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Retrospective Monitoring of Alkylphenols and Alkylphenol Monoethoxylates in Aquatic Biota from 1985 to 2001: Results from the German Environmental Specimen Bank

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Breams (*Abramis brama*) and zebra mussels (*Dreissena polymorpha*) from freshwater, and common mussels (*Mytilus edulis*) from marine ecosystems, archived in the German Environmental Specimen Bank were analyzed for the presence of 4-nonylphenol (NP), 4-tert-octylphenol (OP), nonylphenol monoethoxylate (NP1EO), and octylphenol monoethoxylate (OP1EO). The samples were collected in the German rivers Elbe, Rhine, and Saar, and in Lake Belau between 1992 and 2001, as well as in the North Sea and Baltic Sea between 1985 and 2001. The main purpose of the study was to investigate the effectiveness of imposed reduction measures regarding the use of alkylphenol ethoxylates. NP1EO and OP were detected in all breams. NP was predominantly above the limit of quantification (LOQ, 2 ng/g; all data on a wet weight basis), and OP1EO was mostly below the LOQ (0.2 ng/g). Maximal concentrations of 112 ng/g NP, 259 ng/g NP1EO, 5.5 ng/g OP, and 2.6 ng/g OP1EO were found in Saar breams from 1994. NP was detected in all zebra mussels from the river Elbe (up to 41 ng/g), whereas in rather few samples OP and NP1EO were found at low levels. OP1EO was not detected in any sample. Concentrations in mussels and breams from the reference site Lake Belau were below the LOQ for all compounds. In marine biota NP was found until 1997 with maximum concentrations up to 9.7 ng/g, whereas NP1EO was detected at levels between 1.7 and 12.9 ng/g in very few samples collected at the end of the 1980s. A tendency of the concentrations to decrease was obvious for all sampling sites; it was most pronounced for NP1EO and NP after 1996/1997. The effectiveness of the reduction measures is most evident at the Saar sampling site Güdingen and the North Sea sampling site Eckwarderhörne.

Introduction

The German Environmental Specimen Bank (ESB, www.umweltprobenbank.de) is a valuable tool for the retrospective monitoring of environmental pollutants, as recently demonstrated for organotin compounds. Analyses of marine organisms from the North Sea and Baltic Sea revealed that tributyltin concentrations remained constant, whereas triphenyltin levels decreased over a 15-year-period (*1*).

Here, we report the analysis of concentrations of alkylphenols (AP) and alkylphenol ethoxylates (APEO) in biota sampled in marine and freshwater ecosystems between 1985 and 2001. Because most environmental monitoring programs focus on water concentrations, until now rather few data are available on AP/APEO tissue concentrations of aquatic organisms. Because of their principal use as detergents, APEO are released via wastewater to sewage treatment plants (STPs) where they are degraded by a mechanism involving the stepwise loss of ethoxy groups to form shorter APEO homologues and the respective AP. In surface waters APEO and AP have been detected frequently in the lower μg/L range with peak levels up to 644 μg/L (*2*). These compounds are known to be very toxic to aquatic wildlife and exhibit estrogenic activity at concentrations of a few μg/L (*3*−*7*). In the Risk Assessment Report of the European Union (EU) on NP (*8*) the predicted no-effect concentration (PNEC) to protect aquatic freshwater organisms was derived to be 0.33 μg/L, and in the UK Risk Assessment Draft Report on OP the PNEC is discussed to be 0.12 μg/L (*9*).

The physicochemical properties of the main APEO metabolites AP1EO and AP, in particular their octanol−water-partition coefficients ($\log K_{ow}$) ranging between 4.10 and 4.48 (*2*), indicate that they tend to bioaccumulate in aquatic organisms (*10*). The partitioning coefficients of OP, OP1EO, NP, and NP1EO for water/particulate matter were 5.52, 6.02, 5.85, and 5.6−6.4, respectively, (*11, 12*) revealing that the substances will adsorb strongly to suspended particulate matter, and finally to sediments, resulting in a significant reservoir in the aquatic environment. AP are slowly biodegradable under aerobic conditions, especially in the presence of adapted microorganisms. They are considered as inherently biodegradable, and a half-life for biodegradation in surface water of 150 d has been estimated for NP (*8*). However, they are relatively persistent under the anoxic conditions found in sediments (*8*).

In the EU, 30% of the nonylphenol ethoxylates (NPEO) and their derivatives are used for industrial cleaners (*8, 13*). Out of the 118 000 t of NPEO manufactured in Europe in 1997, around 55 000 t were produced and 12 500 t were processed in Germany (*8, 14*). After deduction of exports, 6800 t remained in Germany, of which 1255 t could be attributed to wastewater-relevant use such as industrial cleaners, leather and textile auxiliaries, flocculating agents, and others (*14*). The common octylphenol ethoxylates (OPEO) are assumed to make up 15−20% of the total APEO production (*15*). However, only 12% of the OP production in Europe is used for the production of OPEO (*13*). For OPEO, detailed information comparable to the data on NPEO is not available.

