Occurrence and behaviour of 4-nonylphenol in river water of Germany

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An analytical method composed of solid-phase-extraction (SPE) followed by gas chromatography-mass spectrometry (GC-MS) has been developed to investigate the occurrence and the behaviour of the endocrine disruptor 4-nonylphenol (4-NP) in river water of Germany. 16 Para-isomers of the complex mixture of 4-NP isomers have been resolved in the SPE-extracts of river water samples. The mass spectra indicate the presence of four distinct groups of isomers. The different groups are characterised by different base peaks of the mass spectra dependent on the substitution of the alpha-carbon on the alkyl chain. The different isomers show (i) alpha-methyl, alpha-ethyl (base peak 149), (ii) alpha-dimethyl (base peak 135), (iii) alpha-methyl, alpha-propyl, alpha-butyl (base peak 121) and (iv) alpha-methyl, alpha-propyl, alpha-butyl (base peak 107) configuration of the alkyl chain. The recovery of the unbranched compound 4-n-NP was $\geqslant 90\%$. Detection limit and determination limit of the whole analytical procedure were 6 ng L^{-1} and 25 ng L^{-1}, respectively with a relative standard deviation of 11.7%. The concentration of the sum of the 4-NP isomers in all samples of river water ranged from 28 ng L^{-1} (Elbe River) to 1220 ng L^{-1} (Oder River). In a period of a higher river water level, a higher amount of 4-NP was detected in the Oder River than in a period of a lower water level. Therefore dilution was not the determinant factor of the 4-NP concentration in river water. The diurnal dynamics of the sewage treatment works (STWs) have to be considered as an influencing factor for the presence of 4-NP in river water.

Introduction

4-Nonylphenol polyethoxylates (NPnEOs) are by far the prevalent subgroup of alkylphenol polyethoxylates (APnEOs) which are widely used as non-ionic surfactants.[1] In 1995, 500,000 metric tonnes of APnEOs were produced world-wide.[2] NPnEOs are synthesised by addition of ethylene oxide to 4-nonylphenol (4-NP) under alkaline conditions.[3] 4-NP is the commercial description for a complex mixture of alkyl-chain-substituted phenols with predominantly nine C-atoms in the carbon chain. This chemical is produced through Friedel–Crafts alkylation of phenol with nonene. Nonene, when exposed to an acid catalyst, forms carbocations that preferentially alkylate at the para-position of phenol.[4] Commercial nonene is not simply a linear C_9H_{18} alpha-olefin; it is a complex mixture of predominantly nine-carbon olefins called propylene trimer. The resultant 4-NP is a complex mixture of isomers which approximately shows the following composition: 3–6% 2-NP, 90–93% 4-NP, and 2–5% decylphenol.

4-NP in the environment originates predominantly from the hydrolysis of NPnEOs. Biodegradation of NPnEOs in sewage treatment plants during anaerobic sludge treatment results in the formation of 4-NP. Discharge of treated waste water is a dominant source of 4-NP in river water.[5] *In vitro* and partly *in vivo* studies have demonstrated an estrogenic activity for 4-NP.[6,7]

Gas chromatographic methods have been successfully applied to resolve 12, 22, and 30 4-NP isomers, respectively in standard solutions.[8–10] These methods have been rarely employed for studies of environmental concern. Previously several methods have been applied for the determination of 4-NP in river water.[11–17] These methods require the use of a high quantity of organic solvents and the necessity of conversion of 4-NP into volatile derivatives before analysis. In most of these studies the quantity of 4-NP in the aquatic environment was not determined by resolving a high amount of isomers. In the present study an improved method based on SPE followed by GC-MS was used for the quantitation of 4-NP isomers in the nanogram per litre range in river water samples from Germany. Furthermore, the Oder River has been sampled at different sites of the river course to study the behaviour of 4-NP downstream a river. The results are compared with data obtained from previous studies to give a broad overview of the trend of the concentrations of 4-NP in river water in Germany during the last two decades.

