Environmental behaviour of onylphenol ethoxylates in coastal waters

Jonkers, C.C.A.
Fate of nonylphenol ethoxylates and their metabolites in two Dutch estuaries: evidence of biodegradation in the field


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Abstract

The environmental behaviour of nonylphenol ethoxylates (A₉PEO) and their metabolites was investigated in field studies in the two Dutch estuaries Western Scheldt and the Rhine estuary. Using liquid chromatography - electrospray mass spectrometry (LC-ESI-MS) analysis after solid-phase extraction, A₉PEO, nonylphenol (NP) and the carboxylated metabolites (A₉PEC) were determined in surface water and sediments. Maximum dissolved concentrations of 2.3, 0.9 and 8.1 µg L⁻¹, respectively, were found. In sediments, maximum concentrations of 242 and 1080 ng g⁻¹ for A₉PEO and NP were observed. In almost half of the sediment samples, concentrations of A₉PEC in sediments were below the detection limit. Occasionally relatively high values were observed, with a maximum of 239 ng g⁻¹. Metabolites of the carboxy alkylphenox y ethoxy acetic acids (CAPEC) type could not be detected in any of the sediment or water samples.

In the Scheldt estuary, dissolved concentration profiles showed non-conservative behaviour for all detected compound groups. While A₉PEO and NP concentrations strongly decreased along the salinity gradient, this decrease was weaker for the A₉PEC metabolites. The increasing concentration ratio of A₉PEC/A₉PEO clearly illustrates the important role that aerobic biodegradation plays in the estuarine fate of these compounds. It is concluded that the oxidative hydrolytic degradation pathway is the main degradation route in this non-stratified estuary.

At high salinities, where concentrations drop to background levels of around 50 ng L⁻¹, this ratio decreases to about unity. Simple model calculations show that this can be explained if continuous diffuse discharges (e.g. from the intensive shipping in the estuary) are assumed. For the stratified Rhine estuary the water concentration profiles are less pronounced, possibly due to more complicated and turbulent water flows and point sources from the Rotterdam harbours.

5.1. Introduction

Alkylphenol ethoxylates (APEO) are the most intensely discussed group of surfactants of recent years. Although they have been replaced in many products, no reports of an actual decrease in production are available yet, and even new fields of application have recently been reported [7].
One of the main nonionic surfactant groups produced since the 1950s, they pose a possible threat to the environment, as they have been shown to partially survive waste water treatment [2], and are omnipresent in the environment [3-5].

Environmental interest in APEO is mainly focused on the possible metabolites of these compounds, as some of them (nonylphenol (NP), octylphenol (OP), A9PEO1 and A9PEO2) have been shown to be weak endocrine disruptors [6]. Several biodegradation pathways of A9PEO have been described in literature. Figure 5.1 shows the main aerobic biodegradation pathway as previously reported from our laboratory studies [7]. Another possible pathway is a hydrolytic shortening of the ethoxylate chain, leading to A9PEO2 and A9PEO1 [8]. NP is believed to be formed only under anaerobic conditions [9, 10].

![Figure 5.1: The main aerobic biodegradation pathway of alkylphenol ethoxylates [7].](image)

Although a substantial amount of data is available on the occurrence of APEO in freshwater environments, the literature on their occurrence and behaviour in estuarine environments is rather limited. Concentrations of APEO are reported between <20 and 25,000 ng L⁻¹ in estuarine and marine waters and <2-30,000 ng g⁻¹ in marine sediments [11-19]. It has been shown that in stratified estuaries, biodegradation rates are up to 8.5 times higher in the upper brackish water layer than in the lower saline layer [20]. In estuarine sediments, the persistence of APEO and NP can be very high, with reported half-lives of 60 years [21]. The occurrence of APEO and metabolites in the marine environment has been reviewed in detail recently [22].

The only data on the metabolites alkylphenoxy ethoxy acetic acids (APEC) in estuarine environments were reported for the Venice lagoon (Italy), with an A9PEC concentration range of 300-6200 ng L⁻¹ [23]. A9PEC/A9PEO concentration ratios of 0.5 in the lagoon and 2-3 in the main river reaching this lagoon were observed. The authors concluded that the main biodegradation mechanisms are different for the estuary and the river. In the estuary, the hydrolytic biodegradation mechanism was suggested to prevail over the hydrolytic-oxidative mechanism, because the formation of A9PEC was observed to be only a minor pathway [23].
The aim of the field study reported in this paper was to identify and quantify the most important metabolites of \( \text{A}_9\text{PEO} \) in two highly industrialized estuarine environments using recently developed LC-MS methods [7]. Trends in the concentration ratios of metabolites and surfactants along the estuaries are explained using the aerobic biodegradation pathway as inferred from laboratory experiments [7].

