Development and validation of a method for the determination of trace alkylphenols and phthalates in sea water and air using GC-MS

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Abstract

An analytical method has been developed for the simultaneous extraction and determination of trace tertiary octylphenol (*t*-OP), technical nonylphenol isomers (NP), nonylphenol monoethoxylate isomers (NP1EO) and seven phthalates in sea water and the atmosphere using gas chromatography-mass spectrometry (GC-MS). Large volume samples were collected using a modified in-situ pump equipped with a PAD-2 resin column for sea water and a high-volume pump with a PUF/XAD-2 column for air. The detection limits of the method for APs and the phthalates ranged from 5 to 200 pg L^{-1} in sea water and from 2 to 100 pg m^{-3} in air, respectively. The recoveries of *t*-OP, NP, NP1EO and the phthalates for the entire procedure were satisfactory (>60%). The method was successfully applied to the determination of the analytes in sea water and the atmosphere. The concentrations of *t*-OP, NP, NP1EO and the phthalates present over land and the North Sea were comparable. It suggested that the atmosphere is a significant pathway for the transport of alkylphenols and the phthalates in the environment.

Keywords: Solid-phase extraction; GC-MS; Nonyphenol; tertiary octylphenol; nonylphenol monoethoxylate; phthalate; atmosphere; sea water

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1. Introduction

In the last two decades, a large number of studies have demonstrated that there are several classes of chemicals that can behave as biologically relevant signals, capable of changing the control of gene expression at the molecular level and interfering with homeostatic feedback loops at the development and function level (Mclachlan, 2001; Myers et al., 2003). Among these chemicals, many, including PCBs, DDT, HCH and dioxins are semi-volatile, persistent, and are subject to long distance transport through atmospheric circulation (Atlas and Giam, 1981; Bidleman, 1988; Eitzer and Hites, 1989; Bright et al., 1995; Kalantzi et al., 2001). However, some of these chemicals, e.g. phthalates and alkylphenols (APs) are still manufactured and consumed worldwide even though they have been clearly proved to be toxic to aquatic organisms and active as endocrine disrupters (Jobling et al., 1996; White et al., 1994). Since 1978, phthalates have been detected in the marine environment and remote regions such as the Arctic, with concentrations comparable to that over land (Giam and Atlas, 1978). As for alkylphenols, they are not typically released directly into the environment, but rather are formed as biological breakdown products of widely used nonionic surfactants, alkylphenol ethoxylates (APEOs) (Giger et al., 1984). The concentrations of APs and their parent compounds have been measured worldwide in all compartments of the environment and even in food products for human consumption (Staples et al., 1997; Dachs et al., 1999; Guidotti et al., 2000; Cincinelli et al., 2001; Kolpin et al., 2002; Fromme et al., 2002; Guenther et al., 2002; Rudel et al., 2003; Toda et al., 2003). The similarities of their environmental persistence and impacts between APs, phthalates and classical persistent organic pollutants (POPs) suggest that there is a need to understand their transport and distribution in the environment.

Several techniques including GC, LC, IR, NMR, and TLC have been used for the analysis of APs and phthalates (Thiele et al., 1997; Gomez-Hens and Aguilar-Caballos, 2003). The most popular techniques for the determination of APs and phthalates in environmental samples are gas chromatography with detection through electron capture, flame ionisation, and mass spectrometry (Stephanou and Giger, 1982; McEvoy and Giger, 1986; Ahel et al., 1985; 1987). Moreover, analysis of the AP, APEO and the phthalates has been performed with HPLC coupled to fluorescence detection and UV detection (Marcomini and Giger, 1987). Recently, as the advantages of liquid chromatography/mass spectrometry (LC-MS) became recognized, several groups developed various LC-MS methods to analyse APs and

phthalates in various environmental matrices (Snyder et al., 1999; Lin et al., 2003). Moreover, tandem mass spectrometry (MS/MS) has been coupled to GC or LC separation systems in order to solve the problems of complicated matrices and improve the identification of complex mixtures. Several research groups have recently reported extremely high sensitivities for estrogenic compounds in environmental samples using LC-MS with electrospray (Jeannot et al., 2002) and atmospheric pressure chemical ionisation (APCI) detection, or LC-MS/MS with electrospray detection (Loyo-Rosales et al., 2003; Jahnke et al., 2004). Ding and Tzing (1998) suggested using an ion trap GC-MS with large volume injection (LVI) techniques to achieve lower detection limits. Additionally, environmental monitoring of NP and OP can be facilitated by bioanalytical techniques such as immunoassays. Zeravik et al. (2004) developed a new method, namely, direct competitive enzyme-linked immunoadsorbent assays (ELISAs) based on polyclonal and monoclonal antibodies. In addition, instrumental analysis and bioassay have been combined in order to quantify the concentrations and identify the endocrine activity of APs and phthalates.

The sensitivity and selectivity of the analytical instruments such as GC or HPLC coupled to MS are usually insufficient for direct determination of these chemicals at very low concentration levels and in environmental samples with complex matrices. Therefore, a sample pretreatment step prior to chromatographic analysis or bioassays is usually necessary. For water samples, liquid-liquid extraction with organic solvents such as dichloromethane or hexane is often used for the pre-extraction of APs and phthalates due to their high polarity. Moreover, solid phase extraction is the most common technique for both water and air samples. Various kinds of materials are used as extraction adsorbents including C_{18} and C_8 silica, polystyrene-divinylbenzene polymer and various carbonaceous sorbents. Solid-phase microextraction (SPME) is also applied for the preconcentration of APs and phthalates based on its attractive advantages, e.g. low solvent consumption, low levels of the analytes in the blanks and time saving (Luks-Betlej et al., 2001; Penalver et al., 2000; Diaz et al., 2002, 2004; Braun et al., 2003). A novel material, namely, mutiwalled carbon nanotubes as a solid-phase extraction adsorbent has been recently introduced and applied for the determination of APs in water (Cai et al., 2003). Although good properties were shown in comparison to the usual material, e.g. XAD-2 copolymer, the extensive use of mutiwalled carbon nanotubes is not yet common in sample preparation as they are extremely expensive.

Although many analytical instruments coupled to novel preconcentration methods, e.g. on line SPE-GC-MS (Brossa et al., 2003), hollow-fibre liquid phase microextraction coupled to GC-MS (Psillakis and Kalogerakis, 2003) and HPLC-MS/MS (Loyo-Rosales et al., 2003)

provided dramatically improved detection power and extremely high sensitivities, the detection of trace APs and phthalates in field samples is still a challenge for the environmental and analytical scientific community. Since APs and phthalates are ubiquitous in the environment, they are present as contaminants in almost all laboratory equipments and reagents (Giam et al., 1975; Williams, 1973; Kuch and Ballschmiter, 2001). While efforts have been made to reduce laboratory contamination, DEHP could still be present in laboratory blanks even with thorough cleaning methods (Giam et al, 1975). In practice, method detection limits are often more than one or two orders of magnitude higher than instrumental detection limits. Therefore, it keeps a need to develop a sensitive and selective method to improve the accuracy of environmental data set for investigating and evaluating of environmental distributions of APs and phthalates.

The purpose of this work is to improve the existing sampling and analytical methods for the determination of alkylphenols and the phthalates at trace levels in the environment. The conditions of a derivatization step for enhancing the selectivity and sensitivity of analysis of alkylphenols were optimised. Sampling equipments are modified to eliminate the potential contaminations from the material. Laboratory instruments were modified to reduce the contamination risk from the indoor air during the sample treatments. The methods were validated with recovery and breakthrough test, blank check and evaluation for the reproducibility. The method developed was applied to quantification of target compounds in the sea water and the atmosphere.

2. Experimental

2.1. Reagent preparation

The solvents (methanol, acetone, hexane, dichloromethane, acetonitrile, diethyl ether (Promochem GmbH, Germany) used were pesticide or HPLC grade, and were distilled prior to use. Milli-Q water (18.2 MΩcm) was generated by a Millipore Ultra-pure water system (Millipore S.A., Molsheim France) and additionally purified with XAD-2 or PAD-2 resins. All glassware was rinsed with Milli-Q water and acetone and then baked at 450 °C for at least 8 hours before use.

Analytical standards (*t*-OP, technical NP and NP1EO, dimethyl phthalate (DMP) diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), di-i-butyl phthalate (DiBP), butylbenzylphthalate (BBP), DEHP and dioctyl phthalate (DOP)), internal standards (4-n-NP *d*8 and dibenzylphthalate) and the surrogates (4-n-OP, 4-n-NP, technical NP1EO *d*2 (NP1EO

*d*2), DMP *d*4, DEP *d*4, DBP *d*4, DEHP *d*4) were supplied by Dr. Ehrenstorfer (Augsburg, Germany). Stock solutions of each chemical or mixture of chemicals were made by dissolving approximately 5-10 µg of the neat chemicals in liquid, solid or in solution into 10 mL of hexane. The standard solutions used in these experiments were made from appropriate dilutions of these stock solutions. Calibration solutions for preparing GC-MS calibration curves were made by diluting 1-200 µl of the standard solutions in hexane (final volume 200 µL). Stock solutions were prepared every half-year; internal standards and surrogates were prepared for the entire sampling campaign and the measurements (in half year).

2.2. PUF/XAD-2 column, PAD-2 column and glass fiber filter (GF/F) preparation

Amberlite XAD-2 resins (particle size: 20-60 mesh) were obtained from Supelco Germany. PAD-2 resins (particle size: 0.3-1.0 mm) were obtained from SERVA Electrophoresis GmbH (Heidelberg, Germany). To prepare the PUF/XAD-2 column, 30 g of XAD-2 resin were packed into a glass column with a glass frit. A piece of polyurethane foam (PUF, 2 cm x 5 cm Ø) was placed on the top to cover the XAD-2 resin. The packed column was cleaned with methanol, acetone and hexane (twice with each solvent) in turn using a modified soxhlet extractor for 72 hours. The residue solvent was removed using purified N_2 (300 mL for 20 min).