Experimental section

Chemicals

Standards of a 4-NP isomer mixture (technical grade with branched isomers) and 4-n-NP were received from Dr. Ehrensdorfer (Augsburg, Germany). A stock solution of squalane (2 g L^{-1}) (Dr. Ehrensdorfer, Augsburg, Germany) in n-hexane was used as internal standard (IS). Stock solutions of 4-NP isomer mixture and 4-n-NP at 2 g L^{-1} were prepared in methanol–acetonitrile (1:1) and stored at 4 °C. Working standard mixtures of 4-n-NP were prepared at 2000 ng L^{-1}, 1000 ng L^{-1}, 200 ng L^{-1}, 100 ng L^{-1}, 50 ng L^{-1}, 20 ng L^{-1}, 2 ng L^{-1} and 1 ng L^{-1} and stored at +4 °C for a maximum of one week. All solvents used in this work were purchased at the

Table 1 Grid values of the monitoring stations

	Easting	Northing
Rhein River, Rüsselsheim	3452500	5536000
Elbe River, Hamburg	3554250	5933500
Elbe River, Dresden	5419750	5652000
Main River Frankfurt/Main	3470400	5550450
Oder River, Küstrin	5446125	5854500
Nidda River, Frankfurt/Main	3473100	5556600
Schwarzbach River, Rüsselsheim	3454750	5532600

highest commercially available purity from Merck (Darmstadt, Germany).

Sampling

In Table 1 the grid values of the river water monitoring stations are shown. River water samples were collected from the six German Rivers Rhein, Elbe, Main, Oder, Nidda and Schwarzbach in March 2000, November 2000 and March 2001. 5 L of river water were obtained from the shore of each river. In July 2001, three river water samples (O_1, O_2, and O_3) were taken at intervals of 20 km distance downstream along the Oder River. All river water samples were collected in methanol-precleaned amber glass bottles and stored at 4 °C for less than one week in the dark until analysis. Between river water sampling points O_2 and O_3, the municipal waste water treatment plant (STW) of the administrative district Man-schnow and the STW of a paper mill discharge their treated waste water into the Oder River. The river mouth of the Polish river Warta is located a few kilometres upstream of sampling point O_3. This river introduces a large fraction of organic compounds into the Oder River.

Solid-Phase-Extraction (SPE)

All river water samples were filtered through folded (Ø 185 mm) filters purchased from Schleicher & Schüll (Dassel, Germany). It was possible to extract 20 water samples at the same time with the applied SPE-method. The SPE-cartridges Bond Elut PPL (100 mg mL^{-1} purchased from Varian, USA) were prepared for analysis by rinsing them sequentially with 1 mL of methanol, 1 mL of methanol–acetonitrile (1:1), and again with 1 mL of methanol. The first and the third cleaning step were carried out under atmospheric pressure, whereas the second step was carried out under vacuum conditions. After the washing steps 1 mL of deionised water was added to each cartridge. Subsequently, the river water samples (5 L volume) were applied to the cartridges at a flow rate of approximately 1000 mL h^{-1}. After the extraction procedure the cartridges were dried under a slight nitrogen stream. Each cartridge was eluted three times with 0.333 mL of a mixture of methanol–acetonitrile (1:1). The resulting extracts were collected in several glass vials (1.1 mL). After removing the solvent from the extracts under a stream of nitrogen at room temperature each residue was dissolved again in 100 μL acetonitrile. Before analysis by gas chromatography, 2 μL of the prepared stock solution of the internal standard squalane was added to each extract.

