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# R&D-Project:

# Identification of Organic Compounds in the North and Baltic Seas

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# R&D-Project: Identification of Organic Compounds in the North and Baltic Seas

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16. Kurzfassung

Kurz- ($C_{10-13,}$ sPCAs) und mittelkettige ($C_{14-17}$, mPCAs) polychlorierte Paraffine sowie Chlordane wurden in Fischleber (Dorsch, Kliesche, Flunder) und Sedimenten aus der Nord und Ostsee bestimmt (Probenahme 2002-2004). Fisch aus dem Nordatlantik (Island, Lofoten, Bäreninsel) wurde zum Vergleich untersucht. Bisher gab es keine Information über die PCA- Belastung dieser Regionen. Verschiedene massenspektrometrische Methoden für Screening und Kongenerenanalyse wurden entwickelt. s+mPCA-Gehalte in Fisch waren für Nordsee (54-3880 ng/g Fett (f), Mittel 985 ng/g f) und Ostsee (90-3170 ng/g f, Mittel 615 ng/g f) vergleichbar. s+mPCA-Gehalte in Dorschlebern aus Hintergrundsgebieten waren bedeutend niedriger (46-265 ng/g fg, Mittel 149 ng/g lw). PCA-Konzentrationen in Sedimenten aus der Ostsee (45-377 ng/g Trockengewicht (tw)) waren höher als für die Nordsee (5-355 ng/g tw), aber vergleichbar auf der Basis von totalem organischem Kohlenstoff. Chlordan-Gehalte waren im niedrigen ng/g Bereich für Fisch und Sedimente.
Ferner wurde das Vorkommen von Chlorpyriphos (-ethyl und –methyl), Endosulfan (I und II), Trifluralin, Dicofol und Pentachlorphenol (PCP) in Wasser-, Sediment- und Biotaproben untersucht. Über diese Pestizide lagen bisher kaum Informationen aus der Nord- und Ostsee vor. Dicofol wurde in keiner Probe gefunden (< 1 ng/L), PCP wurde in der Elbe und in wenigen küstennahen Stationen beobachtet (< 0,2 bis 1 ng/L). Chlorpyrirphos-ethyl, Endosulfan I und Trifluralin wurden in den meisten Proben in sehr geringen, aber ähnlichen Konzentrationen gefunden. Die Medianwerte lagen im Bereich von 15 bis 26 pg/L für Meerwasser und zwischen 9 und 20,5 ng/kg tw für Sedimente. In Fischleber wurden Mediane von 1,6 bis 1,9µg/kg Fett gefunden. Trifluralin zeigte als einzige Verbindung eine saisonale Abhängigkeit– mit stark erhöhten Werten im Winter. Die Konzentrationsverteilungen sind am besten mit einer diffusen Hintergrundbelastung auf sehr niedrigem Niveau zu interpretieren.

17. Schlagwörter
Polychlorierte Paraffine, Chlordane, Nordsee, Ostsee, Nordatlantik, Fisch, Sedimente
Chlorpyriphos-ethyl, Chlorpyriphos–methyl), Endosulfan, Trifluralin, Dicofol, Pentachlorphenol (PCP)
Meerwasser, GC-NCI-MS

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# Report Cover Sheet

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16. Abstract

Short-chained ($C_{10-13,}$ sPCAs) and medium-chained ($C_{14-17}$, mPCAs) polychlorinated paraffins as well as chlordanes were determined in fish liver (cod, dab, flounder) and sediments from the North and Baltic Seas collected during 2002-2004. Fish from the North Atlantic (Iceland, Lofot Islands, Bear Island) was analysed for comparison. So far, information about PCA levels did not exist for these areas. Different mass spectrometric methods for screening and detailed congener analysis were developed as a first step. s+mPCA concentrations were comparable for the North Sea (54-3880 ng/g lipid weight (lw), mean 985 ng/g lw) and the Baltic Sea (90-3170 ng/g lw, mean 615 ng/g lw). The highest s+mPCA levels were far above 1 ppm, which is remarkably high. s+mPCA levels in cod liver from the remote areas were considerably lower (46-265 ng/g lw, mean 149 ng/g lw). PCA levels in sediments from the Baltic Sea (45-377 ng/g dry weight(dw)) were generally higher than in those from the North Sea (5-355 ng/g dw), but were quite equal when expressed on total organic carbon (TOC) basis. Chlordane concentrations were in the low ng/g range for fish and sediments.
In addition, the occurrence of chlorpyrifos (-ethyl and -methyl), endosulfan (I and II), triflurlin, dicofol and pentachlorophenol (PCP) in water, sediment and biota samples was investigated. So far, nearly no information was available on the levels of these pesticides in the North Sea and Baltic Sea. Dicofol was not detected in any sample (< 1ng/L); PCP was found in the river Elbe and in few coastal stations (< 0.2 to 1 ng/L). Chlorpyrifos-ethyl, endosulfane I, and triflurlin were detected in most samples at very low but similar concentrations. Medians were in the range of 15 to 26 pg/L for sea water and of 9 to 29.5 ng/kg dw for sediments. In fish liver medians ranging from 1.6 to 1.9 µg/kg lipid were observed. Trifluralin only exhibited a seasonal variation - with elevated concentrations in winter. The distributions of the concentrations are best explained by a general diffuse background burden at at a very low level. No major input sources were observed.

17. Keywords

Polychlorinated paraffins, chlordanes, North Sea, Baltic Sea, North Atlantic, fish, sediments, mass spectrometry chlorpyrifos-ethyl, chlorpyrifos–methyl), endosulfan, trifluralin, dicofol, pentachlorphenol (PCP)
sea water

| | | |
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| 18. Price | 19. | 20. |

# Table of Content

# 1 Abbreviations and explanations

| | |
|---|---|
| BaP | **B**enzo[**a**]**p**yrene |
| BfA-Fi | **B**undesforschungsanstalt **f**ür **F**ischerei, Federal Research Centre for Fisheries |
| BSH | **B**undesamt für **S**eeschifffahrt und **H**ydrographie, Federal Maritime and Hydrographic Agency |
| BLMP | **B**und/**L**änder-**M**ess**p**rogramm, Federal and State Monitoring Programme |
| $CH_4$ | Methane |
| CID | **C**ollision **I**nduced **D**issociation: A mass spectrometric techniques, where a molecular ion or a fragment ion is further fragmented by collision with e.g. Ar atoms to increase the selectivity of detection. |
| conc. | concentrated |
| congener | This expression means any single PCA compound with the same chain length but a variable number of chlorine atoms. |
| D | Replicate determination |
| DBP | **D**ichloro**b**enzo**p**henone |
| 4,5-DCCD | 4,5-Dichlorchlordene |
| DCM or $CH_2Cl_2$ | **D**i**c**hloro**m**ethane |
| dw | **d**ry **w**eight |
| ECD | **E**lectron **c**apture **d**etector |
| ECNI-MS | **E**lectron **C**apture **N**egative **I**on **M**ass **S**pectrometry: A mass spectrometric technique, where electrons of thermal energy are generated in the ion source. These can only be captured by compounds of high electron affinity such as polychlorinated pesticides. Since most matrix residues do not capture such thermal electron, background is suppressed and selectivity increased. |
| EI | **E**lectron **I**onisation: The standard ionsation technique in mass spectrometry, where compounds are ionised with electrons of high energy (normally 70 eV) |
| EPA | U. S. **E**nvironmental **P**rotection **A**gency |
| ESI | **E**lectro **S**pray **I**onisation |
| EU | **E**uropean **U**nion |
| f | **F**emale |