To prepare the PAD-2 resin column, 50 g of PAD-2 resin were first rinsed with 500 mL Milli-Q water, and then, the water was replaced with acetone. The PAD-2 resins and acetone were packed into a glass column with a glass frit. The column was filled to about 2/3 with PAD-2 resin. The PAD-2 column was rinsed with 200 mL acetone and then cleaned with acetone and DCM (twice with each solvent) using a modified soxhlet extractor for 72 h. Finally, DCM was replaced by purified milli-Q water (200 mL).

Glass fiber filters (GF/F 8 and GF/F 52) were obtained from Schleicher and Schuell Corporation (Dassel, Germany). GF/F 8 (diameter: 155 mm, pore size: 0.45 µm) was used for atmospheric particles and GF/F 52 (diameter: 142 mm, pore size: 0.7 µm) was used for total suspended matter (TSM) in sea water. Filters were wrapped in a single layer of aluminium foil that was sealed around the filter to create a 'bag'. The filters and the aluminium bag were then baked for 12 h at 450 °C in a muffle furnace.

After purification, the PUF/XAD-2 and PAD-2 columns were covered by a pair of pan-like and ball-like caps and sealed by sliding clips. Columns were stored before and after sampling in heat-sealed airtight polypropylene/aluminium/polyethylene bags (PP/AL/PE, Tesseraux, Germany) at 7 °C for water samples and at –20 °C for air samples, respectively. Cleaned

filters were wrapped between aluminium foil in PP/AL/PE bags and used filters were closed in fused test tubing and stored at –20 °C.
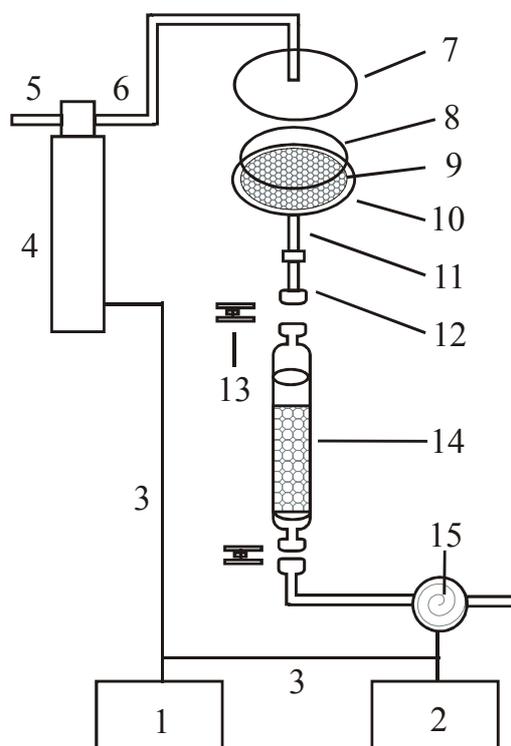
Figure. 1. Schematic of the in-situ pump. 1: flow meter controller; 2: flow meter; 3: cable connections; 4: pump; 5: pump inlet; 6: pump outlet; 7: stainless steel deck of filter holder; 8: GF/F 52 filter; 9: glass plate; 10: filter holder; 11:stainless steel tubing; 12 glass connect; 13 adjustable clip; 14: PAD-2 resins column; 15: counter of flow meter

2.3. Sampling and sample preparation

2.3.1. Water and air sampling

Water sampling was conducted with a modified Kiel In-Situ Pump (KISP) which has been widely applied to the extraction of marine trace organic chemicals (Wodarg et al., 2004; Bruhn et al., 2002; Lakaschus et al., 2002). Petrick et al (1996) described the technical design and principle and tested its performance in the Atlantic Ocean. Although low blanks and extremely low detection limits obtained from KISP samples could satisfy the demands for reliably detecting PCBs and HCHs, the system still presents a blank risk for the determination of trace APs and phthalates as several parts of the KISP are manufactured with or contained PVC material. Therefore, modifications were made to the frame of KISP. All plastic parts were removed and replaced with parts made from stainless steel or glass.

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As shown in Fig. 1, the in-situ pump includes a filter holder, a PAD-2 column, a pump and a flow meter. The pump and the flow meter were operated on board. The pumping rate can be selected from 0.01-2 L min^{-1} by adjusting the power supply. The glass fiber filter (GF/F 52) was placed on the glass filter holder. Stainless steel tubing was used to connect the pump to the filter plate. Glass tubing connects the filter plate to the PAD-2 resin column. Water flowed over the flow counter before being discharged and the flow rate could be read from the flow meter. Sea water samples were taken from beneath the bottom of the ship. In the North Sea, typical water sample volumes were from 20 to 100 L in the area near the coast and from 200 to 400 L in the open sea. In the Atlantic Ocean, up to 1000 L of sea water can be extracted due to the low concentration of total suspended matter (TSM).
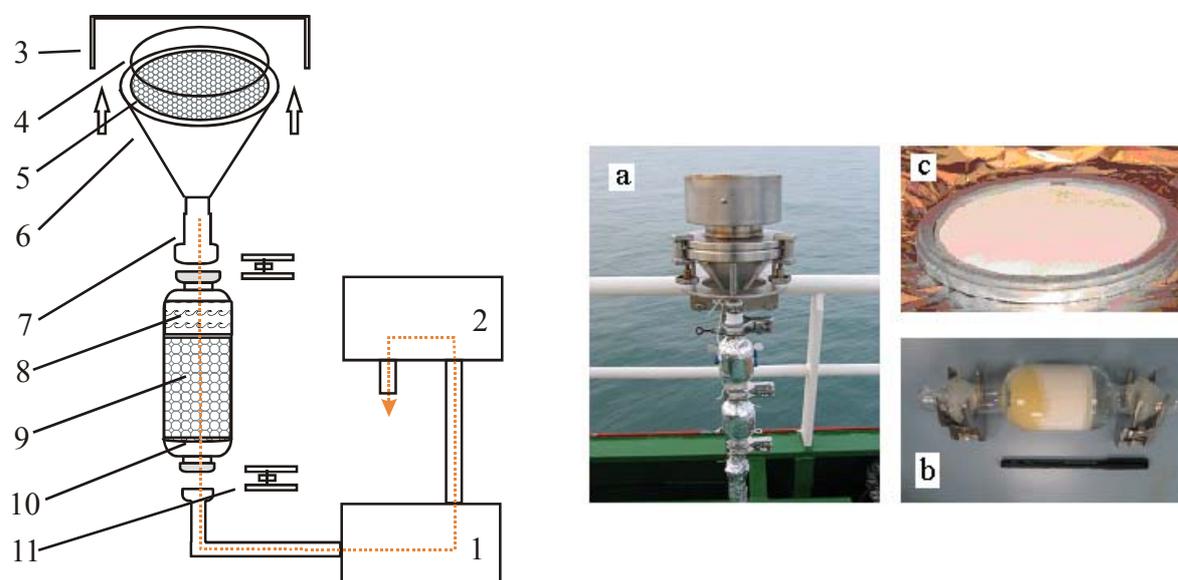
Figure 2. Schematic of the air sampler (left) and operation on board (right). 1: high volume pump; 2: flow meter; 3: filter shelter; 4: GF/F 8 filter; 5: metal frame for holding up glass filter 6: stainless steel filter holder; 7: teflon connector; 8: PUF sheet; 9: XAD-2 resins; 10: glass frit; 11: adjustable clip; a: air sampler; b: PUF/XAD-2 column; C: filter and particles

Air samples were collected using a high-volume air sampler that was operated at a constant flow rate of 200 L min^{-1}. As show in Fig 2. (left), the high volume air sampler consists of a high volume pump (ISAP 2000, Schulze Automation & Engineering, Asendorf, Germany), a digital flow meter, a metal filter holder and a PUF/XAD-2 column. The filter holder and the PUF/XAD-2 were linked with a Teflon connector that could protect the glass column while it works under stormy weather. To eliminate the blank risk from the Teflon, the connector was cleaned ultrasonically, three times with acidified water (pH: 2.0) and three times with acetone,

respectively. All parts of the filter holder were washed with a washing machine and rinsed with acetone. The pump and the flow meter were set up separately in metal boxes. All electronic plugs were wrapped with waterproof stick film for work outside. GF/F 8 was used to collect atmospheric particles. The filter was changed in the laboratory with tweezers pre-cleaned by burning in fire. The ship-borne air samples were collected on the upper deck of the research vessel (see Fig. 2, right). Land air samples were collected at GKSS Research Centre with a sampling position 5 m above the ground. Typical air sample volumes were from 400 to 1000 m^3. As reported by Lohmann et al. (2004), there is always the potential for contamination by air from ship-board samples. In order to avoid emissions from the ship's funnel, therefore, air sampling was performed on headwind and was halted at station or wind speeds lower than 3 m s^{-1}.

2.3.2. Extraction

The PUF/XAD-2 columns were spiked with the internal standards (50 µL of 200 ng mL^{-1} 4-n-NP d8 50 µL of 1.0 µg mL^{-1} NP1EO d2) and extracted for 16 h using 300 mL of 10% (v/v) diethyl ether in hexane solution with the modified Soxhlet extractor. The PAD-2 columns were extracted for 16 h using 250 mL DCM with the modified Soxhlet extractor after spiking with the internal standards (50 µL of 200 ng mL^{-1} 4-n-NP d8 50 µL of 1.0 µg mL^{-1} NP1EO d2). Both air and water filter samples were spiked with surrogate standards (50 µL of 200 ng mL^{-1} 4-n-NP and 4-n-OP, 50 µL of 1.0 µg mL^{-1} NP1EO d2, 50 µL of 0.5-1.25 µg mL^{-1} deuterated phthalates) and extracted for 16 h using 150 mL of DCM with the Soxhlet extractor. After Soxhlet extraction, the samples were stored in the freezer for rotation evaporation. Several PUF/XAD-2 columns, PAD-2 columns and filters were extracted for a second time in order to check the extraction efficiency.

