Alkylphenol Ethoxylates: Trace Analysis and Environmental Behavior

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Contents

I. Introduction

4-Alkylphenol ethoxylates (APEOs) belong to the group of nonionic surfactants (Figure 1). The term surfactant covers surface-active compounds which are characterized by their ability to concentrate at surfaces and to form micelles in solution.

The prerequisite for this surface activity is the amphiphilic structure of the surfactant molecule, which consists of a polar (hydrophilic) part and a nonpolar (hydrophobic) part. In APEOs, the hydrophilic group is a polyethoxylate chain with varying ethoxylation degrees (number of ethoxylate (EO) units = 1−40). The hydrophobic region is provided by alkylphenols (APs) in which the branched hydrocarbon chains most commonly contain eight or nine carbon atoms.

The technical synthesis of APEOs starts from phenol which is alkylated by trimethylpentene yielding 4-(1,1,3,3-tetramethylbutyl)phenol (4-*tert*-octylphenol, OP) or by a mixture of nonene isomers to form 4-nonylphenol (NP) in an acid-catalyzed process. The chemical composition of technical nonene leads to a complex mixture of NP consisting of isomeric compounds with differently branched structures of the nonyl side chains.[1] Ethoxylations are carried out using KOH/ethanol as the catalyst and supplying a known molar ratio of ethylene oxide to alkylphenol.[2] The resulting products are mixtures of oligomer homologues with varying lengths of the polyethoxy chain following a Poisson distribution.[3,4]

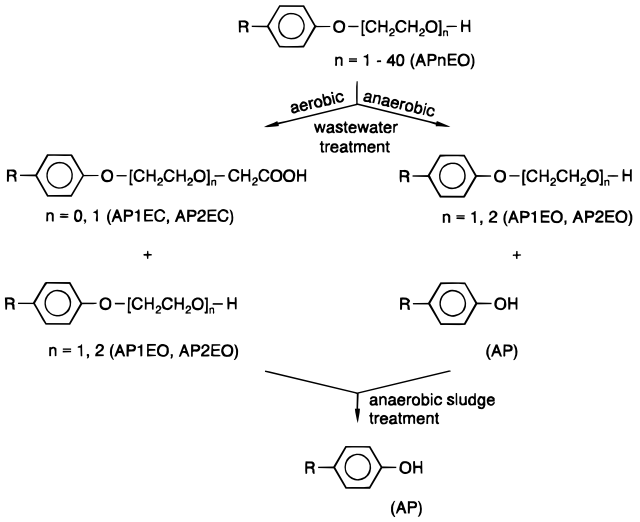

Figure 1. Aerobic and anaerobic biotransformation pathways of alkylphenol polyethoxylates. Reprinted with the permission from ref 83. Copyright 1994 Elsevier.

The physical properties of surfactants, in general, are formed by their amphiphilic molecule structures. Depending on the surfactant concentrations in water, different regions of state have to be distinguished concerning their colloidal and interfacial chemical properties. At very low concentrations surfactants adsorb at interfaces and reduce the surface tension. With increasing surfactant concentration the formation of micelles in the bulky phase starts to occur. The critical surfactant concentration of micelle formation, also called critical micelle concentration (cmc), is strongly dependent on the chemical structure of the surfactant. Cmc values for nonionic surfactants are in the range $10^{-3}-10^{-5}$ mol L^{-1}. They are 1−2 orders of magnitude lower than those of ionic surfactants with the same hydrophobic chain because of the absence of ionic head groups which lead to electrostatic repulsion during micelle formation. Moreover nonionic surfactants exhibit only a small effect in the presence of electrolytes compared to ionic surfactants.[5,6]

APEOs are widely used in cleaning products and as industrial process aids. The spectrum of applications ranges from dispersing agents in paper and pulp production to emulsifying agents in latex paints and pesticides formulations, flotation agents, industrial cleaners (metal surfaces, textile processing, and food industry), cold cleaners for cars, and household cleaners. The majority of APEOs are used in aqueous solutions; therefore, they are discharged into municipal and industrial waste waters which enter sewage

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Klaus Günther (born in 1957) studied chemistry at the Universities of Siegen, Cologne, and Münster. He received his Diploma in 1985 and his Doctoral Degree in Analytical Chemistry in 1988, both under supervision of Professor Fritz Umland at the University of Münster. His thesis work dealt with the speciation of heavy metals in biomatrices. Also in 1988 he joined the Institute of Food Chemistry, University of Bonn, for studies on multielement speciation analysis in foodstuff. In 1992 he was appointed Visiting Professor at Nanchang University, P. R. China. In 1994 he entered the Institute of Applied Physical Chemistry of the Research Centre Jülich. Here he focused his activities on the trace analysis of organic compounds, especially surfactants and their degradation products. In 1996 he finished a Habilitation thesis and became Privat-Dozent at the University of Bonn, where he gives lectures in Analytical Chemistry, in addition to his appointment with the Research Centre Jülich.

treatment plants. During the different steps of sewage treatment a complex biodegradation process of APEOs takes place, leading to the formation of several biorefractory metabolites (Figure 1).[7−12] The degradation products 4-alkylphenol diethoxylate (AP2EO), 4-alkylphenol monoethoxylate (AP1EO), [(4-alkylphenoxy)ethoxy]acetic acid (AP2EC), (4-alkylphenoxy)acetic acid (AP1EC), and 4-alkylphenols formed by shortening of the hydrophilic ethoxy chain are persistent, strongly lipophilic, and more toxic than the parent compounds (Figure 1). Acute toxicity data of NP to aquatic organisms range from 0.18 to 5.0 mg L^{-1} dependent on species and experimental conditions.[13,14] In comparision, toxicities of NP9/10EO to fish are 5.0−11.0 mg L^{-1}.[15] The release of these highly toxic compounds via secondary effluents or sewage sludge could be harmful to the aquatic or terrestrial environment.

Milan Johann Schwuger (born in 1938) studied chemistry at the Rheinisch-Westfälische Technische Hochschule in Aachen and received his Ph.D. 1966. His Ph.D. thesis was in the field of "Adsorption/Desorption kinetics in aqueous solutions". In 1966 he joined Henkel Co. in Düsseldorf. From 1966 to 1977 he was responsible for different interdisciplinary projects of the company. From 1977 to 1989 he was head of the physical chemistry research. For his development of zeolite being included in phosphate-free detergents he received 1979 the Steinkopff Award. Parallel habilitation at the University of Düsseldorf was finished 1984. In 1989 he was appointed as the director of the Institute of Applied Physical Chemistry of the Research Centre in Jülich and full professor of Applied Physical Chemistry at the University of Düsseldorf. In 1996 he was nominated for the degree Doctor of Science Honoris Causa by the Clarkson University, Potsdam. At present he is president of Kolloid-Gesellschaft in Germany.

Due to considerable doubts about the use of APEOs, they have been extensively replaced in laundry detergents in a number of countries. In 1986 the German detergent industry, for example, issued a voluntary agreement leading to APEOs no longer being used in any commercial formulation with a transitional period until 1992.[16] However, in 1990, the annual world APEO production was still 300 000 tons.[17] Two-thirds of this amount was produced in the U.S., largely for domestic sale.[18] In 1995, APEO production slightly decreased to 180 000 tons in the U.S.[19]

The discussion about NP in the environment has recently revived because of its estrogenic activity. Soto et al. identified NP as an estrogenic substance inducing the proliferation of MCF_7 human breast tumor cells.[20] In another study, male rats were exposed to estrogenic OP, resulting in a reduction of testicular size and daily sperm production.[21] Sharpe and Skakkebaek have discussed a possible role of estrogenic chemicals including APs in the induction of male reproductive disorders. On the basis of the information at that time, they were still not in a position to give any confident answer.[22,23]

In this paper, we review today's knowledge on APEOs/APs in the environment. The first part is devoted to the analytical methods for APEO/AP determination in environmental matrices; the second part represents their concentrations in the different compartments of the environment. In the last section we look at the risk potential of APEOs/APs for the aquatic and terrestrial environment. All relevant publications from the two last decades were taken into account for this review. For an overview of the analysis of nonionic surfactants in general, including

APEOs, the reader is referred to other books and reviews.[24−29]

II. Analysis

A. Sampling and Storage

Analytical results significantly depend on the homogeneity of the samples and accurate storage procedures which guarantee that no changes take place in the composition of the samples. The main problem of surfactants in general is their tendency to adsorb on all phase boundaries due to their amphiphilic nature. Consequently losses to surfaces or suspended solids from aqueous solutions are commonplace. Especially for matrices like sewage sludge, sediment, or soils, quantitative recovery of analytes turns out to be very difficult. Therefore, internal standards are added to the samples to correct for nonquantitative recovery during isolation and quantification of the analytes. Giger et al. used *n*-nonylbenzene[11,30] and 2,4,6-tribromophenol[8] in gas chromatographic determinations of APs/APEOs from sludge and water, respectively. 4-*n*-Nonylphenol, which is not included in technical NP, is applied to the quantification of NP in soils by GC.[31] For water analysis by HPLC 2,4,6-trimethylphenol is well suited.[32] This approach, however, is useless for nonspecific methods since they cannot discriminate analytes initially present from added internal standards.

Environmental samples have to be preserved immediately upon collection with chemical biocides to minimize and prevent microbial degradation of the surfactant present. Water samples from sewage treatment plants, rivers, or seas are generally collected in glass bottles, preserved with 1% formaldehyde, and stored at 4 °C.[33−37] A less common preservation method for aqueous samples is the addition of methylene chloride and acidification to pH 2 with hydrochloric acid.[38] Kubeck et al. showed that refrigeration alone was sufficient to stabilize river water samples for up to 4 weeks.[35] Due to diurnal variations of APEO concentrations in the influents and effluents of sewage treatment plants, 24-h and 2-h composite samples should be collected, ideally, with automatic sampling devices.[39]

Sewage sludges are dealt with in the same way as water samples, i.e. preservation with 1% formaldehyde and storage at 4 °C.[39,40] Jobst et al. preferred aluminium vessels to store the sludge samples.[41]

Sediment samples are collected from the upper 2 cm using a grab sampler and frozen at −20 °C until analysis.[33,42−44] In the laboratory, samples are freeze-dried[44] or air-dried at 21 °C[43].

The application of sewage sludges to agricultural land has resulted in the need to monitor concentrations of detergents in sludge-amended soils. Soil samples are collected from the upper 5 cm with a stainless steel corer, dried at 60 °C, pulverized, and stored in the dark at 4 °C.[40]

Biological matrices represent a difficult problem with regard to a representative sampling and a unchanged composition of the samples during storage. The Environmental Specimen Bank (ESB) of Germany has developed a method for collection and preparation of fresh biological materials.[45,46] Different specimen types from the terrestrial and aquatic environment are repeatedly collected at the same sites and times. They are directly frozen below −150 °C with liquid nitrogen at the sampling site. All subsequent preparation steps involving prechrushing, grinding, homogenization, and long-term storage are carried out at the same cryogenic conditions.[47,48] The whole process of the ESB is performed according to Standard Operating Procedures (SOPs).[49]

B. Extraction

1. Solid Phase Extraction (SPE)

In SPE, a hydrophobic solid material is utilized to adsorb surfactants from aqueous solutions via their hydrophobic end. The adsorbed surfactants are later efficiently eluted with small amounts of organic solvents in a concentrated solution. A wide range of SPE resins concerning polarities and functionalities are available today and various ones have been used for APEO/AP extraction from water samples.

Amberlite XAD-2 and -4 resins are based upon a styrene structure cross-linked with divinylbenzene and are highly selective for aromatic compounds such as APEOs/APs. XAD-2 resin is used to extract the analytes from waste water and sea water. Filtered water samples (1 L) saturated with NaCl are passed through a column containing 1.5−2.0 g of resin. The recovery is 91−94% after elution with acetone:water (9:1).[42] In another case, sequential elutions with Et_2O and methanol are applied.[50] For the separation of linear alkylbenzene sulfonate (LAS) from APEOs, water samples are shaken with an anion-exchange resin. The resulting filtrate is shaken with 1 g of XAD-2, and then the APEOs adsorbed on the resin are eluted with 100 mL of methanol with recoveries of 90−100% for 0.5 and 2.0 mg L^{-1} aqueous NP9EO standard solutions.[51] XAD-4 resin turns out to be an useful adsorbent for the extraction of polyethoxylated detergents from river water. A narrow glass column is filled with 5 g of resin and purified by successive washings with acetone:hexane (1:1), acetone, and ethanol prior to use. Up to 50 L of water can be handled with this resin. Elution of the adsorbed compounds is done with four solvent systems with recoveries of 90−100% for 1 mg L^{-1} aqueous APEO standard solutions.[52,53]

The more polar XAD-8 resins have a cross-linked polymethacrylate structure and are used for the analysis of ground water. After passing 300 L of water through a column containing XAD-8 resin the adsorbed APEOs are eluted in a Soxhlet extraction with methanol. Interfering anionic surfactants are removed by trapping on an anion-exchange resin. Recovery after these two steps is 84 ± 5% for 1 mg L^{-1} aqueous APEO standard solutions.[54]

Granular activated carbon (GAC) is useful for processing large amounts of river and drinking water. Water samples (2000 L) are passed through a column with 105 g of GAC at a flow rate of 35 mL min^{-1}. After the GAC is dried, it is extracted in a Soxhlet apparatus with 0.5 L of dichloromethane for 48 h. The extracted surfactants are identified by mass spectrometry.[55−57]

Graphitized carbon black (GCB) is a nonporous material with positively charged active centers on the surface employed for the separation of NPEOs/NP from acidic nonylphenoxy carboxylate (NPEC), linear alkylbenzene sulfonate (LAS), and sulphophenyl carboxylate (SPC). This procedure involves passing 10 and 100 mL of influent and effluent water samples, respectively, through a 1-g GCB extraction cartridge. A stepwise desorption of the analytes from the GCB with three different solvent systems is performed, leading to three fractions. The first fraction contains NPEOs and NP, the second the carboxylated biotransformation products of NPEOs, i.e., NPECs, and, finally, the last fraction LAS and their metabolites, i.e., SPC. NPEOs and NP are obtained with recoveries of 96 ± 4% and 89 ± 7%, respectively, for raw sewage spiked with 200 μg L^{-1} NPEOs and 20 μg L^{-1} NP.[34,58]

Octadecylsilica (C-18) minicolumn cartridges are employed to enrich LAS, NPEOs, and NP from sea water,[37,59] treated and untreated waste water,[60] and river water.[61] Marcomini et al. conditioned the cartridges with 3 mL of acetonitrile, 3 mL of methanol, and 5 mL of distilled water. After the addition of 8% NaCl (alternatively 7 mmol L^{-1} sodium dodecyl sulphate) and filtration, 50 mL of raw sewage water or 250 mL of biologically treated water is passed through the C-18 cartridges. Desorption from the cartridges is carried out with 3 mL of acetone. Recoveries of NPEOs and NP from water containing 8% NaCl and spiked with 56 μg L^{-1} NPEOs and 76 μg L^{-1} NP are 96 ± 2.5% and 92 ± 3.0%, respectively.[60] Blackburn et al. slightly modified this procedure by using ethyl acetate followed by dichloromethane as elution solvents.[61] Kubeck et al. also used a C-18 cartridge to adsorb NPEOs, but first, the water samples are passed through a mixed bed ion exchange resin to remove all ionic species. The adsorbed analytes are eluted from the C-18 cartridge with warm methanol with recoveries of 84% for NPEOs from river water spiked with 2.91 μg L^{-1} NPEOs.[35] For the simultaneous isolation of NPEOs, LAS, and their acidic biodegradation products, i.e., NPECs and SPC, respectively, the water samples are acidified with HCl to pH 2 and the analytes adsorbed on the C-18 column are eluted with methanol. Recovery experiments on NPECs and SPC from effluent water samples showed good results for NP1EC (91%, 96 μg L^{-1} added) and NP2EC (88%, 24 μg L^{-1} added) but were unsatisfactory for SPC with up to six carbon atoms in the carboxylate chain.[36]