In Germany, a voluntary agreement by manufacturers of household detergents on renunciation of APEO has existed since 1986. Industrial cleansing agents which are subject to the German Washing and Cleansing Agents Act (WRMG) (*16*) were added to this agreement in 1992. As a result, the use of APEO in detergents was reduced by nearly 85% from 1985 to 1997 (*14*). Switzerland also has banned the use of NPEO in textile washing agents since 1986, and there was a voluntary renouncement of the producers on the use of NPEO in domestic cleaners (*17*).

A time limit for the substitution of APEO-containing flocculating agents in STPs was voluntarily set by the European manufacturers at the end of 2001 (*18*). A voluntary

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TABLE 1. Sampling Areas and Sample Organisms of the Environmental Specimen Bank Analyzed in this Study.

organism; sampled tissue	functional/ trophic level	sampling	sampled material (per sampling)	sampling areas	sampling sites
Mytilus edulis (common mussel); soft body without shell	consumer (primary; filter feeder)	pooled samples from 6 samplings per year (every 2 months) for the North Sea pooled from 2 samplings per year (every 6 months) for the Baltic Sea	approximately 2500 mussels	North Sea I (Jadebay) North Sea II (near Sylt) Baltic Sea	Eckwarderhörne List Königshafen (until 1992) or List, south of harbor (since 1992) Darsser Ort
Dreissena polymorpha (zebra mussel); soft body without shell	consumer (primary; filter feeder)	sampled between mid-September and late November, after spawning	approximately 3−6 kg	Elbe Bornhöved Lake District	Blankenese Lake Belau
Abramis brama (bream); muscle tissue	consumer (secondary)	sampled between mid-July and mid-October, after spawning	approximately 20−40 fish, aged 8−12 years	Elbe Rhine Saar Bornhöved Lake District	Prossen, Barby Blankenese Weil, Iffezheim, Koblenz, Bimmen Güdingen, Rehlingen Lake Belau

ban on the use of NPEO in domestic detergents was agreed upon by all major European manufacturers of detergents. Recommendation 92/8 of the International Paris Commission (PARCOM) for the prevention of pollution in the maritime area of the North East Atlantic required signatory countries to achieve the phase-out of NPEO in domestic detergents by 1995 and in all detergent applications by 2000 (*19*).

Keeping all voluntary agreements should have resulted in a decrease of the APEO and AP burden of the aquatic environment and should be detectable by retrospective monitoring. It was therefore the main objective of our study to investigate whether imposed reduction measures resulted in decreased levels of AP and APEO. A further objective was to assess whether the detected levels of AP compounds are of ecotoxicological relevance for exposed organisms.

Experimental Section

Sampling of Environmental Specimen Bank (ESB) Material. Sampling and treatment of ESB samples prior to their being archived is described in ref *1*. The sample material, representing different trophic levels of terrestrial, freshwater, and marine ecosystems, is stored as sub-samples of approximately 10 g each at temperatures below −150 °C in an inert atmosphere resulting from evaporating liquid nitrogen which is used as coolant. All procedures are performed according to ESB standard operating procedures (*20*). The applied methods were described previously (*21*−*24*).

Table 1 lists the organisms and the respective ESB sampling areas analyzed in this study. The marine sampling areas are located either in or adjacent to national parks or biosphere reserves (*1*). Only the North Sea site Eckwarderhörne is influenced by larger anthropogenic activities. Maps of the marine sampling sites are shown in ref *1*.

Sampling areas for freshwater ecosystems are the rivers Rhine, Elbe, and Saar, and the Lake Belau (Figure 1). The Rhine drains a catchment area of about 225 000 km^2 along a course of 1300 km, and about 50 million people are living in this region. Since 1995, routine sampling for the ESB has been conducted at the following sampling sites: Weil (upper Rhine valley; km 174), Iffezheim (km 334), Koblenz (km 590, upstream of the confluence with the river Moselle), Bimmen (km 865, at the German−Dutch border).

The Elbe drains a catchment area of 148 268 km^2 on its 1100 km course from the source in the Sudeten Mountains to the estuary at Cuxhaven (North Sea). A length of 728 km and 97 119 km^2 of the catchment area are on German territory.

FIGURE 1. Sampling sites of the freshwater (circles) and marine (triangles) ecosystems. For details of the marine sampling sites see ref *1*.

Since 1994 routine sampling for the ESB has been performed at the sampling sites Prossen (near the German−Czech border; km 13), Zehren (km 96, not analyzed here), Barby (km 296, downstream of the confluence with the rivers Saale and Mulde), Cumlosen (km 470, not analyzed here), and Blankenese (km 634, near Hamburg harbor). In the Saarland conurbation, biota samples from the river Saar are taken at the sites Güdingen (near the German−French border, km 54) and Rehlingen (km 91). This 600 km^2 region has a population density of 1119 inhabitants/km^2. In the Bornhöved Lake District, an agrarian ecosystem in the northern part of Germany, the Lake Belau has been part of the ESB program since 1997. The lake does not receive any discharges from STPs.

Analytical Methods. The following standards were used: 4-tert-octylphenol (OP, CAS 140-66-9), 4-tert-octylphenol monoethoxylate (OP1EO, no CAS no.), 4-nonylphenol (NP branched, CAS 84852-15-3), and 4-nonylphenol monoethoxylate (NP1EO, no CAS no. assigned for this mixture). Because no adequate deuterated standards were available at the time the method development started, unlabeled 4-*n*-nonylphenol (4nNP, CAS 104-40-5) and 4-*n*-nonylphenol monoethoxylate (4nNP1EO, CAS 104-35-8) which are not present in technical isomer mixtures were used as internal standards (IS). All standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg).