2.3.3. Evaporation

The inner system of the rotation evaporator was cleaned with 100 mL of acetone prior to and after use. A self-designed adaptor was used to connect the round flask to the evaporator. The special design prevents condensate solvent flow backward into the round flask to eliminate potential contamination from inner tubing of the evaporator. The volume of the extracts were reduced to ~20 mL using rotation evaporator at 30 °C under reduced pressure (500-600 mPa for DCM, 220-290 mPa for the mixture of hexane and diethyl ether, 340 for acetone). 20 mL hexane was added to the flask and the solution was continually evaporated to

10-20 mL. The extracts were transferred to another 25 mL pear-bottom flask. The volume of the extracts was further reduced to 1-2 mL before clean-up. In order to remove small amount of water that might be present, the extracts were stored overnight in the freezer at –20 °C prior to clean-up.

2.3.4. Silica gel clean-up

All the extracts were purified through a 5% H_2O deactivated silica gel column (2.5 g silica gel packed in a 15 cm x 1 cm i.d. glass column). The silica gel (0.063-0.200 mm, Merck, Darmstadt, Germany) was prepared as follows: extracted using acetone and baking out at 450 °C for 12 h to remove organic contamination and deactivation by addition of 5% (w/w) of milli-Q water (purified by PAD-2 resin). After the extracts were transferred into the column, purification was performed by passing 10 mL of hexane through the column in order to remove non-polar compounds. The column was then eluted with 30 mL of hexane and diethyl ether (3:1 v/v) for the APs and phthalates fraction. It was followed with a 25 mL hexane and diethyl ether (1:1 v/v) fraction for NP_2EO. Eluates were reduced in volume in a rotary evporator and subsequently concentrated in a nitrogen evaporator to 100 µL.

2.3.5. Derivatization

The extracts were derivatized in a glass vial by the addition of N,O-bis(trimethylsilyl)trifluoroacetamide and 1% trimethylcholosilane (TMCS) (BSTFA + 1% TMCS) (Part No. 701 490.201, Macherey-Nagel GmbH, Dueren, Germany). 40 µL of 500 ng mL^{-1} surrogate standard mix 5 were spiked as internal standard (if it is not spiked before extraction). The volume was reduced to 100 µL under a gentle stream of nitrogen (99.999%). 100 µL of BSTFA + 1% TMCS was added to the glass vial. The mixture was allowed to react for 1 h at 70 °C. After cooling for 5 min, the final sample volume was adjusted to 200 µL using hexane. After derivatization, the extracts were ready for GC-MS without further treatment.

2.4. GC-MS analysis

Quantification of APs and phthalates was performed with an Agilent system consisting of a 6890 N gas chromatograph equipped with an Agilent 7683 series autosampler, a 7683 split-splitless temperature and pressure-programmed injector, and an Agilent 5973 quadrapole

mass selective detector (GC-MS). Chemstation Software (2000 version) was used for data processing. The injector was equipped with a deactivate PTV multi-baffle liner. Ions detected were generated by electron impact ionization and monitored in the selective mode (EI-SIM) and total ion scan mode by two injections. A 30 m x 0.25 mm fused silica capillary column (5%-phenyl-95% methylpolysiloxane, HP-5ms) with 0.25 μm film thickness was used for the separation. General conditions for GC-MS analysis are shown in Table 1.

Table 1. GC-MS conditions for the determination of APs and phthalates

GC-MS	APs	Phthalates
Column	HP-5ms (30 m x 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA)	HP-5ms (30 m x 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA)
Injection	1 μL	1 μL
Injector temperature program	280 °C (pulse splitless mode, 20 psi for 2 min) (Program 1) 80 °C (1min), 300°C min^{-1} to 250 °C (10 min)^{b} (Program 2)	300 °C (pulse splitless mode, 20 psi for 2 min)
Carrier gas	Helium, 1.0 mL min^{-1}	Helium, 1.0 mL min^{-1}
Purge gas	Helium, 250 mL min^{-1}	Helium, 250 mL min^{-1}
Oven temperature program	80 °C (1 min), 30 °C min^{-1} to 130 °C, 3 °C min^{-1} to 240 °C, 10 °C min^{-1} to 300 °C, then 300 °C (5 min)	80 °C (1 min), 30 °C min^{-1} to 150 °C, 5 °C min^{-1} to 300 °C (5 min)
Ionization energy	70 eV	70 eV
Interface temperature	280 °C	290 °C
Ion source temperature	230 °C	230 °C
Quadrapole	150 °C	150 °C

2.5. Calibration and quantification

Stock solutions containing all the analytes at accurately defined concentrations were prepared in hexane by dilution in the peak-bottom glass vials. The solvent was removed under a gentle nitrogen stream to 100 μL. These solutions were derivatized as described above. Quantification was carried out using calibration curves based on the peak area of the internal standards 4-n-NP d8 and the surrogate standard mix 5. NP and NP1EO were quantified by each of the isomer peaks. Calibration curves were made with concentrations from 12.5 to 500 ng mL^{-1} for *t*-OP, NP and NP1EO and from 5 to 5000 ng mL^{-1} for the phthalates. The limits of detection (LODs) were set as 3 times the signal to noise ratio. The detection limits of the

method (MDLs) were derived from the blanks and quantified as mean field blanks plus three times the standard deviation (3σ) of field blanks according to the sample volumes (typically, sea water: 200 L, air: 500 m^3). The LODs and MDLs calculated for the analytes are listed in Tab. 2.

Table 2. Instrumental limit of detection (LOD) and method detection limits obtained in this method

Compound	LOD (pg)	Sea water (200 L) (pg L^{-1})		Air (500 m^3) (pg m^{-3})	
		Dissolved	TSM	Vapour	Particle
t-OP	0.4	5	5	5	5
NP	3.5	40	5	15	5
NP1EO	3.7	25	10	5	5
DMP	0.8	65	15	5	5
DEP	1.2	75	125	10	10
DiBP	0.3	40	15	5	5
DnBP	0.3	25	30	5	5
BBP	1.8	5	5	2	2
DEHP	1.8	200	150	100	40
DOP	1.4	5	5	2	2

As compared to those reported in the literature, the instrument detection limits for *t*-OP, NP and NP1EO were quite comparable to those obtained with GC-MS (Berkner et al., 2004; Heemken et al., 2001), GC-MS/MS (Jeannot et al., 2002; Hoai et al., 2003), LC-MS and LC-MS/MS (Loyo-Rosales et al., 2003). For phthalates, it was found that GC-MS provided LODs for single phthalates from 0.03 to 0.5 pg, which are 1-3 orders of magnitude lower than those obtained with LC-ESI-MS. The detection limits of the method were found to be comparable between GC-MS and LC-ESI-MS (Lin et al., 2003). In this work, coupling GC-MS analysis with large volume sampling, except for DEHP, the detection limits for APs and phthalates could reach a few pg L^{-1} in sea water and a few pg m^{-3} in the atmosphere, which are 1 - 2 orders of magnitude lower than the reported MLDs (Teil et al., 2005; Loyo-Rosales et al., 2003; Berkner et al., 2004; Cincinelli et al., 2001; Diaz and Ventura, 2002; Kuch and Ballschmiter, 2001).

