	Bed-sediments	71	223	123
	BCF, water column	----------1000-1995----------		

Degradation

EXAMS allows chemical loss or transformation through oxidation, hydrolysis, photolysis and biodegradation processes. The laboratory degradation measurements made on 17β-oestradiol and oestrone demonstrated that degradation did not occur in sterilised controls. This indicated that the main degradation route other than photolysis, was biologically mediated and this was the only degradation pathway included in the EXAMS models of the three river environments.

Biodegradation half-lives were estimated from the degradation experiments carried out in water samples collected from the Rivers Thames, Calder and Aire. These were only available for 17β-oestradiol and oestrone. Data was available for a ethinyl-oestradiol half-life in river water from the Thames that indicates that it was a more stable compound that the other two oestrogens. The half-life was therefore taken as being twice that of 17β-oestradiol and Oestrone although this may be an underestimate (table 5.5). It should be noted that the half-lives measured were at 20°C and that under more average conditions they would be expected to be greater than this. This can be accounted for in EXAMS by specifying the rate at which the half-life will change over a 10 K temperature change (Q_{10}). For these model applications a Q_{10} value of 2 was used in all cases, i.e. the rate of degradation will halve for every 10 K drop in temperature. It is worth noting that these degradation half-lives were calculated from experiments using several hundred μg/l of the oestrogens and that it has been assumed that these rates would be sustained at much lower environmental concentrations.

EXAMS also required estimates of the degradation half-life on the suspended solids and in the sediments. Although there was some evidence that these compounds were very stable in

anaerobic sediments (see section 4), river sediments in general are aerobic, at least near the surface, and the measured water half-lives were also used for the sorbed oestrogens (table 5.5).

Table 5.5 Half-lives (days) at 20°C for the three oestrogenic substances used for water column and bed-sediment compartments in the EXAMS model of the three river environments.

	Thames	Aire	Calder
17β-Oestradiol	3	2	2
Oestradiol	3	2	2
Ethinyl-Oestradiol	6	4	4

Volatilisation

Each of these three compounds has a very low vapour pressure (table 5.6) and although the losses through volatilisation were included in the model, they were negligible.

Table 5.6 Vapour pressures for the three steroid oestrogens used in the EXAMS models

	17β-Oestradiol	Oestrone	Ethinyl-Oestradiol
Vapour Pressure (Torr)†	2.25×10^{-10}	2.25×10^{-10}	4.5×10^{-11}

†data from Schweinfurth *et al.*, 1996

5.2.3 Model Scenarios

Two scenarios were chosen to represent average conditions and the potentially critical periods of summer low flows. Mean and 95 percentile flows were obtained for appropriate gauging stations from the UK Hydrometric Register (IH and BGS, 1993) and these data are given in table 5.7. Other parameter values that varied between scenarios were the temperature which was taken as 10°C under average conditions and 20°C under low flow conditions and the channel depths (see table 5.1).

Table 5.7 Flow rates used under the two modelling scenarios for the Rivers Thames, Aire and Calder.

River	Gauging Station	Average Flow Conditions (m^3/s)	Low Flow Conditions (m^3/s)
Thames	Days Weir	28.5	3.2
Aire	Fleet Weir	17.3	4.8
Calder	Newlands†	17.8	4.9

† Data only available from 1960-1976

All three river environments were simulated under each of the two scenarios for the three steroid oestrogens. The oestrogen loadings used as inputs to the models were derived from data for 7 sewage treatment works discharges given by Desbrow *et al.* (1996). The average discharge from each of these works was combined with concentration data collected on three occasions to derive a total of 21 loads for each of the three oestrogens. These values were then averaged to give a single value for each compound (table 5.8). For ethinyl-oestradiol the measurements were in many cases below the detection limit of the analytical method used. In these cases a value of one tenth of the 17β-oestradiol concentration was used as suggested by Desbrow *et al.* (1996). Using these loads is equivalent to an effluent dilution factor of 29 and 4 for the River Thames and 17 and 6 for the Rivers Aire and Calder under average and low flow conditions respectively. The dilution factor is the ratio of the river flow rate upstream of the discharge to the discharge flow rate.

Table 5.8 Loads (kg/h) of 17β-oestradiol, oestrone and ethinyl-oestradiol calculated from data for 7 UK sewage treatment works†

	Mean	Standard Deviation	Minimum	Maximum
17β-oestradiol	$2.4x10^{-5}$	$2.8x10^{-5}$	$1.3x10^{-6}$	$9.0x10^{-5}$
Oestrone	$2.9x10^{-5}$	$3.1x10^{-5}$	$1.8x10^{-6}$	$9.0x10^{-5}$
Ethinyl-oestradiol	$2.8x10^{-6}$	$3.3x10^{-6}$	$1.3x10^{-7}$	$1.3x10^{-5}$

† Data derived from Desbrow *et al.* (1996).

5.2.4 Sensitivity Analysis

There is an uncertainty associated with several of the parameters that have been used to define the interactions between the steroid oestrogens and the model environments. It is important to quantify the sensitivity of model outputs to variations in the values of these parameters. Such information helps in two main ways: firstly it can indicate those parameters that should be most closely defined; and secondly it gives an indication of the confidence with which the model output can be used.

Within this analysis a sub-set of model parameters was investigated in which it was known there was a degree of uncertainty and would be likely to have a significant effect on the model results. These were:

- the dispersion coefficient between the bed-sediments and the water column;
- the bioconcentration factor for water column dwelling fauna;
- the chemical degradation rate of the compounds;
- the suspended sediment concentration;
- the effluent dilution factor.

The effect of each of these parameters was studied individually under summer and average flow conditions. However, in order to reduce the number of model runs, sensitivity analysis simulations were only carried out for certain river systems, selected to highlight the expected impact of the parameter variations. The sorption coefficients were not included explicitly in the sensitivity analysis because this was already tested in the Aire and Calder river models where the extent of sorption was the only major difference between them.

5.3 Results and Discussion

5.3.1 Baseline Scenario Simulations

The expected environmental concentrations under the two scenarios for the three river systems modelled are summarised for 17β-oestradiol, oestrone and ethinyl-oestradiol in tables 5.9, 5.10 and 5.11 respectively. The results are given as concentrations in the individual sub-compartments of the water column and the bed-sediment compartments for the reach into which the oestrogen was assumed to be discharged and for a reach 10 km further downstream. The steady-state distribution of the chemical between the water and sediment phases is given over the 10 km stretch modelled together with the amount of degradation that occurred over that distance.