Gas chromatography-mass spectrometry (GC-MS)

GC coupled with MS was used for the trace analysis of 4-NP in the river water extracts. Derivatisation procedures were not necessary. The GC-MS analyses of the extracts were carried out by injection of a 1 μL aliquot of each extract by the auto sampler AS 800 (Fisons Instruments, Milan, Italy) into the injector using the splitless mode. The injector temperature was kept at 280 °C. Separation of the analytes was performed by a gas chromatograph (GC 8000 series, Fisons Instruments, Milan, Italy) using a 30 m FS-Supreme®-5 capillary column (CS, Langerwehe, Germany) with a film thickness of 0.25 μm. Helium served as the carrier gas with a back pressure of 70 kPa. The GC oven temperature was first held at 80 °C for 1 min, then increased to 300 °C at a rate of 4 °C min^{-1} and kept at the final temperature for 20 min.

Mass spectrometric analyses were run on the MD 800 (Fisons Instruments, Milan, Italy) by full-scan acquisition. Data acquisition, processing and instrument control were performed using Masslab software (Fisons Instruments, Milan, Italy). Electron impact (EI) mass spectra were obtained at an electron energy of 70 eV and monitored from 50 to 600 m/z. Concentrations of 4-NP in all water samples were calculated by comparing the sum of the peak areas of all isomer peaks relative to the peak area of the IS squalane.

Blanks were performed on each day of extraction to eliminate laboratory contamination of 4-NP. The eluates of the precleaned cartridges were analysed with GC-MS and 4-NP was not detected in the blanks.

Correction factors c and response factor f

The main problem to determine the exact concentrations of 4-NP in environmental samples is that no available standard mixture of technical 4-NP has a defined composition of isomers. Examination of the spectra obtained from the GC-MS analysis of the river water extracts indicated that four predominating isomer groups of 4-NP were present (Table 2). Identification of each single isomer was done by comparing the resulting mass spectra with the different mass spectra obtained from GC-MS analysis of the technical standard 4-NP and by comparison of the retention times. In groups 1–3a the base peak ions m/z 149, m/z 135, and m/z 121, respectively, and in group 3b the third intense ion m/z 177 were used as quantification ions. Quantification by different mass traces avoided superimposition of single isomer peaks. Therefore a correction factor c has to be introduced which is given by

$$c = A_{TIC}/A_{quantification\ ion} \quad (1)$$

where $A_{(TIC)}$ is the peak area of the isomer peak in the total ion chromatogram, and $A_{quantification\ ion}$ is the peak area of the isomer peak in the equivalent mass trace of the quantification ion. The correction factors c for the different quantification ions were as follows (a) m/z 149, $c = 3.8$, (b) m/z 135, $c = 6.0$, (c) m/z 121, $c = 4.1$, (d) m/z 177, $c = 12.5$.

A standard solution spiked with 2 μg of 4-n-NP and squalane, respectively was analysed by GC-MS to calculate the relative response factor f given by

$$f = A_{4-n-NP}/A_{IS} \quad (2)$$

Table 2 Correction factors c of the four isomer groups of 4-NP

Group no.	No. of isomers	Ion used for quantification	Ions (% relative abunance)	c
1	1–4	Base peak ion: 149	121(>90), 107(>50), 191(>10)	3.8
2	5–11	Base peak ion: 135	107(>25), 121(>5)	6.0
3a	12–14	Base peak ion: 121	163(>36), 177(>15), 149(>3)	4.1
3b	15–16	Fragment ion: 177	121(>90), 177(>45), 149(>40), 163(>25)	12.5

where $A_{4\text{-n-NP}}$ is the peak area of 4-n-NP and A_{IS} is the peak area of squalane. The resulting response factor f was 0.466. To simplify the analytical procedure the response factor for 4-n-NP was used for quantification of all isomers of 4-NP in the river water extracts given by

$$c_{4\text{-NP}} \, [\text{ng L}^{-1}] = c_n \times 4000 \text{ ng L}^{-1} \times 1/f \times A_n/A_{IS} + c_{n+1} \\ \times 4000 \text{ ng L}^{-1} \times 1/f \times A_{n+1}/A_{IS} \quad (3)$$

where $c_{4\text{-NP}}$ is the concentration of 4-NP in the samples, c_{n+1} is the correction factors of each peak group, and n is the number of each peak isomer.