Biota samples (common mussel, zebra mussel, and bream muscle) were mixed in a ratio of 1:6 (W/W) with 1−5 g of anhydrous sodium sulfate and ground in an agate stone mortar. After adding the IS, samples were extracted using an accelerated solvent extraction (ASE) system from DIONEX GmbH (Idstein). A glass fiber filter, 1 g of sodium sulfate, and the homogenized biota/sodium sulfate mixture were packed into an ASE extraction cell of appropriate size. Extractions (2 cycles) were performed with cyclohexane/ethyl acetate (95:5, V/V) at 100 °C and 14 MPa with a preheat equilibration of 5 min and a static extraction time of 10 min. The extract (approximately 35 mL for a 33-mL cell) was dried by shaking it with 1 g of sodium sulfate, and concentrated in a gentle stream of nitrogen to 1 mL. After dilution with an appropriate amount of solvents to yield approximately 2 mL of a mixture of dichloromethane/cyclohexane of 1:1 (V/V), it was cleaned up by gel permeation chromatography (GPC) on 10-mm diameter columns filled up to a height of 400 mm with Bio-Beads S-X3 (Bio-Rad GmbH, Munich) in an automatic GPC system (CleanUp XL, from Gilson, Bad Camberg). Elution was performed at room temperature with dichloromethane/cyclohexane (1:1, V/V) at a flow rate of 1.5 mL/min. The 13−18-min fraction was collected and concentrated to approximately 0.2 mL. After dilution with dichloromethane/ethyl acetate (1:1, V/V) a second GPC chromatography was performed under the same conditions as described above but with dichloromethane/ethyl acetate (1:1, V/V) as eluent. The 12−18-min fraction was evaporated to 0.1 mL, and after addition of 1 mL cyclohexane was again concentrated to 0.2 mL. The second GPC step was not necessary for bream muscle samples. Afterward, a solid phase extraction (SPE) on BakerBond Amino (from Mallinckrodt Baker, Griesheim) with a SPE vacuum workstation spe-12G (Mallinckrodt Baker) was conducted. SPE columns were filled manually with 0.5 g of pre-cleaned BakerBond Amino. After flushing the SPE column with 10 mL of *n*-hexane/2-propanol (1:1, V/V), and conditioning it with 10 mL of *n*-hexane, the sample was injected and rinsed with 10 mL of *n*-hexane. The analyte fraction was eluted with 12 mL of *n*-hexane/2-propanol (95:5, V/V) and concentrated to approximately 0.3 mL, transferred into a GC micro vial, and evaporated to dryness with a gentle stream of nitrogen. Analytes were derivatized with 50 μL of MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoracetamide, CAS 24589-78-4; from Sigma-Aldrich Chemie GmbH, Steinheim) by heating the closed vial up to 70 °C for 10 min. The formed trimethylsilyl ethers were analyzed on a nonpolar capillary column (SGE HT8; 25 m × 0.22 mm; 0.25 μm film) with a MAGNUM ion trap GC/MS/MS system (Finnigan-MAT/Varian SATURN 4D with SATURN GC/MS Version 5.2 software; from Finnigan-MAT, Bremen). GC conditions are given in Table S1 in the Supporting Information. Isomer mixtures of NP and NP1EO were separated into approximately 10 individual peaks with different mass spectra. For the MS/MS process different precursor ions and dissociation parameters (Table S2 in Supporting Information) were selected as a compromise between sensitivity and retaining of the fingerprint of the isomer mixtures. MS conditions were as follows: EI ionization with 70 eV, filament current 70 μA, AGC−PIT 1500 μs, AGC target 5000, multiplier offset +300 V, manifold temperature 200 °C, mass defect 0 mmu/100 amu.

The internal standard method was applied for quantification with 4nNP and 4nNP1EO as IS. For NP and NP1EO all isomer peaks within appropriate retention time windows of approximately 1 min, respectively, were recorded, and the signals of substance-specific product ions (typical mass fragments) were integrated. Representative chromatograms from the NP analysis and a mass spectrum of a typical NP isomer are provided as Supporting Information (Figure S1).

Analyses were performed in 2002, except for the following samples, which were analyzed in 1999: bream muscles from the Saar 1992, 1994, 1995, 1997, 1998; common mussel from Eckwarderhörne 1985, 1986, 1988, 1992−1995; List 1986, 1988, 1992−1995; and Darsser Ort 1992−1995.

Quality Assurance. Under the applied storage conditions (temperatures below −150 °C and exclusion of oxygen in the inert atmosphere from evaporating nitrogen) the analytes are assumed to be stable over decades.

AP and APEO are ubiquitously used chemicals. Traces were found in nearly all of the used chemicals and solvents. To minimize contamination during sample preparation and to achieve low blank values, the following steps were carried out. Glassware, including the SPE columns, was cleaned carefully, heated to 250 °C for at least 24 h, and rinsed with the solvents applied later on. standard ASE cellulose filters were replaced by glass-fiber filters. ASE cells and glass-fiber filters were heated to 250 °C for at least 24 h. The purity of MSTFA was checked routinely and replaced if necessary. Crimp caps and ASE seals were heated to 70 °C for at least 48 h under a reduced pressure of 50 mbar. SPE columns were rinsed with 2-propanol several times in an ultrasonic bath and afterward dried in a drying chamber. All concentration steps were done under a gentle stream of nitrogen or were carried out in an especially cleaned rotary evaporator.