Average Flow Conditions

Within the water column, 17β-oestradiol and oestrone were predicted to appear at concentrations close to or just above the detection limit reported by Desbrow *et al.* (1996) of 0.2 ng/l. In the case of ethinyl-oestradiol the predicted concentrations were an order of magnitude less as would be expected from the input loading used in the simulations. The concentrations predicted for oestrone were highest due to having the largest input load and also because it can be generated by the degradation of 17β-oestradiol. In all cases the dissolved fraction accounted for the majority of the oestrogens in the water column. Despite this, the solid phase concentrations were much higher due to the preference of the compounds for the solid phase over the dissolved phase. The dissolved phase contributes more to the total concentration merely because there is much more water in each compartment than suspended sediments. The differences between rivers reflected the observed differences in the distribution coefficients measured in the laboratory and used in the model.

Table 5.9 Predicted environmental concentrations for 17β-oestradiol under average and low flow conditions for model environments representing conditions similar to the Rivers Thames, Calder and Aire.

			Water Column Concentration				Bed Sediment Concentrations			Chemical Fate (%)		% Degraded over 10 kms
Scenario	River	km†	Total (ng/L)	Dissolved (ng/L)	Suspended Sediments (ng/kg)	Biota (ng/kg)	Total (ng/kg)	Pore Waters (ng/L)	Sediments (ng/kg)	Water	Benthic Sediment	
Average Flow	Thames	1	0.22	0.22	23.0	30.2	0.74	0.036	0.73			
		10	0.22	0.21	22.4	29.3	0.72	0.035	0.71	87.1	12.9	3.0
	Calder	1	0.37	0.35	1180	48.5	2.50	0.071	2.48			
		10	0.36	0.34	1150	47.0	2.42	0.069	2.40	62.7	37.3	3.5
	Aire	1	0.38	0.37	617	50.3	4.88	0.073	4.86			
		10	0.37	0.35	590	48.1	4.67	0.070	4.65	46.9	53.1	4.8
Summer Flow	Thames	1	1.99	1.97	207	270	3.64	0.18	3.57			
		10	1.24	1.22	129	168	2.25	0.11	2.22	92.5	7.5	41.2
	Calder	1	1.34	1.27	4270	174	8.38	0.24	8.29			
		10	1.15	1.09	3650	149	7.17	0.20	7.10	47.4	52.6	15.8
	Aire	1	1.36	1.30	2190	179	16.2	0.24	16.1			
		10	1.08	1.03	1740	141	12.8	0.19	12.7	32.1	67.9	22.9

† Distance downstream from effluent discharge

Table 5.10 Predicted environmental concentrations for oestrone under average and low flow conditions for model environments representing conditions similar to the Rivers Thames, Calder and Aire.

			Water Column Concentration				Bed Sediments Concentration			Chemical Fate (%)		% Degraded over 10 kms
Scenario	River	km†	Total (ng/L)	Dissolved (ng/L)	Suspended Sediments (ng/kg)	Biota (ng/kg)	Total (ng/kg)	Pore Water (ng/L)	Sediments (ng/kg)	Water	Benthic Sediment	
Average Flow	Thames	1	0.27	0.27	28.0	36.5	1.50	0.073	1.47			
		10	0.27	0.26	27.7	36.2	1.48	0.072	1.45	80.2	19.8	3.2
	Calder	1	0.45	0.43	1440	58.8	4.99	0.14	4.94			
		10	0.44	0.45	1415	57.9	4.89	0.14	4.84	50.5	49.5	4.3
	Aire	1	0.46	0.44	746	60.9	9.77	0.15	9.72			
		10	0.45	0.43	728	59.4	9.46	0.14	9.41	34.9	65.1	6.3
Summer Flow	Thames	1	2.50	2.48	260	346	7.79	0.38	7.66			
		10	2.08	2.06	216	283	5.79	0.28	5.70	88.4	11.6	38.7
	Calder	1	1.63	1.55	5190	212	16.9	0.48	16.7			
		10	1.50	1.42	4750	194	15.1	0.43	14.9	35.5	64.5	19.6
	Aire	1	1.66	1.59	2670	218	32.7	0.49	32.5			
		10	1.42	1.36	2290	186	27.2	0.41	27.0	22.5	77.5	29.0

† Distance downstream from effluent discharge

Table 5.11 Predicted environmental concentrations for ethinyl-oestradiol under average and low flow conditions for model environments representing conditions similar to the Rivers Thames, Calder and Aire.

			Water Column Concentration				Bed-sediments Concentration			Chemical Fate (%)		% Degraded over 10 km
Scenario	River	km†	Total (ng/l)	Dissolved (ng/l)	Suspended Sediments (ng/kg)	Biota (ng/kg)	Total (ng/kg)	Pore Water (ng/l)	Sediments (ng/kg)	Water	Benthic Sediment	
Average Flow	Thames	1	0.026	0.025	9.31	251	0.51	0.0071	0.51			
		10	0.025	0.024	9.11	246	0.50	0.0069	0.50	53.4	46.6	2.5
	Calder	1	0.043	0.036	430	363	1.50	0.012	1.49			
		10	0.042	0.035	412	349	1.44	0.016	1.43	24.6	75.4	4.5
	Aire	1	0.045	0.038	228	384	3.00	0.013	2.99			
		10	0.041	0.036	212	357	2.79	0.012	2.78	14.3	85.7	7.8
Summer Flow	Thames	1	0.24	0.23	84.9	229	2.70	0.038	2.68			
		10	0.17	0.16	60.7	164	1.93	0.027	1.92	66.3	33.7	31.1
	Calder	1	0.15	0.13	1530	129	5.02	0.041	5.01			
		10	0.12	0.10	1190	101	3.92	0.032	3.91	14.8	85.2	24.1
	Aire	1	0.15	0.13	793	134	9.82	0.042	9.81			
		10	0.10	0.08	502	84.7	6.23	0.027	6.22	8.1	91.9	39.8

† Distance downstream from effluent discharge

The bed-sediments showed higher concentrations than the water column although the interstitial water had very low concentrations of dissolved oestrogens in all cases. The distribution of the chemical between the water column and the bed-sediments varied between the three model river systems. The River Thames consistently showed the majority of the compounds in the water column while in the Rivers Aire and Calder the compounds favoured the bed-sediments. This difference was mostly due to the differences in the distribution coefficient used for the three rivers. However, the ratio of the volume of the water column to the bed-sediments in each compartment of the Thames model river was several times that of the rivers Calder and Aire, giving the water column more capacity to hold the compounds.