Field et al. investigated the use of strong anion exchange (SAX) disks for NPEC isolation from effluent and river waters. After the disks were rinsed with acetonitrile and deionized water the centrifuged water samples were applied to the disks. NPEC elution from the disks with acetonitrile were simultaneously combined with derivatization with methyl iodide. The recoveries of NPEC spiked into deionized water (40 μg L^{-1}) were 91−100%.[62]

The use of solvent-free solid-phase microextraction (SPME) for the extraction and analysis of nonpolar semivolatile analytes has received increasing attention. Hitherto, SPME was applied in combination with GC, restricting the application to APEOs due to their low volatility. But recently Pawliszyn et al. developed a SPME/HPLC method for the determination of APEOs in water. By replacing the conventional injection loop of a HPLC system with a specially designed desorption chamber the SPME device can be transferred to the injection port where all adsorbed analytes on the fiber are desorbed in the eluent stream. Among several different coated fibers the Carbowax/template resin (CWAX/TR) coated fiber best maintained the same ethoxylate oligomer distribution in extracted samples as was present in the original water sample.[63]

2. Solvent Sublation

Solvent sublation is a technique capable of selectively concentrating surfactants free from non-surface-active materials. In the original procedure by Wickbold a 1-L water sample is placed into a sublation apparatus and the pH is adjusted to 7−8 by adding 5 g of $NaHCO_3$. The aqueous sample is overlaid by 100 mL of ethyl acetate, and solvent-saturated nitrogen is purged through the liquids for 5 min. Surfactants are enriched at the gas−liquid phase boundary and carried by the gas stream into the ethyl acetate where they are dissolved and concentrated. The organic layer is then replaced by fresh ethyl acetate for a second extraction.[64] In addition to the above-mentioned procedure Giger et al. added 40 g of NaCl to the water sample due to the salting-out effect.[12,65] The Wickbold method has now been standardized.[66]

Waters et al.[67] thoroughly investigated the Wickbold procedure[64] for the sublation of water samples. In the optimized procedure, four rather than two sublation steps, each of 10-min duration, in conjunction with unfiltered samples are used to obtain high recoveries of nonionic surfactants especially in raw-sewage samples. A cation/anion-exchange treatment step is necessary to remove potential ionic interferences from the sublation extracts.[67] The optimized Wickbold procedure has been often applied by other authors.[68−70]

The application of the Wickbold method is well suited to APEOs with ethoxy chain lengths of 3−11 which are recovered from spiked water samples (1 mg L^{-1} Marlophen 810/Hüls) with almost 100%.[65] Lower recoveries (<70%) are achieved for the higher oligomers (12−17 EO units)[65] or the biodegradation products AP1EC and AP2EC.[12]

3. Liquid–Liquid Extraction

For hydrophobic organic compounds, liquid−liquid extraction is the method of choice to concentrate them from aqueous solutions. Surfactants, however, do not have such a distinct preference for the organic phase. On the contrary, they are concentrated at phase boundaries. For this reason, direct extraction of APEOs from aqueous solutions is restricted to their less surface-active metabolites, i.e., APEOs with 1−3 ethoxy units, APECs and APs.

Noncontinuous liquid−liquid extraction of water samples using a separatory funnel has been applied

by several work groups. Effluent samples of 200 mL are extracted three times with 20-mL portions of dichloromethane with recoveries of 87% for NP and 79−86% for NP1EO−NP3EO.[8] The addition of NaCl to the water sample should prevent the formation of emulsions and increase the extraction efficiency due to the salting-out effect, leading to recoveries of 98% for NP and 82−100% for NP1EO−NP3EO.[71] Acidification of the water sample to pH 2 by adding H_2SO_4 is indispensable in order to extract NPECs.[12,72]

Continuous liquid−liquid extraction in an apparatus for organic solvents heavier than water was applied by Giger et al. to extract APs, AP1EO, and AP2EO from effluent water samples. By percolating dichloromethane through the aqueous layer, the analytes could be obtained with recoveries of 101%, 87%, and 93%, respectively.[8,72] A modified continuous liquid−liquid extractor is used to extract 200 L of effluent water with 150 mL of hexane.[73] Lee et al. have combined liquid−liquid extraction with in-situ derivatization to extract NP from river water. To 250 mL of effluent were added 1 g of K_2CO_3 and 1 mL of acetic anhydride which were overlaid by 30 mL of petrol ether. After being stirred for 30 min the formed acetic ester was quantitatively transferred to the organic phase. Replicate analyses of lake water at spiking levels of 1 and 10 μg L^{-1} produced recoveries of 97 and 93%, respectively.[74]

Steam distillation/solvent extraction using an apparatus designed by Veith and Kiwus[75] is a sophisticated method to concentrate steam-distillable NP, NP1EO, and NP2EO. After 30 g of NaCl is added, 2-L water samples are distilled and the condensed distillate is extracted by passing through a small layer of 1−2 mL of cyclohexane. Continuous reflux of spiked water samples (21.2 μg L^{-1} NP, 100 μg L^{-1} Marlophen 83/Hüls) for 2 h leads to recoveries of 94% for NP and 105% and 82% for NP1EO and NP2EO, respectively. The extraction efficiency already dramatically decreased for NP3EO to 15%.[32,76−78] Steam-distillable compounds can also be separated by vacuum distillation. In this case the evaporated water is trapped at −80 °C and then extracted with diethyl ether for 24 h with a continuous liquid−liquid extractor.[79]

4. Extraction from Solid Matrices

Mainly two methods have been successful for the extraction of APEOs/APs from solid matrices, namely Soxhlet extraction and steam distillation/solvent extraction. Soxhlet extractions are mostly carried out with dried samples. For this the samples are either dried at 60 °C[80−82] or freeze-dried[42,59] and then pulverized. In a typical procedure 0.5 g of detergent powder, 2 g of sewage sludge, and 20 g of soil or sediment are transferred into the preextracted paper thimble of the Soxhlet apparatus. After solid NaOH (20% (w/w)) is added, the samples are extracted with 80 mL of methanol for 30 min for detergents, 4 h for sludges, and 12 h for soils and sediments. The recovery from detergent powder is 103% for APEOs, from sludge and sediment it is 99% and 93%, respectively, for NP.[80] In addition to methanol, methanol: dichloromethane (1:2),[42] 2-propanol,[38] and hexane[59] are also used as extraction solvents. Marcomini et al. percolated the Soxhlet extract of a dry sediment (spiked with 21 ng g^{-1} NP, 36 ng g^{-1} NP1EO, and 12 ng g^{-1} NP2EO d.w.) through an aminosilica cartridge to remove interfering substances. The adsorbed analytes were eluted with 5 mL of hexane:acetone (3:1), achieving recoveries of 97%, 92%, and 84% for NP, NP1EO, and NP2EO, respectively.[59]

Extraction of NP and NP1EO−NP3EO is also performed with steam distillation/solvent extraction. Sludge, sediment, or soil (10−50 g) is suspended in 1.5 L of water and refluxed for 3 h by using cyclohexane (1−2 mL) as the extracting solvent through which the distillate percolated.[11,30,32,33,83,84] The recoveries for NP from sewage sludge spiked with 0.4, 0.9, and 1.3 g kg^{-1} d.w. are 105, 104, and 93%, respectively.[11] Jobst et al. reported on steam distillation/solvent extraction by suspending 25 g of soil or 50 g of sludge in 400 mL of water containing 36 g of NaCl and 1 mL of H_3PO_4 and steam distilling for 4 h with simultaneous extraction using 20 mL of isooctane. The extraction efficiency varied from 90 to 116% for NP, referring to soils spiked with 0.1−1.0 mg of NP kg^{-1}.[31,41]

Finally, supercritical fluid extraction (SFE) has been investigated for the extraction of NP from sewage sludge and sediment. Dried sludge (250 mg) or dried sediment (1 g) spiked with 30 μL of triethylamine and 100 μL of acetic anhydride is extracted with supercritical carbon dioxide at 80 °C. The extraction times are 15 min static and 10 min dynamic. The NP derivatized in situ is trapped on C-18 silica gel and later eluted with hexane, reaching a recovery of 96−98%.[74]

C. Determination Procedures

1. Colorimetry/Titrimetry

Nonspecific analytical methods like colorimetry and titrimetry for the determination of summary parameters (BiAS: bismuth active substances; CTAS: cobalt thiocyanate active substances) were the earliest attempts to analyze nonionic surfactants at environmental concentrations. The determination methods are based on the formation of donor−acceptor complexes. Hard Lewis acids (alkaline or alkaline earth metal ions) form cationic coordination complexes with the polyethoxylate chain of the nonionic surfactants. Large anions like tetraiodobismuthate or tetrathiocyanatocobaltate function as the corresponding counterions. The properties of these anions are then utilized for the final determination step.

The BiAS procedure has been elaborated by Wickbold et al.[64,85,86] using barium tetraiodobismuthate (modified Dragendorff reagent) to form an orange precipitate with nonionic surfactants in moderately to strongly acidic solutions. The precipitate is then dissolved with ammonium tartrate solution, and the released bismuth ions are determined colorimetrically,[87,88] by potentiometric titration,[64,85] or by X-ray fluorescence spectroscopy.[89,90] In its original scope, the procedure was designed for the analysis of contaminated water samples containing milligrams

per liter levels of nonionic surfactants, after concentration by solvent sublation (see section II.B.2). Waters et al. have shown that an optimized procedure involving unfiltered samples, four 10-min sublation steps, and a cation/anion exchange cleanup of the sublation extract (see section II.B.2) is required to obtain reliable BiAS levels.[67] The BiAS procedure fails to determine APEOs with less than five ethoxy groups because these compounds are not precipitated by the modified Dragendorff reagent.[8] Thus, investigations using the Wickbold method do not detect the metabolites of APEOs, i.e., the shorter chain compounds, and APs themselves.

In the U.S., the CTAS procedure has found wide application. The nonionic surfactants are reacted with ammonium tetrathiocyanatocobaltate in aqueous solution to form a blue water-insoluble complex which is readily extractable into dichloromethane[91] or benzene.[92] The intensity of the extract is measured colorimetrically at 620 nm.[91] Again, a minimum of six ethoxy groups is necessary for complex formation.[92] Cationic surfactants lead to interferences resulting in high values. They therefore have to be separated before complex formation. The CTAS method involves fewer steps in sample preparation than the BiAS method and is thus simpler to perform.

Other complex salts have been used for donor−acceptor complex formation,[85] but none of them has reached a stage of application comparable to BiAS or CTAS.

2. IR and NMR Spectroscopy

IR spectroscopy is used for qualitative identification of nonionic surfactants and differentation between them and nonsurfactant compounds. Prior to IR spectroscopy, separation of the organic compound complex into different fractions performed by the use of thin layer chromatography (TLC)[93] or column chromatography on silica gel[53,89,94] is required to obtain meaningful spectra. The general appearance of IR spectra of NPEO and linear alcohol ethoxylates (AEOs) is very similar with the broad strong peak at 1101 cm^{-1} characteristic of the aliphatic −C−O−C− vibration. The only clearly recognizable difference between them is the very sharp aromatic peaks present in the NPEO spectrum at 1609 and 1512 cm^{-1} which can be used for the identification of APEOs in environmental samples.[53,90,93]

NMR spectroscopy is normally used for the characterization of nonionic surfactants with regard to their molecular structure. A review of NMR analysis of nonionic surfactants appeared in a volume of the *Surfactant Science Series*.[95] Its application to the determination of APEOs in environmental samples is very rare because of concentrations not sufficient for NMR and the lack of pure reference substances. Jones et al. used NMR spectroscopy to qualitatively determine APEOs in water samples without giving any details about the spectra themselves.[53]

3. Mass Spectrometry

Mass spectrometry (MS) is an undeniable method for the determination of the molecular structure of compounds. Mass spectra of the parent ions can directly give the molecular weight distribution of surfactant oligomers. Fragmentation patterns allow statements about the kind of the isomers, such as location of side chains and degree of branching. MS is most widely used in environmental analysis as an on-line detection system coupled to GC or HPLC. However, some new ionization techniques have been applied to the direct identification of APEOs and their degradation products in environmental samples.

Fast atom bombardment (FAB) MS is useful for the identification of APEOs, especially the higher oligomers, and its metabolites in environmental matrices. Ventura et al. analyzed polyethoxylated compounds in raw, river, and drinking water by FAB using thioglycerol saturated with NaCl as matrix.[55−57,96,97] The addition of salt causes the formation of strong $[M + Na]^+$ ions which can be easily identified in the spectra. In the absence of salts the abundance of $[M + H]^+$ ions is very low even when the matrix is adulterated with trifluoroacetic acid.[98] The characteristic appearance of FAB spectra of technical APEO surfactants with a series of $[M + K]^+$ ions separated by 44 units corresponding to different degrees of polyethoxylation (Figure 2) led to the use of APEOs as exact mass internal standards for FAB-MS.[98] Quantitation is far more difficult with FAB-MS. On the one hand, the intensity of the quasimolecular ions decreases with molecular weight, so misleading results for the molecular weight distribution of APEOs can be obtained. On the other hand, accurate quantitative information can only be achieved with the use of isotopically labeled internal standards.[50]

Field desorption (FD) mass spectra of APEOs are dominated by quasimolecular ions $[M + H]^+$, while structure-specific ions are often missing or of low intensity (Figure 3).[50,79,99−104] Therefore, the FD technique is less suited for structure elucidation but well suited to determine the molecular weight distribution of APEOs in technical surfactants and environmental samples. Levsen et al. monitored the biodegradation of NPEOs by FD-MS enabling the temporal changes in the concentrations of the individual NPEO compounds.[99,102] Shiraishi et al. used FD-MS to characterize fractions collected after HPLC separation of APEOs from river water.[103,104] They observed that the FD spectra were significantly affected by the presence of salts in the river water extracts, resulting in $[M +$ alkali metal$]^+$ ions as the major ion species.[79,104] The combination of FD and the method of collisionally activated decomposition (CAD) in a tandem mass spectrometer gives spectra with a typical fragmentation pattern along with strong quasimolecular ions. In this way structure elucidation becomes possible with this technique.[101]

By the application of desorption chemical ionization (DCI) MS, the molecular weight distribution of APEOs and structure information on APEOs are available from the spectra as shown in Figure 4.[99,100] In contrast to the FAB technique, the DCI method leads to the generation of quasimolecular $[M + H]^+$ ions of fair abundance even for APEOs with long ethoxylate chains.[100]

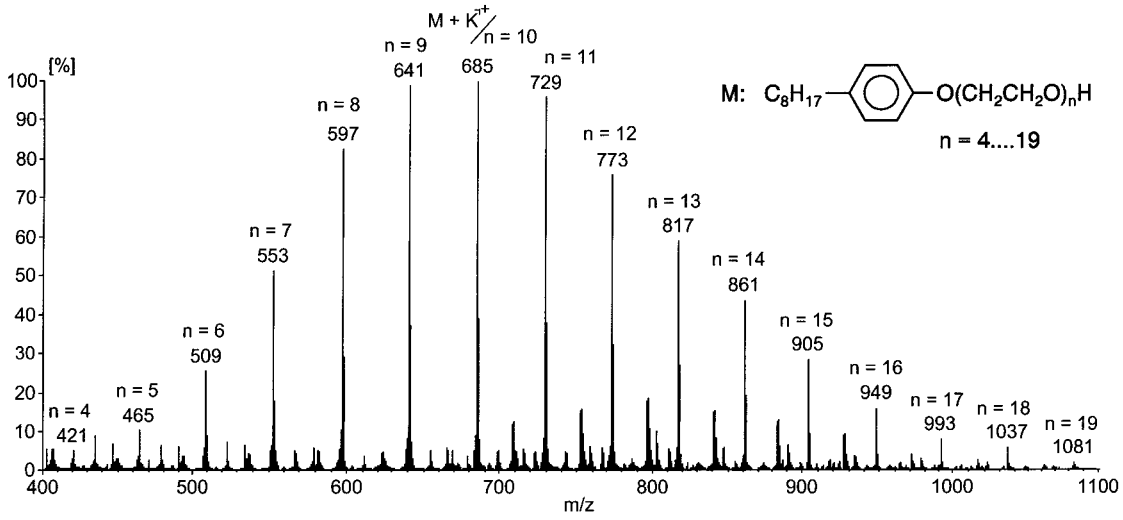

Figure 2. FAB mass spectrum of a technical OPEO surfactant (IGEPAL CA 720) adulterated with KCl in DTT/DTE matrix. Reprinted from ref 98. Copyright 1990 American Chemical Society.

