Environmental behaviour of onylphenol ethoxylates in coastal waters
Jonkers, C.C.A.

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Introduction

The present study focuses on the environmental occurrence and fate of the nonionic surfactants nonylphenol ethoxylates (A₉PEO) and their metabolites (see figure 1). These are surface active agents widely used in many applications such as industrial cleaning agents, emulsifiers or wetting agents.

Surfactant molecules such as A₉PEO have a hydrophobic part (the alkyl chain) and a hydrophilic part (the ethoxylate chain). This is the reason why they tend to accumulate at the interface between hydrophobic and hydrophilic phases, which is the key characteristic used in all applications of surfactants. This is also the reason why their chemical analysis as well as their environmental behaviour differs from ‘classical’ contaminants such as dioxins or polycyclic aromatic hydrocarbons (PAHs).

Due to their high production volumes and relatively high toxicity, these compounds are of environmental relevance. In addition, some of their possible metabolites (nonylphenol, nonylphenol monoethoxylate (A₉PEO₁) and nonylphenol diethoxylate (A₉PEO₂)) are known to be endocrine disruptors (causing adverse effects on the sexual development or reproductive systems of organisms). A number of reports appeared in recent years which suggested that some of these metabolites (breakdown products) of A₉PEO may be more harmful to the environment than the surfactant itself.

The environmental occurrence of A₉PEO in both the water and sediment phase has been reported in a number of scientific publications, and in several cases Maximum Permissible Concentrations are exceeded.

For these reasons, research projects were funded by the European Union (project PRISTINE) and the Dutch National Institute for Coastal and Marine Management (RIKZ, project SURTRANS) to investigate the environmental behaviour of surfactants and their metabolites in detail, and to estimate their potential risk to the European and Dutch aquatic environment. The work described in this thesis was part of both of these projects.

In this thesis, A₉PEO and their metabolites were investigated using a combination of laboratory experiments, sampling in the field, and fate modeling. The general objective was to extend the knowledge of the possible sources, the behaviour, and the fate of A₉PEO in the aquatic environment.
Figure 1: Structures of the alkylphenol ethoxylate (A₉PEOₙ) surfactant and three possible metabolites: nonylphenol (NP), alkylphenoxy ethoxy acetic acid (A₉PEₙC) and carboxy alkylphenoxyn ethoxy acetic acid (CA₉PEₙC). The ethoxylate chain of A₉PEO can have one to about 20 units (n=1-20). In all figures only one alkyl isomer is shown, but many are possible. All compound groups always exist of mixtures of ethoxylate chain lengths and alkyl isomers. For CAPEC, the alkyl chain can also be shortened.

As chemical analytical methods play an important role throughout this study, the analysis of A₉PEO using liquid chromatography – mass spectrometry (LC-MS) was investigated in detail (chapter 2). Several types of signals which were observed were studied using molecular dynamics calculations, and the consequences for the quantification of A₉PEO in environmental samples were investigated.

Further, a study was conducted focusing on the aerobic biodegradation of A₉PEO in freshwater (chapter 3). New metabolites could be identified and a biodegradation route was proposed.

Relatively few data are available on the occurrence of A₉PEO in saline aquatic environments. Therefore, field studies were performed in the Scheldt and Rhine estuaries (chapter 5) as well as in the Dutch coastal zone (chapter 6). The spatial distribution of A₉PEO and metabolites gave insight into the main sources of A₉PEO to the Dutch coastal zone.

Finally, a fate model was applied to determine which are the main processes governing the fate of A₉PEO in the Dutch Scheldt and Rhine estuaries (chapter 7).
LC-MS analysis of A₉PEO

Adduct formation

The chemical analysis of A₉PEO is relatively complicated because these compounds exist as mixtures of oligomers with varying alkyl isomers and ethoxylate (EO) chain lengths. A very suitable analytical method for these polar, nonvolatile compounds is liquid chromatography – mass spectrometry (LC-MS). In LC-MS analysis, A₉PEO are detected as adduct ions (a positively charged complex including a metal ion).

In chapter 2, these adduct formation processes were studied in detail. The electrospray interface was used, and the influence of a number of analytical conditions on adduct formation was investigated, including the mobile phase solvent, mobile phase buffer, LC-column type and ionization mode.

