Environmental behaviour of onylphenol ethoxylates in coastal waters

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General introduction
“Chemistry is everywhere”. With this slogan on a poster in the chemistry classroom, my fellow high school students and me were taught how beneficial chemical products are in all aspects of everyday life. Indeed, it would be difficult to imagine our society without the conveniences that the chemical industry has developed and provided. However, the slogan also has a less profitable side, as “everywhere” potentially harmful chemicals are present in the environment, resulting in adverse effects on organisms in numerous cases. Fortunately, with the scientific advances in chemistry also came the possibilities to study the occurrence and behaviour of these contaminants in detail, in order to describe and hopefully solve the current environmental problems/issues.

In 1948, dr Paul Müller received the Nobel prize in physiology & medicine for his discovery of the extremely effective insecticide DDT (dichlorodiphenyl trichloroethylene), which was applied worldwide in enormous amounts in agriculture, sprays for human use, household products and cloth impregnation [7]. Two decades later, the product was banned, as it had become clear that DDT is also extremely toxic to higher organisms, was widely distributed in the environment and was responsible for the disappearance of many bird and fish populations. With this discovery, the scientific investigation of specific contaminants released into the environment began. Since then many contaminants have been identified and studied in detail, and regulatory actions have been taken. However, our knowledge of contaminants in the environment is far from complete. As an example, currently the chemical identity of less than 5% of the organic material in wastewater treatment plant effluents is known [2]. New types of contaminants as well as new adverse effects on the environment are still discovered regularly.

Connected to this is an ongoing discussion on the definition of “pollution” and the desired regulatory actions. Regrettably, this discussion is often driven by feelings and emotions, instead of facts and figures. A widespread opinion is that the ultimate goal should be to reduce concentrations of contaminants to levels as they were in the ‘natural’ pre-industrialized situation. However, it has become clear that with 6 billion people on the planet, this will never be achieved. The role of environmental chemistry in this discussion is to provide facts and figures, and that is what this thesis aims to do.

**Development of environmental analysis**

The earliest examples of the quantitative analysis of substances in water are the measurements of the total salt contents of sea water by Robert Boyle (1627-1691) [3]. This analysis was possible because only a simple balance was needed to weigh these relatively high concentrations (g L\(^{-1}\) range). Later, Antoine Lavoisier (1743-1794) quantified the
different salts in sea water by fractionated crystallization and the dissolution of salts in different solvents.

In 1855, Michael Faraday reported on the pollution of the river Thames by describing its bad smell and pale brown colour [4]. To test the opacity of the water, he dropped pieces of white paper in the water, and observed that they were indistinguishable before they had sunk an inch below the surface.

It was not until the beginning of the 20th century that oxygen and nutrient concentrations in water could be measured quantitatively by titration and the use of UV spectrophotometers, at concentrations in the mg to µg per liter range.

Several decades later, oil mixtures could be characterized by Infra Red spectroscopy and gas chromatography methods. With the development of gas chromatography – mass spectrometry (GC-MS) methods in the 1970s, low concentrations (ng L\(^{-1}\)) of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) could be detected. A further improvement of this technique was the introduction of capillary GC-MS, which made it possible to separate and quantify individual PCB congeners.

Since the 1990s, it has become possible to also analyze more polar compounds in detail with the development of liquid chromatography – mass spectrometry (LC-MS). Current environmental research often focuses on the quantification of polar pesticides and pharmaceuticals in the environment.

From this brief overview, it is evident that the type of environmental research which is performed is strongly determined by the state of the art of analytical chemistry.

Another type of polar compounds which can be analyzed in detail since the recent advances in LC-MS techniques are surfactants. One group of surfactants which currently gets much attention by environmental scientists consists of the alkylphenol ethoxylates (APEO). These compounds form the subject of this thesis. Their environmental behaviour and possible effects on organisms differ in several important aspects from ‘classical’ contaminants.

**Surfactants**

Alkylphenol ethoxylates are surfactants (surface active agents). The key characteristic of surfactants is that the molecules consist of a hydrophobic and a hydrophilic part. The hydrophobic part is usually an alkyl or alkylaryl chain, while a large variation exists in the identity of the hydrophilic part.