5.2. Experimental Section

Study Areas.
The Rhine estuary reaches the North Sea after passing the harbours of Rotterdam (see map in figure 5.2a). The freshwater flow is around \( 1000 \text{ m}^3 \text{ s}^{-1} \) with a residence time in the estuary of one to three days. The water column is vertically stratified. The whole area is heavily industrialized, and has intensive shipping traffic. About 50-60% of the terrestrial particulate matter entering the estuary is retained there. Heavy dredging activities and strong tidal currents in the estuary result in a well-mixed surface sediment layer [24].

The other investigated estuarine area is the Western Scheldt estuary, which stretches over a distance of 100 km from the Belgian city of Antwerp to the North Sea, crossing some highly industrialized areas (see figure 5.2b). It is a tide-governed estuary with a low fresh water input from the Scheldt river of on average \( 104 \text{ m}^3 \text{ s}^{-1} \). The tidal wave corresponds to an average difference of 3.8 m between high and low tide in Vlissingen and 5.0 m at Antwerp. Other sources of fresh water are the canal Gent-Terneuzen (15 m\(^3\) s\(^{-1}\)) and the drain canal at Bath (11 m\(^3\) s\(^{-1}\)). The harbour areas of Antwerp and Vlissingen are the main sources of industrial wastewater, while the estuary also receives both treated and untreated domestic wastewater [25].

The water in the estuary is vertically well-mixed and has a relatively high residence time of two to three months.

Terrestrial particulate matter entering the estuary is retained there for about 90% [25]. The shipping channel of the narrow estuary is heavily dredged, and the dredging material is dumped elsewhere in the estuary. Vertical profiles of Al and other metals in sediment cores have indicated that the surface layer down to 30 cm is heavily disturbed [26].
Fate of nonylphenol ethoxylates and their metabolites in Dutch estuaries

Figure 5.2: Maps of the two investigated regions: the Rhine estuary (A), and the Scheldt estuary (B), The Netherlands, showing the sediment sampling points (R and S).

Reagents and standards.
Sep-Pak C_{18}-SPE cartridges (500 mg) from Waters were used for extraction of water samples. Nanopure water was obtained from a Barnstead D4742 ultrapure water system and was subsequently further purified by sub-boiling. Methanol was HPLC-grade (Rathburn, Walkerburn, Scotland). Ammonium acetate (p.a.) and sodium acetate were purchased from Merck (Amsterdam, NL) and Baker (Deventer, NL), respectively. The A\textsubscript{9}PEO\textsubscript{2} and NP
Chapter 5

Standards used as internal standards (containing $^{13}$C labeled aromatic rings) were purchased from Cambridge Isotope Laboratories, Andover, USA. Pure $A_9$PEO$_1$, $A_9$PEO$_2$, $A_9$PE$_1$C and $A_9$PE$_2$C standards were synthesized by F. Ventura of AGBAR, Barcelona and characterized by GC-MS. Technical mixtures of on average $A_9$PEO$_{10}$ (with an ethoxylate range of 4 to 15) were provided by Shell Amsterdam.

Sampling campaign.
Both sampling campaigns were conducted in November 1999 in the Scheldt and Rhine estuaries with research vessels of the Dutch Ministry of Transport, Public Works and Water Management (RIKZ). Surface sediments were collected using a box core sampler (average box core depth 30 cm). At each location, three box cores were taken and the complete cores were mixed, to ensure that the sample was representative of the location (see figure 5.2). Water samples were taken from the water surface (at a depth of approximately 0.5 m) using a stainless steel bucket. Water sampling was performed along the salinity gradient, at salinity intervals of 2‰ (except in the coastal part of the Rhine estuary, where the salinity decreased too rapidly). Therefore, water samples were collected at different locations than the sediment samples. Water salinity, pH, temperature, turbidity, and oxygen concentration were monitored on-line during sampling.

To avoid any chance of conservation problems, the water samples were treated immediately after sampling in the laboratory on board the vessel. Sediment subsamples taken from the mixed cores were stored immediately after sampling at $-20$ °C.

The sediments were characterized by organic carbon content, particle size and $^{13}$C/$^{12}$C and $^{15}$N/$^{14}$N stable isotope ratios ($\delta^{13}$C and $\delta^{15}$N, respectively) using an element analyzer and isotope ratio – mass spectrometry (IR-MS). The isotope ratios give an indication of the origin of the sediment, as both ratios are lower for sediment from terrestrial origin than for sediment from marine origin.