For the different A₉PEO oligomers, strong differences in adduct formation behaviour were found. With our ‘base method’ (using sodium acetate and methanol in the mobile phase, a reversed phase column and electrospray ionization in positive mode), several types of adduct were formed for many of the oligomers. Besides common sodium adducts \([A₉PEOₙ+Na]^+\), surfactant dimer adducts \([2×A₉PEO₁+Na]^+\) and \([2×A₉PEO₂+Na]^+\) and adducts including a solvent molecule \([A₉PEO₁+MeOH+Na]^+\) and \([A₉PEO₂+MeOH+Na]^+\) were formed for short chain oligomers, while for long chain oligomers doubly charged adducts \([A₉PEO₋₁₁+2×Na]^₂⁺\) were found.

In order to explain some of these adduct formation phenomena, the theoretical stabilities of some adducts were calculated with molecular dynamics computer simulations. A comparison was made between the stabilities of monomer and dimer adducts of A₉PEO₂ and A₉PEO₅. The calculations resulted in energetically optimized three-dimensional structures of the adducts (lowest energy conformers), which showed that the A₉PEOₙ molecule wraps itself around the sodium ion in a way that negative electronic charges on oxygen have optimum electrostatic interaction with this ion (see figure 2).

**Figure 2:** Orientation of two A₉PEO₂ oligomers around a sodium ion: \([2×A₉PEO₂+Na]^⁺\).
Oxygen atoms (small dark) are coordinated around the sodium atom (large dark).
Van der Waals interactions between alkyl chains are of less importance for the stability of these adducts. Both $[2 \times A_9\text{PEO}_2+\text{Na}]^+$ and $[2 \times A_9\text{PEO}_5+\text{Na}]^+$ dimer and $[A_9\text{PEO}_2+\text{Na}]^+$ and $[A_9\text{PEO}_5+\text{Na}]^+$ monomer adducts turned out to be stable from an energetic point of view with adducts of $A_9\text{PEO}_5$ being more stable than adducts of $A_9\text{PEO}_2$. For the monomer adducts this is in agreement with the responses observed in LC-MS analysis, but for the dimer adducts this is only partially in agreement, as the signal of $[2 \times A_9\text{PEO}_5+\text{Na}]^-$ is not observed at all in LC-MS analysis, while the $[2 \times A_9\text{PEO}_2+\text{Na}]^+$ signal is.

**Quantitative analysis**

The m/z values of the dimer $[2 \times A_9\text{PEO}_1+\text{Na}]^+$ and $[2 \times A_9\text{PEO}_2+\text{Na}]^+$ and disodium $[A_9\text{PEO}_{>11}+2 \times \text{Na}]^{2+}$ adducts happen to be equal to the sodium adducts of some other $A_9\text{PEO}$ oligomers (e.g. $[2 \times A_9\text{PEO}_2+\text{Na}]^+$ and $[A_9\text{PEO}_9+\text{Na}]^+$ both have m/z = 639). Therefore, the consequences of this signal overlap for the quantitative analysis of environmental samples were evaluated. It was found that in clean samples, the presence of $A_9\text{PEO}_{1,2}$ can cause an overestimation of long-chain $A_9\text{PEO}_{>2}$. In real environmental extracts, other processes such as matrix effects have a stronger influence on the quantitative result, and therefore no significant influence of adduct formation processes could be observed.

The inclusion of $[A_9\text{PEO}_1+\text{MeOH}+\text{Na}]^+$ and $[A_9\text{PEO}_2+\text{MeOH}+\text{Na}]^+$ adduct signals in the Selected Ion Monitoring (SIM) mode significantly improves the detection limits of these two short chain oligomers.

The quantitative analysis of $A_9\text{PEO}$ was further studied by comparing $A_9\text{PEO}$ calibration mixtures with different oligomer compositions. It was found that regardless of the exact $A_9\text{PEO}$ composition of the standard, correct quantitative results are obtained as long as $A_9\text{PEO}_1$ and $A_9\text{PEO}_2$ are quantified separately. Longer oligomers must be quantified with a molar calibration followed by correction of the average molar weight of the $A_9\text{PEO}_{>2}$ in the sample.

