



UvA-DARE (Digital Academic Repository)

Assessment of azaarenes and azaarones (oxidized azaarene derivatives) in the Dutch coastal zone of the North Sea

de Voogt, P.; Laane, R.W.P.M.

Published in:
Chemosphere

DOI:
[10.1016/j.chemosphere.2009.04.029](https://doi.org/10.1016/j.chemosphere.2009.04.029)

[Link to publication](#)

Citation for published version (APA):

de Voogt, P., & Laane, R. W. P. M. (2009). Assessment of azaarenes and azaarones (oxidized azaarene derivatives) in the Dutch coastal zone of the North Sea. *Chemosphere*, 76(8), 1067-1074.
<https://doi.org/10.1016/j.chemosphere.2009.04.029>

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.



Assessment of azaarenes and azaarones (oxidized azaarene derivatives) in the Dutch coastal zone of the North Sea

Pim de Voogt^{a,b,*}, Remi W.P.M. Laane^{a,c}

^a IBED, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands

^b KWR Watercycle Research Institute, P.O. Box 1072, 3430 BB Nieuwegein, The Netherlands

^c Deltares, P.O. Box 177, 2600 MH Delft, The Netherlands

ARTICLE INFO

Article history:

Received 5 January 2009
Received in revised form 13 April 2009
Accepted 14 April 2009
Available online 13 May 2009

Keywords:

PAH
PANH
Mineral oil
Marine sediments
Analysis
GC–MS

ABSTRACT

Azaarones (oxidized derivatives of azaarenes) is a group of newly emerging chemical compounds. Little is known about their occurrence in the aquatic environment. Azaarenes are polycyclic aromatic heterocyclic compounds containing one nitrogen atom in one of the aromatic rings. The (photo) oxidized metabolites of the azaarenes are often more toxic than the parent compound.

For the first time the concentration of seven azaarenes and seven primary metabolites have been measured in the surface sediments (fraction < 63 μm) of the Dutch coastal zone of the North Sea. Samples collected in 2000 and 2001 were analyzed using a newly developed method to determine the contents of azaarenes and azaarones simultaneously in a single GC–MS run.

The concentrations of acridine, benz[*a*]acridine, benz[*c*]acridine and 5,6-benzoquinoline varied between 10–63, 3.9–25, 3.3–11 and 3.98–10.84 ng g^{-1} , respectively. Concentrations of 7,8-benzoquinoline and phenanthridine were below the limit of detection. 2-Hydroxyquinoline and 5-hydroxyquinoline, probably metabolites of quinoline, were present in relatively high concentrations: 7.4–949 and 11–188 ng g^{-1} .

A gradient was observed with highest concentrations of the sum of azaarenes and the sum of the concentration of their metabolites close to the coast and lower concentrations further offshore. The concentrations of azaarenes and their metabolites are in the same order of magnitude as those found to induce phototoxicity to algae.

The concentrations of mineral oil and PAHs in the surface sediments of the Dutch coastal zone of the North Sea were, at most locations, above the Dutch chemical targets for environmental protection. Spatial distribution of PAHs and mineral oil were slightly different from those of azaarenes and transformation products.

Simultaneous GC–MS for azaarenes and their degradation products is possible but extraction/clean up can be further improved. Azaarenes as well as their primary metabolic products are present in the marine environment. In sediments the cumulative concentrations of transformation products amount to about four times the cumulative concentrations of the azaarenes. In conclusion, azaarenes and their metabolites constitute a new group of emerging polycyclic aromatic compounds which need more attention in the future.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Azaarenes are polycyclic aromatic hydrocarbons (PAHs) in which a carbon atom in one of the aromatic rings is substituted with a nitrogen atom (polycyclic aromatic nitrogen heterocyclics, PANH). They are naturally produced by organisms in the form of

e.g. mycotoxins, alkaloids and nucleotides (Schmitter et al., 1982; Kaiser et al., 1996). A large part of the azaarenes observed in the environment originate from anthropogenic sources, such as chemical industry with coal tar and oil shale processing operations, wood preserving facilities and chemical manufacturing plants (Wakeman, 1979; Kaiser et al., 1996). Environmental concentrations of azaarenes are known already for about 30 years. Concentrations of azaarenes have been detected in fresh and marine water and sediments and in groundwater (Blumer et al., 1977; Wakeman, 1979; Furlong and Carpenter, 1982; Pereira et al., 1983; van Genderen et al., 1994; Kozin et al., 1997; Osborne et al., 1997; Bleeker et al., 1998; Chen et al., 2008). In general, their

* Corresponding author. Address: IBED, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands. Tel.: +31 20 5256565; fax: +31 20 5257431.

E-mail addresses: w.p.devoogt@uva.nl (P. de Voogt), remi.laane@deltares.nl (R.W.P.M. Laane).

concentrations are lower (1–10%) than those of the PAH analogues (Kozin et al., 1997; Bleeker et al., 1998). However, as the azaarenes are more water-soluble than PAHs they are likely to be more bio-available (Pearlman et al., 1984). An unknown part of the azaarenes is biologically and photochemically oxidized into azaarones in the aquatic environment (Wiegman et al., 2001). De Voogt et al. (1999) concluded that keto-metabolites are major products in the aquatic fate of benzoquinolines. In the atmosphere similar photochemical transformations have been observed (Negrón-Encarnación and Arce, 2007). In contaminated groundwater hydroxylated N-heterocyclics have been reported to be significantly higher than their parent compounds (Pereira et al., 1988; Reineke et al., 2007).

Compared to azaarenes, much more scientific attention has been paid to the possible negative effects of PAHs on organisms (Gissel-Nielsen and Nielsen, 1996; Reynaud and Deschaux, 2006). PAHs have been found nearly everywhere in the aquatic environment (Jones et al., 1989; Wild and Jones, 1995; de Maagd, 1996; Xue and Warschowsky, 2005; Reynaud and Deschaux, 2006) and associated with the occurrence of for instance fish diseases (Vethaak et al., 1996).

A few papers have described the acute and chronic toxicity of azaarenes to several fish species, a few aquatic invertebrates, and marine algae (Gissel-Nielsen and Nielsen, 1996; Wiegman et al., 2001; Neuwoehner et al., 2008). Wiegman et al. (2001) showed that some of the oxidized derivatives of azaarenes are easily produced photochemically in the aquatic environment and that the derivatives are more toxic to algae than the parent compounds.