Sample treatment
Sample treatment procedures were modified from previously described methods [7,18]. One L of water sample was filtered using a GF/C glass fiber filter. The filters (suspended particulate material (SPM) samples) were then stored at $-20$ °C, and extracted using the same methods as for the sediments. Filtered water was immediately acidified to pH=2 with HCl and extracted using Solid-Phase Extraction (SPE). C$_{18}$-SPE cartridges were conditioned with 10 mL of methanol and then 10 mL of nanopure water. Subsequently the cartridge was loaded with 500 mL sample. The cartridge was then dried by a stream of nitrogen, and eluted with 10 mL of methanol. The extract was then evaporated with nitrogen until dryness, and redissolved in 1 mL of methanol/nanopure water 1:1 (v:v). Finally, the extract was filtered through a 0.2 μm Acrodisc filter.

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Sediment samples were extracted using a Soxhlet device, which was prerinsed by refluxing methanol for 5 hours. 9-25 g of wet sediment was extracted overnight with 250 mL of basic methanol. The extract was concentrated to 15 mL, then 80 mL of nanopure water was added and this mixture was acidified to pH=2. To purify the extract, it was passed over a C\textsubscript{18}-SPE cartridge as described above.

All extracts were analyzed using reversed phase liquid chromatography coupled to electrospray mass spectrometry detection (LC-ESI-MS), as described previously [7]. A Thermoquest Navigator LC-MS system was used with a Lichrospher RP-C\textsubscript{18} column (dimensions 125 x 2 mm, 3\textmu m) and a mobile phase flow rate of 0.25 mL min\textsuperscript{-1}. The mobile phase was made up of a mixture of pure methanol (A) and a water-methanol 3:1 (v:v) buffer (B). For the detection of the ethoxylates, positive ionization was used with a sodium acetate buffer (0.1 mM), while all metabolites were analyzed in negative ionization mode, using an ammonium acetate buffer (2 mM).

All analyses were performed in SIM mode, using the deprotonated molecular ions of NP, A\textsubscript{9}PEC and CAPEC during negative ionization, and the sodium adducts of A\textsubscript{9}PEO in positive mode. For A\textsubscript{9}PEO\textsubscript{1}, the mass of the [A\textsubscript{9}PEO\textsubscript{1}+methanol+Na]\textsuperscript{+} adduct (m/z = 319) was also selected. The electrospray interface was set at a probe temperature of 220 °C, and probe and cone voltages of -3.8 kV and -20 V in negative ionization mode, and +4.0 kV and +20 V in positive ionization mode, respectively.

In all samples, A\textsubscript{9}PEO\textsubscript{1} and A\textsubscript{9}PEO\textsubscript{2} were quantified separately with pure standards, and the longer oligomers were quantified using a commercial mixture of on average 10 ethoxylate units (and with A\textsubscript{9}PEO\textsubscript{1} and A\textsubscript{9}PEO\textsubscript{2} below 0.5%). This standardized way of quantifying A\textsubscript{9}PEO\textsubscript{2} with always the same A\textsubscript{9}PEO\textsubscript{10} mixture has the obvious advantage that calculations are simplified and reproducibility is improved. However, in some cases an error may be introduced, when the average ethoxylate chain length in the sample is much lower than that of the standard, leading to an overestimation of the concentration. The maximum error introduced for A\textsubscript{9}PEO\textsubscript{2} concentrations is around 30% for some of the present samples. NP, A\textsubscript{9}PE\textsubscript{1}C and A\textsubscript{9}PE\textsubscript{2}C were quantified using pure standards, and for higher A\textsubscript{9}PEC, the A\textsubscript{9}PE\textsubscript{2}C calibration was used, assuming the response of A\textsubscript{9}PE\textsubscript{2}C to be equal to that of A\textsubscript{9}PE\textsubscript{2}C. It can be expected that the actual response of A\textsubscript{9}PE\textsubscript{2}C is somewhat lower than A\textsubscript{9}PE\textsubscript{2}C, as the response of A\textsubscript{9}PE\textsubscript{2}C is also lower than that of A\textsubscript{9}PE\textsubscript{1}C. This would lead to an underestimation of the A\textsubscript{9}PE\textsubscript{2}C concentrations. However, for A\textsubscript{9}PEO the differences in response between consecutive oligomers tend to decrease with increasing chain length, which is probably not different for A\textsubscript{9}PEC.