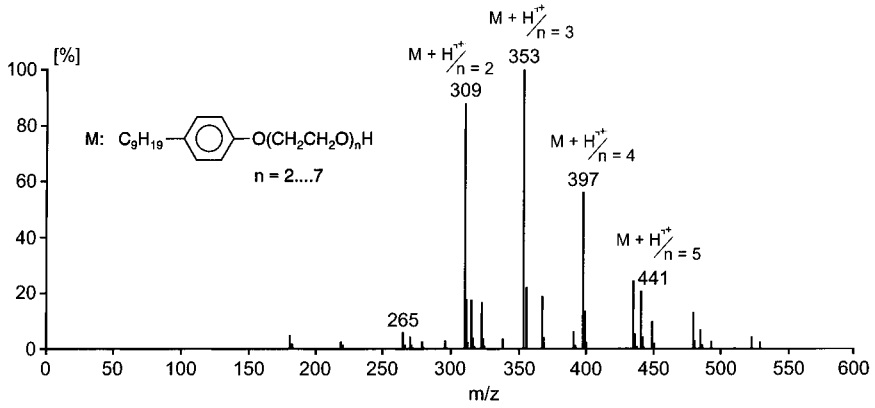

Figure 3. FD mass spectrum of NPEO. Reprinted with permission from ref 100. Copyright 1983 Fresenius' Zeitschrift fuer Analytische Chemie.

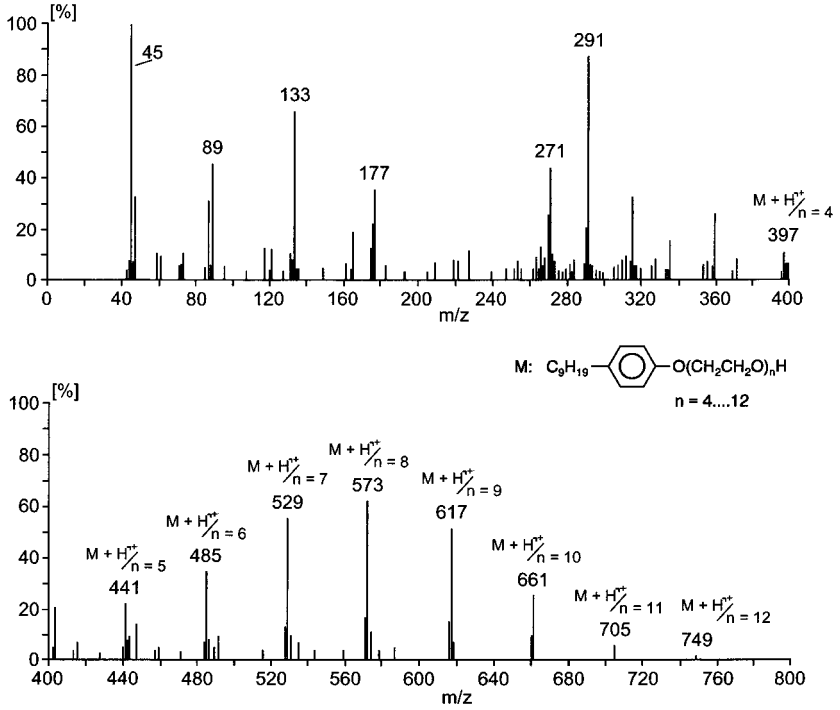

Figure 4. DCI mass spectrum of NPEO. Reprinted with permission from ref 100. Copyright 1983 Fresenius' Zeitschrift fuer Analytische Chemie.

4. High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography (LC) Coupled to MS (LC/MS)

Technical APEO surfactants are complex mixtures consisting of various homologues and oligomers by length of the alkyl and ethoxylate chains. Consequently, HPLC separation of APEOs into individual molecules is a two-dimensional problem best solved by using different HPLC stationary phases. Polar normal-phase columns separate nonionic surfactants by their interaction with the hydrophilic polyethoxylate chain without resolving the hydrophobes (Figure 5), while nonpolar reversed-phase columns separate them by their interaction with the hydrophobic chain only eluting the ethoxymers as a single peak (Figure 6). The ring chromophore in APEO molecules enables direct and sensitive UV or fluorescence detection. Therefore, normal and reversed-phase HPLC provide a quite simple and suitable technique for the environmental analysis of APEOs and their metabolites (Table 1). Recently a review on the analysis of nonionic surfactants with ethoxylate chains by HPLC was published by Miszkiewicz et al.[105]

Normal-phase HPLC is mostly applied to obtain information about the ethoxylate chain distribution of APEOs. Therefore, quantitative determinations of APEOs in samples from waste water, sewage treatment, and the aquatic environment are often performed by normal-phase HPLC to reveal the changes in the APEO composition due to biodegradation. Giger et al. used aminosilica columns with gradient elution coupled to UV detection (277 nm) to isolate APEOs. The application of spherical 3-μm aminosilica material and shorter columns had proved to be more suitable compared to irregular 10-μm material. Elution volumes and retention times were considerably reduced without loss to the separation efficiency (Figure 5).[32,33,65,78,83] Detection limits were 1 and 3 μg L^{-1} for NP3EO and NP18EO, respectively.[65] An increase in sensitivity and selectivity for APEOs is attained by the use of a fluorescence detector. Thus, Holt et al. determined NPEOs in water samples by the use of normal phase HPLC and

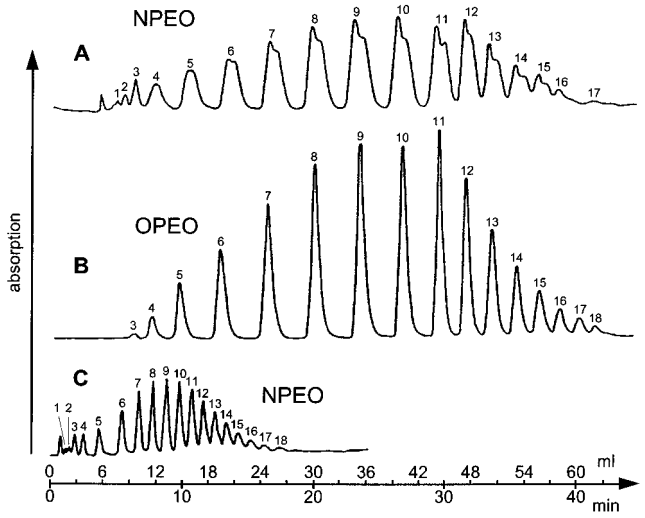

Figure 5. Normal-phase high-performance liquid chromatograms of nonionic surfactants of the APEOs type using different columns: LiChrosorb-NH_2 (250 × 4.6 mm, 10 μm) (A, B) and Hypersil APS (100 × 4 mm, 3 μm) (C). Reprinted from ref 65. Copyright 1985 American Chemical Society.

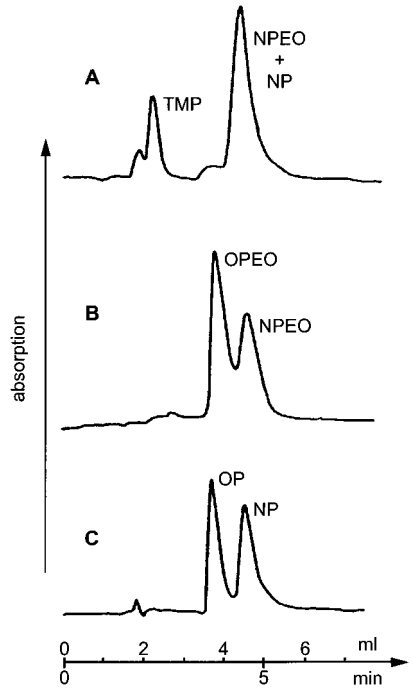

Figure 6. Reversed-phase high-performance liquid chromatograms of different mixtures containing APs and APEOs (TMP: 2,4,6-trimethylphenol). Reprinted from ref 32. Copyright 1985 Amercian Chemical Society.

fluorescence detection at excitation and emission wavelengths of 230 and 302 nm, respectively, with a minimum of detection of 0.2 ng for each individual homologue of APEOs.[69,70] Lee et al. reported detection limits of 0.02 μg L^{-1} and 0.015 μg g^{-1} d.w. for the analysis of NPEOs in river water and sediments, respectively, using a 5-μm NH_2 column with isocratic elution and fluorescence detection.[43] Quantification of APEOs and APs by normal-phase HPLC is generally carried out by the internal or external standard method using response factors generated by suitable standards. For lack of APEOs with polyethoxylate chains of discrete lengths as standard compounds commercial APEOs are separated by preparative normal-phase HPLC and the amount of APEO in these fractions is determined gravimetrically.[32,65,70] Then the response factors for each individual oligomer are calculated directly[70] or in relation to 2,4,6-trimethylphenol as an internal[32] or external standard.[65]

Reversed-phase HPLC is used as a complementary method to normal-phase HPLC to be able to separate the homologous compounds OPEO and NPEO but coeluting the ethoxylate oligomers. Thus, the qualitative analysis of NP and NPEOs from waste water, river water, or sewage sludge performed on 10-μm octylsilica (C8) columns with isocratic methanol/water elution and UV detection at 277 nm results in a single peak (Figure 6A).[11,30,32,33,78,83,84,106] Marcomini et al. described the simultaneous determination of LAS, NP, and NPEOs by reversed-phase HPLC on C8 or C18 columns with acetonitrile and water containing $NaClO_4$ and fluorometric detection. Quantification of LAS and NPEOs was performed by external standard calibration graphs.[37,59] A similar method was developed for determining simultaneously LAS and NPEOs as well as their corresponding metabolites sulfophenyl carboxylate (SPC) and

alkylphenol ether carboxylates (APECs), respectively. These analytes were quantitatively determined using calibration graphs or the standard addition method.[36,81]

Electrospray (ES) LC/MS currently provides the most sensitive and selective analysis of NPEOs. This method allows the determination of NPEOs from influents and effluents of sewage treatment plants, river water, and drinking water with detection limits of 0.6, 0.02, 0.002, and 0.0002 μg L^{-1}, respectively, using an alcohol ethoxylate (C-10 EO_6) as an internal standard.[58] Clark et al. used particle beam (PB) LC/MS for the semiquantitative determination of NPEOs in drinking water.[107] By use of ion spray LC/MS Di Corcia et al. analyzed NPEC in effluents of sewage treatment plants. The mass chromatograms obtained in the selected ion monitoring (SIM) mode with *m/z* values corresponding to protonated molecular ions of $[NPnEC + H]^+$, with $n = 3-10$, clearly demonstrated that NPECs with more than two ethoxy units can also occur in treated sewages.[34]

Thus, LC/MS analysis, in general, provides valuable information on the molecular weight and structure of separated compounds apart from their sensitivity and selectivity.

5. Gas Chromatography (GC) and GC coupled to MS (GC/MS)

As a separation technique GC is inherently more powerful than HPLC, but it is limited by the volatility of the compounds to be analyzed. Only APEOs with a short polyethoxylate chain are amenable to direct determination using GC. Quantitative GC analysis of nonionic surfactants of a higher degree of ethoxylation, therefore, requires derivatization reactions to transform the APEOs into more volatile compounds. Using capillary columns a complex pattern is obvious for every polyethoxylate homologue, indicating that each individual alkyl chain structural isomer is separated (Figure 7). GC coupled to MS becomes more and more the dominant determination method for APEOs/APs in environmental matrices because of its sensitivity and selectivity. Applications of GC for the environmental analysis of APEOs and their metabolites are summerized in Table 2.

APEO analysis by GC without derivatization has been mainly used on the more volatile biodegradation products of APEOs, namely NP2EO, NP1EO, and NP. GC/MS provided the basis for the reliable identification of these analytes by their fragmentation pattern apart from quantitation of these compounds. For the analysis of NP2EO, NP1EO, and NP in waste water[8,12] and river water[12] Giger et al. have successfully applied GC/MS in the electron impact ionization (EI) mode. Quantitation was performed by addition of tribromophenol[8] or *n*-nonylbenzene[12] as internal standards to the extracts just before the GC determination. Detection limits ranged between 1.0 and 10.0 μg L^{-1}.[8,12] Jobst performed the determination of NP from soil samples by GC/MS in the selected ion monitoring (SIM) mode for sensitive detection (0.05−0.1 mg kg^{-1} soil) and selective identification. The internal standard 4-*n*-nonylphenol not included in technical NP was already added to the original samples to correct for nonquantitative recoveries during isolation.[31]

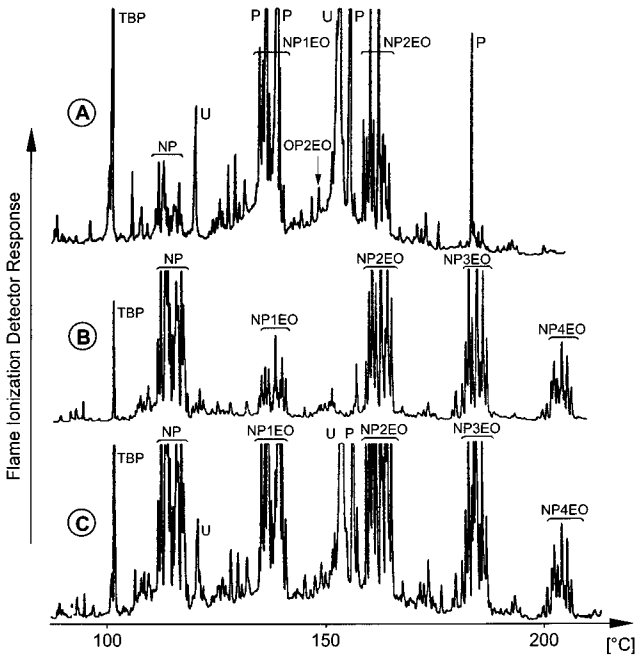

Figure 7. Glass capillary gas chromatograms of an extract of secondary sewage effluent (A), a reference mixture of NP and Marlophen 83 (B), and the coinjection of A and B (C) (P: phthalate, TBP: tribromophenol, U: unknown). Reprinted from ref 8. Copyright 1982 American Chemical Society.

Günther et al. used an off-line coupling of normal-phase HPLC and GC/EI-MS in the SIM mode to determine the different NP isomers in biological matrices in the ultra trace range.[113] Figure 8 shows an example of the obtained total ion current and typical SIM traces of NP from common mussels. NP is separated into 16 peaks corresponding to its isomers which are quantified on different ion traces free from interferences. The internal standard 4-*n*-NP is added to the mussel samples from the beginning. To determine the response factors for individual NP isomers pure standard compounds of these isomers are necessary but currently not available. Therefore, the quantitative distribution of NP isomers in the technical product is determined by GC/FID. Then, the response factors for each NP isomer are determined by analysis of technical NP by GC/EI-MS in the SIM mode and calculated in relation to the internal standard.[113]

GC/EI-MS is also used for the mass spectral characterization of individual nonyl chain isomers of NP, NP1EO, and NP2EO. By use of high-resolution capillary GC/EI-MS Wheeler et al. were able to separate 22 isomers of technical NP. Analysis of the corresponding mass spectra indicated the presence of five distinct groups of isomers.[118] Chemical ionization (CI) induced mass spectra of OPEO and OPEC give very reliable information on the molecular weight of the analytes due to the presence of the adduct ions $[MH]^+$ and $[MC_2H_5]^+$.[119−121] Therefore, GC/CI-MS is a suitable method for the selective identification of these compounds in environmental matrices.

