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Xenoestrogens in the River Elbe and its tributaries

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"Capsule": *High concentrations of organic chemicals in the River Elbe may be detrimental to aquatic organisms.*

Abstract

4-Alkylphenols, 4-alkylphenol ethoxylates, 4-alkylphenoxy carboxylates, bisphenol A, bisphenol F, 4-hydroxyacetophenon, 4-hydroxybenzoic acid and steroid hormones were analyzed in water samples of the River Elbe and its tributaries Schwarze Elster, Mulde, Saale, Havel and Schwinge. Additionally, freshly deposited sediments (FDS, composite samples) of the River Elbe and its tributaries were analyzed. The concentrations in water samples ranged from (in ng/l): bisphenol A 4 to 92, branched nonylphenol 13 to 87, branched nonylphenol ethoxylates <0.5 to 120, 4-*tert*. nonylphenoxy carboxylates <10 to 940 and 4-hydroxybenzoic acid 4 to 12. Steroid hormones were only detected in the Czech tributaries Jizera and Vltava in concentrations near the limit of quantification. In FDS samples the concentrations amounted to (in µg/kg d.w.): bisphenol A 10–380, branched nonylphenol 27–430, branched nonylphenol ethoxylates 24–3700, nonylphenoxy carboxylates <50 and 4-hydroxybenzoic acid 23–4400. Increased bisphenol A concentrations were found in water and FDS samples taken from the Czech-German border at Schmilka and the mouth of the Schwinge (only water sample). According to studies conducted in the Elbe Estuary and the German Bight, the River Elbe must be considered as a major source of pollution for the North Sea in respect of the compounds analyzed. A comparison of bisphenol A concentrations, 4-alkylphenols and the corresponding ethoxylates analyzed in the River Elbe and its tributaries with those found in other German surface waters indicated a low level of contamination. The evaluation of the data based on LOEC-values indicated that the concentrations were well below the effectivity threshold for some 4-alkylphenols. According to recent ecotoxicological investigations, for example, with prosobranch snails, bisphenol A concentrations found in water samples of the River Elbe and its tributaries may well be detrimental to aquatic organisms. On the basis of the monitoring data and its implications for estrogenic potency the inclusion of bisphenol A in the list of priority substances (European Union Directive 2000/60/EC, Annex X) should be considered.

Keywords: Bisphenol A; Alkylphenols; Alkylphenol ethoxylates; Alkylphenoxy carboxylates; River Elbe

1. Introduction

Endocrine system disorders in humans and animals resulting from the influence of synthetic substances have been discussed in both scientific and popular literature (Danish Environmental Agency, 1995; Seibert, 1996; Cameron, 1997). In a field study investigations on rainbow trout (*Oncorhynchus mykiss*) exposed to effluents of sewage treatment works (STW) in the UK indicated that substances present in the water induced the synthesis of the estrogen dependent yolk-precurser vitellogenin in both male and female fish (Purdom et al., 1994). The conclusion of this study was that compounds in the effluents are the reason for the elevated vitellogenin levels and the prevalence of hermaphroditism found in the fish population.

Further field studies and laboratory experiments with different compounds exposed to aquatic organisms confirm these results or at least indicate a connection between the incidence of estrogens and the occurrence of disturbances in the reproductive system (Jobling et al., 1998; Lye et al., 1999; Aerle et al., 2001; Fairchild et al., 1999; Tillmann et al., 2001; Körner et al., 2001). In 1999, investigations with the bream *Abramis brama* were conducted along the River Elbe. The main objective of

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this study was a biomarker directed evaluation of the specific exposure situation to endocrine disrupting chemicals along the River Elbe, based on information of the natural variability of endocrine functions. A remarkably high induction of vitellogenin was observed at the locations Barby and Magdeburg as well as at Meißen—areas that are characterized by considerable STW influences. The sampling site Barby is located in the middle course of the River Elbe, downstream of the mouth of the Saale, and Meißen is just downstream of the STW effluent of the city of Dresden. Nevertheless, compared with the controls significantly elevated vitellogenin-titers were measured in most of the fish caught along the river (Hecker et al., 2002).

Natural and synthetic estrogens such as 17α-ethinylestradiol, 17β-estradiol, estrone and estriol were detected in effluents of STW (Ternes et al., 1999). Further investigations of effluents of STW in southwestern Germany revealed that as well as a variety of natural and synthetic hormones, industrial chemicals such as bisphenol A, benzyl butyl phthalate, dibutyl phthalate and microbial degradation products of non-ionic surfactants such as 4-nonylphenol diethoxylate, 4-nonylphenoxyacetic acid and 4-nonylphenol are to be found (Spengler et al., 2001).

In this study, water samples and freshly deposited sediment (FDS) samples from the River Elbe and some of its tributaries were investigated. The River Elbe is one of the major rivers in Central Europe. From its source in the Riesengebirge (Czech Republic) to its mouth at the North Sea near Cuxhaven (Germany) it flows over a distance of 1091 km and has a catchment area of 148,268 km^2 (Simon, 1993)—with about one third located in the Czech Republic and two thirds in the Federal Republic of Germany (Fig. 1). The most important tributaries are the Jizera, the Vltava, the Ohre and the Bilina. In the Czech Republic, the upper course of the River Elbe is exemplified by about 65 weirs and countless chemical plants located along the River Elbe as well as along its tributaries.

The middle course of the River Elbe runs downstream to the weir of Geesthacht (the only weir in the German section of the river), for the most part traversing the territory of the former German Democratic Republic (GDR). The Schwarze Elster, Mulde, Saale, and Havel are the major tributaries. There are a number of chemical plants and large industrial areas in this section of the river, of which the area of Bitterfeld on the River Mulde achieved the most notoriety for excessive contaminant emissions until 1990.

The lower course of the River Elbe comprises the stretch from the weir at Geesthacht to the river's mouth at Cuxhaven. Running through the international port of Hamburg the river receives a significant load of contaminants and nutrients originating from municipal and industrial sewage, especially in the harbor area (e.g. from shipyards). Flow conditions in the estuary are governed both by the river's discharge and by the tides.

