Occurrence, distribution and fate of pharmaceuticals and further polar contaminants in the marine environment

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Stefan Weigel

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University of Hamburg
Institute of Organic Chemistry
Martin-Luther-King-Platz 6
20146 Hamburg
Germany

http://www.chemie.uni-hamburg.de/oc/hfuss/

1. Reviewer: Prof. Dr. H. Hühnerfuss

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Abbreviations

AHTN	1-(5,6,7,8-Tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)ethanone
amu	Atomic mass units
AR	Additional recovery
BFR	Brominated flame retardants
BHA	*tert*-Butylhydroxyanisole
BHT	Di-*tert*-butylhydroxytoluene
BSH	Bundesamt für Seeschiffahrt und Hydrographie
CI	Chemical ionisation
CV	Coefficient of variation
DBP	Dibutylphthalate
DCPy	Dichloropyridine
DDE	1,1-Dichloro-2,2-bis(4-chlorophenyl)ethene
DDT	1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane
DEET	*N,N*-Diethyl-3-toluamide
DEHP	Di(ethylhexyl)phthalate
DEP	Diethylphthalate
DMP	Dimethylphthalate
DOC	Dissolved organic carbon
EC	European Community
EI	Electron impact ionisation
ESI	Electrospray ionisation
FAME	Fatty acid methyl ester
GC	Gas chromatograph/chromatography
GCB	Graphitised carbon black
GF	Glass fibre
HCB	Hexachlorobenzene
HCH	1,2,3,4,5,6-Hexachlorocyclohexane
HHCB	1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(*g*)-2-benzopyrane
HPLC	High performance liquid chromatography
Kow	Distribution coefficient octanol/water
LC	Liquid chromatography
MAST	Marine Science and Technology
MRM	Multi reaction monitoring
MS	Mass spectrometer/spectrometry
MXR	Multixenobiotic resistance
NIST	National Institute of Standards & Technology (USA)
NP	Normal phase
OSPARCOM	Oslo and Paris Commission for the Protection of the Marine Environment of the North-East Atlantic
PAH	Polycyclic aromatic hydrocarbon
PBDE	Polybrominated diphenylether
PCB	Polychlorinated biphenyl
PCN	Polychlorinated naphthalene
PE	Population equivalents
POPs	Persistent organic pollutants
PPCPs	Pharmaceuticals and personal care products
PRISMA	Prozesse im Schadstoffkreislauf Meer-Atmosphäre
PROFILE	Processes in Regions of Freshwater Influence
PS-DVB	Polystyrene-divinylbenzene
PUF	Polyurethane foam
RSD	Relative standard deviation
RT	Retention time
RR	Recovery rate

SD	Standard deviation
SIR	Selected ion recording
SIM	Selected ion monitoring
SIS	Surrogate internal standard
SOP	Standard operating procedure
SPE	Solid-phase extraction
SPM	Suspended particulate matter
SSRI	Selective serotonine reuptake inhibitor
STP	Sewage treatment plant
TBT	Tributyltin
TCDD	Tetrachlorodibenzodioxin
TCEP	Tris(chloroethyl)phosphate
TCPP	Tris(chloropropyl)phosphate
TIC	Total ion current
TPPO	Triphenylphosphine oxide
TUVAS	Transport, Umsatz und Variabilität von Schad- und Nährstoffen in der Deutschen Bucht
v/v	Volume/volume
ZISCH	Zirkulation und Schadstoffflüsse in der Nordsee
z.R.	zur Rückstandsanalyse (Organic trace analysis grade)

1 Introduction

1.1 Contamination of the marine environment

The North Sea is a semi-enclosed, epi-continental large marine ecosystem. Its area, as defined by the North Sea Task Force, includes the region south of 62° N, the Skagerrak, the Kattegat and the English Channel. It covers 750 000 km^2 and has a volume of 94 000 km^3. Its catchment area (841 500 km^2) comprises some of the most densely populated and highly industrialised regions of Europe. These are drained by the major rivers Tyne, Tees, Humber, Thames, Rhine, Meuse, Scheldt, Ems, Weser and Elbe, resulting in yearly freshwater inputs of approximately 300 - 350 km^3. Since these rivers transport large amounts of chemicals from industrial, agricultural and domestic sources into the North Sea, pollution from contaminants and nutrients has been a major environmental issue for decades. The hydrodynamic situation is characterised by inflow of Atlantic water through the English Channel and between Scotland and the Shetland Islands and a general counter-clockwise water flow (Figure 1). [1]

The analytical detection of organic contaminants such as polychlorinated biphenyls (PCBs) in marine ecosystems started in the 1960's [2]. Since then, a large variety of synthetic organic substances has been detected in biota, sediment and water of the North Sea and other marine ecosystems. The scientific and public concern about observed or potential toxicological and ecotoxicological effects on one hand and the increase in the use of certain chemicals on the other hand are reflected in the number of investigations and the specific compounds included. In the early phase the focus was on the determination of strongly bioaccumulating compounds in biota, as for example PCBs in seals or DDT in predatory birds. One decade later, in the late 1970s, further compound classes beside pure chloro-hydrocarbons were addressed by marine pollution research. In mussels (*Mytilus edulis*) from Dutch coastal waters, methylthiochlorobenzenes were detected besides PCBs, hexachloro-benzene (HCB), DDT, dieldrin and endrin [3]. Giam et al. reported on phthalate plasticisers as a new class of marine pollutants in 1978 [4] and Weber and Ernst identified more than 30 organic compounds in the estuaries of Elbe, Weser and Ems between 1977 and 1983 [5], among them phthalates but also alkyl and aryl phosphate plasticisers. With regard to the applied methodology it remains questionable whether the in some instances high concen-trations (> 500 ng/L for dibutyl- and di(ethylhexyl)-phthalates in the river Elbe estuary) are not mainly artefacts from the ship and laboratory environments. Nevertheless, further identified compounds included organophosphate pesticides (e.g., parathion-methyl), di- to pentachloro-phenols, bis(2-chloro-1-methylethyl)ether and a variety of polycyclic aromatic hydrocarbons (PAHs).

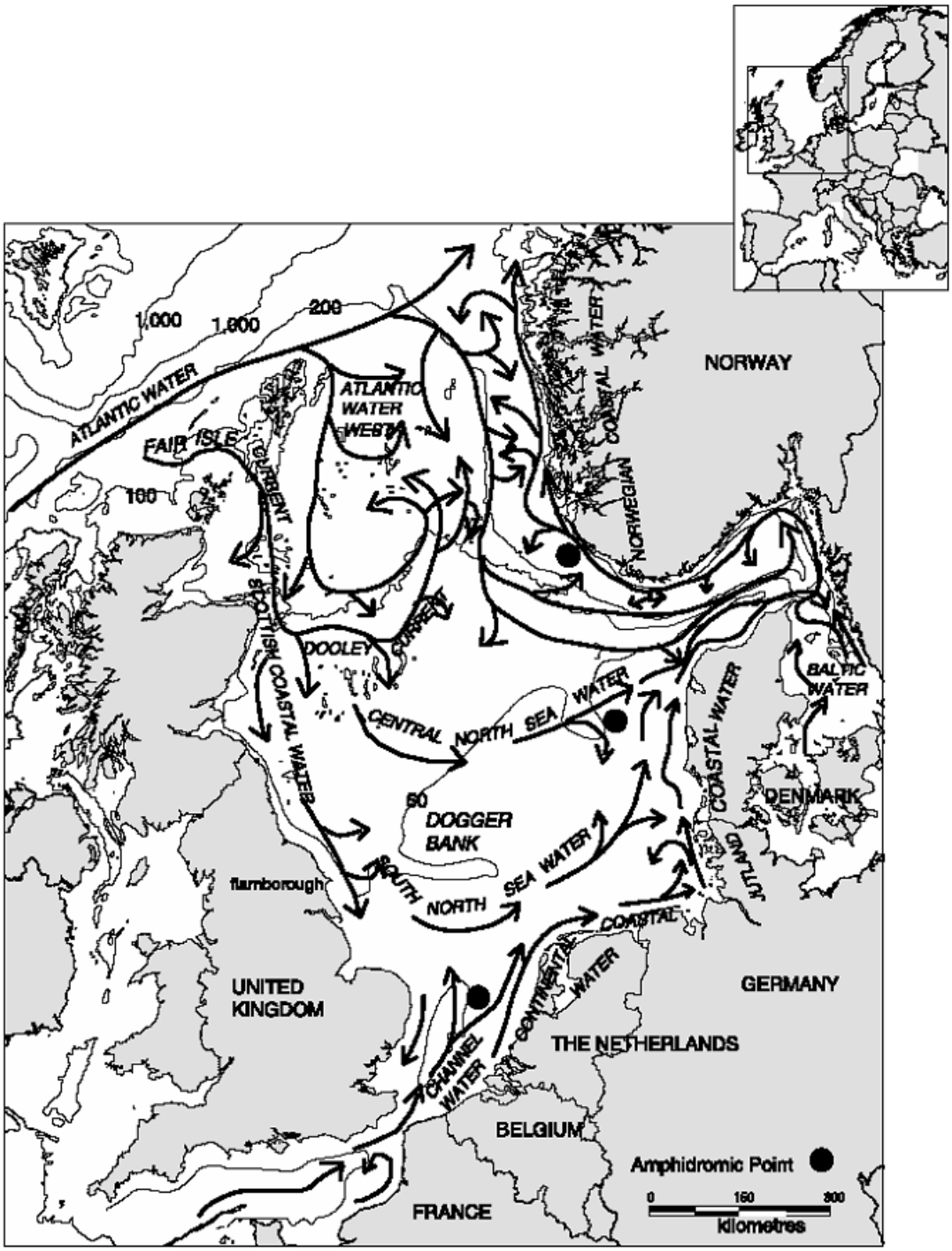

Figure 1: General water circulation in the North Sea [1]

Throughout the 1980s, intensified research into organic contaminants in the North Sea lead to various publications on further substances, e.g. chloronitrobenzenes, nitrotoluenes and hexachlorobutadiene [6] as well as octachlorostyrene [7]. Further investigations concentrated on the fate and behaviour of chlorohydrocarbon compounds, e.g., interaction of PCBs with marine humic substances [8] and sediments [9,10] and on the partitioning of chlorobenzenes and hexachlorocyclohexanes (HCHs) between water and sediment [11,12]. Large scale interdisciplinary experiments substantially contributed to the current knowledge on the distribution and fate of organic contaminants in the North Sea. Within the framework of

ZISCH (Zirkulation und Schadstoffflüsse in der Nordsee, engl.: Circulation and contaminant fluxes in the North Sea; 1985 - 1989) [13] and PRISMA (Prozesse im Schadstoffkreislauf Meer-Atmosphäre, engl.: Processes in the ocean-atmosphere contaminant cycle; 1990 - 1993) [14] multi-ship experiments were conducted. Their aim was to quantify presence, fluxes and budgets of major contaminants in the North Sea, including heavy metals, organic contaminants and nutrients and to determine transport paths and fate of compounds of concern [15]. Simultaneously, distribution data was gathered for PCBs, α- and γ-HCH in seawater, in sediments (plus HCB) and in biota (PCBs, HCB, γ-HCH, DDE) [16-19]. Based on the obtained data, mass balances of HCHs, PCBs and triazines were calculated for the German Bight [20]. North Sea research during this era was complemented by further projects such as TUVAS (Transport, Umsatz und Variabilität von Schad- und Nährstoffen in der Deutschen Bucht, engl.: Transport, turnover and variability of contaminants and nutrients in the German Bight; 1990 - 1992) [21], the English National Environmental Research Council's North Sea project [22] and European Marine Science and Technology (MAST) activities, e.g., the PROFILE (Processes in Regions of Freshwater Influence) project [23]. Since then, further scientific contributions to the knowledge of the distribution and fate of organic contaminants were rather due to single studies than to large-scale integrated experiments. Concentrations of some compounds measured in samples from the German Bight in the 1990s are listed in Table 1. A comprehensive survey on North Sea research concerning input, occurrence, distribution, fate, effects and determination methods of organic contaminants was given in a recent report [24]. As outlined above and in the mentioned report, a considerable knowledge on the contamination status of the North Sea is available. Nevertheless, it can be assumed that a substantial proportion of organic trace compounds present in North Sea water has not been identified yet.

In high latitude marine areas, e.g., the Arctic Ocean, concentration data for contaminants in seawater is extremely limited and restricted to organochlorine persistent organic pollutants (POPs), mainly α- and γ-HCH, PCBs, DDTs and chlordanes. For the Norwegian Sea, only data for α-HCH (2.75 ng/L) and γ-HCH (0.38 ng/L) has been reported [25].

Substance	concentration range [ng/L]	year	ref.
Nitrobenzene	0.6 - 2.5	1993	[26]
Musk xylene	< 0.03 - 0.17	1993	[26]
Musk ketone	< 0.02 - 0.08	1993	[26]
2-Chloronitrobenzene	< 0.02 - 0.45	1993	[26]
2-Chloronitrobenzene	< 0.01 - 0.59	1995	[27]
3-Chloronitrobenzene	< 0.01 - 0.076	1995	[27]
4-Chloronitrobenzene	< 0.01 - 0.61	1995	[27]
2,5-Dichloronitrobenzene	< 0.05 - 0.93	1995	[27]
2,5-Dichloroaniline	< 0.01 - 0.65	1995	[28]
Bis(2-chloro-1-chloromethylethyl)ether	2.4 - 47.8	1991	[29]
O,O,S-Trimethyldithiophosphate	0.1 - 9.4	1991	[30]
Benzothiazole	0.4 - 1.23	1995	[31]
Methylthiobenzothiazole	0.04 - 1.37	1995	[31]
Nonylphenol	0.3 - 63	1999	[32]
Nonylphenolmonoethoxylate	0.7 - 29	1999	[32]

Table 1: Concentrations of some compounds in water from the German Bight in recent years

1.2 Pharmaceuticals and personal care products (PPCPs) in the environment

1.2.1 Background

The development and application of chemically defined pharmaceuticals has apparently changed human societies a lot in the course of the last century. Along with the improvement in nutrition, sanitary and working conditions, pharmaceutical substances contributed to a rise in life expectancy of more than 20 years, at least in western societies. Currently, the provision of European populations with pharmaceuticals is on a high level. In addition to their application in human medicine, a large number of compounds is also applied in veterinary medicine for the prevention and acute treatment of infectious diseases in intensive livestock farming. Additionally, antibiotics are also used as growth promoters. Depending on their use, pharmaceuticals enter the environment on different pathways, as outlined in Figure 2 [33]. Compounds applied in human medicine and their metabolites are excreted with urine and faeces to sewer systems. Expired and surplus drugs are assumed to be disposed off via toilets to the sewer system by the consumer to an unknown extent. Subsequently, they are released via the effluents of sewage treatment plants into the aquatic environment. The proportion of a drug that is retained in sewage treatment either due to transformation or by

adsorption to sludge strongly depends upon its chemical structure and physico-chemical properties, but also on the specific conditions within the respective plant. Water temperature, residence times (corresponding to flow rates), dilution with rainwater and sludge age (and thus adaptation of microbial communities) were found to exert an effect on elimination efficiencies [34,35]. Observed elimination rates ranged from more than 80 % for acetylsalicylic acid, ibuprofen, bezafibrate, metoprolol and propranolol to less than 10 % for carbamazepine and x-ray contrast media [36,37]. In many cases, veterinary pharmaceuticals are directly released into the environment by their use in aquaculture, the dispersion of manure from treated livestock on fields or the therapeutic treatment of livestock on meadows.

Although the aspect of pharmaceutical chemicals in the environment was occasionally mentioned in the late 1970s [38] and mid-80s [39,40], little attention had been paid to these substances as potential environmental pollutants until the early 1990s, when Stan and Linkerhägner [41] identified amazingly high concentrations of clofibric acid, metabolite of the lipid regulating agents clofibrate and etofibrate, in groundwater of the city of Berlin/Germany. Subsequently, investigations carried out by further research groups revealed the presence of a vast array of pharmaceutical residues in sewage treatment plant (STP) effluents and river water [36] in concentrations up to the μg/L-range. Among these were analgesics/ antiphlogistics [42], β-blockers and β-sympathomimetics [43], antibiotics [44] and synthetic estrogens [45,46]. Some of them, especially clofibric acid, were even determined in drinking water [47] as well as in the North Sea [48], where this compound was found in concentrations similar to classical pollutants such as lindane (γ-HCH). As the widespread occurrence of pharmaceuticals demonstrates, they have to be regarded as a new class of priority environmental pollutants.

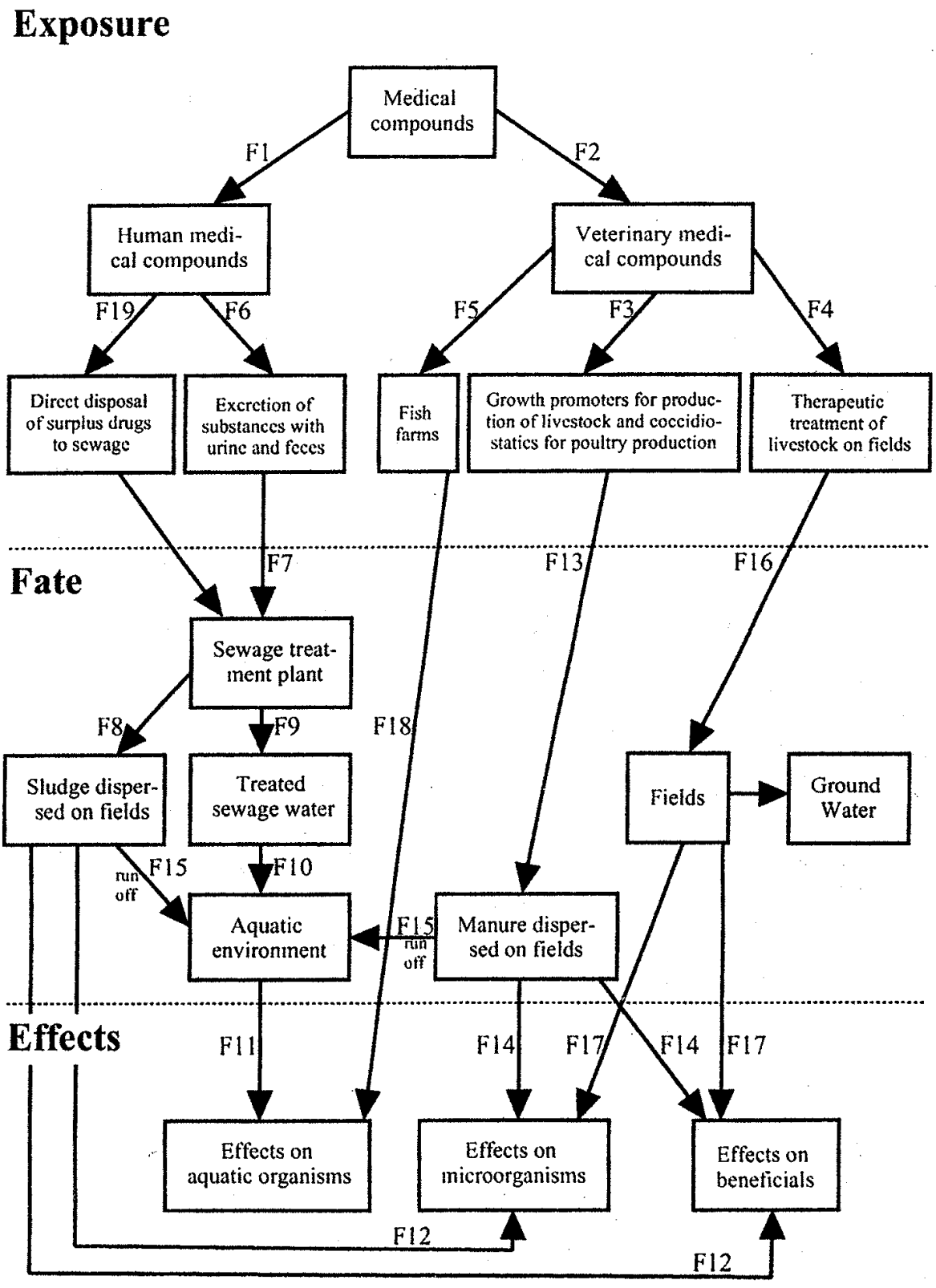

Figure 2: Anticipated exposure routes of pharmaceuticals from use in human and veterinary medicine [33]

1.2.2 Relevance

Production and consumption amounts of pharmaceuticals equal or exceed those of agro-chemicals in several cases. Prescribed amounts of the analgesic ibuprofen for instance summed up to almost 150 t in Germany in the year 2000 [49], not taking into account the presumably higher proportion of this "over-the-counter" drug being sold without prescription. High environmental concentrations can be expected for pharmaceuticals of either high frequency of prescription, high daily doses, long-term intake and/or low elimination in sewage treatment. The release of substantial amounts of pharmaceutical agents into the environment is especially precarious for the following reasons:

a) Pharmaceutical drugs are designed to trigger certain biological effects. Thus, they can be expected to interfere with the respective receptors, enzymes or hormonal systems of unintentionally exposed organisms.

b) In order to avoid the drug from breakdown before it reaches its place of action, or to prolong its residence time in the body, often functional groups are introduced into the molecule to prevent fast metabolisation. For example, this is the case for some synthetic estrogens. This pharmacologically desired effect turns to a threat from an environmental point of view: it raises the persistence of the drug.

c) In contrast to other environmental pollutants as for example pesticides, which are released mainly seasonally, pharmaceutical drugs are introduced continuously and directly into the receiving waters. Even compounds of low persistence could act as if they were persistent due to perpetual life-cycle exposures for aquatic organisms [50].

The main threats to be encountered from the presence of pharmaceutical drugs and related substances in the environment are:

a) Development of antibiotic-resistant bacteria. Alarming evidence is presented of multi-drug-resistant bacteria. Recently, in Denmark two persons died because of drug-resistant salmonella infections [51]. Although often attributed to heavy use of antibiotics in livestock operations, a contribution of antibiotics released into aquatic ecosystems from human and veterinary use can be supposed. Significantly higher amounts of antibiotic-resistant bacteria were observed in marine sediment [52] and fish [53] exposed to sewage effluent.

b) Endocrine disruption. In addition to naturally excreted human estrogens, the therapeutic administration of both synthetic and natural hormones may lead to concentrations in STP effluents that reach effective levels. Purdom et al. [54] observed positive responses in fish down to 17α-ethinylestradiol exposure levels of 0.1 to 0.5 ng/L, which is below the concentrations of this substance typically found in STP effluents (e.g., median value 17 ng/L [46]) and occasionally in rivers (1 - 4 ng/L [46]). Hitherto, 'endocrine disruption' focuses mainly on the sexual/reproductive hormone system. Knowledge about the disruption of other hormone systems is scarce. There is some evidence that for example the thyroid system is disturbed

by environmental pollutants such as triclosan, which is structurally similar to the natural hormone levothyroxin. Additionally, levothyroxin itself is prescribed in considerable amounts. An indication for effects on the reproductive system of aquatic organisms beyond estrogenic activity is the observation that certain antidepressants from the group of selective serotonine reuptake inhibitors (SSRIs) induce spawning in mussels at concentrations in the ng/L to µg/L-range [55].

c) Genotoxic effects. Many antineoplastic drugs are designed to act as alkylating agents. The therefrom arising genotoxic potential poses a high risk to exposed organisms.

d) Human exposure. In some areas, residues of pharmaceutical compounds were detected in drinking water [43,47,56], leading to an uncontrolled exposure to these substances.

e) Chemosensitising. Many organisms, e.g., filter feeders and bottom dwellers, develop a 'multixenobiotic resistance' (MXR) in contaminated areas. This system removes potentially toxic compounds of medium to low lipophilicity (as are many pharmaceuticals) from their body. The MXR system is effectively inhibited by a couple of pharmaceuticals, e.g., verapamil, a cardiac drug, thus raising the susceptibility to other pollutants [50].

In a current and comprehensive review [50] the authors conclude: *"While resources continue to be focused on environmental fate/toxicology of conventional POPs, yielding only incremental enhancement of our knowledge base, a fraction of these same resources could yield significant advancements in the analogous understanding of pharmaceuticals and personal care products in the environment"*. Among their recommendations for priority research needs they pose a more profound knowledge on the occurrence and distribution of these substances in the environment. This requires the development of new, highly sensitive ultra trace analytical methods since many of these compounds and their metabolites are highly polar and thus not accessible by established standard analytical methods.

1.2.3 Metabolism

Following ingestion, most pharmaceuticals undergo substance-specific metabolisation. Before being retrieved from the body with the urine, phase I or phase II metabolites are formed. Phase I reactions usually include oxidation, reduction or hydrolysis, and the products are often more reactive and sometimes more toxic than the respective parent compounds (as known from the metabolisation of PAHs to epoxy- and dihydrodiolepoxide derivatives) [33]. Phase II reactions involve conjugation mainly with glucuronic or sulfuric acid, but also with acetic acid, glutathion and taurine. Both phase I and phase II metabolisation renders the parent compound more water soluble. While phase I metabolites may also possess a pharmacological activity that sometimes is even higher than that of the parent drug [57], phase II metabolites are usually inactive. However, during sewage treatment and in manure cleavage of the conjugates and thus a reactivation was observed [33]. In many cases only a

small proportion of the ingested drug is excreted unchanged. As an example, human metabolism of the anti-inflammatory and analgesic drug ibuprofen is depicted in Figure 3. For an assessment of the overall contamination of the environment with drugs from human and veterinary medicine it is crucial to include the main metabolites in the investigations. While phase II metabolites probably are cleaved during STP passage, phase I metabolites deserve far more attention than they have received so far.

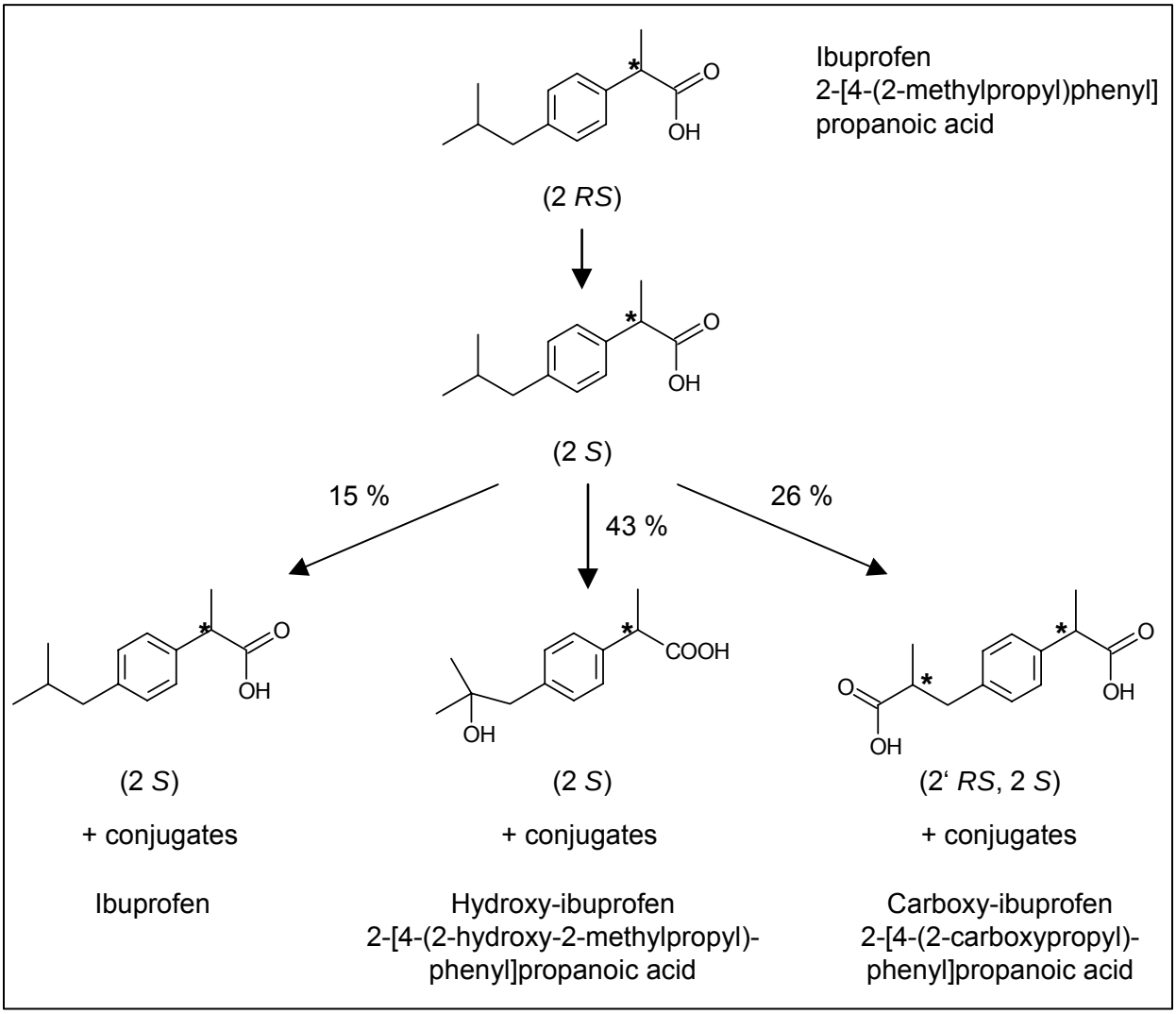

Figure 3: Metabolism of ibuprofen in humans [58], including renal excretion rates [59] and chiral aspects [60]

1.2.4 Caffeine

Caffeine is an alkaloid that is formed in more than 60 plant species. Economically most relevant are coffee (*Coffea arabica, C. robusta*), tea (*Camellia sinensis*) cola (*Cola vera*) and cacao (*Theobroma cacao*), while others are of a more regional relevance, such as mate (*Ilex paraguariensis*) and guaraná (*Paullinia cupana*). Caffeine is in pharmaceutical use as analeptic and is added to several analgesics such as acetylsalicylic acid in order to enhance

their effect. However, the major amount of caffeine is consumed as a natural stimulant. It is considered to be the most widely used drug in the world. Depending on cultural environment, preferred consumption forms are coffee, black, green or mate tea, cacao or caffeinated soft drinks. The daily uptake may reach 400 mg per person per day with a world average of 70 mg, 0.5 to 7 % of the ingested caffeine are excreted unmetabolised via the urine [61]. Besides this, an unknown amount of caffeine-containing beverages is discharged directly to the sewage system. Despite efficient removal in most sewage treatment plants (80 - 99.9 % [35]) the residual loads result in considerable concentrations in rivers and streams. Consequently, caffeine was detected among many other compounds in most non-target screening studies (STP effluent [62], river water [39,63,64]). Systematic research on the distribution of caffeine in the aquatic environment started during the recent years, establishing the ubiquitous character of this compound in surface-, ground- and harbour-water [61,65-67]. The use of caffeine as a tracer for domestic sewage was proposed. A recently published study demonstrated in detail the suitability of this substance for this purpose [35].

1.3 Objectives

The basic idea of this work was to highlight the necessity of an adequate consideration of the contribution of polar contaminants to the overall impact of anthropogenic substances on aquatic environments. The focus was put on marine environments as there is substantially less knowledge of the occurrence and distribution of more hydrophilic compounds in this field as compared to limnic areas. Special emphasis was placed on pharmaceuticals and their metabolites since there was almost no information available on the presence of this emerging class of pollutants for marine waters. In order to achieve the underlying goal, investigations were carried out in four steps:

- Non-target screening of North Sea water samples for the presence of potentially harmful organic compounds, identification by structure elucidation and verification by means of the respective reference compounds.
- Quantification of selected compounds throughout the North Sea.
- Development of highly sensitive extraction and determination methods for pharmaceuticals from environmental water samples.
- Quantification of selected pharmaceuticals and personal care products (PPCPs) in different types of water.

2 Analysis of polar organic compounds from aqueous matrices

2.1 Extraction

Five principles are basically used in various modifications in environmental analysis for the enrichment of organic molecules from aqueous matrices: purge-and-trap, liquid/liquid extraction (LLE), solid-phase extraction (SPE), steam destillation and lyophilisation. While purge-and-trap enrichment is limited to volatile compounds, LLE and SPE are the most widely used methods for the extraction of a large variety of xenobiotics from water samples, e.g., pesticides, PAHs and PCBs. In LLE, highest extraction efficiencies are obtained with solvents of a polarity similar to that of the target analytes. For highly polar analytes this approach is limited by the miscibility of adequate solvents with water, which can only partly be overcome by the addition of salt to the sample. Therefore, aiming at hydrophilic analytes, SPE is the method of choice. Using SPE, a broad variety of organic chemicals, e.g., organophosphorus and –nitrogen pesticides [68,69], chlorophenols [70], explosives [71] and aromatic sulfonates [72], has been enriched from different types of water [73-76]. The sorbents used in SPE include graphitised carbon black (GCB) [77], silica gels modified with alkyl- or functionalised alkyl chains and polymeric materials. The most widely used alkyl-silica material (and SPE sorbent in general) is the octadecyl (C_{18} -) phase, but ethyl-, butyl-, cyclohexyl-, octyl-, phenyl-, propylamino-, dimethylaminopropyl- and cyanopropyl- silica phases have been applied as well [78,79]. With respect to polymeric sorbents, the best known are styrene-divinylbenzene co-polymers (Polysorb S, Amberlite XAD-2 and XAD-4) and polyacrylates (Amberlite XAD-7 and XAD-8). Unsatisfactory recovery rates [78] and poor reproducibility [68] were observed for XAD-resins. Especially for the XAD-resins intensive cleaning procedures are required prior to their use [80]. The development of a new generation of polystyrene-based sorbents with a higher degree of cross-linkage and a larger inner surface did not only overcome the problems associated with XAD-resins. It also enlarged the capacity of these styrene/divinylbenzene or divinylbenzene/ethylvinylbenzene co-polymers immensely. The capacity is specified to be 10-fold higher than that of C_{18}-RP sorbents, which roughly correlates to the carbon content of the sorbents. This is in the range of 10 to 18 % for C_{18}-silicas and approximately 85 % for polystyrene-based sorbents. Even more important than the rise of the overall capacity is the increase in retention power for many analytes due to improved molecular interaction modes. While in C_{18}-silicas retention is achieved by van der Waals forces (and eventually by hydrogen bonding between residual silanol groups of the silica sorbent base and functional groups of the analyte) PS-DVB sorbents additionally offer possibilities for π-π interaction. Interaction possibilities can further be broadened by functionalisation of the polymer (e.g., sulfonation) or co-polymerisation with different (polar)

monomers. The polymeric sorbents have been used successfully for the extraction of the whole range of organic contaminants [72,81]. They proved to be especially suitable for medium to highly polar substances, where they showed substantially higher recovery rates than alkyl-silica sorbents [71,73] or liquid-liquid extraction (LLE). Even acidic and phenolic pesticides such as dicamba (3,6-dichloro-2-methoxybenzoic acid), 2,4,5-T (2,4,5-trichlorophenoxyethanoic acid) and dinoterb (2-*tert*-butyl-4,6-dinitrophenol) [82] as well as chlorophenols [83] were extracted quantitatively without acidification of the sample.

2.1.1 SPE for marine chemistry

In marine analytical chemistry, SPE was mainly applied to estuarine or coastal water samples of around 1 L volume. At the open sea, concentrations of most organic pollutants are low, as compared to limnic systems. Concentrations are typically in the lower ng/L range (e.g., lindane [84]) or even in the low pg/L range (e.g., PCBs [85]). A conceivable possibility to meet the requirements for low detection limits is to rise the volume of the sample to 10, 100 or more litres. Basic needs for large volume SPE are (i) efficient online filtration, (ii) high flow rates (to keep the extraction time within acceptable limits), (iii) low flow resistance (both of the filter and the extraction unit), (iv) mechanical stability of the sorbent package.

Commercially available standard SPE systems are often incapable of handling these volumes. However, some approaches to solid-phase extraction of large volume (> 10 L) seawater samples were reported in the literature [85-89]. In general, the loaded sorbents, commonly Amberlite® XAD resins, polyurethane foams (PUF) or C_{18}- material, were back-extracted in a Soxhlet or Ehrhardt apparatus, which is time consuming, prone to contamination and requires considerable amounts of solvents.

The method used in this work for the extraction of large-volume seawater samples is based on an approach developed within the preceding diploma thesis [90] for 10 L water samples. For the application to 20 L- seawater samples from the North Sea it was further improved and also validated for the quantitative determination of selected target analytes (chapter 2.5.1) within this work. By the use of a hyper-crosslinked polystyrene-divinyl-benzene co-polymeric sorbent of high inner surface (> 1000 m^2/g) high extraction efficiencies were obtained, particularly for polar compounds (log Kow < 3). In a parallel work that used the same method for the extraction of river Elbe water samples with the aim of a subsequent bioassay directed fractionation and chemical identification, in certain fractions even readily water soluble sugars, alcohols (e.g., glycerol) and organic acids (e.g., hydroxy(hydroxy-phenyl)ethanoic acid) were identified after silylation or methylation [91]. This feature impressively demonstrates the suitability of the method for the extraction of a wide range of analytes, from classical lipophilic contaminants such as hexachlorobenzene down to highly hydrophilic compounds. Thus, it was employed with only minor modifications. The superiority of this approach for non-target screening purposes over liquid-liquid extraction or solid-phase

extraction (SPE) with alkylated silica sorbents is discussed in detail in [90]. A schematic overview of the method is presented in Figure 4, details are given in the respective standard operating procedure (SOP) in the annex (SOP 1). Since emphasis was placed on more hydrophilic water constituents that are not expected to sorb to suspended particulate matter (SPM) in a relevant proportion, only the dissolved phase was regarded. The particulate phase retained on the filter candles was separately collected and stored, but not investigated within this work.

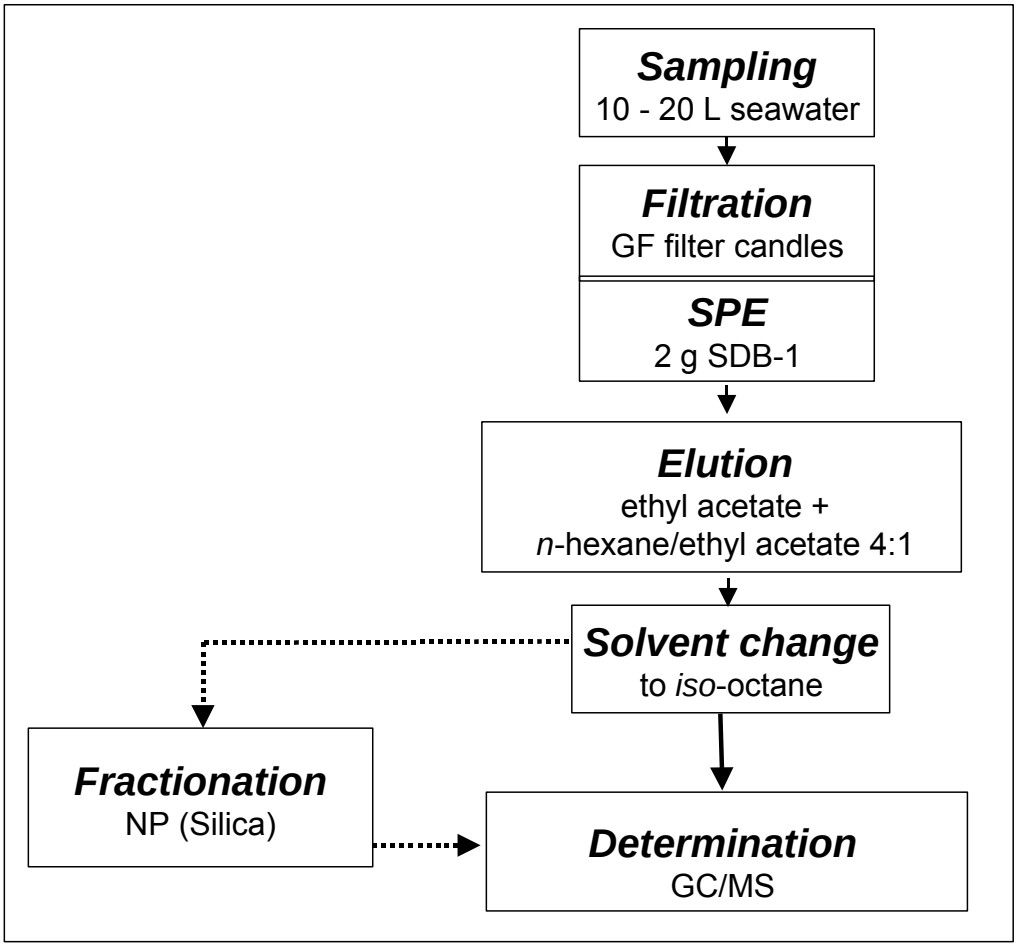

Figure 4: Method for the extraction of large volume North Sea water samples (NP: normal phase)

For the screening of seawater from supposedly higher contaminated areas in the Tromsø Sound/Norway (e.g., harbours), the procedure was down-scaled by a factor of 10 and transferred to standard equipment such as commercially available SPE cartridges. Additionally, the elution protocol was modified, allowing a separation of analytes into three fractions according to their polarity (Figure 5, SOP 4).

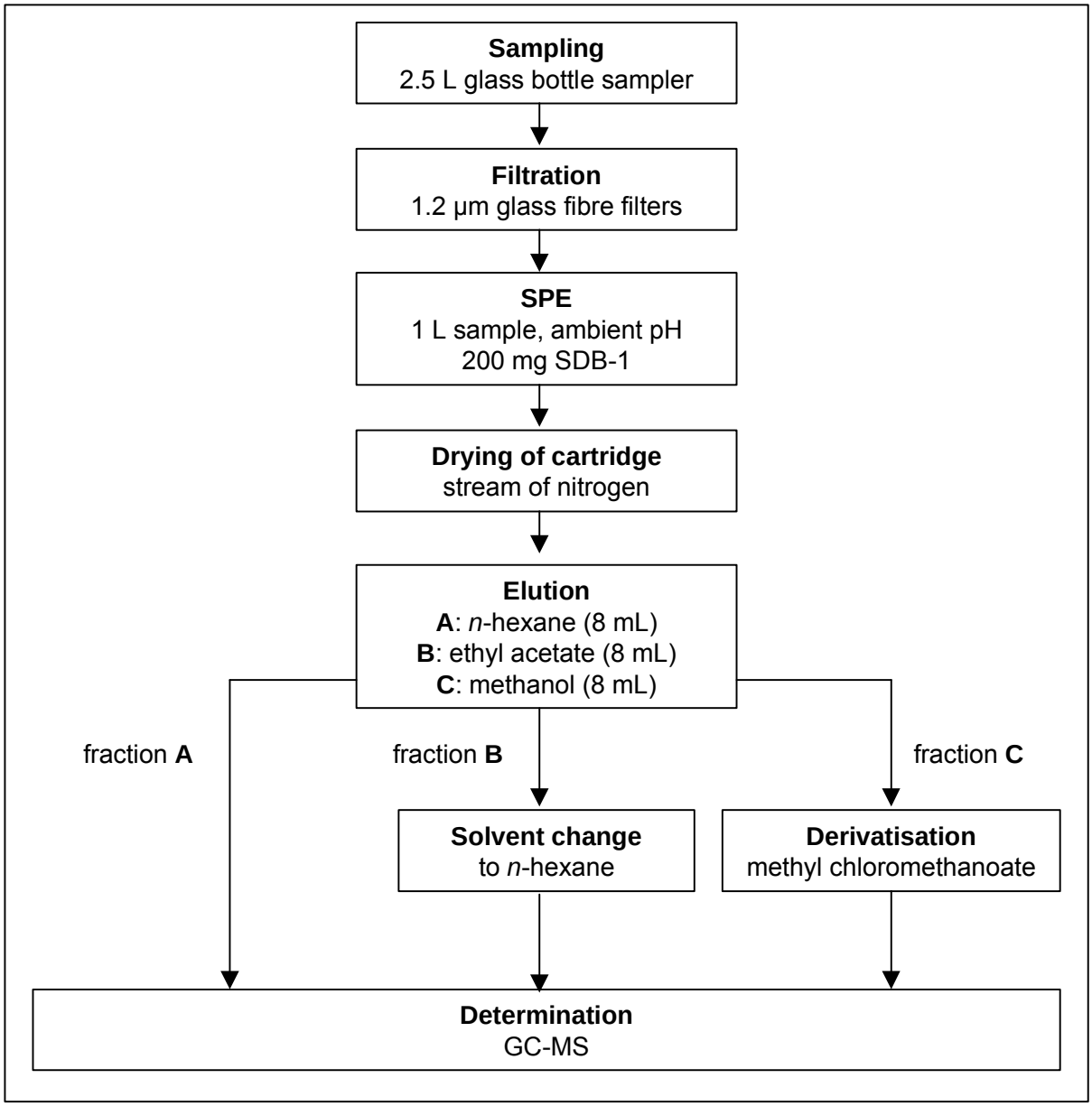

Figure 5: Method for the extraction of 1 L water samples

2.1.2 SPE of pharmaceuticals

From a chemical point of view, pharmaceuticals comprise a complex variety of chemical classes, often combining different moieties in one molecule. A common feature of most pharmaceuticals is their hydrophilic character. Hydroxy-, carboxy- and amino-groups are frequent constituents of pharmacologically active substances, necessary either for the intended effect or the transport to the place of action. One intention of this work was to develop an extraction method that is capable of extracting acidic, hydrophilic neutral and basic pharmaceuticals simultaneously from water samples at ambient pH. As pointed out above, for the extraction of highly polar analytes SPE with polymeric sorbents often proved to be superior to alkyl-bonded silica (e.g. C_{18} -) sorbents and LLE [73,92,93]. A variety of hyper-crosslinked polystyrene-divinylbenzene (PS-DVB) based sorbents is commercially available,

differing in the degree of linkage, porosity and surface area. Higher surface areas have been found to yield higher retention of analytes [82,94]. The exploration of the possibilities of functionalised polystyrenes for analytical SPE was intensified in the beginning of the 1990s with the introduction of acetyl- and hydroxymethyl-groups into PS-DVB resins [92]. Since then, a variety of polymers carrying different functionalities, e.g., carboxybenzoyl moieties [95] was developed. The scope of these sorbents, their preparation and application was reviewed recently [96,97]. In consequence, functionalised polymers became commercially available during the second half of the 1990s. They are either co-polymerisates of styrene and a polar component (e.g., methacrylate or *N*-vinylpyrrolidone) or the functional groups are introduced after polymerisation (e.g., by sulfonation). This functionalisation results in mainly two effects: improved wetting characteristics for better mass transfer and additional possibilities for interactions with functional groups of the analytes and thus a higher retention. Due to these improvements, this generation of SPE-sorbents is increasingly used in the analysis of polar pesticides and pharmaceuticals in environmental water samples [98,99].