**Aerobic biodegradation of $A_9\text{PEO}$**

The aerobic biodegradation of $A_9\text{PEO}$ was investigated in laboratory studies as described in chapter 3. For these experiments a laboratory scale bioreactor (see figure 4) was filled with aerated water from the river Rhine, and spiked with an $A_9\text{PEO}$ mixture. Samples were taken daily and extracted by Solid Phase Extraction (SPE). Using LC-MS analysis in positive and negative mode, concentrations of $A_9\text{PEO}$ and metabolites were quantified.
A relatively fast primary degradation of A₉PEO was observed, with >99% degradation after 4 days. No lag phase was observed, indicating that no acclimatization of the micro-organisms was necessary. This may be explained by the fact that the microbial community in the river Rhine is continually exposed to A₉PEO.

An oxidative hydrolytic degradation mechanism was observed, with the initiating step of the degradation being ω-carboxylation of the individual ethoxylate chains. This resulted in the accumulation of metabolites having long carboxylated EO-chains with chain lengths of 1 to 15 (alkylphenoxy ethoxy acetic acids, A₉PEC (see figure 1)). Further degradation proceeded more slowly. Gradually, the carboxylated EO chain is shortened, with the A₉PE₂C oligomer being formed in the highest abundance. At the same time, further oxidation of the A₉PEC metabolites occurs, leading to metabolites having both a carboxylated ethoxylate and alkyl chain of varying lengths (CAPEC, see figure 1). The identity of the CAPEC metabolites was confirmed by the fragmentation pattern obtained with LC-ESI-MS/MS.

After 20 days, around 90% of the metabolites had been further degraded, but low concentrations of both A₉PEC and CAPEC metabolites were still present in the bioreactor after 30 days.

During the experiment, A₉PEO₂ was formed only to a minor extent and was further degraded in several days. This indicates that the nonoxidative hydrolytic degradation pathway is of minor importance under these conditions. No accumulation of the endocrine disruptor nonylphenol was observed in this study.

The mass balance in the study was incomplete, and therefore it is possible that additional metabolites which were too polar for extraction by C₁₈-SPE or non detectable by LC-MS were formed. Alternatively, a large fraction of the A₉PEO may have been mineralized without the accumulation of any metabolites.
Literature study on the occurrence of nonionic surfactants in marine and estuarine environments

In chapter 4, the literature on the behaviour of nonionic surfactants in saline environments is reviewed. The field data available on surfactants in marine and estuarine environments is considerably less than for freshwater.

In general, dissolved concentrations of $A_9$PEO in estuaries are an order of magnitude lower than those in freshwater. However, in some cases local sources in the estuary lead to high surfactant concentrations. Local ‘hot spots’ with dissolved concentrations exceeding 10 µg L$^{-1}$ were present in some English estuaries, and at some locations close to the shore in the Mediterranean coast of Spain as well as Israel. Land-based sources (industry, waste water effluents or river water containing high concentrations of $A_9$PEO) could be identified in each case.

In stratified estuaries, $A_9$PEO have been reported to accumulate at the two phase boundaries of air-water and freshwater-saline water, resulting in a complex vertical distribution profile in the water column.

Biodegradation of $A_9$PEO is an important process in saline waters, although degradation rates are lower than in freshwater. When $A_9$PEO have entered the sediment, degradation proceeds very slowly.

From the literature overview it was concluded that the processes dominating the environmental fate of nonionic surfactants in estuaries are biodegradation in the water column and sorption/sedimentation. For the relatively volatile NP, volatilisation may also play a significant role.

Field studies on the occurrence of $A_9$PEO and their metabolites in the Dutch aquatic environment

Extensive field studies were conducted in the Dutch estuarine and coastal environment, in which $A_9$PEO and metabolites were analyzed in water, suspended particulate material (SPM) and sediment. The objective of these studies was to obtain insight into the main sources of $A_9$PEO to the Dutch estuarine and coastal environment, as well as to determine which are the main processes governing the fate of $A_9$PEO in these areas.

Filtered water samples were extracted using Solid Phase Extraction (SPE), while for sediments and SPM, sample extraction was performed with Soxhlet extraction methods. Analyses were performed by LC-ESI-MS.
Dutch estuaries

In several sampling campaigns in the Dutch Scheldt and Rhine estuaries, water was sampled along the estuary at certain salinity intervals, while sediments were collected at fixed locations. This study is described in chapter 5.