All quantitations were done using 8-point quadratic calibration curves. Peak areas of sample extracts were always in the lower (linear) part of the calibration curves, and therefore the slight deviation from linearity of the calibration curve observed at higher concentrations will have no significant influence on the result.
No standards of the CAPEC metabolites were available, and therefore a qualitative identification was performed using extracts from our biodegradation experiments as a reference, in which CAPEC were identified using LC-ESI-MS/MS [7].

Quality control
A correction was made for variations in sensitivity of the LC-MS by adding $A_9$PEO$_2$ and NP isotopes with $^{13}$C-labeled aromatic rings as internal standards to the extracts prior to injection. In these internal standards, the alkyl chain is linear, and therefore the internal standards have a slightly longer retention time than the branched $A_9$PEO and NP analytes. Sediment samples were extracted in batches of 4, all containing either a procedural blank or a spiked sediment. Sediments were spiked at environmental levels: for each compound, 3 µg were added, resulting in spiking concentrations between 100 and 200 ng g$^{-1}$ (wet weight). The average recoveries and standard deviations calculated from the spiked sediments were 83±11, 63±10 and 44±14% for $A_9$PEO, NP and $A_9$PEC, respectively. For the relatively volatile nonylphenol, recovery loss probably occurs mainly during the evaporation of the extract. Relatively low NP recoveries compared to $A_9$PEO have been reported before in literature [27, 28].

For the polar $A_9$PEC metabolites, the SPE clean up step is a probable cause of recovery loss, as part of the analyte may remain in the methanol/water during the loading of the cartridge. The low recoveries of $A_9$PEC from sediments inevitably led to higher variations in recovery, and therefore these results could suffer from some inaccuracy. For water samples, average recoveries and standard deviations (n=5) were obtained of 95±13, 90±4, 87±4 and 69±9% for $A_9$PEO$_{10}$, $A_9$PE$_{1}$C, $A_9$PE$_{2}$C and NP, respectively.

Detection limits in water for $A_9$PEO$_{1}$, $A_9$PEO$_{2}$, $A_9$PEO$_{3-15}$, NP and $A_9$PEC were 15, 6, 30, 11 and 13 ng L$^{-1}$, respectively.

5.3. Results and Discussion

Concentrations in sediments of the Scheldt and Rhine estuaries
During LC-ESI-MS analysis, $A_9$PEO$_{1}$ behaves differently from the higher oligomers. The $[A_9$PEO$_{1}$+Na]$^+$ signal intensity is relatively low, but during the analysis of the pure $A_9$PEO$_{1}$ standard we observed an additional, more abundant signal at m/z 319, corresponding to a $[A_9$PEO$_{1}$+methanol+Na]$^+$ adduct. A constant 319/287 ratio of 2 is observed. By selecting both masses in the SIM analysis, satisfactory detection limits are obtained. The $[A_9$PEO$_{1}$+methanol+Na]$^+$ adduct is only observed at low source voltages.

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In the estuarine sediments, recovery corrected concentrations of A₉PEO and NP in both estuaries vary from <LOD to 1000 ng/g dry weight, as shown in table 5.1. These values are consistent with literature data reported for these compounds in estuarine sediments (see introduction, section 5.1). For the Scheldt estuary, the highest concentrations of A₉PEO and NP are found in sediments from the vicinity of the industrial areas of Antwerp and Vlissingen. This illustrates the importance of local industrial sources for these compounds. However, in water no concentration increase is observed near these industrial areas (see figure 5.4). This could indicate that the A₉PEO and NP concentrations found in the sediments are not caused by recent emissions. This would be in agreement with the results of Shang et al., who demonstrated that A₉PEO and NP can be very persistent in estuarine sediments [21].

Table 5.1: Concentrations of A₉PEO, NP and A₉PEC in sediments of the Rhine (R) and Scheldt (S) estuaries, corresponding to the locations in figure 5.2.