Recovery, reproducibility, detection- and determination limit

The analytical parameters recovery rate, standard deviation, detection limit, and determination limit were calculated by spiking 1 L of 10 different river water samples with the prepared standard solutions of 4-n-NP. All spiked samples were extracted by SPE followed by GC-MS analysis described above. Detection limit and determination limit were calculated as reported in DIN 32 645[18] and DIN 45 000,[19] respectively. The detection limit and the determination limit of 4-n-NP were 6 ng L^{-1} and 25 ng L^{-1}, respectively. As a prerequisite for the calculation of the concentrations of 4-NP in river water samples the absolute amounts of 4-NP had to be above the determination limit. The results of the extraction of the spiked river water samples showed an efficient recovery of $\geqslant 90\%$ at the determination limit. The relative standard deviation of the whole analytical procedure was 11.7%.

Results and discussion

Mass spectra interpretation of 4-NP

Mass spectra interpretation of the different isomers of 4-NP obtained from river water samples was done according to laboratory studies from ref. 9. Fig. 2a–d shows the mass traces m/z 149 (a), m/z 135 (b), m/z 121 (c), and m/z 177 (d) respectively obtained by GC-MS analysis of a water extract from the Oder River. It was possible to separate 16 isomer peaks of 4-NP in water extracts with the applied GC method. A mass spectrum from each resolved isomer peak was obtained. Background scans were subtracted to reduce interference from background fragmentation ions. In respect of the characteristic fragmentation ions and their intensities, the distinct peak groups 1, 2, 3a and 3b could be evaluated. Table 2 summarises associated peak numbers, ions used for quantification, and characteristic fragment ions with their relative abundances. All spectra show the molecular ion m/z 220 at different intensities ($\leqslant 2\%$). The fragmentation ion m/z 107 is also a characteristic ion present in all spectra of the several isomer peaks of 4-NP at abundances $\geqslant 20\%$. Fig. 1 demonstrates the fragmentation process generating the ion m/z 107. The breakdown of the benzene ring is common to all phenolic compounds.[20] Primarily there is a formation of an isopropyl phenol ion (m/z 135) followed by an elimination of CO from the benzene

Fig. 2 Mass traces m/z 149 (a), m/z 135 (b), m/z 121 (c), and m/z 177 (d) of a ground water extract with numbers of isomers.

ring. After the chemical breakdown of the benzene ring elimination of a proton produces the fragment ion m/z 107.

Isomer group 1 (peak nos 1–4). Fig. 2a shows the mass trace m/z 149 with the four 4-NP-isomer peaks 1–4 of peak group 1. The corresponding mass spectrum, chemical structure and fragmentation processes are shown in Fig. 3a. All isomers had the base peak at m/z 149 generated by a loss of C_5H_{11}. Another representative ion is m/z 191 with relative abundance $\geqslant 10\%$. The spectra of these isomers indicate the presence of an alpha-methyl, alpha-ethyl configuration (Fig. 3a). The presence of the ethyl group on the alpha-carbon is responsible for generating the fragment ion m/z 191. The occurrence of m/z 121 at a very high abundance ($\geqslant 90\%$) is suggested to result from a higher
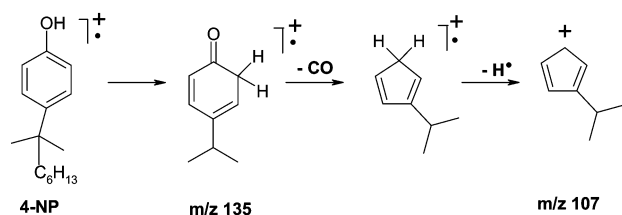