The experimental toxicological findings described above prompted an investigation into the environmental occurrence of these compounds. This paper describes the assessment of the concentrations of azaarenes and azaarones (ranging from two- to four-ringed structures) in the surface sediments of the Dutch coastal zone of the North Sea. For that purpose an analytical method was developed that would be able to detect both azaarenes and their oxygenated transformation products in one analytical run. Simultaneously the concentrations of PAHs and mineral oil in these sediments were determined in order to possibly relate their presence to that of the azaarenes.

2. Materials and methods

2.1. Solvents and chemicals

Kieselguhr GR (Merck, Darmstadt, Germany) was used as received. Aluminiumoxid 90 (Merck) aktiv neutral aktivitätsstufe I. (0.063–0.200 mm, mesh 70–230) was dried overnight in an oven at 200 °C and deactivated with distilled water before use. Silica (Kieselgel 60, 70–230 mesh; Merck) was activated at 160 °C overnight and deactivated with distilled water.

All solvents used were of HPLC grade. Anhydrous sodium sulphate (Sigma–Aldrich Zwijndrecht, The Netherlands) was of analytical grade quality. Nitrogen-containing analytes were obtained from Sigma–Aldrich, and had purities varying between 97% and 99%. Acridine may contain acridinone and was further purified by adsorption chromatography on aluminium oxide. Homocyclic PAHs were obtained from various suppliers and all had a declared purity of 98–99%.

2.2. Sampling

Earlier studies on the distribution of metals and organic compounds (PCBs and PAHs) in the surface sediments (fraction < 63 µm) of the Dutch coastal zone revealed that the first 30–40 cm of the surface sediments are homogeneously mixed (Laane et al., 1999). The

fraction smaller than 63 µm was isolated because most of the hydrophobic chemical compounds are adsorbed on the smaller particles. Standardization of concentrations is necessary because in the Dutch coastal zone there is a gradient in the amount of smaller particles in the surface sediment (Eisma, 1993). Klamer et al. (1990) showed that the fraction < 63 µm could be used to standardize the concentration of the various hydrophobic chemical compounds. Sixty-two surface samples (<20 cm) were sampled with a box core in the Dutch coastal zone of the North Sea from September 2000 to January 2001 (Fig. 1). Sampling locations are clustered into four groups: A ($n = 8$) and B ($n = 10$) off the Dutch mainland coast, C off the island of Texel ($n = 20$) and D near the island of Terschelling ($n = 24$) (Fig. 1). The locations A and B represent transects through the major salinity and suspended matter gradients along the Dutch coast. Locations C and D were part of a larger survey that investigated relationships between occurrence of organotin concentrations in marine sediments and shipping activities along the coast. It is known that sediment concentrations of various organic contaminants do not show large seasonal variations and therefore all locations were sampled once in time.

Sediment samples were stored at –20 °C and sieved over a 2 mm sieve before the fraction smaller than 63 µm was isolated by sieving and continuous flow centrifugation (Laane et al., 1999). The sediments can be characterized as sandy (<63 µm) with relatively low organic carbon contents (Fig. 2).

2.3. Analysis

Limits of detection were defined as three times the standard deviation of the noise level. LODs may vary from sample to sample, even for the same analyte, because of different sample intakes, and different signal to noise ratios caused by sample matrices. Seven azaarenes: quinoline (limit of detection: 4.0 ng g⁻¹), acridine (LOD: 4.2 ng g⁻¹), phenanthridine (LOD: 0.7 ng g⁻¹), 5,6-benzoquinoline (LOD: 4.4 ng g⁻¹), 7,8-benzoquinoline (LOD: 4.4 ng g⁻¹), benzo[a]acridine (LOD: 4.4 ng g⁻¹), benzo[c]acridine (LOD: 4.0 ng g⁻¹) and five oxidized derivatives: 2-, 5-, 6- and 8- hydroxyquinoline (LOD: respectively, 4 (for samples from clusters C and D) and 10 (for samples from clusters A and B), 10, 0.5 and 11 ng g⁻¹) and 6(5H)-phenanthridinone (LOD: 3.9 ng g⁻¹) have been extracted and analyzed.



Fig. 1. Sampling locations of the surface sediments from the Dutch coastal zone of the North Sea. Sampling locations are clustered into four groups A, B, C, and D.

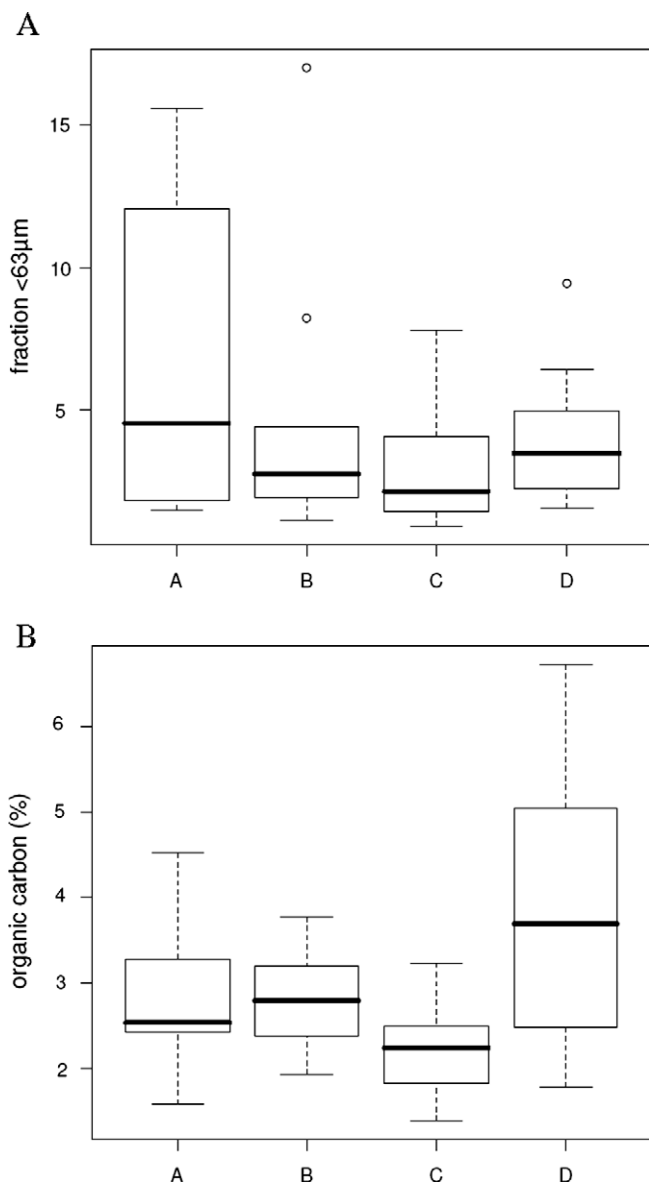


Fig. 2. Box whisker plot of averaged grain size distribution (A; <63 μm) and organic carbon contents (B; %OC) in the surface sediments of the four sampling clusters (A–D) in the Dutch coastal zone in 2000 and 2001.