| | |
|---|---|
| formula and congener groups | This expression describes the total of all compounds in a technical PCA mixture. Both the length of the carbon chain as well as the number of chlorine atoms can vary (see also congener). |
| FS | Research vessel (**F**orschungs**s**chiff) |
| GA | Research vessel **GA**USS |
| *Gadu mor* | *Gadua morhua*, cod |
| GC | **G**as **C**hromatography |
| GPC | **G**el **P**ermeation **C**hromatography |
| HCB | **H**exa**c**hloro**b**enzene |
| HCH | **H**exa**c**hlorocyclo**h**exane |
| HELCOM | **Hel**sinki **Com**mission |
| HFBAA | **H**eptafluoro**b**utyric **a**cid **a**nhydrate |
| HPLC | **H**igh **P**erformance **L**iquid **C**hromatography |
| HRGC | **H**igh **R**esolution **G**as **C**hromatography: Separation with capllary columns. |
| HRMS | **H**igh **R**esolution **M**ass **S**pectrometry: Mass separation is carried out in a magnet field at mass resolutions of 5’000-10’000. This increased selectivity and suppresses disturbances by sample matrix and other organic pollutants. |
| ID | **I**nternal **D**iameter |
| ISTD | **I**nternal **St**andar**d** |
| $K_{OW}$ | Octanol/water distribution coefficient |
| LC | **L**iquid **C**hromatography |
| $LC_{50}$ | **L**ethal **c**oncentration by which 50% of the test organisms die |
| $LD_{50}$ | **L**ethal **d**ose, by which 50% of the test organisms die |
| *Lima lim* | *Limanda limanda*, dab |
| LOD | **L**imit **o**f **D**etection (normally at a signal-to-noise ratio of 3:1): The height of a chromatographic signal must be three times higher than the chemical and electronic noise of the baseline. |
| LOQ | **L**imit **o**f **Q**uantification (normally at a signal-to-noise ratio of 10:1): The height of a chromatographic signal must be ten times higher than the chemical and electronic noise of the baseline. |
| lPCA | **l**ong-chain **p**oly**c**hlorinated *n*-**a**lkanes |
| LRMS | **L**ow **R**esolution **M**ass **S**pectrometry: All mass spectrometric techniques with so-called unit mass resolution. This means that ions with a mass difference of one mass unit can be separated. |

| | |
|---|---|
| lw | **l**ipid **w**eight |
| m | **M**ale |
| MAE | **M**icrowave **A**ssisted **E**xtraction |
| MATC | **M**aximum **a**cceptable **t**oxicant **c**oncentration |
| MC5, MC7, MC8 | Trivial names of chlordane compounds |
| ME | Methyl- |
| mPCA | **m**edium-chain **p**oly**c**hlorinated *n*-**a**lkanes |
| MRM | **M**ultiple **R**eaction **M**onitoring |
| MS | **M**ass **S**pectrometry |
| MS/MS | Tandem mass spectrometry: A mass spectrometric technique which increases selectivity and lowers detection limits. Ions specific for a compound are selected with the first mass filter and then fragmented into smaller structure characteristic ion in a collison cell. One of these ions is the selected with the second mass filter. |
| MUDAB | **M**eeres**u**mwelt**da**ten**b**ank, Marine Environmental Data Base, BSH |
| MW | **M**olecular **w**eight |
| *m/z* | Mass-to-charge ratio: The mass of an ion detected by mass spectrometry compared to its charge z (normally 1). |
| na | **n**ot **a**nalysed |
| nd | **n**ot **d**etectable |
| ni | **n**ot **i**dentified |
| NICI | **N**egative **I**on **C**hemical **I**onisation: A mass spectrometric ionisation technique, which forms anion adducts or fragments ion from molecular ions by gas phase reactions between an reagent gas and a molecule. This increases selectivity and sensitivity for selected compounds. |
| np | **n**ot **p**ossible |
| NPD | **N**itrogen-**P**hosphorus **D**etector |
| NOEC | **N**on **O**bservable **E**ffect **C**oncentration |
| ns | **n**ot **s**pecified |
| OCN | **O**cta**c**hloro**n**aphthalene |
| OSPAR | **Os**lo-**Par**is Convention |
| PAH | **P**olycyclic **A**romatic **H**ydrocarbons |
| PCB | **P**oly**c**hlorinated **B**iphenyls |

| | |
|---|---|
| PCP | **P**enta**c**hloro**p**henol |
| *Plat fle* | *Platichtys flesus*, flounder |
| p,p'-DDT | p,p'-Dichloro-1,1-diphenyl-2,2,2-trichlorethane |
| PCA | **P**oly**c**hlorinated *n*-**A**lkanes |
| PCB | **P**oly**c**hlorinated **B**iphenyls |
| POP | **P**ersistent **O**rganic **P**ollutant |
| PTV | **P**rogrammable **T**emperature **V**aporising Injector |
| Q1, Q3 | Quadrupole 1 or 3 |
| $r^2$, $R^2$ | Coefficient of regression |
| RP | **R**eversed **p**hase |
| RT | **R**etention **t**ime |
| Sec | **sec**ondary |
| SIM | **S**elected **I**on **M**onitoring: Selection of two or more fragments or isotope ions from a mass spectrum, which are specific for an analyte. This increase selectivity and allows to identify a compound further by the given intensity ratio between the selected ions. |
| S/N | **S**ignal-to-**N**oise Ratio: Determination of the peak-to-peak noise of the baseline of a chromatogram and comparison with the signal height of an analyte. |
| sPCA | **s**hort-chain **p**oly**c**hlorinated *n*-**a**lkanes |
| SPE | **S**olid **P**hase **E**xtraction |
| SPM | **S**uspended **P**articulate **M**atter |
| STD | **St**andard **D**eviation |
| TCPy | **T**ri**c**hloro**py**ridinol |
| THF | **T**etra**h**ydro**f**urane |
| TIC | **T**otal **i**on **c**hromatogram |
| TOC | **T**otal **O**rganic **C**arbon |
| Toxaphene | Trade name of a technical mixture of polychlorinated bornanes used as pesticide |
| UK | **U**nited **K**ingdom |
| US | **U**nited **S**tates of America |
| v/v | Volume/Volume |
| WFD | **W**ater **F**ramework **D**irective |

ww **w**et **w**eight

## Measuring units

| | |
|---|---|
| cm | Centimetre |
| eV | Electron Volt |
| g | Gram |
| hPa | Hekto Pascal |
| L or l | Litre |
| m | Metre |
| Min | Minute |
| ml | Millilitre |
| mm | Millimetre |
| mM | Millimolar |
| ms | Millisecond |
| ng | Nanogram |
| pg | Pikogram |
| ppb | Parts per billion |
| ppm | Parts per million |
| µg | Microgram |
| µm | Micrometre |
| µl | Microlitre |