<table>
<thead>
<tr>
<th>location</th>
<th>organic carbon content (%)</th>
<th>concentration (ng g⁻¹ dry weight)</th>
<th>A₉PEO₁</th>
<th>A₉PEO₂</th>
<th>A₉PEO₃₋₁₅</th>
<th>NP</th>
<th>total A₉PEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>0.6</td>
<td>&lt;1.3</td>
<td>3.6</td>
<td>63.5</td>
<td>8.9</td>
<td>&lt;0.3</td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>0.34</td>
<td>4.3</td>
<td>5.5</td>
<td>71.8</td>
<td>11.0</td>
<td>&lt;0.3</td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td>&lt;0.05</td>
<td>6.6</td>
<td>2.9</td>
<td>12.5</td>
<td>1.5</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>R4</td>
<td>0.48</td>
<td>11.2</td>
<td>19.2</td>
<td>183.1</td>
<td>20.9</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>R5</td>
<td>0.65</td>
<td>19.8</td>
<td>31.5</td>
<td>247.3</td>
<td>59.5</td>
<td>21</td>
<td></td>
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<tr>
<td>R6</td>
<td>2.1</td>
<td>34.6</td>
<td>29.1</td>
<td>61.0</td>
<td>92.2</td>
<td>185</td>
<td></td>
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<tr>
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<td>0.09</td>
<td>6.0</td>
<td>5.2</td>
<td>32.3</td>
<td>12.5</td>
<td>2.9</td>
<td></td>
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<td>R8</td>
<td>0.23</td>
<td>2.4</td>
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<td>54.2</td>
<td>10.1</td>
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<tr>
<td>S1</td>
<td>1.4</td>
<td>16.1</td>
<td>5.7</td>
<td>51.5</td>
<td>22</td>
<td>&lt;0.3</td>
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<td>S2</td>
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<td>&lt;0.3</td>
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<td>S3</td>
<td>0.75</td>
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<td>17.3</td>
<td>115.5</td>
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<td>239</td>
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<td>S4</td>
<td>0.21</td>
<td>&lt;1.3</td>
<td>0.39</td>
<td>&lt;0.6</td>
<td>3.9</td>
<td>&lt;0.3</td>
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<td>S5</td>
<td>0.05</td>
<td>7.3</td>
<td>1.0</td>
<td>7.0</td>
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<td>&lt;0.3</td>
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<tr>
<td>S6</td>
<td>0.09</td>
<td>13.0</td>
<td>1.9</td>
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<td>22</td>
<td>&lt;0.3</td>
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<tr>
<td>S7</td>
<td>0.05</td>
<td>&lt;1.3</td>
<td>&lt;0.3</td>
<td>1.7</td>
<td>3.1</td>
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<tr>
<td>S8</td>
<td>0.19</td>
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<td>3.9</td>
<td>15.1</td>
<td>28</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>S9</td>
<td>&lt;0.05</td>
<td>&lt;1.3</td>
<td>&lt;0.3</td>
<td>0.92</td>
<td>0.9</td>
<td>&lt;0.3</td>
<td></td>
</tr>
<tr>
<td>S10</td>
<td>1.7</td>
<td>17.1</td>
<td>26.2</td>
<td>198.9</td>
<td>1080</td>
<td>65</td>
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</tr>
</tbody>
</table>
The A9PEO oligomer distribution patterns in sediments from both estuaries are different: while in the Scheldt estuary all sediments show the same distribution around an ethoxylate chain length of 3, in the Rhine estuary the average ethoxylate chain lengths vary from around 2 to around 9. The higher average ethoxylate chain lengths in the Rhine estuary reflect more recent discharges, or discharges of less treated wastewater. Possibly, local industries in the Rhine estuary use different A9PEO formulations. These results are a confirmation of earlier findings in this area [18].

In both estuaries, no gradient in sediment concentrations of A9PEO or NP is observed when going from the freshwater to the marine environment. Stable isotope ratios in sediments from the Rhine estuary show a clear gradient. The δ13C values decrease from −23.7‰ in marine sediment to −29.8‰ in the most terrestrial sediment, and δ15N values decrease from 8.5‰ to 4.7‰ going from marine to terrestrial sediment. In the Scheldt estuary, δ13C values slightly decrease from −25.1‰ to −29.0‰ from marine to terrestrial sediments, while δ15N strongly varied in the estuary between 3.2 and 10.5‰.

Neither the δ13C nor the δ15N isotope ratios in the sediments correlate well with the A9PEO or NP concentrations. This suggests that the A9PEO contaminated sediment is neither from terrestrial nor marine origin, but rather that the sorption of A9PEO has occurred in situ. Organic carbon normalized concentrations do not give better correlations with isotope ratios or location along the estuary.

As shown in table 5.1, organic carbon contents of the sediments vary from <0.05 to 2.1%, with median values of 0.09% for the Scheldt and 0.34% for the Rhine estuary. The percentage of particles in the size range 16-2000 μm was between 38 and 96%, with median values of 81.8% and 81.6% for the Scheldt and Rhine estuaries, respectively. This indicates that the sediment samples consist mainly of sand.

In both estuaries, sediment concentrations tend to increase with increasing organic carbon content of the sediment, with correlation coefficients of 0.84 and 0.71 for A9PEO and NP in the Scheldt estuary, and 0.42 and 0.91 for A9PEO and NP in the Rhine estuary. This indicates that organic carbon present in sediments is an important factor for sorption of surfactants, similar to non- and slightly polar organics.