It appeared that three azaarenes, viz., 4-OH-acridone, 8-hydroxyquinoline and 4-hydroxyl-quinoline, could not be quantified (see below).

The molecular structures of the azaarenes and azaarones are given in Fig. 3.

For the determination of azaarenes and their derivatives, wet sediment (3–5 g, fraction <63 μm) was mixed with Kieselguhr until a homogeneous free-flowing powder was obtained. One hundred microliters of an internal standard solution were then added and the sample was left to equilibrate (>1 h). Next, the sample was transferred to a glass Soxhlet thimble and extracted in a Soxhlet device during 18 h using 200 mL of a solvent mixture consisting of 8:2 (v/v) *n*-hexane and ethyl acetate (EtAc). Hexane–EtAc was chosen because this mixture provides an appropriate polarity for the extraction of the PANH. The resulting extract was concentrated to 1 mL. EtAc is particularly suited for the transformation products of PANH, e.g. acridone), which have limited solubility in solvents like hexane or methanol. Clean up of the extracts was performed over a glass column (i.d. 5.7 mm) containing 2.2 g of 5% H₂O-deac-

tivated aluminium-oxide topped with 0.4 g of anhydrous sodium sulphate. The column was conditioned with 10 mL of methanol, 1 mL of ethyl acetate and 10 mL of *n*-hexane. The sample extract was then transferred to the top of the column and fractionated according to following elution scheme: fraction 1 and 2, respectively, 15 mL and 20 mL of hexane–EtAc (1:1), fraction 3, 20 mL of MeOH/EtAc (1:1) and the last fraction 10 mL of 100% MeOH. Fractions 1 and 3 were collected; these contain the analytes. Fractions 2 and 4 were not measured. Three azaarenes, viz., 4-hydroxy-acridone, 8-hydroxyquinoline and 4-hydroxyquinoline, were not recovered from the chromatographic column used and could therefore not be quantified.

For extraction of marine water samples, liquid/liquid extraction using 50 mL of ethyl acetate and 500 mL of sample was used in a triple extraction step and concentrated to 2 mL.

Recoveries of extraction and clean up procedure were evaluated by spiking analytes onto dried sediments for which analyte levels had been previously determined. If native levels were present then these values were subtracted from the recovered amounts.

A purified extract of sediment or a concentrated extract of water was analyzed by GC–Electron Impact–single quadrupole MS (Trace GC Ultra, Thermo Scientific) in SIM mode using a 50 m \times 35 μm film thickness DB-wax column and on-column injection. Helium was used as carrier gas and the oven temperature was programmed from 70 $^{\circ}\text{C}$ (2 min hold) to 150 $^{\circ}\text{C}$ at $t = 10$ min and to 250 $^{\circ}\text{C}$ at $t = 35$ min (10 min hold). Deuterated standards of acridine and quinoline were used as surrogate standards. For the other azaarenes no D-standards were available. Ions used for identification and quantification are listed in the [Supplementary information](#) together with typical chromatograms of a sample and a standard of 8-OH-quinoline and a mass spectrum. All D-standards used were of 99% purity or better. For the identification of analytes in the samples a decision scheme was used as shown in the [Supplementary information](#).

PAHs were extracted from the sediments with an acetone/*n*-hexane (10:90 v/v) mixture in an accelerated solvent extractor (Dionex ASE-2000). Three to 5 g (fraction <63 μm) of each sample were weighed into the pressure tubes used for the accelerated solvent extractor. Pure solvent-washed sand was added to the tubes as filler and internal standards were added. The sample, filler and solvent were heated to 100 $^{\circ}\text{C}$, pressure 10.35 MPa using the standard ASE program. After the ASE extraction, the samples were evaporated with a stream of nitrogen gas to a volume of 2–3 mL. Extracts were purified over a 3 g of 15% water-deactivated silica-column. The purified extracts were transferred into acetonitril and PAHs were separated and analyzed by LC (Vydac column, Varian 9065) using a diode-array detector and a Jasco FP-950 fluorescence detector (excitation 254 nm, detection at fluorescence maximum). The mobile phase consisted of acetonitril–water employing a gradient from 70% to 90% ACN in 25 min at a flow rate of 1.0 mL min^{−1}. The sum of the 16 PAHs of EPA have been analyzed: Naphthalene (LOD: 2 ng g^{−1}), Acenaphthene (LOD: 2 ng g^{−1}), Acenaphthylene (LOD: 3 ng g^{−1}), Anthracene (LOD: 0.5 ng g^{−1}), Phenanthrene (LOD: 8 ng g^{−1}), Fluoranthene (LOD: 1 ng g^{−1}, *), Fluorene (LOD: 2 ng g^{−1}), Pyrene (LOD: 1 ng g^{−1}), Benzo[*a*]anthracene (LOD: 0.5 ng g^{−1}), Chrysene (LOD: 0.5 ng g^{−1}), Benzo[*b*]fluoranthene (LOD: 0.5 ng g^{−1}, *), Benzo[*k*]fluoranthene (LOD: 0.5 ng g^{−1}, *), Benzo[*a*]pyrene (LOD: 1 ng g^{−1}, *), Benzo[*ghi*]perylene (LOD: 0.5 ng g^{−1}, *), Dibenzo[*ah*]anthracene (LOD: 0.5 ng g^{−1}), and Indeno[123 *cd*]pyrene (LOD: 3 ng g^{−1}, *); * = Borneff PAHs.