A tempting feature of these high surface PS-DVB, functionalised PS-DVB, and hydrophilic/lipophilic co-polymers is their capability of extracting acidic analytes from water without acidification of the sample, together with neutral analytes of a wide polarity range. A hitherto underestimated aspect of sample preparation is that the commonly performed acidification of samples for the extraction of acidic analytes may lead to hydrolysis or other transformations of target analytes. The lipid lowering drug fenofibrate (1-methylethyl 2-[4-(4-chlorobenzoyl)-phenoxy]-2-methylpropanoate) for example was observed to hydrolyse rapidly to fenofibric acid (2-[4-(4-chlorobenzoyl)-phenoxy]-2-methylpropanoic acid) in an acidic aqueous solution (pH 2) at room temperature [100] and very recently the formation of 1-(2,6-dichlorophenyl)indolin-2-one from diclofenac under acidic extraction conditions was reported [101]. Pichon et al. [82] found recoveries > 80 % for acidic and neutral pesticides extracted jointly from water at pH 7 with the PS-DVB sorbent SDB-1. Furthermore, they showed that the co-extraction of humic and fulvic acids was significantly reduced at pH 7 as compared to extraction at pH 3. Recoveries of 40 % or above for the extraction of acidic pharmaceuticals from alkaline seawater (pH 8.3) using the same sorbent were obtained within this work (chapter 2.5.1). Up to date, few other studies reported on this potential of PS-DVB sorbents [102]. More commonly, simultaneous extractions of acidic and base/neutral analytes, especially pesticides, were carried out with graphitised carbon black (GCB) sorbents [103-105]. However, significant drawbacks (desorption problems, presence of active oxygen complexes [106]) prevented their more widespread application.

Modified PS-DVB sorbents combine the advantages of high retention of polar analytes and reproducible desorption and have recently been used for simultaneous extractions without pH adjustment [98,107]. In sum, the main advantages of the extraction with

polymeric sorbents at neutral pH are: (i) simplified sample handling: no acidification step, no clean-up for the removal of humic and fulvic acids, (ii) possibility of on-line filtration/-extraction, especially of large sample volumes (iii) no enhanced risk of acidic hydrolysis or other transformations of susceptible analytes, (iv) no protonation of basic analytes. The resulting ability to extract a broad range of analytes simultaneously under the same conditions from one sample is essential when sampling and sample extraction are the limiting factors of the analytical procedure.

2.1.3 Comparison of different polymeric sorbents for the simultaneous extraction of acidic, neutral and basic pharmaceuticals from water

The intention of this part was to evaluate various different polymeric sorbents for their ability to extract acidic, neutral and basic analytes from water for a subsequent use in either liquid chromatography-mass spectrometry (LC-MS) or gas chromatography-mass spectrometry (GC-MS) determination. In addition to three non-functionalised PS-DVB sorbents with surface areas of ≥ 1000 m^2/g (Bakerbond SDB-1, LiChrolut EN, Chromabond HR-P), two functionalised PS-DVB sorbents (Isolute Env+, Chromabond EASY) of high surface area (1000 - 1200 m^2/g) and two co-polymers composed of both lipophilic and hydrophilic monomers (Oasis HLB, abselut Nexus) of lower surface area (500 - 700 m^2/g) were included in the comparison. Property details are listed together with the obtained results in Table 2 (page 21). Chemical structures of some of the sorbents are depicted in Figure 6.

The test compounds (Figure 7) were chosen to cover a wide range of chemical properties. Representatives of several environmentally relevant pharmaceutical classes were included: analgesics, lipid lowering and psychopharmaceutical agents, ß-blockers, as well as the stimulant caffeine and two estrogens. The major objective was to find a sorbent yielding recoveries above 80 % at low standard deviations for all of these classes. The extraction experiments were carried out at a concentration level of 2 - 5 µg/L which is at the upper range typically detected in surface waters. The slightly basic pH of the tap water used for spiking (7.8) was not adjusted since two of the target matrices for further method development are also characterised by a pH value above 7 (lower reaches of river Elbe: 7.5 - 8, seawater: 8.3). For the determination of recovery rates, a high performance liquid chromatography (HPLC) method was developed allowing the separation and quantification of all included analytes (Figure 8).

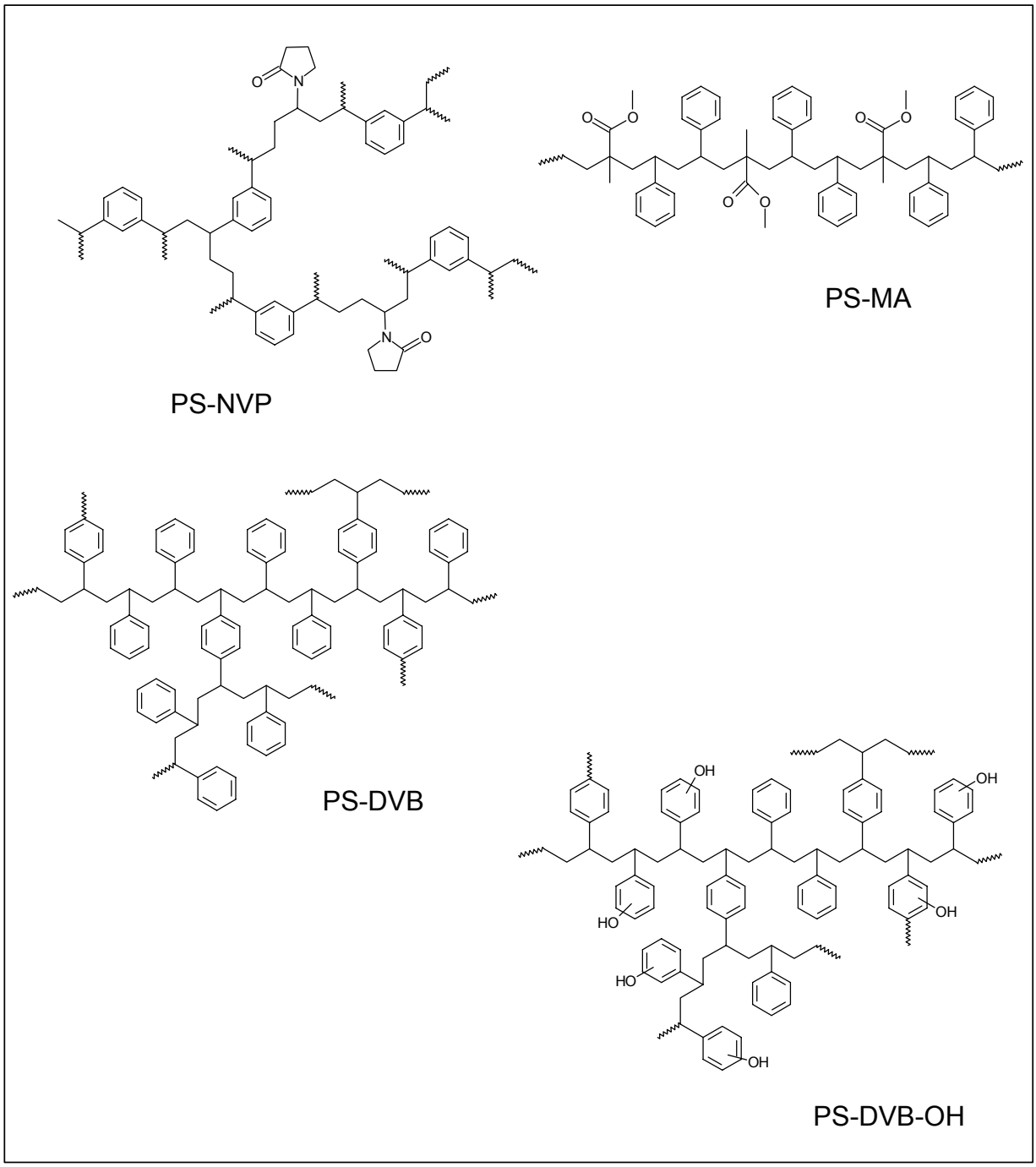

Figure 6: Chemical structures of selected polymeric sorbents (PS: polystyrene, DVB: divinylbenzene, OH: hydroxy, MA: methyl methacrylate, NVP: *N*-vinylpyrrolidone)

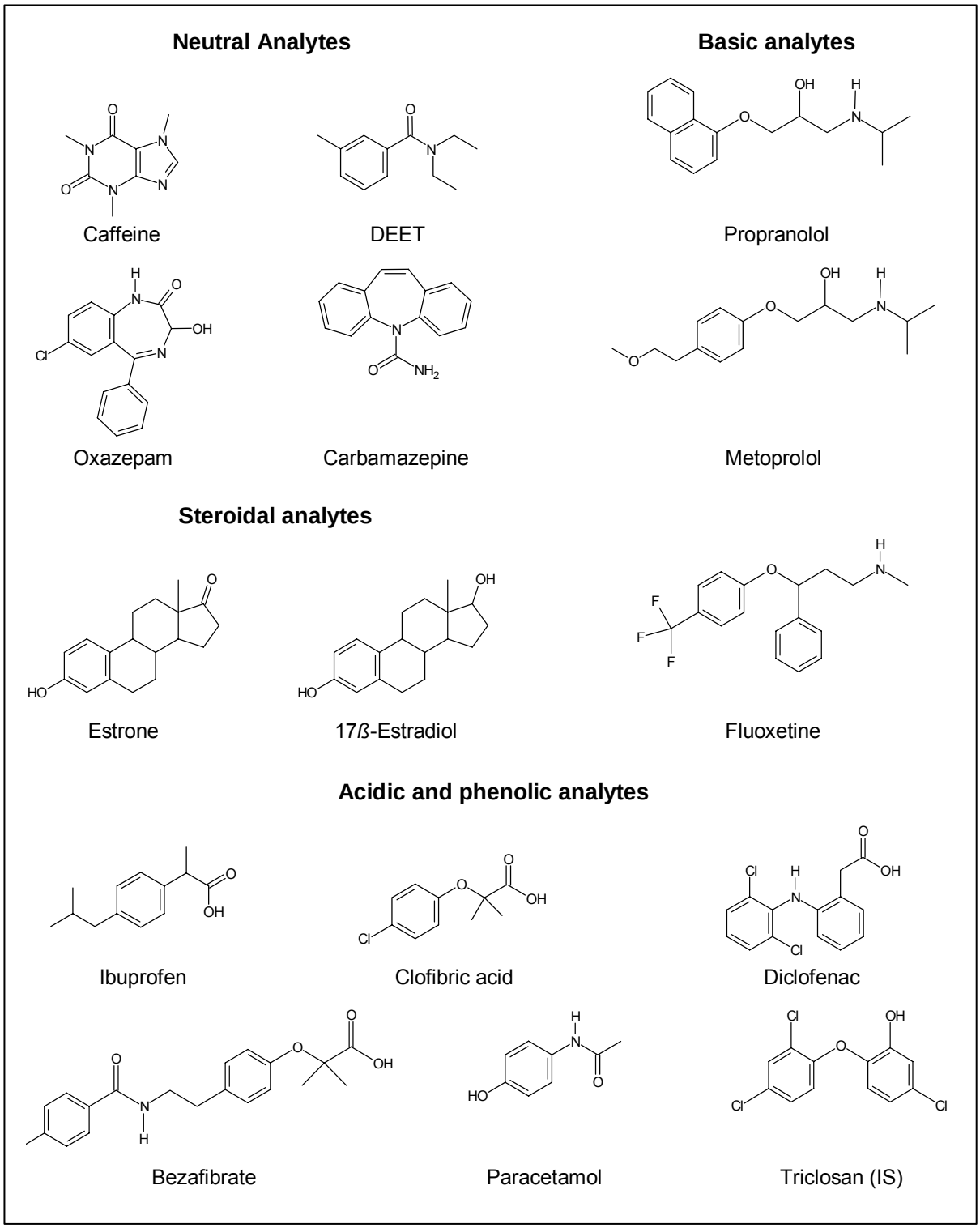

Figure 7: Chemical structures of the compounds included in the sorbent comparison study (IS: internal standard)

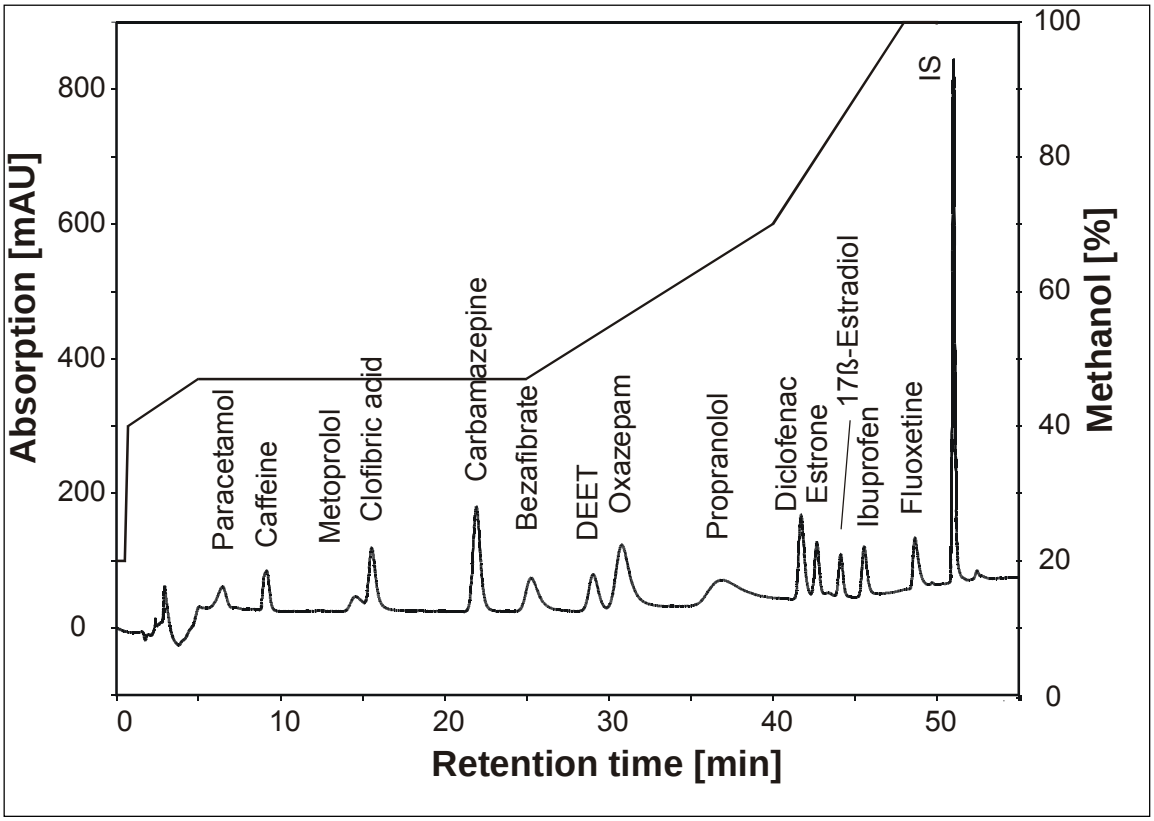

Figure 8: HPLC-chromatogram of a standard solution (c = 10 µg/mL) of the test compounds (stationary phase: C_{18}-silica, MeOH/H_2O gradient, UV-detection at 230 nm)

The results are summarised in Table 2. Carbamazepine and DEET were almost quantitatively (90 - 100 %) recovered on all investigated sorbents. The same holds for caffeine, with one exception (nexus: 14 %). In this case, and in the case of paracetamol, which showed low to acceptable recoveries on all sorbents (0 - 72 %), it can be assumed that their pronounced water solubility limits their retention. This corresponds to the early elution of paracetamol and caffeine in the HPLC-chromatogram (Figure 8). Although retention on the C_{18}-material of the HPLC column cannot be compared directly to that on the polymeric SPE-sorbents, this behaviour gives at least a hint at the strength of the analyte's interaction with organic material. Interestingly, retention was lowest on the two SPE polymers containing hydrophilic monomers. The highest recovery for paracetamol (72 %) was obtained on Chromabond HR-P. It should be noted though that the two Chromabond cartridge types contained 500 mg of sorbent versus only 200 mg in the other test cartridges. Recoveries of the benzodiazepine oxazepam were ranging from 60 to 100 % (except for HR-P: 27 %), being highest on the two hydrophilic/lipophilic co-polymers. The three basic analytes, carrying all a secondary amino-function, were recovered at 70 % or higher (exception: Isolute Env+ and Chromabond HR-P), in the case of the hydrophilic/lipophilic co-polymers at 90 - 100 %. For the two estrogens included in the present study good recoveries were obtained. Except for Chromabond HR-P, they were higher than 75 %. On the PS-DVB

sorbents recoveries for estrone were generally lower than those for 17*ß*-estradiol, whereas on the two hydrophilic/lipophilic co-polymers, both were almost quantitatively recovered. The largest differences in behaviour were observed for the acidic analytes. Best results were obtained with Oasis HLB: quantitative recoveries for bezafibrate, ibuprofen and diclofenac and still 83 % for clofibric acid, the compound with the lowest log Kow (-1.3) under the given conditions. It was followed in performance by the second hydrophilic/lipophilic co-polymer, abselut Nexus, with recoveries of 70 - 90 % for most acids but a clearly lower value for clofibric acid (23 %). This is in accordance with the weak performance of this sorbent for the hydrophilic compounds paracetamol and caffeine. Among the PS-DVB sorbents, Bakerbond SDB-1, Lichrolut EN, and Isolute ENV+ showed a comparable behaviour, with recoveries in the range of 40 to 60 %, also for clofibric acid (except for Lichrolut EN: 29 %). Exceptionally low values were observed for the two Chromabond sorbents HR-P and EASY for the acidic compounds. They were between 1 and 27 % and had extraordinarily high relative standard deviations of up to 110 % in the case of EASY. This sorbent is described by the manufacturer as a ”polarly modified PS-DVB carrying a weak ion exchanger”. It ought to ”be easily water wettable due to the bifunctional modification, thus eliminating the need for column conditioning”, placing it in line with hydrophilic/lipophilic co-polymers such as PS-MA or PS-DVB-NVP. Upon request, the manufacturer provided the information that the sorbent material does not carry a polar modification as stated in the catalogue but a ”weak *anion* exchanger” [108]. With regard to this fact, pure methanol (without pH-adjustment) might not be the most suitable elution solvent for acidic compounds.

The relative standard deviations (RSDs) were below 10 % in most cases (Table 2). RSDs averaged over all test compounds were 2 % (Nexus), 3 % (HLB), 4 % (SDB-1, EN) and 5 % (HR-P). Clearly higher values were determined for Env+ (9 %) and EASY (20 %), the latter mainly due to the problems with the acidic analytes mentioned above.

Typically, manufacturers recommend elution volumes of around 5 mL methanol for 200 mg cartridges. In order to assure a complete elution of all analytes, also those that might be better eluted with other solvents, all cartridges were eluted with 30 mL of methanol. Additionally, for each sorbent type, one cartridge was eluted with further 40 mL of methanol to check whether a certain amount of analytes remained on the cartridge. In several cases this yielded additional recoveries. In Table 3 these additional recoveries are compared to the mean recoveries obtained with 30 mL. No additional recoveries were observed for the two hydrophilic/lipophilic co-polymers Nexus and HLB. Analytes were well retained on these sorbents and also easily desorbed. For the remaining PS-DVB type sorbents, only the polar neutral compounds were completely desorbed with the first 30 mL of solvent. Additional recovery of the comparatively lipophilic estrogens might be related to incomplete removal due to the high polarity of methanol.

Table 2: Properties of the tested SPE-cartridges, recovery rates (RR) and relative standard deviations (RSD) of 3 replicate extractions. Conditions: 1 L tap water samples (pH 7.8) spiked at a concentration of 2 - 5 µg/L. log Kow at pH 8: calculated values [109]. PS: polystyrene, DVB: divinylbenzene, EVB: ethylvinylbenzene, OH: hydroxy, AX: weak anion exchanger, MA: methyl methacrylate, NVP: *N*-vinylpyrrolidone

Sorbent		**Bakerbond SDB-1**		**Lichrolut EN**		**Isolute Env+**		**Chromabond HR-P**		**Chromabond EASY**		**abselut Nexus**		**Oasis HLB**	
Polymer-type		PS-DVB		PS-DVB-EVB		PS-DVB-OH		PS-DVB		PS-DVB-AX		PS-MA		PS-DVB-NVP	
Surface area		1060 m^2/g		1200 m^2/g		1000 m^2/g		1200 m^2/g		650 - 700 m^2/g		500 - 650 m^2/g		810 m^2/g	
Particle size		40 - 120 µm		40 - 120 µm		90 µm		50 - 100 µm		40 / 80 µm		65 - 80 µm		30 µm	
Amount		200 mg		200 mg		200 mg		500 mg		500 mg		200 mg		200 mg	
Recoveries [%]	**lg Kow**	**RR[a]**	**RSD[b]**	**RR**	**RSD**	**RR**	**RSD**	**RR**	**RSD**	**RR**	**RSD**	**RR**	**RSD**	**RR**	**RSD**
Paracetamol	0.3	60	4	37	4	39	22	72	4	50	25	0	0	14	2
Caffeine	-0.1	99	4	91	2	99	9	94	3	99	3	25	2	97	3
DEET	2.0	96	3	100	3	94	6	91	2	100	3	91	3	100	3
Carbamazepine	2.7	100	3	97	2	104	3	95	5	99	3	95	1	101	2
Oxazepam	2.3	65	3	74	2	81	4	27	5	80	4	91	4	98	1
Fluoxetine	-	69	4	80	5	86	7	53	5	86	4	94	4	88	2
Metoprolol	0.6	81	6	79	13	50	14	52	4	79	3	97	2	96	7
Propranolol	1.9	68	4	65	8	36	22	50	6	70	1	90	2	98	4
Estrone	3.7	92	2	75	0	80	3	54	5	71	3	92	1	96	3
17ß-Estradiol	4.1	96	2	89	3	101	5	85	5	95	0	95	1	98	2
Clofibric acid	-1.3	54	3	29	1	48	10	25	4	27	3	23	3	83	6
Bezafibrate	-0.4	55	9	55	5	43	9	23	5	18	110	87	2	95	2
Ibuprofen	0.3	46	2	61	4	55	9	6	10	10	25	68	1	98	1
Diclofenac	-0.4	42	6	62	3	38	7	19	4	1	92	90	3	102	2

a: n = 1; b: RSD determined from an earlier series (elution volume 70 mL, n = 3)

Table 3: Comparison of mean recovery rates (RR) [%] obtained by elution with 30 mL (n = 3) and additional recoveries (AR) by elution with further 40 mL (n = 1) of methanol. A fictitious overall recovery is given as the sum (Σ).

Sorbent	Bakerbond SDB-1			Lichrolut EN			Isolute Env+			Chromabond HR-P			Chromabond EASY			abselut Nexus		Oasis HLB	
	RR	AR	Σ	RR	AR	Σ	RR	AR	Σ	RR	AR	Σ	RR	AR	Σ	RR	AR	RR	AR
Paracetamol	60		60	37		37	39		39	72		72	50		50	0		14	
Caffeine	99		99	91		91	99		99	94		94	99		99	25		97	
DEET	96		96	100		100	94		94	91		91	100		100	91		100	
Carbamazepine	100		100	97		97	104		104	95		95	99		99	95		101	
Oxazepam	65	**4**	68	74	**7**	81	81		81	27	**16**	43	80	**1**	81	91	no additional recovery	98	no additional recovery
Fluoxetine	69	**7**	76	80	**6**	86	86	**9**	95	53		53	86	**15**	101	94		88	
Metoprolol	81	**8**	89	79	**6**	85	50	**17**	67	52	**16**	68	79	**11**	90	97		96	
Propranolol	68	**14**	82	65	**17**	82	36	**22**	58	50	**10**	60	70	**16**	86	90		98	
Estrone	92	**2**	94	75	**11**	86	80	**9**	89	54	**11**	65	71	**10**	81	92		96	
17ß-Estradiol	96	**2**	98	89	**9**	95	101		101	85	**4**	89	95	**4**	99	95		98	
Clofibric acid	54	**6**	61	29	**9**	38	48	**10**	58	25	**11**	36	27	**24**	51	23		83	
Bezafibrate	55	**25**	81	55	**22**	77	43	**14**	57	23	**14**	37	18	**10**	28	87		95	
Ibuprofen	46	**26**	72	61	**11**	72	55	**17**	72	6	**9**	15	10	**21**	31	68		98	
Diclofenac	42	**31**	72	62	**11**	73	38	**14**	52	19	**14**	33	1		1	90		102	

Possible explanations for the hindered desorption of the basic and acidic analytes are assumed to be: (i) specific π-π interactions between the aromatic ring systems of these substances and the styrene-sorbent, although this should affect the respective neutral analytes similarly, (ii) the existence of non-specified modifications of the PS-DVB matrix. Thurman [110] states that some manufacturers equip their ”PS-DVB”-sorbents with a light sulfonation to improve their wetting characteristics. This would explain the difficult desorption of the amino-compounds in case of ion-exchange interactions with protonated analytes. (iii) under the given extraction conditions, the acidic compounds are partly dissociated and retained in this form. Pure methanol might not have a sufficiently high elution strength for the anions. However, this effect should be most pronounced for clofibric acid (lowest pKa). In contrast, clofibric acid had the lowest additional recoveries. In conclusion, these findings highlight the necessity of an elaborated elution protocol (e.g., pH adjustment of solvent) when extracting acidic or basic analytes with PS-DVB sorbents.

Chromabond EASY, abselut Nexus and Oasis HLB are advertised as not requiring a solvent conditioning step. This was checked for the first two sorbents by running an extraction with a non-conditioned cartridge in parallel. For EASY, most recoveries were the same as with conditioning, except for the acidic compounds for which recoveries went down to 0 %. Co-elution of sorbent matrix prevented the proper quantification of clofibric acid and metoprolol and led to erroneously high recoveries for propranolol and fluoxetine (163 %). In the case of Nexus, all recoveries except for that of estrone were reduced, some even drastically (e.g., caffeine, metoprolol, bezafibrate, diclofenac, ibuprofen). Quantification of clofibric acid and also fluoxetin was severely affected by co-elutions. Co-elutions clearly have to be attributed to the lack of a cartridge cleaning that is an important secondary effect of a solvent conditioning. Another important aspect is the significantly higher flow resistance caused by the hydrophobic polyethylene frits when they were not conditioned prior to extraction.

In conclusion, the two hydrophilic/lipophilic co-polymers showed the best overall performance under the test conditions. The only drawback of Oasis HLB was the low recovery of paracetamol (14 %) while for almost all other compounds quantitative recoveries were obtained. Abselut Nexus revealed unacceptable low recoveries for paracetamol, caffeine and bezafibrate and a slight weakness for the other acids compared to HLB, but still within an acceptable range (70 - 90 %). Chromabond EASY yielded good results for most base/neutral compounds but insufficient ones for the acids. Except for paracetamol the results for Chromabond HR-P were below average. Bakerbond SDB-1, Lichrolut EN and Isolute Env+ behaved rather similar to each other, with exceptions for single compounds, and recoveries between 70 and 100 % for the base/neutral compounds and 40 to 60 % for the acidic ones, often going up to 70 % by additional elution.

2.2 Sequential elution

According to the differences in the chemical nature of the analytes, their determination often requires a separation into related groups. Especially in GC, various analytes are only accessible after derivatisation. Optimum sensitivity for different chemical groups, e.g., carboxylic acids, amines or steroids is achieved by specific derivatisation reactions. In LC-MS as well, separation and ionisation conditions can be specifically optimised when similar analytes are separated into groups. A primary separation can be achieved by the sequential elution of loaded SPE sorbents with solvents of different polarity as has been shown for graphitised carbon black sorbents [103-105]. In this work, this concept was explored for the polymeric sorbents utilised herein. In the case of the large volume marine samples, a two step elution was performed, using (i) ethyl acetate and *n*-hexane/ethyl acetate 4:1 v/v to remove compounds of low to medium/high polarity and (ii) methanol to elute substances of very high polarity (e.g., carboxylic acids). This concept was further refined for the SPE of pharmaceuticals, then consisting of three steps: (i) *n*-hexane, removing lipophilic matrix components and lipophilic analytes, which were not within the focus of this work, (ii) ethyl acetate, eluting analytes of medium to high polarity (e.g., N-heterocycles, amines, steroids), and (iii) methanol, eluting acidic and phenolic target analytes.

2.3 Clean-up, derivatisation

In order to perform an efficient screening with the aim of an unambiguous identification of a large number of substances, it is desirable to remove interfering matrix components (e.g., humic and fulvic acids, chlorophyll) from the sample extract and to separate the contained analytes in sub-fractions, thus reducing co-elutions in gas chromatography. This was achieved by an eight step fractionation on mini-silica columns previously established in the research group (SOP 2) [111].

Derivatisation of the acidic and phenolic compounds contained in the methanolic eluate of the SPE (both marine large volume method and PPCP method) was performed with methyl chloromethanoate, according to Butz and Stan [112] and Kuhlmann [113] (SOP 3). The reaction with carboxylic acids yields the mixed anhydrides which are then quantitatively decarboxylated to the respective methyl esters under the given reaction conditions. Phenols are transformed to the corresponding carbonic acid diester derivatives. The reaction schemes are exemplarily shown for ibuprofen and triclosan in Figure 9. The derivatisation procedure (re-extraction of the derivatised analytes with *n*-hexane from the aqueous reaction mixture) additionally served as an efficient clean-up, enabling excellent signal-to-noise ratios and thus very low detection limits.

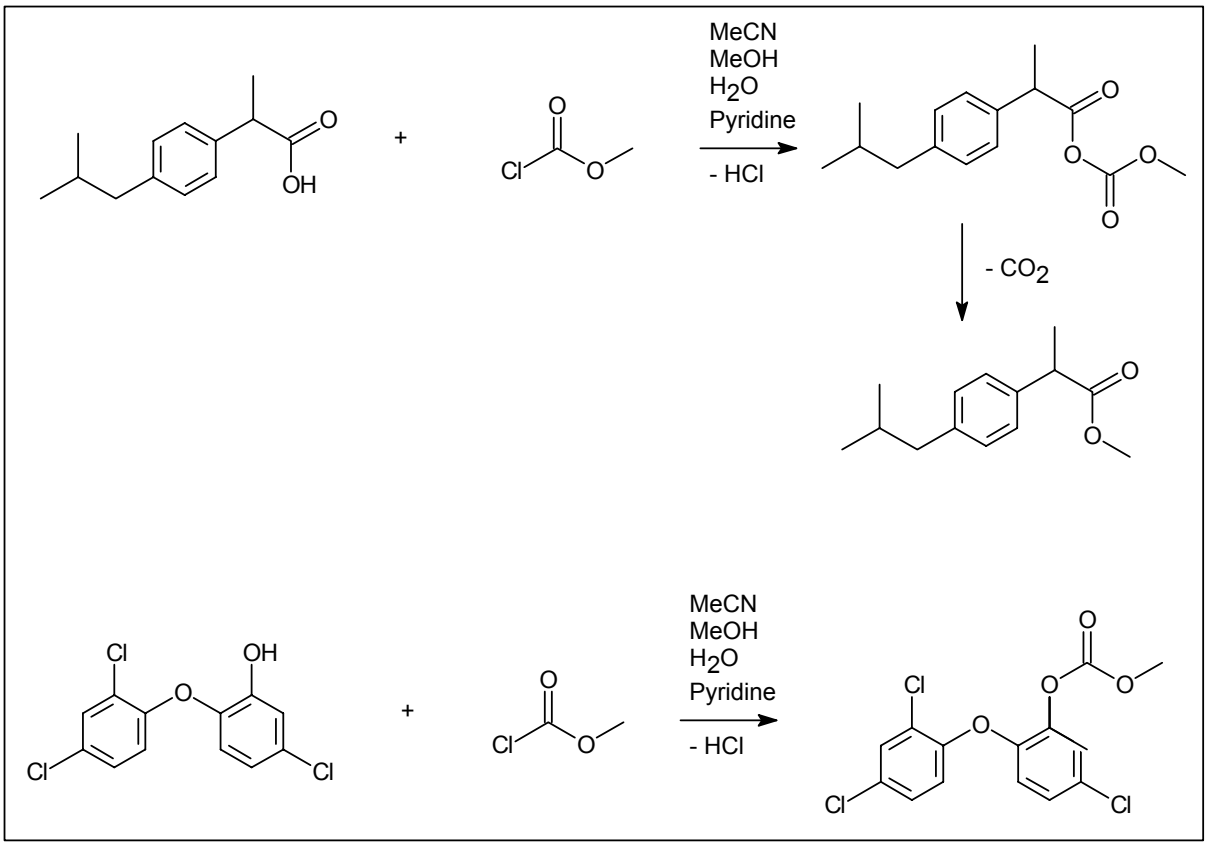

Figure 9: Derivatisation of acidic and phenolic analytes with methyl chloromethanoate: ibuprofen (top) and triclosan (bottom)

2.4 Instrumental analysis

For the identification of unknown compounds from environmental samples mass spectrometry is a suitable approach since it provides considerable structural information already at low absolute analyte amounts. Electron impact ionisation (EI) mass spectrometry (usually coupled to gas chromatography in environmental analytical chemistry) produces spectra of substance-specific fragmentation patterns, while other ionisation methods such as chemical ionisation (CI) or electrospray ionisation (ESI) usually result in quasi-molecular or adduct ion peaks. The nature of these ions depends on the reactand gas (in GC-MS) or the eluent/ buffer composition and ionisation conditions (in LC-MS), respectively, but these ionisation methods usually do not provide characteristic fingerprint spectra. Furthermore, the dependence of the spectra on the type of ion source and its specific parameters does not allow the compilation of instrument independent searchable spectra libraries as available for GC-MS (EI).

Within this work, an ion trap MS was used for the identification of organic compounds in North Sea water extracts. Compared to quadrupole MS, ion trap instruments provide an up to ten-fold higher sensitivity in the full scan mode. Additionally, confirmatory high resolution MS measurements were carried out on a sectorfield instrument for selected substances. Quantification of selected target analytes in the large volume seawater samples was also

carried out on the ion trap MS, thus providing the possibility for the identification of unknown compounds in the same run.

For the quantification of acidic, neutral and basic pharmaceuticals as intended in the second part of this work, gas chromatography often requires derivatisation of the analytes prior to injection, while thermolabile and non-volatile analytes are not amenable to GC at all. Alternatively, HPLC-methods can be applied to overcome these limitations. The coupling of HPLC-separation to MS or MS/MS detection techniques (e.g., selected ion recording - SIR, multi reaction monitoring - MRM) provides highly sensitive and specific possibilities for the quantification of these analytes. In order to achieve an optimum performance for the different targeted groups of analytes (carboxylic acids, neutral N-heterocycles, amines etc.), GC-MS and LC-MS techniques were compared to identify the most suited method in terms of chromatographic (peak shape) and mass spectrometric (sensitivity) performance and to explore the potential for a multiresidue method. Best results in the aforementioned sense were obtained for many of the nitrogen-containing analytes (amines, amides, N-heterocycles), especially the ß-blocking 2-hydroxyalkaneamine-derivatives by LC-MS/MS in the positive electrospray mode (ESI+), while for the acidic compounds GC-MS after derivatisation yielded better separation and lower detection limits. Based upon these results, GC-MS was chosen for the determination of the acidic, phenolic and selected neutral target analytes (e.g., caffeine and DEET). For the determination of ß-blockers and SSRIs LC-MS was preferred and further method development was carried out within a co-operating parallel work [114].

Compound	**LC-MS**		**GC-MS**
	Ionisation	LOQ [ng/L]	LOQ [ng/L]
Propranolol	ESI+	0.7	na
Metoprolol	ESI+	0.7	na
Carbamazepine	ESI+	9.7	td
Propyphenazone	ESI+	0.5	1.6
Caffeine	ESI+	10.7	1.7
Paracetamol	ESI+	5.5	na
DEET	ESI+	0.7	0.4
Bezafibrate	ESI+	4.5	na
Bezafibrate	ESI-	2.5	na
Ibuprofen	ESI-	8.2	0.02
Diclofenac	ESI-	9.7	0.05
Clofibric acid	ESI-	3.4	0.08
Acetylsalicylic acid	ESI-	2.9	na

Table 4: Estimated limits of quantification (LOQs, s/n = 10) for the extraction of 1 L water samples in LC-MS (SIR) and GC-MS (acids after derivatisation); na: not analysed, td: thermal decomposition

2.5 Method validation

Analytical methods for the quantitative determination of defined parameters (in this work concentrations of chemical compounds) are developed with the intention to measure these parameters with sufficient exactness under the expected conditions. Method validation is the process of assuring and documenting that an analytical method or procedure is fit for the intended purpose. “Validation of a method establishes by laboratory studies that the performance characteristics of a method meet the requirements for the intended analytical applications. Performance characteristics are expressed in terms of analytical performance characteristics, i.e.: Precision, Accuracy, Limit of detection, Limit of quantification, Selectivity, Specificity, Range, Linearity, Ruggedness.” [115]

Although there have been several approaches by various authors and institutions, e.g., the International Union of Pure and Applied Chemistry [116], EURACHEM [117] and the Commission of the European Communities [118], to establish harmonised guidelines for the validation of analytical methods, there are still deviations in the definitions of analytical terms. Nevertheless, it is commonly agreed upon that the performance of an analytical method has to be confirmed by objective evidence, i.e., information which can be proved true, based on facts obtained through observation, measurement, test or other means [115]. While the requirements to University research laboratories may not be as strict as for commercial or governmental monitoring laboratories, the performance of a new analytical method should be tested and the results documented to characterise this method. Basic components of the method development and validation process should be (for definitions of the respective analytical terms see [117]):

- calibration of the instrument
- determination of the working range
- calibration over the entire method
- determination of instrument precision
- determination of repeatability
- determination of recovery rates
- determination of detection/quantification limits
- evaluation of ruggedness

Desirable, but eventually not or not fully possible due to the reasons discussed below, are the following components:

- determination of reproducibility
- determination of accuracy

The determination of the reproducibility requires the measurement of the target analyte in distinct subsamples of the same test material under changing conditions, e.g., observer, measuring instrument, location, conditions of use, time [117]. For water samples as investigated in this work the problem arises that the possibility of analysing subsamples of

one sample at different times is limited by the stability of the analytes in water. Therefore, reference samples have to be prepared under comparable conditions. Furthermore, variations in instruments and operators were limited by the available resources. For the determination of the accuracy it is mandatory to compare the value obtained for a test material by the method under validation with the certified or conventional true value of this material. This may be achieved by the analysis of certified reference material or the participation in proficiency testings. Both possibilities were not available for the analytes/ matrix under investigation. Resultantly, for the methods developed and validated within this work the following performance characteristics were determined and are discussed in the respective chapters below: working range/linearity (from the calibration over the entire method), instrument precision, recovery rates, repeatability (expressed as relative standard deviation of replicate recovery experiments), reproducibility (over the time of the current investigations, mainly covering variations in instrument performance, expressed as coefficients of variation), limits of quantification, ruggedness.

2.5.1 Quantification of pesticides, industrial chemicals and pharmaceuticals from marine large volume samples

The extraction method that was originally developed for the qualitative determination of a wide array of non-target analytes was complemented by the methanolic elution and derivatisation of acidic compounds (Figure 10) and was then validated for the quantification of a number of selected target compounds. Studies of the recovery rates, repeatability and linearity were carried out for these substances from spiked 20 L samples of artificial seawater. The obtained recoveries are summarised in Table 5. The outstanding feature of the present method is its ability to extract neutral and acidic analytes from water samples at basic pH simultaneously. This is especially valuable for seawater analyses, because due to its buffering capacity the neutralisation or acidification to a pH of 2, as often proposed for the extraction of acidic analytes, would require the addition of substantial amounts of hydrochloric or sulfuric acid. Therefore, the present method simplifies sample handling, allows on-line extraction directly from the water body (i.e., pumping the sample directly from the sea through the filtration/extraction unit) and avoids the risk of introducing contamination from the added acids. The obtained recovery rates for most neutral analytes were in the range of 70 to 80 %. Lower recoveries (35 - 44 %) were determined for the more volatile neutral compounds (dichloropyridines, nitrobenzene, 3-chloro-4-fluoronitrobenzene, dichlobenil). This is caused by losses during the eluent evaporation and subsequent solvent change to *iso*-octane, a procedure that was not optimised for the determination of volatile compounds. The recoveries were around 40 % for the acidic compounds (except for ketoprofen: 58 %). These low values could partly be attributed to incomplete extraction of the analytes from the water phase in which they are present at least partly in their ionic forms under the given

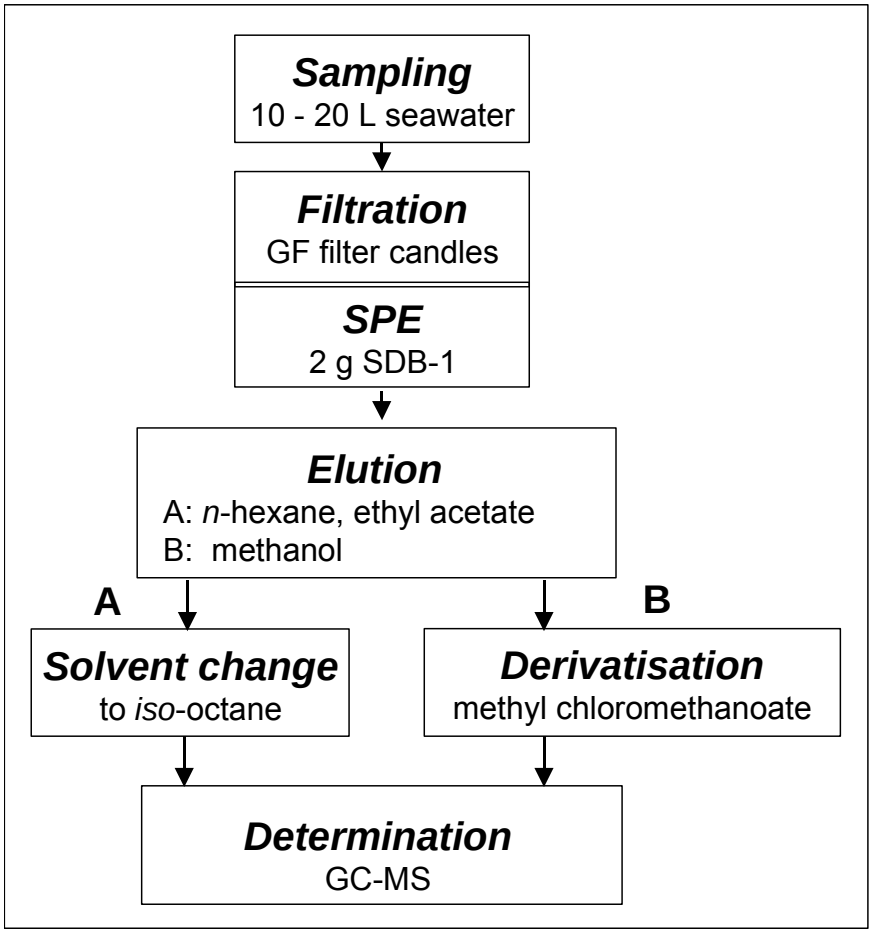

Figure 10: Modified method for the quantitative determination of neutral and acidic analytes from 20 L seawater samples

conditions. Additionally, the elution step, which originally was designed for polar neutral analytes, may also give rise to losses. Elution with relatively large volumes of the medium polar solvent ethyl acetate partly removes the acidic analytes from the sorbent. This amount would remain in the neutral fraction and thus escape detection. Furthermore, recent experiments indicated a slow elution of some analytes by pure methanol. The modification of the elution protocol, e.g., direct elution with a mixture of methanol and acetic acid, would most probably improve the recovery of the acidic target substances. The relatively high standard deviations of the recovery rates can be explained by the mechanical assembly of the extraction unit. Occasionally, irregularities in the packing and the sorbent bed height were observed after extraction, possibly facilitating the breakthrough of analytes. Furthermore, repeated evaporation steps did not only lower the recovery of the more volatile analytes, but also raised the standard deviation in their determination. A strength of the method are the remarkably low limits of quantification under full scan detection, owing to the high enrichment factor (200 000) and the high sensitivity of the ion trap mass spectrometer. Limits of quantification (LOQs), signal to noise (s/n) ratios = 9, were in the range of 0.02 to 0.2 ng/L for the neutral analytes and even lower for most derivatised acidic compounds (0.002 - 0.008 ng/L except for ketoprofen: 0.03 ng/L). The lower LOQs for the acidic substances are a result

of the effective clean-up by the derivatisation procedure and of the lower polarity of the derivatised acids as compared to the investigated polar neutral analytes, resulting in better peak shapes (less tailing) and thus improved s/n ratios. It should be noted though that some LOQs were below the range in which linearity was investigated (0.05 - 50 ng/L). Environmental concentrations below 0.05 ng/L presented herein therefore have to be considered as semi-quantitative. The LOQs were determined by GC-MS measurement of spiked procedural blanks. This provides some matrix, but it cannot account for natural matrices in all cases. For the acidic compounds matrix interferences in real samples were largely eliminated by the derivatisation. In contrast, propyphenazone could not be identified unambiguously in marine samples although it can be expected to be present at least in the plume of the river Elbe. In the non-target screening of sample DB30-3 (off the Eiderstedt peninsula, Figure 13) this compound was identified. The same extraction method was applied, but the extract was subject to a silica clean-up, yielding proper identification and an estimated concentration of 0.2 ng/L (chapter 3.3). Thus, an appropriate clean-up of the neutral fraction would substantially improve the detectability of certain compounds and could further lower their LOQs.