The concentrations of A9PEC metabolites are below detection limits in about half the number of sediment samples, which can be explained by the hydrophilic nature of these compounds. However, the relevance of A9PEC in sediments cannot be completely neglected as at three locations, A9PEC are present at higher concentrations than NP or A9PEO. The two sediments from near industrial areas show relatively high A9PEC concentrations, consistent with A9PEO and NP levels. As the low recoveries of the A9PEC extraction from
sediment lead to higher variations in recovery, these quantitative results should be treated with some caution.

**Dissolved concentrations in the Rhine estuary**

In figure 5.3 the dissolved concentrations of A₉PEO and metabolites are plotted against salinity in the Rhine estuary. In contrast to some of the sediments in this estuary, only short-chain A₉PEO species are found in the water samples, with an average ethoxylate chain length of 3.

Along the salinity gradient, which ranges from 0.2% at location R8 to 19.0% at the North Sea (R1, see figure 5.2), a slightly decreasing trend can be observed, although both A₉PEO and metabolite concentrations fluctuate along this estuary. A variable load into the estuary due to fluctuating river concentrations is one possible explanation of this observation. Another possible reason for the observed variations are the probable local sources of A₉PEO in the Rotterdam harbours. Further monitoring and model studies will be performed to explain these phenomena.

![Figure 5.3: Dissolved concentrations of A₉PEO, NP and A₉PEC along the salinity gradient in the Rhine estuary.](image-url)
Table 5.2: Water bulk parameters and dissolved concentrations of A₉PEO, NP and A₉PEC in the Rhine (R) and Scheldt (S) estuaries.

<table>
<thead>
<tr>
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<th>Rhine estuary</th>
<th>Scheldt estuary</th>
<th>canal Gent-Terneuzen</th>
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<td>salinity (%)</td>
<td>temperature (°C)</td>
<td>pH</td>
<td>oxygen concentration (mg L⁻¹)</td>
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<td>9.4</td>
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The high water flow explains the apparent lack of biodegradation in this region, as the water residence time of only several days is too short for any significant biodegradation to occur. Ratios of A₉PEC/A₉PEO are between 1.8 and 4.3 throughout the estuary, while NP/A₉PEO ratios vary between 0.1 and 0.3. No clear gradient is observed for these ratios through the estuary.

From the analysis of the suspended particulate material samples (SPM), average sorbed concentrations were calculated of 18 μg g⁻¹ for A₉PEO and 4.6 μg g⁻¹ for NP. Average organic carbon normalized observed partition coefficient values were determined using the
Fate of nonylphenol ethoxylates and their metabolites in Dutch estuaries

formula \( K_{oc} = \frac{[\text{SPM}]}{(f_{oc} \times [\text{water}])} \) in which [SPM] is the concentration in SPM, \( f_{oc} \) is the fraction organic carbon in the SPM sample and [water] is the dissolved water concentration. Average \( \log K_{oc} \) values and standard deviations were determined of 5.8±0.3 for A₉PEO and 6.0±0.6 for NP.

Figure 5.4: Dissolved concentrations of A₉PEO, NP and A₉PEC along the salinity gradient in the Scheldt estuary (logarithmic scale). • = total A₉PEO; ■ = NP; ▲ = A₉PE₁C; x = A₉PE₂C; △ = A₉PE₃C; ○ = A₉PE₄C; - = theoretical plot of a compound behaving conservatively. NS = North Sea end of the estuary.

Dissolved concentrations in the Scheldt estuary

Figure 5.4 shows the concentrations of A₉PEO, A₉PEC and NP along the salinity gradient in the Scheldt estuary, starting at a salinity of 2 near Antwerp to salinity 32 at the North Sea. Much more clearly than in the Rhine estuary, a strong gradient can be observed in dissolved concentrations of A₉PEO when going downstream in the estuary, decreasing from 2300 to 41 ng L⁻¹. Figure 5.4 shows that the behaviour of A₉PEO is nonconservative, as the concentration decrease is faster than linear along the salinity gradient (note the log scale of the concentration). This suggests that in addition to simple dilution of the river water,
removal processes like biodegradation or sorption play a significant role in the area. The ethoxylate distribution of the A₉PEO is rather constant throughout the estuary, with a maximum around A₉PEO₃. The ethoxylate distribution pattern is virtually equivalent in samples of sediment and water from this area.

The concentration profile of NP along the salinity gradient shows non-conservative behaviour very similar to that of A₉PEO.