Mineral oil in the samples (20 g) was first extracted with 50 mL of acetone (3 h) and subsequently with a 50–50 mixture of acetone and hexane (16 h). The filtered extract was twice extracted with distilled water to remove the acetone. The hexane extract was dried with anhydrous sodiumsulphate, purified with 10 g of Florisil[®] and concentrated to 1 mL with Kuderna–Danish under a

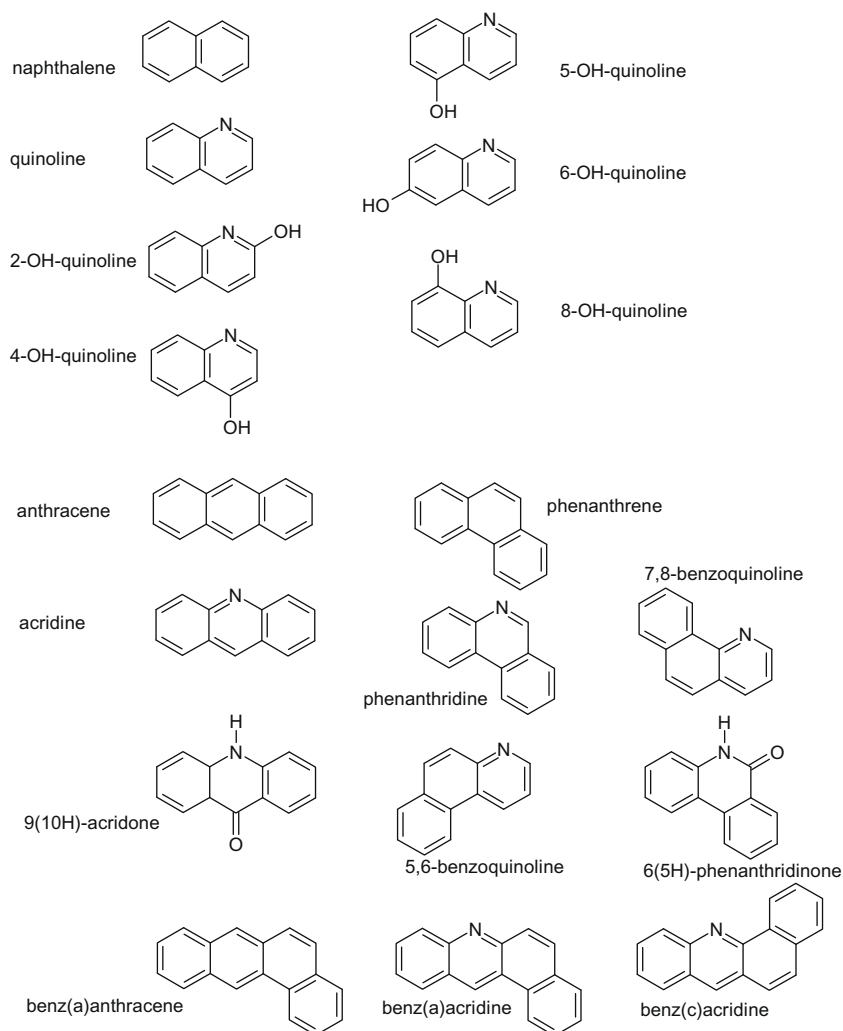


Fig. 3. Molecular structure of azaarenes and azaarones (oxidized derivatives) and corresponding homocyclic polyaromatic hydrocarbons (PAHs).

nitrogen flow. Analysis of mineral oil took place on a Chrompack CP9000 GC with FID and on-column injection (0.5 μ L). The mineral oil concentration was calculated by integrating the part between C₁₀ and C₄₀, an external certified standard and dry solid extraction.

The frozen samples were freeze-dried and ground by a ball mill before the organic carbon content was determined by combustion in an oxygen atmosphere, thus converting any organic carbon present to CO₂, and determining the CO₂ content by non-dispersive Infrared detection.

3. Results and discussion

3.1. Analytical method

In this study an analytical method was optimized and employed to simultaneously determine azaarenes and their oxygenated derivatives in a single GC–MS run. Method optimization included selection of MS fragment ions for confirmation (see [Supplementary material](#)), selection of extraction solvent, and determination of optimum volumes of clean-up fractions. We used different methods for extracting PANHs compared to PAHs because the PAH method (ASE) was an existing procedure validated for PAHs that had been shown previously in our laboratory to be more efficient (in time and volume consumption) than the Soxhlet extraction, whereas the extraction of PANH in our laboratory had been

validated for the Soxhlet procedure only. Extraction solvents tested for PANH in a Soxhlet set up included *n*-hexane:acetone (1:1 v:v), hexane–EtAc (8:2), and methanol. Hexane–EtAc acetate yielded the best results, in particular for the azaarones because of their limited solubility in other organic solvents (de Voogt et al., 1999). Koci et al. (2007) have tested different extraction methods for PANH spiked in soil and found Soxhlet extraction to be somewhat inferior to e.g. SFE or pressurized solvent extraction (ASE). However, the solvents they used are less suitable for the PANH transformation products. In this study we did not avail of SFE, and we are currently proceeding with testing efficiency of ASE for extraction PANH transformation products from sediments.

The clean up elution scheme was optimized by varying solvent amounts used for each fraction. The final fraction volumes selected (indicated in Section 2) were found to provide a suitable separation of analyte and waste fractions. The waste fractions were discarded so as to minimize presence of sample matrix components in the final extracts.

To the best of our knowledge this is the first time that simultaneous determination of azaarenes and their metabolites in sediments by GC–MS without a derivatization step has been reported. With HPLC coupled to DAD or fluorescence detection good separation of NPAH have been reported (see e.g. Svabensky et al., 2007), but so far no transformation products have been included. The simultaneous method turned out to work particularly well for the

parent compounds, 2-OH-quinoline and both metabolites of the three-ringed azaarenes, with overall recoveries >70%. The overall recoveries and a typical chromatogram are shown in the supporting information.

Obviously for some of the hydroxylated quinolines this methodology was suboptimal, leading to non recoveries for 8-OH-quinoline and 4-OH-quinoline, and also 4-OH-acridine. These compounds could not be quantified therefore. In the case of 5-OH quinoline and 6-OH-quinoline relatively low (5% and 18%, respectively), but consistent and repeatable recoveries were found (see supporting information). Both compounds could be quantified therefore, although these results should be taken as indicative. Probably in the case of 8-OH, 5-OH and 6-OH-quinoline the aluminum oxide absorption chromatography step retains most, or all of the analytes. In the course of this study no further optimization of the eluting solvent could be made. However, employing a more polar solvent, e.g. methanol–dichloromethane would probably recover the hydroxylated metabolites. A drawback would be the incompatibility of the resulting methanol extract with GC, requiring additional solvent exchange. In the case of 4-OH-quinoline, also no chromatographic separation could be obtained, i.e. very poor peak shapes were invariably observed, possibly partly caused by its possible tautomerism. Therefore quantification was not possible even if recoveries would have been better.

Analytical results presented below have not been corrected for recovery.

We also analyzed azaarenes and their transformation products in marine and fresh waters by the method described above. The levels observed were in general below the limits of detection.