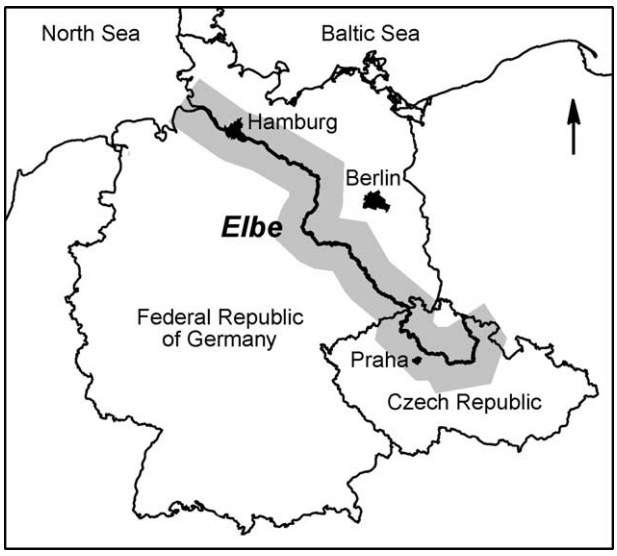

Fig. 1. The River Elbe in the Federal Republic of Germany and in the Czech Republic.

Previously the River Elbe was one of the most polluted rivers in Central Europe as many industrial plants and cities in the Czech Republic and the former GDR conveniently rid themselves of their generally untreated wastewaters discharging into the river. Due to measures taken to reduce the contaminant input (construction of STWs, technical improvements in production processes where possible, cessation of production and the closure of production facilities, etc.) ecological conditions have improved significantly in recent years so that today the river has been repopulated by salmon (Krinitz et al., 2000).

Bisphenol A and two of its main metabolites 4-hydroxybenzoic acid and 4-hydroxyacetophenone (Lobos et al., 1992), bisphenol F, 4-alkylphenols, 4-alkylphenol ethoxylates, phenoxy carboxylates and four steroids were analyzed in water samples and FDS samples from the River Elbe and its tributaries. Sampling sites were located along the whole course of the Elbe from its source in the Czech Republic to its estuary at Cuxhaven.

2. Material and methods

2.1. Sampling

2.1.1. Water samples

Surface water samples were taken at a depth of about 1 m at 10 sites along the course of the River Elbe and at the mouths of its tributaries Schwarze Elster, Mulde, Saale, Havel and Schwinge in July 2000 (Fig. 2). Sampling was performed from the river bank or from a pontoon, using a Teflon device to which a 2-l glass bottle was attached.

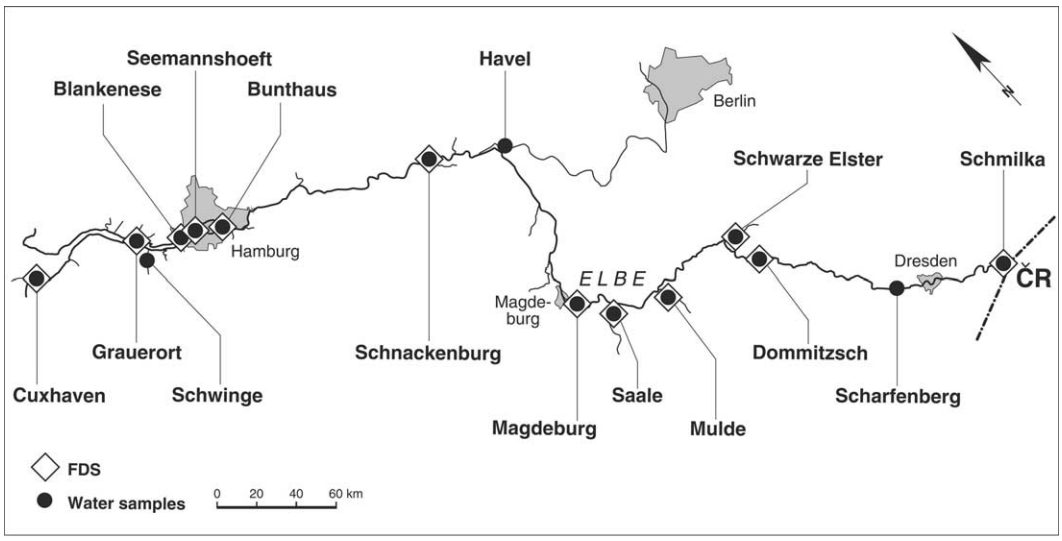

Fig. 2. Sampling sites on the River Elbe and the mouths of its tributaries.

Two additional investigations were carried out in August 1999 and August 2000. Water samples were taken by helicopter from the source (Pramen Labe) of the River Elbe down to the weir Geesthacht (1999) and to Hamburg (2000). Sampling by helicopter (Teflon device, 2-l glass bottles) is a very flexible and rapid method which is not too dependent on the weather. Especially in the upper course in the Czech Republic, where a large number of weirs are encountered (about 65), sampling by helicopter can be coordinated more easily than by ship. In the river's tidal section water samples are taken under similar tidal flow conditions (Krinitz et al., 2000). A map of the sampling locations is given in Fig. 3; sampling by helicopter is described in Krinitz et al. (2000). Water samples were acidified and stored at 6 °C until analysis.

2.1.2. Freshly deposited sediments (FDS)

FDS samples were taken from the River Elbe (nine sites) and at the mouths of its tributaries, Schwarze Elster, Mulde and Saale in July 2000 (Fig. 2). Samples were collected over a period of about 4 weeks (composite samples). A detailed description of the sampling device is given elsewhere (Stachel et al., 1995; Heemken et al., 2000b).

2.2. Analytical method

2.2.1. Substances selected for analysis

2,2′-Bis(4-hydroxyphenyl)propane (bisphenol A; BPA), 4-hydroxyacetophenone (HAP), 4-hydroxybenzoic acid (HBA), 4,4′-dihydroxydiphenylmethane (bisphenol F isomer; BPF), 4-*tert*. butylphenol (BP), 4-*tert*. pentyl-