3. Results and discussion

3.1. GC-MS analysis

APs and phthalates were analysed in different GC-MS programs. It is shown in Fig. 3 that the chromatographic separation of *t*-OP, NP and NP1EO was achieved as expected from Isobe et al. (Isobe et al., 2001). The full-scan mass spectra of silylated APs and NP1EO and phthalates are shown in Fig. 4. The only major ion was found at m/z of 207 for *t*-OP which corresponds to $[(CH_3)_3Si\text{-}O\text{-}C_6H_4\text{-}C(CH_3)_2]^+$; the molecular ion observed at an m/z of 278 was used for the confirmation of *t*-OP. The chromatogram of NP contains more than 15 isomer peaks with various branched structures in the nonyl substitutes. The major ions at m/z values of 235, 221, 207 and 193 were present in the mass spectra of the derivatives of NP isomers by loosing the alkyl chain of C_4H_9, C_5H_{11}, C_6H_{13} and $C_3H_7\text{-}C_4H_9$ or $C_2H_5\text{-}C_5H_{11}$, which have been elucidated by Thiele et al (2004) using GC-MS with a 100 m capillary column. Similarly, NP1EO was also resolved into more than 15 isomer peaks. The major ions were at m/z of 279, 265 and 251. The molecular ions at an m/z of 292 for NP and at an m/z of 336 for NP1EO were very low. The patterns of the mass spectra of NP1EO d2 were very comparable to that of NP1EO with the most abundant ions at m/z values of 281, 267 and 253. For 4-n-OP and 4-n-NP, molecular ions at m/z values of 278, 292 and the ion at an m/z of 179 were present in the mass spectra and 4-n-NP d8 has spectra of the ions at an m/z of 185. The characteristic ions of the derivatives are selected and listed in Tab. 1a (supporting information) and applied to quantify the levels of the analytes. In this work, 13 of the NP and NP1EO isomer peaks with high proportions were selected for the quantification (see Fig. 3). Furthermore, some of the peaks contain several isomers and do not represent pure isomers (Isobe et al., 2001).
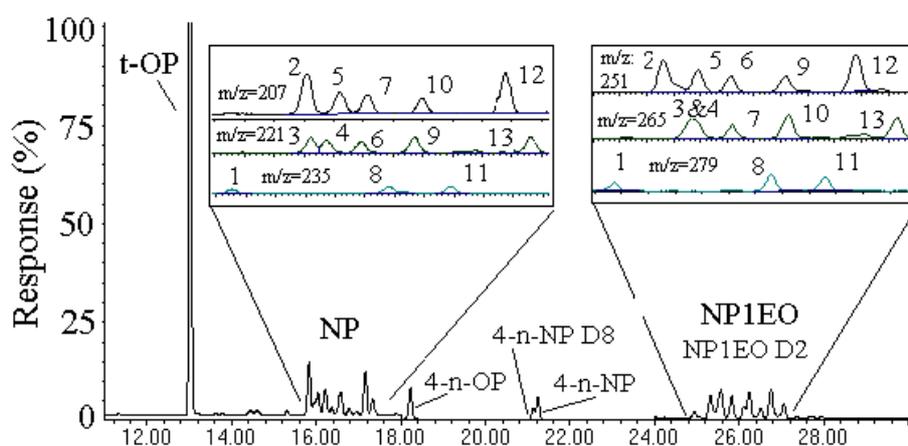
Figure 3. The chromatograms of *t*-OP, NP and NP1EO obtained using GC-MS.
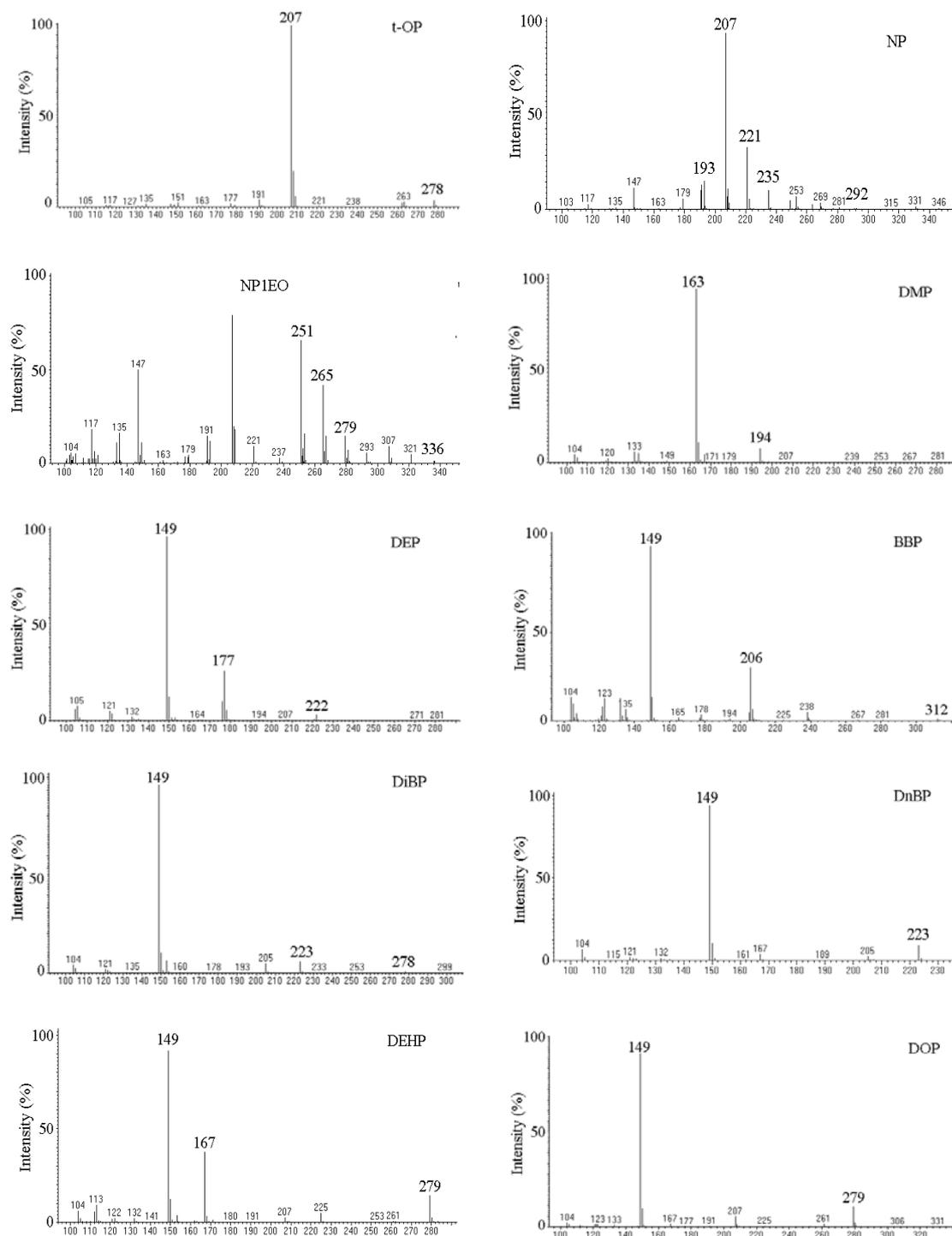
Figure 4. Mass spectra of derivatives *t*-OP, NP, NP1EO and the phthalates.

Except DMP, all phthalates show an intense characteristic base peak at an m/z of 149, resulting from fragmentation with loss of the alkyl ester groups and furan ring formation (David et al., 2003; Earls et al. 2003). As shown in Fig. 4, besides the most abundant ion at an m/z of 149, the spectra were relatively pure and the intensities of molecular ions were too weak to be detected. The second abundant ion was at an m/z of 177 for DEP, an m/z of 223

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for DiBP and DBP, an m/z of 206 for BBP, an m/z of 167 and an m/z of 279 for DEHP and DOP, respectively. The ion at an m/z of 167 results from the further fragmentation of the ion at an m/z of 279 and has an abundance of 40% as compared to that of the ion at an m/z of 149 for DEHP, therefore it can be specially used as quantification ion for DEHP. In the mass spectra of DMP, a molecular ion was detected at an m/z of 194. The most abundant ion was at an m/z of 163 that corresponds to the loss of a methxy group (M-31). The patterns of the mass spectra of the deuterated phthalates were very comparable to those of the original phthalates. The characteristic ions of the analytes are selected and listed in Tab. S1b (supporting information).

3.2. Derivatization for APs

Three derivative reagents were tested in our experiments, namely n-methyl-n-(t-butyldimethylsilyl)trifluoroacetamide (MTBSTFA), BSTFA and BSTFA + 1% TMCS. After optimizing the conditions for the derivatization, the different reagents were compared with regard to detection response, separation of the different NP and NP1EO peaks and procedures. Best results were achieved using BSTFA + 1% TMCS which shows a high detection response and good separation between the isomers of NP and NP1EO. Especially, the responses of the TMS products of the NP1EO isomers were increased by a factor of two orders of magnitude as compared to those without derivatization. The use of MTBSTFA showed adequate results as well. However, the reaction with MTBSTFA was very sensitive to the solvent and extra steps were necessary to dry the extracts and exchange the solvent to acetonitrile, which essentially reduced the recovery of the analytes and increased potential risk for the contaminations. The results with BSTFA were similar to that of BSTFA + 1%TMCS for *t*-OP and NP, whereas, the response enhancement for NP1EO was lower than when using BSTFA + TMCS. Therefore, BSTFA + 1% TMCS was selected as the sylilation reagent for all experiments.

There are a number of parameters that can affect the derivatization: reaction time, temperature, and the amount of reagent and matrix. It has been reported that derivatization could be completed at room temperature, but it always takes more than 3 hours for the reaction. The reaction at elevated temperature has often been conducted at 60°C or 70°C (Berkner et al., 2004). A reaction temperature of 70 °C was selected in our study in order to achieve a high reaction rate. Studies by Li et al. (2001) of the kinetics of the silylation reaction with BSTFA and APs indicated that the polarity of the organic solvent could significantly influence the reaction rate.
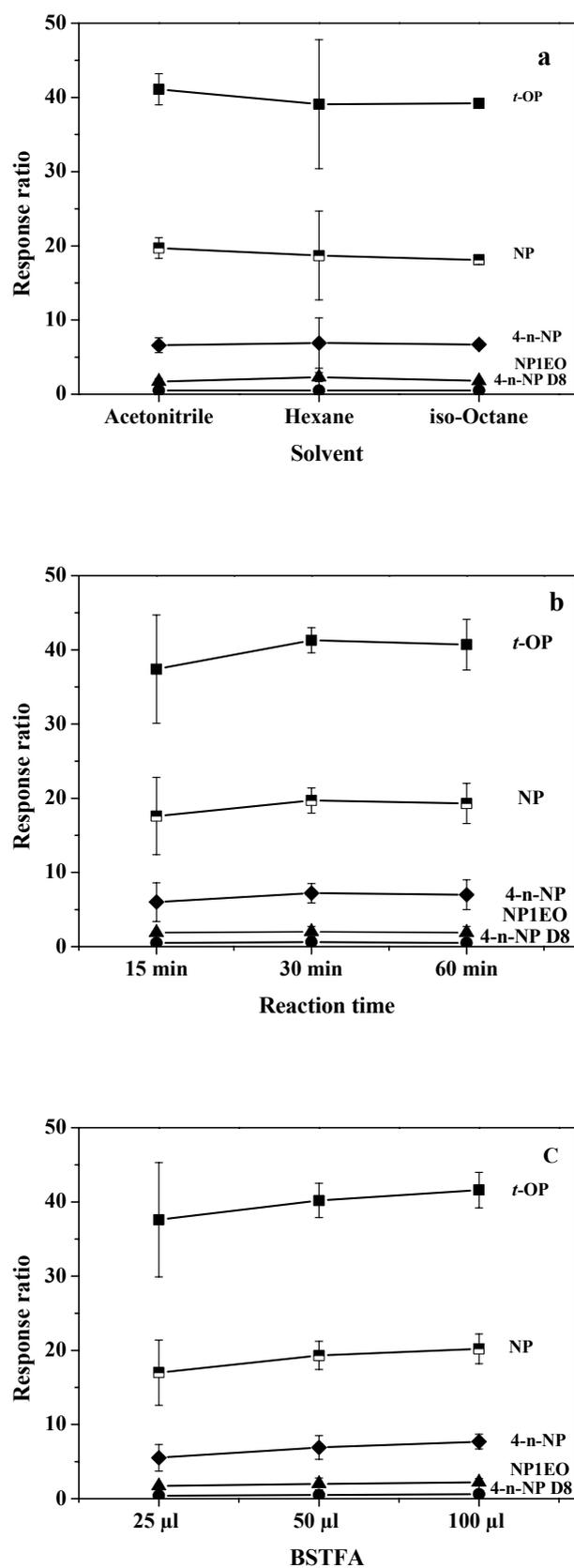
Figure 5. The effects of the solvents (a), reaction time (b) and proportion of BSTFA (c) on the responses of TMS-derivatives of APs

In this work, the parameters for the derivatization, e.g. reaction time, solvent and the amount of BSTFA + 1% TMCS were optimized by orthogonal experiments. The 3 levels of the parameters: reaction time (15, 30, 60 min), solvent (hexane, acetonitrile, iso-octane) and the volume of BSTFA + 1% TMCS (25, 50, 100 μL diluted to 200 μL with the appropriate solvent) in association with the Latin Square are shown in Tab. S2 (supporting information). The procedure of derivatization followed the orthogonal experiments. A relative response for APs compared to that obtained for gamma HCH was calculated for the evaluation of the experiments designed and is shown in Tab. S3 (Supporting information). In order to evaluate the effects of different levels of each factor selected, the average of the relative responses for each factor is plotted in Fig. 5a, b, c.