3.2. Azaarenes and azaarones

The oxidized derivatives of quinoline, 2-hydroxyquinoline and 5-hydroxyquinoline, were present in the highest concentrations in the Dutch coastal zone of the North Sea; ranges were 7.4–980 ng g⁻¹ (mean 132 ± 208 ng g⁻¹, n = 28) and 12–188 ng g⁻¹ (mean 60.5 ± 48.8 ng g⁻¹, n = 31), respectively. The mean concentration of the parent compound quinoline was 11.7 ± 20.6 ng g⁻¹ (range 0.97–66.4 ng g⁻¹, n = 44). The highest concentrations of 2-hydroxyquinoline and of 5-hydroxyquinoline were found in clusters D and A, respectively. Standardized on organic carbon the concentrations of these two compounds at the locations in cluster D were of the same order of magnitude as those in clusters B and C. At 21 sampling locations both concentrations were above the LOD and the ratio between 2-hydroxyquinoline and 5-hydroxyquinoline was not constant and ranged between 0.1 and 35. The mean ratio of 2-hydroxyquinoline to quinoline observed in this study amounts to 12. Values of between 1 and 30 have been reported for the ratio of 2-OH-quinoline to quinoline in contaminated groundwaters (Reineke et al., 2007).

The range in the concentrations of 6-hydroxyquinoline was somewhat lower: 4–16 ng g⁻¹ (mean 9.9 ± 5.2 ng g⁻¹, n = 4).

The concentrations of acridine in the sediments varied between 9.97 and 63.5 ng g⁻¹ (mean = 30.3 ± 15.2 ng g⁻¹; n = 48). Its transformation product 9(10H)-acridone was detected only at one sampling station where it amounted to 11.2 ng g⁻¹.

The concentrations of 7,8-benzoquinoline were below the LOD at all sampling locations. The concentrations of 5,6-benzoquinoline and of 6(5H)-phenanthridinone varied between 3.9–10.8 ng g⁻¹ (mean 6.4 ± 2.8, n = 5) and 4.2–28 ng g⁻¹ (11.6 ± 4.3 ng g⁻¹, n = 37), respectively. The concentration of phenanthridine was below its limit of detection of 0.7 ng g⁻¹ at all sampling locations.

This absence of phenanthridine and the presence of acridine as well as both the 3-ring metabolites may be explained by the differences in transformation rates of phenanthridine and acridine. Van Herwijnen et al. (2004) showed that bacterial transformation rates of azaarenes depend on the concentration of the azaarene, and that

at relatively low concentrations (as is the case in the present study) phenanthridine may be transformed more rapidly than acridine.

The concentrations of benzo[a]acridine and benzo[c]acridine, ranged between 3.76–24.8 ng g⁻¹ (10.1 ± 5.6 ng g⁻¹, n = 29) and 3.3–11.2 ng g⁻¹ (5.6 ± 2.0 ng g⁻¹, n = 22), respectively.

In 41 samples, the concentrations of both quinoline and the sum of the hydroxyquinolines were above the limit of detection. In all samples the summed concentration of the hydroxyquinolines was higher than that of quinoline (ratio of means: ~20). Only in four samples in cluster D the concentration of quinoline was higher than the hydroxyquinolines.

A gradient was found in the mean concentrations of quinoline and mean total concentrations of hydroxyquinolines going from lowest concentrations in location cluster D (59.6 and 55.9 ng g⁻¹, respectively), via C (97.0 and 77.9 ng g⁻¹, respectively), and B (161.6 and 157.9 ng g⁻¹, respectively), to the highest concentrations observed in cluster A (335.43 and 332.0 ng g⁻¹, respectively). A similar, but less steep, gradient was observed in the oxidized phenanthridines: 9.9 ng g⁻¹ in cluster D, 11.37 ng g⁻¹ in C, 13.15 ng g⁻¹ in cluster B and 13.71 ng g⁻¹ in cluster A. As an example, the gradients in the concentration of quinoline and of the sum of the oxidized quinolines along two transects in the Dutch coastal zone (see Fig. 1) are given in Fig. 4. Concentrations below the detection limit were set to zero.

The concentrations of azaarenes and azaarones observed in this study have been compared with concentrations found elsewhere (Table 1). The total azaarenes concentrations in the surface sediments of the Dutch coastal zone are somewhat less than those observed in the estuarine sediments of the Mersey and the sediment of Lake Zurich. Concentrations of acridine in this study are in the same order of magnitude of those found in Puget sound and higher than those reported in the Danshuei river in Taiwan. The

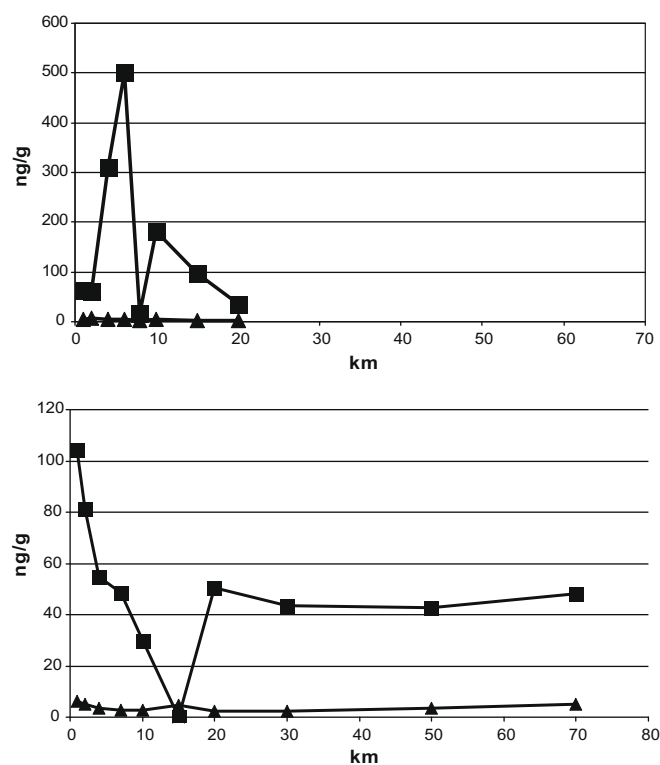


Fig. 4. Gradients in the concentration of quinoline (▲, ng g⁻¹) and the concentration of the sum of the oxidized quinolines (■, ng g⁻¹) in the surface sediments (fraction < 63 μm) at two transects (A, top and B, bottom, see Fig. 1) in the Dutch coastal zone of the North Sea.

Table 1
Comparison of literature data on occurrence of azaarenes and azaarones in sediments with those found in this study.