To identify possible contamination problems within each sample batch, a blank sample (i.e., only sodium sulfate) was analyzed. Despite the described pretreatment, blank values for the analytes were above the limit of quantification (LOQ, calculated according to the respective German standard; *25*). Consequently the LOQ were influenced by the measured blank values. The LOQ was 0.2 ng/g for OP, 0.2 ng/g for OP1EO, 2.0 ng/g for NP, and 1.5 ng/g for NP1EO.

The method was checked for systematical errors with standard addition experiments. The results of basic calibration experiments (only pure standards analyzed) were compared to a calibration procedure obtained by executing the whole method with matrix spiked biota samples. On the basis of these experiments mean recovery rates between 76 and 138% were calculated. Method validation data including magnitude of blank contamination, recoveries in different matrixes, and coefficients of variation of the calibration lines are provided in Table S3 in the Supporting Information.

Results

Freshwater Ecosystems. In the freshwater ecosystems OP was detected in all investigated muscle tissues of breams gathered from the rivers Elbe, Rhine, and Saar. NP1EO concentrations were also above the LOQ in these breams except for the fish from the Rhine sampling site Koblenz. NP was not detected in all samples, due to its higher LOQ of 2.0 ng/g. In contrast to the breams from the Saar, OP1EO was mostly below the LOQ of 0.2 ng/g in breams from Elbe and Rhine. The highest concentrations were found in the samples from the Saar, followed in decreasing order by biota from Rhine and Elbe. Concentrations of all compounds in breams from the reference area, Lake Belau, were <LOQ.

Regarding the Saar, generally higher NP and NP1EO concentrations were detected at the sampling site Güdingen,

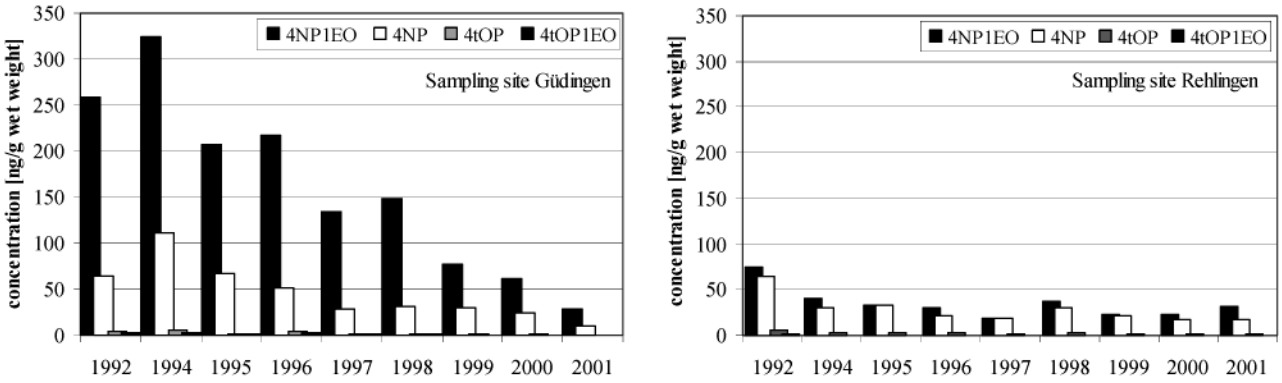

FIGURE 2. Alkylphenols in muscle tissue of breams from the river Saar. No data for 1993. No bar shown: concentrations below the LOQ.

TABLE 2. Alkylphenolic Compounds in Bream Muscles from the River Rhine [ng/g Wet Weight]

year	OP	NP	OP1EO	NP1EO	OP	NP	OP1EO	NP1EO
	Weil				Iffezheim			
1995	0.6	11.0	< 0.2	29.3	0.7	9.3	< 0.2	29.4
1996	0.6	15.3	< 0.2	34.3	1.1	8.7	0.2	24.7
1997	0.5	11.7	< 0.2	34.9	0.6	4.3	< 0.2	15.8
1999	0.3	6.3	< 0.2	15.1	0.4	6.2	< 0.2	15.0
2000	0.4	6.0	< 0.2	11.3	0.4	5.5	< 0.2	14.0
2001	0.6	7.4	< 0.2	17.6	0.5	4.2	< 0.2	6.3
	Koblenz				Bimmen			
1995	0.6	5.0	< 0.2	< 1.5	0.4	< 2.0	< 0.2	1.9
1996	0.3	3.2	< 0.2	< 1.5	0.3	< 2.0	< 0.2	2.1
1997	0.4	4.2	< 0.2	< 1.5	0.2	< 2.0	< 0.2	2.5
1999	0.3	< 2.0	< 0.2	< 1.5	0.3	2.1	< 0.2	2.5
2000	0.4	2.8	< 0.2	< 1.5	0.3	< 2.0	< 0.2	3.4
2001	0.4	3.4	< 0.2	< 1.5	0.3	< 2.0	< 0.2	2.3

located between the French border and the city of Saarbrücken, compared with the downstream site Rehlingen, except for samples collected during 2001. There were only slight differences in the relatively low OP levels between samples from Rehlingen and Güdingen (Figure 2; for detailed data refer to Table S4, Supporting Information). The concentration gradient of the more persistent NP was less pronounced than that of NP1EO along the river during the 1990s.