The presence of the long-chain A₉PE₃₈C is especially interesting, as it is the first time that these specific oligomers are reported to be present in the estuarine environment. The longest-chain APE₅₄C were only present in the upper part of the estuary, as well as in the canal Gent-Terneuzen (see figure 5.2). A chromatogram showing long-chain A₉PEC in a water sample taken near Antwerp is shown in figure 5.5.

![LC-MS chromatogram showing long-chain A₉PEC](image)

Figure 5.5: LC-MS chromatogram (negative SIM) showing both short- and long-chain A₉PEC in estuarine water near Antwerp.
Only very few reports are available on \( A_9 \text{PEC} \) in estuaries. Marcomini found \( A_9 \text{PEC} \) concentration ranges in the Venice lagoon similar to those found in the present study, but it is unclear which ethoxylate chain lengths were present [23]. Short-chain \( A_9 \text{PEC} \) are also mentioned qualitatively to be present in Jamaica Bay, New York, USA [15].

In the Scheldt estuary, the \( A_9 \text{PEC} \) metabolites are present at higher dissolved concentrations than the \( A_9 \text{PEO} \), and their concentration decrease along the salinity gradient is much slower. This difference is illustrated in figure 5.6, in which ratios in dissolved concentrations of metabolite over total surfactant are shown. The \( A_9 \text{PEC}/A_9 \text{PEO} \) ratio strongly increases from 5 at salinity 2 to 43 at salinity 24. A likely explanation for this increase is estuarine formation of \( A_9 \text{PEC} \) out of \( A_9 \text{PEO} \).

![Figure 5.6: \( A_9 \text{PEC}/A_9 \text{PEO}, A_9 \text{PEO}_2/A_9 \text{PEO}_{\text{total}} \) and \( \text{NP}/A_9 \text{PEO} \) water concentration ratios in the Scheldt estuary.](image)

Another explanation for the observed trends can be selective removal of \( A_9 \text{PEO} \) and \( \text{NP} \) by sorption to suspended particulate material (SPM), while negligible sorption of \( A_9 \text{PEC} \) may occur. Analysis of the SPM samples did indeed show higher observed SPM-water partition coefficients for \( A_9 \text{PEO} \) and \( \text{NP} \) than for \( A_9 \text{PEC} \). In many SPM samples, no \( A_9 \text{PEC} \) were detected, but for the five samples in which \( A_9 \text{PEC} \) concentrations were above detection limit, an average organic carbon normalized log\( K_{oc} \) and standard deviation of \( 4.2 \pm 0.5 \) was...
calculated. A₉PEO and NP were detected in all SPM samples, and show a slightly decreasing trend from 28 µg/g upstream to 0.7 µg/g downstream, while the concentration of SPM in the water slightly increases. Both A₉PEO and NP showed the same average logKₒC of 5.9±0.6. No clear trend can be observed in the logKₒC values along the estuary, indicating that sorption does not increase significantly, and no salting out effects are observed. The average logKₒC values in the Scheldt estuary are similar to those determined for the Rhine estuary, which implies that this partitioning behaviour does not necessarily cause the strong concentration decrease observed in the Scheldt estuary.

When both the dissolved A₉PEO and the fraction sorbed to SPM are added to obtain calculated total concentrations for the water column, the trends as shown in figure 5.4 do not change significantly. The thus observed decrease of this total A₉PEO concentration is still steeper than conservative. This proves that removal processes other than sorption are occurring simultaneously. The steeper concentration decrease of A₉PE₃C compared to the lower A₉PE₃₋₅C (see figure 5.4) is an additional indication that the main removal process in this estuary is biodegradation.

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Figure 5.7: Theoretical concentration ratios of A₉PEC/A₉PEO during the biodegradation of A₉PEO assuming consecutive first order reactions. ■ = A₉PEC/A₉PEO without a baseline concentration of both compounds; ▲ = A₉PEC/A₉PEO with a baseline concentration of both compounds. Note that both plots have different scales.
The decrease of the A9PEC/A9PEO ratio at salinities higher than 25 seems illogical at first sight, as one would expect that the ratio would further increase if the degradation reactions proceed. However, when one assumes a very low, constant input of A9PEO all along the estuary, by diffuse sources such as from the tanker cleaning of ships, the resulting low ‘baseline’ concentration would explain why at very low A9PEO and A9PEC concentrations the ratio would drop. Due to e.g. the intensive shipping traffic to and from Antwerp it is not unlikely that this baseline level may be present. The influence of a baseline concentration can be illustrated by simple model calculations, as shown in figure 5.7. Here a theoretical plot of the metabolite/substrate concentration ratio versus time is shown. The assumption is made that both surfactant and metabolite are (further) degraded following consecutive first order reactions [29]. Two plots are made: one showing the ratios when no baseline concentration is present, and the other showing the situation when a very low concentration of both substrate and metabolite is added to the results of the concentration equations. In the example shown, the A9PEO starting concentration is the actual dissolved concentration at Antwerp (2300 ng L⁻¹), and a baseline concentration of 10 ng L⁻¹ A9PEO and A9PEC is chosen, assuming first order rate constants of 0.01 and 0.003 h⁻¹ for the formation and degradation of A9PEC, respectively. These rates are optimized values, chosen so that the maximum ratio reached in the example corresponds to the actual maximum ratio observed in the estuary, on a time scale comparable to the freshwater residence time in the estuary. As can be seen from figure 5.6, neither the A9PEO₂/A9PEO_total nor the NP/A9PEO_total ratio show any significant increase when going downstream in the estuary. This observation indicates that it is unlikely that A9PEO₂ or NP are formed out of A9PEO in this region. The main degradation route of A9PEO in the estuary is direct oxidation of the terminal ethoxylate group, followed by shortening of the ethoxylate chain. This is an important field confirmation of a previously proposed biodegradation route inferred from experimental laboratory work [7].