Concentrations in the 100s of ng/kg were predicted for biota that might live permanently in the river downstream of the input loads modelled. Caution must be used in interpreting these data as they are based on estimates of bioconcentration factors. These factors are based on the assumption that the main driving force for accumulation in the biota is the affinity of the chemicals for fatty tissues within the organisms. Oestrogens are likely to interact with organisms in other ways than this and may be excluded, passed through or accumulated more rapidly. The number reported here should only be considered a reasonable estimate assuming that the oestrogens acted only like organic compounds and did not interact in any way with the metabolism of the stream fauna.

The amount degraded did not exceed 8% over the 10 km river stretches modelled for any of the oestrogens. The amount of degradation is controlled by the half-life of the chemicals in relation to the residence times in the river system. Under average flow conditions for these model environments the mean residence time for a compound in the water phase was between 3 and 5 hours compared to half-lives of several days. Therefore it is not surprising that little degradation was predicted.

Low-Flow Conditions

In general the observations on the differences between the rivers and compounds discussed above hold true for the model results under low flow conditions. In the water column the concentrations were higher than under average flow by a factor of between around 5 and 10, 1 km below the input. This was primarily a dilution effect caused by using lower model flow rates while keeping the oestrogen loads constant. Concentrations of oestrogens predicted to be attached to the suspended solids accumulated in stream fauna were increased similarly.

In the bed-sediments, concentrations were also increased particularly in the Rivers Aire and Calder where a decrease in river depth gave a shorter pathway from the water column. The interstitial water concentrations were also increased to levels that would be at analytical detection limits for 17β-oestradiol and oestrone. The distribution of the compounds between the water column and the bed-sediments followed the same pattern as described above, only more enhanced in favour of the bed-sediments for the Rivers Aire and Calder.

The amount of degradation was much more significant than under average conditions accounting for between 16 and 41% of the input load of oestrogen. This can be accounted for in the increased water residence times (12 hours for the Aire and Calder and 48 hours for the Thames)

and shorter half-lives at the higher temperature. The degradation showed a significant effect in dissolved concentration over the 10 km reach for 17β-oestradiol and ethinyl-oestradiol but less so for oestrone because it is created by the degradation 17β-oestradiol.

5.3.2 Sensitivity Analysis

Water Column to Bed-sediment Diffusion

A range of values have been given for the diffusion coefficient (DSP) that controls this process within the context of the EXAMS modelling system (Burns *et al.*, 1982). The value used in the basic scenario simulations was 1.0×10^{-4} while the upper and lower limits sighted were 2.2×10^{-4} and 1.0×10^{-6} respectively. The sensitivity test for this parameter involved running the model for the River Calder under average conditions and comparing the outputs using these extreme values (table 5.11).

It is clear that the value of DSP had an insignificant effect on the predicted environmental concentrations in the water column. However, there was a dramatic effect on the concentrations predicted for the bed-sediments. The two orders of magnitude change in the value of DSP was magnified into a three order of magnitude change in the bed-sediment concentrations. Using a low value of DSP the concentrations were almost negligible while with a high value they could be significant.

There is a corresponding change in the balance between the distribution of the chemical between the water column and the bed-sediments: from a 50:50 split with a high value of DSP to almost total domination by the water phase with a low value of DSP. It is interesting to note that the similarity in dissolved phase concentrations using either value of DSP, implies that there is less chemical in the modelled river system when the value of DSP is low. Therefore, more chemical must have passed through the 10 km model with implications for concentrations further downstream. It is clear, therefore, that the amount of chemical seen by the sediment is critically dependent on the value of DSP used in this model system. However, the dissolved phase concentrations in the water column were shown to be insensitive to the value of DSP and therefore this parameter was not important in estimating these values.

Table 5.12 Sensitivity of predicted environmental concentrations of 17β-oestradiol and oestrone to variations in the value of the water column/bed-sediment diffusion coefficient (DSP) for the River Calder under average flow conditions.

			Water Column Concentration				Bed-sediments Concentration			Chemical Fate (%)		% Degraded over 10 km
Compound	DSP	km†	Total (ng/l)	Dissolved (ng/l)	Suspended Sediments (ng/kg)	Biota (ng/kg)	Total (ng/kg)	Pore Water (ng/l)	Sediments (ng/kg)	Water	Benthic Sediment	
17β-oestradiol	$2.2x10^{-4}$	1	0.37	0.35	1180	221	4.39	0.12	4.35			
		10	0.36	0.34	1130	212	4.22	0.19	4.17	48.8	51.2	4.5
	$1.0x10^{-6}$	1	0.37	0.35	1180	222	0.032	0.00091	0.032			
		10	0.36	0.34	1160	217	0.032	0.00089	0.031	99.2	0.8	2.2
Oestrone	$2.2x10^{-4}$	1	0.45	0.42	1430	268	8.20	0.23	8.02			
		10	0.44	0.42	1420	263	7.90	0.22	7.81	38.6	61.4	5.6
	$1.0x10^{-6}$	1	0.45	0.43	5120	269	0.070	0.0020	0.070			
		10	0.45	0.42	4510	267	0.070	0.0018	0.069	98.6	1.4	2.2

† Distance downstream from effluent discharge

Table 5.13 Sensitivity of predicted environmental concentrations of 17β-oestradiol and oestrone, 10 km downstream, to increasing the value of the water column suspended sediment concentration to 250 mg/l for the River Aire under average flow conditions.

	Water Column Concentration				Bed-sediments Concentration			Chemical Fate (%)		% Degraded over 10 km
Compound	Total (ng/l)	Dissolved (ng/l)	Suspended Sediments (ng/kg)	Biota (ng/kg)	Total (ng/kg)	Pore Water (ng/l)	Sediments (ng/kg)	Water	Benthic Sediment	
17β-oestradiol	0.36	0.26	435	163	3.44	0.052	3.42	54.7	45.3	4.1
Oestrone	0.45	0.32	533	200	6.94	0.10	6.90	42.3	57.7	5.3

Bioconcentration Factor

Two values of bioconcentration factor (BCF) could be calculated for the oestrogens using the two equations described above (see section 5.2.2). The basic scenario runs were made using the lower value while the sensitivity was on 17β-oestradiol and oestrone for the River Calder under summer and average flow conditions. Changing the value of the BCF produced an approximately linear response in the predicted water column biota concentrations (data not shown). Because the biota make up only a small part of the system modelled in mass terms, there was no significant effect on other predicted concentration values.