Derivatization of NP and NPEOs with pentafluorobenzoyl chloride (PFBCl) or heptafluorobutyric anhydride (HFBA) provides derivatives with high electron affinities which can be specifically and

Table 1. Determination of APEOs, APECs, and APs by HPLC

compd	sample type	enrichment	column (normal, *reversed-phase*)	mobile phase (normal, *reversed-phase*)	detection	detection limit ($\mu g\ L^{-1}$)	recovery (%)/rel std dev (%)	ref
APEO	spiked water		*Waters Bondapak C-18, 120 × 2 mm*	*H_2O (A)/MeOH (B)*	UV absorption (280 nm)		72−101/−	103
APEO	technical surfactants		DuPont Zorbax-NH_2, 250 × 4.6 mm, 6 μm	isooctane:CH_2Cl_2:MeOH (95:5:3)/isooctane:	UV absorption (276 nm)		−/−	3
			LiChrosorb-NH_2, 500 × 4.6 mm, 10 μm	CH_2Cl_2:MeOH (60:40:7.5)				
APEO	technical surfactants		LiChrosorb Si 60, 150 × 4 mm, 5 μm	hexane, after 60 min replaced with EtOH: THF:H_2O (60:40:1)	UV fluores (280/310 nm)[a]	0.2 $\mu g\ g^{-1}$	−/−	108
NPEO	technical surfactants		LiChrosorb-Diol, 250 × 4.6 mm, 5 μm	hexane:CH_2Cl_2 (95:5, A)/ hexane:CH_2Cl_2:*i*PrOH (5:4:1, B)	UV absorption (275 nm)	51−132 ng of individual NPEO	−/2.5−5.6	109
			Perkin-Elmer Amino SIL-X-100, 250 × 4.6 mm, 5 μm	hexane:*i*PrOH (95:5)	UV absorption (275 nm)			
			Perkin-Elmer HS-5 C_{18}, 150 × 4.6 mm, 5 μm	*H_2O:THF:hexane, (3:14:83)*	UV absorption (275 nm)			
OPEO	technical surfactants		CSC-C1,[b] 150 × 4.6 mm, 5 μm	MeOH:H_2O (53:47 or 60:40) + 0.02 g L^{-1} NH_4OOCCH_3	UV absorption (225 nm)		−/−	110
APEO	river water, drinking water	solid-phase extraction	Spherisorb-CN, 250 × 4.6 mm, 5 μm	*i*PrOH (A)/hexane (B)	UV absorption (?)		−/−	50
			Spherisorb-ODS, 250 × 4.6 mm, 5 μm	*MeOH (A)/H_2O + 0.1% acetic acid (B)*	UV absorption (?)			
APEO	river water, drinking water	solid-phase extraction	*LiChrosorb RP18, 250 × 4 mm*	*H_2O:MeOH (9:1, A)/ CH_3CN (B)*	UV absorption (?)		−/−	56, 57
			μ-Bondapack C-18, 300 × 7.8 mm, 10 μm	*H_2O:MeOH (9:1, A)/ CH_3CN (B)*				
APEO	influent and effluent water of a STP	solid-phase extraction	μ-Porasil	hexane (A)/5% DMSO in $CHCl_3$ (B)	UV absorption (280 nm)	2.0	91−97/5−23	69
APEO	influent and effluent water of a STP	sublation technique by Wickbold	Whatman Partisil 5 PAC or DuPont Zorbax NH_2, 250 × 4.6 mm	MeO*t*Bu + 0.1% acetic acid (A)/CH_3CN: MeOH (95:5) + 0.1% acetic acid (B)	UV fluores (230/302 nm)[a]	0.2 ng of each individual APEO	−/−	69, 70
NPEO	treated waste water, river water	solid-phase extraction	Rainin Microsorb CN, 250 × 4.6 mm, 5 μm	THF:hexane (20:80, A)/ H_2O:*i*PrOH (10:90, B)	UV fluores (229/310 nm)[a]	0.1	84/4	35
NPEO	river water, sediment	separatory funnel Soxhlet extraction	Hypersil APS, 100 × 2.1 mm, 5 μm	hexane:*i*PrOH (98:2)	UV fluores (230/300 nm)[a]	0.02 (water), 0.015 $\mu g\ g^{-1}$ d.w.	−/−	43
NPEO	water, sewage sludge	SPME	Supelcosil LC-NH_2, 250 × 4.6 mm, 5 μm	hexane:*i*PrOH (9:1, A)/ *i*PrOH:H_2O (9:1, B)	UV absorption (220 nm)	1.57 for each individual NPEO	−/−	63
NPEO	influents and effluents from river water, STPs, drinking water	solid-phase extraction	*Alltech C-8, 250 × 2.1 mm, 5 μm*	*MeOH (A)/H_2O (B), both containing 0.1 mmol L^{-1} TFA*	ES-MS in the positive ion mode	0.6 (influent), 0.02 (effluent), 0.002 (river water), 0.0002 (drinking water)	97/4.3 (infl.), 98/5.1 (effl.), 96/5.0 (river), 98/7.8 (drink.)	58
NP	water	solid-phase extraction	*Kromasil ODS, 250 × 2 mm, 5 μm*	*0.005 M NH_4OOCCH_3 (A)/ CH_3CN (B)*	UV absorption (280 nm)	−	81/0.3	111
NP	sewage sludge	steam distillation/ solvent extraction	Hypersil APS, 100 × 4.6 mm, 3 μm	hexane (A)/hexane: *i*PrOH (85:15, B)	UV absorption (277 nm)	−	82/16	112
NP	biological matrices	steam distillation/ solvent extraction	Hypersil APS, 125 × 4 mm, 3 μm	hexane (A)/hexane: *i*PrOH (80:20, B)	UV fluores (260/300 nm)[a]	−		113
AP, APEO	mixtures of nonionic tensides		*Nucleosil C_{18}, 250 × 4 mm*	*MeOH:H_2O (9:1)*	differential refractometer	2.0 $\mu g\ mL^{-1}$	−/−	114
AP, APEO	sewage sludge	steam distillation/ solvent extraction	LiChrosorb-NH_2, 250 × 4 mm	hexane:*i*PrOH (9:1)		−	93−105/−	11, 30
			LiChrosorb RP-8, 250 × 3 mm	*MeOH:H_2O (3:1)*	UV absorption (278 nm)			
NP, NPEO	municipal waste water, river water, sewage sludge	steam distillation/ solvent extraction	LiChrosorb-NH_2, 250 × 4.6 mm, 10 μm	hexane (A)/hexane: *i*PrOH (1:1, B)	UV absorption (277 nm)	0.5 (water)	>80/3−7	32, 78, 84, 106
			Hypersil-APS, 60 × 4 mm, 3 μm	hexane (A)/hexane: *i*PrOH (85:15, B)	UV absorption (277 nm)			
			LiChrosorb RP-8, 250 × 3 mm, 10 μm	*MeOH:H_2O (8:2)*	UV absorption (277 nm)			

NP, NPEO	municipal waste water, primary and secondary effluents from STPs	sublation technique by Wickbold	LiChrosorb-NH_2, 250 × 4.6 mm, 10 μm	hexane:*i*PrOH (9:1, A)/ *i*PrOH:H_2O (9:1, B)	UV absorption (277 nm)	1.0 (lower NPEO), 3.0 (higher NPEO)	87/11	65
			Spherisorb-NH_2, 120 × 3 mm, 5 μm	hexane:*i*PrOH (9:1, A)/ *i*PrOH:H_2O (9:1, B)				
			Hypersil-APS, 100 × 4 mm, 3 μm	hexane:*i*PrOH (98:2, A)/ *i*PrOH:H_2O (98:2, B)				
NP, NPEO	waste water	sublation technique by Wickbold	Waters Resolve CN, 100 × 8 mm, 10 μm	CH_3CN:H_2O (95:5)	UV absorption (229 nm)	40.0	71−78/2 (NP1−4EO)	115
			Waters Resolve C 18[b], 100 × 8 mm, 5 μm	CH_3CN:H_2O (98:2)			83−92/2 (NP3−15EO)	
			Nova-Pak C 18, 100 × 8 mm, 5 μm	*MeOH:H_2O (90:10)*				
NP, NPEO	sludge-amended soils	Soxhlet extraction	Aminosilica,3 μm	1% *i*PrOH in hexane	UV fluores (225/304 nm)[a]		−/−	82
NP, NPEO, LAS	laundry detergents, hard surface cleaners, sewage sludge, sludge-amended soils, river sediments	Soxhlet extraction	*LiChrosorb RP-8, 100 × 4 mm, 10 μm*	*iPrOH (A)/H_2O (B)/ CH_3CN:H_2O (45:55) + 0.02 M $NaClO_4$ (C)*	UV absorption (225 nm) or UV-fluores (230/295 nm)[a]	95 ng of NP, 65 ng of NPEO	93−100/1.7−5.6	80, 116
			Octadecylsilica ODS II, 250 × 4 mm, 3 μm	*iPrOH (A)/H_2O (B)/ CH_3CN:H_2O (45:55) + 0.02 M $NaClO_4$ (C)*				
			Hypersil APS, 100 × 4 mm, 3 μm	hexane:*i*PrOH (98.5:1.5)	UV absorption (277 nm)			
NP, NPEO, LAS	marine water, sediment	solid-phase extraction	Hypersil APS, 100 × 4 mm, 3 μm	hexane (A)/hexane: *i*PrOH (98.8:1.2, B)	UV fluores (225/304 nm)[a]		99/4	37, 59
			Spherisorb S3 ODS II, 250 × 4 mm, 3 μm	*CH_3CN (A)/CH_3CN: H_2O (25:75) + 10 g L^{-1} $NaClO_4$ (B)*	UV fluores (225/295 nm)[a]			
			LiChrosorb RP-8, 100 × 4 mm, 10 μm	*CH_3CN (A)/CH_3CN: H_2O (25:75) + 10 g L^{-1} $NaClO_4$ (B)*				
APEC	technical tensides		*LiChrosphere 100 RP-18, 125 × 4 mm, 5 μm*	*MeOH:H_2O:CH_3CN (75:15:10) + 4 mmol L^{-1} NMe_4HSO_4 + 1 mmol L^{-1} NMe_4OH*	UV absorption (276 nm)	100.0	−/0.65	117
NPEC	influent and effluent water from STPs, river water	separatory funnel	LiChrosorb-NH_2, 250 × 4.6 mm, 10 μm	0.25% *i*PrOH in hexane	UV absorption (277 nm)	1.0	83/7.6	12
APEO, APEC	drinking water	liquid/liquid extraction	*LC (Brownlee Labs), 250 × 4.6 mm*	*H_2O + 0.01% NH_4OOCCH_3 (A)/MeOH (B)*	PB-MS in the EI mode		−/−	107
NP, NPEO, NPEC	influent and effluent water of a STP	solid-phase extraction	*Supelco C_8, 250 × 4.6 mm, 0.5 μm*	*1 mmol L^{-1} phosphate buffer (pH 6.5) + 10 mmol L^{-1} NEt_4Cl (A)/MeOH (B)*	UV fluores (225/295 nm)[a]	0.2 (NP), 0.6 (NPEO)	90/7 (NP), 98/5 (NPEO), 97/5 (NPEC)	34
NPEO,NPEC, LAS, SPC	influent and effluent water of a STP	solid-phase extraction	*LiChrosphere RP-18, 250 × 4 mm, 5 μm*	*MeOH or CH_3CN (A)/H_2O containing $NaClO_4$, TFA, or $TBAH_2PO_4$ (B)*	UV fluores (225/295 nm)[a]		88−91/3 (NPEC)	36
NPEO,NPEC, LAS, SPC	influent and effluent water of a STP, sewage sludge	Soxhlet extraction	*LiChrosphere RP-18, 250 × 4 mm, 5 μm*	*CH_3CN (A)/H_2O: CH_3CN (75:25) + 10 mg L^{-1} $NaClO_4$ (B)*	UV fluores (225/295 nm)[a]		92/6 (NPEC)	81
			LiChrosorb RP-18, 100 × 4 mm, 10 μm					

[a] (excitation/emission-wavelength). [b] Reversed-phase column operated in normal-phase mode.

Table 2. Determination of APEOs, APECs, and APs by GC

compd	sample type	enrichment,derivatization	column	temp	detection (reactant gas)	detection limit (μg L^{-1})	recovery (%)/rel std dev (%)	ref
APEO	technical surfactants	−, derivatization with pyridine/HMDS/TMCS	FSOT-column coated with OV-1 (HT-GC), 10 m × 0.53 mm, 0.1 μm		FID		−/−	123
		−, derivatization with pyridine/HMDS/TMCS	FSOT-column coated with SE-54 (CSFC), 10 m × 0.05 mm, 0.1 μm		FID			
APEO	river water	solid-phase extraction	S.G.E. DB-1, 25 m × 0.25 mm	60−275 °C in 4 °C min^{-1}	EI-MS		−/−	57
APEO	effluent of a STP	liquid−liquid extraction	Chrompack CP-Sil 5 CB, 25 m	40−250 °C in 8 °C min^{-1}	FID, CI-MS (CH_4)		−/−	73
APEO	drinking water	liquid−liquid extraction	DB-1, 30 m × 0.32 mm, 0.25 μm	50−320 °C in 4 °C min^{-1}	EI-MS		−/−	107
NPEO	waste water, coastal seawater and sediments	solid-phase extraction, derivatization with diazomethane or BSTFA	SE-54, 25 m × 0.32 mm, 0.25 μm	60−300 °C in 6 °C min^{-1}	FID, SIM-EI-MS, CI-MS (CH_4)		91−94/−	42
NP	technical NP		HP-1, 25 m × 0.32 mm, 0.25 μm		FID		−/−	124
NP	technical NP		Petrocol DH, 100 m × 0.25 mm, 0.25 μm	170, 180, or 190 °C (isothermal)	EI-MS, FTIR		−/−	118
NP	technical NP	solid-phase extraction, derivatization with diazomethane or BSTFA	10% SE 30 packed on Chromosorb-W		FID, EI-MS		−/−	125
			Carbowax-20M, 50 m × 0.25 mm, 0.25 μm					
			PONA, 50 m × 0.2 mm, 0.5 μm					
NP	ground water, drinking water	solid-phase extraction	SE-54, 50 m × 0.32 mm, 0.15 μm	100−270 °C in 4 °C min^{-1}	MS		−/−	126
NP	river and coastal water	solid-phase extraction	J & W DB-5, 30 m × 0.25 mm, 0.25 μm		EI-MS	0.03−0.2	78−110/12	61
NP	effluent and sludge from STPs	liquid−liquid extraction of the effluents, SFE of the sludges, in-situ derivatization with acetic anhydride	HP-5-MS, 30 m × 0.25 mm, 0.25 μm	70−240 °C	SIM-EI-MS	0.1 (effluent), 0.1 mg kg^{-1} (sludge)	93−98/3.2−5.3	74
NP	sewage sludge, sediments	Soxhlet extraction, derivatization with PFBBr	DB-5, 30 m × 0.25 mm, 0.25 μm	70−310 °C	ECD	2.9 pg g^{-1} d.w.	70/10	44
			DB-5 MS, 30 m × 0.25 mm, 0.25 μm	70−300 °C	SIM-EI-MS, NCI-MS (CH_4)	1.4 pg g^{-1} d.w., 0.3 pg g^{-1} d.w.		
NP	sewage sludges	steam distillation/ solvent extraction, derivatization with PFBCl	DB-17, 20 m × 0.18 mm, 0.3 μm	50−240 °C	FID, SIM-EI-MS	0.5 mg kg^{-1} d.w.	105/−	41
NP	sewage sludge	steam distillation/ solvent extraction	OV-73, 20 m × 0.31 mm	80−270 °C in 3 °C min^{-1}	FID		93−105/4	11, 30
NP	soil samples	steam distillation/ solvent extraction	fused silica column coated with OV-1, 30 m × 0.25 mm, 0.25 μm	70−240 °C	FID, SIM-EI-MS	0.05−0.1 mg kg^{-1}	93−102/7−16	31
NP	biological matrices	steam distillation/ solvent extraction	DB-5 MS, 60 m × 0.25 mm, 0.25 μm	70−315 °C	SIM-EI-MS		−/−	113
NP	landfill leachates	separatory funnel	DB-5, 30 m, 0.25 μm	70−300 °C in 4 °C min^{-1}	MS	1.0	−/38	127
NP, NPEO	river water	solid-phase extraction	S.G.E. BP-5, 25 m × 0.22 mm, 0.25 μm	60−260 °C in 4 °C min^{-1}	EI-MS		−/−	55
NP, NPEO	waste water, sewage sludge, biota	liquid−liquid extraction, derivatization with PFBCl	S.G.E. BP-1, 25 m × 0.22 mm, 0.25 μm	80−310 °C	ECD	0.1−1.0 (water), 0.1−2 mg kg^{-1}	84−98/2−7 (NP), 34−100/2−7 (NPEO)	71
			SE-54 15 m × 0.25 mm	80−300 °C	EI-MS, CI-MS (CH_4)	d.w. (sludge), 1−6 μg kg^{-1} (biol. matrices)		
NP, NPEO	waste water, river water, sediment	separatory funnel (water), Soxhlet extraction (sediment)	glass column packed with 3% SP-2100 on Supelcoport, 180 cm × 2 mm		FID		−/−	38
			SE-52, 25 m × 0.25 mm		EI-MS			