Fig. 1 Formation of m/z 107 ion in 4-NP through elimination of C_6H_{13}, breakdown of the benzene ring, and elimination of CHO (adapted from ref. 20).
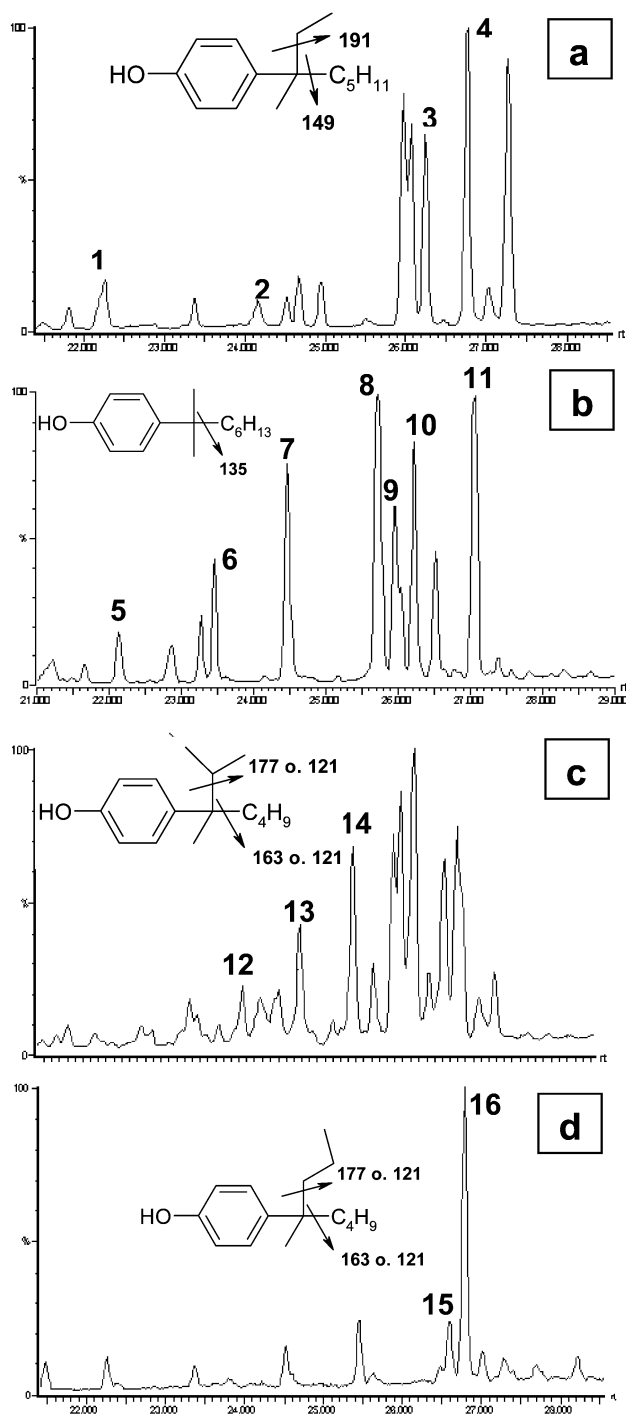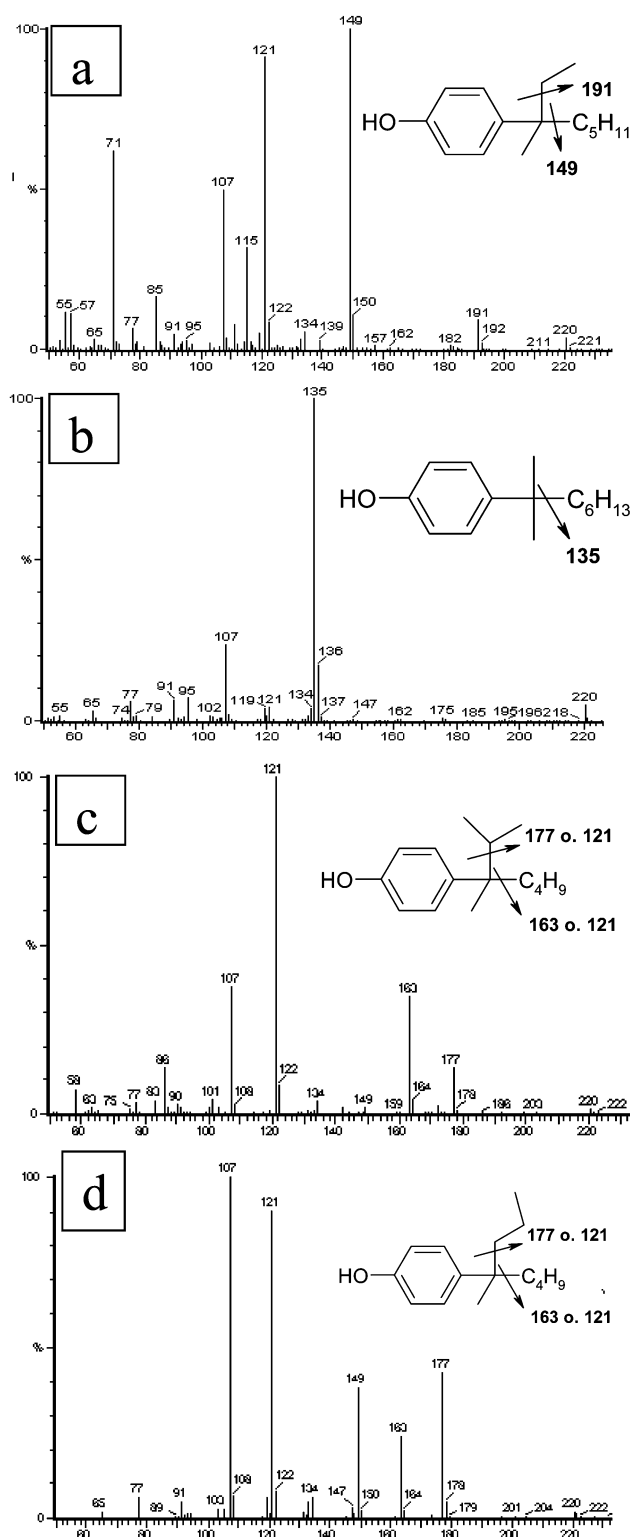
Fig. 3 Mass spectra of different isomer groups of 4-NP. Each spectrum is representative for all isomers of one group (a = group 1, b = group 2, c = group 3a, d = group 3b).