In the observed period, the highest NP and NP1EO concentrations were found between 1992 and 1996 with peak levels in 1994 at Güdingen. After 1996, there was a moderate continuous decrease of the levels with a steep decline from 1998 to 1999. OP1EO and OP contents seem to follow the same trend at concentrations of a few ng/g. At Rehlingen, the highest decline in alkylphenol concentrations could be observed between 1992 and 1994, followed by constant levels until 2001.

Along the Rhine, NP was detected in nearly all samples at concentrations >LOQ except for the most downstream sampling site Bimmen near the Dutch border (Table 2). NP1EO could be found >LOQ in all breams with the exception of the fish sampled at Koblenz. Along the course of the Rhine the NP1EO and NP levels in bream muscles decreased, with largest gradients between 1995 and 1997. Considering the NP and NP1EO tissue levels above the LOQ, a decreasing trend could be observed especially at the sampling sites Weil and Iffezheim during the examined period. OP was detected in breams from all sampling sites at very low concentrations of maximum 1 ng/g, whereas OP1EO concentrations were mostly <LOQ.

In samples from the Elbe the tissue concentrations of breams are roughly comparable with the values found in the samples from the middle and lower course of the Rhine (Table 3).

Because the concentrations of the analyzed compounds were very low, no time-dependent trend could be identified at the sampling sites Prossen and Blankenese. Clearly decreasing concentrations during the sampling period could only be observed at Barby, located in the middle course of the Elbe, downstream from the mouth of the tributaries Saale and Mulde. As observed in samples from the Rhine, the exposure to NP and NP1EO clearly declined after 1997 and decreased below or near the LOQ during the following years. In samples from Blankenese, the concentrations of the alkylphenol compounds varied at low levels of a few ng/g with peak values in 1995. OP1EO was below the LOQ, whereas OP levels were constantly close to the LOQ, except for the sampling site Barby in 1993.

Along the Elbe, NP1EO and NP contents of the bream tissues clearly increased from Prossen to Barby, the inflow region of the river Saale, between 1993 and 1997, and declined again to relatively low levels at Blankenese. Generally, the exposure to alkylphenol compounds has been relatively constant at this low level at all sampling sites since 1999.

In addition to bream, zebra mussels from the sampling site Blankenese collected between 1995 and 2000, and zebra mussels from the reference site Lake Belau sampled between 1990 and 1999, were analyzed (see Table S5, Supporting Information). All compounds investigated were below the LOQ in the samples from the reference area. Compared to the Elbe breams, the NP content of the zebra mussels from Blankenese was clearly higher, ranging from 3.7 to 41.2 ng/g NP with a peak concentration in 1997. NP1EO was only >LOQ in two samples at a comparable level of 2.2 and 2.8 ng/g in 1996 and 1997, respectively. OP1EO could not be detected in the zebra mussels, and OP was only present at concentrations around 0.4 ng/g in samples from 1995 to 1997.

Marine Ecosystems. For the monitoring of marine ecosystems, common mussels (*Mytilus edulis*) from the North Sea and Baltic Sea were analyzed (Figure 3; for detailed data refer to Table S6, Supporting Information). NP was detected in all samples until 1997, with the highest value (9.7 ng/g) at Eckwarderhörne in 1985, whereas NP1EO was only found in very few samples between 1.7 and 12.9 ng/g, especially in samples from Eckwarderhörne collected at the end of the 1980s. Almost all detected NP1EO concentrations were lower than the respective NP concentrations, which is in contrast to the freshwater samples. The concentrations of OP1EO were below the LOQ in all samples; OP could only be determined in some samples in the range of the LOQ. In mussels from Eckwarderhörne NP1EO concentrations declined from 1985 to 1988 and have been below the LOQ since 1990 comparable to the other sampling sites. For the more persistent NP, a temporally delayed decrease to levels at or below the LOQ could be observed with a plateau from 1990 to 1995. The low influence of human activities on the North Sea biosphere reserve (List) and the Baltic Sea National Park (Darsser Ort)

TABLE 3. Alkylphenolic Compounds in Bream Muscles from the River Elbe [ng/g Wet Weight]

year	OP	NP	OP 1EO	NP 1EO	OP	NP	OP 1EO	NP 1EO	OP	NP	OP 1EO	NP 1EO
	Prossen				Barby				Blankenese			
1993	0.4	2.8	< 0.2	3.6	1.4	13.3	0.4	17.6	0.3	3.6	< 0.2	3.5
1995	0.3	< 2	< 0.2	2.2	0.3	6.4	< 0.2	12.3	0.2	5.6	< 0.2	4.0
1996	0.3	3.1	< 0.2	3.3	0.3	6.4	< 0.2	5.8	0.3	2.1	< 0.2	1.5
1997	0.2	< 2	< 0.2	3.1	0.3	9.1	< 0.2	9.2	0.2	3.1	< 0.2	2.2
1999	0.2	< 2	< 0.2	2.8	0.2	3.4	< 0.2	2.5	0.3	4.7	< 0.2	2.1
2000	0.2	2.3	< 0.2	10.7	0.2	2.0	< 0.2	3.3	0.2	2.4	< 0.2	1.9
2001	0.3	2.7	< 0.2	3.8	0.2	< 2	< 0.2	1.8	0.3	3.0	< 0.2	2.3