Suspended Sediment Concentrations

Average values of suspended sediment concentrations were used when making the basic scenario runs under summer and average conditions. They are known to be highly variable and the effect of this variability was tested by running the river model environment for the River Aire under summer and winter conditions with the suspended sediment concentration increased ten fold. (i.e. 250 mg/l). The results are shown in table 5.13 and should be compared with those for the Aire in tables 5.9 and 5.10 for 17β-oestradiol and oestrone.

It is interesting to note that increasing the suspended sediment concentration by ten times has decreased the solid phase concentration in the water column from 587 to 435 ng/kg for 17β-oestradiol and from 725 to 533 ng/kg for oestrone. However, the total amount held on the solid phase and in the dissolved phase in the water column must have increased because the distribution of both chemicals between the water column and the bed-sediments has shifted in favour of the former. The increase in suspended sediments has reduced the dissolved concentrations of both chemicals in the water column by around 0.1 ng/l. This is quite a modest change given the dramatic increase in the suspended sediment concentration simulated, although in the case of 17β-oestradiol the concentration is reduced to close to detection limit.

Biodegradation Rate

The natural purification mechanisms in rivers could play an important part in determining the impact of a point source discharge of oestrogens to downstream environments. The baseline simulations had assumed the same half-life for both the water column and the sediment. The sensitivity test considered the possibility of no degradation in the bed-sediments, which might be the case under anaerobic conditions. This test considered 17β-oestradiol and oestrone with the model for the River Thames under low and average flow conditions. The only significant effects observed were for the bed-sediment concentration (table 5.14). For example, compared to the base line scenario values for the River Thames in table 5.9 the total concentration of 17β-oestradiol in the bed-sediment was increased by a factor of around 7 under average flows and almost 10 under low flows. There was also a corresponding change in the percentage of the chemical distributed between the water column and the bed-sediments from 87/13 to 55/45 under average conditions and from 93/7 to 56/44 under low flow conditions. The effects on the predicted oestrone concentrations were similar.

The river bed-sediments clearly have the potential to be a sink for 17β-oestradiol, oestrone and ethinyl-oestradiol especially when they are anaerobic. The only way the chemicals can be removed from the sediment is through scouring or diffusion processes across the sediment water column interface. Major scouring events will happen only infrequently and therefore sediment dwelling fauna may become exposed to high levels of these oestrogens for several weeks. Whether this exposure will lead to any detrimental effects will depend on, amongst other things, whether the chemicals are bioavailable.

Dilution Factor

Under summer, low-flow conditions many UK rivers have a higher percentage of their flow made up of sewage treatment works effluent than under the low-flow conditions simulated in the base scenario cases. Loads of 17β-oestradiol (5.8 kg/hr), oestrone (7.0 kg/hr) and ethinyl-oestradiol (0.68 kg/hr) were used in the model to give estimated environmental concentrations with dilution factors of 1 for the River Thames and 2 for the Rivers Aire and Calder (table 5.15). The concentrations in all compartments for all three oestrogens have been increased by a factor of over 2 for the Thames and 2.5 for the Aire and Calder. Even concentrations of ethinyl-oestradiol would be detectable over 10 km downstream of the discharge under these conditions. The general distribution of the compounds between the water and the bed-sediments, and the amount of the compounds degraded over the 10 km simulated were almost unchanged from the base scenario simulations.

Comparing these values to the base line scenario values for the River Thames in tables 5.9 and 5.10 shows that there were significant differences in predicted environmental concentrations after 10 km for both chemicals. The concentration of 17β-oestradiol was reduced by 37% from 1.24 to 0.78 ng/l and oestrone was reduced by 22% from 2.08 to 1.62 ng/l. The percentages of both chemicals degraded over this distance was increased by around 50%. There were corresponding changes to the predicted chemical concentrations in all of the model sub-compartments.

It is clear that the effect of degradation rates on the fate of the steroid oestrogens will be most significant in slow flowing rivers whose residence time is at least comparable to the half-lives of these chemicals. Thus, significant effects on predicted concentrations in the model environments were only seen during the low-flow periods typical of summer conditions. They were particularly noticeable in the River Thames where the 95 percentile flow is only 10% of the average compared to 25% for the Rivers Aire and Calder.

Table 5.14 Sensitivity of predicted environmental concentrations of 17β-oestradiol and oestrone to assuming no degradation in the benthic compartments in the River Thames.

			Water Column Concentration				Benthos Concentration			Chemical Fate (%)		% Degraded over 10 kms
Scenario	River	km	Total (ng/L)	Dissolved (ng/L)	Sediments (ng/kg)	Biota (ng/kg)	Total (ng/kg)	Dissolved (ng/L)	Sediments (ng/kg)	Water	Benthic Sediment	
Average Flow	17β-Oestradiol	1	0.22	0.22	23.0	138	4.16	0.20	4.09			
		10	0.22	0.21	22.5	135	4.06	0.20	3.99	54.5	45.5	2.6
	Oestrone	1	0.27	0.27	27.8	167	5.03	0.24	4.94			
		10	0.27	0.27	27.7	167	5.00	0.24	4.92	54.6	45.4	2.6
Summer Flow	17β-Oestradiol	1	0.20	0.20	206	1240	34.5	1.67	33.9			
		10	0.13	0.13	133	796	22.2	1.08	21.8	56.4	43.6	38.7
	Oestrone	1	0.25	0.25	258	1550	43.2	2.10	42.5			
		10	0.21	0.12	222	1340	37.1	1.80	36.5	56.5	43.5	35.3

Figure 5.15 Sensitivity of predicted environmental concentrations of 17β-oestradiol, oestrone and ethinyl-oestradiol to reducing the dilution factor to 1 for the River Thames and 2 for the Rivers Aire and Calder