# 2 Executive summary

The aim of the project „Identification of Organic Pollutants in the North and Baltic Seas" was to identify and quantify toxic organic substances in the marine environment of the North Sea and Baltic Sea for which environmental data are either insufficient or not available. The selection of compounds is based on the lists of substances identified for priority action by the OSPAR and HELCOM Conventions and the European Commissions Water Framework Directive.

## 2.1 Part Polychlorinated paraffins and chlordanes

### 2.1.1 Characterisation of polychlorinated paraffins (PCAs)

Polychlorinated *n*-alkanes (PCAs) are complex technical mixtures containing thousands of different isomers, congeners, diastereomers and enantiomers. The chlorine content of the products varies between 30 and 70 %. PCAs are divided into short chain PCAs ($C_{10\text{-}13}$, sPCAs), medium chain PCAs ($C_{14–17}$, mPCAs) and long chain PCAs ($C_{>17}$, lPCAs) depending on the length of the carbon chain. PCAs are persistent, bioaccumulate and have physical properties, which allow dispersion in the environment by long-range transport. Therefore, they fulfil the properties of a persistent organic pollutant. PCAs are applied as additives in metal working fluids, as plasticizers and/or flame retardants in polymers and as surface coating agents.

Since their first large scale usage in 1932 as extreme pressure additives, the world wide production of PCAs has increased to estimated 300 kt/year in 1993 and is probably at the same level today (estimate of production capacity up to 160'000 t annually in the EU in 2002). However, the application has changed from sPCAs to mPCAs due to the unfavourable toxic properties of sPCAs. sPCAs have low acute toxicity, but are carcinogenic and show high chronic toxicity to aquatic biota, whereas mPCAs and lPCAs do not. Due to their widespread and mainly unrestricted use and due to the properties mentioned above, PCAs are present in aquatic and terrestrial food webs of rural and remote areas at levels comparable to polychlorinated biphenyls (PCBs). For example, levels in fish and marine mammals were reported to be between 100 –

1700 ng/g lipid. However, nothing is known about the input of PCAs into the sea via rivers.

sPCAs and mPCAs are part of the OSPAR List of Substances of Possible Concern and of the HELCOM list of Selected Substances for Immediate Priority Action. However, the OSPAR List of Chemicals for Priority Action and the WFD list of priority substances in the field of water policy contain only sPCAs. In Germany, the production of all PCA was stopped in 1998. Moreover, PCA application is prohibited for metal working and leather treatment since summer 2003 by the Directive 2002/45/EC. Finally, a risk assessment was carried out in the EU for sPCAs and mPCAs.

### 2.1.2 Characterisation of chlordanes

The pesticide technical chlordane was first synthesised in 1945. 147 compounds could be identified in technical chlordane. Main constituents are about 24 % trans-chlordane, 19 % cis-chlordane, 9 % γ-chlordene, 7 % heptachlor, 7 % trans-nonachlor, 3 % α–chlordene, 3 % β-chlordene, 1 % chlordane and 19.5 % related compounds including cis-nonachlor. About 70’000 tons were manufactured between 1960 and 1988. Technical chlordane is a contact and stomach pesticide with a broad spectrum. It was mainly used for building protection, ornamental lawns and trees, drainage ditches, but also to protect crops like corn and potatoes. Technical chlordane was mainly applied for termite control.

Technical chlordane was neither manufactured in Europe nor in Japan, but was commercially available in Germany around 1950. It was hardly used in Europe and no consumption data are available for this region. First restrictions of use were decided in 1978 by the US Environmental Protection Agency. It was then mainly applied for termite control and finally banned in the USA in 1988 and in Central America in 1997.

Chlordane is registered in the OSPAR List of Substances of Possible Concern and of the HELCOM list of Selected Substances for Immediate Priority Action. However, it is not listed in the OSPAR List of Chemicals for Priority Action and the WFD list of priority

substances in the field of water policy. Moreover, no risk assessment has been carried out in the EU.

Chlordane is lipophilic and has a high octanol-water partitioning coefficient $K_{OW}$.Chlordane can be dispersed by atmospheric long-range transport. This has led to a significant chlordane input into the European Arctic. Since hardly any data are available about chlordane levels in Central Europe, this pesticide was included into this investigation.

Oxychlordane and cis-heptachlorepoxide are chlordane metabolites and were never produced. Unlike other chlordane degradation products, they are very persistent, lipophilic and accumulate even more effectively than their precursors in biota. Therefore, they were included into the investigation.

### 2.1.3 Analytical problems of PCAs

Information about environmental levels is still very scarce. The main reason is the complex composition of PCAs, which caused tremendous analytical problems. High resolution gas chromatography cannot resolve them into single compounds. Moreover, mass overlap occurs between PCA compounds of different chain length and degree of chlorination as well as other polychlorinated pollutants requiring normally high resolution mass spectrometry. Electron capture negative ion (ECNI) chemical ionisation mass spectrometry is needed to obtain reasonable low detection limits. However, response factors are highly dependent on the degree of chlorination of PCAs present in samples and require a careful selection of the quantification standards. Otherwise, systematic errors of several hundreds of percents are possible. Moreover, up to thirty (!) analyses by mass spectrometry were needed for a complete pattern of one sample including all PCA compounds of different chain length and degree of chlorination. Finally, so far clean-up techniques were not able to remove other disturbing polychlorinated pollutants as PCBs. Therefore, PCA concentrations published so far are in many cases indicative and hardly comparable between studies.

### 2.1.4 Method development for PCA analysis

The aim of the project was to develop new methods for the determination of PCAs in fish and sediments. They should allow a low cost screening and a less complex determination of formula and congener group patterns. Therefore, the mass spectrometric techniques described below were developed or improved during this project and a full method validation and intercomparison was carried out. The were used to determine PCA concentrations in fish from the North and Baltic Sea as well as from selected sites from the northern North Atlantic to obtain such information for the first time.

***Negative ion chemical ionisation (NICI) with $CH_2Cl_2/CH_4$:*** This technique suppressed strongly interferences from other polychlorinated pollutants, allows even the detection of low chlorinated PCAs and makes the use of low resolution MS possible. Moreover, response factors are quite uniform and independent from the degree of chlorination. The only disadvantage is the limited number of analyses possible, before the mass spectrometer has to be cleaned.