Surfactants are classified according to the charge of their polar head group into anionic, cationic, nonionic and amphoteric. Amphoteric surfactants are both positively and negatively charged. Some examples of these surfactant classes are shown in figure 1.1.
Due to the amphiphilic nature of surfactants, they have a tendency to accumulate at interfaces such as the air-water or oil-water interface. During this process, the hydrophilic head group will orient itself towards the aqueous phase, with the hydrophobic moiety projected into the air or oil. As a result, the surface tension between the two phases will decrease, thereby facilitating the contact between and mixing of the two phases. This principle underlies the many applications of surfactants: mainly as cleaning agents, but also as emulsifiers in e.g. paints, oils or spray additives in pesticides [5].

\[ \text{Figure 1.1: Examples of surfactant structures of the four classes: a) linear alkylbenzene sulfonates (LAS, anionic), b) soap (anionic), c) alcohol ethoxylates (nonionic), d) ditallow dimethyl ammonium chloride (DTDMAC, cationic), e) cocamidopropyl betaine (amphoteric).} \]

History of surfactants
Surfactants have been known since ancient times, and the first recorded accounts of the use of soap-like materials were on Sumerian clay tablets dating back to 2500 B.C. [6]. The word 'soap' originates from ancient Roman legends, which tell of Mount Sapo, on which animals were sacrificially burned. Animal fat and wood ashes washed from the altars into the river Tiber, and people noticed that at this location in the river their wash became cleaner with less effort. Soap became quite popular, as demonstrated by a full-scale soap factory, including finished soap bars, excavated at Pompeii. During the Middle Ages, the interest in soap declined, but by the 17th century, soap had been rediscovered and was heavily taxed as a luxury product.
Until the beginning of the 20th century, soap was produced by the saponification process using as raw materials animal fat or olive oil together with lye or later soda. Food shortages in the World Wars urged the need for other ways of producing cleaning agents, and in the 1930s, the first petrochemical-based synthetic detergents were developed in the form of alkylbenzene sulfonates. By the 1950s, sales of the synthetic detergents had surpassed those of soap [7].

Environmental problems

Environmental problems with surfactants were recognized relatively early in the history of environmental chemistry. This is not surprising, as the detection of this problem did not require any sophisticated equipment. During the 1950s and 1960s, treatment ponds in wastewater treatment plants and many rivers and streams receiving wastewaters were covered by huge layers of foam, caused mainly by the large concentrations of alkylbenzene sulfonate surfactants with highly branched alkyl chains. After the substitution of these surfactants by isomers with a linear alkyl chain (LAS, see figure 1.1a) which are more easily biodegradable, the foaming problem was largely solved [7].

Another historic problem related to surfactants was their contribution to eutrophication due to the phosphates which were added to synthetic detergent products as builders (to improve water softening and maintaining an alkaline pH). The phosphates were banned, and replaced by more environmentally benign components such as nitrilotriacetic acid (NTA) and zeolites [5].

Many of the changes in detergent product formulations in favour of the environment were carried out voluntarily by detergent manufacturers, as especially in this branch of industry public opinion and acceptance are important economical factors.

In 3rd world countries, few of the above mentioned improvements in surfactant formulations have been carried out, due to economical and political reasons [7]. This is illustrated by the recent detection of high concentrations of branched alkylbenzene sulfonates in Philippinan (maximum 75 µg L⁻¹) and Costa Rican surface waters (maximum 350 µg L⁻¹) [8, 9].