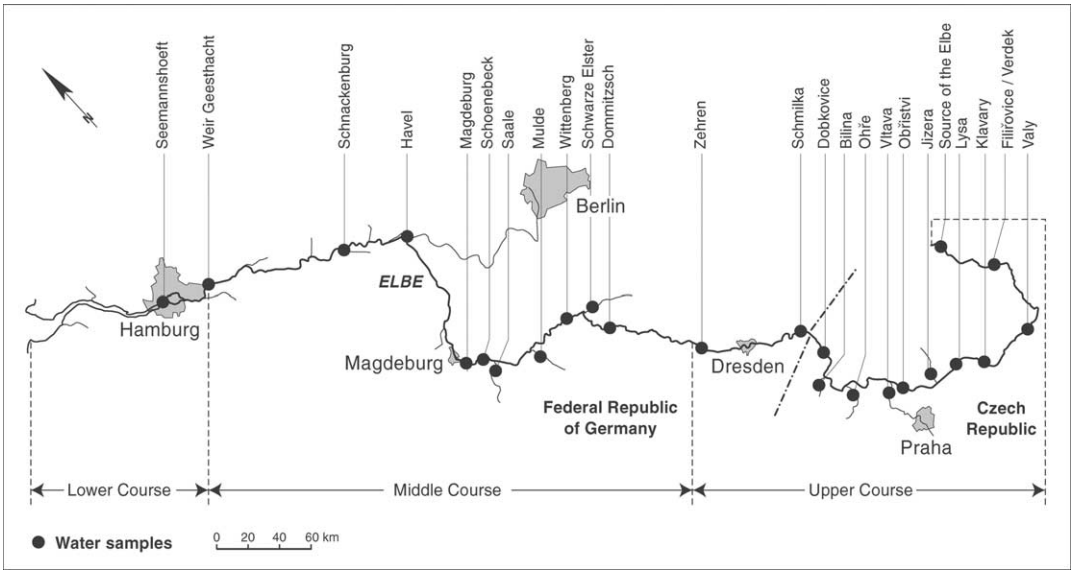

Fig. 3. Sampling sites on the River Elbe from its source to Hamburg and at the mouths of its tributaries (sampling by helicopter).

phenol (PP), 4-*tert*. octylphenol (OP), branched nonylphenols (NP), octylphenol ethoxylates (OPnEO, $n=1$–8), branched nonylphenol ethoxylates (NPnEO, $n=1$–10), octylphenoxy carboxylates (OPnEC, $n=1$–8), nonylphenoxy carboxylates (NPnEC, $n=1$ to 8), estrone, estradiol, mestranol and 17α-ethinylestradiol.

2.2.2. Analytical methods

(A) Water samples and FDS samples (July 2000)
To a 2 l water sample the internal standards BPA-d16, *n*-pentylphenol, *n*-octylphenol and *n*-NP1EO were added and the sample acidified with sulfuric acid (pH 1). The compounds were extracted using dichloromethane (3 × 40 ml). FDS samples (5 g) were extracted with tert. butylmethyl ether (3 × 10 ml). After evaporation to 200–500 µl the compounds were derived by reaction with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) and quantified by GC/MSD (injection port: PTV-solvent vent mode, injection volume 5 µl, column DB 5, length 30 m, i.d. 250 µm, film thickness 0.25 µm; detector: quadrupole MS-EI+, SIM mode).
(B) Water samples (August 1999)
Determination of BPA: a 1 l water sample was prefiltered (0.45 µm glass fiber filter), internal standards BPA-d16, estron-d4, estradiol-d4, 17α-ethinylestradiol-d4 were added and the sample acidified with concentrated sulfuric acid (pH 2–3). The compounds were enriched by solid phase extraction (glass tube with teflon frits filled with 250 mg LiChrolut® RP-18 and 100 mg LiChrolut®-EN) and eluted with acetone. After evaporation to 200 µl (N_2) and clean up on silica gel, the compounds were derived with a mixture of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide/Trimethyliodosilane/Dithioerythritol (MSTFA/TMIS/DTE), and the trimethylsilane (TMS) derivatives were analyzed by GC/MSD (injection port: PTV-splitless mode, injection volume 2 µl, column HP-5MS (Agilent), length 30 m, i.d. 250 µm, film thickness 0.25 µm, detector: quadrupole MS-EI+, SIM mode).
(C) Water samples (August 2000)
Determination of BPA: a 0.5 l water sample was prefiltered (0.45 µm glass fiber filter), internal standard BPA-d16 was added and the sample acidified with concentrated sulfuric acid (pH 2–3). After liquid–liquid extraction with dichloromethane and adding 1 ml 2,2,4-trimethylpentane the extract was evaporated to 500 µl (N_2). The compound was derived with heptafluorobutyric anhydride (HBFA) in the presence of 200 µl K_2CO_3 solution (20% in water). The organic layer was separated and analyzed by GC/MSD (injection port: PTV-solvent vent mode; injection volume 4 µl; column CLPesticides (Restek), length 30 m, i.d. 250 µm, film thickness 0.25 µm, detector: quadrupole MS-EI+, SIM mode).

Three different analysis procedures were used for the detection of BPA in water samples. An intercalibration study (Theobald and Meyer, 2001) indicates that the three procedures (A, B and C) for the determination of BPA in water samples provide comparable results.

3. Results and discussion

3.1. Bisphenol A and metabolites, bisphenol F

In July 2000, water samples collected along the German course of the River Elbe, from Schmilka (Czech–German border) to Cuxhaven (Elbe estuary), and from the mouths of the tributaries Schwarze Elster, Mulde, Saale, Havel and Schwinge were analyzed for several industrial chemicals, metabolites and steroid hormones. The results are shown in Table 1.

BPA concentrations in the Elbe ranged from 4 to 30 ng/l. The BPA level was highest near the Czech-German border whilst in the tidal part it was lowest, due to dilution by increasing water discharge from the tributaries and/or microbial degradation of this compound (biodegradation, half-life 0.5–6 days, Dorn et al., 1987; Klecka et al., 2001). BPA concentrations in the tributaries were generally as high as in the River Elbe, ranging from 4.4 ng/l in the Schwarze Elster to 92 ng/l in the Schwinge. This relatively high BPA concentration at the mouth of the Schwinge might be discharge-related and can probably be attributed to effluents of a local STW. The metabolite HBA was found in low concentrations along the river whilst metabolite HAP was detected only at a few sampling sites (Table 1). BPF concentrations were below the limit of quantification in all samples, except that taken from the mouth of the river Schwinge (0.4 ng/l).

The results of water samples taken by helicopter are shown in Table 2. In August 1999 (15 sites) the BPA concentrations ranged from 16 to 100 ng/l. BPA concentrations in water samples taken in August 2000 (24 sites) along the entire course of the River Elbe, from its source (Pramen Labe) to Hamburg, ranged from <1 ng/l (Valy and also Klavary) to 66 ng/l (Schoenbeck and Magdeburg). In the Czech tributary Ohre the highest concentration of 110 ng/l was found. A high BPA concentration of 51 ng/l also occurred in another tributary of the Elbe, the Bilina, which is known to be polluted by waste water effluents from a chemical plant (Gandrass, 1999; Kurz et al., 1996).