It is shown in Fig. 5a that the reaction rates for APs in acetonitrile are slightly higher than those in hexane and iso-octane. This result reflects the effect of the polarity of the organic solvent on the silylation rate as indicated by Li et al. (2001). Since these differences are mostly within the uncertainties, and in order to prevent potential contamination risk from the solvent exchange for the extract, therefore, hexane was selected as the solvent for the derivatization. Fig. 5 b shows that the reaction rates reached a stable level after 30 min, which indicates that BSTFA was very robust for the silylation of the APs. Although the reaction might be completed after 30 min, we use 60 min in the derivatization procedure to eliminate any effect from the complex matrices. Fig. 5c shows that the concentration of BSTFA + 1% TMCS in the solution could affect the reaction rates. 50 –100 μL BSTFA + 1% TMCS was necessary to achieve a higher reaction efficiency. Especially for the extracts related to water samples, they might contain some other polar organic contaminants that can also react with BSTFA + 1% TMCS. Therefore, 100 μL of BSTFA + 1% TMCS was adapted in the derivatization procedure.

3.3. Recovery and reproducibility

Recoveries and reproducibility of the entire procedure including sampling, extraction and clean-up were checked using field spiked samples. For the vapour phase samples, three PUF/XAD-2 columns were spiked with *t*-OP, NP, 4-n-NP and DEHP d4 and used to collect ca 500 m^3 of ambient air. For the water samples, 4-n-NP, 4-n-OP and the surrogate standard mix 5 were spiked in the PAD-2 columns which were then filtered with 20-1000 L sea water samples. The storage recoveries were incorporated in the field recoveries of surrogates. Matrix spiking recoveries were only checked for the air samples. The recoveries for soxhlet extractions and clean-up were conducted with standard spiking. Recoveries and reproducibility are shown in Tab. 3. Precisions were determined from the relative standard

deviations based on 3 or 5 multiplicate measurements for soxhlet extraction and matrix spiking.

3.3.1. Recoveries of soxhlet extraction, matrix spiking and reproducibility

As shown in Tab. 3, satisfactory extraction recoveries were achieved for all the compounds in the different matrices. The matrix spiking recoveries for phthalates and surrogates ranged from 73% to 141%. The cases of recoveries higher than 100% may be caused by signal enhancement. In this study, deuterated phthalates were spiked in the PUF/XAD-2 or PAD-2 columns in order to monitor the recoveries through the sampling, storage and laboratory treatments. Surrogate standard mix 5 was used as the internal standard for quantification. Generally, the signal enhancement rates differ among the phthalates. When the signal enhancement rate of any phthalate is equal or comparable to that of the surrogate standard mixture 5, the recovery should be lower or close to 100%; if the signal enrichment rate of any phthalate is higher than that of surrogate standard mixture 5, then the recoveries will be more than 100%. As the detailed mechanisms of signal enhancement were not clear, all phthalate concentrations were corrected for deuterated phthalate recoveries in order to overcome this problem. Concentrations of DiBP and BBP were corrected for the recovery of DnBP d4 and concentrations of DOP were corrected for the recovery of DEHP d4.

Table 3. The recoveries of *t*-OP, NP, NP1EO and phthalates for extraction, field sampling and matrix spiking (the relative standard derivations (RSD) are shown in the blanket).

Compound	Recovery of Extraction (%)			Recovery of field spiking (%)		Recovery (%) Matrix spiking
	PUF/XAD-2	PAD-2	GF/F (52&8)	PUF/XAD-2	PAD-2	
t-OP	59 ± 3 (5)	65 ± 5 (8)	109 ± 17 (16)	-	-	64 ± 6 (9)
4-n-OP	-	-	97 ± 16 (16)	70 ± 13 (18)	76 ± 9 (12)	-
NP	81 ± 4 (5)	82 ± 6 (7)	108 ± 9 (8)	-	-	77 ± 8 (10)
4-n-NP	83 ± 1 (1)	98 ± 9 (9)	86 ± 10 (12)	69 ± 15 (22)	71 ± 10 (14)	88 ± 9 (10)
NP1EO	-	116 ± 2 (2)	105 ± 7 (7)	-	-	-
DMP	93 ± 12 (13)	-	87 ± 8 (9)	75 ± 19[a] (25)	64 ± 20 (33)	141 ± 8 (6)
DEP	99 ± 10 (10)	-	92 ± 7 (7)	87 ± 19[a] (22)	73 ± 21 (29)	114 ± 2 (2)
DnBP	95 ± 10 (10)	-	89 ± 5 (6)	120 ± 27[a] (22)	110 ± 24 (22)	135 ± 5 (4)
BBP	85 ± 9 (10)	-	88 ± 2 (2)	-	-	134 ± 5 (5)
DEHP	106 ± 10 (9)	-	117 ± 4 (3)	121 ± 10[a] (8)	99 ± 17 (17)	73 ± 4 (5)
DOP	98 ± 12 (12)	-	118 ± 12 (10)	-	-	82 ± 3 (4)

The low recoveries for *t*-OP may result from its relatively volatile ability and adsorption ability to the surface (Berkner et al., 2004). In order to solve this problem, Berkner et al. (2004) suggested deactivating the glass surface by silanisation using a solution of 5% dimethyldichlorsilane in toluene. Although this procedure reduced the losses and improved recoveries of *t*-OP and NP during sample treatment, it was not employed for this work because the additional treatment with butanol may increase the risk of contamination from indoor air. Because *t*-OP is much more volatile than NP and the phthalates, low recoveries may also be caused by extracts concentration, especially during the final step using a N_2 stream. The commonly used polar solvents e.g. methanol, acetonitrile, and ethylacetate usually take more than 1 h to be removed from the extracts. Therefore, the recoveries of the analytes may decrease according to their partial pressure. In this work, hexane was used as the solvent for the final extracts. The recoveries for *t*-OP and NP were comparable to those reported in the literatures. (Berkner et al. 2004; Lagana et al. 2004). The relative standard deviations (RSD) of the APs and the phthalates ranged from 2 to 16% for the extraction procedure and from 2 to 10% for the matrix spiking experiments, showing the good reproducibility of the procedures. For the field spiking recoveries, the relative standard deviations ranged from 12 to 22% for the APs and from 8 to 33% for the phthalates, respectively. It is suggested that the sampling properties, e.g. sample volume and temperature may affect the recoveries of the phthalates.

3.3.2. Effects of sample volume and temperature on recoveries of sea water sampling

The effects of sampling volumes and temperatures on the recoveries for water sampling were studied by field spiking. 4-n-NP, 4-n-OP and deuterated phthalates were used as surrogates to examine the losses during the sampling. The recoveries ranged from 64 to 110% for the APs and the phthalates in water samples. The recoveries indicated that the sampling method is efficient for the determination of APs and phthalates at ultra trace levels. In order to evaluate the effects of sampling volume and ambient temperature on the recoveries for water samples, the recoveries for individual samples were plotted versus their volumes and the average temperatures. As shown in Fig. 6, the recoveries for DnBP d4 and DEHP d4 were mostly in the range from 75 to 120%. The recoveries of DnBP d4 and DEHP d4 in one sea water sample were as high as 142 and 131%, respectively. The recoveries of DMP d4, DEP d4, 4-n-OP and 4-n-NP were in the range of 45-75%, which indicated certain losses due to the sampling and laboratory treatments. As compared to their matrix spiking recoveries, the

losses of DMP and DEP may result from their relative high solubility in water. However, the field recoveries of 4-n-OP and 4-n-NP were very comparable to their matrix spiking recoveries, indicating that the losses of AP probably happened during the laboratory treatments. There was no clear correlation between the sample volumes and the recoveries. Fig. 7 shows that the recoveries of analytes in samples taken at low temperatures were slight higher than those taken at higher temperatures. This could be an explanation for the high relative standard deviations present in the recoveries for field spiking. This phenomena agrees with that reported by Jara et al. (2000). pH value and salinity were other important parameters which can influence the efficiency for solid phase extraction (Jara et al., 2000). As these two parameters are less variable in open ocean water, their influences were expected to be minor in this work. Based on the overall recoveries, PAD-2 was proved to be an ideal material for large volume sampling for the determination of trace phthalates and APs in sea water.
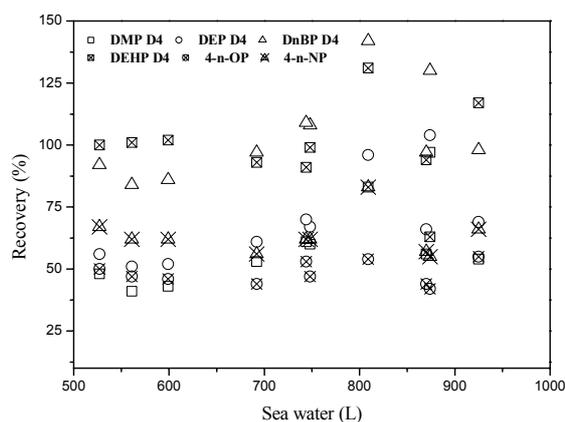
Figure 6. The effects of sample volumes on the recoveries of APs and phthalates in sea water
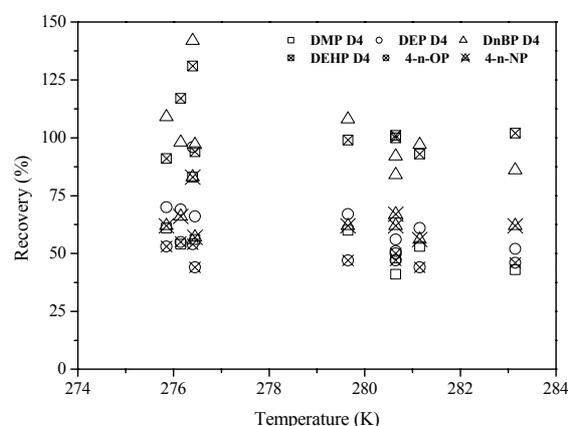
Figure 7. The effects of water temperature on the recoveries of APs and phthalates in sea water

3.3.3. Breakthrough and recoveries of air sampling

For air sampling, the sampling efficiency was examined by recoveries incorporating with breakthrough tests. Surrogate standards 4-n-OP, 4-n-NP, DMP d4, DEP d4, DBP d4 and DEHP d4 were spiked into the PUF/XAD-2 column on site before sampling. A second column was connected in series for breakthrough checking. The recoveries of surrogates are plotted against the sample volumes in Fig. 8. It is shown that the recoveries of surrogates in more than 80% of the samples were within a range from 70% to 140%. The recoveries obtained in the air samples with volumes more than 1000 m^3 were comparable to those obtained in the small volume samples. 4-n-OP and 4-n-NP usually have recoveries from 70% to 98%, whereas deuterated phthalates always present recoveries from 80% to 140%, which indicates that signal enhancements are active for phthalates.