Location	Type	Substances	Ranges observed (ng g ⁻¹ d.w.)	Reference
North Sea	Sediments < 63 μm	Benzacridines	7–36	This study
Dutch Rivers	Sediments	Benzacridines	2.5–95	Kozin et al. (1997)
Lake Zurich	Sediments	Benzacridines	95	Wakeman (1979)
North Sea	Sediments < 63 μm	Total azaarenes (non methylated)	1–125	This study
Mersey	Sediments < 125 μm	Total azaarenes (including methylated)	2–264	Osborne et al. (1997)
Lake Zurich	Sediments	Total azaarenes (not specified)	6000	Wakeman (1979)
North Sea	Sediments < 63 μm	Total benzoquinolines	37	This study
Lake Zurich	Sediments	Total benzoquinolines	88	Wakeman, 1979
North Sea	Sediments < 63 μm	Acridine	30	This study
Puget Sound	Sediments	Acridine	43	Blumer et al. (1977)
Danshuei River	sediments	Total quinolines	3–10	Chen et al. (2008)
		Total 3-ring	0.5–4.1	
		Total 4-ring	0.5–3.0	

concentrations of benzacridines and total benzoquinolines are of the same order of magnitude as found in other areas (Table 1).

3.3. Mineral oil

The mineral oil concentrations in surface sediments in the Dutch coastal zone of the North Sea ranged between <107 and 753 μg g⁻¹. The mineral oil concentration at transects A and B are presented in Fig. 5. The average concentration is higher at transect A than at B. At transect A the highest concentrations of mineral oil are found 6 km offshore, decreasing to lower values at 20 km. The concentration of mineral oil at transect B was below the limit of detection (<107 μg g⁻¹) at 10 km and further offshore. The concentrations of mineral oil observed at transects A and B (1–10 km offshore) in 2000 are similar to those reported in 1996 (Laane et al., 1999; Hegeman and Laane, 2004). The median concentration of mineral oil in the offshore samples of cluster D is 212 μg g⁻¹, whereas nearer to the shore a mean value of 133 μg g⁻¹ was found.

3.4. PAHs

Concentrations of the sum of the 16 EPA PAHs (Σ16 EPA) and the sum of the Borneff PAHs (Σ6Borneff) ranged between

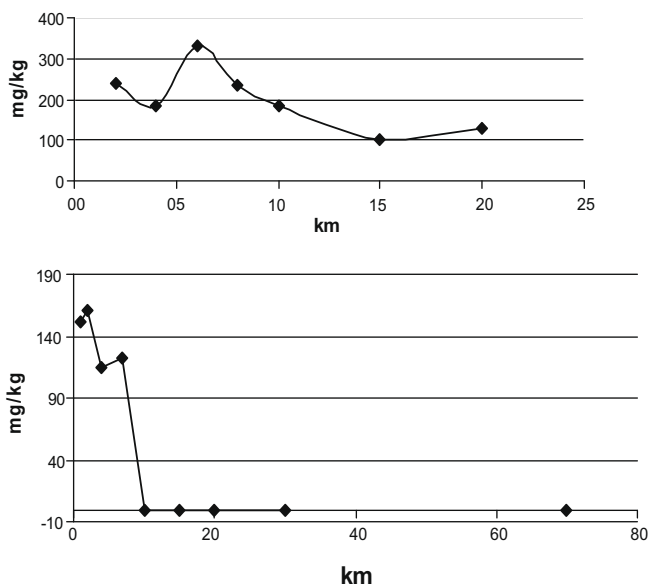


Fig. 5. Mineral oil concentration (mg kg⁻¹) in the surface sediments (fraction < 63 μm) at two transects (A, top and B, bottom, see Fig. 1) in the Dutch coastal zone of the North Sea. Note different distance and concentration scales in both panels.

125.7–1134 ng g⁻¹ (average = 492.7 ng g⁻¹; n = 62) and 77.8–644 ng g⁻¹ (average = 285.7 ng g⁻¹; n = 62), respectively. The gradients in the concentrations of the Σ16 EPA and Σ6 Borneff PAHs at transects A and B in the Dutch coastal zone are presented in Fig. 6.

The highest PAH concentrations were found at the two transects A and B in the area close to the coast (<15 km). Concentrations decrease at the locations more offshore. These values and gradients are in agreement with the PAHs concentrations found in that area in 2000 and 2003 (Hegeman and Laane, 2004; Van Gils, 2007).

The concentrations of the 2-ring compound naphthalene in the surface sediments of the Dutch coastal zone of the North Sea (fraction < 63 μm) was below the LOD at all stations. As shown above, the concentration of the heterocyclic 2-ring azaarene quinoline and of the oxidized quinolines were above detection limit at various locations. Phenanthrene was found in detectable concentrations at

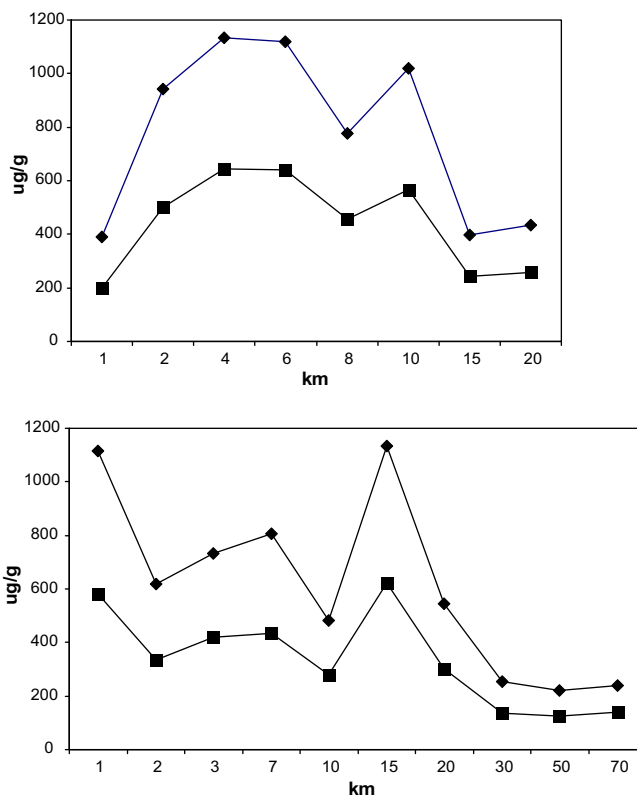


Fig. 6. Concentration of polycyclic hydrocarbons (PAHs; ng g⁻¹) in the surface sediments (fraction < 63 μm) at two transects A and B in the Dutch coastal zone of the North Sea (♦: 16 EPA PAHs, ■: 6 of Borneff). Note different distance scale in both panels.

all locations in the Dutch coastal zone of the North Sea: its concentration ranged between 12 and 140 ng g⁻¹ (average = 53.3 ng g⁻¹). As described above, the concentration of its heterocyclic analogue, phenanthridine, were below the LOD and the concentrations of the oxidized phenanthridines, 5,6-benzoquinoline and of 6(5H)-phenanthridinone (range between 8.2 and 38.8 ng g⁻¹) are in the same order of magnitude as those of the parent homocyclic PAH phenanthrene.