The results for the FDS collected over a 4-week period are shown in Table 3. BPA concentration was highest in the sample from Schmilka (380 µg/kg d.m.). Even BPA, with a reported half-life of 0.5–1 day in the River Elbe (Klecka et al., 2001), was detected in all FDS samples analyzed. Considering that FDS were collected for the duration of about 4 weeks, it can be assumed

Table 1
Concentrations of xenoestrogens and steroids in water samples from the River Elbe and the mouths of its tributaries, in ng/l (July 2000)

Compound	River Elbe									Tributaries				
	Cuxhaven	Grauerort	Blankenese	Seemannshoeft	Bunthaus	Schnackenburg	Magdeburg	Dommitzsch	Scharfenberg	Schmilka	Saale	Mulde	Schwarze Elster	Havel	Schwinge
BPA	3.8	10	21	11	20	18	26	26	29	30	23	11	4.4	10	92
HAP	<0.5	22	0.8	<0.5	<0.5	<0.5	0.7	<0.5	<0.5	1.1	<0.5	<0.5	<0.5	<0.5	2.8
HBA	6.8	5	6.1	7.3	4.4	11	5.2	9.2	11	7.4	12	10	6.5	12	9.5
BPF	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	0.4
BP	<0.5	44	2.0	<0.5	<0.5	<0.5	5.5	51	66	63	<0.5	4.8	<0.5	<0.5	2.4
PP	<0.5	1.7	<0.5	<0.5	2.6	<0.5	3.6	4.9	4.7	2.9	<0.5	4.8	<0.5	<0.5	5.9
OP	1.9	2.1	1.9	1.8	1.2	<0.5	2.5	3.3	2.8	1.2	3.3	4.5	5	3	3.6
NP	24	29	20	22	13	29	35	27	53	48	87	38	32	26	32
OPnEO ($n=1$–8)	7.2	2.5	2	2.2	1	0.6	0.6	1.6	2.3	1.7	3.2	3.9	9.6	<0.5	2.7
NPnEO ($n=1$–10)	40	37	12	38	28	<0.5*	90	62	124	90	73	67	63	<0.5*	51
OPnEC ($n=1$–8)	1.9	<1*	9.7	4.9	<1*	<1*	8.2	7.6	14.8	7.4	16	17	5.3	9.7	34
NPnEC ($n=1$–8)	<10*	187	182	207	233	<10*	436	755	944	831	469	814	<10*	98	166
Estron	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
17β-Estradiol	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
17α-Ethinylestradiol	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2

*Below the limit of quantification for one isomer; "<value" = concentration below the limit of quantification.

Table 2
Concentrations of bisphenol A in water samples from the River Elbe from its source to Hamburg and from the mouths of its tributaries, in ng/l (sampling by helicopter)

Sampling site	1999	2000
Pramen Labe (source)	16	5.4
Filirovice/Verdek	n.s.	12
Valy	19	<1
Klavary	21	<1
Lysa	n.s.	3.7
Jizera	83	23
Obristvi	n.s.	32
Vltava	18	7
Ohre	21	114
Bilina	n.a.	51
Dobkovice	n.s.	19
Schmilka	100	19
Zehren	95	22
Dommitzsch	57	44
Schwarze Elster	17	22
Wittenberg	n.s.	37
Mulde	16	9
Saale	34	56
Schoenbeck	n.s.	66
Magdeburg	31	66
Havel	n.s.	3.3
Schnackenburg	n.s.	32
Weir Geesthacht	38	19
Seemannshoeft	n.s.	14

"<Value" = concentration below the limit of quantification; n.s. = no sample; n.a. = not analyzed.

that the decomposition process of BPA in the particulate phase takes much longer.

In addition, HAP levels detected in FDS were lower than HBA levels. Extremely high HBA concentrations (several thousand μg/kg d.m.) were found in FDS samples from Dommitzsch, Bunthaus and Seemannshoeft (Table 3). These local hot spots might not be primarily due to BPA-degradation, but could result from microbial decomposition of parabens (preservatives used in food production), released to the River Elbe via STW effluents. This hypothesis remains to be substantiated by further studies. Despite the observed high HBA concentrations a threat to the aquatic environment in terms of endocrine disruption is not given (Calabro and Soto, 2001).

BPF was detected in low concentrations in most of the FDS samples, with a maximum concentration of 7 μg/kg d.m. in the Mulde.

When comparing data on concentrations of xenoestrogens from the River Elbe (Tables 1–3) with those from other German surface waters the following limitations have to be taken into account:

- The data to be compared refer to rivers and surface water systems with different water discharge characteristics;
- analyses have been performed by various laboratories using different analytical procedures; and

Table 3
Concentrations of xenoestrogens and steroids in freshly deposited sediments (FDS) from the River Elbe and the mouths of its tributaries, µg/kg d.m. (July 2000)

Compound	River Elbe								Tributaries		
	Cuxhaven	Grauerort	Blankenese	Seemannshoeft	Bunthaus	Schnackenburg	Magdeburg	Dommitzsch	Schmilka	Saale	Mulde	Schwarze Elster
BPA	10	20	21	44	58	70	149	235	379	29	88	51
HAP	3	2.8	4.8	15	8.7	8.1	12	19	21	15	26	28
HBA	26	23	728	2309	4443	124	628	3103	200	103	157	34
BPF	<1	<1	2.7	3.9	2.4	6	4.2	4.6	<1	3	7	2
BP	<1	<1	<1	<1	<1	2.6	40	117	185	3.1	n.a.	<1
PP	<1	<1	1.8	<1	1.9	<1	<1	<1	<1	<1	<1	<1
OP	5.5	25	6.3	24	7.7	13	11	8.3	24	4	36	62
NP	27	32	428	67	56	101	316	43	182	42	367	150
OPnEO ($n=1$–8)	111	813	69	742	77	258	122	27	434	25	579	535
NPnEO ($n=1$–10)	53	60	302	141	24	163	682	153	3667	90	409	197
OPnEC ($n=1$–8)	7.6	9.1	54	18	<5*	25	91	<5*	530	13	428	<5*
NPnEC ($n=1$–8)	<50*	<50*	<50*	<50*	<50*	<50*	<50*	<50*	<50*	<50*	<50*	<50*
Estron	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
17β-Estradiol	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
17α-Ethinylestradiol	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2

n.a. = Not analyzed; *below the limit of quantification for one isomer; "<value" = concentration below the limit of quantification.

- differences in results for a specific river can be caused by collecting samples at different locations and/or different times.

Tables 4 and 5 summarize concentrations of BPA, OP, NP, and NPnEO analyzed in water as well as FDS, suspended particulate matter (SPM) and sediment samples from various German rivers. Minimum and maximum concentrations are given.

The highest BPA concentration of 4400 ng/l was found in the Pleiße (Table 4), a small river which flows through Saxony and Thuringia. The Pleiße is highly contaminated by effluents from a municipal STW. High BPA concentrations of 2600 ng/l also occurred in the Thuringian course of the Weiße Elster and in the Saale (1600 ng/l), and are probably due to industrial and municipal STW effluents. A BPA contamination of the River Elbe near Scharfenberg, as indicated by the concentrations found in 1998 (780 ng/l), was not confirmed in the investigation in year 2000 (Table 1).