Substance	**Recovery [%]**	**SD [%]**	**LOQ [ng/L]**	**r^2**
Pesticides				
Dichlobenil	44	6	0.03	0.9998
Desethylatrazine	90	12	0.11	0.9999
Terbuthylazine	72	11	0.03	0.9998
Pirimicarb	69	13	0.05	0.9999
Parathion-methyl	80	16	0.02	1
Metolachlor	73	13	0.03	0.9999
Industrial chemicals				
3,5-Dichloropyridine	35	9	0.11	1 *
2,5-Dichloropyridine	41	12	0.12	0.9999 *
2,3-Dichloropyridine	42	9	0.09	1 *
2,6-Dichloropyridine	44	10	0.07	1 *
Nitrobenzene	44	13	0.18	0.9999 *
3-Chloro-4-fluoronitrobenzene	44	10	0.04	1 *
PPCPs				
Caffeine	72	12	0.11	0.9996
DEET	68	12	0.03	0.9999
Propyphenazone	71	12	0.1	0.9999
Clofibric acid	40	12	0.008	0.9998
Diclofenac	39	10	0.006	1
Ibuprofen	42	12	0.002	1
Ketoprofen	58	18	0.03	0.9998

Table 5: Recovery rates (n = 4, spiking level 5 ng/L), repeatability as standard deviations (SD), limits of quantification (LOQ, s/n = 9) and linear regression coefficients r^2 (4 point calibration, concentration range 0.05 - 50 ng/L; * 3 points, 0.5 - 50 ng/L) as determined for the extraction from spiked 20 L samples of artificial seawater

2.5.2 Quantification of PPCPs from 1 L samples

For the reasons mentioned above (chapter 2.4), GC-MS was chosen for the determination of the acidic, phenolic and selected neutral target analytes (Figure 11). After identification of Oasis HLB as the most suitable sorbent for the analytes of interest, a method for the extraction, separation and quantification of these compounds from water samples was developed and validated, aiming at quantification limits below 1 ng/L. The resulting method is outlined in Figure 12.

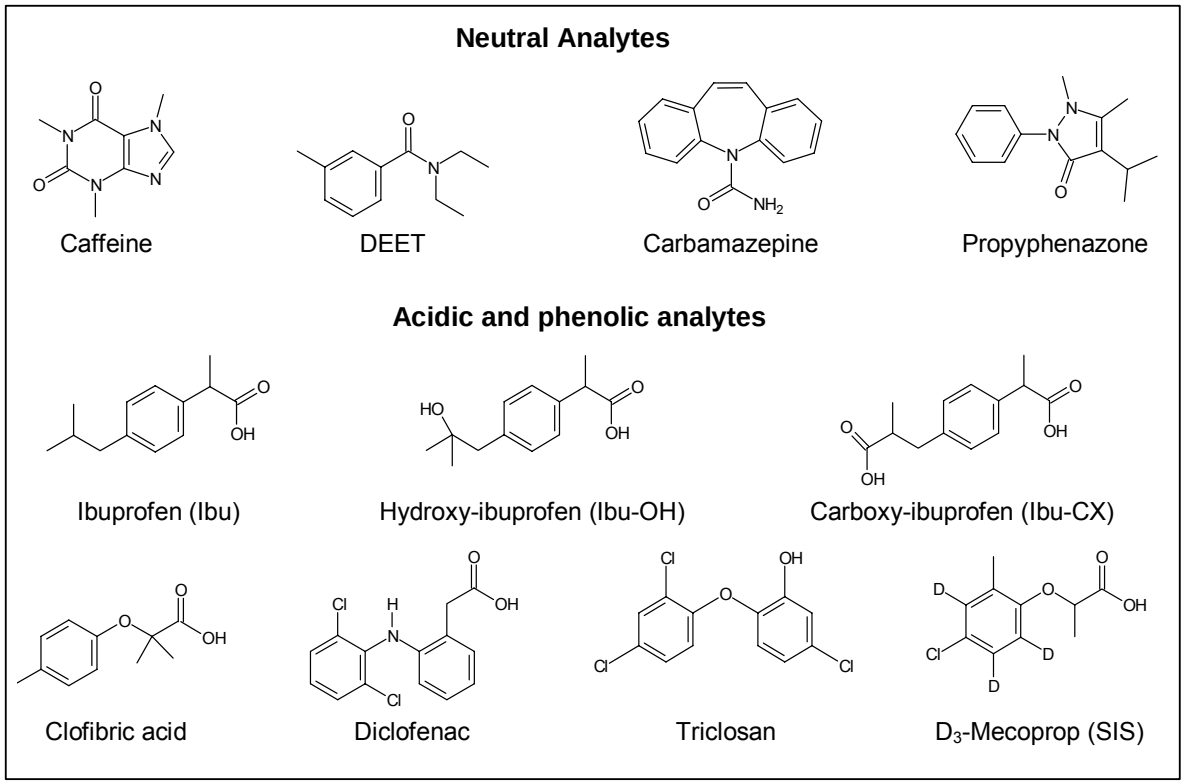

Figure 11: Chemical structures of the neutral, acidic and phenolic target analytes (SIS: surrogate internal standard)

In contrast to the sorbent comparison experiments, the pH of the sample was adjusted to 7 prior to extraction. This additional step became necessary by the inclusion of the ibuprofen metabolites into the set of target analytes. The recovery of carboxy-ibuprofen (ibu-CX) was strongly affected by pH, at values above 7 recoveries were minimal (0.04 % at pH 8). At pH 7 they were around 30 to 40 %, increasing further with decreasing pH to 74 % at pH 2. While this effect is expected for acidic compounds in general, it is interesting to note that in this case it was only observed for ibu-CX, the most hydrophilic compound (log Kow at pH 7: -2.8 [109]). Obviously, the concept of a simultaneous extraction of acidic and base/ neutral analytes at neutral pH reaches its limitations here. For the other acids there was hardly any pH-effect on recoveries within the investigated range. As expected, the neutral compounds were not influenced by variations in pH.

The elution of the loaded and dried cartridges was carried out sequentially with different solvents to divide the target analytes into separate groups. The initial elution with 5 mL of *n*-hexane removed lipophilic matrix components but none of the target analytes from the sorbent and thus served as a clean-up step. The following elution with 5 mL of ethyl acetate removed the neutral/basic analytes such as caffeine and DEET while the final elution with 14 mL of methanol yielded the acidic analytes, e.g., ibuprofen and clofibric acid. Most acidic analytes were eluted within the first 5 mL of methanol, only diclofenac required an additional 9 mL for maximum recovery.

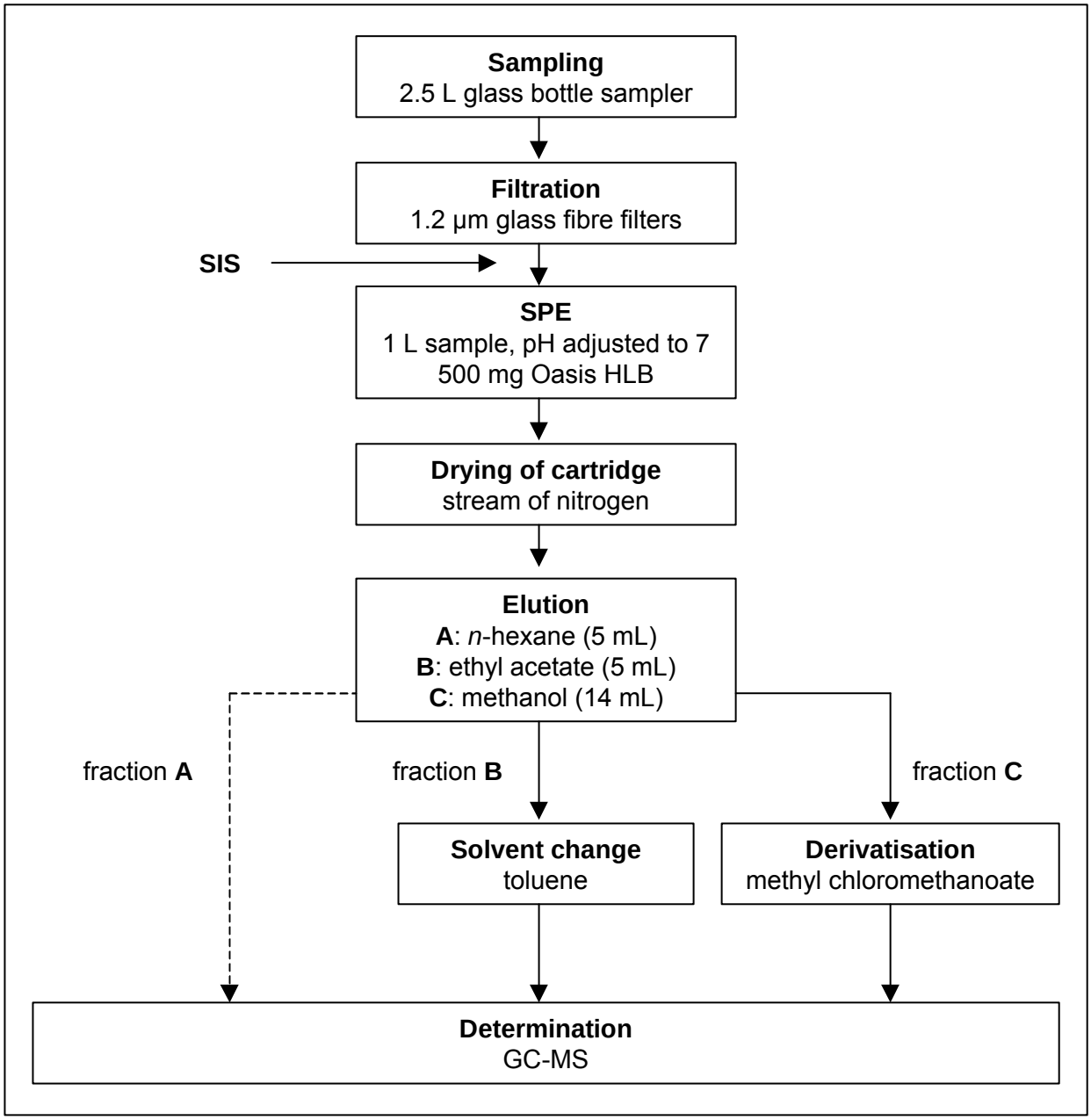

Figure 12: Method for the determination of basic, neutral, and acidic compounds from water samples (SIS: surrogate internal standard)

Recovery rates for the present method were determined at an environmentally relevant concentration level of 20 ng/L and were in the range of 70 to 100 % (Table 6). For triclosan the recovery was slightly lower which is owed to the fact that a small proportion of this analyte is already eluted in the ethyl acetate fraction. Ibu-CX had an exceptionally low recovery due to the reasons discussed above. RSDs were generally low (1 - 6 %) with two exceptions. For ibu-CX the tight control of the pH is crucial for the extraction accuracy. Already small variations alter recovery in a way that affects the RSD. In the case of clofibric acid the high variations originated rather from the derivatisation than from the extraction. In comparison to the results from the sorbent testing experiments, recoveries were lower for some compounds, e.g., ibuprofen (74 % vs. 98 %). The prepacked cartridges used in the sorbent comparison contained 200 mg HLB of a particle size of 30 μm, while the bulk material used for laboratory packing of glass cartridges is exclusively available in a 60 μm quality. Using the same amount of sorbent (200 mg), recoveries were lower for the 60 μm material. So either the two sorbents do not only differ in particle size (and thus in the number of theoretical plates), but also in their extraction properties or the observed effect is caused by charge-to-charge variations. In this context it is interesting to note that for the Oasis HLB sorbent the recoveries obtained under similar conditions for some of the investigated analytes vary considerably in different publications. Farré et al. [119] for example used 300 mg of Oasis HLB (particle size not specified) for 1 L water samples at pH = 7 and flow rates of 10 mL/min and recovered ibuprofen at only 38 %.

Linearity of the method was given in the concentration range expected in environmental samples (0.2 - 200 ng/L). Linear regression coefficients r^2 (four point calibration) were 0.9992 or above. Repeatability of the overall method expressed as the relative standard deviation of parallel extractions (n = 3) was ranging from 1 to 6 % except for clofibric acid (21 %) due to losses in the derivatisation procedure and ibu-CX (12 %) because of the susceptibility of its recovery to small differences in the sample pH. Repeatability expressed as coefficients of variation (n = 6) was in the range of 4 to 14 % except for clofibric acid (19 %) and ibu-CX (34 %) due to the reasons mentioned above. A ruggedness testing was carried out for reasonable variations in the following parameters: pH of the sample, extraction flow rates, extracted volume, cartridge drying times, elution volumes, derivatisation conditions and sample matrix (tap-, river-, lake-water). The method was robust against most variations, while a tight control of the conditions was crucial for the sample pH (only for ibu-CX), the elution volumes, and the derivatisation (only for clofibric acid). Limits of quantification (LOQs; signal to noise ratio of 9) as determined from standard runs and calculated for 1 L-water samples (corrected for recovery rates) were in the range of 0.07 to 0.7 ng/L (Table 6).

Compound	RR [%]	RSD [%]	r^2	CV [%]	LOQ [ng/L]	Ions, m/Z [amu]
Ibuprofen	74	5	0.9999	10	0.07	<u>161</u>, 220 (-Me)
Ibu-OH	92	2	0.9999	10	0.42	<u>119</u>, 178 (-Me)
Ibu-CX	30	12	0.9992	34	0.69	<u>145</u>, 205 (di-Me)
Clofibric acid	108	21	1	19	0.24	<u>128</u>, 228 (-Me)
Diclofenac	87	1	1	4	0.09	<u>214</u>, 242 (-Me)
Triclosan	66	6	0.9992	14	0.24	<u>252</u>, 346 (-COOMe)
D_3-Mecoprop (SIS)	94	2	1	-	-	<u>172</u>, 231 (-Me)
DEET	82	4	0.9996	13	0.20	119, <u>190</u>
Caffeine	95	4	1	9	0.26	109, <u>194</u>
$^{15}N_2$-Caffeine (SIS)	99	4	1	-	-	110, <u>196</u>

Table 6: Recovery rates (RR) for extractions of 1 L of tap water, pH 6.8, spiking level 20 - 30 ng/L, repeatability expressed as relative standard deviations (RSD; n = 3), linear regression coefficients (r^2; concentration range 0.2 - 200 ng/L, 4 point calibration) and reproducibility as coefficients of variation (CV; n = 6) for the extraction method, limits of quantification (LOQ, s/n = 9) calculated for 1 L water samples and corrected for recovery rates, ions used for quantification (underlined) and as qualifiers for GC-MS analysis, for the acidic compounds as the methyl (-Me), dimethyl (-di-Me) esters or the methoxycarbonyl derivative (-COOMe) (SIS = surrogate internal standard)

3 Non-target screening of marine samples

3.1 German Bight

The main emphasis of this work was placed on polar organic chemicals, i.e., substances with an octanol/water distribution coefficient (log Kow) of approximately 3 or lower. In many cases, a prerequisite for only moderate to low enrichment in the organic phase is the existence of hydrophilic functional groups, as for example hydroxy-, carboxy- and amino-groups. As a result, many compounds have either a low volatility or are easily removed from the atmosphere by rain events and are hardly re-volatilised from receiving water bodies. In contrast to lipophilic POPs such as PCBs, atmospheric long-range transport is not expected to contribute major amounts to the concentrations of hydrophilic contaminants in the North Sea. Nevertheless, for triazine and organophosphorous pesticides of medium polarity, atmospheric transport to the North Sea from application areas was observed [120] and wet and dry deposition assumed to be relevant for offshore regions. However, highest concentrations are expected to be present in coastal waters, caused by riverine inputs and eventually atmospheric short to medium range transport from land-based sources (spray drift from pesticide application on agricultural land and forests, industrial emissions into the atmosphere) and is shown for several contaminants, e.g., triazines [121].

A screening for hitherto unnoticed organic contaminants seemed most promising in coastal waters, especially in the plumes of rivers known to discharge significant loads of chemicals to the North Sea. Therefore, water from a location in the German Bight within the river Elbe plume, off the Eiderstedt peninsula (DB30, Figure 13), was chosen for a thorough non-target screening.

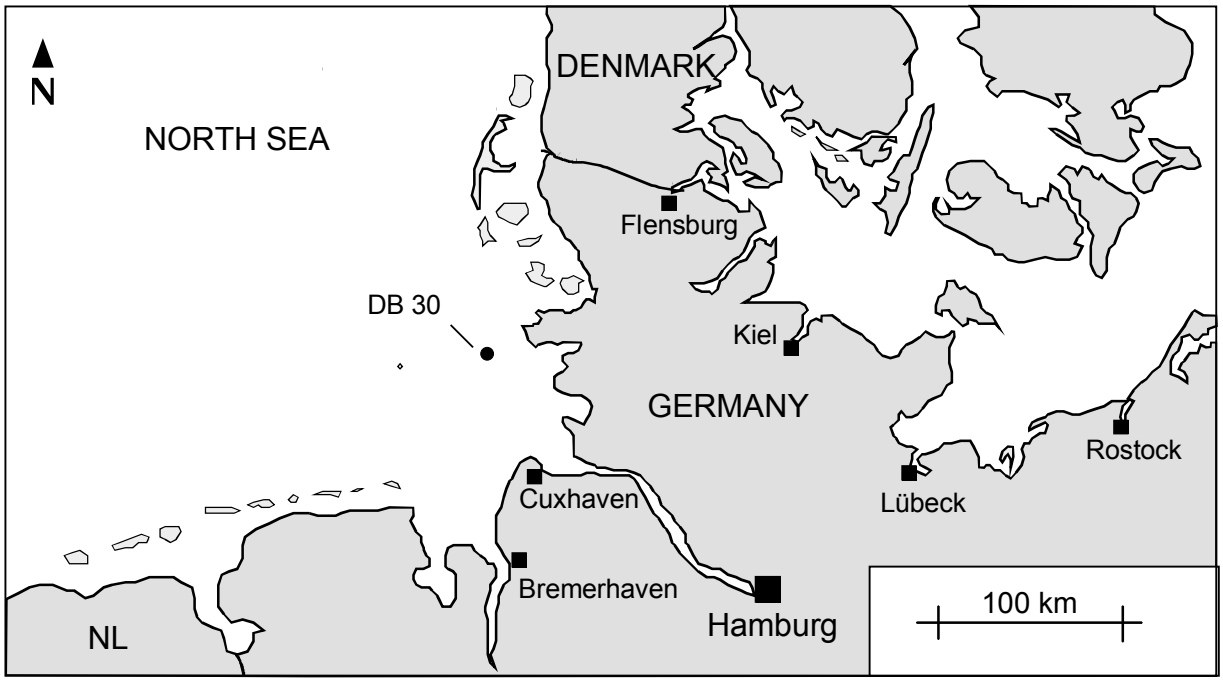

Figure 13: Position of the sampling location DB30 within the German Bight

Experimental procedure

A 10 L sample extract was subject to an eight step silica fractionation (SOP 2) to reduce sample complexity and to gain information on the polarity of unknown compounds according to their elution order. The goal of the present approach was not the elucidation of every peak in the resulting chromatograms but rather the identification of (anthropogenic) chemicals, the presence of which has not or only rarely been reported for the North Sea before. For example, in this work no effort was spent on the in-depth identification of PAHs present in the samples since their occurrence in the North Sea is well documented [122] and regularly monitored [123]. The same holds for further aromatic hydrocarbons, e.g., the large number of alkylbenzenes. Furthermore, all compounds that were detected in the procedural blanks or are known to migrate into the samples from the laboratory environment or vial and injector septa were excluded from further consideration, in spite of possible concentration differences between blanks and samples. This mainly affects plastic additives such as plasticisers (phthalates, alkyl-/arylphosphates), anti-oxidants (di-*tert*-butylhydroxytoluene- BHT, *tert*-butylhydroxyanisole - BHA), flame retardants (tris(chloroethyl)phosphate - TCEP), organo-silicon compounds and further single substances (e.g., naphthalene and some derivatives, dibenzofuran, diethylbiphenyl, *N*-butylsulfonamide). Single phthalates were present in some samples in concentrations by an order of magnitude or more higher than in the blanks. Due to the extremely high fluctuations in phthalate blank concentrations determined in previous experiments [90], it was not attempted to define a maximum blank concentration, above which the environmental presence of a phthalate is unambiguous. The results presented in Tables

7 to 14 were mainly proposals from mass spectral library searches and manually checked for plausibility according to the following criteria: (i) agreement of the spectra of the proposed substances with those from the samples, taking into account specific deviations characteristic of the used ion trap mass spectrometer (MS) as compared to quadrupole instruments, (ii) comparison of the volatility of the proposed compounds with retention times (RT) on the gas chromatograph (GC) column, (iii) comparison of the polarity of the proposed substance with the elution order in the silica fractionation, (iv) GC-amenability of the proposed compound. As a rough criterion for the agreement of the library and sample spectra, fit-, refit- and purity factors are given. The fit-factor describes how well the library spectrum fits into the sample spectrum, the refit-factor is the inverse approach and purity is calculated from both. It is commonly assumed that a high likelihood of identification is given for factors above 500. However, in samples containing a high amount of matrix, the refit-factor will be low despite a good fit-factor, resulting in low purity. On the other hand, for compounds with not very characteristic spectra (e.g., only one or two fragment ions) false positives may be obtained so that a careful interpretation of spectra and proposals is crucial. The chosen procedure is further limited by the fact that for the used instrument a high background was generally observed for masses below 100 amu, mainly due to organosilicons and hydrocarbons stemming from the injector area, but also from sample matrix. For the ion trap detector this leads to a significant decrease in sensitivity since only a limited amount of ions can be stored in the trap. As a compromise between sensitivity and specifity, masses below m/z = 100 amu were rejected from the trap. Thus, the identification and spectra interpretation of substances with significant mass fragment smaller than 100 amu was complicated and in some cases impossible. Furthermore, the use of the ion trap detector does not allow high resolution MS. Information on the elemental composition of unknown compounds from exact mass determinations is thus not available. As a consequence, the identifications obtained by this approach have to be regarded as proposals until their identity is verified by retention time and spectra comparison with the respective reference compounds (chapter 3.3).

In the following paragraph, only a brief overview of the results obtained for the single fractions will be given, while selected compounds (marked in bold italic letters) will be discussed in more detail in chapters 3.3 and 4.

Fraction 1 (*n*-hexane, see Table 7) was dominated by alkylbenzenes as well as PAHs and their alkyl derivatives. Remarkable is the presence of valencene (1,2,3,5,6,7,8,8*a*-octahydro-1,8*a*-dimethyl-7-(1-methylethen)naphthalene), an essential oil from oranges and grapefruit, possibly used as fragrance. Furthermore, chlorohydrocarbons (***dichloro***- and ***trichlorobenzene***) were present in this fraction.

In fraction 2 (*n*-hexane/dichloromethane 9:1 v/v, Table 8) the presence of alkylbenzenes and PAHs was even more pronounced. Two methylbiphenyls were identified as

well as the oxygen-compounds benzophenone and 9*H*-xanthene. With respect to organochlorine compounds, ***chloronaphthalene*** was detected and a trichlorobiphenyl was proposed.

In fraction 3 (*n*-hexane/dichloromethane 4:6 v/v, Table 9) further biphenyls and terphenyl were identified besides some alkylbenzenes and PAHs still eluting in this fraction. Furthermore, ***nitrobenzene*** and ethylquinoline were detected. Identified oxygen-compounds in addition to 9*H*-xanthene that already eluted in the previous fraction were diphenylether and ***propenylmethoxybenzene***, of which the *para*-isomer is known as anethole and used for flavouring, but also in medicine as expectorant. A considerable number of organohalogen compounds eluted in this fraction. Among these there were a ***chlorofluoronitrobenzene***, a ***dichlorobenzonitrile***, two ***bis(dichloropropyl)ethers***, an unknown chlorinated compound (Cl-I) and three isomers of ***HCH***. Remarkable is also the presence of three isomeric bromo-compounds that were identified with a high fit-factor as ***bromodimethoxybenzenes***.

In fraction 4 (dichloromethane, Table 10) three nitrogen-heterocycles were identified: acridine, benzocinnoline and an indazolo-derivative. Aldehydes (propylbenzaldehydes), ketones (diphenylethenone, anthracendione) and esters (fatty acid esters, benzylbenzoate) composed the oxygen-compound group of this fraction, while in the organohalogen group chlorofluoronitrobenzene, ***dichloropyridine***, ***chloroaniline***, ***dichloroaniline***, dichlorobenzonitrile and two bis(dichloropropyl)ethers were found.

In the next fraction 5 (dichloromethane/ethyl acetate 1:1 v/v, Table 11) an increasing number of nitrogen- and oxygen-compounds was identified, among them anilines (***N-ethyltoluidine***, methyl-1-methylethylaniline), amides (***N,N-diethyltoluamide***), carbamates (***propoxur***), ethers, alcohols, phenols, aldehydes, ketones and esters. In the group of the organohalogens, several pesticides were identified (***simazine***, ***atrazine***, ***terbuthylazine*** and ***metolachlor***) in addition to dichloropyridine, bromophenol and two fluorine-compounds (fluor-methoxyethenyl-benzene, fluorotrimethylbenzene). Furthermore, some sulfur-compounds were detected (thiophenes, methoxyphenylisocyanate, ***morpholinylbenzothiazole***).

In fraction 6 (ethyl acetate, Table 12), only a small number of library proposals was deemed sufficiently acceptable. This is not because of a low number of substances present in this fraction. In contrast, a large number of mainly organo-nitrogen and organo-oxygen compounds, presumably of biogenic origin co-eluted and thus hindered a sound identification. Further sub-fractionation of this fraction would improve the elucidation rate. Nevertheless, some organochlorines were identified, among them ***dichlorobenzeneisocyanate***, a known thermal transformation product of *N*-phenylurea herbicides, two triazine herbicide transformation products (***desethylatrazine***, ***desethylterbuthylazine***) and two isomers of the

flame retardant ***tris(chloropropyl)phosphate***, while further organochlorine compounds remained unidentified (Cl-III to Cl-IV).

In fraction 7 (acetone, Table 13), a considerable number of N-heterocycles was detected, among them the pharmaceuticals ***propyphenazone*** and ***carbamazepine***. Further proposals for this fraction were alkylphenols, thiophenes and a tetrafluoroaminoaniline, while the proposal famotidine (3-{[2-(diaminomethylenamino)-4-thiazolyl]-methylthio}- N2- sulfamoylpropamidin) does not appear to be amenable to GC without derivatisation, if thermostable at all.

Finally, in fraction 8 (methanol, Table 14), among other N-heterocycles, ***caffeine*** was detected. Oxygen-compounds comprised alkylphenols, ethers, aldehydes and esters. Several chlorinated compounds were detected in this fraction and proposed to be chloroethylmethylpyrimidine, chlorophenyl-chlorobenzyl alcohol and chlorobenzoic acid methyl ester. Proposed organofluorine compounds were methoxyfluorbenzyl alcohol, fluoromethoxyethenylbenzene and difluoroanisole. Furthermore, two organophosphorus compounds were identified (mevinphos, ***triphenylphosphine oxide*** - TPPO).

Table 7: Compounds identified by mass spectral library search in fraction 1 (*n*-hexane) of sample DB30-3

RT [scan nr.]	**Compound**	Ions m/z [amu]	Purity	Fit	Rfit
	Hydrocarbons				
	Alkylbenzenes				
1026	Ethyldimethylbenzene	119, 134, 117, 105	722	752	919
1169	(Dimethylpropyl)benzene	105, 119, 148	263	636	360
2080	Di-*tert*-pentylbenzene	105, 218, 189	246	575	341
2149	(Methylnonyl)benzene	105, 218	515	742	669
2328	(Methyldecyl)benzene	105, 232	608	792	737
2498	(Methylundecyl)benzene	105, 106, 256	502	793	611
2662	(Methyldodecyl)benzene	105, 260	389	712	471
	PAHs (+ derivatives)				
1756	Ethenylnaphthalene	154, 153, 152	673	835	785
1788	Ethylnaphthalene	141, 156, 115	601	805	717
1825	Valencene	161, 105,107,189	557	707	673
1839	Dimethylnaphthalene	141, 156, 155, 115	573	837	665
1848	Dimethylnaphthalene	141, 156, 155, 115	564	845	638
1969	Acenaphtene	153, 154, 152	516	909	553
2158	Fluorene	165, 166	503	785	631
2512	Anthracene	178, 152	543	759	654
	Organohalogens				
	Cl-Compounds				
1016	Dichlorobenzene	146, 148, 111, 113	373	549	473
1341	Trichlorobenzene	111, 125, 180, 182, 145, 147	252	586	331

Table 8: Compounds identified by mass spectral library search in fraction 2 (*n*-hexane/dichloro-methane 9:1 v/v) of sample DB30-3

RT [scan nr.]	**Compound**	Ions m/z [amu]	Purity	Fit	Rfit
	Hydrocarbons				
	Alkylbenzenes				
928	Trimethylbenzene	105, 120	548	722	733
946	Trimethylbenzene	105, 120	638	696	862
973	Trimethylbenzene	105, 120	718	739	944
1005	Diethylbenzene	113, 119, 105, 131, 134	272	639	401
1016	Diethylbenzene	119, 134	293	596	352
1029	Ethyldimethylbenzene	119, 134	389	591	555
1054	Propenylbenzene	117, 115, 118	516	689	728
1085	Diethylbenzene	119, 105, 134	641	763	823
1104	Methylpropylbenzene	105, 134	432	717	590
1127	Methyl(methylethyl)benzene	119, 134	298	750	379
1238	Diethylmethylbenzene	119, 105, 133, 148	313	594	500
1254	Diethylmethylbenzene	119, 105, 133, 148	456	628	658
1261	Ethenyldimethylbenzene	117, 115, 132	548	810	631
1280	Ethyldimethylbenzene	119, 117, 115, 134	484	589	655
1290	Diethylmethylbenzene	119, 105, 133, 148	500	629	704
1303	Ethyl(methylethyl)benzene	105, 119, 148, 134	524	664	646
1390	Ethyl(methylethyl)benzene	134, 105, 148	368	756	454
2202	(Butylheptyl)benzene	105, 142, 175, 232	286	409	668
2330	(Methyldecyl)benzene	105, 232	613	774	753
2393	(Propylnonyl)benzene	133, 105, 203, 246	277	388	662
2500	(Methylundecyl)benzene	105, 246	633	801	760
2599	(Ethylundecyl)benzene	273	465	532	
2664	(Methyldodecyl)benzene	105, 260	522	784	631
	Biphenyls				
1960	Methylbiphenyl	168, 167, 165, 152, 153	678	798	830
1980	Methylbiphenyl	168, 167, 165, 152, 153	588	799	711
	PAHs (+ derivatives)				
1524	Tetrahydromethylnaphthalene	131, 118, 117, 146	463	831	533
1593	Ethylidene-1*H*-indene	141, 142, 115	844	870	960
1757	Ethenylnaphthalene	153, 152, 151	800	864	916
1840	Dimethylnaphthalene	156, 141, 115	776	881	871
1888	Dimethylnaphthalene	156, 141, 115	271	695	339
1971	Acenaphthene	153, 154, 152	588	907	628
2024	Tetrahydrodimethyl(methylpropenyl)naphthalene	159, 131, 202	737	864	824
2091	Trimethylnaphthalene	155, 170, 119	294	851	334
2159	1*H*-Phenalene	165, 166			
2172	Methylpropylnaphthalene	155, 184, 115	362	631	455
2510	Anthracene	178, 152	662	788	801
	O-Compounds				
2151	Benzophenone	105, 182	352	625	474
2252	9*H*-Xanthene	181, 182, 152	364	780	440
	Organohalogens				
	Cl-Compounds				
1768	Chloronaphthalene	127, 162, 164,	248	869	274
2636	Trichlorobiphenyl	186/188, 256/258/260	238	658	323

Table 9: Compounds identified by mass spectral library search in fraction 3 (*n*-hexane/dichloro-methane 4:6 v/v) of sample DB30-3

RT [scan nr.]	**Compound**	Ions m/z [amu]	Purity	Fit	Rfit
	Hydrocarbons				
	Alkylbenzenes				
1319	Bis(methylethenyl)benzene	128, 143, 157	403	647	523
1472	Ethyl(methylethyl)benzene	128, 148, 158	301	676	413
1546	Trimethyl(propadienyl)benzene	143, 128	356	894	383
2150	Methyl(phenylmethyl)benzene	182, 165, 105	573	736	679
2189	Methyl(phenylmethyl)benzene	105, 165, 167, 181, 182, 196	420	672	515
	Biphenyls etc.				
1970	Biphenyl	153, 154, 152	431	714	543
1980	Methylbiphenyl	168, 167, 165, 152, 153, 115	754	857	870
2170	Dimethylbiphenyl	182, 167, 165,	557	784	674
2219	Dimethylbiphenyl	182, 181, 167, 152	628	755	767
2489	Diphenylmethylpenten	119, 180, 236	269	492	494
2549	Diphenylmethylpenten	143, 127, 221, 236	593	780	719
2646	Terphenyl	230, 231, 229, 215	274	615	378
	PAHs (+ derivatives)				
1338	Dihydromethylnaphthalene	139, 138, 144	398	770	494
1499	Dihydromethylnaphthalene	129, 128, 144	293	766	365
1511	Dihydromethylnaphthalene	129, 128, 144	472	761	589
1593	Ethylidene-1*H*-indene	141, 142, 115	802	872	911
1756	Ethenylnaphthalene	154, 153, 152	840	877	946
1839	Dimethylnaphthalene	141, 151, 150, 115	673	843	780
1850	Dimethylnaphthalene	141, 151, 150, 115	558	832	649
2158	Fluorene	165, 166	803	869	913
2508	Anthracene	178, 176, 152	718	774	888
2689	Methylphenanthrene	192, 191, 165	551	874	604
2893	Dimethylphenanthrene	206/207, 191/190	397	799	457
2949	Pyrene	202/200	793	949	827
	N-compounds				
1156	Nitrobenzene	123, 107			
2063	Ethylquinoline	157, 142, 141, 156, 200	437	785	521
	O-compounds				
1371	Anethole (methoxypropenylbenzene)	148, 147, 117, 121	602	761	767
1794	Diphenylether	141, 170, 142, 115	588	736	782
2248	9*H*-Xanthene	181, 182, 152	602	833	698
	Organohalogens				
	F-Compounds				
1404	Chlorofluoronitrobenzene	129, 175, 109, 117, 145	635	717	874
	Cl-Compounds				
1665	Dichlorobenzonitrile	113, 115, 171, 173, 136, 138	425	811	507
1895	Bis(dichloropropyl)ether	189/191, 111/113, 141/143			
1934	Bis(dichloropropyl)ether	189/191, 111/113, 141/143			
2310	Unknown compound Cl-I	195/197, 167, 210/212			
2340	HCH	181/183/185, 109/111, 219/217	466	760	591
2432	HCH	181/183/185, 109/111, 219/217	285	644	420
2451	HCH	181/183/185, 109/111, 219/217	773	864	883
	Br-Compounds				
1393	Bromodimethoxybenzene	218/216, 203/201	363	719	439
1452	Bromodimethoxybenzene	216/218, 201/203	331	708	413
1394	Bromodimethoxybenzene	216/218, 201/203	361	738	439

Table 10: Compounds identified by mass spectral library search in fraction 4 (dichloromethane) of sample DB30-3

RT [scan nr.]	**Compound**	Ions m/z [amu]	Purity	Fit	Rfit
	Hydrocarbons				
	PAHs				
1337	Methyldihydronaphthalene	129, 139	588	779	722
2062	Trimethyldihydronaphthalene	158, 142	416	762	487
2159	Fluorene	165	418	761	499
	N-Compounds				
2249	Acridine	179, 152	314	547	477
2431	Benzocinnoline	152, 180	509	831	578
2648	6*H*,12*H*-Indazolo[2,1-*a*]indazolo-6,12-dione	208, 236	462	844	545
	O-Compounds				
1423	(Methylethyl)benzaldehyde	105, 133, 148	459	717	612
1623	(Methylethyl)benzaldehyde	105, 133, 148	433	683	565
2352	Methyl dimethyldodecanoate	143, 199, 242	457	628	677
2456	Benzylbenzoate	105, 194, 212	409	600	651
2569	Diphenylethenone	165, 194	536	844	571
2672	Methyl methylpentadecanoate	143, 227, 270	244	453	488
2805	Anthracendione	152, 208, 180	297	782	335
	Organohalogens				
	F-Compounds				
1403	Chlorofluoronitrobenzene	129/131, 175/177, 145/145	531	701	744
	Cl-compounds				
1170	Dichloropyridine	112/114, 147/149	355	725	458
1233	Chloroaniline	127/129, 100	371	654	561
1417	Unknown compound Cl-II	133/134, 171/173			
1656	Dichloroaniline	161/163, 126	613	747	804
1665	Dichlorobenzonitrile	171/173, 136/138	605	825	725
1894	Bis(dichloropropyl)ether	189/191			
1933	Bis(dichloropropyl)ether	191/189, 141/143, 111/113			

Table 11: Compounds identified by mass spectral library search in fraction 5 (dichloromethane/ ethyl acetate 1:1 v/v) of sample DB30-3

RT [scan nr.]	**Compound**	Ions m/z [amu]	Purity	Fit	Rfit
	N-Compounds				
1332	(Dimethylamino)nitropyrimidine	139, 168, 153	345	511	492
1430	Methylmercaptoaniline	139, 124	464	562	766
1592	*N*-Ethyltoluidine	120, 135	695	778	824
1799	*N*-Ethylisoindoldione	160, 175, 132	502	712	620
2108	*N,N*-Diethyltoluamide (DEET)	119, 190	373	848	406
2163	Propoxur	110, 135, 152			
2444	Methyl-1-methylethylaniline	134, 149, 204	257	683	340
	O-Compounds				
1252	Dihydroxybenzaldehyde	148, 109	417	750	488
1279	Methylphenoxyethanol	108/107, 152	567	642	774
1468	Methylpropenylphenol	105/107, 133, 148	589	712	744
1536	(Hydroxy-methoxyphenyl)ethanone	151, 166	482	680	667
1842	Trimethylphenylethanone	147, 119	623	772	732
1887	Dimethylethylmethoxyphenol	165, 180	608	774	712
2059	Dimethyl-3(2*H*)-benzofuranone	133, 105, 162	475	686	624
2157	Tetramethylphenylpropanol	134/133/132/131, 119, 159	471	930	504
2197	Methoxynaphthalenol	159, 103, 131, 174	368	723	452
2429	9*H*-Fluorenol	152, 180	652	743	791
2771	Xanthone	196, 168, 139	344	612	430
2805	9,10-Anthracendione	152, 180, 208	698	864	772
3028	Ethylhexyl methoxyphenylpropenoate	178, 161, 132/133	440	774	548
3055	Eicosyl methoxybenzoate	152	209	864	221
	Organohalogens				
	F-Compounds				
1258	Fluoro(methoxyethenyl)benzene	152, 109, 147	644	813	764
1806	Fluorotrimethylbenzene	123, 138	446	742	572
	Cl-compounds				
998	Dichloropyridine	147/149, 112/114	364	579	488
2394	Simazine	201/203, 173/175, 186/188	355	603	546
2410	Atrazine	200/202, 215/217	440	517	833
2454	Terbuthylazine	204/206, 173, 229/231	458	681	662
2763	Metolachlor	162, 238	323	715	422
	Br-Compounds				
1208	Bromophenol	172/174			
	S-Compounds				
1309	Acetyldimethylthiophene	139, 154	747	837	848
2067	Dibutylthiophene	153, 196, 111	496	735	620
2076	Methoxyphenylisothiocyanate	165, 122	250	629	354
2340	Morpholinylbenzothiazole	136, 220, 164	604	742	767
	or Methabenzthiazuron		437	715	543

Table 12: Compounds identified by mass spectral library search in fraction 6 (ethyl acetate) of sample DB30-3

RT [scan nr.]	**Compound**	Ions m/z [amu]	Purity	Fit	Rfit
	N-Compounds				
2297	Tetramethylquinoxaline	186, 145, 104	405	821	482
2393	Diacetylaminobenzoquinone	138, 222	473	809	537
2649	Ethyl(propylbenzimidazole)	145, 188, 159	372	755	466
	O-Compounds				
1414	Hydroxybenzeneethanol	138, 107	339	757	433
1437	Diethylhydroxycyclopentenone	126/125, 111, 108, 154	556	822	665
2404	Cyclohexyl hydroxybenzoate	135, 153	423	790	474
	Organohalogens				
	Cl-Compounds				
1596	Dichlorobenzeneisocyanate	124/126, 159/161, 187/189	426	824	495
1659	Methyl chloropicolinate	113/115, 123/125, 141	284	588	459
1739	Unknown compound Cl-III	125/127, 107	495	671	641
1900	Cl-IV (chlorophenoxyalkanoic acid?)	177/179, 220/222, 141, 135			
2259	Desethylatrazine	172/174, 187/189			
2289	Desethylterbuthylazine	186/188, 145			
2474	Tris(chloropropyl)phosphate (TCPP)	277/279, 201/203, 155/177, 157/159			
2493	Tris(chloropropyl)phosphate (TCPP)	277/279, 201/203, 155/177, 157/159			
	S-Compounds				
1375	Ethylisopentylthiophene	125, 110	415	647	591
1698	Ethylbutylthiophene	125, 108, 155	423	707	548

Table 13: Compounds identified by mass spectral library search in fraction 7 (acetone) of sample DB30-3

RT [scan nr.]	**Compound**	Ions m/z [amu]	Purity	Fit	Rfit
	N-Compounds				
1372	Butenylpyrrolidine	110, 125	320	580	516
1478	Aminohydroxybenzoic acid	153, 115/117, 123, 135	549	760	635
1679	Methylquinazoline	144, 129, 103, 117	666	784	827
1772	Dimethylquinazoline	158, 166, 143, 120, 117	354	757	452
1772	Ethyl ethylmethylpyrrolecarboxylate	166, 120, 181	424	647	569
1648	2-Oxo-methyl-isopropylpyrazine	109, 125, 137	333	698	435
1991	*N*-Phenylethylacetamide	104, 163	306	543	540
2053	Dihydromethyl-2*H*-benzimidazolone	148/147, 119	523	836	609
2103	Methoxyindole	132, 109, 147	303	704	389
2297	Methylquinolinole	130, 159	328	765	409
2749	Propyphenazone	215, 230	285	705	369
3369	Carbamazepine	193, 236			
	O-Compounds				
1963	Bis(methylethyl)phenol	121, 136, 117	461	618	584
	Organohalogens				
	F-Compounds				
2429	Tetrafluorooaminoaniline	180, 152, 221	432	773	502
	Cl-Compounds				
1348	Unknown compound Cl-V	141/139, 111, 157/159			
	S-Compounds				
1797	Heptanoylthiophene	121, 111, 108	454	761	499
1851	Heptanoylthiophene	111, 121, 116	333	640	384
2094	Famotidine	121, 155, 188, 113	237	849	279

Table 14: Compounds identified by mass spectral library search in fraction 8 (methanol) of sample DB30-3

RT [scan nr.]	**Compound**	Ions m/z [amu]	Purity	Fit	Rfit
	N-Compounds				
1448	Aminohydroxybenzoic acid	135, 153	229	670	280
1479	Dimethylhydroxypyridinemethanol	153, 123, 135	303	444	553
1495	Methyl(methylpropyl)imidazolidinedione	113, 114	442	747	566
2573	Caffeine	194, 109	529	681	763
	O-Compounds				
951	Acetylmethylfuran	109, 142, 124	307	744	375
1051	Dimethylphenol	122, 121, 123, 138, 139,109	226	603	293
1071	Hydroxybenzaldehyde	122, 121, 207	415	716	571
1280	(Dimethylethyl)benzenediol	109, 103, 151, 132	231	418	343
1293	Hydroxymethylbenzaldehyde	136, 135, 107, 101	377	655	523
1543	Dimethylethylphenol	135, 150, 107	734	798	910
1890	Pentadecyl methoxybenzoate	152, 135	322	857	340
1900	Nonylphenol	135, 220	307	803	328
1995	Ethoxyanisaldehyde	180, 112, 109, 110, 139	289	593	366
2291	Diethoxymethoxypropenylbenzene	193, 236, 165	292	658	400
	Organohalogens				
	F-Compounds				
1187	Methoxyfluorobenzylalcohol	109, 156, 103, 123	322	558	490
1257	Fluoro(methoxyethenyl)benzene	109, 152, 143, 137	433	767	549
1679	Difluoroanisole	101, 144	200	581	283
	Cl-Compounds				
2141	Chloroethylmethylpyrimidine	155, 156, 157	319	782	397
2416	(Chlorophenyl)chlorobenzylalcohol	139/141, 249/251/253	346	726	418
2484	Methyl chlorobenzoate	139, 141	280	558	408
	P-Compounds				
1318	Mevinphos	127, 119, 109, 102	250	630	324
3716	Triphenylphosphine oxide	277, 199, 201	861	912	926
	S-Compounds				
1922	Butylethylthiophene	125, 126, 110	401	589	603
2223	Butyl methylbenzenesulfonate	173, 111, 155	284	571	444

3.2 North Sea

After having screened river Elbe plume water in detail, further samples from the entire North Sea were investigated without prior fractionation. The objectives were to identify on a North Sea-wide basis substances with a widespread distribution and sufficiently high concentrations to be detected without prior clean-up, as well as to gain information about potential local differences in the composition of organic water constituents. From the large number of identified substances that included PAHs, oxo-PAHs, alkylbenzenes, organohalogens, ethers, alcohols, aldehydes, ketones, esters, anilines, amides, nitro-compounds, N-heterocycles, sulfonamides, thiophenes, benzothiazoles, alkyl- and chloroalkyl phosphates selected ones that are of particular interest because of either their appearance in a number of samples or their potential ecotoxicological impact are summarised in Table 15. The positions of the sampling sites within the North Sea are given in Figure 14. The findings will be discussed in chapter 3.3. No positive entry in the table does not necessarily mean the absence of a compound at this position. The screening of the total ion chromatograms is inherently less sensitive than the determination on extracted ion traces.