In the Scheldt estuary, dissolved concentrations were highest upstream near Antwerp (maximum dissolved concentration of A₉PEO 2.3 µg L⁻¹), while in the Rhine estuary, the highest concentrations were observed halfway in the estuary near the Rotterdam harbours. At several locations in both estuaries, concentrations are above the Maximum Permissible Concentrations (MPC) proposed for The Netherlands. The A₉PE₁₈C metabolites which had been found in the laboratory experiments were also found in the field samples. In most cases, dissolved A₉PEC concentrations were higher than those of A₉PEO.

The dilution of river water with sea water could be described using the salinity profile of the water. By comparing the A₉PEO concentration profiles with these salinity profiles, it was observed that in the Scheldt estuary, dissolved concentrations decreased more sharply than could be explained by simple dilution of the river water (non-conservative behaviour). This decrease was less sharp for the A₉PEC metabolites (see figure 5). The increasing concentration ratio of A₉PEC/A₉PEO when going downstream in the Scheldt estuary illustrates the important role that the oxidative hydrolytic degradation pathway plays in the estuarine fate of these compounds. At high salinities, where concentrations dropped to background levels of around 50 ng L⁻¹, the A₉PEC/A₉PEO ratio decreased to about unity.

The A₉PEO oligomer distribution remained constant throughout both estuaries, indicating that degradation by nonoxidative shortening of the EO chain does not occur to a high degree.
For the Rhine estuary, the water concentration profiles were less pronounced, and no clear trend in $A_9$PEC/$A_9$PEO ratios was observed. The relatively short residence time of the water (several days in the Rhine estuary compared to 3 months in the Scheldt estuary) is apparently too short for significant biodegradation to occur. In addition, in the vertically stratified Rhine estuary more complicated and turbulent water flows could possibly disturb any longitudinal concentration profiles. Finally, significant point sources may be present in this area, such as the Rotterdam harbours.

An additional sampling campaign in the Rhine estuary confirmed that dissolved $A_9$PEO is vertically stratified in both the freshwater and saline part of the estuary, with concentrations being 1.5 to 4 times higher at the water surface than at several meters depth.

In almost all of the sediments of both estuaries, $A_9$PEO and NP were detected. In about half of the sediment samples, concentrations of $A_9$PEC were below the detection limit. Relatively high concentrations of $A_9$PEO and NP in sediment were observed near possible sources such as the Antwerp and Vlissingen harbours.

Metabolites of the carboxy alkylphenoxy ethoxy acetic acids (CAPEC) type have not been detected in any of the sediment or water samples.

North Sea
Chapter 6 of this thesis describes the results of two large sampling campaigns performed in the Dutch coastal zone of the North Sea. Sampling locations were chosen along transects starting at possible sources of $A_9$PEO, such as freshwater discharges (e.g. Rhine and Scheldt) and production platforms.

In sediments, relatively high concentrations of $A_9$PEO and NP were observed near the shore, with maximum concentrations of 277 and 86 ng g$^{-1}$ d.w., respectively. These concentrations rapidly decreased with distance from the shore until below 50 ng g$^{-1}$ at about 10 km offshore. From the spatial distribution of $A_9$PEO in sediments, the main routes of $A_9$PEO to sediments in the Dutch coastal zone were identified as the Rhine and Scheldt estuaries, dump sites for harbour dredge and in some cases production platforms. These sources have only a limited spatial influence, as the concentrations in sediments drop to almost the open sea background levels at 10 km from the source.

Figure 6: Research ship ‘Mitra’ of the Directorate North Sea of the Dutch Ministry of Transport, Public Works and Water Management.

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Concentrations of A₉PEO in water showed a different spatial profile. In one of the two sampling campaigns, unusually high dissolved A₉PEO concentrations were observed at a number of off shore locations. These locations coincide with the main shipping lanes in the Dutch coastal zone. No trend of decreasing dissolved concentrations with distance from the shore was observed, which suggests that the main sources of A₉PEO to the marine water are at sea and not freshwater or land based. The intensive shipping activities in the Dutch coastal zone would be a likely source of A₉PEO. At present, no specific legislation exists on the discharge of surfactants from ships during e.g. cleaning activities.

At several locations in the Dutch coastal zone, dissolved concentrations exceeded proposed Maximum Permissible Concentrations. Concentrations in sediment exceeded the MPC only at one location (inside the North Sea canal).