***Electron ionisation combined with MS/MS:*** Further fragmentation of fragment ions in common for all PCAs allow the determination of total PCAs with low cost instrumentation such as an ion trap MS (€ 60'000) with very high selectivity and detection limits comparable to high resolution mass spectrometry. Response factor differences are eliminated. The only disadvantage is the missing differentiation between sPCA and mPCA and that formula/ congener group specific results are not obtained. The technique has even lower detection limits using more expensive triple quadrupole MS.

***Optimisation of quantification by ECNI:*** Response factors depend strongly on the degree of chlorination of PCAs and mass overlap between formula/congener groups occur with low resolution MS. However, methods could be developed which compensated the influence of the degree of chlorination and which allowed eliminating mass overlap interferences by a careful retention time range selection and isotope ratio control of registered masses. This enabled the use of low resolution MS.

Finally, a newly developed clean-up procedure removed other interfering pollutants such as PCB completely. A full validation of the methods and intercomparison including high resolution MS showed, that all methods were comparable within 10–20 %, which is excellent for trace analysis of a extremely complex compound class. Limits of detection were around 1 ng PCA absolute corresponding to ca. 1 ng/g fish liver and 2–8 ng/g sediment. Linear ranges of two orders of magnitude were achieved. Recoveries were typically between 80–100 %.

Commercial technical PCA mixtures were characterised as a first step. $C_{11}$ and $C_{12}$-compounds contribute ca. 67–82 % to the total sPCA amount. Precision of the formula/congener group determination was about 1 %. The average chlorine content and molecular weight were the main factors of differentiation. ECNI overestimated the chlorine content compared to available supplier information. However, NICI with CH4/CH2Cl2 confirmed it within 1–2.5 %.

### 2.1.5 Quantification of chlordanes

The analysis of chlordane compounds is well established and mainly carried out by ECNI mass spectrometry with low resolution instruments. Usually, the four representatives cis/trans-chlordane and cis/trans-nonachlor as well as the main metabolites of cis/trans-chlordane are quantified. Some unusual chlordane representatives such as MC5 and MC7 were also determined in this project. Chlordane analysis contained the following elements: The extraction and clean-up method was identical to those for PCAs. All main chlordane compounds were present in the PCA fraction. Gas chromatographic separation was carried out on the same capillary as PCAs. Quantification was carried out by conventional ECNI according to a validated method.

A method based on EI-MS/MS was also developed to check for possible systematic errors concerning quantification of sediments. It had comparable detection limits for most compounds. The same ECNI quantification technique for sediment analysis was used on two mass spectrometers from different supplier. No significant difference

within the measuring uncertainty (10–15 %) was observed. The same was valid for ECNI and EI-MS/MS.

Detection limits were less than 0.1ng/g for biota and sediments. The linear range was at least three orders of magnitude and typical recoveries between 75–90 %. Method blanks were equal to the detection limit.

### 2.1.6 PCAs in biota from the Baltic/North Sea and the northern North Atlantic

Fish livers (cod, dab, flounder) were collected during two monitoring expeditions at five to six sites in the North and Baltic Sea in August/September 2002/2004. Livers were pooled to obtain minimum 5 g of sample. Moreover, six cod liver samples were obtained from the north and south coast of Iceland and the Lofot Islands via the Norwegian Institute for Air Research. Liver and muscle tissue of fish (Arctic char) and sea birds (little auk, kittiwake, glacous gull) were obtained from Lake Ellasjøen at Bear Island, which is known as a site of elevated concentrations of polychlorinated compounds (PCBs, DDT, toxaphenes) due to long range transport and on-site bioaccumulation. Sampling was carried out in 2001 by Aquaplan-NIVA (Norway).

PCA levels were well detectable in all samples by screening using EI-MS/MS. Quantification and formula/congener group analysis were carried out by ECNI to enable a comparison with former studies using mainly the response factor correction mode.

s+mPCA levels in fish liver from the North and Baltic Sea showed no species-specific concentration dependence. Concentration ranges were comparable for the North Sea (54–3880 ng/g lw, mean 985 ng/g lw) and the Baltic Sea (90–3170 ng/g lw, mean 615 ng/g lw). The highest s+mPCA levels were far above 1 ppm, which is remarkably high. s+mPCA levels in cod liver from remote areas (Lofot Islands/Iceland) were considerably lower (46–265 ng/g lw, mean 149 ng/g lw) than in cod liver from the North and Baltic Sea.

Arctic char (200–2500 ng/g lw, mean 1005 ng/g lw) from the remote region Bear Island had comparable levels as cod from the North and Baltic Sea. Similar concentrations were also reported for PCB, DDT-compounds and toxaphenes. Main reasons for such an exposure are long range transport, condensation effects and a high precipitation rate around the sampling site Lake Ellasjøen and the breeding sites of thousands of sea birds close by. Birds from Bear Island had also a considerable s+mPCA burden, which was in the same range as for DDT-compounds, PCB and toxaphenes.

The mPCA concentrations were higher than those for sPCA in many samples from the Baltic Sea and in birds. No clear relation between s- and mPCAs was observed for the North Sea and northern North Atlantic. $C_{14}$ chains were dominant (mean 69 %, range 59–100 %) in the mPCA-pattern of all samples. This is also typical for technical mixtures.

The situation was more complex for sPCA-patterns. C11 and C12 congeners were most abundant in the biota from the Baltic and North Sea. The distribution resembled those in technical sPCA, but showed a larger variability. However, cod livers from the northern North Atlantic showed a difference. Here, C10 and C12 congeners were most common. The C10 fraction and C10/C12 ratio increased to 28.4% and 0.76, respectively, in cod liver from the northern North Atlantic compared to 13.6% and 0.43 in technical mixtures or 16.8% and 0.53 in cod liver from the Baltic Sea. The change of C10 congeners from a minor to a major fraction of sPCA was also observed in marine mammals from the Northern North Atlantic such as beluga, walrus and seals. It indicates a fractionation and enrichment of the more volatile C10 congeners during long range transport to this remote region.

### 2.1.7 PCAs in sediments from the Baltic and North Sea and additional sites in Europe

Sediments were collected during monitoring expeditions in August/September 2001, August/September 2002, May/June 2003 and spring 2004 (13 sediments from 7 sites in the Baltic sea and 20 sediments from 16 sites in the North Sea). Moreover, 8 river and sea sediments and suspended particulate matter (11 samples) were obtained from

different institutions for comparison (Laboratoire d`Etudes ed d`Analyses, Le Havre, France; Behörde für Wirtschaft und Arbeit Hamburg, Germany; Landesanstalt für Umweltschutz, Karlsruhe, Germany; Akva-plan niva, Tromsø, Norway; BSH, Hamburg, Germany).