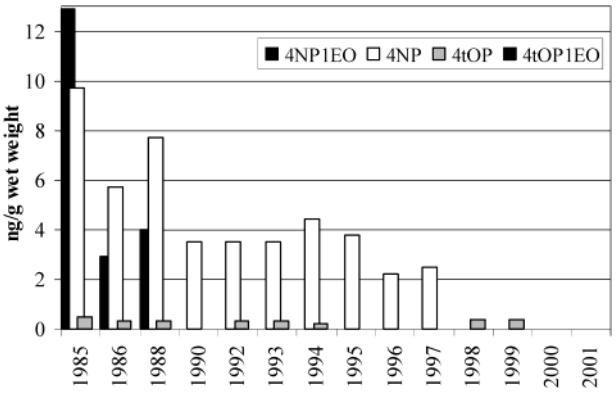

FIGURE 3. Alkylphenols in soft tissue of common mussel from the North Sea, sampling site Eckwarderhörne. No data for 1987, 1989, and 1991. No bar shown: concentrations below the LOQ.

compared to Eckwarderhörne is documented by the NP1EO concentrations <LOQ in mussels of these sampling sites, except in one year each.

Discussion

Monitoring Results. Using the archived biota samples of the German Environmental Specimen Bank, temporal changes and regional differences in the exposure of aquatic organisms to alkylphenols during the past decade could be identified. The data reflect the efficiency of the different voluntary agreements and restrictions on the phase-out of APEO in domestic and industrial washing and cleansing agents. The tissue concentrations of the breams reflect more or less the actual exposure concentrations, as 95% of the steady-state concentration is reached after 175 h and 50% elimination is expected after 40 h. These values were calculated using the log K_{ow} of 4.48 according to OECD Guideline 305 (*26*) and are in agreement with reported half-lives for NP in fish muscles between 24 and 99 h (*27, 28*)

Alkylphenol exposure in the rivers was highest in the Saar followed in decreasing order by the Rhine and Elbe. The differences between the rivers can be attributed mainly to the structure of the catchment areas. The Saar region is characterized by high population density, and the sampling site Güdingen, which showed the highest exposure concentrations during the 1990s, is strongly influenced by STPs effluents from France, where no early ban on the use of NPEO in domestic detergents was implemented as agreed upon in Germany in the mid 1980s. Because of this ban, the AP and APEO discharges into the German part of the Saar were constantly lower as indicated by the lower body burden of the breams from Rehlingen (Figure 2). In the Rhine, the early ban of NPEO in Switzerland and Germany resulted in lower alkylphenol concentrations in the biota of the upper Rhine downstream of the Swiss border as compared to those of the upper Saar. In the Elbe, low exposure levels were also observed at the German−Czech border, probably indicating minor influence of STPs. The contributions of STP effluents' rich tributaries to the alkylphenol load of the Elbe is documented by the increased concentration at the sampling site Barby especially up to 1997.

Along the rivers there was a more or less pronounced decrease in the body burden of the breams. Generally, where high levels were detected at upstream sites, a decreasing concentration trend could be observed downstream. This is probably the result of dilution by elevated water flow due to the inflow of tributaries in combination with biodegradation as well as adsorption to particulate matter. Partition into sediment and aerobic biodegradation are important removal processes for NP1EO and NP (*11, 12*), but they are of different relevance. Adsorption should be of similar importance as the compounds have comparable log K_{oc} values (see Introduction), whereas NP1EO can be more easily biodegraded than NP. Where a reduction of NP1EO can be observed along the river the decrease of the more persistent NP was less pronounced resulting in a decreasing concentration ratio of NP1EO/NP over time. Therefore, it can be assumed that a decreasing NP1EO/NP ratio along the river indicates that there were no additional discharges of APEO between the respective sampling sites, and that an observed decline of the NP level can mainly be attributed to dilution. If the decrease of both NP1EO and NP is comparable, removal by adsorption seems to play a significant role. From the above, it could be concluded that no significant discharges of NP1EO had occurred between the Rhine sampling sites Iffezheim and Koblenz.

Although the Rhine crosses a region with a high population density between the sampling sites Koblenz and Bimmen, only low levels of the discharged NP1EO could be found and NP concentrations were further decreased <LOQ, probably due to biodegradation of NP1EO as well as overall dilution and adsorption processes. In the Elbe, the NP1EO concentrations in breams revealed minor variations along the river after 1997 indicating a balanced ratio between APEO input and removal as well as dilution processes at these sampling sites. The generally higher NP concentrations found in zebra mussels compared to fish (Table S6, Supporting Information) are consistent with findings from Ekelund et al. (*29*) reporting higher bioaccumulation for mussels as compared to fish, possibly caused by different feeding behaviors. Because of NP adsorption to suspended solids, an elevated exposure of filter-feeding zebra mussels is expected in contrast to fish, as mussels filter out large quantities of particles using their enlarged gills and completely subject the particles to digestion, whereas breams feed on small animals.