Alkylphenol ethoxylates

The surfactant group investigated in this thesis consists of the alkylphenol ethoxylates (APEO, see figure 1.2a). This surfactant group belongs to the class of nonionic surfactants. Currently, APEO are probably the most controversial of all surfactants. Demands for a complete ban of these products by environmental organizations are countered by industry with statements that many of the harmful effects attributed to APEO are not scientifically
proven. (Greenpeace: “Gender bending” chemicals found in children’s bodies and consumer products’ [10] versus the European Council for Alkylphenols and Derivatives (a sector group of CEFIC): ‘Nonylphenols and endocrine effects: risks not substantiated’ [11]). Besides the considerable toxicity of these surfactants themselves, the environmental relevance of APEO lies especially with some of their possible metabolites. The metabolites octylphenol (OP) and nonylphenol (NP), and to a lesser extent A₉PEO₁ and A₉PEO₂, are relatively persistent and have been shown to cause endocrine disruption in laboratory tests (adverse effects on the sexual development or reproductive systems of organisms) [12, 13]. Even when endocrine disruption is not considered, metabolites of APEO are environmentally relevant, as a recent risk assessment of these compound groups as a whole in Swiss surface waters demonstrated [14]. In that study, the toxicity of the mixture of APEO and metabolites was estimated under the assumption of concentration addition (similar modes of action). A comparison of predicted environmental concentrations (PEC) and predicted no effect concentrations (PNEC) showed that although for the individual components the PEC values did not exceed the corresponding PNEC values, the PEC/PNEC ratio for the complete mixture was higher than 1. Therefore, APEO together with their metabolites must be considered potentially harmful. Interestingly, the metabolites accounted for 89% of the overall risk [14].

![Figure 1.2: Structures of (a) the alkylphenol ethoxylate (APEO) surfactant and (b) the APEO metabolite nonylphenol (NP). The ethoxylate chain of APEO can have one to about 20 units (n=1-20), while the alkyl chain can be a nonyl or octyl chain. In both figures only one alkyl isomer is shown, but many are possible. Commercial APEO always exists of a mixture of many ethoxylate oligomers and alkyl isomers.](image)

A voluntary ban of APEO in household products began in Europe in 1995 [15]. The replacement in industrial applications is proceeding slowly, mainly because of the excellent performance and low production costs of APEO. Recently (July 2003), the European Commission published a Marketing and Use Directive that restricts the major uses of NP and APEO. The Directive states that NP and APEO "may not be placed on the market or used as a substance or constituent of preparations in concentrations equal to or higher than
0.1% by mass” in a number of applications in which APEO and NP may end up in wastewater [16].
No regulatory action on the restriction of APEO usage has been taken in the USA.

**Production and application of APEO**
The raw materials for the industrial production of APEO are an alkylphenol and ethylene oxide. The alkylphenol mainly used is nonylphenol (~80%), and to a lesser extent octylphenol. Nonylphenol (see figure 1.2b) is produced by the alkylation of phenol using nonene (tripropylene), which is a highly branched olefin [7]. In the base-catalyzed addition of ethylene oxide to the alkylphenol, a range of ethylene oxide derivatives can be produced, with different numbers of ethoxylate units, depending on the desired properties of the final APEO product [7]. The final product will be a complex mixture of branched alkyl chain isomers and ethoxylate chain lengths. This complexity often has a favorable influence on the effectiveness of the product (as the different components can have synergistic effects) [5], but also complicates their chemical analysis. In addition, environmental risk assessment is complicated as different oligomers have different toxicities and environmental behaviour. Among the nonionic surfactants, APEO hold a second place in production volumes, after the alcohol ethoxylates (see figure 1.1c). The annual global production of APEO is estimated to be 700 000 tons. APEO consumption in Western Europe was around 90 000 tonnes in 1998 [7]. In the Netherlands, no production of APEO takes place, but the annual consumption is estimated around 1500 tonnes [17].

As the production of APEO containing a nonyl group (A₉PEO) is about 10 times higher than that of APEO having an octyl group (A₈PEO), the research described in this thesis focused only on A₉PEO. Several reports confirm that environmental levels of A₈PEO are at least an order of magnitude below those of A₉PEO [18, 19].

APEO are extremely effective products in a wide range of applications. The main application of APEO is as industrial and institutional (30%) or household (15% worldwide, but less in the Netherlands) cleaning agent [6, 20]. The remaining APEO is used in many industrial applications, e.g. as wetting agents, dispersants, emulsifiers, solubilizers, foaming agents and polymer stabilizers [20].