All samples were first quantified by EI-MS/MS to obtain the total PCA content. In addition, the sPCA and mPCA content was determined in those samples exceeding ca. 50 ng/g dw total PCA. Below this level, quantities were too low for this analytical procedure based on single formula and congener groups. $CH_4$/$CH_2Cl_2$-NICI was mainly used. ECNI became sensitive enough, when new equipment was bought in 2003.

EI-MS/MS allowed detecting PCAs in all sediments (5–377 ng/g dry weight (dw)). PCA levels in sediments from the Baltic Sea (45–377 ng/g dw) were generally higher than in those from the North Sea (5–355 ng/g dw, ten of sixteen samples below 50 ng/g dw). However, they were quite equal when expressed on TOC basis (North Sea 2.3–33.1 ng/g TOC, Baltic Sea 2.1–9.4 ng/g TOC). The TOC content was a good marker for PCA concentrations. Concentrations of mPCA (C14–16, 42–303 ng/g dw) were always higher than for sPCA (C10–13, 18–128 ng/g dw). The ratio mPCA/sPCA varied between 1.7 and 3.2. Higher TOC levels indicated usually also a higher PCA burden. The highest PCA concentrations in the North Sea were found at sites in the Elbe estuary, where chemical waste and sewage sludge had been dumped several years ago.

PCA concentrations in sediments have only been reported from very few sites world-wide. They were in the same range as in sediments from the North and Baltic Sea at non-contaminated sites. A limited number of additional sediments were analysed from different regions of Europe to increase the data set for comparison. The PCA levels in these river and sea sediments were comparable with those from the North and Baltic Sea.

PCAs with $C_{13}$ and $C_{14}$ chain and 4 to 6 Cl atoms were usually the main components in the marine sediments. $C_{17}$ chains were not detected in any sediment. sPCAs in marine sediments consisted to 50–87% of $C_{12}$- and $C_{13}$-compounds and mPCAs between 56–

81 % of $C_{14}$-constituents. The content of $C_{16}$ chains length was maximum 12% in mPCAs.

Differences were found between marine and river sediments as well as suspended particulate matter (SPM). The chlorine content of sPCAs was lower in marine sediments (51–59 %) than in river sediments/SPM (58–63 %). Main compounds in all sediment types were $C_{11}$ and $C_{12}$ chains with 7–8 Cl. However, the fraction of $C_{11}$ compounds was somewhat lower in marine sediments (19–34%) than in river sediments/SPM (24–43 %). Moreover, the chlorine content of mPCAs and their composition was comparable for both sea and river sediments as well as SPM.

### 2.1.8 PCA in sea water

Only two sea water samples were analysed due to the expected very low concentrations. Samples of 100 l were taken and extracted. No PCAs could be detected by EI-MS/MS at a detection limit of 0.4 ng/l water, which corresponds to an absolute detection limit of 0.2 ng for the most abundant fragmentation.

### 2.1.9 Chlordanes in biota from the Baltic and North Seas and the northern North Atlantic

Sum concentrations (Σchlordane) on lipid weight basis (11–47 ng/g lw) were comparable to other studies of fish from the Baltic and North Sea. No significant concentration difference (t-test) was observed between the Baltic (Σchlordane 11.3 ± 5.1 ng/g ww) and the North Sea (10.4 ± 6.0 ng/g ww) or between the two measuring campaigns. However, compared to cod liver from the Lofot Islands, the chlordane compound level in the North and Baltic Sea was about one order of magnitude lower.

*trans*-Nonachlor contributed most to the sum concentrations (37 ± 6 %) as it is typical for most studies. cis-Chlordane dominates in fish recently exposed to technical chlordane. Therefore, any trans-nonachlor/cis-chlordane ratio >1 indicate no new and recent chlordane input. The cod livers of this study had a mean ratio of 1.8 ± 0.6, which

point to old sources and no recent chlordane exposure. The two chlordane compounds MC5 and MC7 were hardly or not detectable (limit of detection ca. 0.1 pg/g).

### 2.1.10 Clordanes in sediments from the Baltic and North Seas and additional sites in Europe

*trans*-Nonachlor was most dominant in the majority of sediments. *cis/trans*-Nonachlor concentrations were about ten times higher in the Baltic Sea (0.12–0.46 ng/g dw) than in the North Sea (0.001–0.34 ng/g dw). *cis/trans*-Chlordane levels were partly higher in the North Sea than in the Baltic Sea, when compared on a wet weight basis. It was vice versa expressed on TOC content. Concentrations of cis-and of trans-chlordane were comparable.

Very little information about chlordane concentrations in sediments is available in the literature. Overall, chlordane concentrations in the sediments analysed in this project were within the same order of magnitude as those published. *trans*-Nonachlor was most dominant in the majority of sediments. *cis/trans*-Nonachlor concentrations were about ten times higher in the Baltic Sea (0.12–0.46 ng/g dw) than in the North Sea (0.001–0.34 ng/g dw). *cis/trans*-Chlordane levels were partly higher in the North Sea than in the Baltic Sea, when compared on a wet weight basis. It was vice versa expressed on TOC content. Concentrations of cis-and of trans-chlordane were comparable.

## 2.2 Part: Sampling and analysis - selected pesticides

### 2.2.1 Characterisation of the target compounds

The target compounds of this project part - chlorpyrifos (-methyl and -ethyl), dicofol, endosulfan (I and II), pentachlorophenol (PCP) and trifluralin - are members of entirely different chemical compound classes and have different applications. Chlorpyrifos, dicofol, and endosulfan are used as insecticides, trifluralin is a herbicide, and PCP has been used for various pesticidal applications. All of them are listed as hazardous substances for priority action by either the OSPAR Commisson or under the WFD due to their suspected persistence and toxic properties as well as their bioaccumulation potential. They were included in this investigation, since the information about their occurrence in the marine environment was considered insufficient.

The structures of these target compounds are very different, but their feature in common is an intermediate polarity (log $K_{OW}$ 3.5 to 5) and stability against biological degradation. However, they are considered less persistent and bioaccumulative than classical pollutants such as DDT, PCB, PAH etc. Moreover, the more polar properties make a prediction of their environmental behaviour more difficult.

### 2.2.2 Chlorpyrifos

Chorpyrifos is a phosphorus ester insecticide. Both the methyl- and ethyl esters are in use (chlopyrifos-methyl, chlorpyrifos-ethyl). They are applied as a broad-spectrum insecticide for the treatment of grain, cotton and vegetable crops. The compound was introduced in 1965 and is primarily produced by Dow AgraSciences (Indianapolis, USA). European manufacturers are Frunol (Unna, Germany) and Point Enterprises (Switzerland). Annual consumption in Europe is about 1000 t.