The breakthrough tests show that 77% of the NP and more than 80% of the phthalates are retained on the first column and thus indicate that no significant breakthrough happens for these compounds. Furthermore, the recoveries present on the first column were very comparable between the target compounds and their surrogates, so that the losses of target analytes during sampling, storage and laboratory treatment could be well corrected using the recoveries of the corresponding surrogates. As an exception, the recoveries of *t*-OP show significant differences for the breakthrough tests conducted under various conditions. Two breakthrough tests were performed during the cruise ARK XX1/2 in the North Atlantic Ocean. The recoveries on the first column were 42% and 30%, respectively, which indicates that strong breakthrough happened. However, in another sampling campaign done in the GKSS Research Centre, *t*-OP shows a recovery of 99% on the first column with no evidence for breakthrough. As the sampling temperatures were very comparable for these samples, it was supposed that the humidity in the air might be the possible reason for the sampling efficiency of atmospheric *t*-OP. Another hypothetical explanation is a possible interference with similar chemical structure and properties. As the concentrations in these samples were low, it is quite difficult to confirm this hypothesis. Moreover, although the recoveries of *t*-OP were quite variable, those of 4-n-OP were quite similar in these samples. Therefore, we just take the masses determined in the first column into account for the calculations. It should be noted that the concentrations of atmospheric *t*-OP reported might be underestimated. In order to overcome this disadvantage, deuterated *t*-OP and individual NP isomers are in preparation for a subsequent study.

The field air samples were collected at temperatures ranging from −1 to 15 °C. It was found that the samples taken at high temperatures had slightly lower recoveries for 4-n-NP and 4-n-OP and DMP d4, but it is not significant for DEP d4, DBP d4 and DEHP d4. Therefore, if the sampling is performed at ambient temperature above 20 °C, we suggest collecting air samples for a volume approximately 500 m^3 or less to prevent losses from breakthrough or potential degradation.
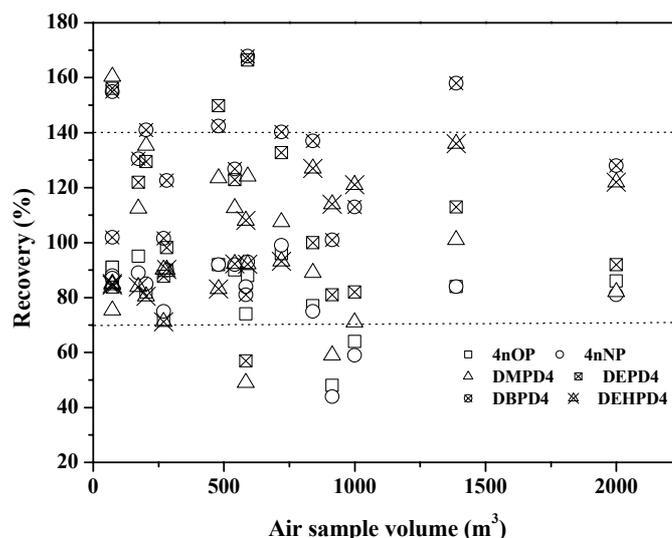
Figure 8. The recoveries obtained for 4-n-OP, 4-n-NP and deuterated phthalates in air samples

3.3.4. Recoveries of filter extraction

Extraction recoveries for the analytes in atmospheric particles and the TSM phase (see Tab. 3) were in the range from 86% to 118% for the APs and the phthalates, respectively. The extraction recoveries for the particles may strongly depend on the particle composition and the extraction methods. Berkner et al. (2004) have compared extraction procedures, e.g. ultrasonic treatment, accelerated solvent extraction and soxhlet extraction. Only accelerated solvent extraction gave lower extraction recoveries for APs. The extraction recoveries with ultrasonic treatment and soxhlet extraction were comparable and satisfactory for glass fiber filter extraction. In order to shorten the exposure to the indoor air and simplify the extraction procedure, soxhlet extraction with DCM was applied for glass fiber filter extraction in this work. During the extraction, it was observed that the broken filter with organic matters adsorbed onto the surface of the glass flask which may adsorb the analytes andthus lead to low recoveries for *t*-OP and NP. However, there was no significant difference for NP1EO and the phthalates. To prevent this drawback, some glass wool was put under the bottom of the

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soxhlet extractor to filtrate the extracts flowing back to the round bottom flask. Although the losses of particle-bound APs and phthalates during sampling were not evaluated, as based on their vapour pressure, *t*-OP, DMP, DEP might be underestimated for their particulate fractions.
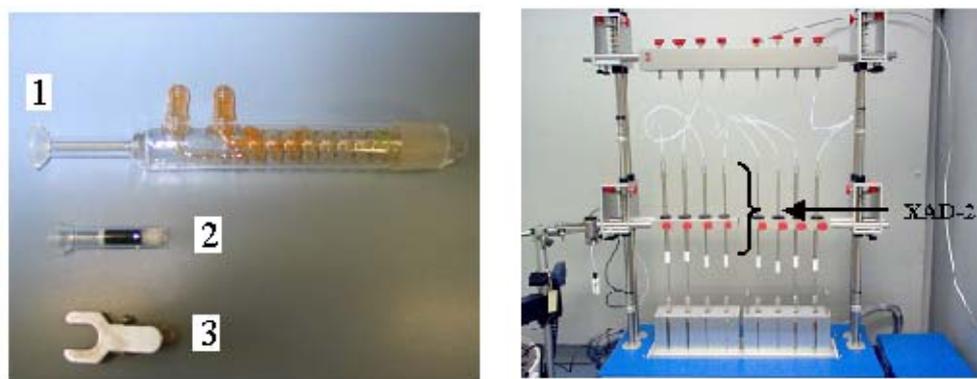
Figure 9. The modification made on the glass cooler and design for the active carbon cartridge (left) and the nitrogen evaporator (Right). 1: modified glass cooler; 2: active carbon cartridge; 3: adjustable clip
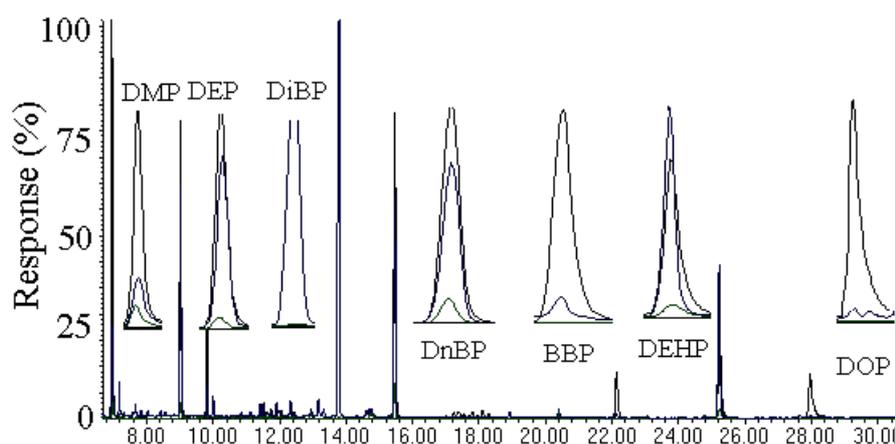
Figure 10. GC-MS chromatogram of phthalates in standard solution (black), blank (green) and air sample (blue).

3.5. Blanks

APs and phthalates are ubiquitous in the environment, laboratory material and instruments (Loyo-Rosales et al., 2003; Kuch and Ballschmiter, 2001). For blank controls, all the solvents used through the procedures were distilled for purification. Distillation was performed with a

modified soxhlet extraction unit. The vent of the glass cooler was closed with an active carbon packed cartridge and the metal tubes of the nitrogen evaporator were filled with XAD-2 resin (see Fig. 9). Usually the blanks of APs and phthalates are quite low in the residue analysis grade solvents. However, the screw caps might be potential contamination sources for these analytes. After distillation, the solvents were therefore stored in full glass bottles. In the chromatograms for blank checks, it is shown that there are no detectable APs, BBP, and DOP present in the solvents and the signals of DMP, DEP, DiBP, DnBP and DEHP were reduced by a factor of 5-10 as compared to the solvent without distillation. The estimated concentrations for DMP, DEP, DiBP, DnBP, DEHP were less than 10 ng L^{-1}, which is much less than the laboratory blank levels and satisfactory for the sample treatments. Chromatograms of phthalates in standard solutions, blanks and air samples are presented in Fig. 10. It shows that the blanks of the phthalates are at a low level based on the blank control procedures.

Field blanks of the water samples were obtained by attaching a PAD-2 column spiked with surrogate standards including 4-n-OP, 4-n-NP, DMP d4, DEP d4, DBP d4 and DEHP d4 to the water pump and putting a glass fiber filter on the filter plate, followed by passing 100 mL of sea water through the column. Field blanks of the air samples were prepared by putting a glass fiber filter on the filter frame and attaching a PUF/XAD-2 column spiked with the same surrogates to the pump. These field blanks were stored together with other samples and transported back to the laboratory. Laboratory and field blanks were incorporated in the analysis to quantify possible contamination due to collection, transport and extraction, as shown in Fig. 11a, b. There were no detectable BBPs and DOPs in all field blanks. Except 4 ng of NP1EO was found in the PAD-2 column, it was not found in the PUF/XAD-2 column and glass fiber filter blanks. The blanks of t-OP and NP were comparable to those reported by Berkner et al. (2004) for air sampling with an XAD-2 column. It is shown in Fig. 11a that DEHP was found in all of the materials with high blank values ranging from 20 to 50 ng, and DMP, DEP and DBP were in the range from 2 to 20 ng.