**Physicochemical properties**
Table 1.1 reports some physicochemical properties of A₉PEO and the metabolite NP. Compared to ‘classical’ environmental contaminants such as PCB or PAH (two examples are listed in table 1.1), APEO are more water soluble and less volatile. With increasing ethoxylate chain lengths, the polarity of the molecule increases, resulting in (slightly) lower $K_{ow}$ values and vapour pressures and higher solubilities.
Chapter 1

Table 1.1: Physicochemical properties of some A₉PEO and NP. Properties of PCB-77 and fluoranthene are given for comparison.

<table>
<thead>
<tr>
<th>compound studied in this thesis</th>
<th></th>
<th></th>
<th></th>
<th>'classical' contaminant</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS number</td>
<td>molar mass (g/mole)</td>
<td>logK_{ow}</td>
<td>water solubility (mg L⁻¹)</td>
<td>melting point (°C)</td>
</tr>
<tr>
<td>NP</td>
<td>C₁₅H₂₄O₁</td>
<td>84852-15-3 (branched)</td>
<td>220.56</td>
<td>4.5 a</td>
</tr>
<tr>
<td>A₉PEO₁</td>
<td>C₁₇H₂₈O₂</td>
<td>25154-52-3 (linear)</td>
<td>264.41</td>
<td>4.2 a</td>
</tr>
<tr>
<td>A₉PEO₂</td>
<td>C₁₉H₃₂O₃</td>
<td></td>
<td>308.47</td>
<td>4.2 a</td>
</tr>
<tr>
<td>A₉PEO₃</td>
<td>C₂₁H₃₆O₄</td>
<td></td>
<td>352.52</td>
<td>4.2 a</td>
</tr>
<tr>
<td>PCB-77</td>
<td>C₁₂H₆Cl₄</td>
<td>23598-13-3</td>
<td>291.99</td>
<td>6.6</td>
</tr>
<tr>
<td>fluoranthene</td>
<td>C₁₆H₁₀</td>
<td>306-44-0</td>
<td>202.26</td>
<td>5.2</td>
</tr>
</tbody>
</table>

a = data from [21]; b = data from [22]; c = data from [23]; d = data from [24]; e = data from [25].

Toxicity and bioaccumulation of APEO

Data on toxicity show that NP and APEO are acutely and chronically toxic in laboratory tests [26]. Acute toxicity of APEO and NP proceeds by the general narcosis mode of action. For NP, 96-h LC₅₀ values are reported for different fish species ranging from 17 to 3000 μg L⁻¹, with most values between 100 and 300 μg L⁻¹ (see figure 1.3) [27]. Short chain A₉PEO₁,₄ show acute toxicities with LC₅₀ values in the range of 148-3000 μg L⁻¹, and toxicity steadily decreases for longer chain oligomers [27]. Overall, the toxicity data show that NP and APEO must be classified as very toxic to aquatic organisms [6].

The ability of NP and APEO to bioaccumulate in aquatic biota is low to moderate [27]. Bioconcentration factors in mussels and fish range from 0.9 to 3400 (NP), 1 to 170 (A₉PEO₁) and 2 to 100 (A₉PEO₂) [22, 27].

Endocrine disruption

In addition to the narcotic type of toxicity, some of these compounds have other effects on organisms in the form of endocrine disruption. They have been shown to mimic the female sex hormone 17β-estradiol, and are able to bind directly to the estrogen receptor [12, 22, 27]. The APEO metabolite mainly responsible for this effect is nonylphenol, and to a lesser
extent A₉PEO₁ and A₉PEO₂ [13]. Which of the nonyl isomers in the industrial nonylphenol mixture actually causes the endocrine disruption is not yet known.

The endocrine disruptor effects of NP were discovered by accident by Soto et al. (1991), when NP leaking from polystyrene centrifuge tubes caused proliferation of human estrogen sensitive MCF-7 breast tumor cells [28]. Since then, many in vitro and in vivo studies have shown NP and A₉PEO₁₊₂ to produce estrogenic responses, in the form of e.g. luciferase reporter gene expression in the ER-CALUX assay [29], induction of the production of vitellogenin (a fish egg protein, only produced in large amounts by female fish) in male trout [12], and inhibition of testicular growth in trout [30]. In comparative tests, NP was found to be 10⁴ to 10⁵ times less potent than 17β-estradiol [27]. The estrogenic activity of A₉PEO₁ and A₉PEO₂ is 10⁴ to 10⁶ times lower than 17β-estradiol [13, 27]. A₉PEO oligomers with ethoxylate chains longer than 2 units were shown not to cause endocrine disrupting effects [12, 31].