NP, NPEO	effluents from STPs, surface waters	(a) separatory funnel, (b) continuous liquid−liquid extraction	DB-5 (CI), 30 m × 0.25 mm	70−280 °C in 3−4 °C min^{-1}	CI-MS (CH_4)		−/−	72
			ORION SE-54 (EI), 25 m × 0.25 mm	70−280 °C in 3−4 °C min^{-1}	SIM-EI-MS			
NP, NPEO	effluents from STPs	(a) separatory funnel, (b) continuous liquid−liquid extraction	glass capillary column coated with OV-73, 15 m × 0.3 mm	50−280 °C in 2 °C min^{-1}	FID, EI-MS	10.0	79−87/4−8 (sep. funnel), 87−101/3−10 (cont. liq.−liq.)	8
NP, NPEO	effluents from STPs	steam distillation/solvent extraction	glass capillary column coated with OV-1, 20 m × 0.3 mm	50−270 °C in 3 °C min^{-1}	EI-MS	semiquantitative	−/−	76, 77
OPEO, OPEC	technical tensides		DB-5, 30 m × 0.25 mm,0.25 μm	70−300 °C in 3 or 10 °C min^{-1}	EI-MS, CI-MS (CH_4)	1.0	−/−	119−121
APEO, APEC	technical tensides	(a) separatory funnel, (b) continuous liquid−liquid extraction derivatization with BSTFA	ORION SE-54, 25 m × 0.25 mm	50−280 °C in 3 °C min^{-1}	EI-MS		−/−	122
NP, NPEO, NPEC	influents and effluents from STPs, river water	(a) separatory funnel, (b) sublation, derivatization with BF_3 or HCl/MeOH	glass capillary column coated with SE-54 or PS-255, 19 m × 0.3 mm	50−280 °C in 4 °C min^{-1}	FID or EI-MS	1.0	82/3 (sep. funnel)	12
APEC, BrAPEC	effluents from STPs	solid-phase extraction, derivatization with diazomethane	glass capillary column coated with SE-54, 50 m × 0.3 mm	50−300 °C in 3 °C min^{-1}	EI-MS		−/−	9

Figure 8. Total ion current chromatogram and SIM traces of characteristic ions of NP from common mussels obtained by GC/EI-MS in the SIM mode. Numbers are referring to the different NP isomers; the internal standard 4-*n*-NP is not shown.

sensitively analyzed by GC coupled to an electron capture detector (ECD), EI-MS, or negative chemical ionization (NCI) MS. Wahlberg et al. successfully used this method for the determination of these analytes in waste water, sewage sludge, and common mussel. Standard solutions of NPEO were prepared by liquid chromatographic separation of technical NPEOs into discrete oligomers on silica gel columns (250 × 20 mm). Decachlorobiphenyl was used as an internal standard.[71] Chalaux et al. applied pentafluorobenzyl bromide (PFBBr) to derivatize NP from sewage sludge and sediments. Their intercomparison between different detection systems (ECD, EI-MS, and NCI-MS) showed that the lowest detection limits can be achieved by the use of NCI-MS in the SIM mode (0.3 pg g^{-1} d.w.).[44] Lee et al. developed an in-situ derivatization procedure whereupon NP from effluent water and sewage sludge was simultaneously extracted and converted into the corresponding acetyl derivatives (see also section II.B.3.). Quantification of NP in the sample extracts was carried out by the external standard method using technical NP and OP as calibration standards which were acetylated in the same manner as the samples. Furthermore, the mass spectra of each single peak were interpreted in terms of the structure of the nonyl chain.[74] The acidic biodegradation products of APEOs, namely APECs, have been also identified by GC/MS after derivatization. Methylation of APECs with diazomethane,[9] 10% solution of BF_3 in methanol,[12] or 1 M solution of HCl in methanol[12] or silylation of APECs with BSTFA[122] yields derivatives which can be easily chromatographed on fused silica capillary columns.

6. Supercritical Fluid Chromatography (SFC) and Capillary Electrophoresis (CE)

In SFC gases above their critical temperatures and pressures are used as mobile phases. Under these conditions the supercritical fluids have the densities of liquids while retaining the diffusion coefficients of typical gases. Because of the high solvent strength of many supercritical phases, SFC gives higher resolution and higher speed than HPLC for many analyses, while not requiring that the compounds be volatile, as GC does. Nevertheless, SFC allows use of the sensitive GC detector, the FID, which has a linear response over a wide range of concentrations and is easier to handle than conventional HPLC detectors.

To the best of the authors' knowledge only applications in technical surfactant analysis have been published. Sandra et al.[123] and Wang et al.[128,129] described capillary SFC methods for the separation and identification of OPEOs and compared the results with those obtained by high-temperature GC and HPLC, respectively. It was shown that GC gives a higher resolution and better reproducibility of retention times and peak areas than SFC, but with SFC, OPEOs with more than 22 EO units are eluted at relatively low temperatures.[123] Compared to HPLC, SFC provides a better separation efficiency but a lot of parameters that can affect the SFC performance have to be optimized.[128] For more detailed information on SFC the reader is referred to Schmitt.[27]

CE of technical OPEO and NPEO using a fused-silica capillary (57 cm × 75 μm i.d.) and UV detection were performed to separate the surfactants into individual EO oligomers.[130, 131] In systematic investigations the separation efficiency could be considerably improved by addition of sodium dodecyl sulfate and high amounts of acetonitrile (20−40%) to the electrophoretic buffer. Despite these results the applicability of CE to the determination of nonionic surfactants is limited because of the insufficient peak resolution of lower and higher ethoxylates and relatively low detection sensitivity. Therefore, the authors recommended CE for product control.[131]

III. Environmental Concentrations

In the last two decades APEO/AP concentrations were intensively analyzed in samples from the different compartments of the environment. Therefore, there is a good understanding of the fate of APEOs in both sewage treatment plants and the aquatic environment. Holt et al. have already reviewed the APEO/AP concentrations in papers published up to 1990.[24] Therefore, the publications cited there are briefly treated here and supplemented by papers published after that date.

A. Sewage Treatment Plants

Alkylphenol polyethoxylates are primarily used in aqueous solutions as nonionic surfactants in detergents and cleaners. After use they are mainly discharged into sewage treatment plants (STPs) before they enter the aquatic environment. Table 3 gives an overview of the concentrations of APEOs and their metabolites in STP samples.

Giger et al. have thoroughly investigated the fate of NPEOs during the different steps of sewage treatment in the past decade. By comparison of the total concentrations of all NPEO oligomers and NP in influents (844−2250 μg L^{-1}) and secondary effluents (40−369 μg L^{-1}) of five STPs in Switzerland an average elimination rate of NPEOs/NP during the process of biological sewage treatment of approximately 80% was calculated.[65] Calculations on a molar basis, however, show that the average elimination rate of nonylphenolic compounds is even 59 ± 10% as a consequence of the biorefractory nature of NPEO biodegradation products.[83] Figure 1 illustrates the aerobic and anaerobic biodegradation pathways of NPEOs to their persistent metabolites. Specific analytical methods allow investigation of the changes in the NPEO composition during sewage treatment. Raw waste waters typically contain higher proportions of the lower NPEO oligomers than commercial NPEO products.[4,39,65,83] During activated sludge treatment, the higher oligomers (nEO > 8) totally disappear in favor of their metabolic products NP, NP1EO, and NP2EO and the carboxylated compounds NP1EC and NP2EC which are resistant to further microbial transformation and, therefore, are partially discharged into the aquatic environment via secondary effluents. For example, treated waste water from six STPs in Switzerland contained 1−14 μg L^{-1} NP, 20−158 μg L^{-1} NP1EO/NP2EO, and 71−330 μg L^{-1} NPEC.[12] Most of the NP, NP1EO, and NP2EO, however, is removed from the treated waste water by adsorption on the sludge because these metabolites have lost their hydrophilic moieties and have become more lipophilic. During anaerobical sludge stabilization NP1EO and NP2EO are further biodegraded to NP, resulting in very high NP levels of 450−2530 mg kg^{-1} d.w. in digested sludge.[11] Giger et al. estimated the mass flows of nonylphenolic compounds in 11 STPs in Switzerland on a molar basis. Starting from 100% NP-c (70% NPnEO, 20% NP1EO/NP2EO) in the raw sewage 60% of these compounds are released into the environment via secondary effluents (40%) and digested sludge (20%). The secondary effluents consist of approximately 50% NPECs and 25% NP1EO/NP2EO, whereas the digested sludges contain 95% NP each relative to the total amount of NP-c.[4,83]

In a comprehensive study on the determination of NPEOs/NP in effluents entering the Fox river of Wisconsin (U.S.) seasonal effects could be observed. NPEO influxes from STP were half as much in summer (10.4 kg day^{-1}) as in winter (22.1 kg day^{-1}). Discharges from paper mills, however, were essentially the same in winter and summer (34.8 and 32.9 kg day^{-1}).[139]

Two independent studies on the NPEO concentrations in influent and effluent water samples from various STPs in England and Italy, respectively, showed that the highest concentrations were found in samples from STPs receiving wastewaters from textile processing. The NP concentrations in effluents from a STP in England treating wastewaters from a textile-based industrial area were 330 μg L^{-1}. The majority of investigated effluents, however, contained 1−5 μg L^{-1} NP.[61] NPEO concentrations

Table 3. Concentrations of APEOs, APECs, and APs in Sewage Treatment Plant Samples

location of the STP	analytical method	influent (μg L^{-1})	effluent (μg L^{-1})	sewage sludge (anaerobically stabilized) (mg kg^{-1} d.w.)	ref (year)
Toronto, Canada	in-situ acetylation; GC/SIM-EI-MS		1.0−15.1/NP	137−470/NP	74 (1995)
Hösel-Dickelsbach, Germany	SPE; HPLC	March: 770 ± 60/APEO; September: 730 ± 60/APEO	March: 230 ± 20/APEO; September: 170 ± 20/APEO		69 (1987)
Germany	liquid−liquid extraction; GC/EI-MS	10−15/NP, 2−10/NP1EO, 2−10/NP2EO	1−2/NP, 1−7/NP1EO, ≤10/NP2EO	130−400/NP, 5−40/NP1EO, ≤3/NP2EO	132 (1987)
Germany	steam dist./solvent extr.; GC/SIM-EI-MS			22.1−1193/NP	41 (1995)
Germany	Soxhlet extraction; GC/MS			90−1300/NP	133 (1997)
Four STPs/Italy	Soxhlet extraction; HPLC	5700−9000/NPEO, 600−1100/NPEC	400−480/NPEO, 500−1600/NPEC	210/NP, NPEO[a] 40/NPEC[a]	81 (1993)
Rome, Italy	SPE; HPLC	2.7−7.5/NP, 64−115/NPEO	0.7−2.6/NP, 4.7−9.7/NPEO, 1.5−3.9/NP1EC, 5.1−9.4/NP2EC		34 (1994)
Three STPs/Rome, Italy	SPE; HPLC	127−221/NPEO	2.2−4.1/NPEO		58 (1995)
Barcelona, Spain	SPE; GC/SIM-EI-MS	37−123/NPEO			42 (1990)
Four STPs/Barcelona, Spain	Soxhlet extraction; GC/NCI-MS			20−350/NP	44 (1994)
Eight STP/Sweden	liquid−liquid extraction; GC/EI-MS or CI-MS			400−1200/NP, 20−190/NP1EO, 1−50/NP2EO	71 (1990)
Six STPs/Switzerland	liquid−liquid extraction; GC/EI-MS		<10−35/NP, <10−133/NP1EO, <10−70/NP2EO		8 (1982)
Three STPs/Switzerland	steam dist./solvent extr.; GC/EI-MS		8−35/NP, 24−130/NP1EO, 3−70/NP2EO		77 (1982)
33 STPs/Switzerland	steam dist./solvent extr.; HPLC			450−2530/NP	11 (1984)
Two STPs/Switzerland	steam dist./solvent extr.; HPLC		5.2−13.6/NP, 13−63/NP1EO, 23−72/NP2EO		78 (1984)
STP/Zürich, Switzerland	steam dist./solvent extr.; HPLC	14/NP, 18/NP1EO, 18/NP2EO	8/NP, 49/NP1EO, 44/NP2EO	1000/NP, 79/NP1EO, not detectable/NP2EO	32 (1985)
Five STPs/Switzerland	sublation; HPLC	844−2250/NPEO + NP	40−369/NPEO + NP		65 (1985)
Switzerland	liquid−liquid extraction; GC/SIM-EI-MS		36−69/NP, 25−189/NP1EO, 37−198/NP2EO		72, (1985)
Zürich, Switzerland	steam dist./solvent extr.; HPLC			1520−1900/NP, 340−410/NP1EO	134 (1985)
13 STPs/Switzerland	steam dist./solvent extr., sublation; HPLC	20−47/NP, 400−2200/NPEO	1−13/NP, 12−120/NPEO	150−2200/NP	4, 39, 135 (1986)
Six STPs/Switzerland	liquid−liquid extraction; HPLC	21−57/NP, 81−254/NP1,2EO, 7−14/NPEC	1−14/NP, 20−158/NP1,2EO, 71−330/NPEC		12 (1987)
Switzerland	SPE; HPLC	280 ± 30/NP, 1920 ± 140/NPEO	30 ± 4/NP, 230 ± 30/NPEO		60 (1987)
Two STPs/Switzerland	Soxhlet extraction; HPLC			1200/NP, 220/NP1EO, 30/NP2EO	80 (1987)
29 STPs/Switzerland	Soxhlet extraction, SPE; HPLC			640−2200/NP, 90−680/NP1EO, 20−220/NP2EO	136, 137 (1988)
11 STPs/Switzerland	steam dist./solvent extr., sublation; HPLC	1090−2060/NP-c[b]	240−760/NP-c[b]		83 (1994)
U.K.	sublation; HPLC	126−410/APEO	40−228/APEO		70 (1986)
Two STPs/U.K.	steam dist./solvent extr.; HPLC			256−824/NP	112 (1994)
12 STPs/U.K.	SPE; GC/EI-MS		<0.2−330/NP		61 (1995)
Two STPs/North Carolina,U.S.	SPE; HPLC	1600−2500/NPEO	1−2.5/NP, 50−100/NPEO		35 (1990)
U.S.	SPE, steam dist./solvent extr.; HPLC	1130−2400/NPEO	43−103/NPEO	1.8−2.8/NP	138 (1992)
Los Angeles, CA, U.S.	Soxhlet extraction; GC/NCI-MS			370/NP	44 (1994)
U.S.	SPE; GC/CI-MS		143−272/NPEC		62 (1996)
Six STPs/Wisconsin, U.S.	steam dist./solvent extr.; HPLC		March: 0.83−14.0/NP, 47.1−353/NP-c;[b] July, September: 0.18−15.9/NP, 8.77−78.8/NP-c[b]		139 (1996)
15 paper mills/Wisconsin, U.S.	steam dist./solvent extr.; HPLC		March: <0.02−21.2/NP, 3.13−729/NP-c;[b] July, September: 0.08−28.6/NP, 1.28−712/NP-c[b]		139 (1996)

[a] Concentrations in activated sewage sludge. [b] NP-c: sum of all nonylphenolic compounds.

in samples from STP near Prato, Italy, dealing with textile waste waters ranged from 5700 to 9000 μg L^{-1} in influent water and from 400 to 480 μg L^{-1} in effluent water, while NPEC concentrations were 600−1100 μg L^{-1} in influents and 500−1600 μg L^{-1} in effluents. NPEOs, therefore, was remarkably biodegraded before reaching the STP since NPECs was already present in the influents.[81] The situation was different in case of sewages of prevailing domestic origin. Thus, the NPEO contents in effluents from STPs in the area of Rome in 1994[34] and 1995[58] ranged from 4.7 to 9.7 and from 2.2 to 4.1 μg L^{-1}, respectively, suggesting that NPEOs plays only a secondary part in household formulations.