### 2.2.3 Dicofol

Dicofol is a dichlorodiphenylmethane insecticide with a strong structural similarity to DDT. However, its hydroxyl group makes it more polar and less stable. Dicofol is banned in Germany, and has not been sold since 1995. It is not registered in most

neighbouring countries of the North Sea and Baltic Sea – Denmark, Netherlands, Sweden, Norway, and Finland – or in Switzerland. The main application countries of dicofol in Europe (about 290 t) are Spain (100–150 t), France (14 t), Portugal (4.8 t), and UK (1 t).

### 2.2.4 Endosulfan

Endosulfan is a sulphite ester of a chlorinated cyclodienediol. The technical mixture contains two parts of endosulfan I – and 1 part of endosulfan II – isomer. Its main application areas are temperate, sub-tropic and tropic climatic zones. In Europe, it has been registered since 1956. World-wide production was about 5,000 to 10,000 t in 1992. One of the main producers is Bayer CropScience. In 1999, the total consumption of the compound in Europe was 469 t mainly in southern Europe (431 t). In most north European countries, endosulfan has not been used since the mid-1990s. This led to a decline of consumption from about 400 t in 1995 to only 38 t in 1999. Only Belgium, France, UK, and Switzerland confirmed applications for 1999.

### 2.2.5 Pentachlorophenol (PCP)

Pentachlorophenol is a chlorinated phenol. PCP production in Europe has been banned since the early 1980s. Nevertheless, 100 t of imported PCP was used as an algaecide and bactericide in the timber and textile industry of the European Union in 1997. Use of PCP in Germany was banned in 1989.

### 2.2.6 Trifluralin

Trifluralin is a dinitroaniline derivative which is used as a selective herbicide. It is marketed under different trade names (e.g. TREFLAN) for use as a pre-sowing or pre-emergence herbicide to control grasses and dicotyledonous weeds. It was first registered in the US in 1963. In the European Union, there is only one manufacturer of trifluralin (Manerbio, Northern Italy). The annual production volume is about 6,000 t; thereof about 3,200 t are used in the European Union.

### 2.2.7 Analytical problems and method development

The aim of the project was to develop methods for the determination of these target compounds at ultra-trace levels in marine water, sediments, and biota. Very sensitive methods were developed for chlorpyrifos, endosulfan and trifluralin with detection limits of <10 pg/L for water, <10 ng/kg for sediment, and <0.5 µg/kg for biota. Sea water extraction was performed by solid phase extraction. Sediments and biota were extracted by microwave assisted hot solvent extraction with a mixture of hexane/acetone. GC-MS in the negative ion chemical ionisation mode was used for the determination.

The limits of detection (LODs) for PCP and dicofol were not quite as low but appropriate for this study and comparable with LODs reported for terrestrial surveys. A fast HPLC-MS method with detection limits of 0.2 and 0.4 ng/L was developed for PCP and the chlorpyrifos degradation product trichloropyridinol (TCPy) in sea water. This method was sufficient for screening purposes in river estuaries and at coastal stations, but not selective enough for sediment and biota samples. Therefore, additionally a GC-NCI-MS method was developed for these matrices, which, however, required a derivation of the phenol group.

Dicofol degraded thermally very quickly during GC-MS analysis. Therefore, a limit of quantification (LOQ) of only 1 ng/L could be achieved, which is only sufficient for a screening of local hot spots. Since dicofol was found to be unstable in sea water and due to time constraints, no method was developed for sediment or biota.

### 2.2.8 Chlorpyrifos in the marine environment of the North and Baltic Seas

This is the first time that chlorpyrifos has been detected in quantifiable amounts in the marine environment. Chlorpyrifos-methyl was not detectable in the North Sea and Baltic Sea. However, the homologue ester chlorpyrifos-ethyl was found in most water and biota samples. It was less frequently detected in sediment samples. Observed concentrations were very low with a median of 26 pg/L in water samples and 9 ng/kg

dry weight (dw) in sediments. Considerable bioaccumulation was observed in fish liver with median concentrations of 0.36 µg/kg wet weight (ww) corresponding to 1.6 µg/kg lipid weight (lw). The enrichment from water to sediment was calculated to a factor of about 350, from water to biota (fish liver) to about 14'000 (ww) and 62'000 (lw). This relatively low enrichment is in accordance with the moderate log $K_{OW}$ value of 3.6–4.5. Compared to the EQS value of 30 ng/L, which is proposed for the evaluation of inland and transitional waters by the WFD, the observed sea water concentrations for chlorpyrifos are quite low. It is remarkable that chlorpyrifos has been detected at all in the marine environment of the North and Baltic Seas considering the short half-live, the low volatility and low emissions from local sources.

The low concentrations and the distribution patterns observed for North Sea and Baltic Sea water are best explained by a generally low background level with some minor local sources. Concentrations in the river Elbe - generally the most important input source of pollutants to the German Bight - are relatively low and consequently its impact on chlorpyrifos input. It is not surprising that TCPy was not found in sea water taken the low chlorpyrifos concentrations and the moderate environmental persistence of TCPy into account.

Water concentrations of chlorpyrifos-ethyl were above those of the classical lipophilic pollutants such as HCB, DDT, PCBs or PAHs, but below those of HCHs. Sediment levels were below that of classical pollutants. In biota concentrations are in a range of HCHs and HCB, but lower than the more lipophilic DDT-group and PCBs.

### 2.2.9 Endosulfan in the marine environment of the North and Baltic Seas

This is the first time that quantifiable conencentration of endosulfan have been detected in the North Sea and Baltic Sea. Endosulfan II levels were generally lower than for endosulfan I and often not detectable in the marine samples of the North Sea and Baltic Sea. The isomer endosulfan I was found in many water samples and in some sediments and biota. However, concentrations were very low with a median of 25 pg/L for water and 20.5 ng/kg dw for sediments. In fish liver, median concentrations of 0.44 µg/kg ww

and 1.9 µg/kg lw were observed. The measured sea water concentrations of endosulfan were about ten times lower than the proposed WFD EQS of 0.5 ng/L.

The observed concentrations and distribution patterns are best explained by a low general background load by e.g. atmospheric deposition or only minor local sources. Concentrations in the river Elbe - the most important source of pollutant input to the German Bight - are often around or below the LOQ. Therefore, no distinct concentration gradients have been observed in the German Bight. Considering the low concentrations of local sources, it is astonishing that endosulfan has been detected in the North and Baltic Seas at all.

Concentrations of endosulfan in water were higher than for classical lipophilic pollutants such as HCB, DDT, PCBs or PAHs but below those for HCH. Compared to classical chlorinated pollutants, the endosulfan concentration in sediment is about 10 to 100 times lower. The bioaccumulation potential of endosulfan becomes apparent when comparing concentrations in the three compartments investigated. The estimated "enrichment" of endosulfan in biota compared to the water phase is about 17,600 based on wet weight, and 76000 based on lipid weight.