Endocrine disrupting effects occur at lower concentrations than the effects of normal acute toxicity. The threshold concentration of NP for the induction of vitellogenin production is approximately 10 μg L⁻¹ [30].

Endocrine disruption in the field was first discovered in the 1980s by anglers in England who observed hermaphrodite fish near locations where wastewater treatment plant (WWTP) effluent was discharged. Experiments later showed that vitellogenin levels were highly elevated in fish which had been caged for 3 weeks near this WWTP outlet [32]. Chemical analysis of the effluent revealed high A₉PEO and NP concentrations to be present, however other chemicals present in the effluent, such as natural and synthetic hormones, were also partly responsible for the observed estrogenic responses [32].

**Environmental occurrence**

As surfactants are mostly used in aqueous solutions, in the developed world their main route to the environment is via wastewater treatment plants. Removal efficiencies of APEO in these plants have been estimated in the range 47-99%, and are generally above 90% [20]. In the Netherlands, an estimated 500 tonnes per year end up in wastewater [17]. Another relevant route of APEO to the environment is their direct discharge into surface waters after application in crop protection agents [17].

The environmental behaviour of APEO differs from ‘classical’ contaminants because of the amphiphilic nature of surfactants. As a consequence of their affinity for both hydrophobic and hydrophilic phases, they are detected in significant concentrations in both water and sediment. Due to the low volatility of APEO, air is considered as a less relevant environmental compartment for these compounds. For the more volatile metabolite NP, some studies exist in which NP was detected in air at maximum concentrations of 81 ng m⁻³ [33, 34].
Figure 1.3: Maximum observed dissolved concentrations of APEO and NP in water samples of wastewater treatment plant effluents, freshwater and marine/estuarine water from different studies, taken from the review by Bennie [39]. In addition, LC50 acute toxicity values for different organisms are shown, taken from the review by Servos [27]. ED = threshold concentration above which endocrine disrupting effects can occur, taken from [27, 30]; MPC = proposed maximum permissible concentration (MPC\(^1\) = value for A\(_9\)PEO\(_{3,8}\); MPC\(^2\) = value for A\(_9\)PEO\(_{1,2}\)).
In the literature, concentration ranges of \( A_9 \)PEO in wastewater effluents in Western Europe and the US of <0.1 to 369 \( \mu g \) L\(^{-1} \) are reported [20]. Sewage sludge sometimes contains high concentrations of especially short chain \( A_9 \)PEO and NP, with reported concentrations of both \( A_9 \)PEO and NP up to around 2500 \( \mu g \) g\(^{-1} \) [35-37]. In surface waters, maximum reported concentrations amount to 70 \( \mu g \) L\(^{-1} \) (\( A_9 \)PEO) and 180 \( \mu g \) L\(^{-1} \) (NP) [38]. In general, surface water concentrations of \( A_9 \)PEO are lower in Northwest-Europe than in southern Europe and the USA [38]. In the few data available for the Netherlands and Germany, \( A_9 \)PEO concentrations are typically below 1 \( \mu g \) L\(^{-1} \) [38]. The occurrence of APEO in sediments has been reported in concentrations up to 45 and 72 \( \mu g \) g\(^{-1} \) for \( A_9 \)PEO and NP, respectively [38].

In figure 1.3, environmental concentration ranges are shown of \( A_9 \)PEO and NP in several types of water. Maximum dissolved concentrations of a number of studies from literature are indicated [38-40]. In addition, the concentration ranges are given at which acute toxic effects are observed (LC50 values) [27]. The figure shows that only in wastewater treatment plant (WWTP) effluents APEO concentrations sometimes exceed threshold values for endocrine disruption (ED in the figures) and acute toxic effects. NP in both WWTP effluents and surface waters is sometimes present at concentrations which can cause endocrine disruption and acute toxic effects. In addition, maximum permissible concentrations (MPC) are indicated in the figures, as calculated by the National Institute of Public Health and the Environment (RIVM) for the Netherlands [22]. It can be concluded that these values are regularly exceeded in various fresh and saline surface waters.