Table 5 shows BPA, OP, NP and NPnEO concentrations in FDS, SPM and sediment samples. The maximum BPA concentrations in composite FDS samples collected over a 4-week period were found in the River Elbe, near Schmilka (340 and 380 µg/kg d.m., respectively), and are probably caused by effluents of a chemical plant situated nearby, about 30 km upstream the Czech–German border. High BPA concentrations also occurred in SPM samples from the Weser (300 µg/kg d.m.) and the Fulda (210 µg/kg d.m.).

Exposure experiments with BPA on prosobranch snails showed LOEC, NOEC and EC_{10} values of 48.3, 7.9 and 13.9 ng/l, respectively (Schulte-Oehlmann et al., 2001b). This indicates that BPA concentrations as low as found in water samples from the River Elbe might affect wild populations of prosobranch snails.

Under Directive 2000/60/EC specific measures must be adopted at Community level against pollution of water by individual pollutants or groups of pollutants presenting a significant risk to or via the aquatic environment. Such measures are designed to achieve progressive reduction and, for hazardous priority substances, the cessation or phasing out of discharges within 20 years after adoption of the Directive. Annex X of the Directive refers to Article 16(5), the list of 33 priority substances (including hazardous substances) as established in November 2001 (decision No. 2455/2001/EC). Nonylphenols are identified as priority hazardous substances, and octylphenols are subject to review for inclusion in this category within the next 12 months. On the basis of recent results on the ecotoxicological effects of BPA on aquatic organisms, for example, prosobranch snails, and monitoring data on BPA from German and other European rivers the inclusion of this compound into Annex X of the Directive (list of priority substances) should be given consideration.

Table 4
Concentrations of xenoestrogens in water samples from German surface waters (min.–max. values), in ng/l

Compound	Elbe	Saale	Weiße Elster	Pleiße	Mulde	Schw. Elster	Havel	Schwinge	Alster	Weser	Werra	Fulda	Berlin/ Brandenburg	Rhein	Hessen
BPA	17–776[a]	125[a]	24–75[a]	<40–4410[b]	11[a]	8.9[a]	10[c]	92[c]	74; 221[d]	<15 to 190[e]	<15 to 88[e]	<15 to 186[e]	0.5–135[f]	42–229[f]	<25 to 130[g]
	4–30[c]	23[c]	<40–2620[b]		23[c]	4.4[c]	3–12[f]				<40 to 180[b]				
		<40–1620[b]													
OP	0.4–1.3[a]	2[a]	1.5–6[a]	n.v.	2.1[a]	1[a]	3[c]	4[c]	17[d]	<5 to 101[e]	7–13[e]	<5 to 19[e]	<50 to 270[h]	n.v.	<80[g]
	<0.5 to 3.3[c]	3.3[c]			4.5[c]	5[c]									
NP	7.3–52[a]	32[a]	78–220[a]	<60–3990[b]	13[a]	1[a]	26[c]	32[c]	26[d]	<10 to 569[e]	105–280[e]	76–280[e]	<80 to 2720[h]	n.v.	<60[g]
	13–53[c]	87[c]	0.06–2970[b]		38[c]	32[c]									
		<60–470[b]													
NpnEO	14–289[a]	108[a]	39–183[a]	n.v.	51[a]	17[a]	51*[c]	51*[c]	98[d]	n.v.	n.v.	n.v.	<50 to 3270[h]	n.v.	<80[g]
	<0.5 to 124*[c]	73*[c]			67*[c]	63*[c]									

NPnEO: sum of $n=1$ and 2; * sum of $n=1$–10; n.v. = no value; < "value" = concentration below the limit of quantification.
[a] No. of sampling sites: Elbe = 10; Weiße Elster = 6; Saale, Mulde and Schwarze Elster = 1 in 1998 (Heemken et al., 2000a).
[c] No. of sampling sites: Elbe = 10; Saale, Mulde, Schwarze Elster, Havel and Schwinge = 1 in 2000 (this investigation).
[b] No. of sampling sites: Weiße Elster = 4; Saale and Pleiße = 1 in 2000 (Geiß, 2001).
[d] One or two sampling sites in 1998 (Umweltbehoerde Hamburg, 2000).
[e] No. of sampling sites: Weser = 3; Werra and Fulda = 1 in 1999 (Weserguetebericht, 1999).
[f] No. of sampling sites = 27; surface waters in Berlin/Brandenburg, 1997–1998 (Böhmer et al., 1999).
[g] No. of sampling sites = 20–25; surface waters in Hessen in 2000 and 2001 (Gihr, 2001).
[h] No. of sampling sites = 32; surface waters in Berlin in 1997 (Fromme et al., 1998).

3.2. Alkylphenols, -ethoxylates and -carboxylates

Elevated BP concentrations were found in samples from the Czech–German border (Schmilka, 63 ng/l), Scharfenberg (66 ng/l) and at Grauerort (44 ng/l). These might be caused by influences of the River Bilina (Schmilka), the municipal STW of Dresden (near Scharfenberg), the Schwinge and/or an industrial site at the tidal part of the River Elbe (Grauerort). PP concentrations ranged from below the limit of quantification up to 6 ng/l (Schwinge). OP levels ranged from <0.5 to 3.3 ng/l in the River Elbe, with a maximum of 5 ng/l at the mouth of the River Schwarze Elster. NP concentrations are generally higher than those of OP in all rivers studied. NP concentrations ranged from 13 to 53 ng/l in the River Elbe (maximum of 87 ng/l at the mouth of the Saale). Among the substances being investigated, alkylphenol ethoxylates and alkylphenoxy carboxylates NPnEO and NPnEC were the dominant compounds (Table 1). The maximum NPnEC concentration of 940 ng/l was found near Scharfenberg (Elbe). The highest concentration of NPnEC in samples collected in the tributaries of the River Elbe was detected at the mouth of the River Mulde (NPnEC 810 ng/l). In general, observed concentrations were slightly lower in samples taken in 2000 than in those from 1998 (Heemken et al., 2000a).

Maximum NP concentrations of 3400 and 3000 ng/l were found in the Pleiße and Weiße Elster, respectively. High NPnEO concentrations (3300 ng/l) occurred in one Berlin surface water sample (Table 4). A comparison of 4-alkylphenols and the corresponding ethoxylates in the River Elbe and its tributaries with those found in other German surface waters indicated a low level of contamination. Only small rivers within the catchment of the River Elbe which have a low water discharge and relatively high influx of STW effluents show high concentrations of xenoestrogens, for example, the River Pleiße and the River Weiße Elster.