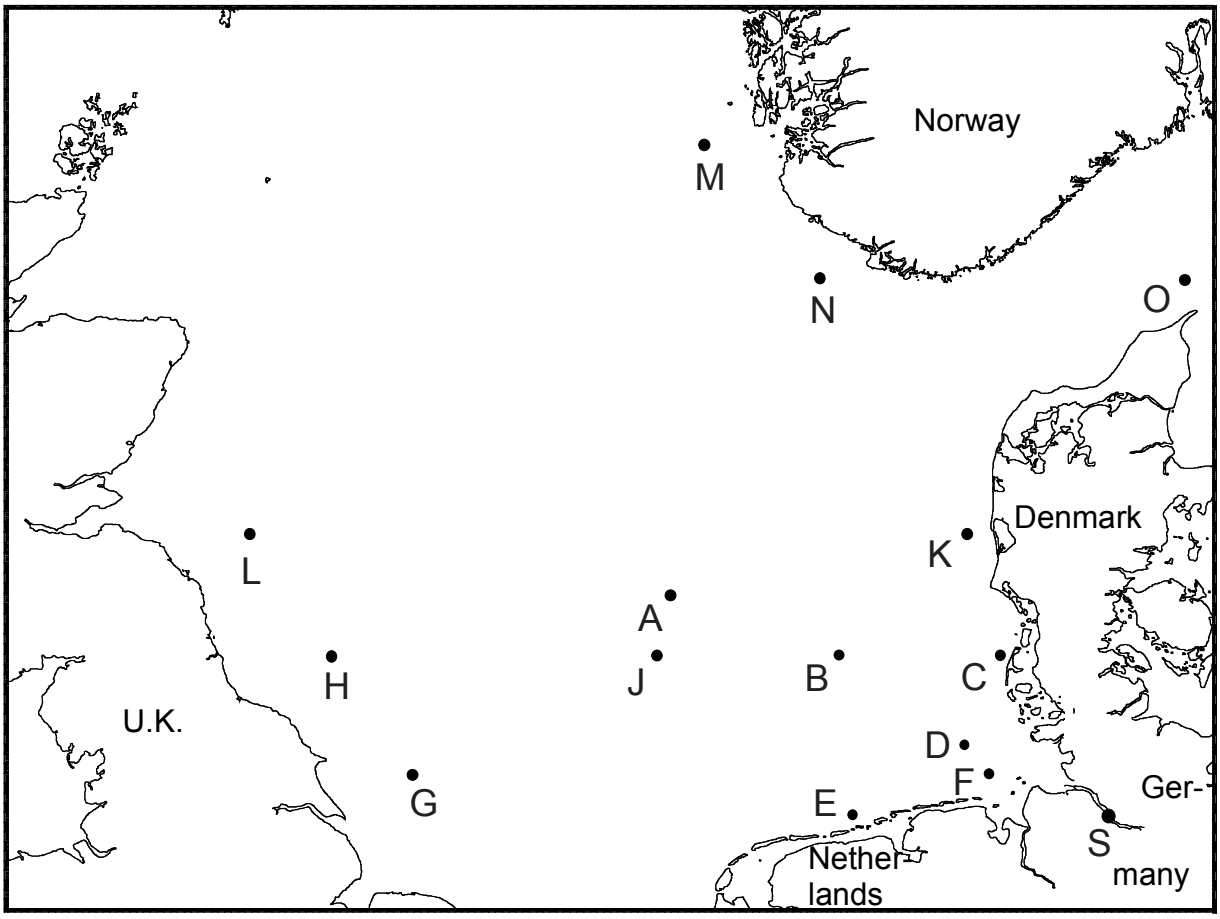

Figure 14: Sampling positions within the North Sea

Table 15: Occurrence of certain non-target substances in selected samples (+: identified by mass spectra, ++ : verified by reference compounds; substances quantified in chapter 4 not included)

Area	Off British coast		German Bight				Skagerrak	Off Norwegian coast	Southern Central North Sea
Sample	H	G	E	F	D	C	O	M	J
γ-HCH		++	++	++		++	++	++	++
Atrazine					++	++		++	
Simazine								++	
Ethofumesate				+				+	
Diuron[a]	++	++	++	++	++	++	++	++	++
1,2-Dichlorobenzene	++								
1,3-Dichlorobenzene	++	++		++		++			
1,4-Dichlorobenzene	++	++				++			
1,2,4-Trichlorobenzene	++								
Trichloroanisole			+						
PCBs (Cl_5-Cl_8; 21 congeners)								+	
Fluorotoluidine	+		+				+		
Bromotrichloropropane						+		+	
Tris(chloropropyl)phosphate TCPP-1		++	++	++	++	++	++	++	
Tris(chloropropyl)phosphate TCPP-2		++	++	++	++	++		++	
Tris(dichloropropyl)phosphate				+	+				
Triphenylphosphine oxide	++				++	++			
N-Ethyltoluidine			+	+		+		+	+
Morpholinylbenzothiazole	+	+	+	+	+		+	+	+
Monobromoindole (n isomers)		+ (2)							
Dibromoindole (n isomers)	+ (1)	+ (2)		+(1)		+(1)			
Tribromoindole		+							
Bromoaniline	+	+	+			+		+	
Dibromophenol		+				+	+		
Dibromoanisole								++	
Bromotoluene	+	+				+			

a: determined as its GC-artefact dichlorobenzeneisocyanate

3.3 Verification and relevance of identified compounds

Since identification by (low-resolution) spectra alone may lead to false assignments, library proposals for a couple of substances were verified by injection and measurement of the respective reference compounds. For some of the substances hitherto not reported to occur in the North Sea, concentrations were estimated by comparison with external standards of these compounds (not corrected for recovery rates). None of them were detected in the procedural blanks. An overview of the results obtained for station DB30 (see Figure 13) is given in Table 16, while details are presented in the respective categories below.

Substance	Verification
Pesticides	
α-HCH	qualitatively
β-HCH	qualitatively
γ-HCH	qualitatively
Atrazine	qualitatively
Simazine	qualitatively
Terbuthylazine	quantitatively (0.7 ng/L)
Desethylatrazine	quantitatively (1.6 ng/L)
Desethylterbuthylazine	qualitatively
Metolachlor	quantitatively (0.3 ng/L)
Dichlobenil	quantitatively (0.1 ng/L)
DEET (*N,N*-diethyl-3-toluamide)	quantitatively (0.6 ng/L)
Industrial chemicals	
1,3-Dichlorobenzene	qualitatively
1,4-Dichlorobenzene	qualitatively
1,2,4-Trichlorobenzene	qualitatively
1-Chloronaphthalene	qualitatively
2,6-Dichloropyridine	quantitatively (0.2 ng/L)
3,5-Dichloropyridine	quantitatively (0.1 ng/L)
Nitrobenzene	quantitatively (0.7 ng/L)
Chloronitrobenzene (*o*- and/or *p*-isomer)	qualitatively
3-Chloro-4-fluoronitrobenzene	quantitatively (1.2 ng/L)
2-Chloroaniline	qualitatively
2,5-Dichloroaniline	quantitatively (0.7 ng/L)
Triphenylphosphine oxide	quantitatively (53 ng/L)
Pharmaceuticals	
Propyphenazone	quantitatively (0.6 ng/L)
Carbamazepine	quantitatively (2 ng/L)
Caffeine	quantitatively (2 ng/L)
Brominated compounds	
2,4-Dibromoanisol	qualitatively

Table 16: Compounds identified in sample DB30-3 by library search (NIST) and verified by comparison with reference substances. Concentrations of selected compounds were determined by comparison with external standards.

3.3.1 Pesticides

A couple of pesticides that are well known to be present in the North Sea [84,121,123] were also identified in this work. The insecticide lindane (γ-HCH) was confirmed besides its isomers α– and ß-HCH in sample DB30-3, while in the unfractionated samples only γ-HCH was apparent at all positions except H and D. Nevertheless, γ-HCH could also be detected in these two samples using the specific extracted ion traces so that these observations are in accordance with the cited references. The identity of the triazine herbicides atrazine, simazine and terbuthylazine was verified (Figure 15), as well as the identities of some of their transformation products (desethylatrazine, desethylterbuthylazine, Figure 17). The occurrence of these transformation products besides the parent compounds is expected since it has already been observed in contributing rivers, e.g., in the river Elbe [124] and Rhine [125], as well as in coastal waters [126] and sediments [127] of the North Sea. A profound investigation on the question whether the parent triazines are further transformed within the sea or whether the observed metabolites originate from biotic or abiotic transformation processes within terrestrial or limnic ecosystems would require detailed quantitative measurements, which were not within the scope of this work. Obviously, triazine herbicides remain major contaminants of the North Sea, despite the ban of the use of some representatives of this class. Furthermore, the herbicide metolachlor and the insect repellent DEET (Figure 15) as well as the insecticide propoxur were verified by reference compounds. The detected dichlorobenzonitrile was identified as the 2,6-isomer and thus as the herbicide dichlobenil. Quantitative investigations on the distribution of desethylatrazine, metolachlor, dichlobenil and DEET in the North Sea were carried out upon their identification in sample DB30-3 and samples from other areas. The results are presented and discussed in chapters 4.1, 4.2 and 4.3, respectively.

Dichlorobenzeneisocyanate, a thermal transformation product known to be formed in the GC from the *N*-phenylurea herbicides diuron and linuron [128], was detected in all samples. In this case, the observed dichlorobenzenisocyanate most probably stems from diuron as derived from the different decay patterns (initial peak, continuous elution of the cyanate due to ongoing transformation on the column, final peak) observed for the two parent compounds under the given GC conditions. Besides the well known application in agriculture, diuron is increasingly used in antifouling paints, following legislation limiting the use of tributyltin (TBT). During the yachting season, diuron was detected in concentrations of up to 6740 ng/L in British marinas, at much higher levels than Irgarol 1051 (1421 ng/L) [129]. The same trend was observed for Dutch marinas and coastal waters [130]. Obviously, diuron contamination of the North Sea is not limited to estuarine and coastal areas, but is also a relevant issue in offshore waters as shown by the findings of this study.

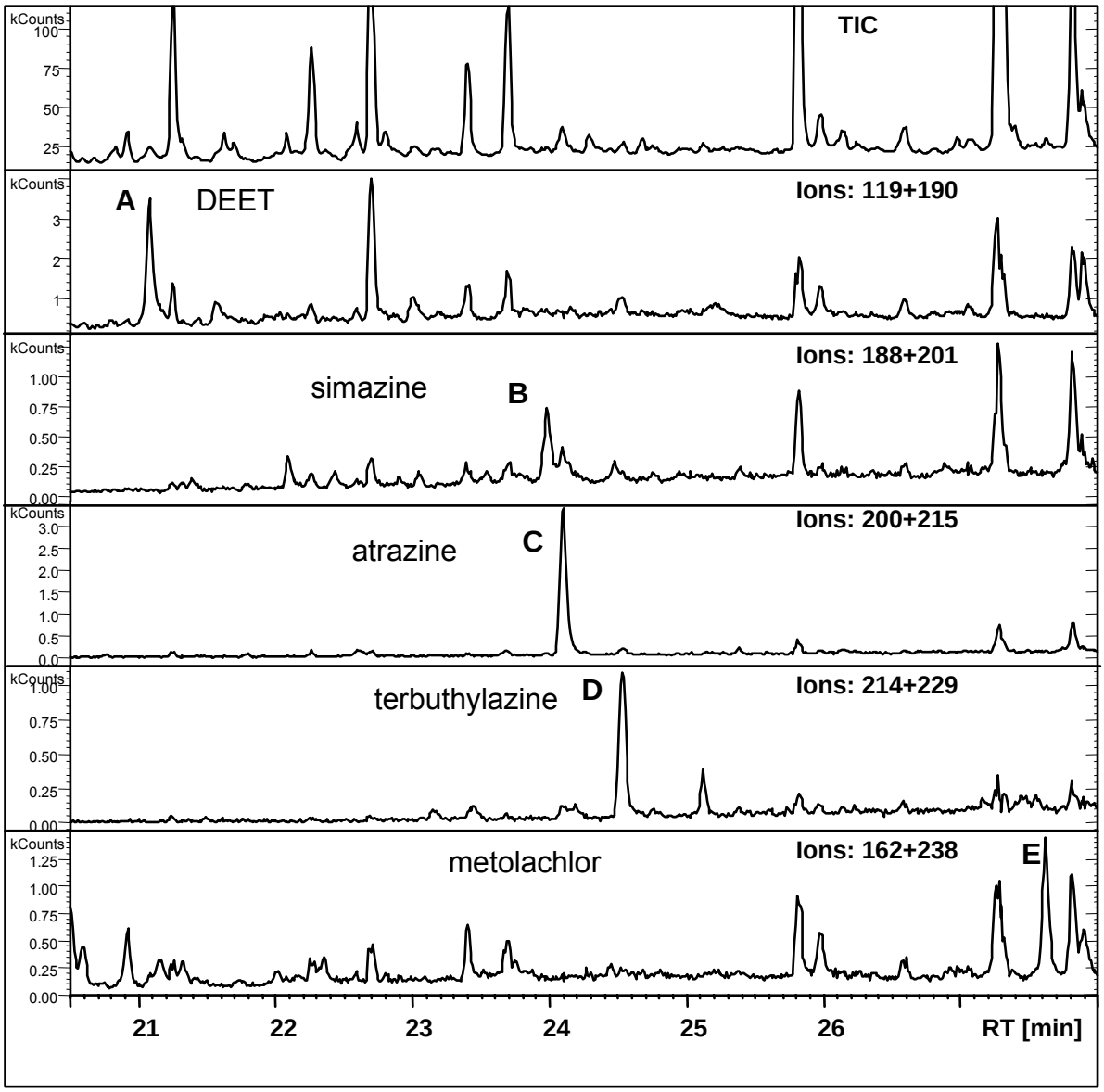

Figure 15: Chromatograms (GC-MS, full scan, TIC and extracted ion traces) of pesticides identified in fraction 5 of sample DB30-3

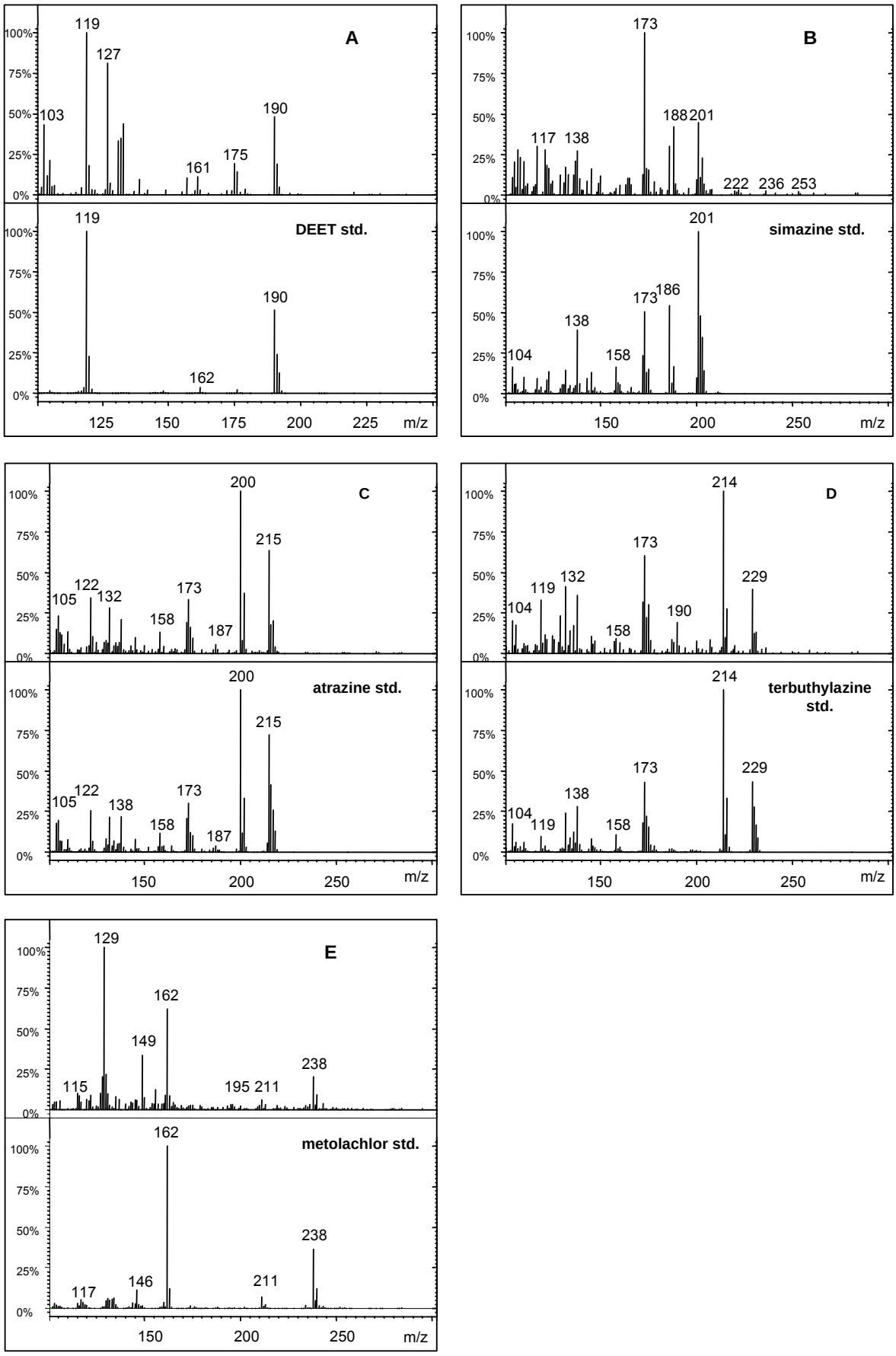

Figure 16: Mass spectra (EI, 70 eV) of pesticides identified in fraction 5 of sample DB30-3 and from the respective standards (std.)

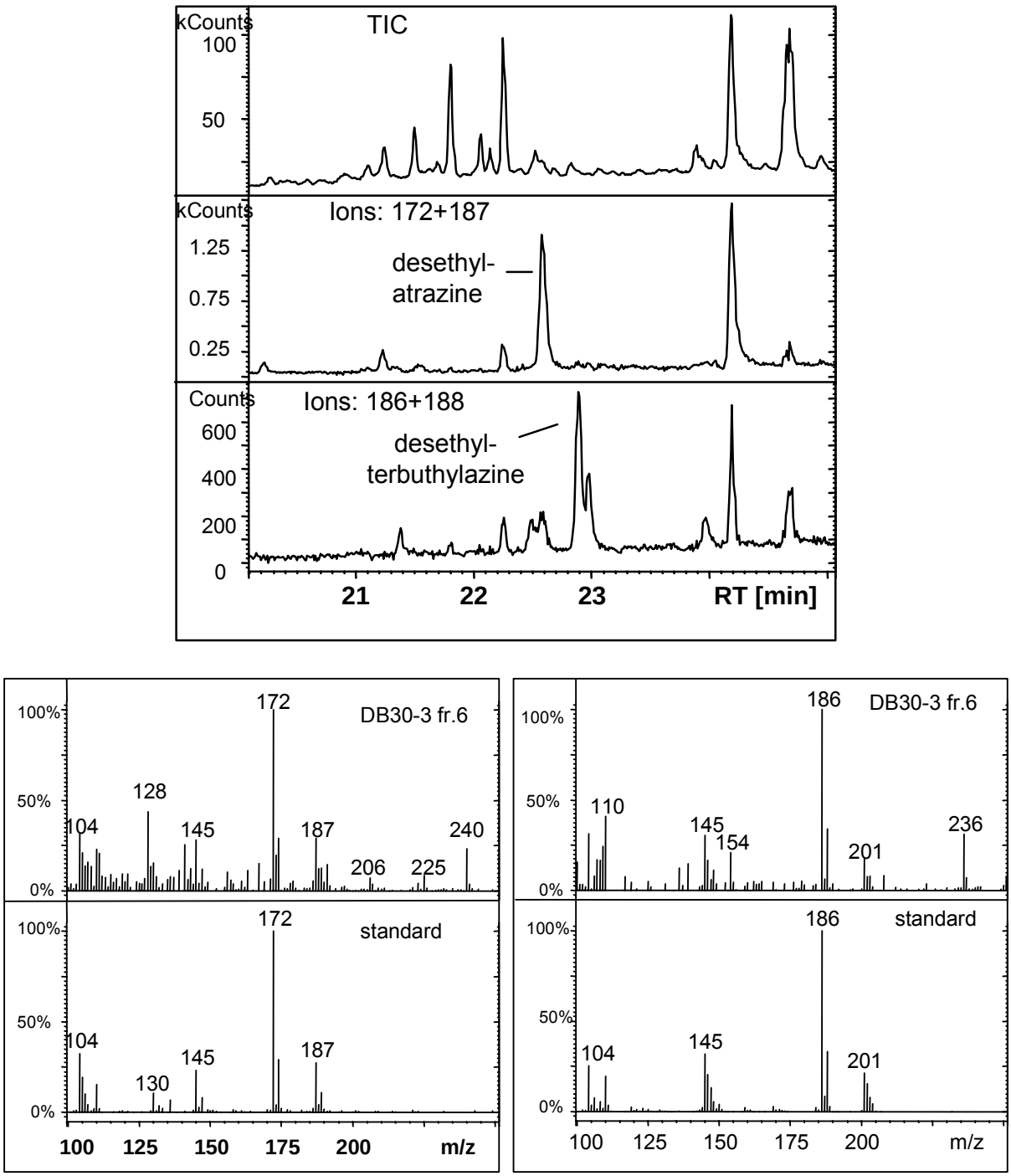

Figure 17: Chromatograms (GC-MS, full scan, extracted ion traces) and spectra (EI, 70 eV) of desethylatrazine (left) and desethylterbuthylazine (right) in fraction 5 of sample DB30-3 and in a standard solution

3.3.2 Industrial chemicals

By comparison with reference compounds, 1,2-, 1,3- and 1,4-dichlorobenzene and 1,2,4-trichlorobenzene were identified in various samples (Figure 18). Chlorobenzenes are important intermediates in the production of dyes, pesticides and pharmaceuticals. In the Elbe river in the year 2000, concentrations were measured in the range of 2 to 20 ng/L (13

single samples, maximum concentrations) at Hamburg (Seemannshöft, river km 628.8), while at the stations Grauerort (km 660.5) and Cuxhaven (km 725.2) all di- and trichlorobenzenes were below the limits of quantification (LOQs) which were between 0.2 and 10 ng/L, depending on the analyte [131]. Thus far, only few data for the North Sea is available. In 1983/84 all six di- and trichlorobenzenes were detected in Dutch coastal waters at median concentrations of around 1 ng/L (trichlorobenzenes), 10 ng/L (1,2- and 1,3-dichlorobenzene) and 100 ng/L (1,4-dichlorobenzene) [6]. In the German Bight, the Bundesamt für Seeschiffahrt und Hydrographie (BSH) measured trichlorobenzenes in seawater in 1997 [132], but no concentration data is retrievable. In view of the dermato-, hepatotoxic and carcinogenic properties of some chloroaromatics, the observed widespread distribution of at least 1,3- and 1,4-dichlorobenzene along the British and Dutch coasts and within the German Bight poses a reason of concern. Their presence in the North Sea cannot be explained by the comparatively low concentrations in river water. Atmospheric input may be a significant route for the more volatile dichlorobenzenes. A systematic investigation of sources, levels and fate appears necessary.

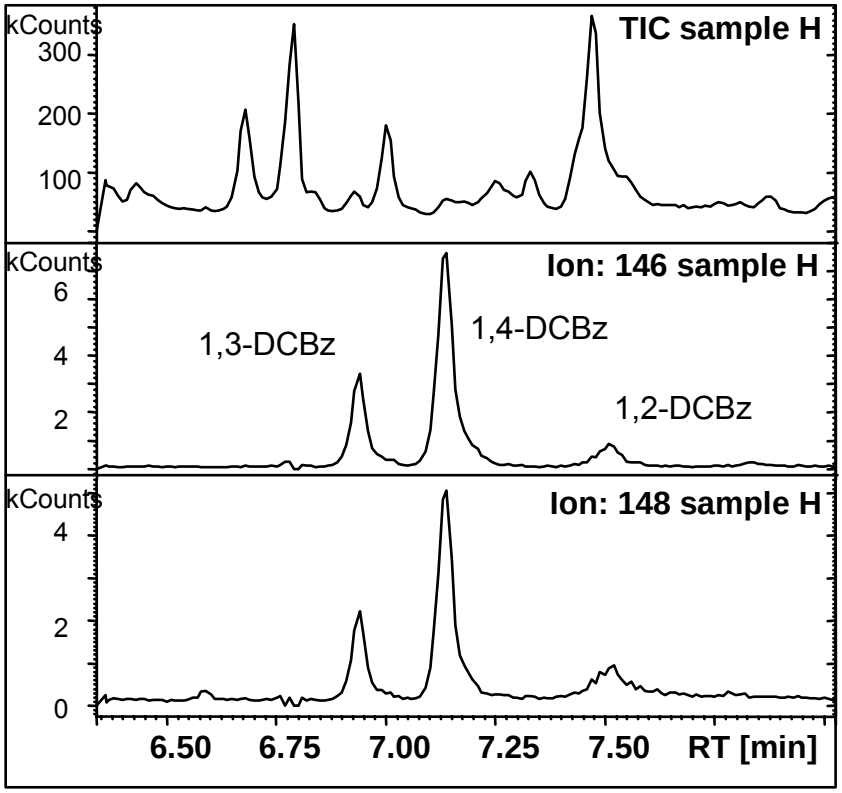

Figure 18: Chromatograms (GC-MS, TIC and extracted ion traces 146 and 148) of dichlorobenzenes (DCBz) in sample H

The detected chloronaphthalene was identified as 1-chloronaphthalene (Figure 19). This compound is used as solvent for oils, fats and DDT [133], while polychlorinated naphthalenes (PCNs), carrying 1 to 8 chlorine atoms, were produced for cable insulation, as

wood preservatives, engine oil additives, capacitors and as a feedstock for dye production, i.e., applications analogous to those of PCBs. Their production started at the beginning of the 20th century and ceased in 1977 in the USA and in the mid-1980s in Western Europe [134]. All congeners are planar compounds, structurally similar to the highly toxic 2,3,7,8-tetrachloro-dibenzodioxin (TCDD) and can contribute to an Ah receptor mediated mechanism of toxicity [135]. They share several POP characteristics with PCBs: persistence, long-range transport properties, widespread distribution in the environment and bioaccumulation potential. Thus, PCNs have been detected in various matrices (air, water, sediment, biota) [136], also in marine particulate matter and sediments [137], but have not been reported for the marine water phase yet. The identification of the most water-soluble congener 1-chloronaphthalene (log Kow = 3.9 [134]) might be an indication for the presence of further PCNs at lower concentration in the water phase and enrichment in suspended particulate matter.

The identity of nitrobenzene, chloronitrobenzene (*ortho*- and/or *para*-isomer), 2-chloroaniline and 2,5-dichloroaniline was verified by comparison with standard solutions. Their presence in North Sea water has previously been described by other authors [6,26-28], while 2-chloroaniline was only reported for sediments [111]. Obviously, nitroaromatics, chloro-nitrobenzenes and chloroanilines remain frequent contaminants of the North Sea. The same holds for chloroalkyl ethers. The two respective compounds detected in the river Elbe plume sample were identified as bis(dichloropropyl)ethers, presumably bis(2-chloro-1-chloromethyl-ethyl) ether and (2-chloro-1-chloromethylethyl)-2,3-dichloropropyl ether. The occurrence of chlorinated bispropylethers in North Sea estuaries was first reported by Weber and Ernst in 1983 [5]. Systematic investigations of this contaminant class were carried out by Franke and co-workers for the Elbe river system [138] and the German Bight [29], establishing this compound class as by-products of the industrial epichlorohydrine synthesis and rather specific for the river Elbe. Nevertheless, they were also present in river Rhine water [64], although at much lower concentrations compared to the Elbe.

The dichloropyridines (DCPys) in the coastal water sample were identified as the 2,6- and 3,5- isomer (Figure 20), while in estuarine water four isomers (2,3-, 2,5-, 2,6-, 3,5-DCPy) were present (Figure 21). The role of this compound class as contaminants of the aquatic environment has not been mentioned yet. Only a monochloropyridine was reported in a screening study of the river Rhine [64]. Therefore, they were investigated in more detail within this work (chapter 4.2). The detected chlorofluoronitrobenzene was identified as the isomer 3-chloro-4-fluoronitrobenzene (Figure 22).

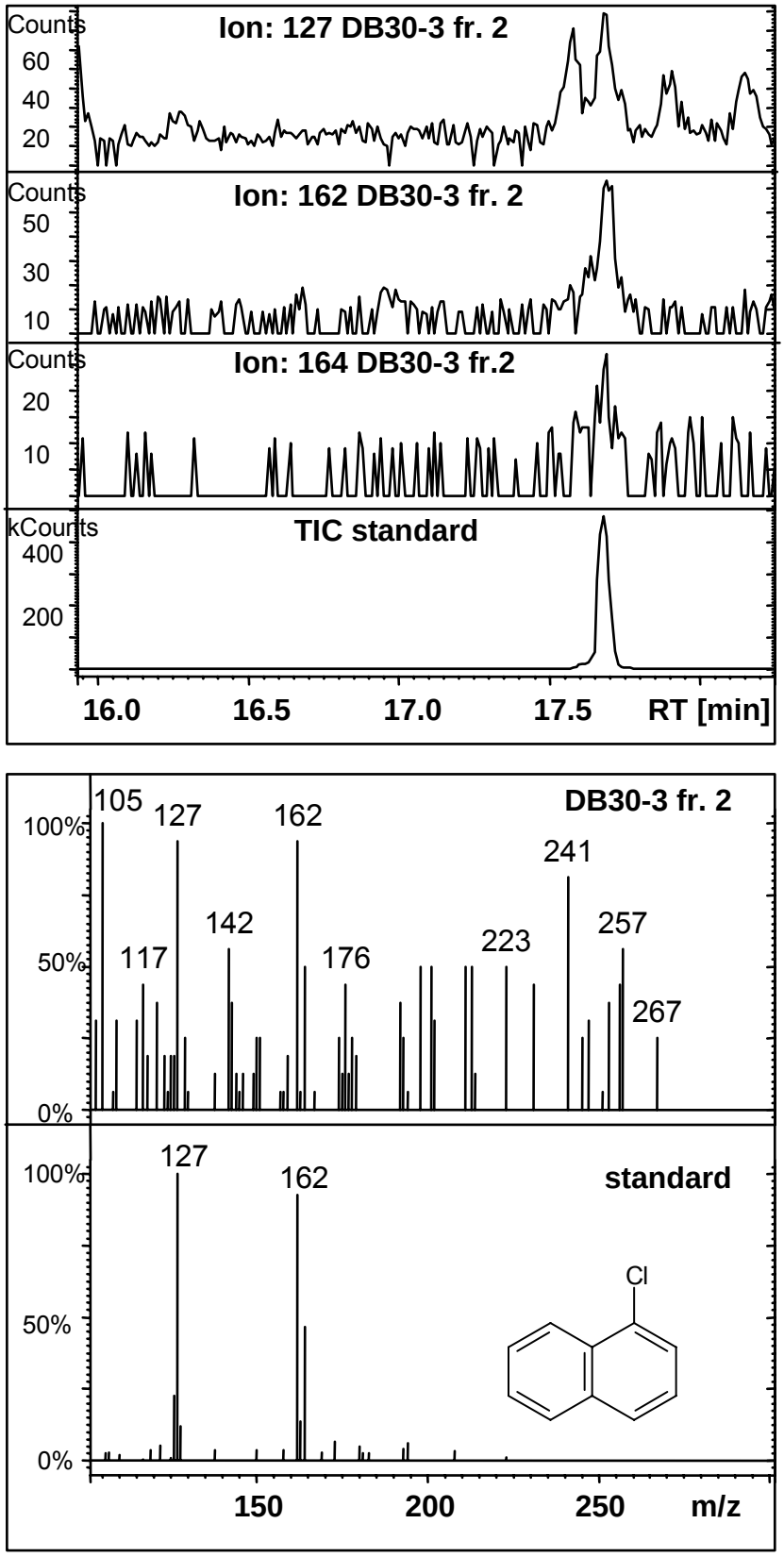

Figure 19: Verification of 1-chloronaphthalene in sample DB30-3 by chromatogram (GC-MS) and spectra (EI, 70 eV) comparison with the pure compound

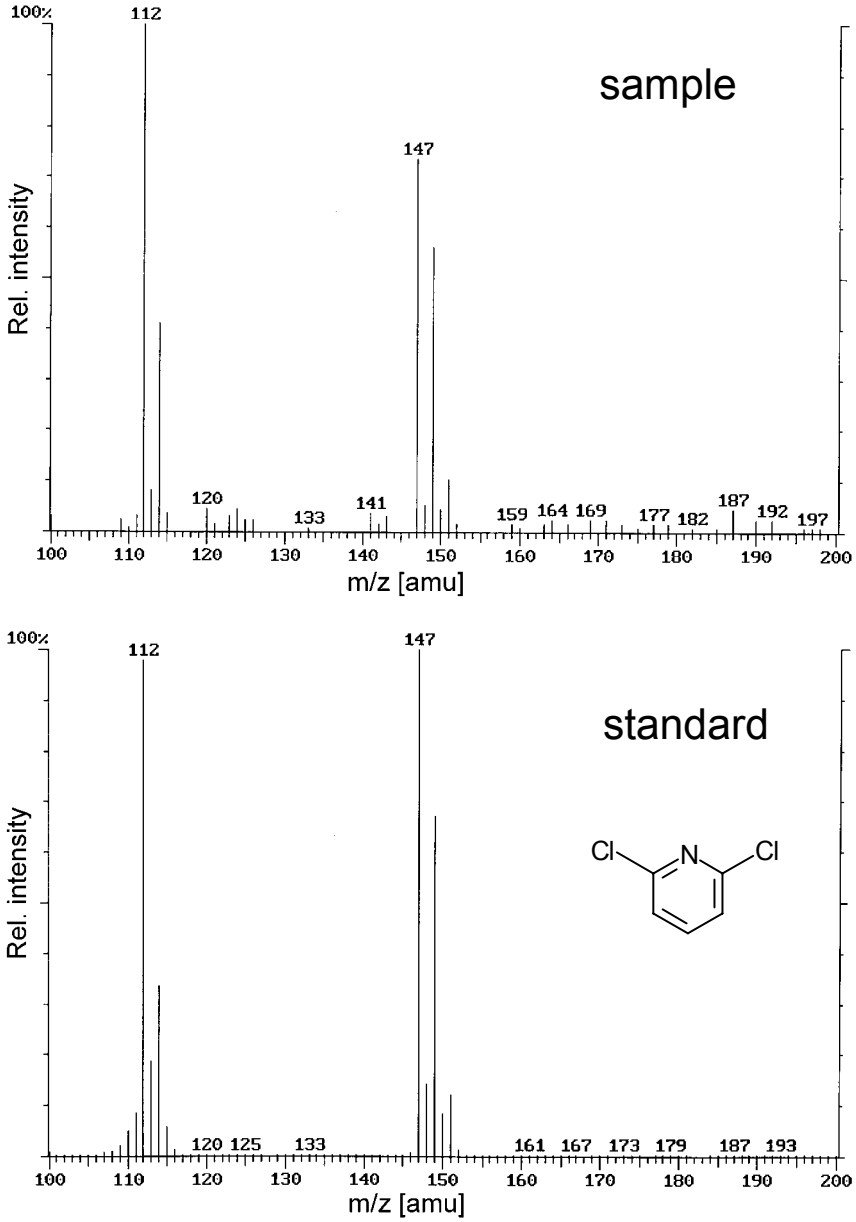

Figure 20: Mass spectra (EI, 70 eV) of 2,6-dichloropyridine obtained from a North Sea water sample (DB30-3) and from a standard solution

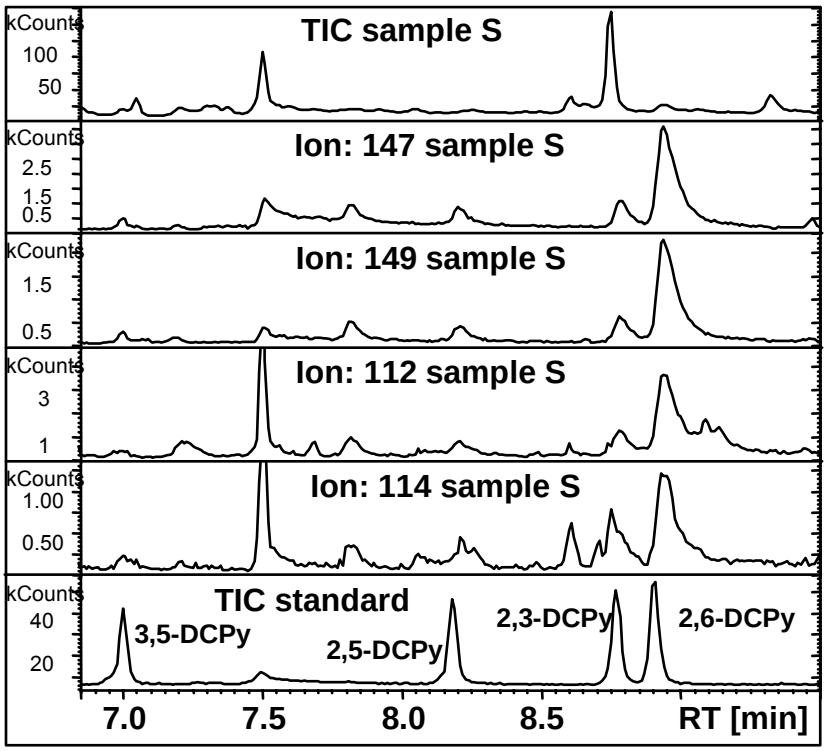

Figure 21: GC-MS chromatogram (full scan, extracted ion traces) of an estuarine water sample (S) and a dichloropyridines (DCPy) standard solution

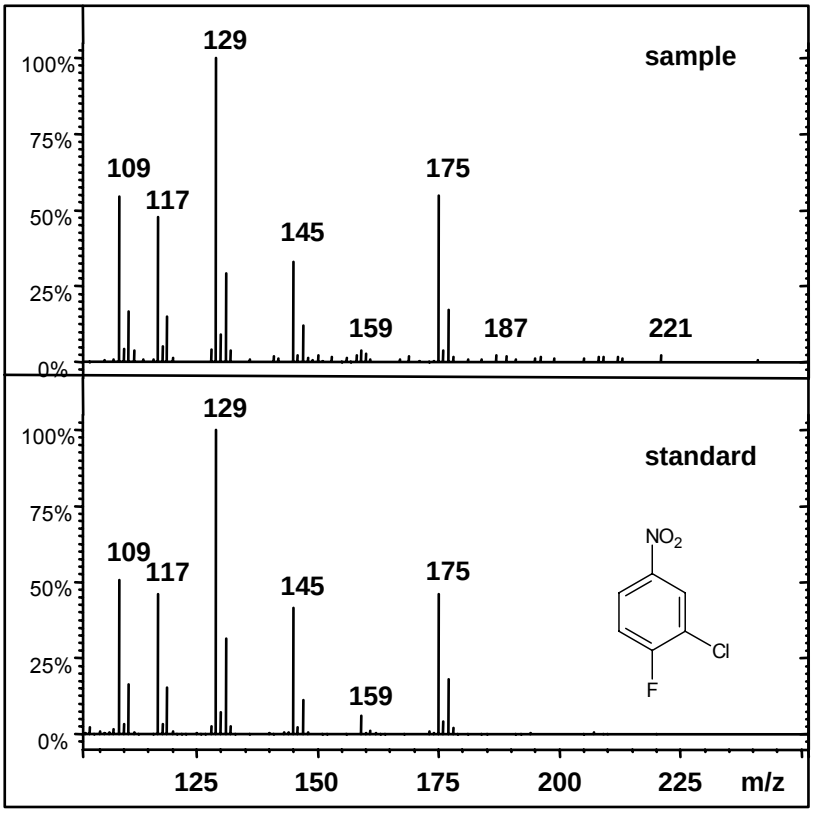

Figure 22: Mass spectra (EI, 70 eV) of 3-chloro-4-fluoronitrobenzene from sample DB30-3 and a standard solution

Tris(chloroalkyl)phosphates, being widely used as flame retardants and plasticisers, were detected in most samples. Since tris(chloroethyl)phosphates were present in blanks (although at lower levels than in most samples) they were not further regarded within this study. No tris(chloropropyl)phosphates (TCPPs; log Kow = 1.5) were detected in the blanks and, therefore, it is assumed that the amounts measured in the samples originate exclusively from the extracted seawater. Their identity was verified by comparison with spectra and retention times of a solution of a technical mixture of TCPP (Figure 23 and 24). The technical product contained two isomeric TCPPs, namely tris(2-chloro-1-methylethyl)phosphate (TCPP-1) and presumably bis(2-chloro-1-methylethyl)-2-chloropropylphosphate (TCPP-2). Both compounds were also detected in the marine samples, although in slightly different proportions.