Regarding OP and OP1EO concentrations no great variations were observed during the observed period, indicating no significant changes in the consumption figures of these compounds.

In the marine environment exposure to alkylphenols was also detected but at lower concentrations than in the rivers. This especially applies for the end of the 1980s and the first half of the 1990s. Because of the further degradation of AP1EO and the reduced discharge of APEO into marine water, there is a higher exposure to OP and NP relative to the AP1EO as compared to the freshwater ecosystems. The body burden

TABLE 4. Calculation of Water Concentrations Based on Maximal and Latest Maximal Tissue Concentrations Found in this Study and Published Bioconcentration Factors (BCF)[a]

sampling area	organism	year	NP (ng/g wet weight)	extrapolated water NP concn. (ng/L)	OP (ng/g wet weight)	extrapolated water OP concn. (ng/L)
Saar	bream	1994	112	996−1273	5.5	55−229
		2001	9.8	84−111	0.65	6−27
Rhine	bream	1996	15.3	132−174	0.64	6−27
		2001	7.4	64−84	0.55	5−23
Elbe	bream	1993	13.3	115−151	1.4	14−58
		2001	3.0	26−34	0.33	3−14

[a] BCFs (L/kg): for NP in fish muscle 88−116 (*41*), for OP in fish muscle 24 (*30*) − 101 (*31*).

TABLE 5. Concentrations of AP and APEO in Surface Water [ng/L][a]

sampling area	year	NP	NP1EO	OP	OP1EO	ref.
Germany, river Elbe	1998 and 2000	10−53		0.4−3.3		*11, 42*
Germany, rivers	2000−2002	67−485		0.8−270		*11, 43, 44*
Germany, river influenced by STP	1998	2720	10−3270	0.4−270		*45*
Austria, rivers	2001	<LOQ−810	<LOQ−170	<LOQ−40	<LOQ−2	*46*
Belgium, rivers	1999	<LOQ	<LOQ−2450	<LOQ	<LOQ	*47*
The Netherlands, rivers	1999−2002	<LOQ−4100	<LOQ−2600[b]	<LOQ−600	<LOQ	*48*
United Kingdom, rivers	1998	<LOQ−30,000	<LOQ−46,000[b]			*49*
Spain, rivers	1999	<LOQ−51	<LOQ			*50*
Spain, river influenced by STP	1999	644,000	up to 100,000[b]			*50*
Turkey	2001		<LOQ			*51*
USA	1998−2000	<LOQ−95,000	<LOQ−330	<LOQ − 7		*2*
Taiwan		1800−10,000	2800−25,700[b]			*2*

[a] Examples from monitoring studies. Blank entries indicate no data available. [b] Sum of NPEO.

of the mussels is influenced by human activities. Of all the sampling sites, Eckwarderhörne, which is considerably influenced by anthropogenic activities (*1*), showed highest exposure in the 1980s. However, between 1998 and 2001, NP could be detected only at concentrations close to the LOQ in samples from List, where the sampling site is near a residential area and a marina. In mussels from the Baltic Sea National Park none of the compounds could be found. The concentration trend observed for mussels from the sampling site Eckwarderhörne documents very well the decrease of the NP burden as a result of the effectiveness of (i) the first German voluntary agreement on the ban of NPEOs in household detergents from 1986, (ii) the inclusion of industrial cleaners in the ban in 1992, and (iii) the respective Europe-wide agreements from 1995 (Figure 3).

In Germany, a further but probably less pronounced reduction of the NP burden can be expected for the time period after the year 2000 due to the consequences of the PARCOM agreement (*19*). It is assumed that the decrease will be most apparent at locations where industrial cleaners and flocculating agents so far have been the main source for the pollution of the aquatic environment. However, as APEO compounds are also used in applications other than detergents, they are expected to be present in the environment further on.

Relation between Body Burden and Effect. Water-borne alkylphenols are rapidly conjugated and eliminated via the liver/bile route in fish, whereas the parent compounds can accumulate in a variety of other fish tissues (*30, 31*). NP tissue concentrations between 5 and 55 ng/g wet weight were reported to induce vitellogenin in male flounder (*32*). Therefore, estrogenic exposure indicated by vitellogenin induction may have occurred in the monitored rivers causing a disturbance of reproduction processes (*5, 33−37*), mainly as a result of NP exposure (*38*). As only few data are available on a correlation between effects in organisms and body loads, which would allow an assessment of the ecotoxicological relevance of the body burdens, more information is needed. Therefore, a project is being initiated in the frame of the ESB monitoring which aims at the concomitant determination of bream plasma vitellogenin and tissue contaminants.