It is not surprising that t-OP, NP and some phthalates have been often detected from the blanks. Kuch and Ballschmiter (2001) found t-OP and NP in a 1 L blank sample of bidistilled or reverse osmosis water with concentrations at levels of 0.2-0.4 ng L^{-1}. Loyo-Rosales et al. (2003) claimed that traces of NP and NPEOs could be determined in the solvents, e.g. DCM and acetone. Although much effort has been dedicated to rule out the potential blanks from all solvents and laboratory material, as for APs and phthalates, they have been widely used in building material, PVC products, paints and cosmetics additives and are present in indoor air with concentrations ranging from several nanogram to lower microgram. Based on our

existing knowledge, we suppose that indoor air is the dominant contamination source for the blanks of APs and phthalates.
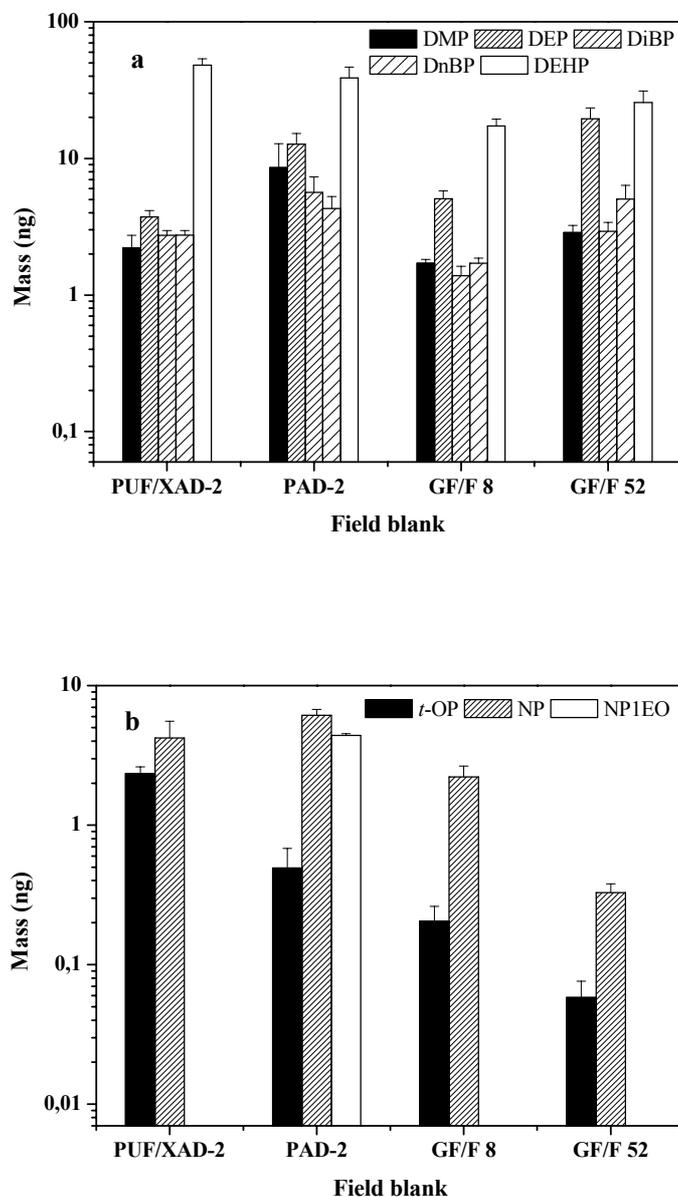
Figure 11a,b Field blanks of *t*-OP, NP, NP1EO (a) and phthalates (b) in the sampling media

Laboratory air samples were collected using an XAD-2 cartridge (5g XAD-2) spiked with surrogate standards. The sampling method and analytical procedures have been described in detail elsewhere (Selzer, 2005). As shown in Fig. 12, the concentrations of *t*-OP and NP were 64.4 ± 8.4 and 102.8 ±12.5 ng m^{-3} respectively, which are in the same order as that determined in American houses (Rudel et al., 2003). However, the NP1EO concentration was

below the detection limit (2 ng m^{-3}). As compared to their environmental concentrations, these results suggest that the degradation of APEOs was not the input source for t-OP and NP in indoor air. On the contrary, it seems that the dominant t-OP or NP were directly leached out from the material or instruments present in the laboratory.
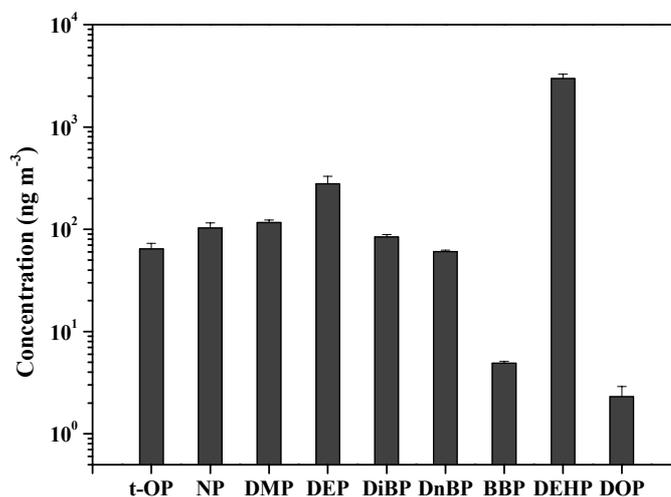
Figure 12. Concentrations of t-OP, NP and phthalates in the laboratory (the error bars were calculated from three parallel experiments).

The concentrations of DEP, DiBP and DnBP were lower by a factor of 2-5 than those reported by Rudel et al. (2003). However, the concentration of DEHP was 2972 ng m^{-3}, which is much higher that that determined in indoor air (Rudel et al., 2003). The concentrations of BBP and DOP were at relatively low levels, which were even comparable to those reported in the atmosphere in Paris (Teil et al., 2005). Contamination could reach the sampling material while the columns were open for sampling or extraction or via the air-solvent exchange during the soxhlet extraction or rotation evaporation. In order to reduce the contamination from laboratory air, the columns and filters should be changed quickly for sampling. For the soxhlet extraction, an active carbon cartridge was used to filtrate the air entering the units. To eliminate contamination during the rotation evaporation, an active carbon cartridge was connected to the vent valve for filtering the air. These specific designs could significantly reduce the contamination occurring during extraction and rotation evaporation. However, potential contamination could still occur during solvent change, extracts transfer and clean up. Therefore, it is not surprising that apart from NP1EO, BBP and DOP, the other analytes could be detected in blank samples even after careful operations control. Comparing the masses of

APs and phthalates determined in the field blanks to the concentrations found in laboratory air, it is found that the blanks were equal to the masses contained in 0.01 to 0.1 m^3 of indoor air. Because the masses of analytes in the field blanks were usually constant and reproducible, therefore, the average masses of field blanks were subtracted from the masses found in the samples.

Table 4. Concentrations of *t*-OP, NP, NP1EO and the phthalates determined in the atmosphere and sea water

Substance	North Sea				GKSS	
	Dissolved (pg L^{-1})	**TSM** (pg L^{-1})	**Vapour** (pg m^{-3})	**Particle** (pg m^{-3})	**Vapour** (pg m^{-3})	**Particle** (pg m^{-3})
t-OP	50 (13-300)	2 (<MDL-11)	18 (5-39)	-	109 (32-364)	6.2 (0.7-16)
NP	305 (90-1400)	15 (<MDL-86)	59 (30-110)	-	170 (55-421)	35 (3.6-116)
NP1EO	368 (25-1660)	11 (<MDL-68)	12 (4-31)	28 (14-50)	20 (5-56)	69 (22-164)
DMP	160 (<MDL-660)	27 (<MDL-70)	300 (120-620)	<MDL	244 (79-821)	3 (<MDL-100)
DEP	670 (30-4000)	200 (<MDL-4130)	1590 (470-4560)	62 (<MDL-280)	1570 (341-8600)	91 (3-260)
DiBP	-	-	-	-	854 (69-2430)	1150 (170-3750)
DnBP	1740 (450-6550)	40 (<MDL-200)	530 (130-1260)	530 (20-1290)	550 (63-1970)	1260 (220-6850)
BBP	50 (<MDL-260)	20 (<MDL-30)	20 (10-70)	52 (40-60)	2 (<MDL-4)	37 (13-88)
DEHP	2220 ((520-4430)	1580 (<MDL-5830)	290 (210-450)	1020 (810-1160)	123 (46-250)	1110 (630-1850)
DOP	<MDL	<MDL	<MDL	<MDL	<MDL	2 (<MDL-7)