**Biodegradation of \( A_9 \)PEO**

Biodegradation is the main process by which \( A_9 \)PEO disappear from the environment (as for most organic contaminants), but also the process by which metabolites more harmful than the surfactant itself may be formed. Although primary degradation in water occurs relatively fast for \( A_9 \)PEO, the metabolites which are formed are more persistent. Degradation of \( A_9 \)PEO in the anaerobic environment, such as in sediments, proceeds slowly [41, 42]. Complete scientific agreement on the exact \( A_9 \)PEO degradation routes does not yet exist, and several mechanisms have been proposed. These routes include nonoxidative and oxidative hydrolytic degradation mechanisms, leading to short-chain \( A_9 \)PEO\(_{1,2}\), nonylphenol (NP) and intermediates with an oxidized terminal ethoxylate group (alkylphenoxo ethoxy acetic acids, \( A_9 \)PEC). Little has been reported on the further degradation of these intermediates. Surprisingly, for the formation of NP out of \( A_9 \)PEO only indirect proof is available. Only one publication is known which reports an actual slight increase in NP concentration during
A₉PEO degradation [43]. Generally, NP is believed to be formed only under anaerobic conditions [36, 44].

Sorption
Especially the more hydrophobic short-chain A₉PEO oligomers and NP are prone to sorption to suspended particulate material and sediment. Organic carbon normalized partition coefficients between suspended material and water (logKₒC) in the field have been reported in the ranges 5.4-6.0 (NP), 5.5-5.6 (A₉PEO₁), 5.2-6.4 (A₉PEO₂) and 4.9 (A₉PEO₃) [18, 45].

Analytical chemistry of A₉PEO
A variety of sample pretreatment methods are available for the extraction of A₉PEO from environmental matrices. For water samples, the method most often used is solid phase extraction (SPE) using C₈, C₁₈ or graphitized carbon black materials, but other methods like Solid Phase Microextraction (SPME) and liquid-liquid extraction (LLE) have also been reported [46-49]. As degradation of A₉PEO in water samples is known to occur relatively easily, it is essential to process water samples as quickly as possible, even if preservation agents have been added to the sample. The extraction of A₉PEO from solid matrices can be performed by Soxhlet extraction, sonication, accelerated solvent extraction (ASE), or supercritical fluid extraction (SFE) [46, 48, 50-52]. The raw extract from the solid matrix is usually cleaned up using SPE, silica or alumina columns [50].

Although high extraction recoveries can be obtained with these methods, they are often not very substance specific. This results in interferences ending up in the final extract, and calls for sophisticated analytical methods for the detection and quantification of A₉PEO in these samples.

Although NP and the shortest A₉PEO₁₂ oligomers can be analyzed by gas chromatography (GC), the longer A₉PEO are not volatile enough for this method and therefore the preferred separation technique is liquid chromatography (LC). Usually, a reversed phase LC column (mostly C₁₈) is applied for A₉PEO analysis, on which all A₉PEO ethoxylate oligomers elute as a single, relatively broad peak. This facilitates the quantification of total A₉PEO concentrations. Alternatively, the application of a normal phase LC column results in separation of the different ethoxylate oligomers, providing insight into the A₉PEO oligomer distribution present in the sample.

Classically, for the analysis of surfactants this chromatographic method was most often coupled to fluorescence detection (LC-Flu). In recent years, this detection method has been replaced gradually by mass spectrometric detection (LC-MS), which has opened whole new fields of research on surfactants. With LC-MS analysis, a high reliability of identification,
low limits of detection and detailed information on the exact composition of the A₉PEO mixture can be obtained.