In 1998 and 1999, water samples were taken from the Elbe Estuary, the German Bight and remote waters of the North Sea using the research vessel "Gauss". The results of the analyses show a declining concentration gradient for BPA, NP and NPnEO from the estuary towards the North Sea. There was no BPA detected in the samples from the coastal areas and the open sea (limit of quantification 50 pg/l). The NP- and NPnEO-concentrations were approximately one order of magnitude less in the coastal samples compared with those from the estuary. The investigations show that the River Elbe represents a significant source of input for industrial chemicals with endocrine potency into the North Sea (Heemken et al., 2001).

4-Alkylphenols and 4-alkylphenol ethoxylates have a pronounced lipophilic character and, therefore, bioaccumulate in aquatic organisms (Lye et al., 1999; Bennett

Table 5
Concentrations of xenoestrogens in samples of freshly deposited sediments (FDS), suspended particulate matter (SPM) and sediments from German surface waters (min.–max. values), in µg/kg d.m

Compound	Elbe	Saale	Mulde	Schwarze Elster	Alster	Weser	Werra	Fulda	Berlin/ Brandenburg	Neckar	Rhein	Donau	Hessen
BPA	66–343[a] 10–379[h] 128–311[c]	121[a] 29[h]	76[a] 88[h]	132[a] 51[h]	143; 192[b]	< 2 to 295[c]	< 2 to 162[c]	< 2 to 208[c]	< 0.2–190[d] < 5–150[i]	< 10[e,f,g]	< 10[e,f,j]	< 10[e,f,g]	n.v.
OP	21–77[i] 6–25[h]	86[a] 4[h]	41[a] 36[h]	38[a] 62[h]	n.v.	< 2 to 17[c]	< 2 to 294[c]	< 2 to 6[c]	< 10[i]	n.v.	n.v.	n.v.	12–50[j] < 5 to 30[k]
NP	367–997[a] 27–428[h]	1378[a] 42[h]	507[a] 367[h]	705[a] 150[h]	n.v.	< 30 to 1724[c]	< 30 to 4012[c]	< 30 to 594[c]	< 20 to 12700[i]	20–4400[e] 24–1400[f]	70–4100[e] 57–940[f]	220–9900[g]	220–4890[j] < 0.05 to 230[k]
NPnEO	869–2578[a] 24–3667*[h]	2402[a] 90*[h]	1562[a] 409*[h]	1745[a] 197*[h]	n.v.	n.v.	n.v.	n.v.	< 10 to 1900**[i]	n.v.	n.v.	n.v.	n.v.

NPnEO: sum of $n = 1$ and 2; * sum of $n = 1$–10; **only NP1EO; SPM = suspended particulate matter; FDS = freshly deposed sediments (monthly mixed samples); n.v. = no value; "< value" = concentration below the limit of quantification.

[a] FDS, No. of sampling sites: River Elbe = 8; Saale, Mulde and Schwarze Elster = 1 in 1998 (Heemken et al., 2000a).
[h] FDS, No. of sampling sites: River Elbe = 9; Saale, Mulde and Schwarze Elster = 1 in 2000 (this investigation).
[b] No. of sampling sites: River Elbe = 4 (FDS) in 1998/1999; Alster = 2 (sediment) in 1998 (Umweltbehoerde Hamburg, 2000).
[c] SPM, No. of sampling sites: Weser = 3; Werra and Fulda = 1 in 1999 (Weserguetebericht, 1999).
[d] Sediment, No. of sampling sites = 12; surface waters in Berlin/Brandenburg in 1996 (Böhmer et al., 1999).
[i] Sediment, No. of sampling sites = 23; surface waters in Berlin 1997 (Fromme et al., 1998).
[e] SPM, No. of sampling sites: Neckar = 3; Rhein = 1 in 1999 (Landesanstalt fuer Umweltschutz Baden-Wuerttemberg, 2001).
[f] SPM, No. of sampling sites: Neckar = 3; Rhein = 1 in 1998 (Landesanstalt fuer Umweltschutz Baden-Wuerttemberg, 2001).
[g] SPM, No. of sampling sites: Donau = 1 in 1997 (Landesanstalt fuer Umweltschutz Baden-Wuerttemberg, 2001).
[j] SPM, No. of sampling sites: Schwarzbach and Rodau = 6 in 2000 (Gihr, 2001).
[k] SPM, No. of sampling sites = 17; surface waters in Hessen in 2000 (Gihr, 2001).

& Metcalfe 2000; Ferrara et al., 2001). The lowest observable effect concentrations (LOEC-values) reported are 5 μg/l for OP, 20 μg/l for NP and 30 μg/l for NP2EO (endpoint: vitellogenin synthesis in male trout; Gülden et al., 1997). Concentrations of OP, NP and NPnEO analyzed in water samples from the River Elbe and its tributaries were all well below these levels.

Generally, NP was found in higher concentrations in FDS samples from the River Elbe and its tributaries than other 4-alkylphenols (Table 3). Maximum NP concentrations were reached at Blankenese (430 μg/kg d.m.) and in the Mulde (370 μg/kg d.m.). OP was detected in all samples at low levels (6–25 μg/kg d.m.). Concentrations increased at Schmilka (24 μg/kg d.w.), Seemannshoeft and Grauerort (24 and 25 μg/kg d.m., respectively). Among alkylphenol ethoxylates NPnEO concentrations are not higher than OPnEO concentrations in general (Table 3). Detailed analyses of chromatograms of FDS samples demonstrated the predominance of OPnEO compounds possessing one to three ethoxy groups. In contrast to the water samples in which NPnEC levels were higher than OPnEC, the only carboxylate detectable in FDS was OPnEC. A maximum OPnEC concentration of 530 μg/kg d.m. was found in the FDS sample from the sampling site at Schmilka.

Extremely high NP concentrations were analyzed in sediment samples from a Berlin surface water (12,700 μg/kg d.m.) and from the upper course of the river Donau (9900 μg/kg d.m.). NPnEO was detected in some German rivers. A maximum level 3700 μg/kg d.m. was found in a composite FDS sample from the River Elbe collected at Schmilka. Effluents of industrial and/or municipal STW appear to be the cause of the enhanced NPnEO concentrations at this site (Table 5).

As shown in the previous sections, all compounds analyzed were detectable in both the aqueous and the particulate phases. For this reason partitioning coefficients of BPA, 4-alkylphenols, and alkylphenol ethoxylates were determined in order to estimate the partitioning between the aqueous phase and SPM. Water samples were collected from the River Elbe at the weir at Geesthacht. The partitioning coefficients (log K_{OC}) were calculated from the analyzed concentration in both phases as: 4.2–4.6 for BPA, 5.5–5.6 for 4-alkylphenols and 5.6–6.4 for 4-alkylphenol ethoxylates (Heemken et al., 2000a).