An additional sampling exercise was performed to compare different types of water sampling. It was found that in all types of samples taken at the water surface, A₉PEO concentrations were about an order of magnitude higher than in the samples collected from several meters depth. This indicates that in the Dutch coastal zone a vertical stratification of A₉PEO in the water column is present, probably caused by the surface active properties of the surfactant.

Both the oxidative and non-oxidative hydrolytic biodegradation mechanisms occur in the Dutch coastal zone. However, concentration ratios of metabolite to A₉PEO are lower in the coastal zone than in the estuaries, indicating that the extent of biodegradation is lower in the coastal zone. This difference is highest for the A₉PEC/A₉PEO ratios, indicating that in the marine environment the oxidative degradation route is less important than in the adjacent Scheldt and Rhine estuaries.

**Fate model for A₉PEO in estuaries**

In order to gain a more quantitative insight into the main processes determining the fate of A₉PEO in the Dutch Scheldt and Rhine estuaries, their behaviour in these areas was simulated using a fate model. This study is described in chapter 7.

The shape of the estuaries was taken into account by dividing them in a number of segments of known actual sizes. Then the hydrodynamic behaviour of the water was modeled by optimizing the exchange between the segments using the observed salinity profile along the estuary. This type of model is called a hydrodynamic advection-dispersion fate model. As it was known that the water column in the Rhine estuary is vertically stratified, each segment in this estuary was divided into a surface and bottom layer.

Since the actual concentrations of both A₉PEO and their metabolites were known, biodegradation processes were incorporated into the model, which could be validated using
both the concentration profiles of the surfactants and their metabolites. Biodegradation rate constants of the consecutive reactions $A_9\text{PEO} \rightarrow A_9\text{PE}_{1,2}C \rightarrow A_9\text{PE}_{1,2}C$ were fitted to the actual concentration profiles, yielding a quantitative estimation of the biodegradability of these compounds in the field.

In addition, sorption of $A_9\text{PEO}$ and metabolites to SPM and exchange of the SPM with sediment (sedimentation/erosion) were incorporated into the model. Sorption coefficients as determined from the actual field data were used. The measured dissolved concentration profiles as well as salinity and concentrations of SPM could be successfully described by the model. The concentrations in SPM and sediment calculated by the model were of the same order of magnitude as the actual concentrations.

In the Rhine estuary, the concentration profiles could only be described correctly when additional sources of $A_9\text{PEO}$ in the middle of the estuary were introduced into the model. This suggests that in this region the Rhine estuary receives significant input of $A_9\text{PEO}$, probably from the Rotterdam harbours as well as the river Oude Maas.

The biodegradation rate constants derived from the optimization of the model were in agreement with values reported in literature. For the Scheldt estuary, the fit of the model could be further improved by using salinity dependent biodegradation rates (higher rates at lower salinities).

A sensitivity analysis of the models showed that in the Scheldt estuary, the environmental process with the strongest influence on the dissolved concentration profiles is biodegradation. In the Rhine estuary, the dissolved concentration profiles were mainly influenced by the additional $A_9\text{PEO}$ sources.

**Final remarks**

The research presented in this thesis shows that the present state of the art in analytical chemistry allows detailed investigations into the behaviour of polar contaminants in the environment. Reliable quantitative analysis is possible for the relatively complex $A_9\text{PEO}$ mixtures and their metabolites.

A number of metabolites which were found in laboratory biodegradation studies were also found in relevant concentrations in the Dutch estuarine and marine environment. From the field data, biodegradation processes of $A_9\text{PEO}$ could be shown to occur in the Scheldt estuary. Biodegradation rates, estimated using a fate model, were in accordance with literature values.
Summary

Important sources of A9PEO to sediment in the Dutch coastal zone include the Scheldt and Rhine discharges, the dump site for harbour dredge and production platforms. For A9PEO in the water phase, off shore sources such as shipping activities are important. Concentrations are in some cases above the proposed Dutch Maximum Permissible Concentrations, and therefore regulatory action should be taken to reduce the load of A9PEO to the environment.
“Zoek de zeep, zoek zoek de zeep!”
Ernie & vriendjes in bad (Zomerkriebels met Bert & Ernie - 1998)