			Water Column Concentration				Benthos Concentration			Chemical Fate (%)		% Degraded over 10 kms
Scenario	River	km	Total (ng/l)	Dissolved (ng/l)	Sediments (ng/kg)	Biota (ng/kg)	Total (ng/kg)	Dissolved (ng/l)	Sediments (ng/kg)	Water	Benthic Sediment	
17β-oestradiol	Thames	1	4.81	4.75	499	3000	8.75	0.42	8.60			
		10	3.00	2.95	316	1860	5.44	0.26	5.35	92.5	7.5	41.1
	Calder	1	3.24	3.05	10200	1920	20.1	0.57	19.9			
		10	2.77	2.61	8760	1650	17.2	0.49	17.0	47.6	52.4	15.7
	Aire	1	3.29	3.14	5270	1980	38.9	0.58	38.7			
		10	2.60	2.48	4180	1570	30.8	0.46	30.7	32.2	67.8	22.8
Oestrone	Thames	1	6.03	5.96	625	3760	18.7	0.91	18.4			
		10	5.02	4.96	521	3130	14.0	0.68	13.7	88.5	11.5	38.5
	Calder	1	3.94	3.71	12400	2340	40.4	1.15	40.0			
		10	3.61	3.40	11400	2140	36.1	1.02	35.7	35.7	64.3	19.4
	Aire	1	4.00	3.82	6410	2410	78.6	1.18	78.2			
		10	3.43	3.27	5490	2060	65.3	0.98	65.0	22.5	77.5	28.9
Ethinyl-oestradiol	Thames	1	0.57	0.56	206	557	6.56	0.09	6.52			
		10	0.41	0.40	148	398	4.70	0.07	4.67	66.3	33.7	31.1
	Calder	1	0.38	0.31	3710	314	12.2	0.10	12.2			
		10	0.29	0.25	2900	245	9.52	0.08	9.50	14.8	85.2	24.1
	Aire	1	0.38	0.32	1280	324	23.9	0.10	23.8			
		10	0.24	0.21	1220	206	15.1	0.06	15.1	8.1	91.9	39.8

6. CONCLUSIONS

6.1 Laboratory Studies

1. The majority of sorption to the bed-sediments occurred within 24 hours but increased sorption was still apparent after 5 days. A hysteresis effect was evident on desorption with less compound released than was sorbed and gave distribution coefficients (K_d) 2-3 times higher than on sorption.

2. Values of K_d (l/kg) for sorption of 17β-oestradiol to the bed-sediments were estimated to be 13-46 for the Thames, 43-67 for the Aire, 34-56 for the Calder, 20-34 for the Tees and 54 for the Tyne. There was a positive correlation with organic carbon content and with decreasing particle size. However, K_{oc} values calculated showed a wide variation (610-2,650, (l/kg)). These values indicated that sediment characteristics were likely to be important factors in determining the fate of 17β-oestradiol in rivers and potentially its bioavailability.

3. K_d values for the sorption of 17β-oestradiol to suspended sediment exceeded those for the bed-sediments. For the Aire and the Calder this represented a 100 fold increase on the K_d of the bed-sediment. Assuming that the organic carbon content is primarily responsible for the extent of sorption, this implies at least a 5 fold increase in sorption efficiency of the organic carbon in the suspended sediment over the bed-sediments. For the River Thames the change was less dramatic. This maybe attributable to the nature of the suspended sediment, which for the Thames was predominantly live algae and for the Aire and Calder was decaying organic matter.

4. Under aerobic conditions 17β-oestradiol was degraded at high concentrations (3-400 µg/l) in river waters from all five river environments. The half-lives which varied from <3 days in the Aire and Calder samples to 27 days in the sample from the Tyne estuary are relatively long compared to river residence times typical of many UK rivers below major sewage treatment works discharges. 17β-oestradiol was shown to be transformed to oestrone, which was then degraded at a similar rate. Ethinyl-oestradiol was shown to be much more persistent with a half-life about ten times that of 17β-oestradiol. Under normal average flow conditions, degradation in the water column would not be expected to be an important loss route for oestrogens.

5. Under anaerobic conditions, degradation of 17β-oestradiol in river water was still rapid in samples from the Aire and Calder although the oestrone so formed was highly persistent. Both compounds showed greater persistence in the other river samples. Ethinyl-oestradiol showed no degradation under anaerobic conditions in samples from the Thames. This has implications for oestrogen residues that become entrained into sediments. If they are buried it is possible that they will enter anaerobic zones where they will persist for a long time. This may become a source from within the river when sediments get stirred up during storm flow conditions.

6.2 Modelling Studies

6. The EXAMS model has been set up for river systems based on the River Thames, Aire and Calder for 17β-oestradiol, oestrone and ethinyl-oestradiol. The model has been used to estimate likely environmental concentrations in the water column, and bed-sediments using the best available data on the physico-chemical properties of the steroid oestrogens in these systems. However, the concentrations predicted were based on average loadings of oestrogens and may not reflect absolutely those concentrations that might be observed in water samples from these rivers.

7. Total concentrations in the water column were predicted to be similar for 17β-oestradiol and oestrone and an order of magnitude less for ethinyl-oestradiol. This simply reflected the difference in the input loads of the chemicals used in the models.

8. The vast majority of the steroid oestrogens within the water column were predicted to be in the dissolved phase. Concentrations under average conditions (dilution factors of 26 for the Thames and 17 for the Aire and Calder) were predicted to vary between 0.21 and 0.37 ng/l for 17β-oestradiol, 0.27 and 0.44 ng/l for oestrone and 0.024 and 0.038 ng/l for ethinyl-oestradiol.

9. Under low-flow conditions predicted concentrations increased by a factor of between 4 and 10 times the average concentrations at the point of discharge. This effect was diminished 10 km down stream due to increased degradation. When effluent dilution factors were reduced to values of 1 or 2, concentrations were more than doubled again.

10. Degradation processes were shown to be unimportant under average flow conditions, accounting for only 2-8% of the input loading. Under low-flows the degradation became a significant loss term accounting for between 14 and 41% of steroid oestrogens entering the rivers.

11. Bed-sediments were shown to account for between 13% (17β-oestradiol in the Thames under average conditions) to 92% (ethinyl-oestradiol in the Aire under low-flow) of the chemical loads in the river systems. This range was controlled by the distribution coefficient for the chemical/sediment system; higher percentages were associated with higher distribution coefficients.

12. Because the chemicals show an affinity for the solid phase, bed-sediment concentrations were predicted to be higher than those in the water column. The interstitial waters were, however, all of very low concentration. In the bed-sediments the majority of the steroid oestrogens were found on the solid phase. Thus studying degradation in the bed-sediments becomes an important consideration in the context of long term accumulation.

13. The model predictions for concentrations in the bed-sediments were shown to be very sensitive to the value of the dispersion coefficient used to describe the interaction between the bed-sediments and the water column. However, the effects on the water column concentrations were negligible.