In 2000, a biological effect monitoring study with two species of prosobranch snails *Potamopyrgus antipodarum* and *Nassarius reticulatus* was performed (Schulte-Oehlmann et al., 2001a). The snails were exposed to sediment samples mostly taken from small ports on the River Elbe and from the river itself. Sampling started at the port Prossen (near the Czech–German border), and the last one was taken in the Wadden Sea at Duhner Watt (Estuary). The aim of this investigation was to analyze the extent and intensity of biological effects caused by endocrine active compounds, which are present as complex mixtures in sediment samples. The results showed androgenic effects on *Nassarius reticulata* mainly caused by tributyl tin compounds in the sediment samples and reproductive toxicity effects on *Potamopyrgus antipodarum* (number of embryos in the posterior oviduct section, referred to as the brood pouch). An estrogenic effect in both snails was shown for the sample from Sandfurth (Elbe), which is located downstream of Magdeburg. These results in respect of the snails correspond with the estrogenic effects found in *Abramis brama* (Hecker et al., 2002) and indicate that the region of Magdeburg is an area polluted with endocrine disrupting chemicals. Further chemical analyses of sediment and water samples are needed to identify the sources of estrogenic compound input in this region.

3.3. Steroid hormones

Since estrone, 17β-estradiol and 17α-ethinylestradiol have a significantly higher estrogenic potency than the compounds previously discussed, synthetic and natural steroid hormones were analyzed in water samples from the River Elbe and its tributaries. The concentrations of these steroids in the River Elbe and five tributaries were below the limit of quantification (0.2 ng/l, Table 1).

In 1999 the steroid hormones estrone, 17β-estradiol, mestranol and 17α-ethinylestradiol were analyzed additionally in 18 water samples from the source down to the weir at Geesthacht. Of these compounds only estrone was detected in two samples from the Czech tributaries Vltava (1 ng/l) and Jizera (4 ng/l). The limit of quantification for samples from this investigation was 1 ng/l.

In all FDS samples the concentrations of the steroids estrone, 17β-estradiol, mestranol and 17α-ethinylestradiol were below the limit of quantification (Table 3).

4. Conclusions

BPA was detected in water and FDS samples from the River Elbe and several of its tributaries. BPA concentrations in the Elbe were between 4 and 66 ng/l, with highest concentrations near the Czech–German border at Schmilka and the mouth of the River Schwinge. In terms of pollution, HBA was the most frequently found metabolite of BPA. BPF was less prominent.

In composite FDS samples, BPA concentrations were also highest near Schmilka (380 μg/kg d.m.). At some sampling sites, HBA concentrations in FDS samples were about one order of magnitude higher than BPA concentrations, and cannot be attributed to BPA degradation only. Steroid hormones were only detected in the Czech tributaries Jizera and Vltava, and in concentrations near

the limit of quantification. A comparison of BPA, 4-alkylphenols and the corresponding ethoxylates analyzed in the River Elbe and its tributaries with those found in other German surface waters indicated a low level of contamination. Only small rivers within the catchment of the River Elbe which have a low water discharge and relatively high influx of STW effluents show high concentrations of xenoestrogens, for example, the River Pleiße and the River Weiße Elster.

Separate analyses of the SPM and water phase showed that all studied compounds tend to be adsorbed to SPM. This is also the case for BPA, which has a rather high solubility in water and is very prone to microbial decomposition in the water phase. Yet, according to recent ecotoxicological investigations, BPA concentrations found in water samples from the River Elbe and its tributaries may well be of concern for aquatic organisms. In view of recent results on the ecotoxicological effects of BPA on prosobranch snails and monitoring data on BPA from German and other European rivers the inclusion of this compound into Annex X of the Directive 2000/60/EC (list of priority substances) should be given consideration.

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References

Aerle, van R., Nolan, M., Jobling, S., Christiansen, L.B., Sumpter, J.P., Tyler, C.R., 2001. Sexual disruption in a second species of wild cyprinid fish (the Gudgeon, *Gobio Gobio*) in United Kingdom freshwaters. Environmental Toxicology and Chemistry 20, 2841–2847.

Arbeitsgemeinschaft zur Reinhaltung der Weser, 1999. Weserguetebericht, Sondermessprogramm.

Bennett, E.R., Metcalfe, C.D., 2000. Distribution of degradation products of alkylphenol ethoxylates near sewage treatment plants in the lower great lakes, North America. Environmental Toxicology and Chemistry 19, 784–792.

Böhmer, W., Bruckert, H.J., Rüdel, H., Wenzel, A., 1999. Verfolgung von Umweltbelastungen durch Alkylphenole, Bisphenol A und organische Zinnverbindungen in repräsentativen Umweltproben. Umweltbundesamt F&E-Vorhaben 297 63 155

Calabro, J., Soto, A., 2001. Personal Communication. Tufts University School of Medicine, Boston.

Cameron, P., 1997. Umweltgifte mit hormoneller Wirkung. WWF-Umweltstiftung, Frankfurt.

Danish Environmental Protection Agency, Copenhagen, 1995. Male reproductive health and environmental chemicals with estrogenic effects. Miljoeprojekt No. 290, 15-30.

Dorn, P.B., Chou, C.S., Gentempo, J.J., 1987. Degradation of bisphenol A in natural waters. Chemosphere 16, 1501–1507.

Fairchild, W.L., Swansburg, E.O., Arsenault, J.T., Brown, S.B., 1999. Does an association between pesticide use and subsequent declines in catch of Atlantic salmon (*Salmo salar*) represent a case of endocrine disruption? Environmental Health Perspectives 107, 349–357.

Ferrara, F.F., Fabietti, F., Delise, M., Bocca, A.P., Funari, E., 2001. Alkylphenolic compounds in edible molluscs of the Adriatic Sea (Italy). Environmental Science and Technology 35, 3109–3112.

Fromme, H., Lahrz, T., Führig, D., 1998. Expositionsmonitoring endokrin wirksamer Substanzen in verschiedenen Umweltkompartimenten. Institut fuer Umweltanalytik und Humantoxikologie, Berlin. Report Umweltbundesamt 216 02 011/12.

Gandrass, J., 1999. Monitoring Data of Bisphenol A (BPA) and Metabolite 4-Hydroxyacetophenone (HAP). Umweltbundesamt Berlin, Texte IV 2.2, 97355-6/8.