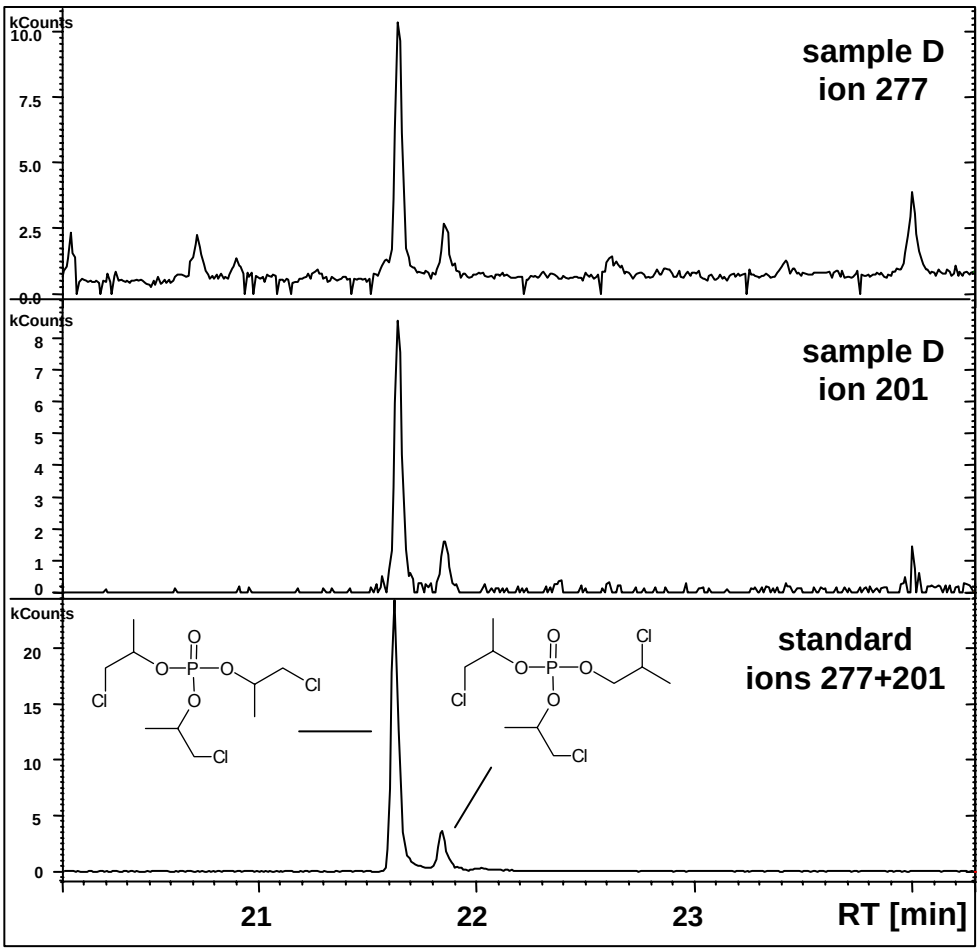

Figure 23: Chromatograms (GC-MS, full scan, extracted ion traces) of tris(chloropropyl)-phosphates (TCPPs) in a North Sea water extract (sample D) in comparison to a standard solution of technical TCPP (left peak: TCPP-1, right peak: TCPP-2 assumed structure)

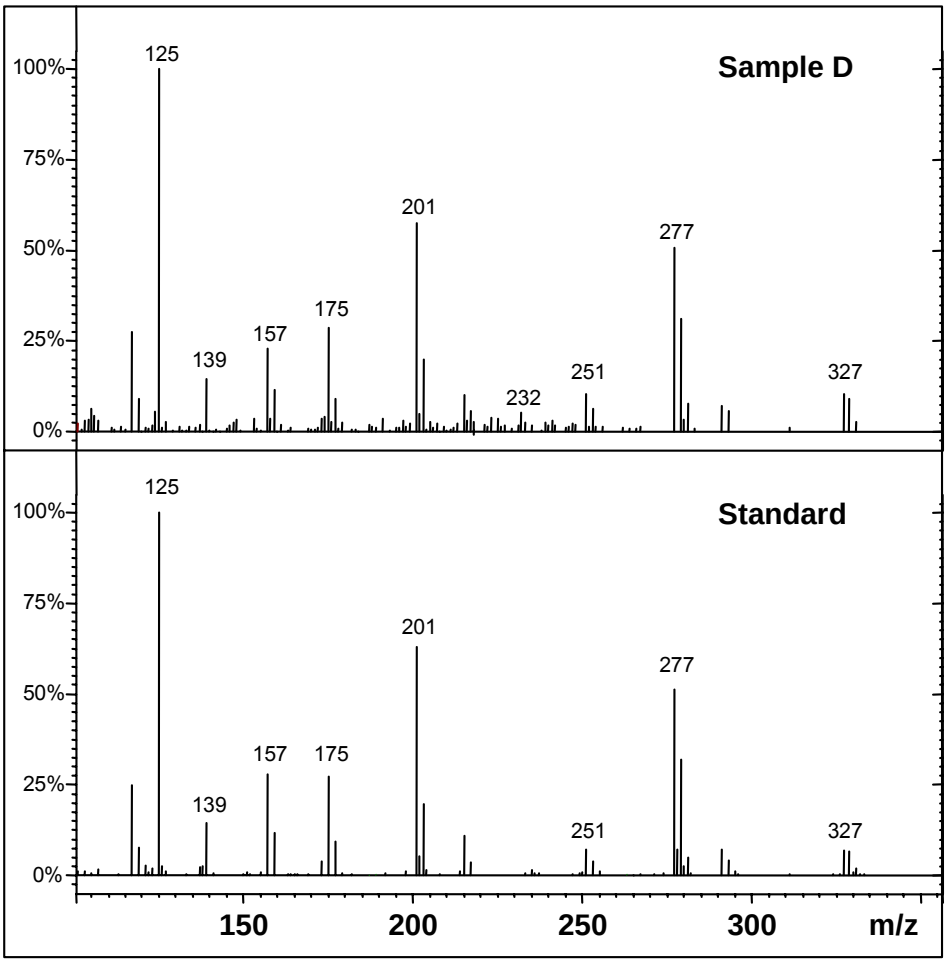

Figure 24: Mass spectra (EI, 70 eV) of tris(chloropropyl)phosphate (TCPP-1) from sample D and from a standard solution

Triphenylphoshine oxide (TPPO) was identified in several samples, mainly from the German Bight, e.g., in sample DB30-3 (Figure 25). TPPO is discharged into the Rhine at Ludwigshafen as an industrial by-product of the Wittig-reaction and consequently enters the North Sea via riverine input. Concentrations determined in the river Rhine monitoring programme were between 100 and 500 ng/L in the year 2000 [139]. In 1996, a concentration of 1 µg/L was detected in river Rhine water in the Netherlands and even in tap water of Amsterdam 50 ng/L were measured [74]. Information on the ecotoxicological impact of these relatively high concentrations is sparse. The potential of TPPO to substitute the banned triphenyltin acetate in the control of the golden apple snail (*Pomacea canaliculata* L.) was explored by Lo and Hsieh [140]. The LC_{90} of TPPO was found to be by the factor 2 higher as compared to triphenyltin acetate. The implications for marine snails remain to be investigated.

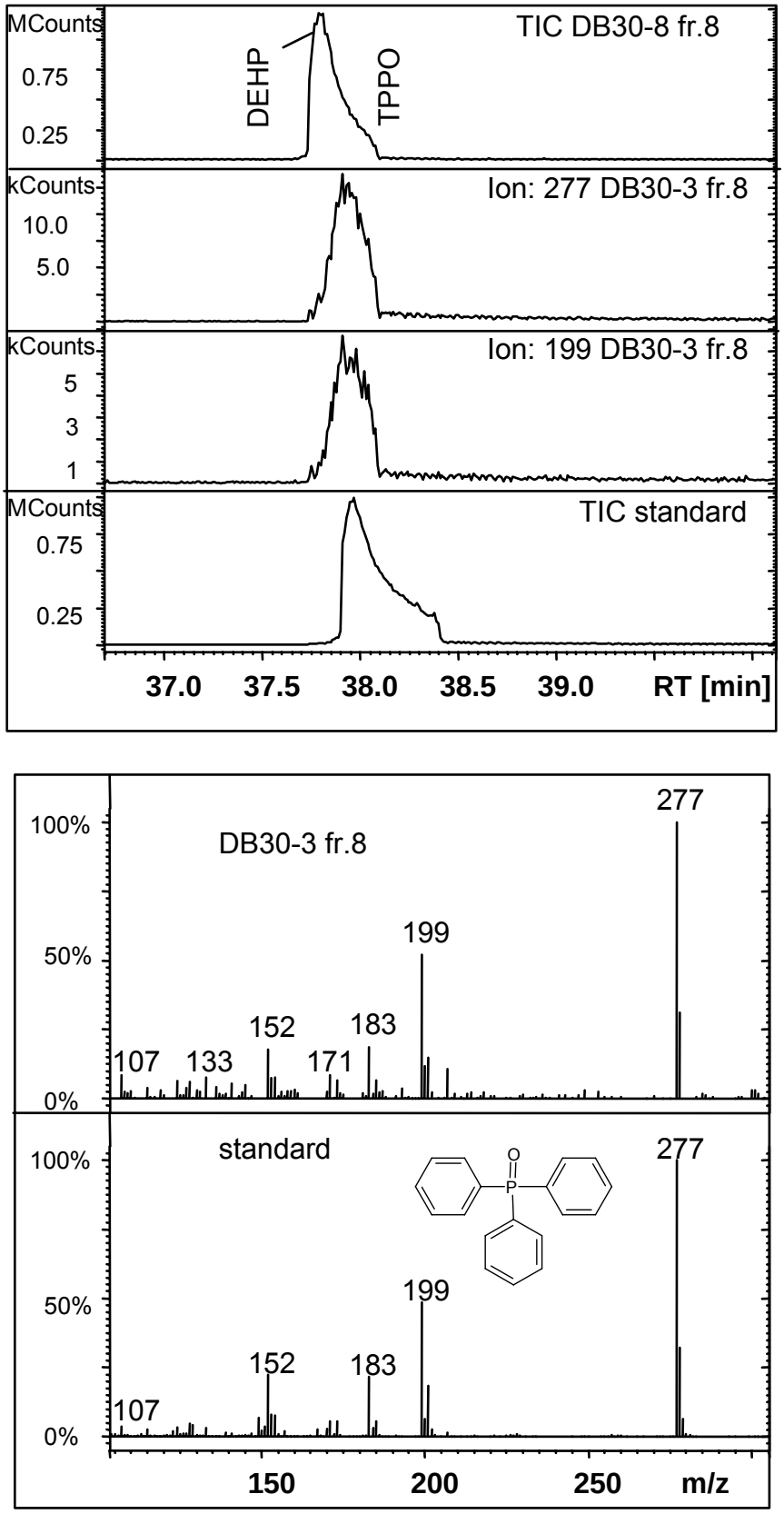

Figure 25: Comparison of GC-MS chromatograms (TIC and extracted ion traces; co-elution with di[ethylhexyl]phthalate DEHP) and spectra (EI, 70 eV) of TPPO from sample DB30-3 and a standard solution

In one sample from the Norwegian part of the North Sea considerable amounts of penta- to octachlorobiphenyls were found (Figure 26). The origin of the PCBs detected in this samples remains unknown. Shipboard or laboratory contamination can be ruled out since no PCBs were detected in any other sample including procedural blanks. Therefore, local discharge or illegal dumping from ships might be a possible source. Furthermore, *N*-ethyltoluidine and morpholinylbenzothiazole were detected frequently.

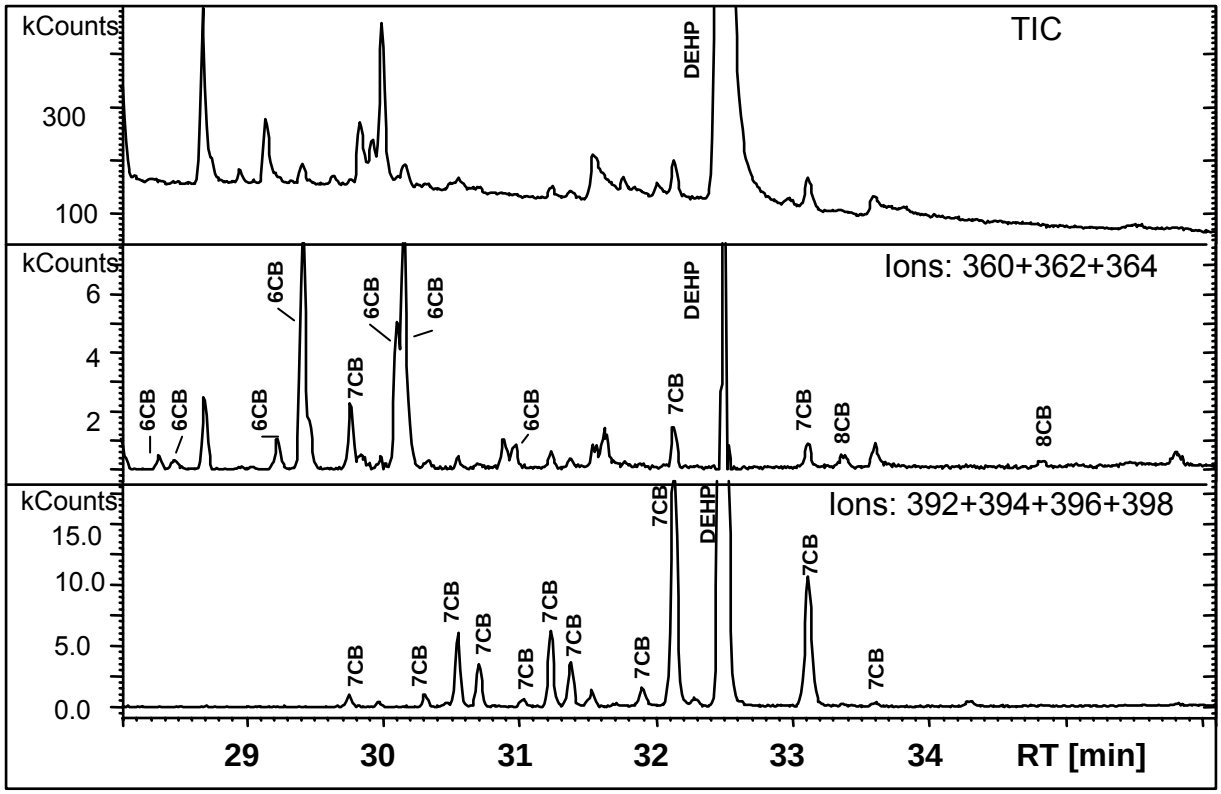

Figure 26: GC-MS chromatogram (TIC and extracted ion traces) of some hexa- and hepta-chlorobiphenyls (6CB and 7CB) in sample M

3.3.3 Pharmaceuticals

Caffeine which was unambiguously identified in most samples is treated in depth in chapter 4.3. The analgesic propyphenazone and the antiepileptic carbamazepine were only identified in sample DB30-3, close to the detection limit (Figures 27 and 28). Obviously, in the case of these compounds the clean-up that is achieved by the silica fractionation is necessary for the identification. Carbamazepine is known to be present in considerable concentrations in river Rhine water (e.g., 50 - 300 ng/L in the year 2001 at Cologne [139]), while concentrations in the lower and middle Elbe only ranged between 60 and 70 ng/L in 1998 [141]. This compound is not very prone to degradation in sewage treatment plants [36] and thus not assumed to undergo extended transformation under environmental conditions. Therefore, it is expected to be present also in the Rhine plume. However, carbamazepine was not detected in the (unfractionated) sample E, neither in the total ion chromatogram nor in the specific extracted ion chromatograms (m/z = 193 and 236 amu). Taking into account the dilution resulting from the distance of sampling position E from the mouth of the river Rhine and the fact that the concentration of carbamazepine in sample DB30-3, right in the inner river Elbe plume, was already very close to the limit of detection, it is not surprising that this substance cannot be detected in sample E without an adequate clean-up step. The compound identified in fraction 3 of sample DB30-3 as the expectorant anethole (1-methoxy-4-propenylbenzene) with a very high match of sample and library spectra could not be verified in comparison to the retention time of the original substance. Most likely, the detected compound is a different isomer of this anisole. Furthermore, qualitative investigations of the derivatised methanolic SPE-eluate revealed the presence of clofibric acid in sample DB-30-3 and of clofibric acid and diclofenac in a sample from the Elbe estuary at Stade [113].

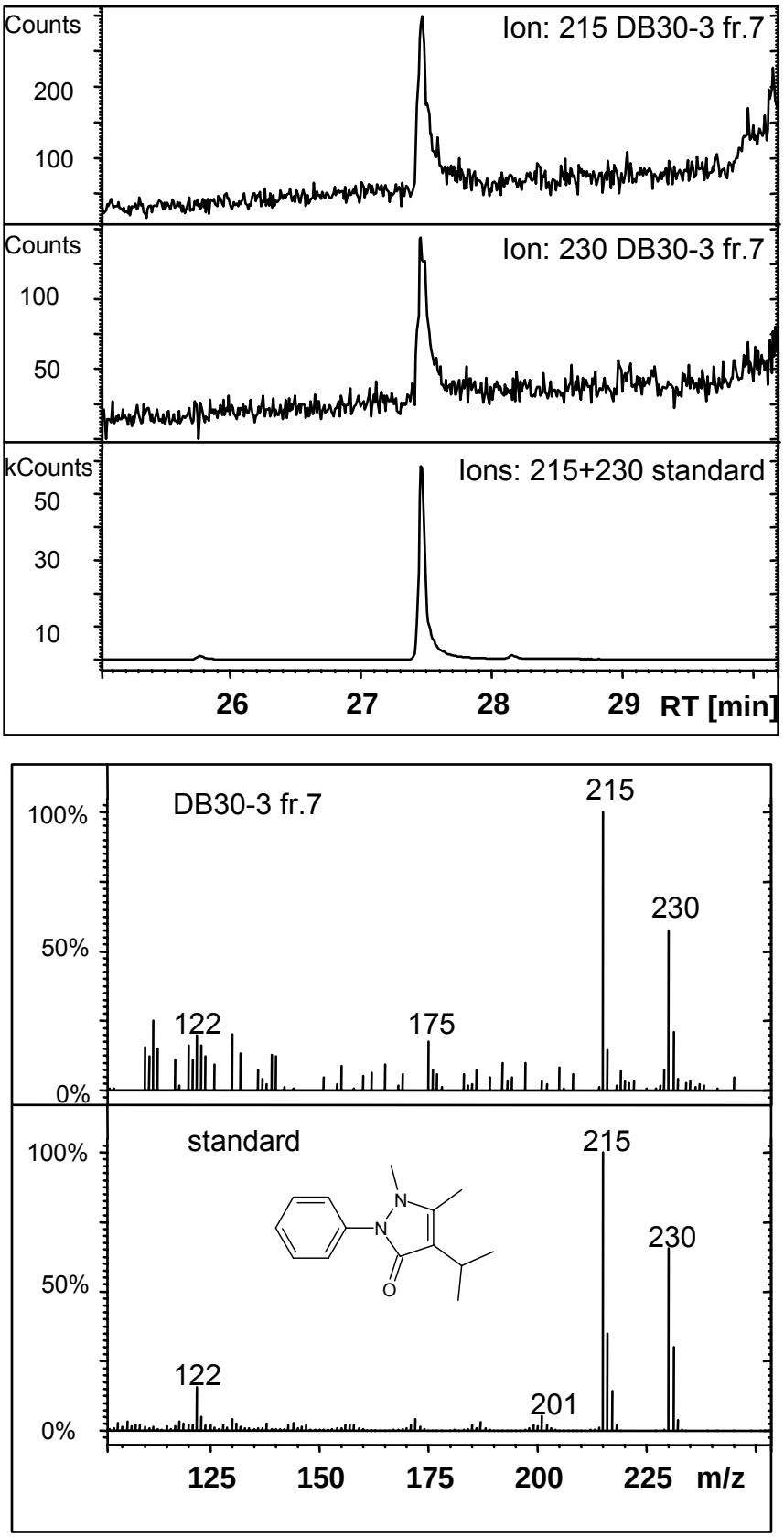

Figure 27: Chromatogram (GC-MS, full scan, extracted ion traces) and spectrum (EI, 70 eV) of propyphenazone identified in fraction 7 of sample DB30-3 in comparison to a standard solution

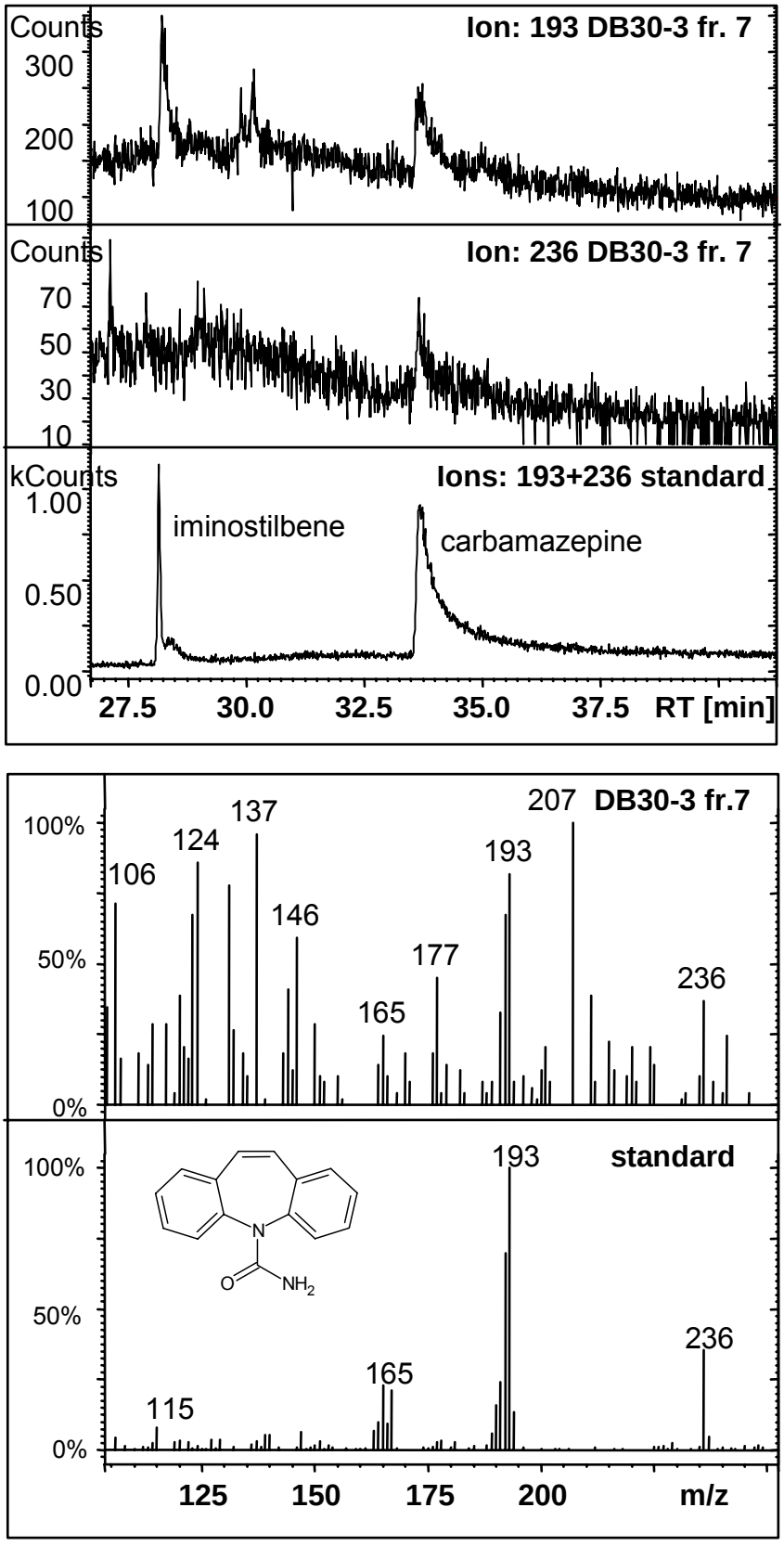

Figure 28: Chromatogram (GC-MS, full scan, extracted ion traces) of carbamazepine and its GC-artefact iminostilbene and spectrum (EI, 70 eV) of carbamazepine in fraction 7 of sample DB30-3 in comparison to a standard solution

3.3.4 Bromoorganic compounds

Anthropogenic organobromine compounds, mainly brominated flame retardants (BFRs) as for example polybrominated diphenylethers (PBDEs) have come into the focus of scientific and public discussions because of their bioaccumulation properties and toxicological potential [142]. However, organobromine chemicals are also produced naturally, mainly by marine animals (sponges, tunicates, worms, corals), plants (seaweed, algae), bacteria and fungi. More than 1600 different biogenic organobromine compounds are known so far [143,144]. Obviously, a substantial part of these organobromine substances is emitted into the surrounding water. In some of the samples investigated in this work, organobromines which were identified by their spectra as mono-, di- and tribromoindoles (Figure 29) contributed major peaks to the corresponding chromatograms (Figure 30). Further identified compounds comprised bromophenols and -anisoles, while others are still to be elucidated, for example a series of three isomeric compounds with spectra strongly resembling bromodimethoxy-benzenes (Figure 32), but with different retention times. In some cases, a distinction between anthropogenic and biogenic origin is difficult since some compounds (e.g., bromophenols) are produced both naturally and industrially, anthropogenic compounds might be cleaved to naturally occurring substances (e.g., PBDEs to bromophenols) or anthropogenic organochlorine compounds might be transhalogenated to their bromo-derivatives under marine conditions (and vice versa). Assessing the contributions and the ecotoxicological impact of biogenic, anthropogenic and mixed bio/anthropogenic organobromine compounds has started to become an important issue in marine research, with first results being currently presented [145,146].

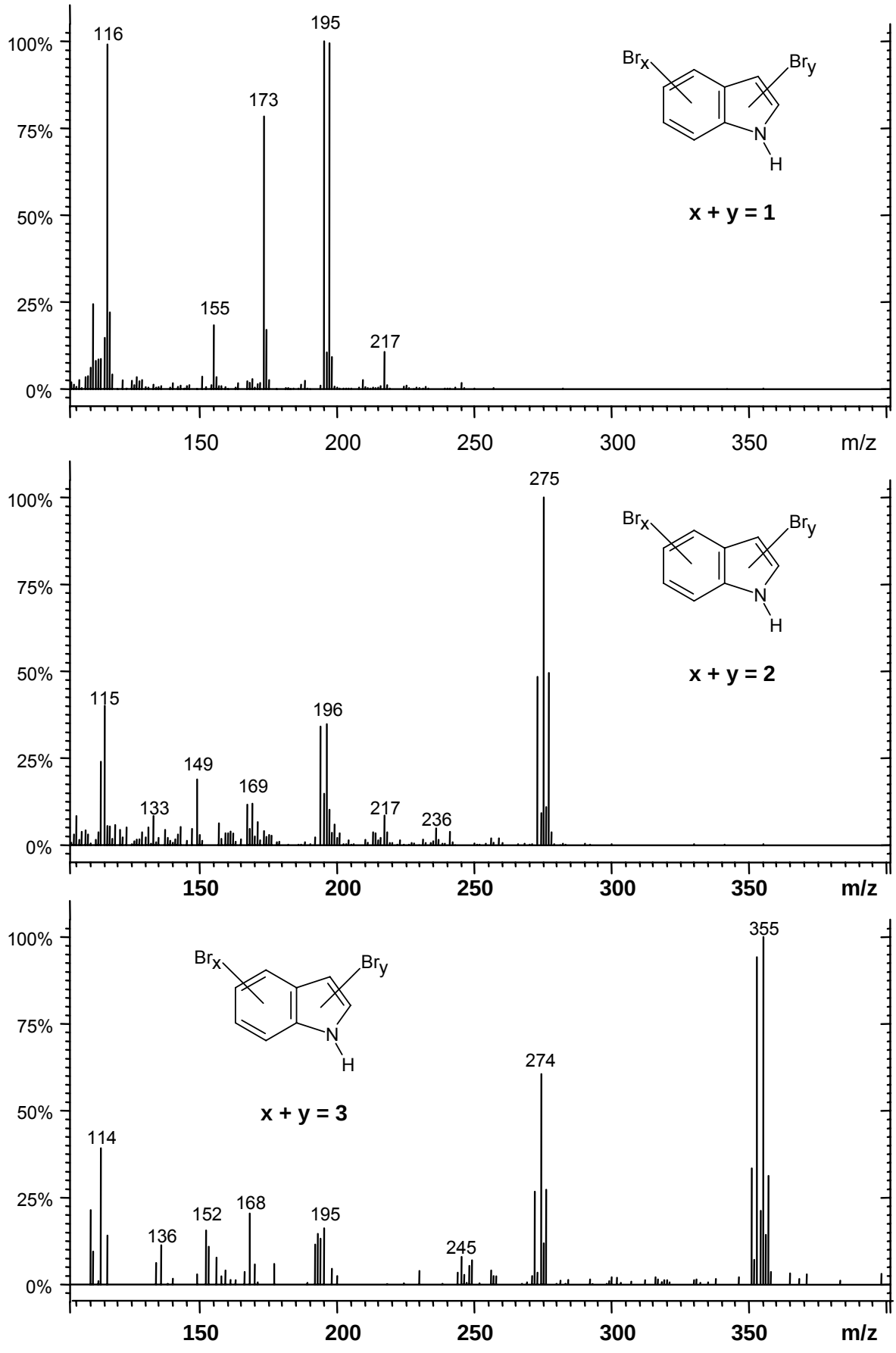

Figure 29: Mass spectra (EI, 70 eV) of mono-, di- and tribromoindoles in a North Sea water extract (sample G)

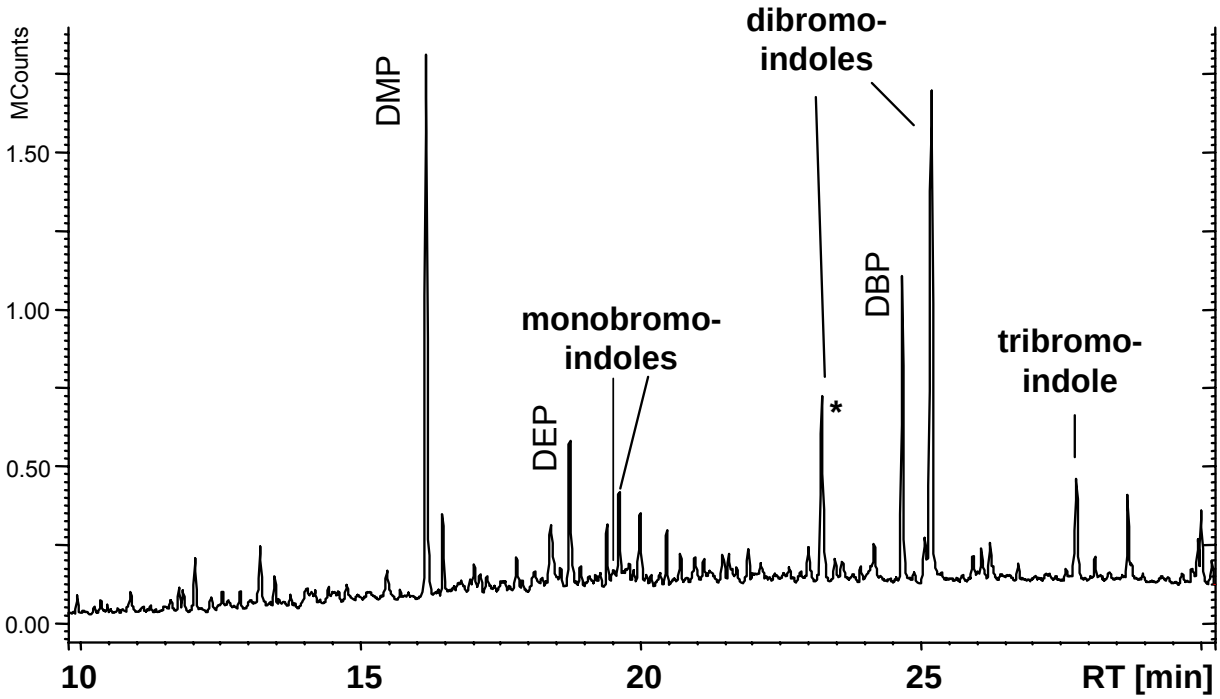

Figure 30: Bromoindoles in the GC chromatogram of the extract of the sample G (* co-elution with di[2-methylpropyl]phthalate, peak area ratio phthalate/dibromoindole = 2:1; DMP = dimethyl-, DEP = diethyl-, DBP = dibutyl-phthalate)

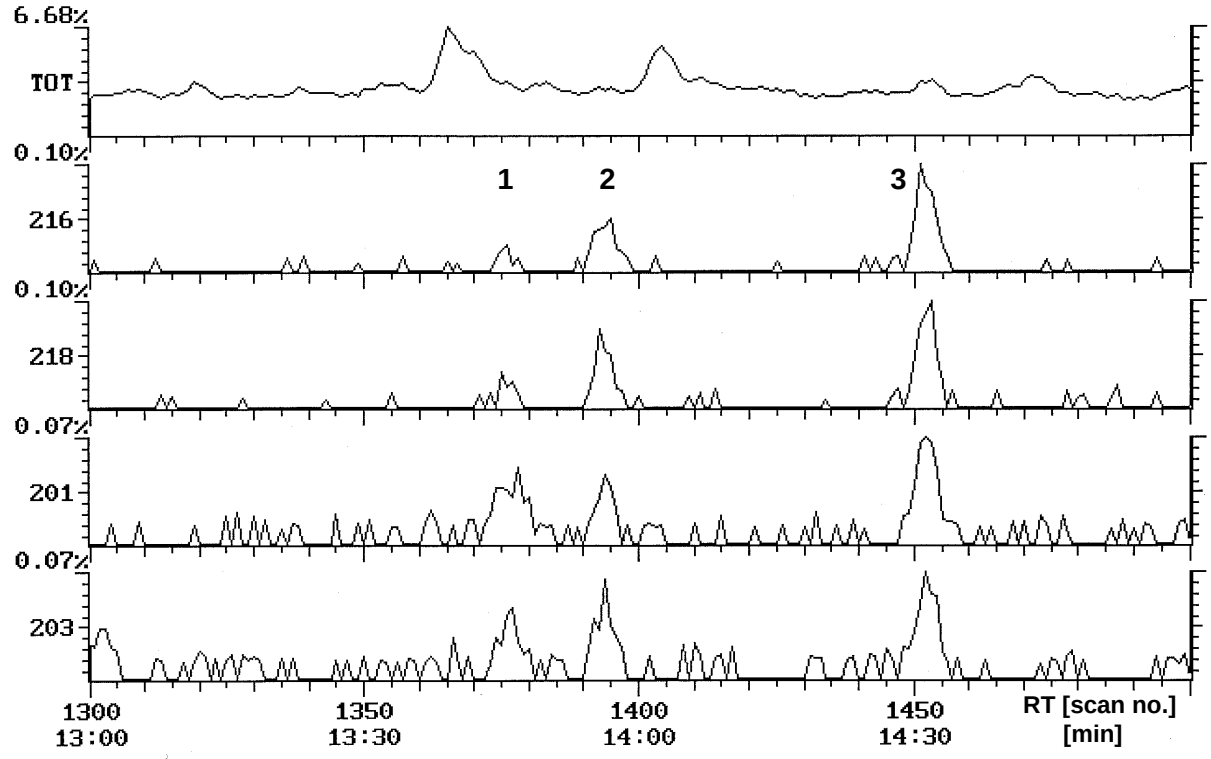

Figure 31: GC chromatogram (TIC) of a sample extract (DB30-3) from the German Bight including extracted ion traces of three unknown isomeric organobromine compounds

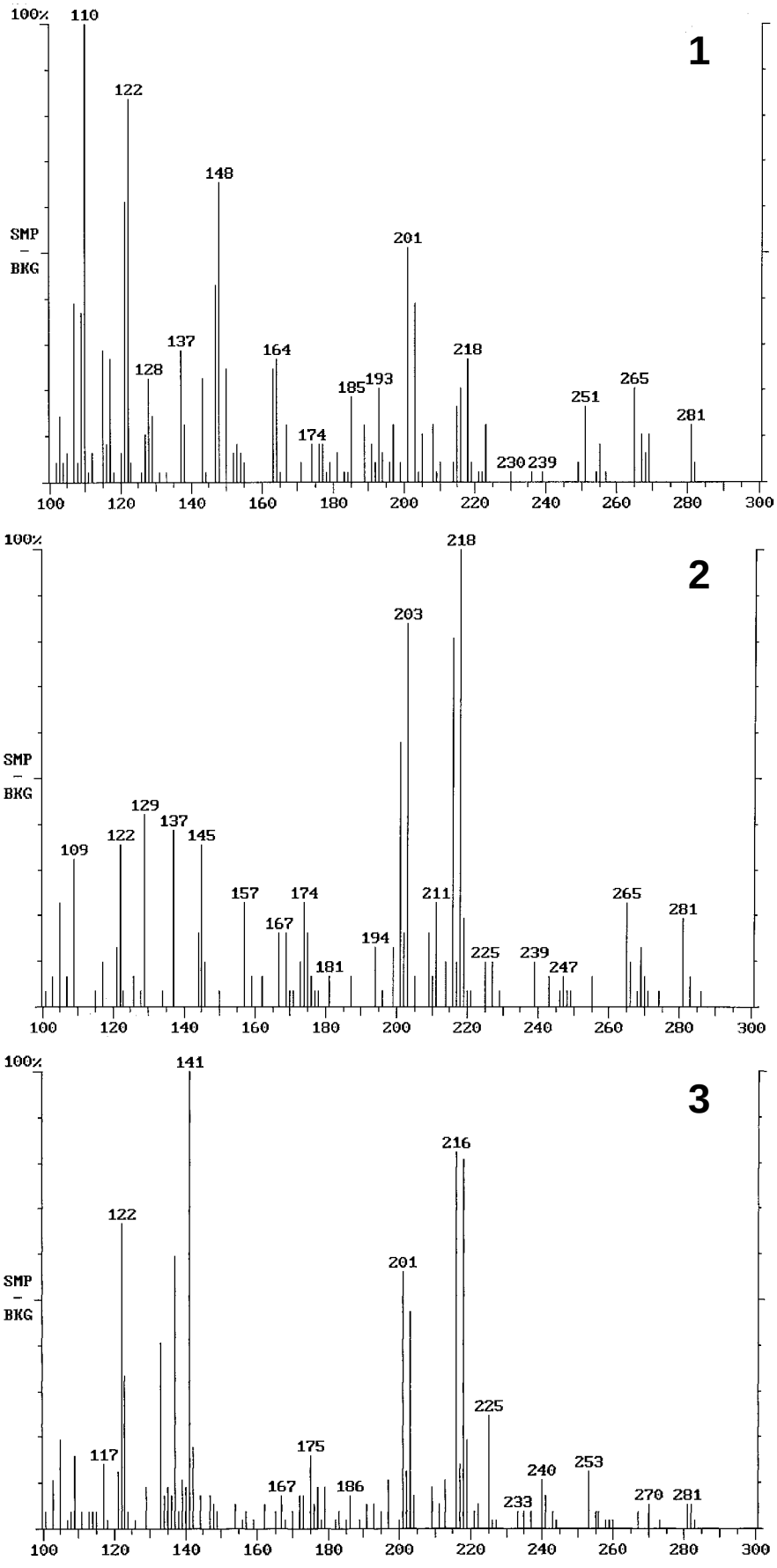

Figure 32: Mass spectra (EI, 70 eV) of three unknown isomeric organobromine compounds in a sample extract (DB30-3) from the German Bight (numbering according to chromatogram in Figure 31)

3.4 Tromsø-Sound

The situation in the Tromsø-Sound strongly differs from that in the German Bight/North Sea. It is characterised by tidal exchange with relatively clean water from the North Atlantic/Arctic Ocean and direct emissions of communal sewage and from industrial sources (fish processing, harbour/shipyard activities, brewery, dairy, cement production) into the Sound. Water temperatures, suspended particulate matter content and biological activity are lower than in the North Sea. Therefore, the composition of the organic fraction can be expected to be quite different. A limited non-target screening was performed for selected samples from areas expected to be influenced by sewage emissions. These were the Breivika harbour area (samples KHA and KHB,) in the vicinity of the outfall of the sewer pipe that discharges mixed communal, hospital and commercial wastewaters, the central harbour (mixed marina and commercial port, shipyard), exposed to effluents and partly protected from the tidal current (sample HC) and an harbour area closer to the outfall of the central sewage treatment plant (sample HS). The main goal was the identification of potential target analytes for the subsequent study on PPCPs in the marine environment (chapter 5).

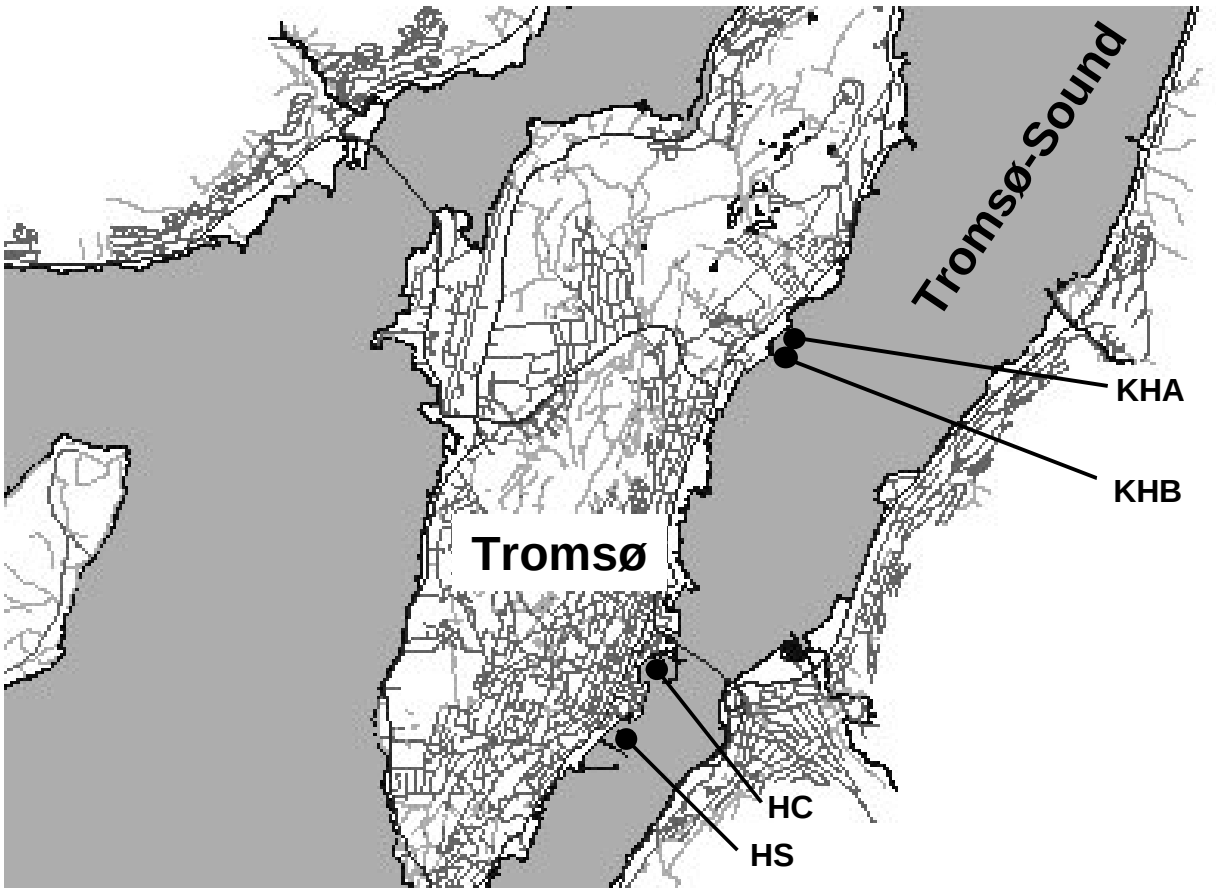

Figure 33: Sampling points of samples KHA, KHB, HC, HS within the Tromsø-Sound/Norway

The samples HS, HC, KHA were treated as outlined in Figure 5, whereas sample KHB after SPE was directly eluted with methanol to obtain a maximum yield of potentially present hospital related acidic drugs. The obtained sample extracts were subjected to GC-MS full scan measurements. Identification results based on mass spectral library search (NIST; TOX) are presented in Table 17. The sample composition was dominated by hydrocarbons (alkylbenzenes, aliphatic hydrocarbons, PAHs) presumably originating from shipping/port activities and by a class of substances that were not properly identified, probably alkanones. These compounds led to a high matrix in the GC-MS chromatograms, which disturbed the identification of other substances at ultra-trace levels. An appropriate clean-up would have to be carried out for the enhanced elucidation of the sample composition. However, since the aim of the screening was the identification of pharmaceutical related compounds present at outstanding concentrations no further clean-up was undertaken. Among the library proposals, paracetamol and dichlorobenzoic acid are of interest with regard to the current project. Paracetamol is a heavily used over-the-counter analgesic. In contrast to central Europe, where paracetamol is eliminated rather completely during sewage treatment and easily biodegraded in surface water, no biological sewage treatment is performed in Tromsø and biodegradation in the seawater around the island is expected to be low because of low water temperatures (around 10 °C/274 K in summer). This might lead to considerable concentrations. The presence of dichlorobenzoic acid was verified by comparison with pure reference compounds. The compound was identified by spectrum and retention time as the 2,4-isomer, which is used as herbicide and as intermediate in the chemical industry. Both sources are not very likely at the sampling area. 2,4-Dichlorobenzoic acid was also reported to be formed naturally [147] and to be released from silicone tubing as a transformation product of bis(2,4-dichlorobenzoyl)peroxide used in silicone production [148]. Since the samples did not get in contact with silicone tubing and dichlorobenzoic acid was neither detected in procedural blanks nor in any samples from other areas within this sampling campaign, the substance is likely to originate from the seawater at this spot. The exclusive presence in the samples taken close to the sewage outlet near the hospital might indicate a correlation. An interesting feature is the obvious presence of several benzoic acid derivatives, among them parabenes which are used as preservatives in personal care products, in the investigated samples.