7. RECOMMENDATIONS FOR FUTURE RESEARCH

7.1 Laboratory Studies

General

Laboratory and field investigations of 17β-oestradiol, oestrone and ethinyl-oestradiol should be conducted using either stainless steel, glass or PTFE equipment if accurate measurements are to be made.

The fate and behaviour of natural steroids other than 17β-oestradiol and oestrone should be investigated, since these two compounds together account for only 30 % of the normal oestrogen excretion (Fotsis *et al.*, 1980 and 1987).

Degradation

The biodegradation studies should now be repeated using the low concentrations that would be expected in the water column. Winter as well as summer temperatures should be simulated.

Due to the possibility of accumulation, the fate of oestrogens in bed-sediments, particularly in anaerobic sediments should be urgently examined. This will require more time and resources then employed in these initial experiments.

17β-oestradiol has been shown to undergo microbial induced oxidation to oestrone, which is then further biodegraded. Preliminary results have also shown that ethinyl-oestradiol is degraded although more slowly than the other two compounds. The degradation was not a complete mineralisation of the steroids and the possibility that the degradation products are themselves oestrogenic should be investigated and if so at what stage is oestrogenicity lost.

The current study has minimised the possibility of photodegradation by conducting experiments in the dark. It should be determined whether this is an important process in river systems.

Sorption

The sorption of oestrogens to suspended sediments has been shown to be a potentially important process within certain rivers. Since this may have an important influence in mediating the oestrogenicity of these compounds, further research is merited. It seems likely that the nature of the organic carbon in the sediments is responsible for this effect. The nature of the organic carbon seems to be different between rivers particularly in respect of live and decaying carbon substrates. Such changes in the nature of the organic carbon might in some circumstances be seasonal (e.g. algal blooms). For predictive purposes, it would be important to know the consistency of such effects within and between rivers.

The extent of sorption of 17β-oestradiol varies between sediments in a manner that cannot be predicted solely by the organic carbon fractions of the sediment. Further work is required to identify the other constituents of the sediment that influence the sorption of steroid oestrogens.

7.2 Monitoring

The modelling carried out in this study has suggested that the steroid oestrogens will be detected in low levels in the water column downstream of sewage treatment works discharging them at the average rate. Methods of measuring steroid concentrations in the low ng/l range are now available and carried out by a number of laboratories. A monitoring programme should be put in place to measure river concentrations of the steroid oestrogens at locations downstream of known discharge points. The discharge concentration and flow rate should also be measured. In the first instance it would be sufficient to take filtered and unfiltered water samples. The ultimate aim would be to construct a mass balance accounting for the distribution of oestrogens downstream of such discharges. Bed-sediments should be tested for the presence of oestrogens to examine whether these compounds are accumulating in this potential sink.

The importance of re-suspended sediment to water column concentrations under high flow conditions should be investigated. This could be achieved by using automatic samplers to take samples over high flow events. Ideally sediment concentrations of oestrogens would be measured before and after the event and suspended solids measured during the event.

Some thought should be given to other possible inputs to the river system, such as where farms involved in battery hen systems may be operating near important water courses.

7.3 Modelling

A dynamic, mechanistic water quality model should be set up for a river system downstream of a known steroid oestrogen discharge. This should be based on field measurement of model parameters made for the river in question and a measurement programme specifically designed to provide boundary condition data and testing data for the model used. This would enable a test of how well the fate of these chemicals is understood in river systems and thus provide a tool for assessing the impacts of discharges, setting effluent standards and designing monitoring programmes to test compliance.

7.4 Ecotoxicology

It is essential to know at what level steroid oestrogens cause an unacceptable effect on stream organisms. For this water column concentrations as well as suspended and bed-sediments loads have to be taken into account. Especially important questions are to what extent sorbed oestrogens are bioavailable and whether the uptake route alters the observed effects on river

fauna. Without this information it is impossible to set consent conditions for steroid oestrogen discharges other than zero.

8. REFERENCES

ArcandHoy, L. D., Nimrod, A. C., and Benson, W. H. (1998) Endocrine-modulating substances in the environment: Estrogenic effects of pharmaceutical products. *International Journal Of Toxicology* **17**, 139-158.

Ahel, M. and Giger, W. (1993) Partitioning of alkylphenols and alkylphenol polyethoxylates between water and organic solvents. *Chemosphere*, **26**, No.8, 1471-1478

Aherne, G.W. and Briggs, R.J. (1989) The relevance of the presence of certain synthetic steroids in the aquatic environment. *J. Pharm. Pharmacol,* **41**, 735-736.

Belfroid, A.C., Van der Horst, A., Vethaak, A.D., Schafer, A.J., Rijs, G.B.J., Wegener, J. & Cofino, W.P. (1999) Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands. *Science Of The Total Environment*, **225**, 101-108.

Brownbill, V.R., Jackson, D.H. and Douglas, S.I. (1992) Impact of sediments released from a combined sewer overflow. In *Proceedings of 5th International Symposium on River Sedimentation*, Karlsruhe, 1992, pp. 589-595.

Brennicke, A. (1993) Hormonverseuchtes Wasser, *Naturwissenschaftliche Rundschau* **46**, 447

Burns, L.A. (1996) Exposure Analysis Modeling System: Users Guide for EXAMS II, Version 2.96, US EPA, Athens, Georgia, USA. 104pp.

Burns, L.A., Cline, D.M., Lassiter, R.R. (1982) Exposure Analysis Modeling System (EXAMS): User Manual and System Documentation, US EPA publication EPA 600/3-82/023, US EPA, Athens, Georgia, USA. 443pp.

Colborn, T. and Coralie, C. (eds.). The Wildlife/Human Connection. Princeton Scientific Publications, Princeton, New Jersey, 1992

Coombe, R.G., Tsong, Y.Y., Hamilton, P.B. and Sih, C.J. (1966) Mechanisms of steroid oxidation by microorganisms. *J. Biol. Chem.* **241**, 1587-1595

Cousins, I.T., Watts, C.D. and Freestone, R. (1995) Field Measurement and Modelling the Fate of Aniline and Lindane in a UK Lowland River, *Environmental Technology*, 16, 515-526.

Desbrow, C., Routledge, E., Sheenan, D., Waldock, M. and Sumpter, J. (1996) The identification and assessment of oestrogenic substances in sewage treatment works effluents. MAFF Fisheries Laboratory and Brunel University : research and development project report P2 - i490/7 published by the Environment Agency, Bristol, UK.