The concentrations of anthracene (range 1.9–35 ng g⁻¹, average 9.42 ng g⁻¹; *n* = 62) were below the concentrations found for the sum of the heterocyclic analogues acridine and its derivative 9(10H)-acridone (21–63 ng g⁻¹, average 31 ng g⁻¹). The concentrations of benzo[*a*]anthracene in the Dutch coastal zone of the North Sea (range between 7.98 and 82 ng g⁻¹, average 30.5 ng g⁻¹, *n* = 62) were somewhat higher than the sum of the heterocyclic analogues of benzo[*a*]anthracene, viz., benzo[*a*]acridine and benzo[*c*]acridine (range between 7 and 36.1 ng g⁻¹, average 15.4 ng g⁻¹).

It can be concluded that the sum of the concentrations of the azaarenes and azaarones range from almost equal to up to one order of magnitude higher than their homocyclic analogues.

As already stated, the concentrations of the oxidized derivatives of quinoline are higher than the concentration of the parent compound quinoline. Along the transects A and B the concentrations of quinoline are rather constant. For transect A the highest concentrations of the oxidized derivatives of quinolines are found around 6 km offshore. At transect B, the highest concentrations of oxidized quinolines are found also in the first kilometers offshore.

In general, concentrations of chemical substances in the first 10 km of transect A are strongly influenced by dumping of dredged material from the Rotterdam harbors and by the export of riverine material from the Rhine and Meuse (Laane et al., 1999, 2006). The spatial gradients observed for the concentrations of PAH and oil in this area are in agreement with these general observations. For PAH this has been established quantitatively by Van Gils (2007); the relative contribution from the rivers and dumping of dredged material to the total PAH concentration in the Dutch coastal zone is between 30% and 50% (Sonneveldt and Laane, 2001).

Although for most of the detectable concentrations of azaarenes and azaarones the concentrations are highest in the Dutch coastal zone, the observed spatial gradients differ from those of PAH and mineral oil. For quinoline this could be caused by its oxidation to hydroxyquinolines with the remaining low level of quinoline representing a non-available threshold concentration as found also at transects C and D. A comparable situation is found for acridine, with non-significant differences in the concentrations found at all locations. The levels of phenanthridine in marine sediments are below its LOD, the latter being probably higher than a possible threshold concentration of phenanthridine.

4. Conclusions

We conclude that simultaneous GC–MS for azaarenes and their degradation products is possible albeit that extraction and clean up can be further improved. In marine and fresh waters levels of azaarenes and their transformation products are generally below the limits of detection. Azaarenes and azaarones have been detected for the first time simultaneously with a GC–MS method in relatively high concentrations in the surface sediments of the Dutch coastal zone. Detectable concentrations of azaarenes and azaarones ranged from 3.3–63 and 7.4–949 ng g⁻¹, respectively. Almost invariably the cumulative concentrations of the transformation products (azaarones) were higher than the cumulative concentrations of the parent compounds (azaarenes). The concentrations of the azaarenes and azaarones together are comparable with those of the PAHs and with levels that induce phototoxicity in algae.

Spatial concentration patterns of the azaarenes and azaarones slightly differ from those of total oil and PAHs in the Dutch coastal zone. Further monitoring of the emerging transformation products of azaarenes is warranted.

Acknowledgements

We thank Rob Bovelandt and Wim Hegeman for the statistical work and for preparing the graphs and box whisker plots, and Peter Serné and Frans van der Wielen for performing the analytical chemical work. We thank Rijkswaterstaat for financial support and the shipping and laboratory facilities provided.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.chemosphere.2009.04.029](https://doi.org/10.1016/j.chemosphere.2009.04.029).

References

- Bleeker, E.A.J., Van der Geest, H.G., Klamer, H.J.C., de Voogt, P., Wind, E., Kraak, M.H.S., 1998. Toxic and genotoxic effects of azaarenes: isomers and metabolites. *Polycycl. Arom. Compd.* 13, 191–203.
- Blumer, M., Dorsey, T., Sass, J., 1977. Azaarenes in recent marine sediments. *Science* 195, 283–285.
- Chen, H.-Y., Su, C.-C., Hung, C.-C., Yeh, T.-C., Shen, Y.-H., Tsai, C.-H., Chen, L.-D., Gong, G.-C., 2008. Occurrence of azaarenes in sediments of the Danshuei River, Taiwan. *Environ. Toxicol.* 23, 25–35.
- De Maagd, P.G.-J., 1996. Polycyclic aromatic hydrocarbons: fate and effects in the aquatic environment. Thesis, University of Utrecht, Utrecht, The Netherlands, pp. 1–167.
- de Voogt, P., Bleeker, E.A.J., van Vlaardingen, P.L.A., Fernández, A., Slobodnik, J., Wever, H., Kraak, M.H.S., 1999. Formation and identification of azaarene transformation products from aquatic invertebrate metabolism. *J. Chromatogr. B* 724, 265–274.
- Eisma, D., 1993. Suspended Matter in the Aquatic Environment. Springer Verlag, Berlin, Germany.
- Furlong, E.T., Carpenter, R., 1982. Azaarenes in puget sound sediments. *Geochim. Cosmochim. Acta* 46, 1385–1396.
- Gissel-Nielsen, G., Nielsen, T., 1996. Phytotoxicity of acridine, an important representative of a group of tar and creosote contaminants, N-PAC compounds. *Polycycl. Arom. Compd.* 8, 243–249.
- Hegeman, W.J.M., Laane, R.W.P.M., 2004. Concentraties en normtoetsing van stoffen in het oppervlakte sediment van het Nederlandse Continentale Plat (1981–2003). Rijkswaterstaat Report RIKZ 2004.138, The Hague, The Netherlands, pp. 1–72.
- Jones, K.C., Stratford, J.A., Tidridge, P., Waterhouse, K.S., Johnston, A.E., 1989. Polynuclear aromatic hydrocarbons in an agricultural soil; long-term changes in profile distribution. *Environ. Pollut.* 56, 337–351.
- Kaiser, J.-P., Feng, Y., Bollag, J.-M., 1996. Microbial metabolism of pyridine, quinoline, acridine and their derivatives under aerobic and anaerobic conditions. *Microbiol. Rev.* 60, 483–498.
- Klamer, J.C., Hull, R.N., Laane, R.W.P.M., Eisma, D., 1990. The distribution of heavy metals and polycyclic aromatic hydrocarbons in the sediments of the Oyster grounds (North Sea). *Neth. J. Sea Res.* 26 (1), 83–87.
- Koci, K., Petrovska, H., Simek, Z., Varadova, E., Syslova, A., 2007. Extraction of polycyclic aromatic nitrogen heterocycles from spiked soil samples. *Intern. J. Environ. Anal. Chem.* 87, 111–123.
- Kozin, I.S., Larsen, O.F.A., de Voogt, P., Gooijer, C., Velthorst, N.H., 1997. Isomer-specific detection of azaarenes in environmental samples by Shpol'skii luminescence spectroscopy. *Anal. Chim. Acta* 354, 181–187.
- Laane, R.W.P.M., Sonneveldt, H.L.A., Van der Weyden, A.J., Loch, J.P.G., Groeneveldt, G., 1999. Trends in the spatial and temporal distribution of metals (Cd, Cu, Zn and Pb) and organic compounds (PCBs and PAHs) in Dutch coastal zone sediments from 1981–1996: a model case study for Cd and PCBs. *J. Sea Res.* 41, 1–17.
- Laane, R.W.P.M., de Voogt, P., Bik, M.H., 2006. Assessment of organic compounds in the Rhine estuary. In: Knepper, T. (Ed.), *The Rhine Handbook Environmental Chemistry*, vol. 5. Springer, Berlin, pp. 307–368.
- Negrón-Encarnación, I., Arce, R., 2007. Light-induced transformations of azaaromatic pollutants adsorbed on models of atmospheric particulate matter: acridine and 9(10-H) acridone. *Atmos. Environ.* 41, 6771–6783.
- Neuwöhner, J., Reineke, A.-K., Hollender, J., Eisentraeger, A., 2008. Ecotoxicity of quinoline and hydroxylated derivatives and their occurrence in groundwater of a tar-contaminated field site. *Ecotoxicol. Environ. Safe* (ASAP available at 11.06.08).
- Osborne, P.J., Preston, M.R., Chen, H.Y., 1997. Azaarenes in sediments, suspended particles and aerosol associated with the River Mersey estuary. *Mar. Chem.* 58, 73–83.