Sample	Library proposals
KHA *n*-hexane-fraction	- various PAHs, alkyl-PAHs, alkylbenzenes, alkylphenols, alkanones - parvifuran - ethylphenoxybenzene - tetrachloronaphtalene
KHA ethyl acetate-fraction	- various alkylbenzenes, some PAHs - benzoic acid alkyl esters - phenylbutenone - trimethylphenylethanone - difluorohydroxybenzeneethanamine - paracetamol - butylhydroxyanisol (BHA) (significantly more than in blanks) - alkylphenols - thiophene derivative - isopropylmyristate - caffeine - parvifuran
KHA methanol-fraction, derivatised with methyl chloromethanoate (methylation)	- methyl dichlorobenzoate - methyl acetylaminophenylethanoate - methyl alkylbenzoate - indolecarboxylic acid derivative - alkylpentanedioic acid ester - alkylphenol - propylparaben methyl ester - various fatty acid methyl esters (FAMEs)
KHB methanolic total eluate, derivatised with methyl chloromethanoate (methylation)	- methylbenzeneethanol - paracetamol - methyl dichlorobenzoate - butylhydroxyanisol (BHA) (significantly more than in blanks) - alkylphenols - chloromethylbenzothiazole - alkylthiophene - methyl acetylaminophenylethanoate - FAMEs - butylcitrate
HC *n*-hexane-fraction	- various PAHs, alkyl-PAHs, alkylbenzenes, alkylphenols, alkanones - parvifuran
HC ethyl acetate-fraction	- some PAHs, alkyl-PAHs, alkylphenols, alkanones - dimethylbutenylfluorobenzene - dibrominated compound (not identified) - triacetine - methyl dichlorobutanoate - thiophene derivative - chlorinated compound (not identified) - methyltryptamine - caffeine - dimethylaminophenylpyridine - alkyl benzoate - *N*-propylbenzamide

HC methanol-fraction, derivatised with methyl chloromethanoate (methylation)	- bromomethyldihydronaphthalene - methyl chlorohydroxybenzoate - pentachloropropane - benzoic acid derivative - nonylphenols - FAMEs
HS *n*-hexane-fraction	- various PAHs, alkyl-PAHs, alkylbenzenes, alkylphenols, alkanones - parvifuran - ethylphenoxybenzene - nitroethylcarbazole
HS ethyl acetate-fraction	- some PAHs, alkyl-PAHs, alkylphenols - dibrominated compound (same as in HC) - difluorohydroxybenzeneethanamine - *N*-acetyl-*N*-hydroxyphenylbutaneamide - caffeine - diphenylbutandione
HS methanol-fraction, derivatised with methyl chloromethanoate (methylation)	- methyl *tert*-butylbenzoate - benzoic acid derivative - hexanedioic acid alkyl ester - several FAMEs - alkylbenzenesulfonate methyl ester

Table 17: Substances identified by mass spectral library search in different fractions of seawater samples from Tromsø-Sound

3.5 Conclusions

As shown by the results presented in this chapter, the composition of the dissolved organic fraction of the investigated seawater samples is expectedly of a high complexity, resulting from biogenic, anthropogenic and geogenic contributions. This holds even though the proportion of the dissolved organic carbon (DOC), that is accessible by the applied methodology hardly represents more than 10 % of the substances actually present in the samples. Thermolabile, non-volatile and macromolecules cannot be covered by GC-MS measurements. Nevertheless, the applied extraction technology extends the coverage of organic substances in terms of hydrophilicity far beyond what was accessible by the formerly used liquid/liquid extraction and also first SPE approaches with alkylsilica sorbents. A first step to reduce the complexity of the samples was an eight step fractionation. While for the non-polar fractions a sufficient separation was achieved, the more polar fractions still showed a high matrix composed of a variety of organo-nitrogen and organo-oxygen compounds, thus masking a significant number of substances expected to be present in the samples and detectable using target analyte specific extracted ion traces, but not in the TIC. Nevertheless, the aim of the conducted screening was a first survey on anthropogenic chemicals, especially substances of high polarity, that were excluded because of their hydrophilic nature by former approaches or simply ignored in previous investigations. In view of this objective, the screening revealed the presence of a substantial number of compounds which have not

been reported before for the North Sea or marine waters in general. Some of these were then chosen for detailed quantitative investigations. It is important to note that in the fractionated and even in the non-fractionated extracts a large number of substances was detected with sufficiently pure spectra but not identified yet, among them a substantial number of organochlorine and -bromine compounds that are more easily accessible to identification by low resolution mass spectrometry than organonitrogen compounds due to their characteristic isotope patterns. While for some limnic systems rather comprehensive screening studies have been presented (e.g., Rhine [64], Elbe [63], Odra [149]), the data situation for the North Sea is far from being complete.

Besides a number of single compounds noticeable in the samples from the German Bight and the North Sea, several classes of organic chemicals were encountered to contribute to the contamination of the North Sea, deserving further attention. These were: chlorobenzenes, polar pesticides and their transformation products (triazines, acetanilides, phenylureas), phthalate and alkyl/aryl-phosphate plasticisers, chloroalkylphosphates, chloroalkylethers, nitro- and chloronitrobenzenes, chloro- and alkylanilines as well as caffeine and pharmaceuticals. Furthermore, also presumably biogenic organobromine compounds significantly contributed to the DOC at some sampling locations. While for the triazines, the chloroalkylethers and the nitro- and chloronitrobenzenes some data is available, priority research need is indicated for the other classes. Out of these, caffeine and pharmaceuticals were selected for further investigation of their presence and behaviour in the (marine) environment (chapter 5).

4 Quantification of selected compounds in the North Sea

Out of the large number of organic compounds identified in seawater extracts as described in chapter 3, or known to be present in the North Sea from other investigations and those assumed to enter this marine area according to published emission, stability and riverine monitoring data, only some representatives from the groups of pesticides, industrial chemicals and pharmaceutical agents were chosen for a study on their concentration levels and distribution in the North Sea. The selection did not aim at a complete and systematic coverage of certain chemical or application classes. One aim was the demonstration of the suitability of the applied analytical methodology for the detection and quantification of a wide range of chemical contaminants, especially those of comparatively high polarity (log Kow < 3). A second aim was to highlight the presence and distribution of contaminants that have not yet gained much attention within marine analytical chemistry, also serving as input for the development of priority substance lists within the framework of international agreements as the Oslo and Paris Commission for the Protection of the Marine Environment of the North-East Atlantic (OSPARCOM) and the European Community (EC) Water Framework Directive. The following compounds were submitted to detailed quantitative investigations:

- **Pesticides**: dichlobenil, metolachlor, terbuthylazine, desethylatrazine, parathion-methyl and pirimicarb
- **Industrial chemicals**: dichloropyridines, nitrobenzene, 3-chloro-4-fluoronitrobenzene and tris(chloropropyl)phosphates
- **Pharmaceuticals and personal care products**: clofibric acid, diclofenac, ibuprofen, ketoprofen, propyphenazone, caffeine and DEET.

4.1 Pesticides

Dichlobenil (log Kow = 2.5) was detected in all analysed samples (Table 18). The highest concentration (1.42 ng/L) was observed at station E, then decreasing in the course of further dilution of the river Rhine plume with less contaminated Central North Sea water (D: 1.14 ng/L, K: 0.42 ng/L) (Figure 34). At the other investigated North Sea stations, dichlobenil was rather evenly distributed at concentrations in the range of 0.3 to 0.4 ng/L. Only at station L, highly influenced by inflow of North Atlantic water, the measured concentration was below the LOQ, but is increasing along the British Coast to 0.33 ng/L. The observed distribution pattern indicates that the river Rhine might be a major source of dichlobenil in the North Sea, while at the same time this compound appears to be rather stable under marine conditions - or that atmospheric inputs contribute to the observed concentrations. In the latter case a possible source might be waste incineration. Chlorinated benzonitriles were reported as constituents of flue gases [150-152]. However, in this case the presence of further isomers could be expected. The analysis of the respective chromatograms on the dichlorobenzonitrile-specific ion traces gave no hints for the presence of further isomers. Volatilisation due to agricultural application and from soil was also observed [153,154]. Being used as herbicide in fruit and wine yards and as total herbicide on non-agricultural land it was reported to be present in groundwater of application areas - but mainly in the form of its more stable trans-formation product 2,6-dichlorobenzamide. Within the river Rhine monitoring programme of German Rhine waterworks dichlobenil was not detected above the LOQ of 50 ng/L during recent years [155]. Hendriks et al. [64] reported dichlobenil concentrations between 20 and 26 ng/L in the year 1989 from the river Rhine delta, while in an extensive non-target screening of the river Elbe and its tributaries [63] the presence of dichlobenil was not mentioned so that the sources of the widespread distribution of this compound in the North Sea remain uncertain. Dichlobenil was even registered as a constituent of an antifouling coating in a patent [156], but is not approved for use in antifouling coatings in UK for example [129]. Apparently, dichlobenil concentrations have only slightly declined within the last 20 years. The compound was reported to be present in the German Bight in 1982 in concentrations of 0.8 (East Friesian coast), 0.6 (North Friesian coast) and 1.8 ng/L (north-east of Heligoland) [157] and thus at similar levels and distribution patterns as determined in this work.

Table 18: Concentrations [ng/L] of quantified pesticides and industrial chemicals in the North Sea, corrected for recovery rates (nd = not detected, na = not analysed, co = co-elution)

Area	Off British coast			German Bight				Danish coast	Skagerrak	Off Norwegian coast		Southern Central North Sea			River Elbe (Stade)	
Sample	L	H	G	E	F	D	C	K	O	N	M	B	A	J	S	Blank
3,5-DCPy	nd	nd	nd	na	nd	nd	nd	nd	na	nd	na	nd	nd	na	< 0.11	na
2,5-DCPy	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.86	nd
2,3-DCPy	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.76	nd
2,6-DCPy	< 0.07	0.13	0.10	nd	nd	nd	nd	nd	0.11	< 0.07	< 0.07	< 0.07	nd	< 0.07	8.76	nd
Nitrobenzene	nd	0.26	1.60	1.02	0.13	3.53	4.37	2.55	1.91	1.00	1.47	2.48	0.67	0.75	co	nd
3-Chloro-4-fluoronitrobenzene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.21	nd	nd	0.47	nd	0.04
Dichlobenil	< 0.03	0.17	0.33	1.42	0.14	1.14	0.46	0.42	0.40	0.24	0.25	0.39	0.34	0.33	co	nd
Desethylatrazine	nd	nd	< 0.11	nd	nd	nd	nd	nd	0.31	nd	0.38	0.23	nd	nd	13.53	nd
Terbuthylazine	nd	nd	< 0.03	0.69	0.19	0.83	0.64	0.50	0.12	0.20	0.16	0.05	nd	nd	14.55	nd
Pirimicarb	nd	nd	0.70	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.67	nd
Parathion-methyl	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Metolachlor	nd	nd	nd	0.36	0.25	0.61	0.26	0.20	nd	0.10	0.07	< 0.03	nd	nd	4.15	nd

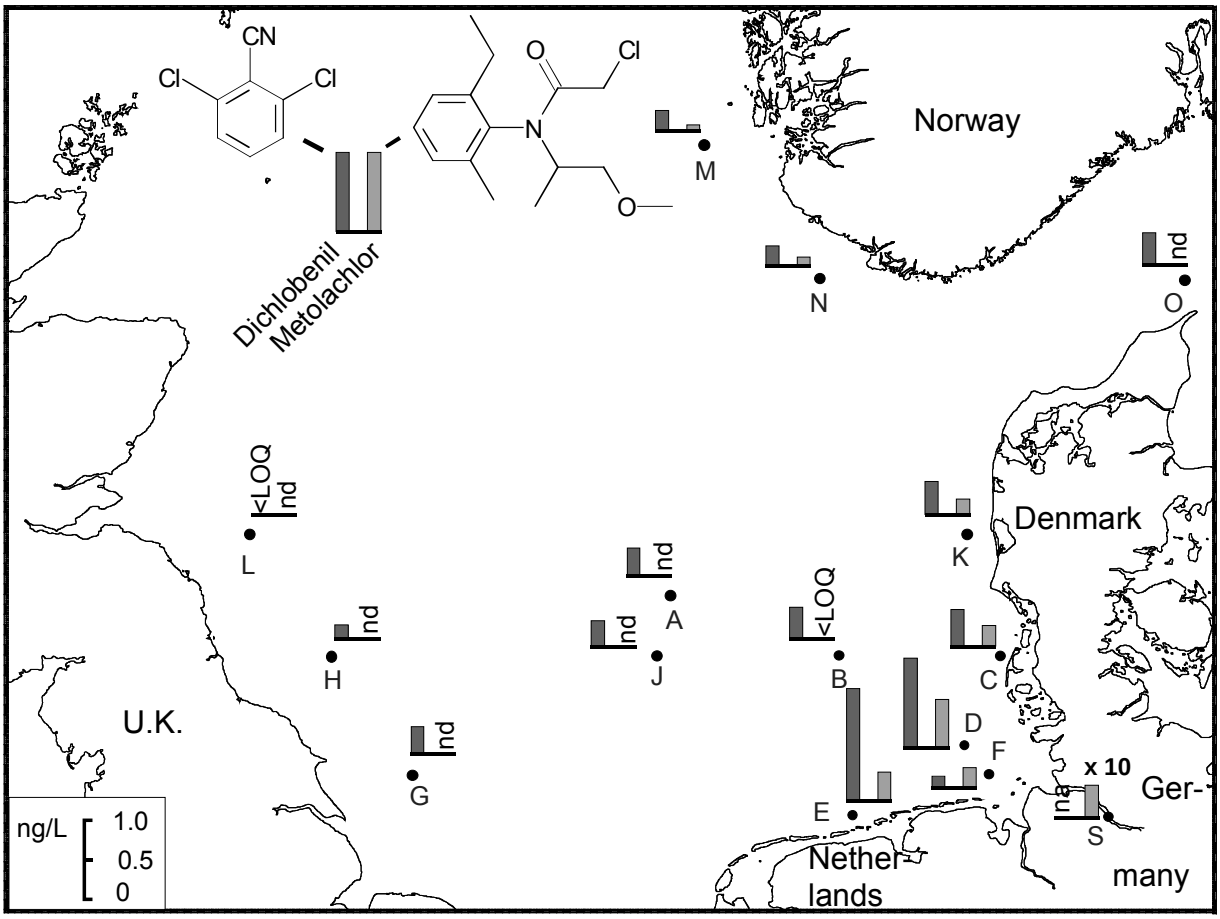

Figure 34: Distribution of dichlobenil and metolachlor in the North Sea (nd: not detected, na: not analysed, LOQ: limit of quantification)

The acetanilide herbicide metolachlor (log Kow = 2.9) was mainly present in the German Bight (0.25 - 0.6 ng/L) and at lower concentrations off the coasts of Denmark and Norway (0.07 - 0.2 ng/L) but not in the central North Sea and off the British coast (Figure 34). The distribution pattern indicates riverine inputs as the main source and transformation within the sea. As two representatives from the class of the triazine herbicides that have not gained as much attention as atrazine in the marine environment yet, terbuthylazine and desethyl-atrazine were chosen for a closer investigation. The ratios desethylatrazine/atrazine that were estimated from peak heights (major ion trace) for the samples in which desethylatrazine was detected above the LOQ were found to be 0.25 (sample B), 0.3 (M) and 0.4 (O), respectively. Terbuthylazine (log Kow = 3.0) showed a distribution pattern similar to metola-chlor, but was present at slightly higher concentrations (0.2 - 0.8 ng/L in the German Bight), while desethylatrazine (log Kow = 1.5) was only detected occasionally (Figure 35). Of the two insecticides, the thiophoshate parathion-methyl (log Kow = 2.8) was not detected in any sample, while the carbamate pirimicarb (log Kow = 2.2) was present in the Elbe estuary (0.67 ng/L) and in one sample off the British coast at a similar concentration (0.7 ng/L).

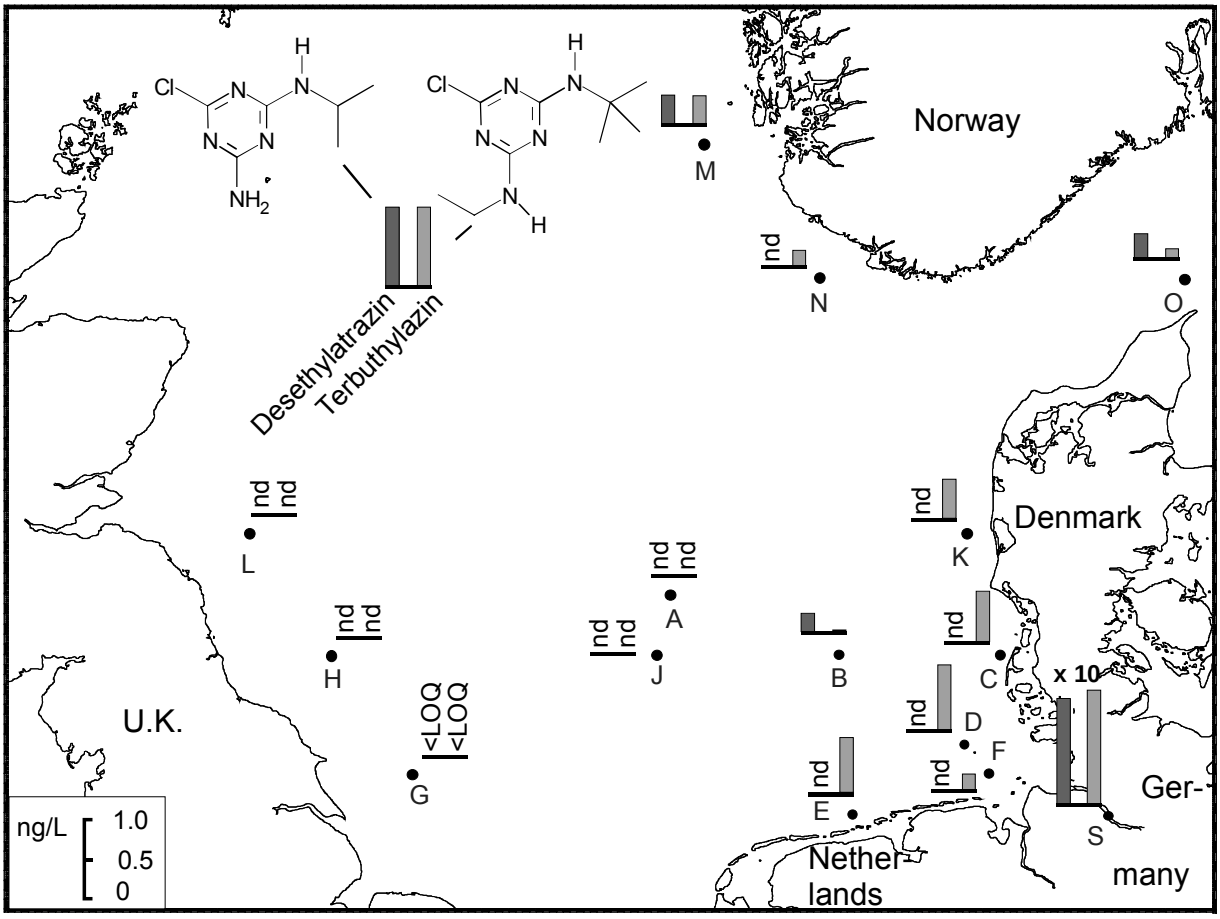

Figure 35: Distribution of terbuthylazine and desethylatrazine in the North Sea (nd: not detected, LOQ: limit of quantification)

4.2 Industrial chemicals

Four isomers of dichloropyridine (DCPy) were detected in the estuarine sample (S), 2,6-DCPy (log Kow = 2.1) being the dominant one (8.8 ng/L). In the sea, 2,6-DCPy was detected in several samples, though only in few above, but still close to the quantification limit of the method at concentrations around 0.1 ng/L (Figure 36). The distribution does not follow an obvious pattern, thus complicating the interpretation. DCPys are industrially used as intermediates in the synthesis of pesticides and pharmaceuticals or are formed as by-products, e.g., in the synthesis of chlorpyrifos from pyridine [158]. Janssens and Schepens [151] reported the presence of chlorinated pyridines in vapour phase samples of municipal waste incinerators, presumably due to *de novo* formation. The knowledge on the environmental fate and effects of DCPys is limited. Liu [159] investigated the transformation of the four DCPys under anaerobic conditions. While 2,3- and 3,5-DCPy were reductively dechlorinated to the monochloropyridines, 2,5- and 2,6-DCPy were not transformed under the test conditions. Halogenated pyridines were reported to possess mutagenic activity [160]. However, the low observed concentrations might not be of ecotoxicological relevance for the marine environment.

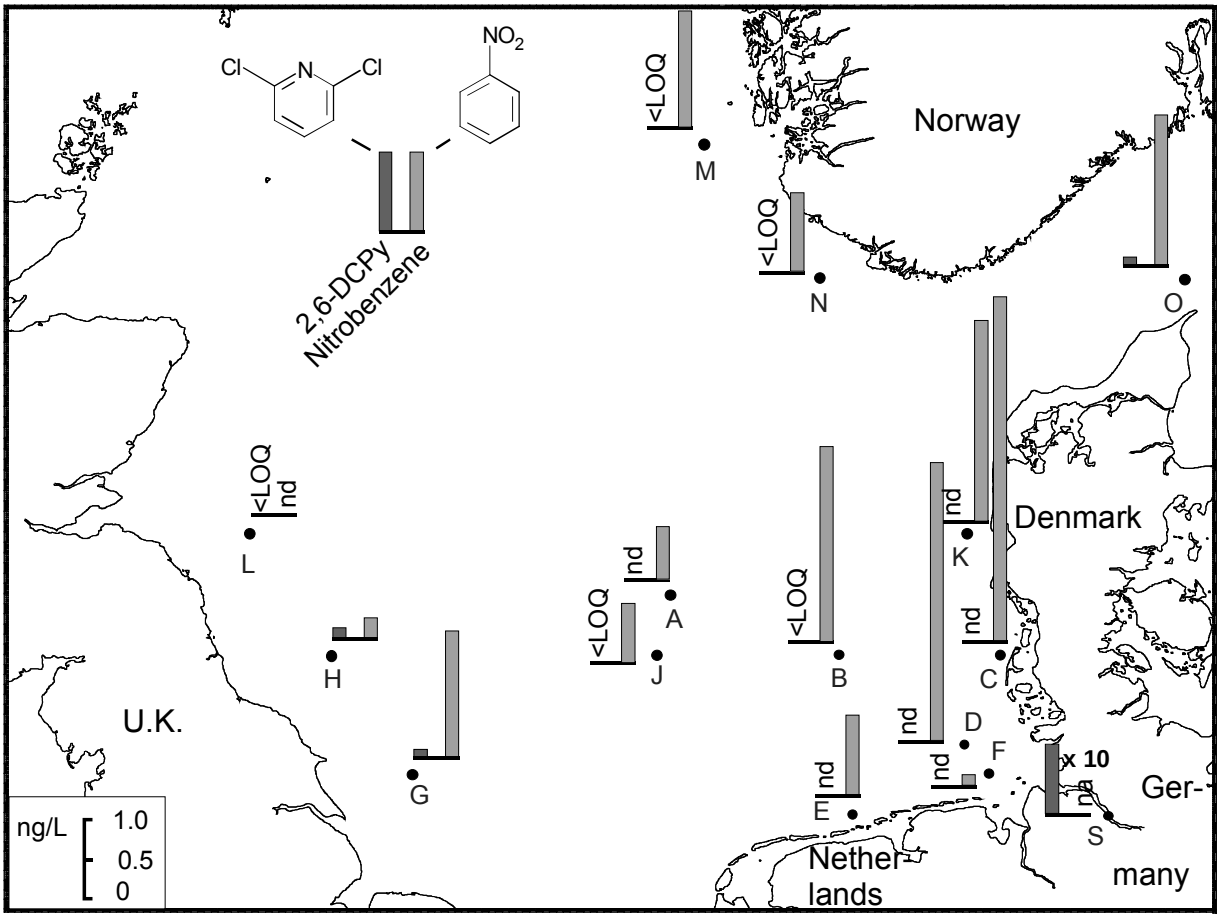

Figure 36: Distribution of 2,6-dichloropyridine (DCPy) and nitrobenzene in the North Sea (nd: not detected, na: not analysed, LOQ: limit of quantification)

Nitrobenzene (log Kow = 2.0) is used in large quantities as industrial raw material, intermediate and as solvent. This is reflected by the higher concentrations in comparison to the other compounds investigated in this work. Concentrations are highest at stations B, C, D and K (2.5 - 4.4 ng/L), areas that are subject to the influence of the larger river Elbe plume (Figure 36). Along the British east coast the typical increase in concentration is observed, resulting from the increasing contamination of North Atlantic water flowing into the North Sea north-east of Scotland with chemicals from riverine inputs. Even in central regions of the North Sea the concentrations of nitrobenzene showed values around 0.7 ng/L. An extraordinarily low concentration (0.13 ng/L) was measured at station F, an effect also observed for dichlobenil and terbuthylazine. Although slightly higher, the nitrobenzene concentrations obtained within this work are comparable to those determined by Gatermann et al. [26] for the German Bight in summer 1993 (0.5 - 2.5 ng/L). The differences are not pronounced enough to derive a temporal trend. 3-chloro-4-fluoronitrobenzene, having been detected in coastal water (sample DB30-3, chapter 3.3.2), was only found in two samples at rather low concentrations (M: 0.2 ng/L, J: 0.5 ng/L) and with no apparent explanation for its occurrence at these spots.

Concentrations of TCPP were estimated for a subset of samples by external quantification with the technical mixture. This mixture contained approximately 50 % of TCPP, mainly consisting of the two isomers tris(2-chloro-1-methylethyl)phosphate (TCPP-1) and presumably bis(2-chloro-1-methylethyl)-2-chloropropylphosphate (TCPP-2) [161]. The relation of TCPP-1 to TCPP-2 in the mixture was determined as 6:1 from GC peak area ratios. Concentrations given in Table 19 were calculated based upon the weight of the mixture. They were in the range of 3 to 8 ng/L for the technical product in the German Bight and lower (ca. 1 ng/L) in the Skagerrak and off the Norwegian west coast. Concentrations in the contributing rivers were in the range of 50 - 150 ng/L (Rhine at Cologne in 2000) [139] and 70 - 300 ng/L (Elbe in 1996) [162].

Sample	Technical TCPP	E	F	D	O	M
concentration TCPP	-	5.9	3.1	7.9	1.1	0.9
ratio TCPP-1/ TCPP-2	6	2.8	2.4	2.3	4.0	- [a]

a: TCPP-2 not quantified

Table 19: Estimated concentrations [ng/L] of tris(chloropropyl)phosphate (TCPP) calculated as the technical mixture in selected samples and peak area ratios of the two isomers

4.3 Pharmaceuticals and personal care products

In contrast to limnic waters, the knowledge on occurrence and distribution of pharmaceuticals and personal care products (PPCPs) in marine environments is very limited. Synthetic musk fragrances are used in large quantities in cosmetic and laundry products and are released upon usage via the sewer systems into the aquatic environment. The presence of the nitroaromatics musk xylene and musk ketone was shown for the German Bight [26], as well as that of the polycyclic musks HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(*g*)-2-benzopyrane; galaxolide®) and AHTN (1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)ethanone; tonalide®) [163]. With regard to pharmaceuticals, Buser demonstrated the presence of clofibric acid [48] and the absence of ibuprofen [164] in two water sample extracts from the German Bight. In order to shed more light on the behaviour of PPCPs in the environment in general and especially on their presence and distribution in the sea, an effort was undertaken to quantify selected PPCPs in water sample extracts from the North Sea. Based upon the findings described in chapter 3.3.3 and literature research on usage amounts and on the prevalence of single compounds in the contributing rivers, the following compounds were chosen for quantification: clofibric acid (active metabolite of the lipid regulating drugs clofibrate and etofibrate); diclofenac, ibuprofen and ketoprofen (analgesics, antiphlogistics); propyphenazone (analgesic); caffeine (analeptic, drink constituent); DEET (insect repellent).

4.3.1 Distribution in the North Sea

Clofibric acid was detected in the estuary of the River Elbe at a concentration of 18 ng/L. The compound was present in most samples from the North Sea as well. Concentrations ranged from 0.28 to 1.35 ng/L off the coasts of the Netherlands, Germany, Denmark and Norway and the inner German Bight. Lower concentrations (0.01 – 0.04 ng/L) were observed in the outer German Bight and off the English east coast. Only in two samples from the central North Sea (A, J) and in one off Scotland clofibric acid was absent (Figure 37).

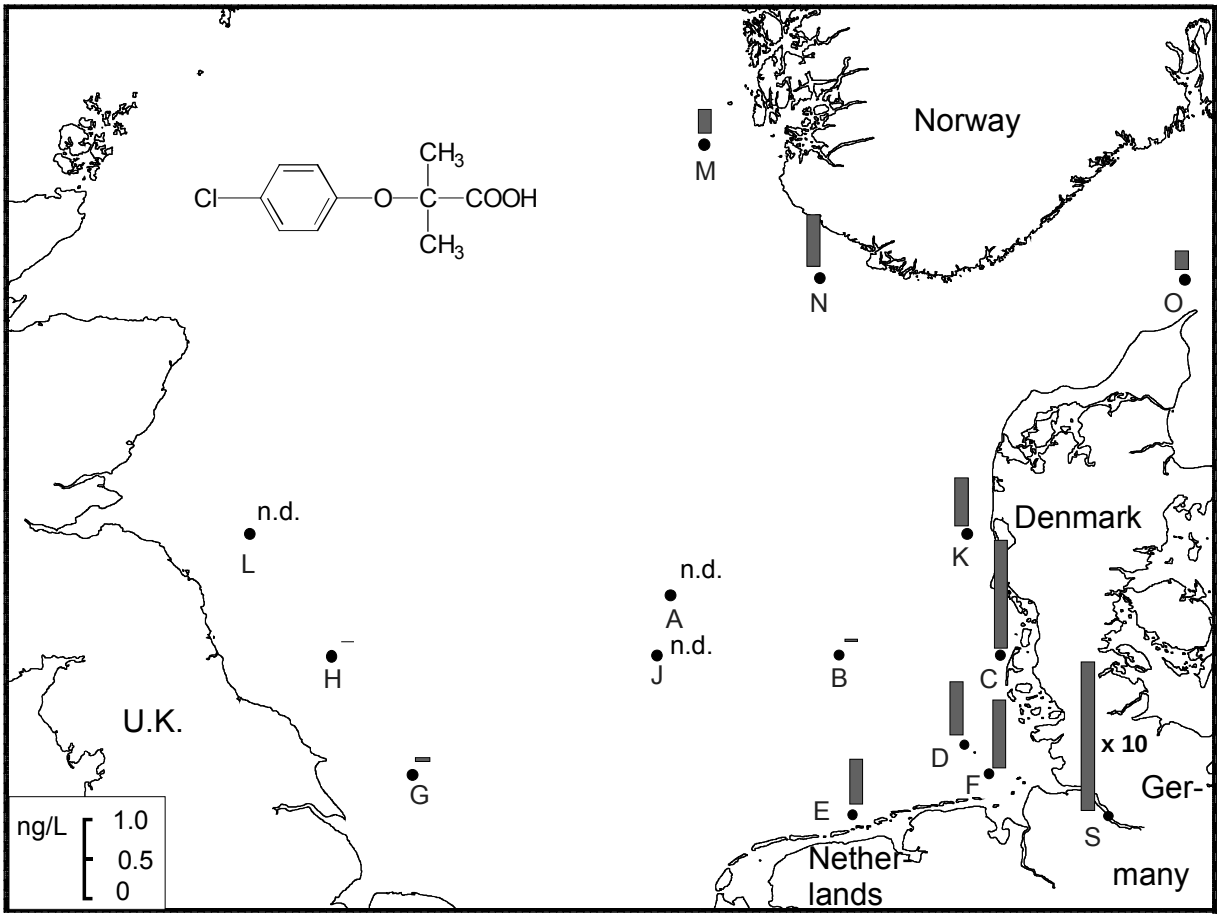

Figure 37: Distribution of clofibric acid in the North Sea (n.d. = not detected)

Diclofenac and ibuprofen were determined in the Elbe estuary at concentrations of 6.2 and 0.6 ng/L, respectively, but in none of the marine samples. Ketoprofen was neither detected in estuarine nor in marine water. Propyphenazone could not be identified unambiguously in the marine samples.

Caffeine was detected in all samples as depicted in Figure 38. Concentrations were lowest in the Central North Sea and off Scotland (2 - 5.4 ng/L). However, these concentrations are not very well distinguished from other locations, where values between 4.9 and 16.1 ng/L were found. Some of the higher values were detected along the coast lines of England (H: 13.1; G: 15 ng/L), Germany (E: 9, C: 8 ng/L) and Denmark (K: 9.7 ng/L).

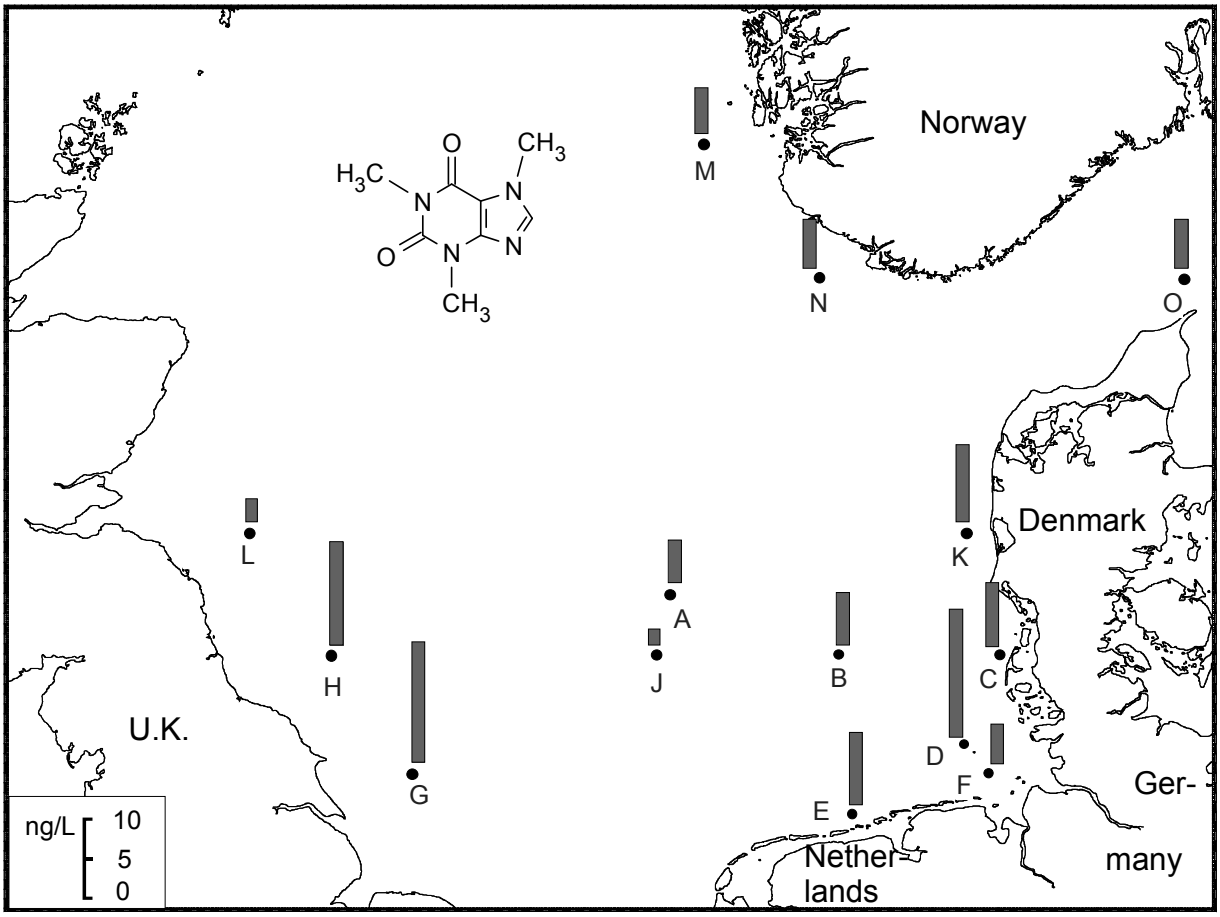

Figure 38: Distribution of caffeine in the North Sea

Residues of the insect repellent DEET were present in concentrations around 1 ng/L in the inner German Bight and in declining concentrations along the Danish (0.6 ng/L) and Norwegian (0.29 - 0.25 ng/L) coasts. Low concentrations were observed in the outer German Bight (n.d. - 0.1 ng/L) and off the British east coast (n.d. - 0.07 ng/L) (Figure 39).

When interpreting pollution gradients in the North Sea one should bear in mind the dominant water currents in this shelf sea. Basically, they are characterised by inflow of fresh North Atlantic water north-east of Scotland and a counterclockwise movement along the British, Dutch, German, Danish and Norwegian coasts (Figure 1). The distribution patterns of clofibric acid and DEET are rather similar to each other and are related to the afore-mentioned water currents. Furthermore, their distribution patterns are typical of contaminants which are transported to the North Sea mainly via rivers. A steep gradient from the Elbe estuary to the sea was observed for clofibric acid due to dilution with seawater. Relatively high concentrations occur in the plumes of the contributing rivers, slowly declining upon mixing with cleaner water masses from the central North Sea. This can be observed well in the German Bight. Station E reflects the outflow from the river Rhine. The stations D and C show the influence of the river Elbe, with declining concentrations along the Danish coast. The same holds for the Norwegian coast, where concentrations are influenced by Norwegian

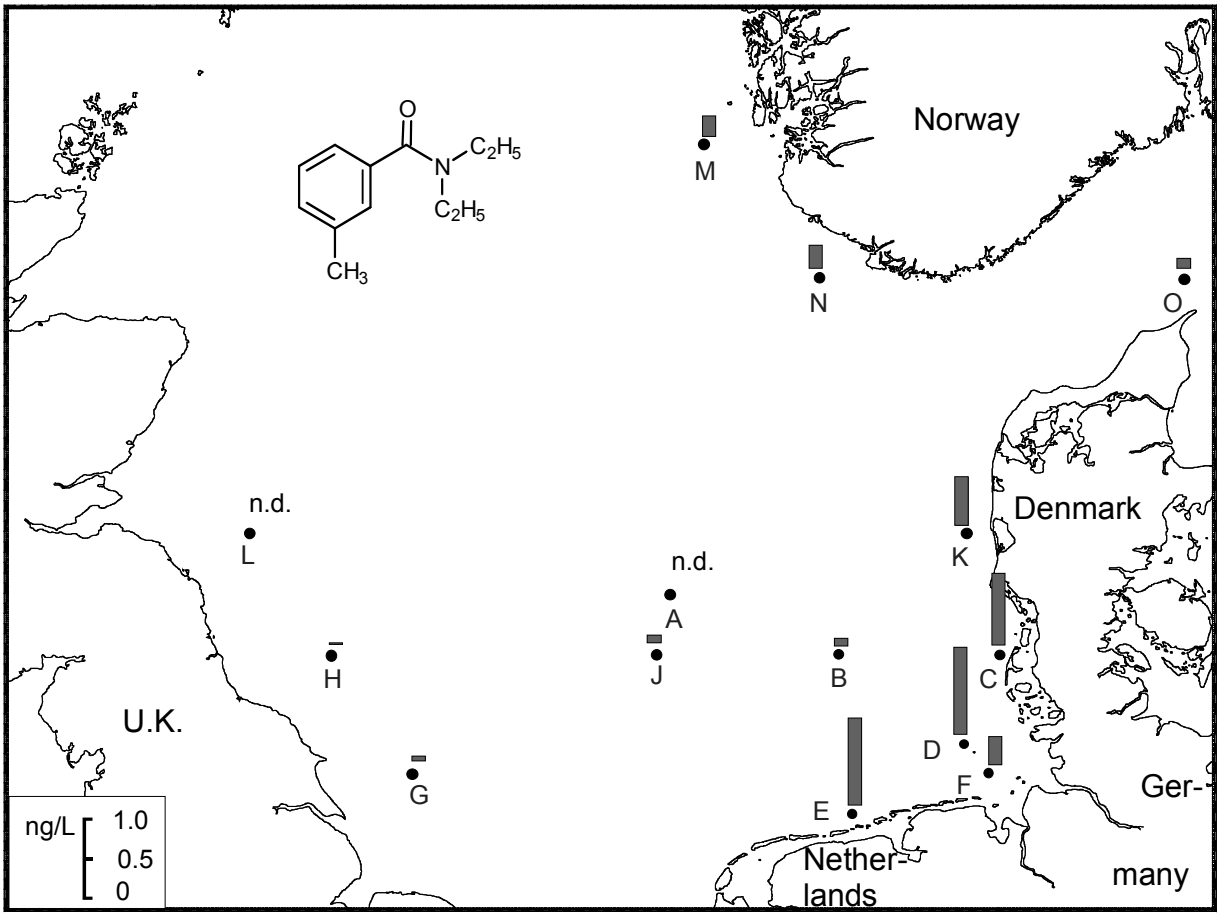

Figure 39: Distribution of DEET in the North Sea (n.d. = not detected)

rivers and fjords, outflow of Baltic Sea water and the current from the German Bight. Along the British east coast it is clearly visible how clean North Atlantic water (L) is slowly mixing with the contaminated plumes of the Firth of Tay, the Firth of Forth and the rivers Tyne, Tweed, Wear and Tees. Concentrations rise from station L (n.d.) over H to G. It can be inferred from the ratios of the concentrations at stations E (river Rhine plume) and D (mixed influence of Rhine and Elbe) to those at stations C (river Elbe plume) and F (mixed influence of Elbe and Weser) that the input of clofibric acid to the German Bight was higher from the river Elbe, whereas in the case of DEET the Rhine had a higher impact. The concentrations of clofibric acid and DEET were in the same order of magnitude as known marine pollutants such as α-HCH, which was detected in the German Bight at levels around 1 ng/L [165].

For caffeine, the picture was slightly different. Concentrations were generally at least by one order of magnitude higher than those of clofibric acid and DEET. Although the pattern described above was visible in the distribution of caffeine as well, the trends were not as pronounced as in the former case. For example, the caffeine concentration was higher at K than at C, there was hardly an increase off the English coast from H to G and considerable amounts were detected in the central North Sea. Since atmospheric deposition of this highly polar compound is not to be expected, three explanations have to be considered. A side current delivers water from the British coast to areas in the central North Sea. Since these

coastal waters obviously contain considerable amounts of caffeine, a portion of this might thus have reached the area in question. Moreover, the North Sea is known to exhibit one of the highest shipping densities in the world and additionally, some 500 offshore platforms are installed at the oil and gas fields of the North Sea. Both sources can be expected to release notable amounts of caffeine directly into the sea, leading to a specific distribution pattern. This hypothesis should be checked by a sampling strategy that compares areas highly affected by offshore installations and shipping routes with rather pristine areas. Another explanation might be an impact of the research vessel itself which could be ruled out by taking samples in greater depths.

It is difficult to draw conclusions on the ecotoxicological impact from the present distribution patterns of clofibric acid, caffeine and DEET for the North Sea. Nothing is known yet about the potential impact of clofibric acid (an isomer of the herbicide mecoprop) on phytoplankton and higher marine plants. Acute toxicity of DEET to some fish species (LC_{50} > 100 mg/L; [162]) and to mammals (LD_{50} > 2000 mg/kg [166]) is low, but oral uptake may lead to damages of the central nervous system. DEET has been associated to neurotoxic symptoms known as the Gulf War syndrome [167]. Subsequent studies found that DEET enhances the activity of cholinesterase (ChE) inhibitors [168,169]. Both studies conclude that DEET might facilitate the passage of the investigated compounds (e.g., organophosphorus pesticides) through the blood-brain barrier. This phenomenon would raise the effective concentration of these compounds, which are present in many parts of the North Sea, in marine organisms.

Summarising, the widespread distribution of these anthropogenic chemicals extends their ubiquitous character that has already been established for rivers, lakes and groundwater to marine ecosystems. It underlines, at least for clofibric acid and DEET, their stability under environmental conditions and, also for caffeine, their relevance as notable marine contaminants.

4.3.2 Transition river - sea

An interesting aspect of the environmental fate of the acidic pharmaceuticals is their behaviour along the transect from the estuary to the sea. In the Elbe estuary, some 70 km from the mouth (station S), clofibric acid (18.6 ng/L), diclofenac (6.2 ng/L) and ibuprofen (0.6 ng/L) were present. Among these three compounds only clofibric acid was detected in coastal and marine waters (Figure 40). Assuming that the decrease in clofibric acid concentration was only due to dilution (factor 0.07 from S to C) and applying the same factor to diclofenac and ibuprofen the concentrations of the latter two compounds would still be above their LOQs. Thus, it remains to be investigated whether clofibric acid is more persistent, while diclofenac and ibuprofen are more readily transformed on their way to the sea. Diclofenac is known to undergo rapid phototransformation [170] but at this stretch, the river water is very rich in particulate matter so that phototransformation may only occur in the

surface layer. The removal of organic compounds from the aqueous phase by adsorption to particles and subsequent precipitation (scavenging) in the estuary is known for lipophilic chemicals such as PCBs. For the water soluble acidic compounds this seems unlikely. Further explanations might be specific (ionic) interactions with particles or a different chemical behaviour under estuarine and marine conditions (alkaline pH), e.g., enhanced hydrolysis. The absence of ketoprofen is not surprising since its concentrations in limnic systems are already low, as compared to the other three acidic drugs investigated herein.