3.6. Application to environmental samples

3.6.1. t-OP and NP

The sampling techniques for air and water samples and material used for laboratory treatments were applied and developed during several sampling campaigns in the North Sea and at a land station in the GKSS Research Centre. The concentrations of APs and phthalates

are shown in Tab. 4. As compared to the existing data, the atmospheric concentrations of *t*-OP and NP determined at GKSS Research Centre were comparable to those determined in a forest area in the Southeast of Germany (Berkner et al., 2004). However, they were lower by 1-2 orders of magnitude than that determined by Van Ry and Dachs et al. (Van Ry et al., 2001; Dachs et al., 1999) in New Brunswick, a more densely populated and more polluted urban area. The average of atmospheric concentrations of *t*-OP and NP present over land were higher than that present over the North Sea. Based on the inter-comparison for the concentrations determined in different samples over the North Sea (Xie et al., 2005b) and over land, an obvious concentration gradient was indicated from land to the open sea.
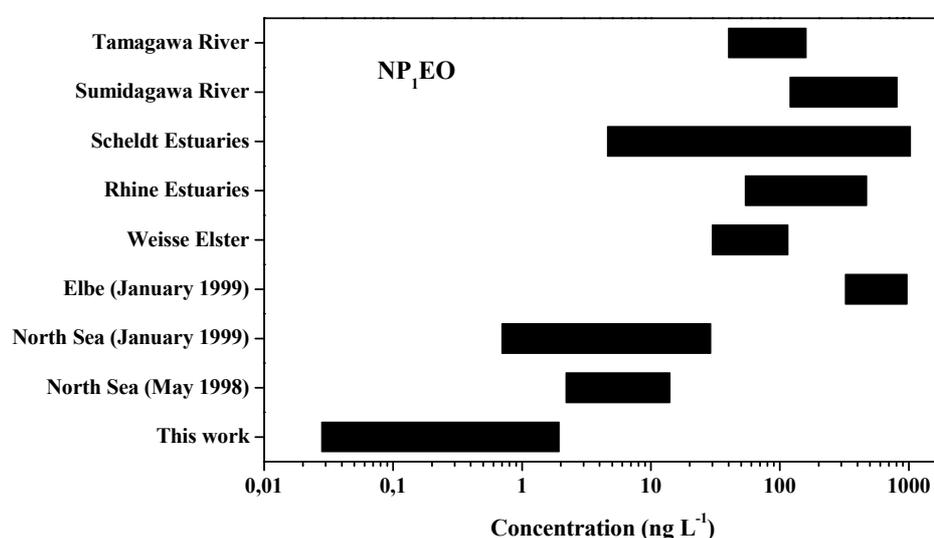
Figure 13. Comparison of concentrations of NP1EO determined in different rivers, estuaries and in the North Sea

3.6.2. NP1EO

NP1EO has been clearly proved to be a metabolite of NPEOs under anaerobic conditions during waster water treatment or in sediments. The concentrations determined in the North Sea were similar to those of NP. Moreover, the concentrations of NP1EO have been determined in many types of water body, e.g. in the Rivers, and related estuaries. These are summarized in Fig. 14. NP1EO concentrations determined in this work were at a surprisingly low level, 1-2 orders of magnitude lower than those determined in the German Bight in 1998 and 1999 (Heemken et al., 2001), and 2-3 orders of magnitude lower than those found in the estuaries of the Rhine and the Scheldt (Jonkers et al., 2003). The differences may partly be

related to the decreasing consumption of alkylphenols and their ethoxylates in the EU member countries (Wenzel et al., 2004).

For the atmospheric occurrence of NP1EO, because the physicochemical properties of NP1EO are unclear, it is quite difficult to estimate the contribution of the emission from the water surface. The concentrations of NP1EO determined at the GKSS Research Centre ranged from 5 to 56 pg m^{-3} in the vapour phase and from 22 to 164 pg in the particles, respectively. Compared to NP, the concentrations of NP1EO were lower by a factor of 3-5 in the vapour phase, and by contrast, are higher by a factor of 2 in the particles. The average particle-bound fraction of 66% indicates that NP1EO strongly partitions to the particles.

3.6.3. Phthalates

The concentrations of phthalates show that DEP, DiBP, DnBP and DEHP are the dominant species of phthalates in the environment. The concentrations of DOP were mostly below the detection limit of the method and BBP was at a low concentration level. The concentrations of phthalates present in the atmosphere and in the sea water of the North Sea have been discussed in (Xie et al., 2005b). There were no obvious differences observed for the concentrations in the terrestrial and coastal atmospheres. The concentrations reported in the previous study on the identification of phthalates in the marine and atmospheric environment were generally similar to those determined in this work. Moreover, the concentrations in total air samples were quite comparable to those determined in the remote area, e.g. over the Atlantic (Giam et al., 1978). As compared to the atmospheric concentrations of phthalates in a recent report (Teil et al., 2005), our concentrations are lower by a factor of 10, which indicates that the urban area is generally polluted much more than the suburban area.

4. Conclusions

The comprehensive studies presented in this work demonstrate that large volume sampling methods with a PAD-2 resin column for sea water and a PUF/XAD-2 column for air are powerful and suitable for the collection of trace APs and phthalates in the environment. The field blanks were significantly eliminated with self-designed glass connectors for the in-situ pump and active carbon cartridges for the soxhlet extractor and the rotation evaporator. These developments are not only beneficial for reducing the blanks for APs and phthalates, but also suitable for controlling the blank levels of other organic pollutants e.g. PCBs, PAHs and

fluorinated compounds. BSTFA + 1% TMCS was selected for the derivatization of t-OP, NP and NP1EO. The products of derivatization were more sensitive to GC-MS by a factor of 1-2 orders of magnitude than that without derivatization or with other reagents. The instrumental detection limits reach picogram (absolute). Furthermore, BSTFA does not react with phthalates under optimized conditions, which allows the detection of t-OP, NP, NP1EO and phthalates simultaneously. Silica gel clean-up is very efficient for the purification of APs and phthalates and no significant losses happen during the clean-up. Extraction with the modified soxhlet extractor combined with the active carbon cartridge and the distilled solvent is very convenient in operation and ensures low contamination in the extraction step. Although the large volume sampling and soxhlet extraction procedures are time consuming and labour – intensive, they eliminate matrix effects, feature high enrichment capacity and allow detection limits in the pg L^{-1} and pg m^{-3} range for sea water and air samples.

The recoveries of t-OP, NP, NP1EO and phthalates achieved for the entire procedure were satisfactory. The losses of phthalates during sampling and laboratory treatments could be well recovered using the deuterated compounds. NP and t-OP show different behaviour as compared to their surrogates 4-n-OP and 4-n-NP. As a solution, in a subsequent study, deuterated t-OP and certain NP isomers will be synthesized for method improvement and for use as surrogate to monitor the losses of t-OP and NP. Moreover, it is supposed that degradation may happen during the air sampling that leads to low recoveries for t-OP and NP. Therefore, it will need further study to make clear the mechanism for the losses of t-OP and NP during the air sampling.

The concentrations of t-OP, NP and NP1EO present over land and the North Sea suggest that both APs and phthalates may undergo long distance transport via the atmosphere and accumulate in the cold region. In a further study, the sampling and analytical methods have been applied for an expedition cruise carried out in the North Atlantic and the Arctic to evaluate the states of APs and phthalates in the remote region and provide evidence for the evaluation of their potential risk to the polar ecosystem.

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Supplementary material

Table S1a. Ion masses, retention time for the quantification of *t*-OP, and NP and NP1EO isomers

APs	m/z	Retention time (min)	Composition (%)
t-OP	207	13.25	-
NP1	235	15.55	2.2 ± 0.1
NP2	207	16.06	16.6 ± 0.1
NP3	221	16.09	6.8 ± 0.1
NP4	221	16.20	5 ± 0
NP5	207	16.28	10.7 ± 0.2
NP6	221	16.43	4.8 ± 0
NP7	207	16.47	9.3 ± 0.1
NP8	235	16.60	3.1 ± 0.4
NP9	221	16.80	6.7 ± 0.1
NP10	207	16.84	7.4 ± 0.1
NP11	235	17.02	3.2 ± 0.1
NP12	207	17.40	17.2 ± 0.1
NP13	221	17.58	7.0 ± 0.1
4-n-OP	179	18.47	-
4-n-NP	179	21.50	-
4-n-NP d8	185	21.39	-
NP1EO1	279	24.96	2.7 ± 0.3
NP1EO1 d2	281	24.94	
NP1EO2	251	25.34	14.9 ± 0.3
NP1EO2 d2	253	25.32	
NP1EO3&4	265	25.54	11.3 ± 0.2
NP1EO3&4 d2	267	25.52	
NP1EO5	251	25.60	8.7 ± 0.1
NP1EO5	253	25.58	
NP1EO6	251	25.84	6.6 ± 0.1
NP1EO6 d2	253	25.82	
NP1EO7	265	25.85	5.0 ± 0.2
NP1EO7 d2	267	25.83	
NP1EO8	279	26.13	6.2 ± 0.1
NP1EO8 d2	281	26.11	
NP1EO9	251	26.24	6.2 ± 0.1
NP1EO9 d2	253	26.22	
NP1EO10	265	26.26	8.7 ± 0.1
NP1EO10 d2	267	26.24	
NP1EO11	279	26.53	5.2 ± 0.0
NP1EO11 d2	281	26.51	
NP1EO12	251	26.77	16.5 ± 0.5
NP1EO12 d2	253	26.75	
NP1EO13	265	27.07	8.0 ± 0.1
NP1EO13 d2	267	27.05	

Table S1b. Ion masses, retention time for the quantification of phthalates

Phthalate	m/z	Retention time (min)
DMP	163, 194	6.96
DMP d4	167, 198	6.94
DEP	149, 177	9.02
DEP d4	153, 181	9.00
DiBP	149, 223	13.49
DnBP	149, 223	15.47
DnBP d4	153, 227	15.45
BBP	149, 167	22.14
DEHP	149, 167, 279	25,23
DEHP d4	153, 171	25,21
DOP	149, 167, 279	27.96
Dibenzyl phthalate	225, 149	25.24
Diphenyl phthalate	149, 225	
Diphenyl isophthalate	149, 225	

Table S2. Orthogonal experiments designed for the optimization of derivative conditions

Experiment	Solvent	BSTFA (µL)	Reaction time (min)
1	Hexane	25	15
2	Hexane	50	30
3	Hexane	100	60
4	Acetonitrile	25	30
5	Acetonitrile	50	60
6	Acetonitrile	100	15
7	iso-Octane	25	60
8	iso-Octane	50	15
9	iso-Octane	100	30

Table S3. Results of experiments according to the orthogonal method. (Relative response to that of gamma HCH)

Experiment	t-OP	NP	NP_1EO	4-n-NP	4-n-NPd8
1	31.6	13.6	1.85	3.95	0.32
2	42.1	21.0	2.54	8.16	0.62
3	43.6	21.6	2.57	8.61	0.64
4	42.2	19.7	1.57	6.27	0.49
5	39.4	18.6	1.48	5.93	0.46
6	41.7	20.8	2.06	7.49	0.59
7	39.0	17.6	1.66	6.33	0.47
8	38.9	18.4	1.88	6.62	0.52
9	39.6	18.2	1.89	7.09	0.54