A number of studies were carried out about the concentrations of NP in sewage sludges. Sweetman et al. found between 256 and 824 mg kg^{-1} d.w. NP in sludges from two STPs in England.[112] Sewage sludges originating from all western German states from 1987 until 1989 have been analyzed for NP. In almost all 149 samples NP could be found in the range from <0.5 to 1193 mg kg^{-1} d.w., and 50% of the samples contained NP up to 83.4 mg kg^{-1} d.w.[41] Lee et al. collected sludge samples from two STPs at Toronto (Canada) for determination of NP. The concentrations were 137 and 470 mg kg^{-1} d.w., respectively.[74]

B. Water

1. River Water

After sewage treatment of APEO-containing wastewaters a number of persistent metabolites have been formed which are released to the aquatic environment. A summary of their concentrations in river waters are given in Table 4.

Giger et al. extensively examined the concentrations of all relevant metabolic products of NPEO in the Glatt river located between Greifensee and the Rhine river in Switzerland for the last 15 years.[12,32,33,77,78,141,142] Longitudinal concentration profiles of NP1EO and NP2EO showed increases from 0.5 μg L^{-1} to 13 and 16 μg L^{-1}, respectively, with increasing the distance from the outflow of Greifensee due to STP discharge points along the river. In contrast, NP showed only a small increase to an upper level of 2.0 μg L^{-1}.[32,78] Studies about the occurrence of NP-c in the Glatt river indicate that NPECs are the most abundant compounds among them.[12,33] The concentrations of NP1EC and NP2EC ranged between 1.0−45 and 2.0−71 μg L^{-1}, respectively, followed by lipophilic NP1EO (<0.3−69 μg L^{-1}), NP2EO (<0.3−30 μg L^{-1}), and NP (<0.3−45 μg L^{-1}). This distribution profile of NP-c in river water was similar to that of secondary effluents.[4,83] More-

Table 4. Concentrations of APEOs, APECs and APs in River Water

river/country	analytical method	concn (μg L^{-1})	ref (year)
35 sites/Canada	in-situ acetylation, liq.-liq. extr.; GC/EI-MS	<0.01−0.92/NP, <0.02−7.8/NP1EO, <0.02−10/NP2EO, <0.005−0.084/OP	43 (1997)
Main/Germany		0.038−0.05/NP	140 (1992)
14 sites/Bavaria, Germany	liq.-liq. extraction; GC/MS	0.01−0.4/NP	133 (1997)
rivers near Rome/Italy	SPE; LC/MS	0.64−4.3/NPEO	58 (1995)
−/Japan	CTAS	50−70/NPEO	51 (1982)
Glatt/Switzerland	steam dist./solvent extr.; GC/EI-MS	3.0/NP, not detectable/NP1EO, 25.0/NP2EO, 9.0/NP3EO	77 (1982)
Glatt/Switzerland	steam dist./solvent extr.; HPLC	0.5−1.8/NP, 0.5−12.7/NP1EO, 0.5−15.7/NP2EO	32, 78 (1984)
Glatt/Switzerland	steam dist./solvent extr.; HPLC	0.7−26.0/NP, 2.0−20.0/NP1EO, 0.8−21.0/NP2EO	141, 142 (1986)
Glatt/Switzerland	liq.-liq. extr.; HPLC	<0.5−4.0/NP, <0.5−24.5/NPEO, 2.0−116.0/NPEC	12 (1987)
Glatt/Switzerland	steam dist./solvent extr., sublation, liq.-liq. extr.; HPLC	<0.3−45.0/NP, <0.3−69.0/NP1EO, <0.3−30.0/NP2EO, <1.0−45.0/NP1EC, 2.0−71.0/NP2EC	33 (1994)
Lake Geneva/Switzerland	liq.-liq. extr.; GC/SIM-EI-MS	1.2−3.4/NP, 1.1−4.1/NP1EO, 1.3−5.8/NP2EO	72 (1985)
six rivers/U.K.	SPE; GC/EI-MS	0.2−180.0/NP	61 (1995)
Colorado/Texas, U.S.	SPE; HPLC	1.1−1.9/NPEO	35 (1990)
30 rivers/U.S.	steam dist./solvent extr.; HPLC	<0.11−0.64/NP, <0.06−0.6/NP1EO, <0.07−1.2/NP2EO	143 (1992)
Fox/Wisconsin, U.S.	steam dist./solvent extr.; HPLC	0.12−0.29/NP, 0.04−0.37/NP1EO, 0.88−3.38/NP2−17EO	139 (1996)
Sava/former Yugoslavia	steam dist./solvent extr., liq.-liq. extr.; HPLC	0.7/NP, 0.4/NP1EO, 0.2/NP2EO, 5.0/NPEC	106 (1991)

over, compositional changes in the river water were calculated by evaluating all inputs from STPs to the Glatt river and comparing these data with the output mass flow which was determined on the basis of the results of the sample location just before the river mouth. The greatest changes were observed for NPECs, which increased from 51 to 85% of the total, and for NP*n*EO ($n = 3-20$), which decreased from 21 to 3.5% of the total. Aerobic biotransformation of NP*n*EO causes the significant increase of NPECs. By comparing the total output of NP-c (82 mol day^{-1}) to the total input load (108.2 mol day^{-1}), an overall elimination of NP-c of 24% could be calculated in the river water.[33]

A comprehensive monitoring study measured the levels of NP, NP1EO, and NP2EO in 30 rivers in the U.S. NP and NP1EO/NP2EO concentrations were mostly (60−75%) below their detection limits (0.1 μg L^{-1}). The highest levels found were 0.6 μg L^{-1} for NP and NP1EO and 1.2 μg L^{-1} for NP2EO.[143] A survey of the Fox river of Wisconsin, (U.S.) in 1995 showed similar NP1EO (0.04−0.37 μg L^{-1}) and NP levels (0.12−0.29 μg L^{-1}) compared to those of the 30-river study. The obtained data also indicated extensive biodegradation of NPEOs in river water during summer, little during the cold winter months.[139] Another extensive study dealt with the determination of NP2EO, NP1EO, NP, and OP in surface water from 35 sites in Canada in 1994 and 1995. Twenty-four percent of the water samples had detectable levels of NP and OP in the ranges from <0.01 to 0.92 μg L^{-1} and from <0.005 to 0.084 μg L^{-1}, respectively. Detectable concentrations of NP1EO and NP2EO were more frequent (58 and 32%, respectively) and the measurable levels were higher. The occurrences of these chemicals were closely related to areas of effluent discharge from pulp and paper mills.[43]

River water from 14 sites in Bavaria, Germany, were analyzed for NP in 1995. Near the outlets of STPs the highest NP concentrations were found ranging from 0.1 to 0.4 μg L^{-1}. At less contaminated sites the NP concentrations were between 0.01 and 0.08 μg L^{-1}.[133]

Six rivers in the U.K. were sampled to establish environmental concentrations of NP. The locations of the rivers ranged from predominantly agricultural to heavily industrialized areas. The highest concentrations of NP (up to 180 μg L^{-1}) were detected in a river receiving high inputs of surfactants derived from textile plants. Elsewhere, concentrations were much lower ranging between 0.2 and 12 μg L^{-1} NP.[61]

2. Marine Water

Marcomini et al. investigated the NPEO levels in the Lagoon of Venice, Italy. Surface marine water contained 0.2 μg L^{-1} NP, 0.73 μg L^{-1} NP1EO, 1.1 μg L^{-1} NP2EO, and 17.5 μg L^{-1} NPnEO ($n = 3-13$).[37] In a second study concentrations of NPEO in marine water from five locations in the Lagoon of Venice at various times of the year were reported. Average NPEO concentrations in April, July, and October were 2.3, 1.6, and 1.4 μg L^{-1}, respectively. The highest values were systematically found at a station facing an industrial zone.[59]

Sea water from the Mediterranean sampled 1.5 km offshore of Barcelona, Spain, was reported to contain 0.85 μg L^{-1} NPEOs.[42]

A comprehensive survey of concentrations of NP in estuaries in England and Wales was undertaken by Blackburn et al. Six estuaries and one harbor around the English coast were sampled. The majority of the samples (80%) contained <0.1 μg L^{-1} NP, and the maximum concentration was at 5.2 μg L^{-1}, probably resulting from inputs from surfactant manufacture.[61]

3. Ground Water and Drinking Water

The fate of NP/NPEOs during infiltration of river water to ground water was studied in Switzerland. Samples of infiltrated water were taken at various distances (2.5−14 m) from the Glatt river. With increasing distance from the river the average concentrations of NP (from 4.1 to 0.3 μg L^{-1}), NP1EO (from 7.5 to 0.1 μg L^{-1}), and NP2EO (from 8.2 to 0.1 μg L^{-1}) decreased.[141, 142] Ahel et al. analyzed NP, NPEOs, and NPECs in ground waters from three field sites near Zagreb, Croatia, a highly industrialized region. These compounds could be detected in all ground waters (<0.1 μg L^{-1} NP, NP1EO, and NP2EO; 0.05−0.2 μg L^{-1} NPEC), but the concentrations compared to those in river water indicated relatively efficient elimination during infiltration.[106]

NP was found at concentrations of 0.002 and 0.001 μg L^{-1} in ground water and drinking water, respectively, sampled in Schleswig Holstein, Germany.[126] Ground water samples taken near the river Main in Germany showed decreasing NP concentrations from 0.15 to 0.047 μg L^{-1} with increasing distance from the river.[133] New Jersey (U.S.) drinking water was analyzed by GC/EI-MS and found to contain 0.077 μg L^{-1} NP1EO and 0.147 μg L^{-1} NP2EO.[107] Marcomini et al. used the very sensitive LC/MS method to determine NPEOs in drinking water. The investigated drinking water from Rome contained 0.061−0.12 μg L^{-1} NPEOs.[58] Ventura et al. reported the occurrence of brominated NP in drinking water from Barcelona, Spain, due to high levels of bromide ions in the water which enter the waterworks and lead to the formation of brominated compounds during bromination.[55, 56]

C. Sediments and Soils

The biodegraded products of APEOs are more lipophilic than their parent compounds and, therefore, tend to adsorb on particulate matter in sludges and sediments. Table 5 gives a summary of APEOs/APs concentrations in river and marine sediments.

Due to the low solubility of NP, OP, NP1EO, and NP2EO in water and log K_{OW} values, greater than 4.0 sediments might be heavier contaminated than the surrounding water. In fact, concentrations of these analytes found in sediments are generally 1−3 orders of magnitude higher than the aqueous levels (see Table 4). Sediment samples from nine heavily industrialized sites in Canada were analyzed for NP, OP, NP1EO, and NP2EO. Values ranged from 0.17 to 72 mg kg^{-1} d.w. and from 0.01 to 1.8 mg kg^{-1} d.w. for NP and OP, respectively. NP1EO concentrations varied from <0.015 to 38 mg kg^{-1} and NP2EO

Table 5. Concentrations of APEOs and APs in Sediments

sampling site/country	analytical method	concn (mg kg^{-1} d.w.)	ref (year)
Canada	SFE; GC/SIM-EI-MS	0.29−41.1/NP, <0.005−0.91/OP	74 (1995)
nine sites/Canada	SFE, Soxhlet extraction; GC/SIM-EI-MS	0.17−72/NP, <0.01−1.8/OP, <0.015−38/NP1EO, <0.015−6/NP2EO	43 (1997)
Nile estuary/Egypt	Soxhlet extraction; GC/NCI-MS	0.019−0.044/NP	44 (1994)
Rhine/Germany	Soxhlet extraction; HPLC	0.9/NP, 0.8/NP1EO, 0.7/NP2EO	80 (1987)
Main/Germany		0.7/NP	140 (1992)
10 sites/Bavaria, Germany	Soxhlet extraction; GC/MS	0.1−10/NP	133 (1997)
Lagoon of Venice/Italy	Soxhlet extraction; HPLC	0.1−5.6/NP, 0.2−6.6/NP1EO, 0.1−1.5/NP2EO	59 (1990)
Besos estuary/Spain	Soxhlet extraction; GC/FID	6.6/NP	42 (1990)
offshore Barcelona/Spain	Soxhlet extraction; GC/NCI-MS	0.006−0.069/NP	44 (1994)
Glatt/Switzerland	steam dist./solvent extr.; HPLC	0.19−13.1/NP, 0.1−8.85/NP1EO, 0.08−2.72/NP2EO	33 (1990)
30 rivers/U.S.	steam dist./solvent extr.; HPLC	<0.003−2.96/NP, <0.002−0.175/NP1EO	143 (1992)
Fox/Wisconsin, U.S.	steam dist./solvent extr.; HPLC	0.026−1.04/NP, 0.004−0.22/NP1EO	139 (1996)

concentrations from <0.015 to 6 mg kg^{-1}. These results are comparable to the values obtained by other groups who analyzed the NP, NP1EO, and NP2EO concentrations in sediments in Germany,[80,140,133] Switzerland,[33] and the U.S.[143] Giger et al. also observed that river mud, rich in organic matter, contained considerably higher concentrations of NP, NP1EO and NP2EO than sand. The differences were much more pronounced for NP (differing by a factor of 15) than for NP1EO (differing by a factor of 3) due to the stronger lipophilicity of NP (log $K_{OW} = 4.5$).[33]

Marcomini et al. quantitatively determined NP, NP1EO, and NP2EO in sediments and resuspended sediments taken from the Lagoon of Venice at various times of the year. The artificial resuspension of the sediments served the purpose of simulating the natural disturbance in the lagoon caused by winds and currents. The sum of NP, NP1EO, and NP2EO in resuspended sediments was in the range 0.15−13.7 mg kg^{-1} d.w., at least 5 times higher than in the underlying 5 cm of sediment. Concentrations measured in February were approximately 1 magnitude higher than in April and July. A major factor for this trend was related to the proliferation of macroalgae during the spring and summer months which accumulated NP, NP1EO, and NP2EO (0.25 ± 0.15 mg kg^{-1} d.w.).[59] Marine sediments collected offshore Barcelona, Spain, contained remarkably lower NP concentrations in the range from 0.006 to 0.069 mg kg^{-1} d.w. than river sediments.[44]

Field investigations on the fate of NP-c in sludge-amended soils were carried out by a number of authors. Thus, Marcomini et al. presented one year's monitoring data of NP, NP1EO, and NP2EO in soil starting immediately after sludge application. The initial concentrations of NP, NP1EO, and NP2EO were 4.7, 1.1, and 0.1 mg kg^{-1} d.w., respectively. During the first 3 weeks the compounds rapidly decreased to approximately 20% of their initial levels, followed by a period in which the concentrations slowly leveled off. The residual mean concentrations, 320 days after the last sludge application, were 0.5, 0.12, and 0.01 mg kg^{-1} d.w. for NP, NP1EO, and NP2EO, respectively.[40,82] A Swedish paper deals with the decomposition of NP in soil. After 10 days an elimination of NP greater than 90% was detected. After 20 days the concentration reached the detection limit of 0.02 mg kg^{-1} d.w.[144] Diercxsens et al. also established an elimination rate >90% for NP in soil after 3 months of sludge application.[84] A sludge-amended soil sample from the Swiss Federal Research Station for Agricultural Chemistry and Environmental Hygiene contained 1.6, 0.4, and 0.07 mg kg^{-1} d.w. for NP, NP1EO, and NP2EO, respectively.[80]

D. Naturally Occurring Biological Matrices

Despite numerous investigations on the determination of bioconcentration factors (BCFs) for NPEOs and NP in organic matrices using field or laboratory experiments (see section IV.B), only a few reports exist relating to concentrations of NPEOs/NP in naturally occurring biological matrices.