Extrapolation from Tissue Concentration to Exposure Level. To assess whether exposure concentrations may have caused an effect, an indirect approach can be used as well. For this purpose, the water concentrations are extrapolated using the tissue concentrations of the organisms together with the respective bioconcentration factors for bream muscle tissue (Table 4). Up to 1999, an impact on aquatic organisms in the Saar due to NP concentrations exceeding the PNEC of 330 ng/L (*8*) can be postulated. Concerning fish populations, especially at the beginning of the 1990s, an impact on the performance of the populations cannot be excluded due to the small safety factor of partly <10 between the extrapolated water concentrations and the reported NOEC values for effects on reproduction between 1 and 10 μg/L (*7, 8, 35, 39*). In the other rivers, the extrapolated NP water concentrations were below the PNEC value. Because of the small amounts released into the environment, OP concentrations in the rivers were far below the proposed PNEC for aquatic organisms of 0.12 μg/L (*9*) except for the river Saar between 1992 and 1994. In 2001, estimated exposure concentrations to NP and OP were below the respective PNEC values in all freshwater sampling sites, and below the current known threshold concentrations for reproductive disorders resulting from a disturbance of the endocrine system (*5, 40*).

However, it has to be considered that NP and OP have the same mode of estrogenic action and consequently will act additively. Therefore, a common quality standard should be developed.

Comparison with Other Monitoring Studies. Water concentrations monitored in the Elbe sampling areas of the ESB study in 1998 and 2000 (Table 5) support the approach of estimating water concentrations by using body burden and BCF values, even though it has to be clearly stated that the approach depends on the quality of the BCF values. Recent monitoring data of several German rivers also revealed

TABLE 6. Concentrations of AP and APEO in Fish Tissue [ng/g Wet Weight][a]

sampling area	year	NP	NP1EO	OP	ref.
Japan	1998−1999	<LOQ−110		<LOQ−6	*53*
Turkey	2001	100−600			*51*
UK, influenced by STP	1994−1995	<LOQ−800	<LOQ−4200[a]	<LOQ	*41*
UK, estuary	1997	5−55	<LOQ	<LOQ	*32*

[a] Examples from monitoring studies. Blank entries indicate no data available. [b] NP1EO + NP2EO.

concentrations comparable to the extrapolated levels of this study. However, in rivers strongly influenced by STP effluents high NP concentrations can occur. From other countries surface water concentrations are reported up to the μg/L range (Table 5).

High NP levels were found in fish from rivers with high fractions of sewage effluents in recent monitoring studies (Table 6). NP levels up to 15 ng/g wet weight in breams from Rhine and Elbe sampled in this study between 1993 and 2001 are comparable with the reported concentrations in fish from a U.K. estuary (*32*). The maximal NP level of 112 ng/g NP in breams from the Saar River in 1994 indicates a relatively high influence of sewage effluents. After 1996, i.e., after the voluntary ban of NPEO in household detergents, NP levels were in the lower ng/g range and suggest lower exposure concentrations in the German sampling areas compared to those of other countries.

A biomarker-directed evaluation of the specific exposure situation to endocrine-disrupting chemicals along the river Elbe detected highest vitellogenin induction in the middle course of the Elbe in 1999, including the site Barby which is characterized by considerable STP influences (*52*). This effect cannot attributed to AP and APEO alone, as the analyzed concentrations in this study in 1999 did not indicate an extremely high alkylphenol load in the area of Barby. Therefore, other highly potent compounds, such as steroidal estrogens released by the STP effluents, may be mainly responsible for the observed effects.

Relevance for Human Consumption. Although the BCF for NP and OP is relatively low in muscle tissue, the compounds accumulate in muscle tissue and may be of importance for exposure of the consumer (*31*). For food consumption, no TDI (tolerable daily intake) values for OP or NP or the respective APEOs are available because of inadequate data (*54*). A provisional TDI value for NP of 0.005 mg/kg body weight was derived by the Danish Institute of Safety and Toxicology (*55*) allowing a daily intake of NP for a 60 kg adult of 30 μg/day. A worst-case assumption based on the consumption of 300 g of fish filet with maximal NP and OP concentrations found in this study for 1994 results in an NP and OP intake of 23.5 μg/day contributing 78% to the provisional TDI. These values decline to around 3.1 μg/day and 11% for fish and 0.8 μg/day and 2.5% for mussels, respectively, in 2001. For comparison, the average daily intake of NPs via mixed food for a German adult was calculated to be 7.5 μg/day in 2000 (*56*) corresponding to 25% of the respective TDI value.

NP amounts determined between 50 and 100 ng/g (*41*) in marine fish intended for human consumption were higher than most of the respective concentrations found in this study. In herring, haddock, and dab sampled around North Sea offshore installations as part of a preliminary UK Food Quality Assurance Monitoring Program, OP concentrations in liver and muscle were below the LOQ, which varied between 100 and 4 ng/g depending on the species and tissue type tested (*57*). However, in the scope of a fish-rich diet where fish of high fat content is consumed, fish can contribute to the daily human exposure to alkylphenols to a considerable extent.

Outlook. This retrospective monitoring study on alkylphenol compounds illustrates the usefulness of the Environmental Specimen Bank as a tool for characterizing temporal changes and regional differences in ecosystem contamination as well as for the persecution of the effectiveness of environmental policy measures. The approach allows the application of today's advanced analytical methods for samples taken in the past. Further retrospective monitoring studies are currently being performed addressing levels of synthetic fragrances and the disinfectant triclosan in aquatic biota.

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Supporting Information Available

Parameters for GC analysis and MS/MS quantification and additional tables referenced in the text (pdf). This material is available free of charge via the Internet at http://pubs.acs.org.

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