The observed endosulfan levels in biota are comparable to classical pollutants like HCH and HCB but below the more lipophilic DDT and PCB group. In 2000, typical HCH concentrations in the German Bight ranged from 0.2 to 0.6 µg/kg ww; HCB had a median of 0.84 µg/kg ww, and the sum of DDTs one of 3.7 µg/kg ww. The low concentrations of endosulfan in the North Sea and Baltic Sea is well explainable by its pattern of use in Europe. According to the OSPAR commission's background paper on endosulfan, endosulfan is used mainly in the south of Europe, while only 38.1 t/a were used in the countries bordering the North Sea and Baltic Sea in 1999. In most north European countries, endosulfan has not been used any more since the mid-1990s.

### 2.2.10 Dicofol in the marine environment of the North and Baltic Seas

Dicofol was not detectable in North Sea and Baltic Sea water (LOD: 1 ng/L). Degradation experiments in sea water showed a half-life of less than one day.

Therefore, no further investigations were carried out in sediments or biota. It was also not considered to be of priority interest to decrease the LOD to the lower pg/L range. In the river Elbe - generally the most important source of pollutant input to the German Bight - no dicofol was found, but the degradation product dichlorobenzophenone (DCB) was detected at concentrations up to 3.8 ng/L. However, DCB is not a specific indicator of dicofol, since other compounds like DDT degrade to DCB as well. Dicofol is mainly used in southern regions such as Portugal, Spain, France, Italy and Greece. Therefore, it may be considered to be of less priority concern for the North Sea and Baltic Sea (unless inputs of Russia, Poland or the Baltic states are of importance). The detection limits of the analysis method applied in this study were not low enough to detect diffuse inputs by atmospheric deposition via long range transport.

### 2.2.11 Pentachlorophenol in the marine environment of the North and Baltic Seas

PCP was found in sea water of the North Sea at concentrations between <0.2 and 1 ng/L. Most values were below the LOQ. Concentrations currently observed in the German Bight are much lower than those reported for 1988, which varied between 0.1 to 6.4 ng/L. Low values (0.1 ng/L) comparable to the results presented here had been observed in the outer German Bight. However, 15 years ago, PCP concentrations in coastal areas were much higher than today, which shows that the pollutant loads of rivers (especially the Elbe) have decreased considerably during the past years. Nevertheless, the river Elbe still is an input source of PCP to the North Sea.

Whether other input sources to the North Sea are relevant at present cannot be derived from the data, since PCP was non-detectable in most samples. Therefore, no spatial distribution pattern could be obtained for the North Sea. Moreover, no high mean concentrations of PCP of 6 to 100 ng/L were found for estuarine regions as in 1983–1997. The observed concentrations of PCP are well below the proposed WFD EQS of 200 ng/L.

### 2.2.12 Trifluralin in the marine environment of the North and Baltic Seas

Since trifluralin had a very low LOQ, it was measurable in most water, sediment and fish liver samples from the North Sea and Baltic Sea. Concentrations are very low in summer, with a median of 15 pg/L for water samples, 10 ng/kg dw for sediments and 0.42 µg/kg ww for fish liver. In winter, concentrations in water are about 10 times higher. This can be explained by the preferred use of this herbicide as a pre-seed agent in winter. The detected trifluralin concentrations in sea water are considerably lower than the proposed WFD EQS value of 30 ng/L.

The concentrations found demonstrate that trifluralin is stable enough in the marine environment of the North Sea and Baltic Sea. Concentrations are highest during and after the main application season in winter. The tenfold lower concentrations in summer demonstrate that trifluralin degrades moderately fast in the (marine) environment. The distribution patterns are best explained by a general diffuse burden (e.g. by atmospheric deposition) with only minor local sources.

Trifluralin concentrations in the river Elbe are relatively low and, consequently its influence on the German Bight. Summer concentrations of trifluralin in sea water are above those of the classical lipophilic pollutants such as HCB, DDT, PCBs or PAHs but below those for HCHs. In winter, levels exceed HCH concentrations. Therefore, it will be worthwhile continuing to study the behaviour of trifluralin in marine waters.

Compared to classical pollutants, trifluralin concentrations in sediment are 10 to 100 times lower. For example, HCH isomer concentrations ranged from 10 to 200 ng/kg at the most polluted station in the German Bight (KS 11). The more lipophilic DDD and CB153 were in the range of 1000 to 5000 ng/kg. PAHs such as benzo(a)pyrene had sediment levels between 40–240 µg/kg.

The enrichment factor of trifluralin based on the median concentrations in water and fish liver is 28’000 (ww) and 113’000 (lw), respectively. The trifluralin levels in biota are comparable to those of HCH and HCB, but lower than the concentrations of DDT-metabolites and PCB. Typical HCH concentrations in the German Bight varied between

0.2 to 0.6 µg/kg ww in 2000. HCB had a median of 0.84 µg/kg ww and the sum of DDTs of 3.7 µg/kg ww.

**In summary,** chlorpyrifos, endosulfan and trifluralin have very similar concentrations in the different marine matrices. The slightly higher median levels of endosulfan compared to chlorpyrifos and trifluralin may have statistical and analytical causes, since the limit of detection and the limit of quantification (LOD/LOQ) of endosulfan were higher in this study than those of the other compounds and values < LOD/LOQ were not taken into account. The observed low concentrations of the compounds studied are in accordance with their limited persistence in the environment compared to classical POPs and their relatively low consumption volume in northern Europe (chlorpyrifos, endosulfan, dicofol, PCP). With the exception of PCP and possibly trifluralin, major local input sources have not been detected. The enrichment factors for sediments and biota samples are similar for all compounds and reflect a comparable intermediate polarity of these pesticides. They are lower than for most classical POPs, since the investigated compounds are less lipophilic and stable.

### 2.2.13 Recommendations for future monitoring activities

Based on the observed environmental concentrations in the North Sea and Baltic Sea, available data on the use of these substances, and their physical, chemical and biological behaviour in the marine environment, the following recommendations are made:

**Polychlorinated paraffins** should be monitored at least with a screening method, which quantifies the total amount of short and medium-chained compounds in fish and sediment. Such techniques require relatively inexpensive equipment and only one separation by gas chromatography. However, the determination of complete formula and congener group pattern for at least selected samples should be part of any specifically designed monitoring project.

**Chlordanes** occur in concentrations in the very low ng/g range in fish and sediments from the North and Baltic Sea and are therefore not recommended for routine monitoring. Concentrations are about one order of magnitude lower than in the

European Arctic, which is significantly exposed to long range atmospheric transport from North America. However, *trans*- and *cis*-nonachlor could be implemented into routine analysis by mass spectrometry as indicator compounds for chlordanes. This is easy, since only two additional masses have to be included into the standard measuring procedure.

**Dicofol** should not be included in marine routine monitoring programmes because of its restricted use and low stability in sea water.