The LC-MS interface most often applied in the analysis of A₉PEO is electrospray ionization (ESI), a soft ionization technique, which is especially suitable for highly polar analytes. During ESI ionization in positive ionization mode, adduct ions including a sodium, ammonium or proton ion are formed, depending on the mobile phase buffer used. Negative ionization mostly results in deprotonated (quasi-)molecular ions. In addition, cluster ions or multiply charged ions can be formed. Another LC-MS interface which is frequently applied in the analysis of A₉PEO is atmospheric pressure chemical ionization (APCI).

For even more detailed studies, tandem mass spectrometry (LC-MS/MS) is a powerful tool in the identification of unknown analytes such as surfactant metabolites, as the fragments obtained in the MS provide detailed information on the molecular structure of the analyte [53].

Some challenges still remain in the LC-MS analysis of A₉PEO, which are related to the effect of the sample matrix on the sensitivity of the system, and the formation of doubly charged or dimer surfactant adducts in the electrospray interface.

In the research described in this thesis, most analyses were performed using an LC-MS system equipped with an electrospray interface and a reversed phase C₁₈ LC-column. In one study (chapter 3), additional analyses were done using LC-MS/MS.

**Why investigate A₉PEO?**

As described above, the presence of A₉PEO and their metabolites in the aquatic environment can have serious consequences for organisms living there. They have a relatively high toxicity, and especially their endocrine disrupting effects have raised public concern. With their high production volumes and wide applications, the emission of A₉PEO to the environment is considerable. A number of publications exist which show that MPC values are sometimes exceeded in the aquatic environment. Therefore, the environmental behaviour of these compounds must be investigated in detail, in order to be able to take appropriate regulatory action to reduce the emission of APEO to the environment.

As explained earlier in this chapter, only since the recent development of sophisticated LC-MS methods this detailed investigation of polar contaminants such as surfactants has become possible. The identification and quantification of these compounds in environmental samples is quite complicated, and the development of LC-MS/(MS) techniques provide the analytical chemist with many possibilities as well as problems. Further development and optimization of these analytical methods is a challenging scientific task.
In addition, there is a need for more detailed investigations into the exact biodegradation processes by which possibly harmful metabolites may be formed out of A₉PEO. The environmental occurrence of these metabolites must be assessed.

Although in some other countries the occurrence of APEO and metabolites has been investigated, no detailed studies exist yet for Dutch estuarine and marine waters. Local endocrine disrupting effects have been observed in Dutch waters, and could in some cases be related to specific contaminants, such as the reproductive failure (imposex) in marine snails, caused by tributyltin compounds (TBT) [54]. A National Survey of Estrogenic Substances (Dutch: Landelijk Onderzoek Oestrogene Stoffen, LOES) in fresh and marine waters of the Netherlands revealed that among the investigated endocrine disruptors (including phthalates, bisphenol-A, brominated flame retardants, natural and synthetic estrogenic hormones) the hormones, the alkylphenols and alkylphenol ethoxylates were the main compound groups responsible for the endocrine disrupting effects observed in the environment during the study [19].

There is a wide international interest in the occurrence and risks of A₉PEO in freshwater and marine environments. In a number of national and international activities, the environmental risks and uncertainties concerning alkylphenol ethoxylates and alkylphenols are recognized and discussed. An important example is the Water Framework Directive of the European Union, in which nonylphenol appears on the list of 33 priority substances which represent a significant risk to or via the aquatic environment at the European Community level [55]. The Danish Environmental Protection Agency issued a List of Undesirable Substances in 2000, in which a high priority was assigned to special action on alkylphenols and APEO [56]. In large surveys in the United Kingdom, besides natural and synthetic hormones, alkylphenols were identified as causal agents for endocrine disruption observed in small streams [57]. In several countries, including Germany, Sweden, Switzerland and Italy a ban of A₉PEO in certain applications is currently in progress [17]. Nonylphenol and APEO appear also on the List of Chemicals for Priority Action of the OSPAR Commission. This commission is currently taking initiatives aimed at reducing discharges of hazardous substances which reach, or could reach, the marine environment, with the goal to reduce their presence in marine areas to concentrations close to zero before 2020 [58].