Ahel et al. analyzed freshwater organisms from the surface waters in the Glatt Valley, Switzerland. NP, NP1EO, and NP2EO were extracted employing steam distillation/solvent extraction and determined by normal-phase HPLC. The concentrations of NP, NP1EO, and NP2EO in various macrophytic algae were in the ranges 2.5−38.0, 0.9−4.7, and 0.6−4.3 mg kg^{-1} d.w., respectively. The analyses of NP, NP1EO, and NP2EO in several fish species showed a higher accumulation of these compounds in liver (1.0, 1.8, and 1.4 mg kg^{-1} d.w. for NP, NP1EO, and NP2EO in *Squalius cephalus* Heck.) than in muscle (0.18, 0.18, and 0.13 mg kg^{-1} d.w. for NP, NP1EO, and NP2EO in *S. cephalus* Heck.). Lipophilic degradation products of NPEOs were also found in all of the tissues and organs of a wild duck. The highest concentrations of NP, NP1EO, and NP2EO were measured in the muscle tissue, respective values being 1.2, 2.1, and 0.35 mg kg^{-1} d.w.[145]

Günther et al. retrospectively investigated the occurrence of NP in common mussels (*Mytilus edulis*

L.) from Eckwarderhörne/North Sea in Germany over a period of 10 years (1985−1995).[113] The collection, homogenization, and storage of the mussels were performed in a special program of the Research Center Jülich according to the SOPs of the Environmental Specimen Bank (ESB) of Germany (see section II.A.).[49] After steam distillation/solvent extraction of the mussel samples and clean-up of the extracts on normal-phase HPLC the NP concentrations were determined by GC/EI-MS in the SIM mode in relation to 4-*n*-NP as the internal standard. The NP concentrations in mussels significantly decreased from 1985 to 1995.[113] This important result can be attributed to the voluntary commitment of the German surfactant industries[16] no longer using APEOs in any formulation of laundry or household surfactants from 1986.

IV. Risk Potential

An ecological risk assessment of the obtained data on APEO/AP concentrations in the different compartments of the environment will be only feasible if they are compared to data on bioaccumulation, toxicity, and estrogenicity. Another interesting aspect is the biodegradability of APEOs to gain a better insight into the biodegradation pathways and the parameters which influence it.

A. Biodegradability

A number of internationally standardized test methods have been established for assessment of the biodegradability of surfactants. To obtain a complete survey of the biodegradability of surfactants, the primary and ultimate degradations have to be analyzed. The methylenblue active substances (MBAS) and bismuth active substances (BiAS) methods are used for the determination of the primary biodegradability of the most common anionic and nonionic surfactants, whereas the ultimate degradation of surfactants to CO_2, H_2O, mineral salts, and biomass is determined by dissolved organic carbon (DOC) analyses. The different pathways for aerobic and anaerobic biodegradation of APEOs to their metabolites are shown in Figure 1. The biodegradability of surfactants, in general, is comprehensively reviewed by Swisher[29] and Karsa/Porter[146] and the biodegradability of nonionic ethoxylates, in particular, by Kravetz[147] and Holt et al.[24]

Mann and Reid evaluated the biodegradability of OPEOs by field trials with a trickling filter sewage treatment plant. Biodegradability rose from 26% in March to a level of about 80% in late August and September. During November and December biodegradation fell until by the end of January it was only 20−25%. The decrease in biodegradation was attributed to low biological activity during the cold winter months.[148] Stiff et al. studied the effect of temperature on the removal of OPEOs on a laboratory scale. Tests were carried out at 8, 11, and 15 °C using the "porous pot" activated sludge technique over a period of 122 days. At 20 mg L^{-1} the percentage removal was high during the period at 15 °C but at 11−12 °C and 8 °C varied between 40−95% and 20−80%, respectively, indicating that at low temperatures a stable population of microorganisms adapted to OPEOs could not be maintained.[149] Rudling and Solyom reported the biodegradation of NPEOs with the Organisation for Economic Cooperation and Development (OECD) screening test. NP*n*EOs (n = 8, 10, 14) were degraded to an extent of more than 90% within 12 days. Fifty percent of the predominantly formed degradation product NP2EO was eliminated at 20 °C after 28 days, while at 15 °C, no degradation was observed.[7] The dependence of ultimate biodegradation on temperature was shown in degradation experiments with labeled NPEOs. The conversion of ^{3}H-labeled aromatic ring of NPEO into ^3H_2O decreased from 20 to 2% by changing the temperature from 25 to 8 °C. Degradation of the ^{14}C-labeled ethoxylate chain to $^{14}CO_2$ decreased from 60% at 25 °C to 50% at 12 °C and to only 10% at 8 °C.[150] In another study by Kravetz et al. the ultimate biodegradation of NPEOs applying ^{3}H- (aromatic ring) and ^{14}C-labeled (EO chain) NPEO substrates in activated sludge aeration units was investigated.[150,151] The low release of tritium as ^3H_2O indicated that the ultimate degradation was slow; however, evolution of $^{14}CO_2$ from the EO chain of NPEOs was comparable with that from a labeled alcohol ethoxylate. Around 35−50% of the ^{3}H-labeled NPEOs were discharged into the effluent in form of soluble ^{3}H-labeled metabolites, whereas only 8−14% ^{14}C-labeled metabolites were detected in the effluent.[151] In an early report Patterson et al. showed that degradation of APEOs in aerated sewage effluent was also affected by the pH. An increase of the pH to 9.2 resulted in a faster degradation.[152]

Evidence for the presence of significant amounts of AP, AP1EO, AP2EO, and their carboxylated equivalents in sewage treatment is given in the section on environmental concentrations. The conversion of APEOs into several metabolites during aerobic and anaerobic biodegradation of sewage treatment, however, has been proved in laboratory-scale biodegradation experiments. Thus, Schöberl et al. investigated the ultimate biodegradation of NPEOs using the Coupled Units test or the OECD confirmatory test. In experiments with activated sludge inoculation the degradation rate of NPEOs was 76% within 40 days. The remaining part consisted of 18% NP2EO/NP3EO and 6.5% NP.[153] Brüschweiler et al. demonstrated the formation of NP2EO and also NP2EC as a second relatively stable intermediate in laboratory simulation tests.[10] Brunner et al. showed that during anaerobic sludge digestion NP2EO and NP1EO were transformed into nondegradable NP.[134] Ball et al. reported on the aerobic and anaerobic degradation of halogenated and nonhalogenated OPEOs and OPECs. Biodegradation of OP*n*EO (n = 1−5) by activated sludge inoculum resulted in the rapid transformation to the carboxylated OP*n*EC (n = 1−3) homologues with OP2EC as the main product within 24 h. OPEOs as well as OPECs were almost completely degraded by primary effluent inoculum after 127 days, whereas halogenated OPEOs and OPECs were insufficiently transformed into recalcitrant metabolites such as halogenated OP2EC under the same conditions. An anaerobic bioassay showed that OPEOs were completely degraded to OP as the

predominant product; however, OPECs and halogenated OPECs did not degrade under anaerobic conditions.[154]

Schöberl et al. investigated the degradation of NPEOs in pond water and sea water at different temperatures over a period of 50 days. The primary biodegradation of NPEOs at 20−23 °C were 33−36% in pond water and 95% in sea water. At 3−4 °C they were degraded to a maximum of 37% in pond water and to only 15% in sea water.[155] Schneider et al. monitored the biodegradation of NPEOs using the river die-away test. NPEOs were degraded to NP2EO as the predominant product within 3 days, while further biodegradation was considerably slower.[102] Ekelund et al. determined the ultimate biodegradation of labeled [^{14}C]NP in sea water by the collection and quantification of the formed $^{14}CO_2$. During the first 4 weeks degradation was very slow but increased to about 50% after a further 28 days. The degradation rate in the presence of sea water and sediment was high from the beginning due to a larger number of microorganisms.[156] Hellmann showed that the biodegradation of NPEOs in river water took place primarily through oxidation of the EO chain resulting in the formation of NPECs with 0−3 EO units.[157]

Trocme et al. studied the biodegradability of NP in sludge-amended soils[158] and in a compost−sandstone mixture[159] spiked with 100 and 1000 mg kg^{-1} technical NP. In both cases biodegradation of NP was approximately 90% after 100 mg kg^{-1} treatment but only approximately 60% after 1000 mg kg^{-1} treatment within 40 days. A rapid decrease in the respiration rates of the soils in 1000 mg kg^{-1} samples by the fourth day indicated a toxic effect of NP on the microorganisms.[158,159] Kirchmann et al. spiked soil samples with 10 and 500 mg kg^{-1} NP and determined the soil respiration by measurement of the CO_2 evolution. At high concentrations of NP respiration was significantly higher compared with the untreated control, whereas no effect was measured at the lower concentration of 10 mg kg^{-1}. In both cases the concentrations of NP reached the detection limit of 0.02 mg kg^{-1} after 20 days.[144] These results are apparently contrary to those of Trocme et al., but a reason for that may be the different concentration levels and experimental conditions.

Schöberl et al.[153,160] and Swisher[29] drew up a scheme for the biochemical pathway for biodegradation of APEOs. According to them biodegradation of APEOs occurs by sequential shortening of the EO chain caused by a hydrolase system. A pathway for the metabolism of the nonylphenol nucleus was not given.

B. Bioaccumulation

The transformation products of NPEOs such as NP, NP1EO, and NP2EO have a pronounced lipophilic character and, therefore, bioaccumulate in aquatic organisms. The quantitative measure of bioaccumulation is the bioconcentration factor (BCF), which is defined as the ratio between the concentration of the chemical in the tissues of an organism and the concentration of the chemical in water at steady state.[161−163] As a rule of thumb, the BCFs of organic compounds increase with increasing octanol/water partition coefficient $K_{O/W}$ and with decreasing solubility in water. A review on surfactant bioconcentration by Tolls et al. gives a collection and critical evaluation of BCF data for surfactants in general.[164]

Estimated values of the BCF for NP in common mussels (*M. edulis* L.) differ considerably in the literature. McLeese et al. exposed mussels to sea water containing NP at different concentrations and reported a BCF of 10.[165] Granmo et al. investigated the bioaccumulation of NP and its short-chained ethoxylates in caged mussels by exposure to sea water near the waste water outlet of a chemical plant on the Swedish west coast. In parallel to the field studies, accumulation experiments were performed with known concentrations of wastewater. The average BCF value for NP calculated on the basis of three separate concentrations in wastewater and mussels was 320. The BCF for the ethoxylates decreased with increasing the length of the EO chain (160 for NP1EO, 120 for NP2EO, and 40 for NP3EO) due to the increasing hydrophilicity.[166] In another investigation by this group performed with common mussels exposed to ^{14}C-labeled NP in running sea water under controlled laboratory conditions the BCF value of NP in common mussels was as much as about 3430. The BCF value had to be calculated by extrapolation because the uptake of NP in the mussels had not reached steady state at the end of the exposure period after 16 days. The bioaccumulation period was followed by an elimination period of 32 days in clean sea water. Within 30 days the NP concentration in the mussels decreased by 92%.[167] The great discrepancy between BCF values for mussels could be due to inaccurate determinations of NP concentrations and different experimental conditions.

Several other aquatic species were also the subject of BCF determinations. Ekelund et al. obtained BCF values for NP in shrimp (*Crangon crangon*) of 100 and in sticklebacks (*Gasterosteus aculeatus* L.) of 1250 by performing laboratory experiments with ^{14}C-labeled NP in running sea water.[167] McLeese et al. determined a BCF value of 280 for NP in laboratory experiments with salmon (*Salmo salar*).[168] Weeks et al. measured the tendency for NP to bioconcentrate in experiments with the fathead minnow (*Pimephales promelas*) at 4.9 and 22.7 μg L^{-1}. The BCF values were 271 and 344 for the two concentrations, respectively. They also determined the rate of uptake of NP and the rate of clearance after transferring the fish to clean water. Both rates were rapid with half-lives of 1.2−1.4 days.[169] Ahel et al. determined the contents of NP, NP1EO, and NP2EO in different freshwater organisms from the Glatt river, Switzerland. On the basis of the average concentrations in different fish species and in water the BCFs for NP, NP1EO, and NP2EO were in the ranges 13−408, 3−300, and 3−326, respectively. The considerable range of these values is attributed to different concentrations in different organs and tissues of the fish species. An interesting aspect here is that the concentration of NP reaches the highest values in liver compared to relatively low concentrations in muscle.[145] Granmo et al. observed the predominant accumulation of NPEOs in organs of fish as well. In

experiments concerning the uptake and elimination of [^{14}C]NP10EO in cod (*Gadus morrhua* L.) the highest concentrations were found in the gall bladder (4000 μg g^{-1}) and liver (500 μg g^{-1}) after 8 h of exposure to 5 mg L^{-1}.[170]

Studies on accumulation of NP in soils and in cultures showed that NP is rapidly degraded in soil.[171] Kirchmann et al. carried out field experiments to evaluate the uptake of organic pollutants by crops. The level of NP in soil did not influence the level in barley grains; therefore, NP was rapidly decomposed in soil or NP was not transported through the roots to the grains.[172]

C. Ecotoxicity

The discussion on the environmental acceptability of APEOs has arisen on the one hand from their persistence and on the other hand from the high toxicity of their biodegradation products, especially APs. For this reason only the toxicity data of the biodegradation products of APEOs are presented. For an overview on the toxicity data of long-chained APEOs the reader is referred to Holt al.,[24] Lewis,[173,174] and Schöberl et al.[15,175]