degree of fragmentation with a loss of both functional groups C_2H_5 and C_5H_{11}, respectively. Phthalic esters are ubiquitous in river water and show their base peak also at m/z 149. However, phthalic esters can be easily distinguished from 4-NP due to a different fragmentation pattern.

Isomer group 2 (peak nos 5–11). Fig. 2b shows the mass trace of the base peak m/z 135 with the isomer peaks 5–11 combined in the second group of peaks. Fig. 3b illustrates a mass spectrum typical for all isomers of group 2. The formation of

the base peak m/z 135 indicates an alpha-dimethyl configuration. This group is represented by seven isomers, yielding the most abundant type of isomers of 4-NP. Apart from m/z 107 there are no other major fragment ions in these spectra.

Isomer group 3a (peak nos 12–14) and isomer group 3b (peak nos 15–16). Fig. 2c and d show the mass traces m/z 121 with the isomer peaks 12, 13 and 14 and m/z 177 with the isomer peaks 15 and 16, respectively. The representative mass spectra, the chemical structures and fragmentation processes of the five isomer peaks are illustrated in Fig. 3c and d, respectively. The mass spectra of all five isomer peaks had the same kind of characteristic ions but with different relative abundances. The isomer peaks 12, 13 and 14 of group 3a showed the base peak at m/z 121. The relative abundances of the fragment ions were $\geqslant 35\%$ (m/z 163), and $\geqslant 15\%$ (m/z 177), respectively (Fig. 3c). Both isomer peaks 14 and 15 of group 3b had the base peak at m/z 107. The relative abundances of the fragment ions m/z 163 and m/z 177 were $\geqslant 25\%$ and $\geqslant 40\%$, respectively. The chemical structures of all isomers of groups 3a and 3b are characterised by an alpha-methyl, alpha-propyl and alpha-butyl configuration. The presence of the propyl group and the butyl group on the alpha-carbon atom results in a loss of 43 amu and 57 amu, respectively to form the m/z 177 and m/z 163 fragments. The loss of both groups generates the prominent m/z 121 ion. Branching in organic compounds results in lower boiling points and therefore branched isomers elute earlier than linear isomers of the same carbon chain length.[21] It is likely that peak isomers 12–14 had a highly branched alkyl chain, more precisely the alpha-propyl group, due to their lower retention times (Fig. 2c).

Occurrence of 4-NP in river water

The concentrations of 4-NP measured in the German rivers Rhein, Elbe, Main, Oder, Nidda and Schwarzbach are listed in Table 3. The mean value of 4-NP in all river water samples was 403 ng L^{-1} ($n = 23$). The concentrations of 4-NP in river water showed a wide range of variation from levels below the determination limit (<25 ng L^{-1}) in the Nidda River to 1220 ng L^{-1} in the Oder River. With a mean concentration of 567 ng L^{-1} ($n = 2$), 4-NP was a major compound in the Rhein River. In the Elbe River, 4-NP was detected at 28 ng L^{-1}. The mean value of 4-NP in the Main River was 410 ng L^{-1} ($n = 3$). The highest mean concentration of 4-NP at 737 ng L^{-1} compared to all investigated rivers was found in the Oder River ($n = 3$). 4-NP was also detected in the Nidda and the Schwarzbach River at mean concentrations of 194 ng L^{-1} ($n = 3$) and 384 ng L^{-1} ($n = 3$), respectively.

Seasonal variations in the Oder river

In March 2000, the mean concentration of 4-NP was 227 ng L^{-1} in all river water samples from the Oder. In November 2000, a higher mean concentration of 308 ng L^{-1} was measured. In March 2001, the mean value of 4-NP increased to 691 ng L^{-1}. The environmental fate of 4-NP can be expected to differ due to seasonally influenced processes such as rainfall, temperature and the variation in use of non-ionic surfactants. One possible reason for the seasonal concentration variation can be dilution during a period of a higher river water level. In March 2000, the water level of the Oder River was at 6.8 m above sea level representing the highest point of all sampling times. In November 2000 and March 2001, the water levels of the Oder River decreased to 4.3 m and 5.0 m above sea level, respectively.[22] Nevertheless, in March 2000 the concentration of 4-NP in the Oder River was higher than in November 2000. Since waste water discharge of STWs is reported to be a source of 4-NP in river water,[11,23] the diurnal dynamics of these STWs

Table 3 Concentration of 4-NP [ng L^{-1}] in river water of Germany (n.d. = not detected, the concentration was below the determination limit of 25 ng L^{-1}, n.i. = not investigated)

	Rhein, Rüsselsheim	Elbe, Hamburg	Elbe, Dresden	Main, Frankfurt/Main	Oder, Küstrin	Nidda, Frankfurt/Main	Schwarzbach, Rüsselsheim	Mean value
March 2000	n.i.	28	n.i.	140	628	308	32	227
November 2000	588	n.i.	112	224	363	n.d.	563	308
March 2001	546	n.i.	n.i.	865	1220	273	551	691
Mean value	567	28	112	410	737	194	382	
O_1	373							
O_2	54							
O_3	858							

have to be considered as an influencing factor for the presence of 4-NP in river water.