Di Toro *et al.* (1991) Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environmental Toxicology and Chemistry* **10**, 1541-1583

Elzerman A.W. and Coates J.T. (1987) Hydrophobic organic compounds on sediments: equilibria and kinetics of sorption. In: Advances in Chemistry Series **216**, 263-317

European workshop on the impact of endocrine disrupters on the human health and wildlife, 2-4 December Weybridge, UK, report on proceedings (EUR 17549)

Fishman, J and Martucci, C. (1980) Dissociation of biological activities in metabolites of estradiol. In McLachlan J.A. (ed) Estrogens in the Environment, Elsevier North Holland Inc., 1980

Fotsis, T. and Adlercreutz, H. (1987) The multicomponent analysis of estrogens in urine by ion exchange chromatography and GC/MS-I. Quantifications of estrogens after initial hydrolysis of conjugates. *Journal of Steroid Biochemistry* **28**, 203-213

Fotsis, T., Järvenpää, P. and Adlercreutz, H. (1980) Purification of urine for quantification of the complete estrogen profile, *Journal of Steroid Biochemistry* **12**, 503-508

Gaudette, H.E., Flight, W.R., Toner, L. and Fulger, D.W. (1974) An inexpensive method for the determination of organic carbon in recent sediments. *Journal of Sedimentary Petrology*, **44**, 249-253.

Gimeno, S., Gerritsen, A., Bowner, T. and Komen, H. (1996) Feminization of male carp, *Nature,* **384**, 221-222

Guerngerich F.P. (1990) Minireview, Metabolism of 17a-ethinylestradiol in humans. *Life sciences 1990.* **47**, 1981-88.

Harries, J.E., Sheanan, D.A., Jobling, S., Matthiessen, P. Neall, P., Sumpter, J.P., Tylor, T. and Zaman, N. (1997) Estrogenic activity in five UK rivers detected by measurement of vitellogenesis in caged male trout. *Environmental Toxicology and Chemistry,* **16**, No.3, 534-542

Harrison, P.T.C., Humfrey C.D.N., Shuker, L.K., Smith, L.L. (1995) IEH assessment on environmental oestrogens: consequences to human health and wildlife, Institute of Environment and Health, University of Leicester, 107 p.

Hope, D., Billet, M.F. and Cressor, M.S. (1994) A review of the export of carbon in river water: fluxes and processes. *Environmental Pollution,* **84**, 301-324.

Jobling, S. and Sumpter, J.P. (1993) Detergent components in sewage effluent are weakly estrogenic to fish: An in vitro study using rainbow trout hepatocytes. *Aquatic Toxicology,* **27**, 361-372.

Johnson, A.C., Hughes, C.D., Williams, R.C., Chilton, P.J. (1998) Potential for aerobic isoproturon biodegradation and sorption in the unsaturated and saturated zones of a chalk aquifer. *Journal of Contaminant Hydrology,* in press.

Johnson, A.C., White, C., Besien, T.J. and Jürgens, M.D. (1998) The sorption of octylphenol a xenobiotic oestrogen, to suspended and bed-sediments collected from industrial and rural reaches of three English rivers. *The Science of the Total Environment,* **210/211**, 271-282.

Kalbfus (1995) Belastung bayrischer Gewässer durch synthetische Östrogene. Vortrag bei der 50. Fachtagung des Bayrischen Landesamtes für Wasserwirtschaft (Institut für Wasserforschung, München, 7/8.Nov.1995): Stoffe mit endokriner Wirkung im Wasser (Abstract), quoted from Römbke *et al.* (1996)

Kan, A.T., Gonmin, F. and Tomsen, M.B. (1994) Adsorption/desorption hysteresis in organic pollutant and soil/sediment interaction. *Environmental Science and Technology,* **28**, 859-867.

Karickhoff, S.W. (1981) Semi-empirical estimation of sorption to hydrophobic pollutants on natural sediments and soils. *Chemosphere* **10**, 833-846.

Koelmans, A.A., Anzion, S.F.M. and Lijklema, L. (1995) Dynamics of organic xenobiotics among different particle size fractions in sediments. *Chemosphere,* **32** 1063-1076.

Lewis, D.R., Williams, R.J. and Whitehead, P.G. (1997) Quality simulation along rivers (QUASAR): an application to the Yorkshire Ouse. *The Science of the Total Environment,* **194/195**, 399-418.

Lebietzka, B. (1996) Untersuchungen zur Abbaubarkeit von Ethinylöstradiol in Abwasserreinigungsanlagen, Diploma Thesis at *Intermationales Hochschulinstitut Zittau.* Germany (supervisors: Prof. B. Markert, Dr. J. Oehlmann)

Lick, W., Chroneer, Z. and Rapaka, V. (1997) Modelling the dynamics of the sorption of hydrophobic organic chemicals to suspended sediments, *Water Air and Soil Pollution* **99:** 225-235.

McLachlan J.A., Korach K.S., Newbold R.R. and Degen G.H. (1984): Diethylstilbestrol and other estrogens in the environment. *Fundamental and Applied Toxicology* **4**, 686-691

Montagnani, D.B., Puddefoot, J., Davie, T.J.A. and Vinson, G.P. (1996) Environmentally persistent oestrogen-like substances in UK river systems, *J.CIWEM 1996,* **10** December, 399-406

Norpoth K., Nehrkorn A., Kirchner M., Holsen H. and Teipel H. (1973) Untersuchungen zur Frage der Löslichkeit und Stabilität ovulationshemmender Steroide in Wasser, Abwässern und Belebtschlamm. *Zbl. Bakt. Hyg., I Abt. Orig.* **B 156,** 500-511.

Oliver, B.G. and Niimi, A.J. (1983) Bioconcentrations of Chlorobenzenes from Water by Rainbow Trout: Correlations with partition coefficients and Environmental Residues. *Environmental Science and Technology*, **17**, 287-291.

Patlak, M. (1996) A testing deadline for endocrine disrupters, *Environmenal Science & Technology / News* **30**, No. 12, 540A-544A

Pignatello, J.P. and Xing, B. (1996) Mechanisms of slow sorption of organic chemicals to natural particles. *Environmental Science and Technology,* **30**, 1-11.

Pinder, L.C.V., Marker, A.F.H., Pinder, A.C., Ingram, J.K.G., Leach, D.V. and Collet, G.D. (1997) Concentrations of suspended chlorophyll a in the Humber rivers. *The Science of the Total Environment,* **194/195**, 373-378.