- Pearlman, R.S., Yalowsky, S.H., Banerjee, S., 1984. Water solubility of polynuclear aromatic and heteroaromatic compounds. *J. Phys. Chem. Ref. Data* 13, 555–562.
- Pereira, W.E., Rostad, C.E., Carbarino, J.R., Hult, M.F., 1983. Groundwater contamination by organic bases derived from coal-tar wastes. *Environ. Toxicol. Chem.* 2, 283–294.
- Pereira, W.E., Rostad, C.E., Leiker, T.J., Updegraff, D.M., Bennett, J.L., 1988. Microbial hydroxylation of quinoline in contaminated groundwater: evidence for incorporation of the oxygen atom of water. *Appl. Environ. Microbiol.* 54, 827–829.
- Reineke, A.-K., Göen, T., Preiss, A., Hollender, J., 2007. Quinoline and derivatives at a tar oil contaminated site: hydroxylated products as indicator for natural attenuation. *Environ. Sci. Technol.* 41, 5314–5322.
- Reynaud, S., Deschaux, P., 2006. The Effects of polycyclic aromatic hydrocarbons on the immune system of fish: a review. *Aquat. Toxicol.* 77, 229–238.
- Schmitter, J.M., Colin, H., Excoffier, J.L., Arpino, P., Guiochon, G., 1982. Identification of triaromatic nitrogen bases in crude oils. *Anal. Chem.* 54, 769–772.
- Sonneveldt, H.L.A., Laane, R.W.P.M., 2001. prediction of sediment quality in the Dutch coastal zone: model validation and uncertainty analysis for Cd, Cu, Pb, Zn, PCBs and PAHs. In: Rainbow, P.S., Hopkin, S.P., Crane, M. (Eds.), *Forecasting the Environmental Fate and Effects of Chemicals*. John Wiley & Sons, Chichester, UK, pp. 177–189.
- Svabensky, Y., Koci, K., Simek, Z., 2007. The study of properties of HPLC determination of polycyclic aromatic nitrogen heterocycles. *Int. J. Environ. Anal. Chem.* 87, 337–349.
- Van Genderen, J., Noij, T.H.M., Van Leerdam, J.A., 1994. Inventory and toxicological evaluation of organic micropollutants. RIWA Report, Association of Rhine and Meuse Water Supply Companies (RIWA), Amsterdam, The Netherlands.
- Van Gils, J., 2007. Afwenteling in de Nederlandse kusttrivier: paden en lotgevallen van prioritair stoffen in de Noordzee. Delft Hydraulics Report Z2182, Delft. pp. 1–103pp. + annex.
- Van Herwijnen, R., de Graaf, C., Govers, H.A.J., Parsons, J.R., 2004. Estimation of kinetic parameter for the biotransformation of three-ring azaarenes by the phenanthrene-degrading strain *Sphingomonas* sp. Lh128. *Environ. Toxicol. Chem.* 23, 331–338.
- Vethaak, A.D., Jol, J.G., Meijboom, A., Eggens, M.L., ap Rheinallt, T., Wester, P.W., Van de Zande, T., Bergman, A., Dankers, N., Ariese, F., Baan, R.A., Everts, J.M., Opperhuizen, A., Marquenie, J.M., 1996. Skin and liver diseases induced in flounder (*Platichthys flesus*) after long-term exposure to contaminated sediments in large-scale mesocosms. *Environ. Health Perspect.* 104, 1218–1229.
- Wakeman, S.G., 1979. Azaarenes in recent lake sediments. *Environ. Sci. Technol.* 13, 1118–1123.
- Wiegman, S., Van Vlaardingen, P.L.A., Bleeker, E.A.J., de Voegt, P., Kraak, M.H.S., 2001. Phototoxicity of azaarenes isomers to marine flagellate *Dunaliella tertiolecta*. *Environ. Toxicol. Chem.* 20, 1544–1550.
- Wild, S.R., Jones, K.C., 1995. Polynuclear aromatic-hydrocarbons in the United-Kingdom environment – preliminary source inventory and budget. *Environ. Pollut.* 88, 91–108.
- Xue, W.L., Warschowsky, D., 2005. Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: a review. *Toxicol. Appl. Pharmacol.* 206, 73–93.