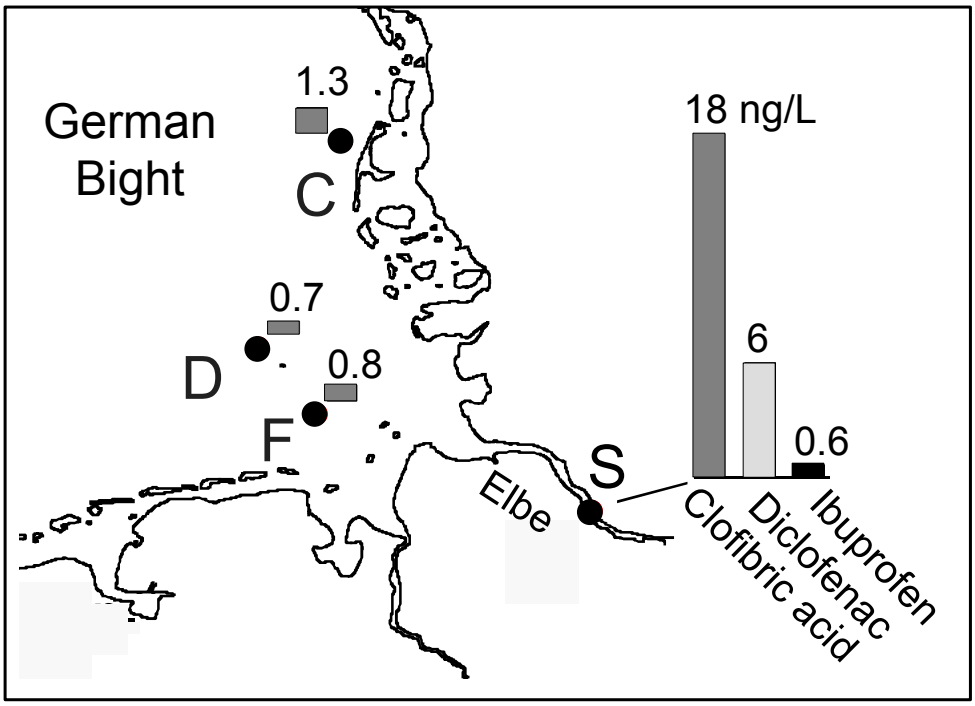

Figure 40: Concentration gradient of the investigated acidic drugs from the Elbe estuary to the German Bight

In order to elucidate the behaviour of the acidic analytes during the transition from the river to the sea further, a higher resolved sampling campaign was carried out. Due to logistic limitations, only 2 L water samples were collected (at station S, at Cuxhaven and at station DB30) and stored frozen in aluminium bottles until extraction on land. The results are given in Table 20. The differences in the concentrations of clofibric acid were much less pronounced compared to the samples from June 1998 (Figure 40). In fact, there was virtually no difference between the samples CUX and DB30 and at Stade the concentration was only twice as high. This reflects a rather homogeneous distribution between the mouth of the river and the river plume within the inner German Bight. The only slightly higher value at Stade indicates that the rising tide strongly contributed with German Bight water to the concentration at Stade. Diclofenac was only detected at Stade, confirming its more rapid transformation and thus no backflush from the mouth/the Elbe plume. Ibuprofen was detected in none of the samples which is in line with the assumption that also at Stade the water body consisted of older water that had been moving back up the river since ibuprofen

is known to be the least stable compound of the three acids, especially under the climate conditions (summer) with water temperatures around 19 °C. DEET also showed similar values at CUX and DB30 and a higher concentration at Stade. For caffeine, present at the highest concentration, a gradient was observed with concentrations declining from 151 ng/L at Stade to 14 ng/L at station DB30. In this case it can be assumed that there is a significant release into the river from point (communal sewage) and diffuse sources (boats, leisure activities along the river).

	S (Stade)	CUX (Cuxhaven)	DB30 (Eiderstedt)
Clofibric acid	4.0	1.9	2.4
Diclofenac	6.5	nd	nd
Ibuprofen	nd	nd	nd
Caffeine	151	50	14
DEET	31	3	2

Table 20: Concentrations [ng/L] of clofibric acid, diclofenac, ibuprofen, caffeine and DEET along the river Elbe into the German Bight (July 2001, nd = not detected)

4.4 Conclusions

Organic contaminants remain an important issue for the North Sea. While concentrations of some classical pollutants, e.g., HCHs, have decreased over the last decades and stabilised on a certain level, there is no significant decrease for others, e.g., dichlobenil. Agricultural and other biocides largely contribute to the contamination of the North Sea. Relative amounts of single agents reflect changes in application patterns following regulatory restrictions. Examples are the shift in triazine herbicide concentrations from atrazine to, e.g., terbuthylazine or the partial substitution of TBT in antifouling coatings by Irgarol 1051. The contribution of industrial chemicals, raw materials, intermediates, by-products and end products released from open uses, is still largely underestimated. One reason for this is the hydrophilic nature of many of these compounds (e.g., aromatic sulfonic acids), which prevented their detection by methods traditionally applied in marine analytical chemistry: gas chromatographic analysis of biota sample extracts or liquid/liquid extracted water samples. The SPE method applied in this work has shown to broaden the spectrum of potential target analytes largely towards higher polarity. Thereby, a number of PPCPs was established as widespread marine contaminants, among them the acidic drug metabolite clofibric acid.

5 Pharmaceuticals in the environment

5.1 Introduction

After having shown the relevance of residues of pharmaceuticals and personal care products (PPCPs) as contaminants of the marine environment, a more detailed investigation was carried out with the intention to elucidate the behaviour of selected compounds from this class under marine conditions. Ideally, one would like to perform fate studies at concentrations of the investigated compounds sufficiently high to be detected reliably. In many cases of riverine input of PPCPs into the sea, concentrations are already low (low ng/L range) in the estuary due to transformation and dilution within the river. For many PPCPs this results in concentrations below the detection limit in coastal and offshore waters as seen in the case of ibuprofen and diclofenac. In contrast to this scenario as given for example for the river Elbe input to the German Bight, direct emissions into the sea would facilitate distribution and fate studies. This situation is given in Tromsø/Norway, where the sewage of the approximately 60 000 inhabitants is released after minimal mechanical treatment directly into the Tromsø-Sound.

The present investigation was carried out in two steps. During a sampling campaign in summer 2001, samples were taken and analysed applying the large-volume SPE method used for North Sea water samples (chapter 2.1.1) modified for 1 L samples from the Tromsø-Sound (SOP 4). The sample extracts were screened for potentially further relevant PPCPs not included in the target list and the concentration levels of important target analytes were determined to gain information on the required enrichment factors to be reached by the specific PPCP SPE method to be developed. After development and validation of the method for the simultaneous extraction of acidic, neutral and basic analytes (SOP 5), the comprehensive second phase of the investigation was carried out. Concentrations of the selected analytes were determined not only in different areas of the Tromsø-Sound and the open Atlantic, but also in sewage samples before release into the sea, in order to assess possible transformation processes. An additional sampling campaign was carried out for sewage, river and lake water in Hamburg/Germany for comparison of the obtained results concerning concentration and transformation patterns at these marine and sub-arctic conditions with temperate regions and limnic conditions.

Out of the list of target analytes (Figure 11), emphasis was placed upon ibuprofen and its primary metabolites hydroxy- and carboxy-ibuprofen (Figure 3) since ibuprofen had already been detected in Tromsø-Sound water in preparatory screening experiments (chapter 5.2.1). The analgesic, antipyretic and non-steroidal anti-inflammatory drug ibuprofen is among the most widely used pharmaceuticals in the world. In Germany, prescribed

amounts already summed up to almost 150 t in the year 2000 [49]. Despite efficient elimination in sewage treatment plants (STPs), residues of ibuprofen are frequently detected in rivers, lakes and streams. However, little attention has been paid so far to the human metabolites of ibuprofen, which are excreted in higher amounts than the original drug. Stumpf et al. [171] quantified ibuprofen (ibu) together with its metabolites hydroxy-ibuprofen (ibu-OH) and carboxy-ibuprofen (ibu-CX) in STP in- and effluent and in rivers. Buser et al. [164] detected the metabolites in addition to ibu in sewage, but they did not quantify them.

5.2 Determination of PPCPs in samples from Tromsø/Norway

5.2.1 Sampling campaign 2001: Seawater

In summer 2001, a sampling campaign was carried out in the Tromsø-Sound, applying the methodology described in chapter 2.1.1 (Figure 5). The resulting sample extracts were not only analysed in the full scan mode for unknown analytes of potential relevance (chapter 3.4). Additionally, a target screening was carried out in the more sensitive selected ion monitoring (SIM) mode for a limited set of target compounds. Out of the analysed compounds, caffeine and ibuprofen were detected in the seawater samples (Table 21). Estimated concentrations of caffeine ranged between 18 and 64 ng/L, while ibuprofen was present at a 10-fold lower concentration level (0.2 to 0.7 ng/L) as depicted in Figure 41. Caffeine is a well suited tracer for communal sewage. Its presence in the investigated samples indicates that the Sound water is considerably influenced by human wastewater despite the strong tidal current and thus dilution within the Tromsø-Sound. This was underlined by the presence of the target compound ibuprofen in all investigated samples. Interestingly, the ibuprofen concentration was higher by a factor of 2 in the hospital related samples, whereas the highest concentration of caffeine was found in the central harbour area that is surrounded by cafés and restaurants and partly protected from the tidal current.

Compound	monitored ions m/z [amu]	detected
Caffeine	194, 109	+
Propyphenazone	215, 230	-
Carbamazepine	193, 236	-
Clofibric acid	128, 228	-
Diclofenac	214, 242	-
Ibuprofen	161, 220	+

Table 21: Target analytes detetcted in Tromsø-Sound water extracts by GC-MS (SIM) measurements, acidic compounds after methylation with methyl chloromethanoate

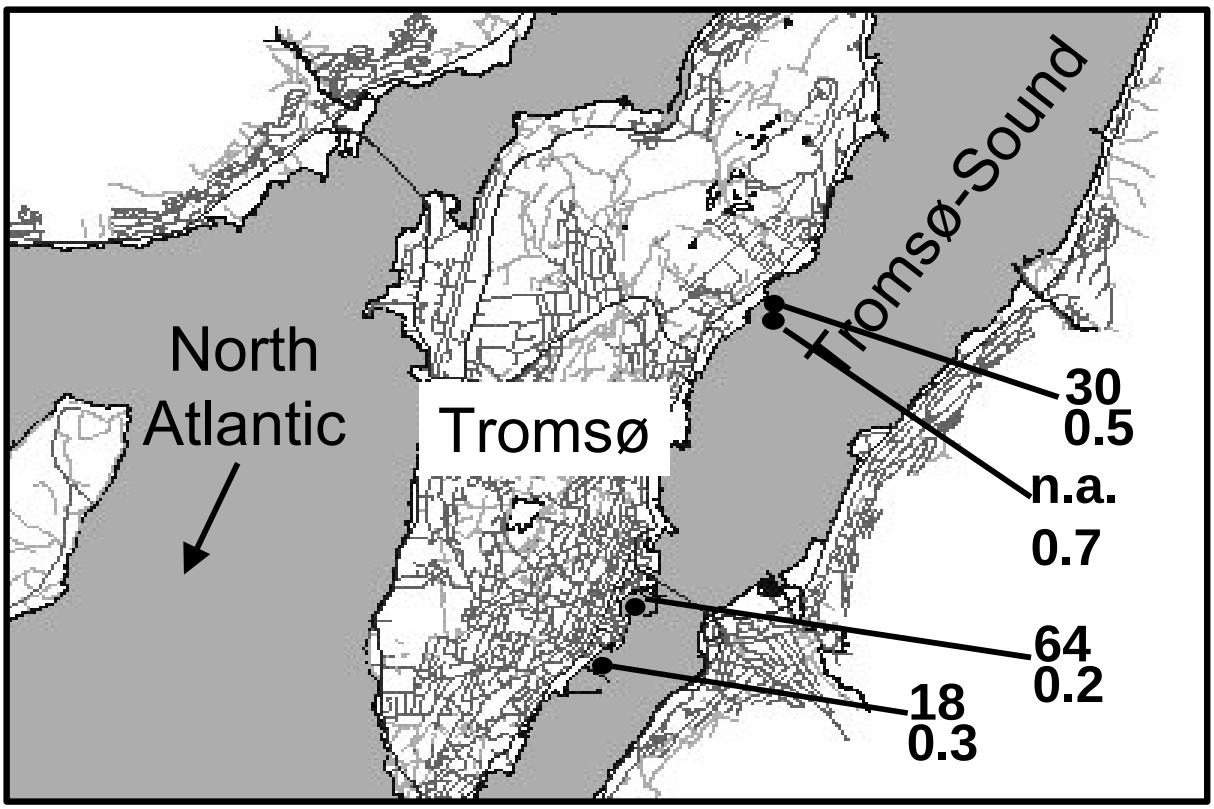

Figure 41: Estimated concentrations [ng/L] of caffeine (upper value) and ibuprofen (lower value) in samples from Tromsø-Sound in summer 2001

5.2.2 Sampling campaign 2002: Sewage

Sewage in Tromsø is collected in sewers and, depending on the geographical location, discharged either directly into the sea or after processing in one of the four sewage treatment plants that cover 25 000 population equivalents (PE) of the 60 000 inhabitants. In this case, sewage treatment consists of a mechanical filtration, but does not include any biological treatment. The two hospitals in the city discharge their wastewater into the public sewer system without prior treatment. Sewage samples were taken at the main STP, receiving effluents from private households and commerce of the major area of the inner city, corresponding to 15 500 PE, 3 500 of which being of commercial origin. Additional samples of raw sewage were collected from sewers receiving hospital effluents. All samples were taken around 9:00 a.m. as grab samples. For comparison, samples were taken at a German STP after primary clarification (being equivalent to the filtration step in Tromsø STPs) and after biological treatment.

Caffeine, ibuprofen, ibu-OH, ibu-CX and triclosan were detected in all investigated samples (Table 22). Caffeine provided the dominant peak in the ethyl acetate fraction of the Tromsø sewage samples (Figure 42), although the observed concentrations were comparable to that in the Hamburg STP sample (after primary clarification) and similar to levels reported from other STPs (e.g., 147 ± 76 µg/L in influents of a German STP [172]). A

clearly higher concentration of caffeine in Tromsø STP effluent was observed in October. This might be explained by the fact that the volume flow in October was only one third of that in April, resulting from the contribution of melting snow to the overall sewage flow in spring. However, no such correlation was observed for the other analytes of interest. Ibuprofen concentrations ranged from 0.2 to 0.7 µg/L, the sum of ibu and its metabolites from 0.7 to 3.5 µg/L. The respective concentrations for Hamburg sewage were considerably higher and comparable to those found by Stumpf et al. [171] who determined concentrations of 4.3 µg/L for ibu, 6.7 µg/L for ibu-OH and 8.9 µg/L for ibu-CX in 24 h composite samples (average concentrations of 5 days in influent). First load estimations for the Tromsø STP yielded values of 10 - 90 g ibuprofen (as Σ ibu + ibu-OH + ibu-CX) per day. This is in the order of magnitude of the 92 g/day that are expected from average ibuprofen consumption in Norway (6.4 defined daily doses/1000 inhabitants per day in 1997 according to Øydvin [173]).

In April, diclofenac was detected only in the two samples that received effluents from the hospitals, but in none of the samples from the STP. However, in October diclofenac was also detected in the STP effluent. The presence of selected SSRI antidepressants and ß-blockers was investigated semi-quantitatively in two sewage samples from the STP and from a sewer that received effluents from the psychiatric hospital Åsgård [114]. The two included ß-blockers metoprolol and propranolol were detected in both samples, with higher concentrations in the sample affected by hospital effluents. The SSRI antidepressants paroxetine and sertraline as well as the anti-epileptic carbamazepine were exclusively detected in the sample affected by hospital effluents. The third analysed SSRI, fluoxetine, was not detected. The active metabolite clofibric acid was not detected in the Norwegian samples since its parent compounds clofibrate and etofibrate are not prescribed in Norway. Concentrations of the antibacterial triclosan ranged from 0.16 to 0.48 µg/L in STP samples and are comparable to the Hamburg STP and to reported values from Swedish STPs [62].

Considerable deviations from the concentration data for STP samples were observed in the samples directly taken from the sewers. It is assumed from the different levels of caffeine in these samples that the variations are mainly resulting from dilution of sewage originating from human excretion with wastewater of different origin (e.g., laundry, shower-bath, dish-washing, commercial/industrial effluents, melting snow). However, even after normalisation to caffeine, elevated concentrations of the ibu-metabolites were found in the Breivika sewer, downstream of the Breivika Hospital sewer (characterised by lower Σ ibu-group concentrations).

Table 22: Concentrations [µg/L] of the investigated compounds in sewage (grab samples collected around 9:00 a.m.; carbamazepine, metoprolol, propranolol, paroxetine, sertraline: semi-quantitative estimations; na: not analysed, nd: not detected, co: interfering co-elutions)

Sample	STP influent	STP effluent	STP effluent	STP effluent	STP effluent	Breivika Hospital sewer	Breivika mixed sewer	Åsgård sewer	STP Hamburg influent S [a]	STP Hamburg effluent [b]
Sampling date	18/04/2002	18/04/2002	23/04/2002	25/04/2002	08/10/2002	18/04/2002	18/04/2002	18/04/2002	18/11/2002	19/11/2002
Ibu	0.60	0.68	0.15	0.16	0.31	0.38	0.09	0.02	1.66	0.03
Ibu-OH	1.32	1.13	0.21	0.38	0.59	5.01	1.59	0.05	6.84	0.09
Ibu-CX	1.63	1.27	0.23	0.50	0.07	10.6	18.4	0.004	23.0	nd
Σ Ibu-group	3.55	3.08	0.66	1.04	0.96	16.0	20.1	0.07	31.5	0.12
Diclofenac	nd	nd	nd	nd	0.03	4.47	nd	0.006	1.23	1.68
Clofibric acid	nd	nd	nd	nd	nd	nd	nd	nd	0.17	0.11
Triclosan	0.43	0.48	0.44	0.16	0.47	0.69	2.38	1.68	0.38	0.18
Caffeine	54.7	47.7	39.1	30.2	126	293	179	20.3	104	0.07
DEET	co	co	0.01	co	0.06	co	co	0.01	0.21	0.13
Carbamazepine	nd	nd	nd	nd	nd	nd	nd	0.27	na	na
Metoprolol	na	na	0.07	na	na	na	na	0.34	na	na
Propranolol	na	na	0.01	na	na	na	na	0.02	na	na
Paroxetine	na	na	nd	na	na	na	na	0.02	na	na
Sertraline	na	na	nd	na	na	na	na	0.10	na	na
Fluoxetine	na	na	nd	na	na	na	na	nd	na	na

a: after coarse filtration and particle sedimentation (comparable to Tromsø STP effluent)
b: after biological treatment, combined effluent from the two influent streams N (draining the northern and western part of the city) and S (draining the southern and eastern part of the city)

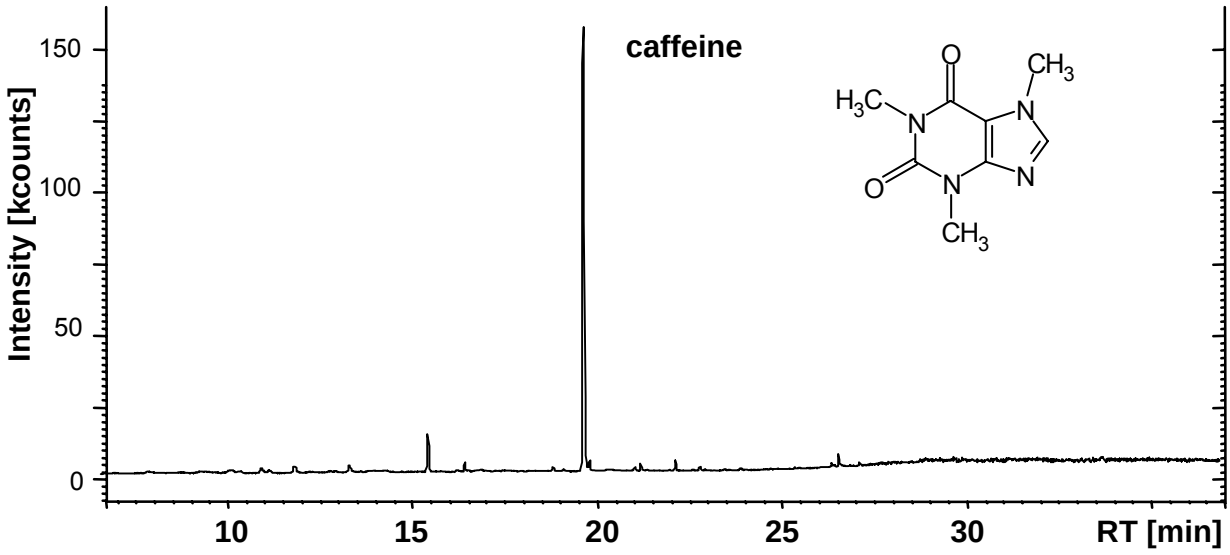

Figure 42: GC-MS total ion chromatogram of the ethyl acetate eluate of a sewage sample (effluent 23.04.2002) from Tromsø (dilution of the final extract, enrichment factor ~ 40)

5.2.3 Sampling campaign 2002: Seawater

STP effluents and non-treated sewage are directly discharged into the Tromsø-Sound at various locations. Despite the strong tidal current and the resulting dilution with presumably non-contaminated North Atlantic water, caffeine and DEET were detected in all and ibu and/or its metabolites in most seawater samples (Figure 43, Table 23). Caffeine concentrations ranged from 17 to 87 ng/L. This substance is rather evenly distributed throughout the Sound, with highest concentrations in a harbour basin that is partly protected from tidal current and surrounded by restaurants and cafés. A transect through the outfall of the central STP showed no significant differences in caffeine concentrations upstream and within the sewage plume. These distribution patterns point to an intense mixing of the water body by tides rather than to a fast exchange with the open sea or a rapid transformation under these conditions. Even at two reference sites at the coastline of the open North Atlantic/Arctic Ocean (island of Kvaløya, Vengsøyfjorden, 10 km from a small village of ca. 500 inhabitants [TOS-42], and Kaldfjorden [TOS-43]), caffeine was present at concentrations of 9 and 7 ng/L, respectively, pointing to its ubiquitous distribution even at remote areas.

Although the concentrations of ibu and its metabolites in seawater from the Sound were low (Σ ibu-group < LOQ to 7.7 ng/L) and sometimes close to or below the LOQ, their presence was unambiguously confirmed by mass spectra (Figure 44) and retention times. Their occurrence is remarkable in view of the fact that these compounds were found to be easily eliminated under STP and limnic conditions. Apparently, low temperatures and low biological activity in the Sound hinder their rapid transformation.

The quotient of the concentrations of caffeine and the sum concentration of the ibu-group ($Q_{c/i} = c_{caffeine}/c_{ibu\text{-}group}$) was very similar for all seawater samples taken around noon on April 18th ($Q_{c/i}$ = 7 - 11). The quotients $Q_{c/i}$ were higher within the samples taken in the

evening on April 23rd ($Q_{c/i}$ = 23 in the harbour, 30 and 32 in the transect up- and downstream the sewage plume, but 211 within the plume). While it remains to be elucidated whether the variations are of diurnal or of day-to-day nature, it is obvious that the water body in the Sound is rather homogenous in terms of source-specificity of these contaminants, apart from dilution effects.

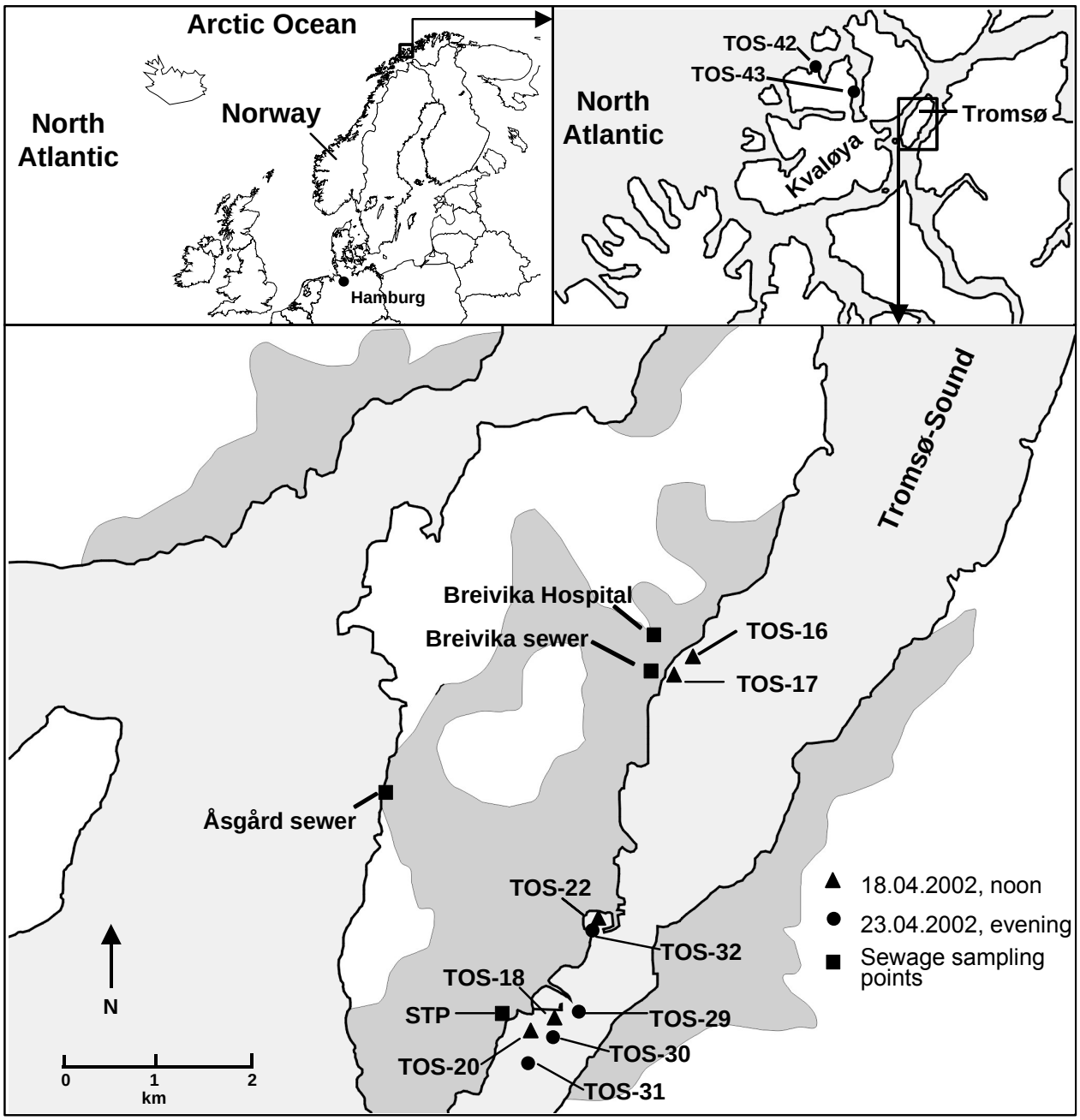

Figure 43: Sampling locations around Tromsø/Norway (more densely populated areas depicted in darker gray in the lower map)

sample number	sampling location, sampling date	caffeine	DEET	ibu	ibu-OH	ibu-CX	Σ ibu-group
TOS-16	Breivika Harbour, 18/04/02	44	0.5	0.3	1.3	3.6	5.2
TOS-17	Breivika Harbour, 18/04/02	56	0.5	0.7	1.5	5.3	7.5
TOS-20	off STP, 18/04/02	29	0.8	0.1	0.5	3.2	3.8
TOS-18	300 m north of STP, 18/04/02	35	0.4	0.1	0.5	3.2	3.8
TOS-40	300 m north of STP, 07/10/02	30	7.4	nd	nd	< 0.69	-
TOS-22	Central Harbour/Marina, 18/04/02	87	0.5	< 0.07	0.8	7.0	7.8
TOS-32	Central Harbour/Marina, 23/04/02	52	10	0.3	0.7	1.3	2.2
TOS-29	downstream sewage plume, 23/04/02	22	13	nd	< 0.42	0.7	0.7
TOS-30	off STP, in sewage plume, 23/04/02	17	7.4	nd	< 0.42	nd	-
TOS-31	upstream sewage plume, 23/04/02	24	5.5	< 0.07	< 0.42	0.8	0.8
TOS-42	Vengsøyfjorden, 08/10/02	9	4.9	nd	nd	nd	nd
TOS-43	Kvaldsund, 08/10/02	7	4.3	nd	nd	nd	nd

Table 23: Concentrations [ng/L] of the compounds detected in seawater (nd: not detected)

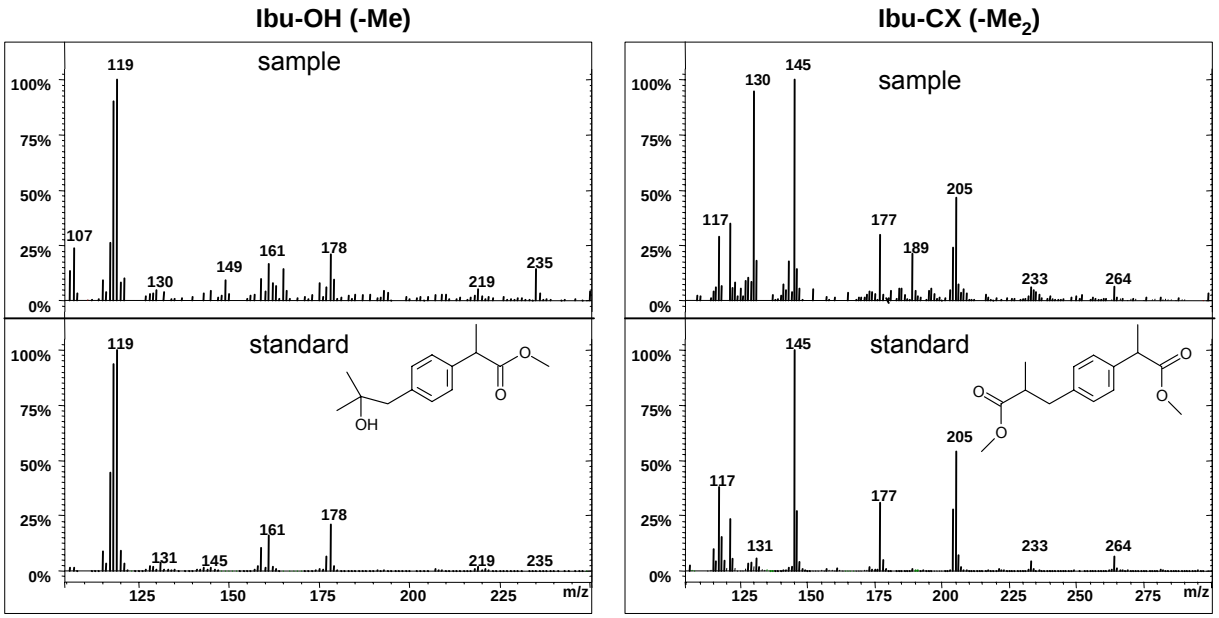

Figure 44: Mass spectra (EI 70 eV, ion trap) of ibu-OH and ibu-CX (after methylation) from a seawater sample (TOS-17) in comparison to spectra obtained from a standard solution (Me = methyl)

5.3 Determination of PPCPs in samples from Hamburg/Germany

In order to compare distribution and transformation patterns observed under sub-arctic conditions to those under temperate climate, a set of surface water samples from the river Elbe and the lake Alster at Hamburg/Germany was taken (Figure 45) and analysed for the target compounds. The results are given in Table 24.

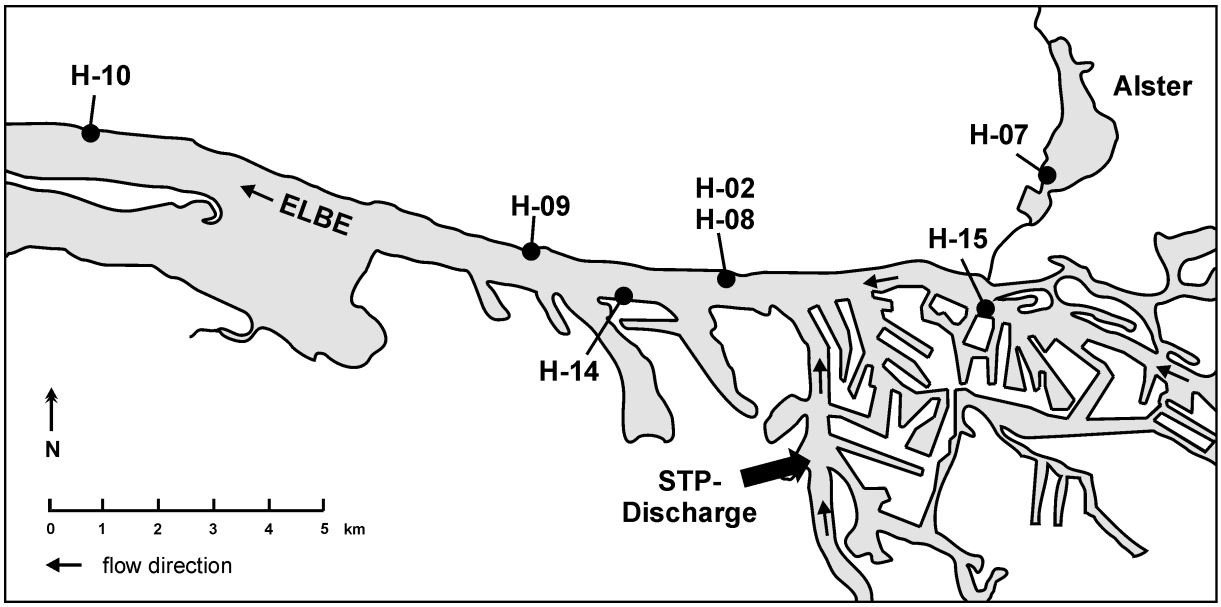

Figure 45: Sampling positions at the river Elbe and the lake Alster at Hamburg/Germany

Sample	H-02	H-08	H-09	H-10	H-15	H-14	H-07
Position	right bank km 626.7	right bank km 626.7	right bank km 630	right bank km 637.7	left bank km 622.3	left bank km 628.6	lake Alster
Date	23.10.	07.11.	07.11.	07.11.	19.11.	19.11.	05.11.
Clofibric acid	4.0	6.3	4.7	4.7	7.6	3.2	2.4
Ibuprofen	5.6	6.0	5.1	11	32	8.7	4.9
Ibu-OH	31	41	23	50	101	32	18
Ibu-CX	< 0.69	15	12	21	32	11	9.5
Diclofenac	38	32	31	33	67	42	26
Triclosan	nd	nd	nd	< 0.24	4.1	< 0.24	nd
Mecoprop	7.6	6.6	6.8	6.7	6.3	7.0	22
Caffeine	98	104	103	104	148	150	176
DEET	38	26	25	24	20	16	7.0

Table 24: Sampling positions, dates, and concentrations [ng/L] of the investigated analytes in surface water samples from Hamburg/Germany in autumn 2002 (nd = not detected)

The majority of the analytes was detected in all samples as shown in the chromatogram of a typical river water sample (Figure 46). Only triclosan, which is prone to adsorption to particles due to its more lipophilic character (log Kow = 5.8 [109]) was not detected in more than one sample above the LOQ since the method covers dissolved analytes only. On the right bank of the river (samples H-08, H-09, H-10), opposite the discharge of the central STP of the city, the concentrations of most target analytes were rather similar to each other within the investigated distance. This indicates a homogeneous water body with no significant transformation processes occurring within this stretch. Only the concentrations of the ibuprofen group were deviating at the three sampling locations. One reason for this finding might be local inputs from the dense traffic of cargo and passenger boats at this part of the river, being situated close to a large seaport. The variation in concentrations between two samples taken at the same location at an interval of two weeks (H-02 and H-08) was low for most compounds. On the left bank of the river, one sample was taken upstream (H-15) and a second one downstream (H-14) of the confluence of the southern arm of the river, bearing the discharge of the municipal STP, into the main course. Interestingly, concentrations were significantly higher in the upstream sample, except for caffeine, DEET and mecoprop, which were present in similar concentrations in both samples. This in the first place contradictory result is explained by the fact that this part of the river is strongly influenced by tides. This means that the flow direction is changing according to tides and the same water body is moving back and forth various times before it reaches the sea. In this way, higher concentrations are observed upstream of the discharge in case that the sampled water body (from

the previous tidal cycle) has received higher inputs. However, since caffeine is expected to originate from the same source as the investigated pharmaceuticals, namely communal sewage discharge, the same trend in concentrations should be expected. The observed similarity in caffeine concentrations either points towards additional sources as for example coffee processing companies situated in the port area, direct discharges from ships or to differences in degradability of caffeine in comparison to the pharmaceuticals.

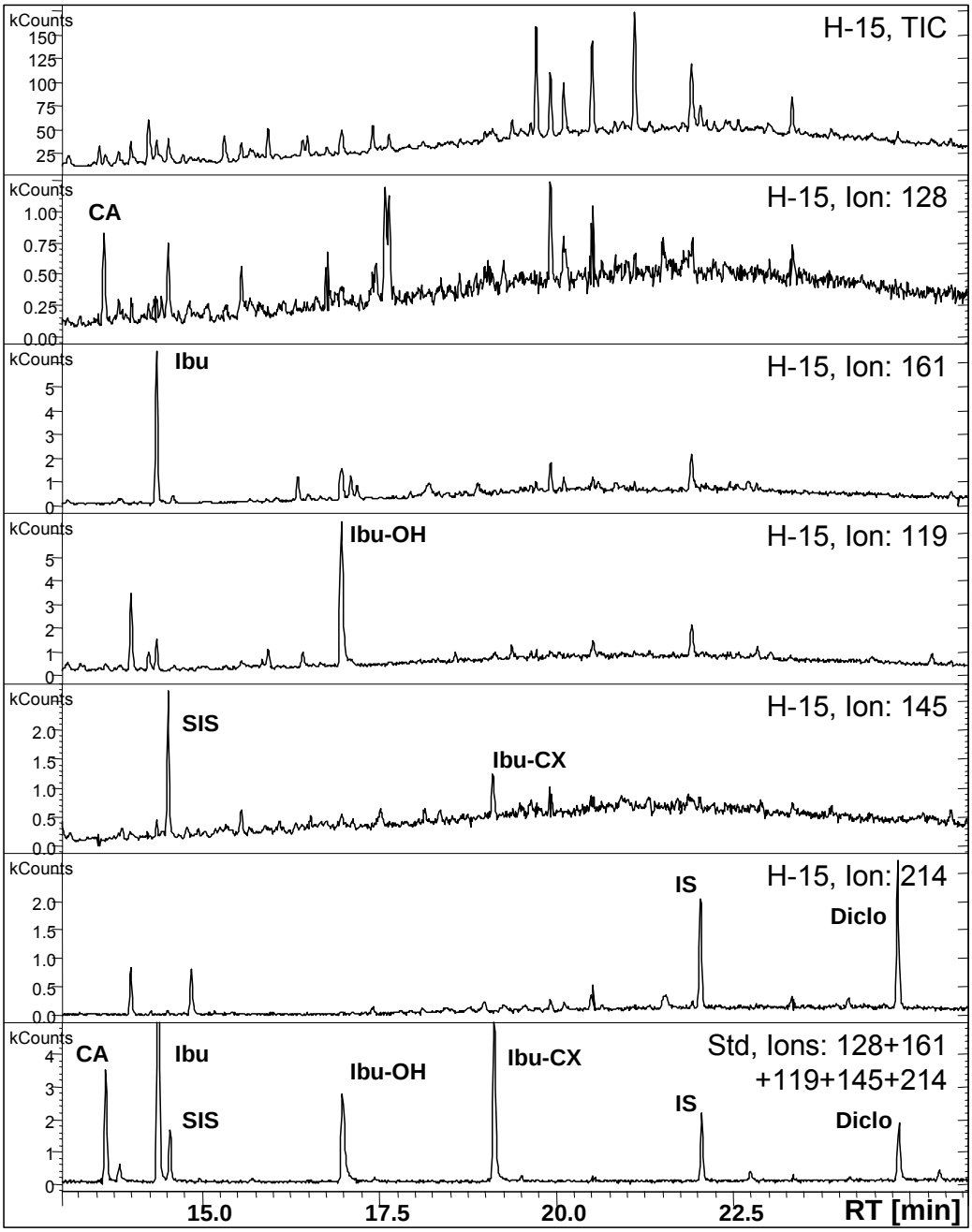

Figure 46: GC-MS chromatogram of the methanolic fraction of a river water sample (H-15) in comparison to a standard solution (Std; c = 200 ng/mL) after derivatisation, displaying the total ion current (TIC) and extracted ion traces (CA: clofibric acid, Ibu: ibuprofen, Diclo: diclofenac, SIS: surrogate internal standard, IS: volumetric internal standard)

Compared to the samples from the right bank of the river Elbe, concentrations of some compounds appeared to be up to 50 % higher on the left bank (e.g. caffeine and diclofenac), while for the herbicide mecoprop they were in the same range. Since the samples on the left and on the right bank were taken on different days, this finding can only serve as an indication for the influence of the STP discharge on PPCP concentrations. Mecoprop, that was included as an indicator for non-STP-derived emissions, was rather evenly distributed in all river samples. This implies that no agricultural or industrial emissions of mecoprop contribute to the river burden within the city area.

In lake water (H-07), concentrations of the pharmaceuticals were lower than in the river, but not as much as could be expected. No regular sewage emissions are reported for the lake and its tributaries. Only rarely, as a result of heavy rain events, the communal sewage system is overloaded so that raw sewage is discharged into channels connected to the lake. Usually, this does not happen more than once or twice a year during summer. It is rather unlikely that these amounts account for the detected concentrations. The concentration of caffeine in lake Alster is even higher than in Elbe river water that is directly affected by the STP-discharge. Even taking into account the low water exchange of the lake, these findings are an indication for additional sources. The concentration of mecoprop was three-fold higher in the lake than in river Elbe water. In addition to agriculture in the upper reaches of the contributing river the application of mecoprop on lawns in the vast gardens and parks along the lake and its tributaries is a likely source.

5.4 Concentration and metabolite patterns of ibuprofen

The metabolism of ibu in the human body is well known from pharmaco-kinetic studies. Main excretion products (including possible conjugates) are: ibu (15 %), ibu-OH (26 %), ibu-CX (43 %) and carboxy-hydratropic acid (2-phenylpropanoic acid) in minor amounts [59] (Figure 3). Under environmental conditions, these compounds have different transformation kinetics. Stumpf et al. [171] found that the excretion pattern is hardly changed on the way to the STP and only slightly during primary clarification. Major changes occurred during biological (activated sludge) treatment. Ibu-CX was almost quantitatively eliminated, while ibu-OH was hardly affected and thus was the dominant compound in STP effluents and rivers. This indicates that ibu-OH is the most stable of the three compounds under these conditions (if it is not continuously formed from ibu or from conjugate cleavage). Zwiener et al. [174] reported that ibu-OH was formed from ibu under aerobic conditions in activated sludge, while carboxy-hydratropic acid was formed under anaerobic conditions. In both cases, these transformation products did not account for more than 10 % of the initial ibu concentration, suggesting that the major amounts in sewage stem from human excretion. In order to compare the behaviour of ibu under North Norwegian and Central European conditions, ratios of ibu and its

metabolites were calculated from the concentrations in the investigated samples from Tromsø and Hamburg and compared with literature data (Figure 47).

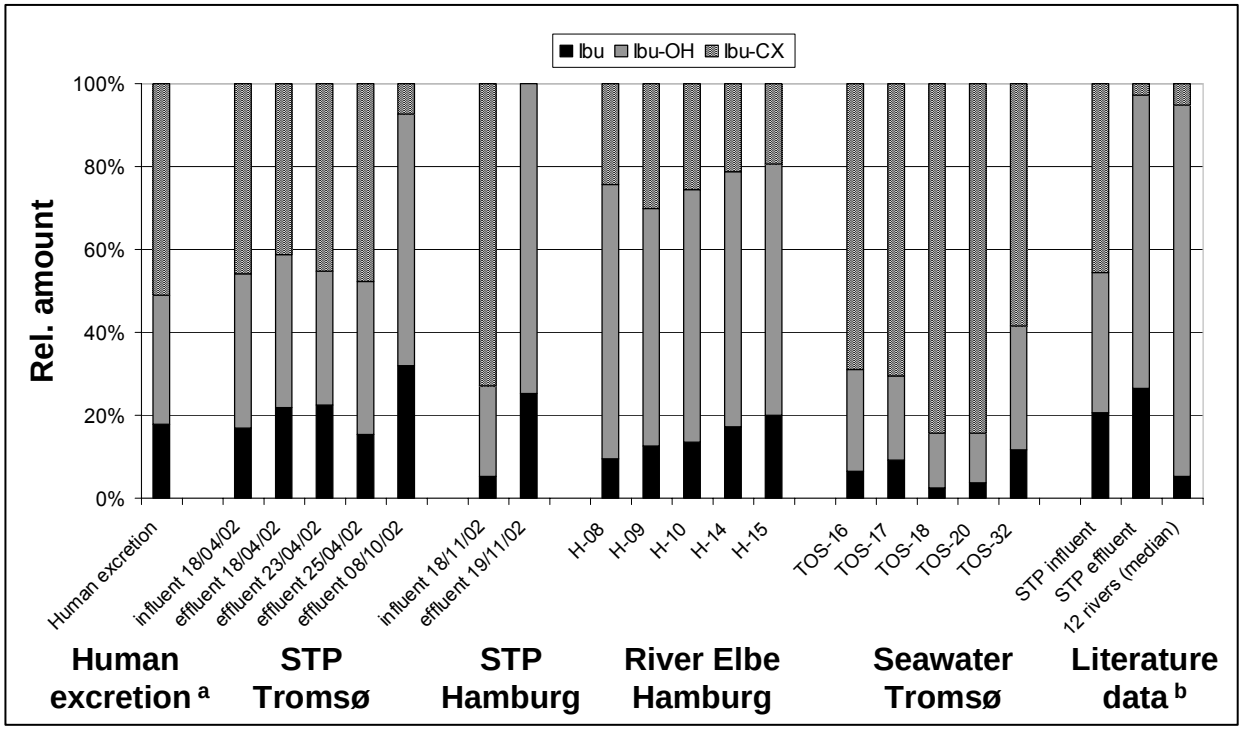

Figure 47: Relative amounts of ibu, ibu-OH and ibu-CX in sewage and seawater from Tromsø/ Norway in comparison to sewage and river water from Germany ([a] von Bruchhausen et al. [59]; [b] Stumpf et al. [171])

The pattern of the relative amounts of ibu and its metabolites in the sewage samples was the same that is observed in human excretions, indicating that no significant transformation processes took place in the sewer system between excretion and STP. This is in accordance with the observations of Stumpf et al. [171]. In contrast to German STPs, the pattern was not changed by STP passage in Tromsø. This is simply due to the fact that no activated sludge treatment is carried out in addition to particle filtration. In river water samples from Hamburg/Germany, ibu-OH was the dominant compound, although not as pronounced as in the river water samples analysed by Stumpf et al. [171]. It remains to be investigated whether sample pre-treatment (acidification to pH 2 vs. pH 7 in this work) excerts an influence on the conjugate cleavage in the sample. Nevertheless, in both cases the proportion of ibu-CX was higher in the rivers than in the contributing STP effluents. A completely different pattern was observed in the seawater samples from Tromsø. Ibu-CX was the major component of the ibu-group. Possible explanations are: (i) higher stability of ibu-CX under marine conditions as compared to ibu and ibu-OH, (ii) formation of ibu-CX from ibu or ibu-OH in the marine environment, (iii) alkaline hydrolysis specifically of ibu-CX conjugates in seawater. However, when interpreting the seawater results it has to be taken into account that several of the observed concentrations were close to the method LOQs and that the deviations in the recovery of ibu-CX at neutral pH are higher than for the other

compounds, resulting in elevated uncertainties. Nonetheless, the differences in patterns were sufficiently pronounced to state a clear difference between limnic and marine conditions and to investigate the environmental behaviour of this compound group further.