Concentration profile in the river course

The variation of the concentration of 4-NP in the river course is demonstrated in Table 3. Since there was no significant waste water discharge, the concentrations of 4-NP decreased downstream in the Oder River from 373 ng L^{-1} (O_1) to 54 ng L^{-1} (O_2). An input of 4-NP by waste water discharge from the municipal STW Manschnow, by the industrial STW of a paper mill located along the Oder River and by the Warta River, respectively between point O_2 and O_3 cause higher concentration of 4-NP (858 ng L^{-1}) at point O_3.

Concentration trends of 4-NP in German rivers

In Fig. 4 the maximum values of 4-NP detected in river water of the German states Hesse, Bavaria, Baden-Wuerttemberg and Brandenburg are illustrated from 1984–2001. Since high concentrations of the degradation product 4-NP in river water are consistent with the frequent use of NPnEOs 19 years ago 4-NP occurred at ppm-levels in German rivers. BUA[24] reported a maximum concentration of 4-NP in rivers of Hesse at 10,000 ng L^{-1} in 1984. In 1986, an unsolicited notice of abandonment for the use of NPnEOs in household detergents was made by the German industry. The maximum values of 4-NP in river water of Bavaria decreased continuously from 3,300 ng L^{-1} in 1990 to 1,300 ng L^{-1} in 1991, and to 400 ng L^{-1} in 1995.[25,26] In the state of Baden-Wuerttemberg in 1998, 4-NP was still measured in river water at a maximum concentration of 458 ng L^{-1}.[27] In the state of Brandenburg, a maximum concentration of 4-NP at 2,720 ng L^{-1} was measured in river water in 1998.[28] In the years 2000 and 2001, the maximum concentrations of 4-NP in river water of Brandenburg at 628 ng L^{-1} and 1220 ng L^{-1} remained still high. In both years, the maximum concentrations of 4-NP in Hessian rivers at 588 ng L^{-1} and 865 ng L^{-1}, respectively were much lower than in 1984, but not negligible.
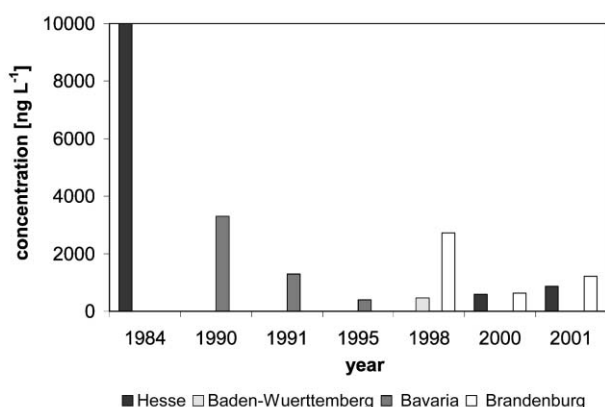
Although the German industry reduced the application of the mother product NPnEO in agents in 1986 the degradation product 4-NP occurs still at relevant levels in the aquatic environment of Germany. The results demonstrate that the control of the occurrence of 4-NP in German rivers should be continued in the future.

Conclusions

With the applied analytical method composed of SPE followed by GC-MS it was possible to resolve 16 isomers of 4-NP in river water samples. 4-NP was determined in the six German rivers Rhein, Main, Elbe, Oder, Nidda and Schwarzbach at ppt–ppb levels. At the Oder River, the discharge of treated waste water into the Oder was demonstrated to be still an important source of 4-NP in river water. Results obtained in this study indicate that concentrations of 4-NP in German rivers decreased during the last two decades. Because of its toxicity to aquatic organisms and its estrogenic activity the actual concentrations of 4-NP in German rivers are still of environmental concern. The results demonstrate the necessity of a continuous monitoring of 4-NP in German rivers. Although in the past the use of non-ionic surfactants was reduced in Germany the results point to the necessity of further restrictions of 4-NP in the aquatic environment.

Acknowledgements

Fig. 4 Concentration trends of 4-NP in river water in Hesse, Bavaria, Baden-Wuerttemberg and Brandenburg (maximum values). 1984–2001.

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