Marcomini et al. [23] found an average A9PEC/A9PEO ratio of only 0.5 in the Venice lagoon, and suggested that the oxidative-hydrolytic degradation was of less importance in the estuarine environment than in freshwater. However, no NP was found in the lagoon water, and A9PEO₂/A9PEO_total ratios were not given, which makes it difficult to compare the importance of the oxidative and non-oxidative routes [23].

The explanation of the A9PEC/A9PEO ratio gradient in the Scheldt estuary is somewhat complicated by the fact that near the location with the highest A9PEC/A9PEO ratio, a canal discharges its freshwater into the estuary. The canal Gent-Terneuzen (see map in figure 5.2b) was found to contain a high dissolved total A9PEC concentration of 9270 ng L⁻¹, with an A9PEC/A9PEO ratio of 43. This water input will have some contribution to the ratio gradient, as this water with its high metabolite concentrations will spread in both directions in the estuary. However, as the water discharge from the canal is about 7 times less than the
freshwater flow in the estuary, it can only partially explain the concentrations observed in the estuary, and it can certainly not explain the different shapes of the A₀PEO and A₀PEC concentration profiles along the salinity gradient (A₀PEO decreasing very rapidly and A₀PEC decreasing more slowly).

In all samples, CA₅₋₉PE₁₋₂C metabolites were analyzed (this notation signifies alkyl chains of 5 to 9 carbons, including the oxidized one, and 1 to 2 ethoxylate units, including the oxidized one). Although in some water samples series of peaks of the correct masses were observed, the retention times deviated too much from the reference extracts (several minutes) to allow a positive identification. In our previous laboratory degradation experiments with spiked Rhine river water, the observed CAPEC concentrations reached a maximum around a level corresponding to 5% of the A₀PEC concentrations. If this ratio would be similar in the present environmental samples, this would correspond to values well above the detection limit. Apparently, the CAPEC metabolites are more quickly (further) degraded in these estuaries than under laboratory conditions. The present results do not confirm the recent findings of DiCorcia, who reported CAPEC as the main A₀PEO metabolites in Italian sewage effluents [30].

Concentrations of A₈PEO are below the detection limit (< 31 ng L⁻¹) in all samples. This finding can be explained by the consumption figures for A₈PEO, which are generally 10 times lower than those of A₉PEO [18, 31]. Reported surface water concentrations of A₈PEO are usually also one order of magnitude lower than for A₉PEO. A recent survey conducted in Dutch estuarine waters concluded similarly about dissolved concentrations of A₈PEO [32]. Ferguson reported maximum A₈PEO₁₋₃ concentrations of 34 ng L⁻¹ [15].

The present study shows that the Scheldt estuary has an efficient self-cleaning capacity concerning alkylphenol ethoxylates, in which aerobic biodegradation is of principal importance. We conclude that the oxidative hydrolytic degradation pathway is the main degradation route in the vertically well-mixed Scheldt estuary. Sorption is less important for the fate of A₈PEO in the water column than degradation. The influence of local sources of AP₈EO on their fate in the water column of the Scheldt is also limited. Our future efforts employing estuarine fate models will investigate the contributions of the different processes more quantitatively.

For stratified estuaries with relatively high water flows like the Rhine estuary, general trends are difficult to distinguish. In the latter type of estuaries, more elaborate sampling strategies including sampling at different depths should be conducted for accurate descriptions of such systems.
Acknowledgments

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References


"How inappropriate to call this planet Earth, when clearly it is Ocean."