5.5 Conclusions

The present work has shown that pharmaceuticals and their metabolites have to be regarded as relevant contaminants also in Norwegian sewage. The observed patterns are differing from findings in other countries because of specific usage profiles. For example, the elsewhere ubiquitous clofibric acid was not detected and carbamazepine only in sewage receiving inputs from a psychiatric hospital. This hospital also specifically contributed the SSRI antidepressants paroxetine and sertraline.

Caffeine was a dominant constituent of the investigated sewage samples. Resulting from the direct emissions into the sea, it was distributed throughout the Tromsø-Sound in the range of 10 - 100 ng/L. Furthermore, caffeine residues were even detected at remote locations hardly affected by human settlements. In addition to their occurrence in all sewage samples, ibuprofen and its hydroxy- and carboxy-metabolites were also present in seawater from Tromsø-Sound in concentrations of up to 7.7 ng/L (sum concentrations). Comparison of the relative amounts of these three compounds in samples from Norway and Germany revealed similar patterns in sewage, but a notably different behaviour in limnic and marine waters. This indicates that the fate of these substances strongly depends on environmental conditions (temperature, salinity, pH, biological activity). The investigation of parent compound/metabolite concentration patterns will be a valuable tool in the further assessment of the environmental fate of pharmaceuticals.

6 Summary

Within the first part of the present work, a specially designed filtration/extraction device was used for the extraction of large volume water samples from different parts of the North Sea. The choice of solid-phase extraction by means of a polymeric sorbent enabled the extraction of a broad range of organic compounds from the water phase, far beyond the scope previously covered in analytical marine chemistry by liquid/liquid extraction or by the use of alkyl-silica based solid-phase sorbent. The thus obtained extracts were screened for the presence of organic contaminants by means of gas chromatography-mass spectroscopy. In the course of these investigations PAHs, PCBs, alkylbenzenes, chlorobenzenes, chloronitrobenzenes, bis(dichloropropyl)ethers, chloroanilines, dichlobenil, HCHs, and triazine herbicides were detected. Furthermore, a number of compounds was identified, which had not been reported before to be present in the water of the North Sea, including 1-chloronaphthalene, dichloropyridines, *N*-ethyltoluidine, DEET, tris(chloropropyl)phosphates, triphenylphosphine oxide and presumably biogenic mono-, di-, and tribromoindoles. The detection of caffeine and some pharmaceutically active compounds as for example propyphenazone, carbamazepine and clofibric acid was of special interest, since no information had been available before on the occurrence of this class of substances in marine ecosystems.

Based on the results of the GC-MS screening of the North Sea water extracts the applied extraction and determination method was validated for the quantification of a set of selected target analytes. This resulted not only in high recovery rates for the polar neutral analytes. Also the investigated acidic compounds such as clofibric acid, ibuprofen and diclofenac were recovered at remarkable 40 % or higher under the given conditions (pH 8.3). Characteristic distribution patterns of the target analytes were obtained by the application of the method to North Sea water samples. Caffeine was detected in relatively high concentrations (2 - 16 ng/L) at all stations. The distribution pattern points to rivers as important sources, with indications for additional contributions from shipping and offshore installations of the oil and gas industry. Clofibric acid, active metabolite of the lipid lowering agents clofibrate and etofibrate, was clearly detectable in the German Bight and off the Danish and Norwegian coasts. Concentrations of this compound ranged between 0.3 and 1.3 ng/L in these areas and thus in the same order of magnitude as classical organic pollutants, e.g., γ-HCH. In the Central North Sea and off the British east coast clofibric acid was either not detected or present below the quantification limit. The rivers Elbe and Rhine appear to be major sources for this compound. The insect repellent DEET showed a distribution pattern similar to that of clofibric acid at concentrations around 1 ng/L in the German Bight. In the case of DEET, the Rhine seems to be more relevant for the input into the North Sea than the

Elbe. Among the investigated pesticides, the distribution of the herbicide dichlobenil is noteworthy. Elevated concentrations (1.1 - 1.4 ng/L) were only observed at the sampling stations influenced by the River Rhine plume, while concentrations at all other stations ranged between 0.3 and 0.4 ng/L. This may be an indication for the contribution of atmospheric inputs to the contamination of the North Sea with this compound, which remains to be verified by the investigation of deposition samples. Further pesticides (e.g., metolachlor and terbuthylazine) showed a distribution pattern typical for predominant riverine input with highest values in the German Bight and along the Danish and Norwegian west coasts and concentrations that were non-detectable or below the limit of quantification in the Central North Sea and off the British east coast. Out of the six isomers of dichloropyridine, which have not been reported as contaminants of the aquatic environment to date, four isomers were identified in samples from the Elbe estuary. 2,6-Dichloropyridine was the dominant isomer, present in a concentration of 8.8 ng/L. In the North Sea, only the 2,6-isomer was detected occasionally, in most cases below or around the quantification limit of 0.1 ng/L. The observed distribution pattern of this compound does not allow unequivocal estimations of its sources, an atmospheric input in addition to the observed contribution of the River Elbe cannot be excluded. For the flame retardant tris(chloropropyl)phosphate first values for its distribution in the water of the North Sea were presented. Estimated concentrations calculated for the technical mixture ranged between 1 and 8 ng/L.

The second part of the present work focussed on the development and application of an analytical method for the simultaneous extraction of acidic, polar neutral and basic pharmaceuticals from environmental water samples at neutral pH. Under these conditions, recoveries of 70 - 100 % were obtained for most target analytes. Only the highly hydrophilic metabolite carboxy-ibuprofen, having a log Kow of -2.8 at pH 7 [109], showed an unsatisfactory recovery (30 %) under these extraction conditions. The developed method was applied to the determination of the concentrations of relevant pharmaceuticals (including some of their metabolites) as well as of caffeine in communal sewage and seawater from Tromsø/Norway and sewage and surface water from Hamburg/Germany. In Tromsø sewage caffeine proved to be a dominant component (concentration range 30 - 300 µg/L). Ibuprofen and its main metabolites hydroxy- and carboxy-ibuprofen were present in all samples in concentrations of up to 20 µg/L (sum of the three single compounds), while diclofenac, the antidepressants paroxetine, sertraline and fluoxetine as well as the ß-blocking agents propranolol and metoprolol were predominantly detected in sewage samples with contributions of hospital effluents. In seawater from the Tromsø area, exclusively caffeine and the compounds of the ibuprofen group were detected. Caffeine was measured in amazingly high concentrations. These were between 17 and 87 ng/L in the Tromsø-Sound and still just below 10 ng/L at the very sparsely populated coast of the open Arctic Ocean. Ibuprofen, hydroxy- and carboxy-

ibuprofen were identified for the first time in seawater, their concentrations in the Tromsø-Sound reached values of up to 7.5 ng/L (sum of the three single compounds). Most of the target analytes were detected in surface water from Hamburg (river Elbe and lake Alster). In Elbe water, caffeine was determined in concentrations of 100 - 150 ng/L, clofibric acid 3 - 8 ng/L, ibuprofen 5 - 32 ng/L, hydroxy-ibuprofen 20 - 100 ng/L, carboxy-ibuprofen from below the quantification limit to 32 ng/L, diclofenac 30 - 70 ng/L, DEET 16 - 38 ng/L. The more lipophilic compound triclosan was only detected occasionally in the water phase (up to 4 ng/L). In the water of the lake Alster the concentrations of the investigated pharmaceuticals were generally lower compared to the Elbe (in the range of 2 - 25 ng/L), while the concentration of caffeine (176 ng/L) was slightly higher than in the river. The concentration of the herbicide mecoprop was even three-fold higher in lake water (22 ng/L).

An interesting aspect revealed the comparison of the relations of the concentrations of ibuprofen and its two main metabolites in the different types of investigated water samples. While in the non-biologically treated effluent of the Tromsø sewage treatment plant (STP) and in the influent of the Hamburg STP the relative amounts were comparable to those known from human urine (pharmacokinetic studies), deviations from this pattern were obvious in biologically treated sewage as well as in river water and seawater. These variations have to be attributed to the differences in transformability of the three compounds under the prevailing conditions in the different types of sampled water. The determination of the characteristic relative amount patterns may be a valuable tool for the elucidation of the environmental behaviour and fate of residues of pharmaceuticals in aquatic ecosystems. This holds especially when it is supplemented by the determination of enantiomeric ratios in the case of chiral compounds as for example ibuprofen, which may contribute information for a distinction between biotic and abiotic transformation.

7 Zusammenfassung

Im ersten Teil der Arbeit wurde eine speziell entwickelte Filtratrations-/Extraktionsvorrichtung während einer Forschungsfahrt auf der Nordsee eingesetzt, um großvolumige Wasserproben (20 L) aus unterschiedlichen Teilen dieses Meeres zu extrahieren. Der Einsatz der Festphasenextraktion unter Verwendung eines Sorbens auf Polymerbasis erlaubte es, ein weites Spektrum organischer Verbindungen aus der Wasserphase zu extrahieren, das weit über das bisher in der organischen marinen Analytik durch Flüssig/Flüssig-Extraktion und Festphasenextraktion mit Alkylsilica-Phasen abgedeckte hinausgeht. Die gewonnen Extrakte wurden gaschromatographisch-massenspektrometrisch auf das Vorhandensein organischer Kontaminanten untersucht. Dabei wurden zahlreiche Verbindungen gefunden, deren Auftreten in der Nordsee bereits beschrieben ist, wie zum Beispiel polycyclische aromatische Kohlenwasserstoffe, PCB, Alkylbenzole, Chlorbenzole, Chlornitrobenzole, Bis(dichlorpropyl)-ether, Chloraniline, Dichlobenil, HCH und Triazinherbizide. Darüber hinaus wurde eine Reihe von Verbindungen identifiziert, deren Präsenz in der Nordsee bisher nicht bekannt bzw. belegt war. Dazu zählen 1-Chlornaphthalin, Dichlorpyridine, *N*-Ethyltoluidin, DEET, Tris-(chlorpropyl)phosphate und Triphenylphosphinoxid sowie vermutlich biogene Bromindole. Besonders bemerkenswert ist der Nachweis von Coffein und einigen pharmazeutischen Wirkstoffen wie Propyphenazon, Carbamazepin und Clofibrinsäure, da über das Auftreten dieser Substanzgruppe in marinen Ökosystemen bisher noch nicht berichtet wurde.

Ausgehend von den gewonnenen Erkenntnissen wurde die verwendete Extraktions- und Bestimmungsmethode für die Quantifizierung ausgewählter Zielanalyten validiert. Dabei ergaben sich nicht nur hohe Wiederfindungsraten für die polaren neutralen Verbindungen. Auch die untersuchten sauren Substanzen wie z.B. Clofibrinsäure und Ibuprofen wurden unter den gegebenen Bedingungen (pH 8,3) zu immerhin 40 % wiedergefunden. Aus den mit der Methode bestimmten Konzentrationen ergaben sich jeweils typische Verteilungsmuster der untersuchten Substanzen in der Nordsee. Coffein wurde in vergleichsweise hohen Konzentrationen (2 - 16 ng/L) an allen Stationen nachgewiesen, wobei die Verteilung auf Flüsse als bedeutendste Eintragsquelle hindeutet, aber auch Indizien für einen Beitrag aus Seeschifffahrt und von Ölförderplattformen enthält. Clofibrinsäure, der aktive Metabolit der Lipidsenker Clofibrat und Etofibrat, war sehr deutlich im Bereich der Deutschen Bucht und vor der Norwegischen Küste nachweisbar. Die Konzentrationen bewegten sich hier zwischen 0,3 und 1,3 ng/L und damit in ähnlichen Größenordnungen wie klassische organische Schadstoffe, z.B. γ-HCH. In der zentralen Nordsee und vor der Britischen Küste war Clofibrinsäure gar nicht oder nur in Spuren nachweisbar. Elbe und Rhein scheinen demnach die bedeutendsten Eintragsquellen zu sein. Das Insektenrepellent DEET zeigte eine ganz ähnliche Verteilung wie Clofibrinsäure mit Konzentrationen um 1 ng/L in der Deutschen Bucht. Allerdings scheint der Rhein für den Eintrag dieser Substanz in die Nordsee

bedeutender als die Elbe. Von den untersuchten Pestiziden ist besonders die Verteilung des Herbizids Dichlobenil auffällig. Erhöhte Konzentrationen (1,1 - 1,4 ng/L) wurden an den von der Rheinfahne beeinflußten Stationen gefunden, während sich die Konzentrationen an fast allen übrigen Stationen im Bereich von 0,3 - 0,4 ng/L bewegten. Dies könnte ein Indiz für den Beitrag eines atmosphärischen Eintrags zur Belastung der Nordsee mit dieser Substanz sein, was durch Untersuchung von Depositionsproben überprüft werden müßte. Weitere Pestizide (z.B. Metolachlor und Terbuthylazin) zeigen eine für überwiegenden Flußeintrag typische Verteilung mit den höchsten Werten in der Deutschen Bucht und entlang der Dänischen und Norwegischen Küsten sowie nicht nachweisbaren oder unterhalb der Bestimmungsgrenze liegenden Konzentrationen in der zentralen Nordsee und vor der Britischen Küste. Von den sechs Isomeren des Dichlorpyridins, dessen Auftreten in der aquatischen Umwelt bisher nicht beschrieben war, konnten vier im Elbeästuar nachgewiesen werden, 2,6-Dichlorpyridin in einer Konzentration von 8,8 ng/L. In der Nordsee hingegen war nur das 2,6-Isomer vereinzelt nachweisbar, meist unter oder um die Bestimmungsgrenze von 0,1 ng/L. Dabei läßt die Verteilung keine eindeutigen Schlüsse auf die Herkunft zu, ein atmosphärischer Eintrag kann nicht ausgeschlossen werden. Erstmals wurden auch Werte für die Verteilung des als Flammschutzmittel eingesetzten Tris(chlorpropyl)phosphats im Wasser der Nordsee vorgelegt. Die abgeschätzten Konzentrationen, bezogen auf die technische Mischung, bewegten sich zwischen 1 und 8 ng/L.

Im zweiten Teil der Arbeit standen die Entwicklung und Anwendung einer Methode zur gleichzeitigen Extraktion saurer, neutraler und basischer Pharmazeutika aus Wasserproben bei neutralem pH-Wert im Vordergrund. Dabei konnten für die meisten Verbindungen Wiederfindungsraten von 70 - 100 % erzielt werden. Lediglich Carboxy-Ibuprofen (log Kow bei pH 7 = -2,8 [109]) zeigte eine unzureichende Wiederfindung (30 %) unter den gegebenen Extraktionsbedingungen. Die Methode wurde zur Bestimmung der Konzentrationen relevanter pharmazeutischer Wirkstoffe (und einiger ihrer Metabolite) sowie Coffein in kommunalem Abwasser und Meerwasser in Tromsø/Norwegen und Abwasser und Flußwasser aus Hamburg/Deutschland eingesetzt. Im Tromsøer Abwasser stellte Coffein eine dominierende Komponente dar (Konzentrationen im Bereich 30 - 300 µg/L). Ibuprofen und seine Hauptmetabolite Hydroxy- und Carboxy-Ibuprofen waren in allen Proben anzutreffen (bis zu 20 µg/L als Summe der drei Einzelverbindungen), während Diclofenac, die Antidepressiva Paroxetin, Sertralin und Fluoxetin sowie die ß-Blocker Propranolol und Metoprolol überwiegend in Abwasserströmen detektiert wurden, die auch Krankenhausabwasser enthielten. In Meerwasser waren ausschließlich Coffein und die Verbindungen der Ibuprofen-Gruppe nachweisbar. Coffein wurde dabei in erstaunlich hohen Konzentrationen detektiert. Diese lagen zwischen 17 und 87 ng/L im Tromsø-Sund und immerhin noch knapp unter 10 ng/L an der kaum besiedelten Küste des offenen Arktischen Ozeans. Ibuprofen,

Hydroxy- und Carboxy-Ibuprofen wurden erstmalig in Meerwasser nachgewiesen, die Konzentrationen im Tromsø-Sund erreichten Werte bis zu 7,5 ng/L (als Summe der drei Einzelverbindungen). In Oberflächenwasser aus Hamburg (Elbe und Alster) waren erwatungsgemäß die meisten untersuchten Verbindungen nachweisbar. In der Elbe wurde Coffein in Konzentrationen von 100 - 150 ng/L bestimmt, Clofibrinsäure von 3 - 8 ng/L, Ibuprofen von 5 - 32 ng/L, Hydroxy-Ibuprofen von 20 - 100 ng/L, Carboxy-Ibuprofen von unterhalb der Bestimmungsgrenze bis 32 ng/L, Diclofenac von 30 - 70 ng/L, DEET von 16 - 38 ng/L, während das stärker lipophile Triclosan nur vereinzelt in der Wasserphase nachweisbar war (bis 4 ng/L). Im Wasser der Alster lagen die Konzentrationen der Pharmazeutikarückstände durchweg niedriger als in der Elbe (im Bereich 2 - 25 ng/L), während die Coffeinkonzentration darüber (176 ng/L) und die Konzentration des Herbizids Mecoprop sogar dreimal so hoch lag (22 ng/L).

Einen interessanten Aspekt offenbarte der Vergleich der Verhältnisse der Konzentrationen von Ibuprofen und seiner beiden Metabolite zueinander in den unterschiedlichen untersuchten Typen von Wasserproben. Während die Verhältnisse im (nicht biologisch behandelten) Abwasser von Tromsø und im Zulauf des Hamburger Klärwerkes Köhlbrandhöft in etwa denen aus der Humanpharmakokinetik bekannten entsprachen, traten in biologisch behandeltem Abwasser sowie in Fluß- und Meerwasser deutliche Verschiebungen auf, die auf der unterschiedlichen Abbaubarkeit der drei Verbindungen unter den jeweiligen Bedingungen beruhen. Die Bestimmung dieser Verhältnisse kann wichtige Informationen über das Umweltverhalten von xenobiotischen Pharmazeutikarückständen liefern, insbesondere wenn durch die Bestimmung von Enantiomerenverhältnissen im Falle chiraler Verbindungen wie etwa Ibuprofen zusätzlich Informationen zur Unterscheidung zwischen biotischer und abiotischer Transformation gewonnen werden können.

8 Experimental

8.1 Instruments

a) Chromatography

GC-MS (Hamburg)

GC: Varian 3400 (Varian Associates, Sunnyvale, USA)

Split/splitless injector 1075 (60 s splitless, 523 K (250 °C))

Carrier gas: helium 5.0 (Linde, Hamburg) 75 kPa

Transfer-line: 523 K (250 °C)

Columns: a) DB5-MS (length 30 m, I.D. 0.25 mm, film thickness 0.25 μm)
(J&W Scientific, Folsom, USA)
b) HP5-MS (length 30 m, I.D. 0.25 mm, film thickness 0.25 μm)
(Agilent Technologies, Palo Alto, USA)

Autosampler: A 200 SE (CTC Analytics, Zwingen, Switzerland), injected volume 2 μL

Temperature programmes:

333 K (60 °C) [2 min] → (7 K/min) → 533 K (260 °C) [20 min]

333 K (60 °C) [2 min] → (10 K/min) → 533 K (260 °C) [20 min] for recovery studies

MS: Magnum ITD ion trap mass spectrometer (Finnigan, MAT, Bremen, Germany)

Ionisation: EI, 70 eV, emission current 10 μA, source temperature 473 K (200 °C)

GC-MS (Tromsø)

GC: Mega II 8065 (Fisons, Milan, Italy)

On-column injector

Autosampler: AS800 (Fisons), injected volume 2 μL

Carrier gas: helium 5.0, 80 kPa column head pressure

GC-MS interface: 523 K (250 °C)

Column: DB5-MS (length 30 m, I.D. 0.25 mm, film thickness 0.25 μm)
(J&W Scientific, Folsom, USA)

Temperature programme: 343 K (70 °C) [2 min] → (7 K/min) → 533 K (260 °C) [20 min]

MS: MD800 quadrupole mass spectrometer (Finnigan MAT, San Jose, CA, USA)

Ionisation: EI, 70 eV, source temperature 493 K (220 °C)

HPLC-DAD

HPLC: Gynkotek Pump M480, Autosampler GINA 50

Detector: UVD 3405 (DAD, UV-VIS)

Column: LiChrocart 125-4 (Merck), filled with LiChrosper 100 RP-18 (5 μm)

Gradient programme: methanol/water (10 mM ammonium acetate, 0.1 % triethylamine, acetic acid to pH 5) (0 - 30 s: 20 % methanol, 1 min: 40 %, 5 - 25 min: 47 %, 40 min: 70 %, 48 - 55 min: 100 %), flow 0.5 mL/min, injected volume 50 µL

HPLC-MS

HPLC: 2690 HPLC (Waters, Milford, USA)

Column: SymmetryShield RP18, 3.5 µm, 2.1 * 50 mm (Waters)

Gradient programme: methanol/water (2.5 mM ammonium acetate) (0 - 1 min: 30 % methanol, 2 min: 40 %, 15: min 62 %), flow 0.2 mL/min, injected volume 10 µL

MS: Quattro LC (triple quadrupole) mass spectrometer (Micromass, Manchester, UK)

Ionisation: ESI and APCI

b) Filtration/Extraction

Filtration unit: Stainless steel 4301 (Mechanical workshop, Department of Chemistry, University of Hamburg)

Extraction unit: PTFE (Mechanical workshop, Department of Chemistry, University of Hamburg)

Pump: Gear pump MCP-Z with pump head Z-120 (Ismatec, Wertheim, Germany)

c) Water purification

Deionised and organically purified water for recovery studies and preparation of artificial seawater was prepared with a Seral-Pur 90 C apparatus (Seral, Ransbach, Germany).

8.2 Preparation of artificial seawater

Artificial seawater of a salinity of 35 ‰ was prepared according to Dietrich [175], but modified in the way that all salts of monovalent metal ions were calculated as NaCl and all salts of bivalent metal ions were calculated as $MgSO_4$. Prior to use, the respective salts (in p.a. quality) were cleaned from organic contaminants and dried by heating them overnight at 873 K (600 °C), except for $NaHCO_3$: 523 K (250 °C). 280 g NaCl and 77 g $MgSO_4$ were dissolved in 10 L of deionised and purified water. The pH was adjusted to 8.3 by the addition of $NaHCO_3$.

8.3 Chemicals

Substance	Supplier	Hazard symbols	CAS number
Solvents			
Acetone z.R.	Merck, Darmstadt	F	[67-64-1]
Dichloromethane z.R.	Merck, Darmstadt	Xn, F	[1665-00-5]
Ethyl acetate z.R.	Merck, Darmstadt	F	[141-78-6]
n-Hexane z.R.	Merck, Darmstadt	Xn, F	[110-54-3]
Methanol z.R./gr. grade	Merck, Darmstadt	T, F	[67-56-1]
iso-Octane z.R.	Merck, Darmstadt	F	[540-84-1]
Toluene z.R.	Merck, Darmstadt	Xn, F	[108-88-3]
Water gr. grade	Merck, Darmstadt	-	[7732-18-5]
Reference compounds			
Acetylsalicylic acid	Merck, Darmstadt	Xn	[50-78-2]
Atrazine	Promochem, Wesel	Xn	[1912-24-9]
Bezafibrate	Sigma-Aldrich, Steinheim	A	[41859-67-0]
Carbamazepine	Synopharm, Barsbüttel	A	[298-46-4]
Carboxy-Ibuprofen	synthesised	-	[15935-54-3]
Clofibric acid	ICN Biomedicals, Eschwege	Xn	[882-09-7]
Caffeine	Merck, Darmstadt	Xn	[58-08-2]
$^{15}N_2$-Caffeine	Dr. Ehrenstorfer, Augsburg	Xn	
2-Chloroaniline	Merck, Darmstadt	T	[95-51-2]
3-Chloro-4-fluoronitrobenzene	Sigma-Aldrich, Steinheim	Xn	[350-30-1]
1-Chloronaphthalene	Merck, Darmstadt	Xn	[90-13-1]
1-Chloro-2-nitrobenzene	Merck, Darmstadt	T	[88-73-3]
1-Chloro-4-nitrobenzene	Merck, Darmstadt	T	[100-00-5]
Desethylatrazine	Promochem, Wesel	Xn	[1007-28-9]
Desethylterbutylazine	Promochem, Wesel	Xn	[30125-63-4]
2,5-Dichloroaniline	Merck, Darmstadt	T	[95-82-9]
1,2-Dichlorobenzene	Merck, Darmstadt	Xn	[95-50-1]
1,3-Dichlorobenzene	Merck, Darmstadt	Xn	[541-73-1]
1,4-Dichlorobenzene	Merck, Darmstadt	Xn	[106-46-7]
2,6-Dichlorobenzonitrile	ABCR, Karlsruhe	Xn	[1194-65-6]
2,3-Dichloropyridine	Sigma-Aldrich, Steinheim	Xi	[2402-77-9]
2,5-Dichloropyridine	Sigma-Aldrich, Steinheim	Xi	[16110-09-1]
2,6-Dichloropyridine	Sigma-Aldrich, Steinheim	T	[2402-78-0]
3,5-Dichloropyridine	Sigma-Aldrich, Steinheim	Xi	[2457-47-8]
Diclofenac-sodium	Synopharm, Barsbüttel	A	[15307-86-5]
2,4-Dibromoanisole	ICN Biomedicals, Eschwege	-	[21702-84-1]
Diuron	Promochem, Wesel	Xi	[330-54-1]
17*ß*-Estradiol	Sigma-Aldrich, Steinheim	Xn	[50-28-2]
Estrone	Sigma-Aldrich, Steinheim	Xn	[53-16-7]]
Fluoxetine-HCl	Sigma-Aldrich, Steinheim	A	[56296-78-7]
α-HCH	Riedel de Häen, Seelze	T, N	[319-84-6]
ß-HCH	Dr. Ehrenstorfer, Augsburg	T, N	[319-85-7]
γ-HCH	Dr. Ehrenstorfer, Augsburg	T, N	[58-89-9]
Hydroxy-Ibuprofen	synthesised	A	[51146-55-5]
Ibuprofen	Synopharm, Barsbüttel	Xn	[110-54-3]
Ketoprofen	ICN Biomedicals, Eschwege	A	[22071-15-4]

Linuron	Promochem, Wesel	Xn	[330-55-2]
Mecoprop-d3	Dr. Ehrenstorfer, Augsburg	Xn	[352431-15-3]
Metolachlor	Dr. Ehrenstorfer, Augsburg	Xn	[51218-45-2]
Metoprolol tartrate	Sigma-Aldrich, Steinheim	A	[56392-17-7]
N,N-Diethyl-3-toluamide	Merck, Darmstadt	Xn	[134-62-3]
Nitrobenzene	Merck, Darmstadt	T^+	[98-95-3]
Oxazepam	Sigma-Aldrich, Steinheim	A	[604-75-1]
Paracetamol	Aldrich, Taufkirchen	-	[103-90-2]
Parathion-methyl	Promochem, Wesel	T	[298-00-0]
Pirimicarb	Promochem, Wesel	T	[23103-98-2]
Propoxur	Promochem, Wesel	T	[114-26-1]
Propranolol-HCl	ICN Biomedicals, Eschwege	Xn	[525-66-6]
Propyphenazone	Synopharm, Barsbüttel	-	[479-92-5]
Simazine	Promochem, Wesel	Xn	[12234-9]
TCPP	Akzo Nobel, Amersfoort, NL	Xn	[13674-84-5]
Terbuthylazine	Promochem, Wesel	Xn	[5915-41-3]
1,2,4-Trichlorobenzene	Merck, Darmstadt	Xn	[120-82-1]
Triclosan	Promochem, Wesel	A	[3380-34-5]
Triphenylphosphine oxide	Sigma-Aldrich, Steinheim	Xn, N	[791-28-6]
Salts			
Magnesium sulfate p.a.	Merck, Darmstadt	-	[7487-88-9]
Sodium chloride p.a.	Merck, Darmstadt	-	[7647-14-5]
Sodium hydrogen carbonate p.a.	Merck, Darmstadt	-	[144-55-8]
Sodium sulfate p.a.	Merck, Darmstadt	-	[7757-82-6]
Synthesis reagents and solvents			
N-Bromosuccinimide	Merck, Darmstadt	Xn	[128-08-5]
3-Chloroperbenzoic acid (85%),	Lancaster, Mühlheim	O, Xi	[937-14-4]
Dibenzoylperoxide	Merck, Darmstadt	E, Xi	[94-36-0]
Iodomethane	Merck, Darmstadt	T	[74-88-4]
Methylmalonic acid diethylester	Aldrich, Taufkirchen	A	[609-08-5]
Potassium *tert*-butoxide	Lancaster, Mühlheim	F, C	[865-47-4]
2-*p*-Tolylmalonic acid diethylester	Aldrich, Taufkirchen	-	[29148-27-4]
Lithium bromide	Lancaster, Mühlheim	-	[16949-15-8]
Palladium/carbon (10%)	Lancaster, Mühlheim	-	[7440-05-3]
Sodium	Merck, Darmstadt	F, C	[7440-23-5]
Sodium hydride	Merck, Darmstadt	F, C	[7646-69-7]
Sodium hydroxide	Merck, Darmstadt	C	[1310-73-2]
Sodium thiosulfate	Merck, Darmstadt	-	[7772-98-7]
Acetic acid	Merck, Darmstadt	C	[64-19-7]
Hydrochloric acid	Merck, Darmstadt	C	[7647-01-0]
Sulfuric acid	Merck, Darmstadt	C	[7664-93-9]
Dichloromethane	Merck, Darmstadt	Xn, F	[1665-00-5]
Diethyl ether	Merck, Darmstadt	F	[60-29-7]
N,N-Dimethyl formamide	Merck, Darmstadt	T	[68-12-2]
Ethanol	Merck, Darmstadt	F	[64-17-5]
n-Hexane	Merck, Darmstadt	Xn, F	[110-54-3]
Petroleum ether	Merck, Darmstadt	F	[8032-32-4]
Tetrachloromethane	Merck, Darmstadt	T, N	[56-23-5]
Tetrahydrofuran	Merck, Darmstadt	F, Xi	[109-99-9]

Table 25: Chemicals and solvents used in the present work

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Annex

SOP 1: Filtration and extraction of large volume water samples (20 L)

SOP 2: Silica fractionation

SOP 3: Derivatisation with methyl chloromethanoate

SOP 4: 1 L SPE (non-target)

SOP 5: PPCP SPE method (1 L samples)

Eidesstattliche Versicherung

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SOP 1: Filtration and extraction of large volume water samples (20 L)

Preparation

Prior to the extraction the complete device is rinsed with acetone p.a., *n*-hexane z. R. (organic trace analysis grade), methanol z.R. and water. A clean glass cartridge (∅ 47 mm; in-house constructed) is fitted into the PTFE extraction unit „TT2“ (in-house constructed). The bottom of the cartridge is covered with a glass fibre filter sheet (∅ 47 mm, GF/C; Whatman, Maidstone, UK). 2 g of the sorbent SDB-1 (Baker, Griesheim, BRD) are suspended in 30 mL *n*-hexane z.R. in a beaker and poured into the cartridge. Sorbent residues remaining in the beaker are transferred into the cartridge with another 2 x 10 mL *n*-hexane. The solvent is removed by applying a gentle vacuum to the exit of the extraction unit. The still wet sorbent is covered with a glass fibre filter sheet. The packed sorbent is fixed by introduction of the PTFE-cylinder (and a suitable number of PTFE distance rings according to the sorbent bed height) and the PTFE screw ring. The sorbent is then washed and conditioned by sequential rinsing with 3 x 50 mL n-hexane, 3 x 50 mL ethyl acetate, 50 mL methanol and 50 mL HPLC-grade water. Care should be taken to avoid the cartridge running dry. Thereupon, the exit of the extraction unit is closed with a blind screw, the unit filled with water and the lid is screwed onto the unit.

Afterwards, a cleaned glass fibre filter candle is set into the filtration unit which is then connected via PTFE tubing with the pump (gear pump MCP-Z; Ismatec; Wertheim, BRD). Another piece of PTFE tubing is connected to the exit of the pump. The filtration unit is filled with water until it reaches the upper end of the tubing from the exit of the pump. The end of the tubing is then connected to the extraction unit. All of the dead volume of the two units should be filled with water. After the filtration unit is completely filled with water the lid is placed on the filtration unit and closed with screws. To the upper exit of the filtration unit the sampling tube is now connected, filled with water and dipped into the sample.

Extraction

After exchange of the blind screw at the exit of the extraction unit for the effluent tubing, the pump is started at a rate of 500 mL/min. After the sample had been pumped completely from the water sampler, the residual water between pump and sorbent is sucked through the sorbent by means of a water jet pump. After opening of the extraction unit and removal of the screw ring and the fixation cylinder the loaded cartridge is removed, wrapped in aluminium foil and stored in a screw lid glass container at 255 K (-18 °C) until elution.

Elution

After the cartridge has been brought to room temperature it is inserted into the extraction unit and fixed as described above. The sorbent is then eluted with 90 mL ethyl acetate z.R. and 50 mL *n*-hexane z.R./ethyl acetate z.R. 4:1 (v/v) by applying a gentle vacuum (850 mbar). Both eluates are collected in a 250 mL round bottom flask and frozen at 255 K (-18 °C) to achieve a better phase separation. Immediately after thawing, the aqueous phase is transferred to a 8 mL sample vial and extracted twice with 1 mL of *n*-hexane. The combined organic phases are dried over sodium sulfate p.a. (granulated) and then transferred to a 250 mL round bottom flask. The remaining sodium sulfate is extracted three times with 10 mL *n*-hexane z.R./ethyl acetate z.R. 4:1 (v/v). The combined organic phases are reduced in volume to ca. 10 mL on a rotary evaporator, transferred to a 25 mL tapered flask with an extended tip and reduced to 150 µL after addition of *iso*-octane as a keeper, assuring complete removal of ethyl acetate. The extract is transferred to a 2 mL sample vial containing a 200 µL insert and stored at 255 K (-18 °C) until measurement.

SOP 2: Silica fractionation

8 mL glass cartridges (Bakerbond SPE) are filled with 2 g silica (Baker, Griesheim, BRD) between PTFE frits (pore size 20 µm; Baker). The silica had been activated 15 h at 393 K (120 °C). The dry packed column is conditioned with 2 bed volumes of *n*-hexane. The sample (in *n*-hexane or *iso*-octane) is added on top of the column and eluted according to the following scheme:

Fraction 1: 6 mL *n*-hexane
Fraction 2: 6 mL *n*-hexane/dichloromethane 9:1 (v/v)
Fraction 3: 6 mL *n*-hexane/dichloromethane 4:6 (v/v)
Fraction 4: 6 mL dichloromethane
Fraction 5: 6 mL dichloromethane/ethyl acetate 1:1 (v/v)
Fraction 6: 6 mL ethyl acetate
Fraction 7: 6 mL acetone
Fraction 8: 12 mL methanol

In each fraction the solvent is changed to *iso*-octane by repeated addition and evaporation of 200 µL portions of *iso*-octane after reduction of the sample volume to approximately 500 µL in a rotary evaporator under reduced pressure at 313 K (40 °C). Finally , the sample is reduced to a volume of 150 µL, transferred to a 2 mL vial containing a 200 µL insert and stored at 255 K (-18 °C) until measurement.

SOP 3: Derivatisation with methyl chloromethanoate

The methanolic SPE fraction is condensed to 1 mL either by rotary evaporator or in a Turbo-vap unit, transferred to a 2 mL vial and evaporated to dryness by a gentle stream of nitrogen. The dry extract is dissolved in a mixture of 100 µL acetonitrile/methanol/water/pyridine (5:2:2:1, v/v) and then 7 µL methyl chloromethanoate are added. The vials are closed and left for 10 minutes at room temperature. After addition of 500 µL *n*-hexane the solution is washed twice with 50 µL of purified water. After drying over Na_2SO_4 and addition of the internal volumetric standard (large volume method: 100 µL ε-HCH in *n*-hexane, 250 ng/mL; PPCP 1 L method: 50 µL mecoprop 2,2,4-trimethylpentylester in toluene, 500 ng/mL, according to 25 ng absolutely), the extract is reduced under nitrogen to a final volume of approximately 100 µL or 50 µL, respectively.

SOP 4: 1 L SPE (non-target)

Commercially available SPE cartridges (Bakerbond SDB-1, 200 mg, 6 mL, polypropylene; Baker, Griesheim, BRD) are used with a vacuum operated extraction column processing system (Baker spe-12G). The cartridges are placed on the Luer lock connectors of the extraction manifold and washed/conditioned by rinsing them according to the following scheme under a gentle vacuum (900 mbar):

1. 2 x 6 mL *n*-hexane
2. 2 x 6 mL ethyl acetate
3. 2 x 6 mL methanol
4. 2 x 6 mL water

Caution must be taken to prevent the cartridges from running dry (from the methanol conditioning onwards). After conditioning, the cartridges are filled with water (sample) and connected to the sample bottles via large volume adaptors, consisting of polypropylene plugs and PTFE tubing (Baker). The extraction is carried out by application of vacuum to the manifold, assuring a flow rate of approximately 15 mL/min (resulting in an extraction time of ca. 1 h/1 L sample). After the extraction is finished, the adaptors are removed and the cartridges are rinsed with 5 mL of deionised water each. The cartridges are then connected to nitrogen supply (at 2 - 3 bar) and dried within 15 to 30 min. The drying is complete when the colour of the sorbent has changed from dark orange to a light orange. If necessary, the dry cartridge is wrapped in aluminium foil and stored in a screw lid glass container at 255 K (-18 °C) until elution.

The elution is carried out (if necessary after equilibrating the frozen cartridges to room temperature) sequentially on the extraction manifold. The elution solvents are sucked

through the cartridges according to the following scheme under a gentle vacuum (900 mbar) and collected in 8 mL sample vials:

1. 8 mL *n*-hexane
2. 8 mL ethyl acetate
3. 8 mL methanol

Fraction 1 (*n*-hexane) is directly evaporated to a final volume of 100 µL under a gentle stream of nitrogen and then transferred to a 2 mL vial equipped with a 150 µL insert. Fraction 2 (ethyl acetate) is condensed to approximately 100 µL under a stream of nitrogen. A solvent change is then performed by addition of 100 µL of toluene and repeated evaporation to 100 µL. This procedure is performed twice in order to remove ethyl acetate completely from the sample extract. The extract is then transferred to a 2 mL vial equipped with a 150 µL insert. Fraction 3 (methanol) is evaporated to 0.5 mL in a Turbovap Closed Cell Concentrator (Zymark, Hopkinton, USA), transferred to a 2 mL vial (the Turbovap glass is rinsed with 0.5 mL of methanol afterwards, which is also transferred to the vial) and derivatised according to SOP 3.

SOP 5: PPCP SPE method (1 L samples)

Filtration

1 L water samples are filtered prior to extraction with GF/C glass fibre filters, 47 mm diameter, 1.2 µm exclusion size (Whatman, Maidstone, UK), using a modified filtration apparatus (Sartorius, Göttingen, Germany). Afterwards, pH is adjusted to 7 by addition of sulphuric acid (25 %) and 100 µL of the surrogate standard mix (0.12 µg/mL D_3-mecoprop + 0.65 µg/mL $^{15}N_2$-caffeine in methanol) is added.

Extraction

Clean 6 mL glass SPE cartridges (IST/Separtis, Grenzach-Whylen, Germany) are filled with 500 mg of Oasis HLB 60 µm bulk sorbent (Waters, Eschborn, Germany) between two PTFE frits (20 µm pore size; IST). The prepared cartridges are placed on the Luer lock connectors of the vacuum operated extraction column processing system Baker spe-12G (Baker, Griesheim, BRD) and washed/conditioned by rinsing them according to the following scheme under a gentle vacuum (900 mbar):

1. 5 mL *n*-hexane
2. 5 mL ethyl acetate
3. 10 mL methanol
4. 10 mL water

Caution must be taken to prevent the cartridges from running dry (from the methanol conditioning onwards). After conditioning, the cartridges are filled with water (sample) and connected to the sample bottles via large volume adaptors, consisting of PTFE plugs and PTFE tubing (IST). The extraction is carried out by application of vacuum to the manifold, assuring a flow rate of approximately 15 mL/min (resulting in an extraction time of approximately 1 h/1 L sample). After the extraction is finished, the adaptors are removed and the cartridges are rinsed with 5 mL of deionised water each. The cartridges are then connected to nitrogen supply (at 2 - 3 bar) and dried within 15 to 30 min. The drying is complete when the colour of the sorbent has changed from light yellow to almost white. If necessary, the dry cartridge is wrapped in aluminium foil and stored in a screw lid glass container at 255 K (-18 °C) until elution.

The elution is carried out (if necessary after equilibrating the frozen cartridges to room temperature) sequentially on the extraction manifold. The elution solvents are sucked through the cartridges according to the following scheme under a gentle vacuum (900 mbar) and collected in 8 mL sample vials:

1. 5 mL *n*-hexane
2. 5 mL ethyl acetate
3. 14 mL methanol

Fraction 1 (*n*-hexane) is discarded. Fraction 2 (ethyl acetate) is condensed to ca. 100 µL under a stream of nitrogen. A solvent change is then performed by addition of 100 µL of toluene and repeated evaporation to 100 µL. This procedure is performed twice in order to remove ethyl acetate completely from the sample extract. 50 µL of the volumetric internal standard (25 ng mecoprop 2,2,4-trimethylpentylester in toluene) are added and the extract is condensed to a final volume of 50 µL. The extract is then transferred to a 2 mL vial equipped with a 150 µL insert. Fraction 3 (methanol) is evaporated to 0.5 mL in a Turbovap Closed Cell Concentrator (Zymark, Hopkinton, USA), transferred to a 2 mL vial (the Turbovap glass is rinsed with 0.5 mL of methanol afterwards, which is also transferred to the vial) and derivatised according to SOP 3.

Eidesstattliche Versicherung

Hiermit erkläre ich, daß ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfaßt, andere als die angegebenen Hilfsmittel und Quellen nicht benutzt und die den benutzten wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Stefan Weigel Hamburg, im Oktober 2003

List of papers

Results of the present thesis are published in the following articles:

S. Weigel, K. Bester, H. Hühnerfuss: New method for rapid solid-phase extraction of large volume water samples and its application in the screening of North Sea water for organic contaminants, J. Chromatogr. A **2001**, 912, 151-161.

S. Weigel, J. Kuhlmann, H. Hühnerfuss: Drugs and personal care products as ubiquitous pollutants: occurrence and distribution of clofibric acid, caffeine and DEET in the North Sea, Sci. Tot. Environ. **2002**, 295, 131-141.

S. Weigel, R. Kallenborn, H. Hühnerfuss: Simultaneous solid-phase extraction of acidic, neutral and basic pharmaceuticals from aqueous samples at ambient (neutral) pH and their determination by gas chromatography-mass spectrometry, J. Chromatogr. A **2004**, 1023, 183-195.

S. Weigel, U. Berger, E. Jensen, R. Kallenborn, H. Thoresen, H. Hühnerfuss: Determination of selected pharmaceuticals and caffeine in sewage and seawater from Tromsø/Norway with emphasis on ibuprofen and its metabolites, submitted for publication in Chemosphere.

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