Purdom, C.E., Hardyman, P.A., Bye, V.E, Eno, N.C., Tyler, C.R. and Sumpter, J.P. (1994) Estrogenic effects of effluents from sewage treatment works. *Chemistry and Ecology,* **8,** 275-285.

Rathner, M and Sonnenborn, M. (1979) Biologisch wirksame Östrogene in Trink- und Abwasser. *Forum Städte-Hygiene* **30/3** 45-49

Ren, L., Lattier, D., Lech, J.J. (1996) Estrogenic activity in rainbow trout determined with a new cDNA probe for vitellogenesis, pSG5Vg1.1. *Bull. Environ. Contam. Toxicol.* **56**, 287-294

Richardson, M.L. and Bowron, J.M. (1985) The fate of pharmaceutical chemicals in the aquatic environment. J. Pharm. Pharmacol. **37**, 1-12

Römbke, J., Knacker, Th., Stahlschmidt-Allner, P. Studie über Umweltprobleme im Zusammenhang mit Arzneimitteln, Umweltbundesamt TEXTE 60/96

Rurainsky, R.D., Theiss H.D., Zimmermann, W. (1977) Über das Vorkommen von natürlichen und synthetischen Östrogenen im Trinkwasser. *gwf-wasser/abwasser.* **118,** 288-291

Schlett, C. and Pfeiffer, B. (1996) Bestimmung von Steroidhormonen in Trink- und Oberflächenwässern. *Vom Wasser.* **87**, 327-333

Schweinfurth, H., Länge, R. and Günzel, P. Environmental fate and ecological effects of steroidal estrogens. In: Proceedings of the IBC Conference "Oestrogenic chemicals in the environment" held at 1 Whitehall Place, London SW1. May 1996.

Shore, L.S., Gurewitz, M. and Shernesh, M. (1993) Estrogen as an environmental pollutant. *Bull. Environ. Contam. Toxicol.* **51**, 361-366.

Stumpf, M., Ternes T.A., Haberer, K., Baumann W. (1996) Nachweis von natürlichen und synthetischen Östrogenen in Kläranlagen und Fließgewässern. *Vom Wasser* **87**, 251-261

Sumpter, J.P. and Jobling, S. (1995) Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment, *Environmental Health Perspectives* **Vol. 103**, Supplement 7, 175-178

Tabak, H.H. and Bunch, R.L. (1970) Steroid hormones as water pollutants. I. Metabolism of natural and synthetic ovulation inhibiting hormones by micro-organisms of activated sludge and primary settled sewage. *Developments in Industrial Microbiology.* **11**, 367-376

Tabak, H.H., Bloomhuff, R.N. and Bunch, R.L. (1981) Steroid hormones as water pollutants. II. Studies on the persistence and stability of natural urinary and synthetic ovulation-inhibiting hormones in untreated and treated waste-waters. *Developments in Industrial Microbiology.* **22**, 497-519

Ternes, T.A., Stumpf, M., Mueller, J., Haberer, K., Wilken, R.-D., and Servos, M. (1999a) Behavior and occurrence of estrogens in municipal sewage treatment plants – I. Investigations in Germany, Canada and Brazil. *Science of the Total Environment.* **225,** 81-90

Ternes, T.A., Kreckel, P., and Mueller, J. (1999b) Behaviour and occurrence of estrogens in municipal sewage treatment plants – II. Aerobic batch experiments with activated sludge. *Science of the Total Environment.* **225,** 91-99

Turan, A. (1996) in: Expert round: Endocrinically active chemicals in the environment, Berlin 9-10. March 1995, Umweltbundesamt TEXTE 3/96 (German version available as: TEXTE 65/95)

United States Environmental Protection Agency (1997) Special report on environmental endocrine disruption: an effects assessment and analysis. EPA/630/R-96/012

Veith, G.D. *et al.* (1980) An evaluation of Using Partition Coefficients and Water Solubility to Estimate Bioconcentration Factors for Organic Chemicals in Fish. Aquatic Toxicology. (ASTM STP 707), 116-129.

Waggot. A. (1980) Trace organic substances in the River Lee in: Cooper W J, Chemistry in water reuse vol.20 p.55-99, Ann Arbour Science

Zacharewski T. (1997) In vitro bioassays for assessing estrogenic substances. *Environmental Science & Technology* **31**, No. 3, 613-623c

9. APPENDIX

Tables

Table A.1 Distribution coefficients [l/kg] after sorption

sorption time / Kd 1(sorption)	0.1 hr	0.5 hr	4.2 hrs	1 day	2 days	6 days
Thames (5 g aerobic)	5.1	8.0	9.1	19.6	26.9	-----
Thames (1 g anaer.)	---	---	----	19.9	33.7	37.4
Aire (1 g anaer.)	---	---	----	74.4	128.9	152.4

Table A.2 Distribution coefficients [l/kg] after sorption and 30 min desorption

sorption time / Kd 2 (desorpt.)	0.1 hr	0.5 hr	4.2 hrs	1 day	2 days	6 days
Thames (5 g aerobic)	23.8	22.7	30.3	36.7	52.4	-----
Thames (1 g anaer.)	---	---	----	52.7	70.4	89.7
Aire (1 g anaer.)	---	---	----	154.1	217.2	437.0

Table A.3 Distribution coefficients [l/kg] after sorption and 24 h desorption

sorption time / Kd 3 (desorpt.)	0.1 hr	0.5 hr	4.2 hrs	1 day	2 days	6 days
Thames (5 g aerobic)	29.3	27.8	29.9	31.9	76.0	-----
Thames (1 g anaer.)	---	---	----	42.7	56.8	132.2
Aire (1 g anaer.)	---	---	----	154.8	148.8	319.2

List of Acronyms

C18	C18-column for solid phase extraction of hydrophobic compounds
DOC	dissolved organic carbon
E1	oestrone
E2	17β-oestradiol
EE2	ethinyl-oestradiol (17α-ethinyl-oestradiol)
ELISA	enzyme linked immunosorbent assay
EXAMS	Exposure Analysis Modelling System
GC/MS	or GC/ion-trap-MS gas chromatography with (ion-trap-)mass spectrometer as "detector"
HRT	hormone replacement therapy
K_d	partition coefficient (between sediments and water)
K_{oc}	organic carbon normalised partition coefficient
K_{ow}	octanol water partition coefficient
NGR	National Grid Reference
SD	standard deviation
STW	sewage treatment work
TLC	thin layer chromatography
TOC	total organic carbon