Table 6 contains data on the toxicity of NP for aquatic organisms. McLeese et al. determined the LC_{50} of NP for various species living in sea water. It was shown that the acute toxicity was in the range 0.2−5.0 mg L^{-1} although both clams (*Anodonta*, *Mya*) were less sensitive.[13] In two independent studies the LC_{50} of NP for salmon[168] and fathead minnow[176] were determined in flow-through systems. The obtained toxicity data of 0.13−0.19 and 0.135 mg L^{-1} for salmon and fathead minnow, respectively, were almost identical but lower than those obtained for fish species in static tests. Comber et al.[177] determined the 24 h EC_{50} value of NP for *Daphnia magna* as 0.30 mg L^{-1}, which was in line with the 24 h EC_{50} of 0.18 mg L^{-1} reported by Bringmann et al.[14] Bringmann et al. tested altogether 183 inorganic and organic substances with respect to their acute toxicity for *D. magna*. In the group of organic chemicals NP was among the most toxic compounds along with hydroquinone (EC_{50} = 0.12 mg L^{-1}) and pentachlorophenol (EC_{50} = 0.8 mg L^{-1}).[14] Granmo et al. ascertained a LC_{50} value of 3.0 mg L^{-1} for the common mussel in semistatic tests. Sublethal effects, manifested as decreased byssus strength and change of scope for growth, were obtained even at 0.056 mg L^{-1}.[178]

Prasad investigated the phytotoxicity of NP for the macrophytes *Lemna minor* L. and *Salvinia molesta* Mitchell. Treatments of cultures of *Salvinia* with four different concentrations of NP (0, 2.5, 10, and 25 mg L^{-1}) reduced frond production in all cases of NP additions by day 3, and by days 6 and 9, the cultures started dying. Daily additions of NP in low concentrations (0.5, 1.25, and 2.5 mg L^{-1}) to cultures of *Lemna* caused significant reductions in growth, bleaching, and mortality of cultures. Electron microscopic investigations of treated fronds of *Lemna* showed that the chloroplast membranes were completely disrupted and the cellular contents were scattered all over the cells.[179] Guenther et al. evaluated the phytotoxicity of NP, LAS, and distearyldimethylammonium chloride (DSDMAC) with higher plant species. LAS and NP caused a stronger inhibition of growth and germination of the test plants than DSDMAC.[180] Bokern et al. obtained a 50% growth inhibition of 12 different plant species in experiments with suspensions of cell cultures containing different NP concentrations between 11 and 220 mg L^{-1}. NP was obtained after the isolation of polar metabolites from cell cultures and their hydrolysis with 4 N HCl and enzymes like β-glucosidase or β-glucoronidase. This result indicated that NP is probably bound to sugars or sugar acids.[181]

Risk assessments for NP based on the comparision of toxicity data and NP levels in river water (Figure 9) show that almost all of the reported NP levels are substantially lower than published LC_{50} values. An exception is a sampling site at the Aire river (U.K.)[61] exposed to effluents from textile plants with NP concentrations up 180 μg L^{-1} exceeding the LC_{50} values for salmon, fathead minnow, and *D. magna*. Extensive studies of rivers in Canada[43] and the U.S.[143, 139] verified low NP contaminations which were 2−3 orders of magnitude below the LC_{50} values. Weeks et al. comprehensively evaluated the risk of NP and its ethoxylates to the aquatic environment in the U.S. According to their calculations margins of safety between observed concentrations in river water and the lowest level known to cause adverse effects are adequate.[169]

Table 6. Reported Acute Toxicities of NP for Aquatic Organisms

test species	duration of the test (h)	test conditions	LC_{50} (mg L^{-1})	ref
freshwater clam (*Anodonta cataractae*)	144	static, 10 °C	5.0	13
shrimp (*Crangon septemspinosa*)	96	static, 10 °C	0.4	13
soft-shelled clam (*Mya arenaria*)	144	static, 10 °C	>1.0	13
lobster (*Homarus americanus*)	96	static, 10 °C	0.2	13
salmon (*Salmo salar*)	96	static, 10 °C	0.9	13
salmon (*Salmo salar*)	96	flow-through test	0.13−0.19	168
shrimp (*Crangon septemspinosa*)	96	static, 10 °C	0.3	168
fathead minnow (*Pimephales promelas*)	96	flow-through test	0.135	176
Daphnia magna	24	static, 20 °C	0.18	14
Daphnia magna	24	static, 20 °C	0.30	177
common mussel (*Mytilus edulis*)	96	semistatic, 17 °C	3.0	178
water flea (*Ceriodaphnia dubia*)	96	flow-through test	0.069	169
amphipod (*Hyalella azteca*)	96	flow-through test	0.15	169
midge (*Chironomus tentans*)	96	flow-through test	0.16	169
shrimp (*Mysidopsis bahia*)	96	flow-through test	0.043	169
sheepshead minnow (*Cyprinodon variegatus*)	96	flow-through test	0.31	169

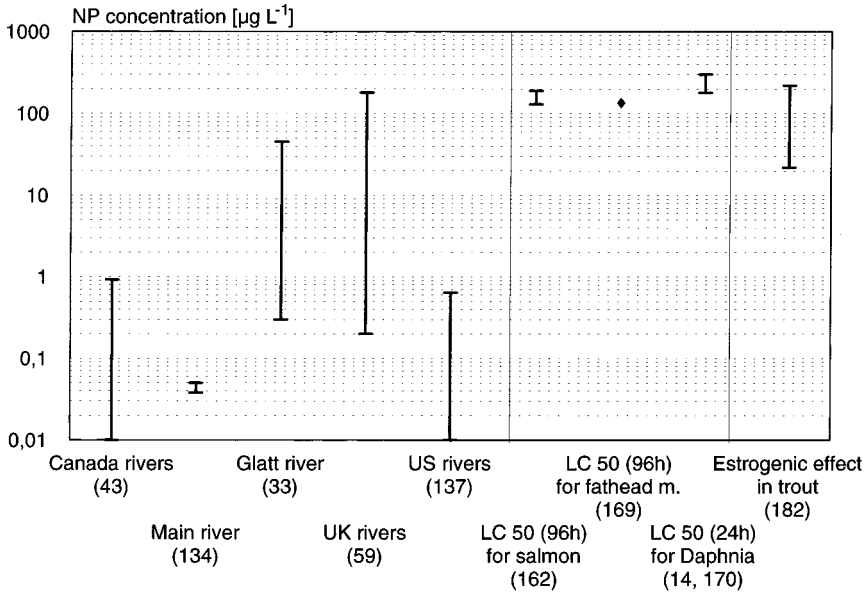

Figure 9. Environmental concentrations of NP and levels for toxicity as well as estrogenic activity.

D. Estrogenic Behavior

An unambiguous hallmark of estrogen action is the induction of mitotic activity in the female genital tract.[182] Estrogen bioassays are therefore designed to measure this proliferative event. In addition to steroid hormones like 17β-estradiol (see Figure 10), nonsteroidal substances of widely diverse chemical structure mimic estrogen action.[183−185] Historical prototypes of xenobiotic estrogens are 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2,2-trichloroethane (*o,p′*-DDT)[186] and chlordecone[185] (see Figure 10). Recently APs are also being discussed with regard to their estrogenic activity. The structural relationship between one of the NP isomers and 17β-estradiol becomes apparent in Figure 10.

Dodds et al. reported for the first time on the estrogenic activity of a *p*-*n*-alkylphenol, namely *p*-*n*-propylphenol.[183] Forty years later Mueller et al. tested various APs for their ability to displace ^{3}H-labeled 17β-estradiol from estrogen receptors of uterine cytosols. *p*-*sec*-Amylphenol showed the highest effectiveness in displacing prebound [^{3}H]estradiol, whereas *o*-alkylphenols were almost inactive.[187] Soto et al. accidentally discovered the estrogenic activity of NP in mammals. NP was released from plastic centrifuge tubes used in their laboratory and unintentionally caused the proliferation of estrogen-sensitive MCF_7 breast tumor cells. This effect was verified in rat endometrium as an alternative bioassay as well.[20] Jobling et al. determined the estrogenic potencies of different APs, NP*n*EO (n = 2, 9, 40) and NP1EC, by use of an in-vitro bioassay based on the estrogen-dependent synthesis of vitellogenin by rainbow trout (*Oncorhynchus mykiss*) hepatocytes. After 4 days of exposure at a single concentration of 10 μM, 4-*tert*-butylphenol was most active (vitellogenin concentration 100-fold above the control value) closely followed by 4-*tert*-octylphenol (90-fold). NP2EO and NP1EC caused a 40−50-fold increase in the vitellogenin concentration compared to the control, while NP and NP9EO showed an 18−20-fold increase. NP40EO and 2- and 3-*tert*-butylphenol had no effect on the vitellogenin synthesis.[188] In a second study by this group it was shown that OP, NP, NP1EC, and NP2EO were estrogenic not only in fish cells but also in avian and mammalian cells. All these compounds were 10^3−10^4 less potent than 17β-estradiol itself. The order of estrogenicity was OP > NP1EC > NP = NP2EO regardless of the bioassay system used. Moreover, these compounds were able to compete with ^{3}H-labeled 17β-estradiol for binding to the trout estradiol receptor except NP2EO. This result led to the assumption that the weak estrogenic activity of APEOs is not triggered by itself but by APs formed within the cells by degradation.[189] Recently Jobling et al. reported on in-vivo experiments with male rainbow trout exposed to four different alkylphenolic chemicals (OP, NP, NP2EO, and NP1EC). All compounds caused synthesis of vitellogenin, a process normally dependent on endogenous estrogens, and a concomitant inhibition of testicular growth. The magnitude of these estrogenic effects was dependent on the estrogenic potency of the chemicals (OP > NP = NP2EO = NP1EC).[190] Sharpe et al. reported that exposure of male rats to xenoestrogens (OP, OP5EO, butylbenzylphthalate) during gestation or during the first 21 days of postnatal life resulted in a significant

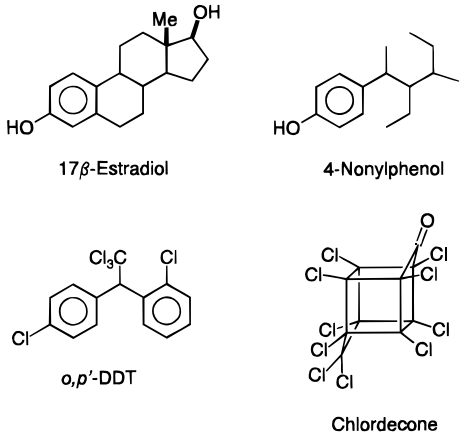

Figure 10. Structures of the steroidal estrogen, 17β-estradiol, and the nonsteroidal estrogens NP (one possible isomer), *o,p′*-DDT, and chlordecone.

reduction in testicular size (5−13%) and daily sperm production (10−21%).[21] Routledge et al. used a recombinant yeast screen to test different anionic, cationic, and nonionic surfactants and some of their degradation products for estrogenic activity. None of the parent surfactants were estrogenic, whereas OP, NP, NP1EC, NP2EC, and NP2EO induced positive responses in the yeast screen.[191]

APEOs appear in a different light due to the weakly estrogenic activities of their degradation products. Figure 9 shows the NP level at which estrogenic activity in rainbow trout becomes evident. NP concentrations in the Aire river and Glatt river exceed this level. Concentrations in other rivers are generally 1−2 orders of magnitude lower than the lowest concentration of NP required to induce a significant elevation of vitellogenin in rainbow trout, but Routledge et al. pointed out that estrogen assays measure short-term responses. They do not reflect the real situation in the aquatic environment, where organisms are probably chronically exposed to estrogenic compounds.[191]

V. Summary

A number of powerful methods are available today for the quantitative determination of APEOs and their degradation products at concentrations relevant to environmental occurrence. Chromatographic methods like HPLC and GC are preferred compared to nonspecific methods for the determination of summary parameters (BiAS and CTAS). In the field of gas chromatography, the trend toward coupling to mass spectrometry is unambiguously observable.

A lot of work has been done on the determination of APEOs and their metabolites in the aquatic and terrestrial environment. On the basis of these data, a good evaluation of the fate of these compounds during sewage treatment and in river and marine water as well as in soil is possible. APEOs entering STPs are aerobically degraded to APECs and short-chained APEOs (1−2 ethoxy units). Then, APECs are discharged into the aquatic environment while the more lipophilic AP1EO and AP2EO are predominantly adsorbed on the sludge and transformed into APs during anaerobic sludge stabilization. Therefore, rather high AP concentratrions have been determined in sewage sludge. The composition of alkylphenolic compounds in river water with APECs being the most abundant is found to be similar to that of effluents from STPs but 1−2 orders of magnitude lower in concentration due to dilution. Particular high concentrations of APEOs metabolites are recorded in river water receiving wastewaters from paper and textile plants. Due to the lipophilic character of AP, AP1EO, and AP2EO their concentrations in river sediments are higher than aqueous levels. Biodegradability proposed on the basis of STP and river surveys has been proved by different laboratory test methods. In addition, their tendency to accumulate in aquatic organisms has been intensively studied, as well as their toxic and estrogenic behavior for animals. Although the use of APEOs in detergents is restricted in many countries and concentrations of the metabolites of APEOs in river water are far below acute toxicity thresholds there may be cause for concern with regard to long-term estrogenic effects on aquatic organisms.

Moreover, the level of information on the APEO/AP concentrations in biological matrices occurring in nature is still incomplete, indicating that the continuation of research in this area is imperative. In addition, no methods for determining each single isomer of technical NP with regard to its concentration and structure have been reported. However, it seems possible that the biodegradability and the estrogenic effect of NP vary with the degree of branching of its isomers. The different biodegradability of branched and linear alkylbenzene sulfonates supports this assumption.

VI. List of Abbreviations

AEO	alcohol ethoxylates
AP	4-alkylphenol
APEC	4-alkylphenoxy carboxylate
APEO	4-alkylphenol ethoxylate
BCF	bioconcentration factor
BiAS	bismuth active substances
BSTFA	bis(trimethylsilyl)trifluoroacetamide
CAD	collisionally activated decomposition
CI	chemical ionization
CTAS	cobalt thiocyanate active substances
DCI	desorption chemical ionization
DMSO	dimethyl sulfoxide
DOC	dissolved organic carbon
DSDMAC	distearyldimethylammonium chloride
d.w.	dry weight
EC_{50}	medium effective concentration
ECD	electron capture detection
EI	electron impact ionization
ES	electrospray
ESB	Environmental Specimen Bank
Et_2O	diethyl ether
FAB	fast atom bombardment
FD	field desorption
FID	flame ionization detection
GAC	granular activated carbon
GCB	graphitized carbon black
GC	gas chromatography
HMDS	hexamethyldisilazane
HPLC	high-performance liquid chromatography
*i*PrOH	isopropyl alcohol
LAS	linear alkylbenzene sulfonate
LC	liquid chromatography
LC_{50}	medium lethal concentration
MBAS	methylenblue active substances
MeOH	methanol
MeO*t*Bu	*tert*-butyl methyl ether
MS	mass spectrometry
NEt_4Cl	tetraethylammonium chloride
NP	4-nonylphenol
NP-c	sum of all nonylphenolic compounds
NPEC	4-nonylphenoxy carboxylate
NP1EC	(4-nonylphenoxy)acetic acid
NP2EC	[(4-nonylphenoxy)ethoxy]acetic acid
NPEO	4-nonylphenol ethoxylate
NP*n*EO	4-nonylphenol ethoxylates, n = number of the ethoxy units
OECD	Organisation for Economic Cooperation and Development
OP	4-*tert*-octylphenol

PB	particle beam
SFC	supercritical fluid chromatography
SFE	supercritical fluid extraction
SIM	selected ion monitoring
SOPs	Standard Operating Procedures
SPC	sulphophenyl carboxylate
SPE	solid phase extraction
STP	sewage treatment plant
$TBAH_2PO_4$	tetrabutylammonium dihydrogen phosphate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMCS	trimethylsilyl chloride

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