Projects involved

The research in this thesis was performed within the framework of three projects. The first project was funded by the European Union (Environment & Climate program), and was called “Priority surfactants and their toxic metabolites in waste effluent discharges: an integrated study” (PRISTINE, ENV4-CT97-0494). The project combined researchers from
Spain, Germany and The Netherlands in the field of analytical environmental chemistry, toxicology, industry specialized in wastewater treatment and industry producing surfactants. The objective of this project was to improve the understanding of the fate of a wide range of surfactants in wastewater treatment plants and receiving surface waters. This involved the development of analytical methodology, performance of degradation studies and toxicological tests, as well as field studies.

The other two projects involved in this work were joint studies between the Dutch National Institute for Coastal and Marine Management (RIKZ) and University of Amsterdam (UvA-MTC) named “Nonionic surfactants and their transformation products in the Dutch coastal zone and North Sea: determination, fate and risk assessment” (SURTRANS) and “Modeling nonionic surfactants in Dutch estuaries” (MONOS). The aims of the SURTRANS project were to measure A9PEO and its metabolites in the Dutch coastal waters, estimate the environmental load of A9PEO to the Dutch coastal zone, determine their transformation rates along the salinity gradients in Dutch estuaries, and assess the ecotoxicological risks of A9PEO in these environments. In the MONOS project, the main objective was the implementation of a hydrodynamic fate model to describe and explain the behaviour of A9PEO and its metabolites in the Scheldt and Rhine estuaries.

Objectives and outline of this thesis

The general objective of this thesis is to extend the knowledge of the possible sources, the behaviour and fate of A9PEO in the aquatic environment. A combination of laboratory experiments, field sampling and modeling studies were applied to achieve this goal.

The main questions asked are:

• what is the most suitable way to sample and analyze A9PEO in the aquatic environment using LC-MS techniques?

• which are the main metabolites of A9PEO and what is their relative persistence in the aquatic environment?

• which are the possible sources of A9PEO to the Dutch coastal zone?

• which processes mainly govern the fate of A9PEO in the estuarine environment?

• do A9PEO form a potential environmental risk to the Dutch coastal zone?
To these aims, A₉PEO and their metabolites were investigated in systems of different scales ranging from $10^{-9}$ to $10^5$ m in size.

At the smallest scale, the behaviour of A₉PEO during LC-MS analysis was investigated in detail (Chapter 2). This involved a study of the types of adduct signals which are formed under different LC-MS conditions, and the calculation of the stabilities of several observed A₉PEO adducts with sodium using molecular dynamics. In addition, different ways of quantification of A₉PEO in environmental samples were evaluated.

On a larger scale, the aerobic biological degradation of A₉PEO in freshwater was studied in laboratory experiments using a lab scale bioreactor (Chapter 3). The biodegradation was followed in time and emerging metabolites were identified and quantified using LC-MS and LC-MS/MS techniques.

At the ecosystem scale, the existing literature on the occurrence and behaviour of nonionic surfactants and their metabolites in the marine and estuarine environment is reviewed in Chapter 4.

Chapter 5 describes the results of sampling campaigns performed in the Scheldt and Rhine estuaries in the Netherlands. A₉PEO and their metabolites were analyzed in water, suspended particulate material and sediments. Sorption coefficients were determined, and the biodegradation routes in the field were determined.

The occurrence of A₉PEO in the Dutch coastal zone of the North Sea was studied in Chapter 6. Two large sampling campaigns in this area were performed with water, suspended particulate material and sediment samples taken along transects starting at suspected sources of A₉PEO, such as the main freshwater discharges, production platforms and dump sites for harbour sludge. With these results, the main sources of A₉PEO to the Dutch coastal zone were assessed.

At the highest scale, the behaviour of A₉PEO and their metabolites in estuarine ecosystems are studied in computer simulations (chapter 7). With a hydrodynamic fate model (ECoS3), the main environmental processes determining the fate of A₉PEO in the Scheldt and Rhine estuaries were assessed, and actual biodegradation rates in the field could be estimated.

The different results of the various laboratory, field and modeling studies are linked in the general discussion and conclusions in chapter 8.
General introduction

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Chapter 1