Geiß, S., 2001. Thueringer Landesanstalt fuer Umwelt und Geologie.

Gihr, R., 2001. Hessisches Landesamt fuer Umwelt und Geologie.

Gülden, M., Turan, A., Seibert, H., 1997. Substanzen mit endokriner Wirkung in Oberflächengewässern. Report Umweltbundesamt Berlin, 102 04 279 (UBA-Texte 46/97).

Hecker, M., Tyler, C.R., Hoffmann, M., Maddix, S., Karbe, L., 2002. Plasma Biomarkers in fish provide evidence for endocrine modulation in the Elbe River, Germany. Environmental Science and Toxicology 36, 2311–2321.

Heemken, O.P., Stachel, B., Theobald, N., Wenclawiak, B.W., 2000b. Temporal variability of organic micropollutants in suspended particulate matter of the River Elbe at Hamburg and the River Mulde at Dessau, Germany. Archives of Environmental Contamination and Toxicology 38, 11–31.

Heemken, O.P., Theobald, N., Hebbel, H., Stachel, B., Reincke, H., 2000a. Endokrin wirksame Stoffe in der Elbe, in Nebenflüssen und in der Nordsee. Report Arbeitsgemeinschaft fuer die Reinhaltung der Elbe, Hamburg.

Heemken, O.P., Reincke, H., Stachel, B., Theobald, N., 2001. The occurrence of xenoestrogens in the Elbe river and the North Sea. Chemosphere 45, 245–259.

Jobling, S., Nolan, M., Tyler, C.R., Brighty, G., Sumpter, J.P., 1998. Widespread sexual disruption in wild fish. Environmental Science and Technology 32, 2498–2506.

Klecka, G.M., Stanley, J.G., West, R.J., Goodwin, P.A., Markham, D.A., 2001. Biodegradation of bisphenol A in aquatic environments: river die-away. Environmental Toxicology and Chemistry 20, 2725–2735.

Körner, W., Spengler, P., Bolz, U., Schuller, W., Hanf, V., Metzger, J.W., 2001. Substances with estrogenic activity in Effluents of sewage treatment plants in South-Western Germany. 2. Biological analysis. Environmental Toxicology and Chemistry 20, 2142–2151.

Krinitz, J., Stachel, B., Reincke, H., 2000. Stoffkonzentrationen in mittels Hubschrauber entnommenen Elbewasserproben (1979 bis 1998). Report Arbeitsgemeinschaft fuer die Reinhaltung der Elbe, Hamburg.

Kurz, J., Rao, S.S., Wilken, R.D., Carey, J.H., 1996. Chemical, toxicological and genotoxicological characterization of sediments from the River Elbe, Germany. NWRJ Contribution No. 20-96, CCIW, Burlington, Ontario, Canada. Cited in: Gülden, M., Turan, A., Seibert, H., 1997. Substanzen mit endokriner Wirkung in Oberflächengewässern. Report Umweltbundesamt Berlin 102 04 279 (UBA-Texte 46/97).

Landesanstalt fuer Umweltschutz Badem-Wuerttemberg, 2001. Untersuchungen zum Vorkommen von Xenobiotika in Schwebstoffen und Sedimenten Baden-Wuerttembergs—Oberirdische Gewaesser/Gewaesseroekologie, Bd. 67, Karlsruhe.

Lobos, J.H., Leib, T.K., Su, T.M., 1992. Biodegradation of bisphenol A and other bisphenols by gram-negative aerobic bacterium. Applied and Environmental Microbiology 58, 1823–1831.

Lye, C.M., Frid, C.L.J., Gill, M.E., Cooper, D.W., Jones, D.M., 1999. Estrogenic alkylphenols in fish tissues, sediments, and waters from

the U.K. Tyne and Tees estuaries. Environmental Science and Toxicology 33, 1009–1014.

Purdom, C.E., Hardiman, P.A., Bye, V.J., Eno, N.C., Tyler, C.R., Sumpter, J.P., 1994. Estrogenic effects of effluents sewage treatment works. Chemistry and Ecology 8, 275–285.

Schulte-Oehlmann, U., Tillmann, M., Casey, D., Duft, M., Markert, B., Oehlmann, J., 2001b. Östrogenartige Wirkungen von Bisphenol A auf Vorderkiemenschnecken (Mollusca: Gastropoda: Prosobranchia). Umweltwissenschaften und Schadstoff-Forschung—Zeitschrift für Umweltchemie und Ökotoxikologie 13, 1–15.

Schulte-Oehlmann, U., Duft, M., Tillmann, M., Markert, B., Stachel, B., Reincke, H., 2001a. Biologisches Effektmonitoring an Sedimenten der Elbe mit *Potamopyrgus antipodarum* und *Hinia (Nassarius) reticulata* (Gastropoda: Prosobranchia). Report Arbeitsgemeinschaft fuer die Reinhaltung der Elbe.

Seibert, H., 1996. Disorder of development and function of the male reproduction system. Umweltwissenschaften und Schadstoff-Forschung—Zeitschrift fuer Umweltchemie und Ökotoxikologie 8, 275–284.

Simon, M., 1993. Die Elbe und ihr Einzugsgebiet. Wasserwirtschaft-Wassertechnik 7, 16–23.

Spengler, P., Körner, W., Metzger, J.W., 2001. Substances with estrogenic activity in effluents of sewage treatment plants in South-Western Germany. 1. Chemical analysis. Environmental Toxicology and Chemistry 20, 2133–2141.

Stachel, B., Elsholz, O., Reincke, H., 1995. Investigation on sample pretreatment for the determination of selected metals and organochlorine compounds in suspended particulate matter of the River Elbe. Fresenius Journal of Analytical Chemistry 353, 21–27.

Ternes, T.A., Stumpf, M., Mueller, J., Haberer, K., Wilken, R.D., Servos, M., 1999. 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.

Tillmann, M., Schulte-Oehlmann, U., Duft, M., Markert, B., Oehlmann, J., 2001. Effects of endocrine disrupters on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part III: cyproterone Acetate and Vinclozolin as antiandrogens. Ecotoxicology 10, 373–388.

Theobald, N., Meyer, C., 2001. Optimierung und Validierung einer GC–MS-Analysenmethode zur Bestimmung alkylierter Phenole und Bisphenol A in Meerwasser und Schwebstoffen. Umweltforschungsplan des Bundesministers fuer Umwelt, Naturschutz und Reaktorsicherheit—Bund/laender-Messprogramm Nord- und Ostsee. Umweltbundesamt Berlin, Forschungsbericht FKZ 2999 22 281.

Umweltbehoerde Hamburg, 2000. Hamburger Umweltberichte 59/2000.