**Chlorpyrifos** and **Endosulfan**, too, should not be monitored in the North and Baltic Seas on a routine basis. However, additional data on chlorpyrifos-ethyl and endosulfan I should be collected in the next two years in order to increase the statistical significance of the results obtained so far. The surveys should include the matrices water, sediment, and biota.

**Pentachlorophenol** should be monitored at least in the coastal waters of the North Sea. The screening survey in the Baltic Sea covering the matrices sediment and biota should be finalised; it should be followed by a final statement on monitoring requirements.

**Trifluralin** is recommended to be routinely monitored in water and biota, with a focus on seasonal variations.

# 3 Introduction

Due to the huge amount of anthropogenic organic contaminants, it is difficult to obtain a comprehensive overview of pollution by hazardous substances in German rivers and especially in the marine environment. The routine monitoring programme mainly covers well known lipophilic substances as PAH, PCBs and some other chlorinated hydrocarbons. In recent year, studies on several other organic contaminants have been performed, but most investigation had been limited in space and time and did not provide a broad picture of contamination.

The OSPAR-Commission, who is implementing the Convention on the protection of the marine environment of the North-Atlantic Ocean, has developed a Hazardous Substance Strategy with the objective of preventing pollution of the maritime area by continuously reducing discharges, emissions and losses of hazardous substances, with the ultimate aim of achieving concentrations in the marine environment near background values for naturally occurring substances and close to zero for man-made synthetic substances by the year 2020. OSPAR has identified 48 chemicals for priority action and a list of about 325 further substances of possible concern. For most of these substances it is not known, if they reach the marine environment and in which concentrations they might occur in water, sediment and organisms.

Therefore, it is the aim of this project, to identify at least for some of the chemicals for priority action and the substances of possible concern their environmental concentrations in marine waters of Germany and to compare these concentrations with selected samples from other areas of the Northeast Atlantic Ocean. By this, the relevance of these substances for the marine environment should be checked.

Not only OSPAR has identified lists of hazardous substances to the marine environment, but also the Helsinki Commission, who is implementing the Convention on the protection of the marine environment of the Baltic Sea. The Water Framework Directive (WFD) has also identified 33 priority substances for coastal waters. Out of these lists, the following substances have been investigated in this project:

| Substance | OSPAR Lists | HELCOM List | WFD | POP Convention |
|---|---|---|---|---|
| Short chained chlorinated paraffins | x | x | x | |
| Medium chained chlorinated paraffins | x | | | |
| Long chained chlorinated paraffins | | | | |
| Chlordane | x | x | | x |
| Chlorpyrifos | | | x | |
| Dicofol | x | | | |
| Endosulfan | x | x | x | |
| Pentachlorophenol (PCP) | x | x | x | |
| Trifluralin | x | x | x | |

Since the analytical approach is quite different for these substances, different laboratories have been involved according to their analytical specialities. Chlorinated paraffins and chlordane have been investigated by the University of Basel, while the selected pesticides have been studied by the Federal Maritime and Hydrographic Agency (BSH). In connection with this project, polybrominated diphenylethers (PBDE) have been analysed by the Federal Environmental Agency, but the results are not available yet and will be published separately.

The analytical methods for the determination and quantification of these substances had to be developed or improved during the project. For polychlorinated paraffins and chlordane, this included low resolution mass spectrometry combined with negative ion chemical ionisation (NICI-LRMS), electron ionisation tandem mass spectrometry (EI-MS/MS) and electron capture negative ion detection (ECNI-LRMS). It was the aim of the project to lower the limit of detection and to develop a methodology for use in routine monitoring for the identification of polychlorinated paraffins in sediment and biota. Methodologies for the quantification of the selected pesticides had to be developed for identification in water, sediment and biota. These methods had to have much lower limits of detection than the methods published so far. A further goal of the project is the development of recommendations for the OSPAR monitoring programme.

Sampling was carried out jointly for all substances during routine monitoring cruises in the North Sea and Baltic Sea by the BSH for water and sediment samples and by the

Federal Research Centre for Fisheries (BFA-Fi) for biota/fish. Additionally, a few fish and seabird samples from the European Arctic, kindly provided by colleagues from Norway, were included to study the occurrence of polychlorinated paraffins and chlordanes in biota.samples from remote areas with little anthropogenic impact.

# 4 Sampling procedure and sampling sites

## 4.1 Water and sediment sampling

Water and sediment samples from the North Sea and the Baltic Sea were taken by the Federal Maritime and Hydrographic Agency (Bundesamt für Seeschifffahrt und Hydrographie, BSH). Sampling was performed in the course of routine monitoring cruises in 2003, 2004 and 2005. Five of the 7 cruises took place in summer (May to September), and two in winter (February). The main survey area for water and sediment sampling covers the German Bight and the western Baltic Sea (up to the Baltic Proper). In addition, water samples were taken during a 2003 survey covering the entire North Sea and during a 2004 research cruise to the Arctic Atlantic. The sampling stations are shown in Figures 2 to 4.

The cruises are designed mainly for the German national monitoring program BLMP (Bund-Länder-Messprogramm) and for international programmes of OSPAR (Coordinated Environmental Monitoring Programme [CEMP]) and HELCOM (Cooperative Monitoring in the Baltic Marine Environment (COMBINE)).

As sampling is performed according to the relevant guidelines, the results of this project can be fully integrated into existing monitoring programmes and used in the assessments. This is particularly important with respect to the occurrence and spatial distribution of hazardous substances included in the list of hazardous substances for priority action under the OSPAR hazardous substances strategy. The sampling design chosen also allows such new substances to be assessed in relation to pollutants that have already been monitored for several years.


**Figure 1 :** Research vessel "Gauss"

Most of the cruises were carried out by the research vessel FS Gauss (Figure 1). The cruise in February 2004 was carried out by FFS Walther Herwig, that to the Arctic by FS Polarstern. The geographical positions of the samples collected for this project are shown in Figure 2 to 4. Relevant parameters for sample characterisation (salinity for water samples and TOC values for sediments) have been summarised in Table 1 and 2.

The stations were selected to obtain the most comprehensive and representative coverage of the sea areas under investigation. Water sampling stations were selected taking into account oceanographic parameters such as currents and river inputs. In sediment sampling, typical sediment parameters (e.g. TOC content) were taken into account as well. However, due to time restraints and limited resources, optimum sampling conditions have not always been achieved.

Water samples were taken using a 10 l glass bowl. In-line filtration followed by solid phase extraction (SPE) was carried out on board. The SPE cartridges were wrapped in aluminium foil and kept in the refrigerator until elution at the land-based laboratory.Sediment samples were taken using a box corer; the upper 2 cm was transferred to an aluminium box by means of a metal spatula and frozen at -20 °C. It was kept at that temperature in a freezer until analysis. Insulated containers equipped with frigistors were used to transport the samples from the ship to the institute

laboratory, and later to distribute them to the analysing laboratories (UBA, University of Basel).

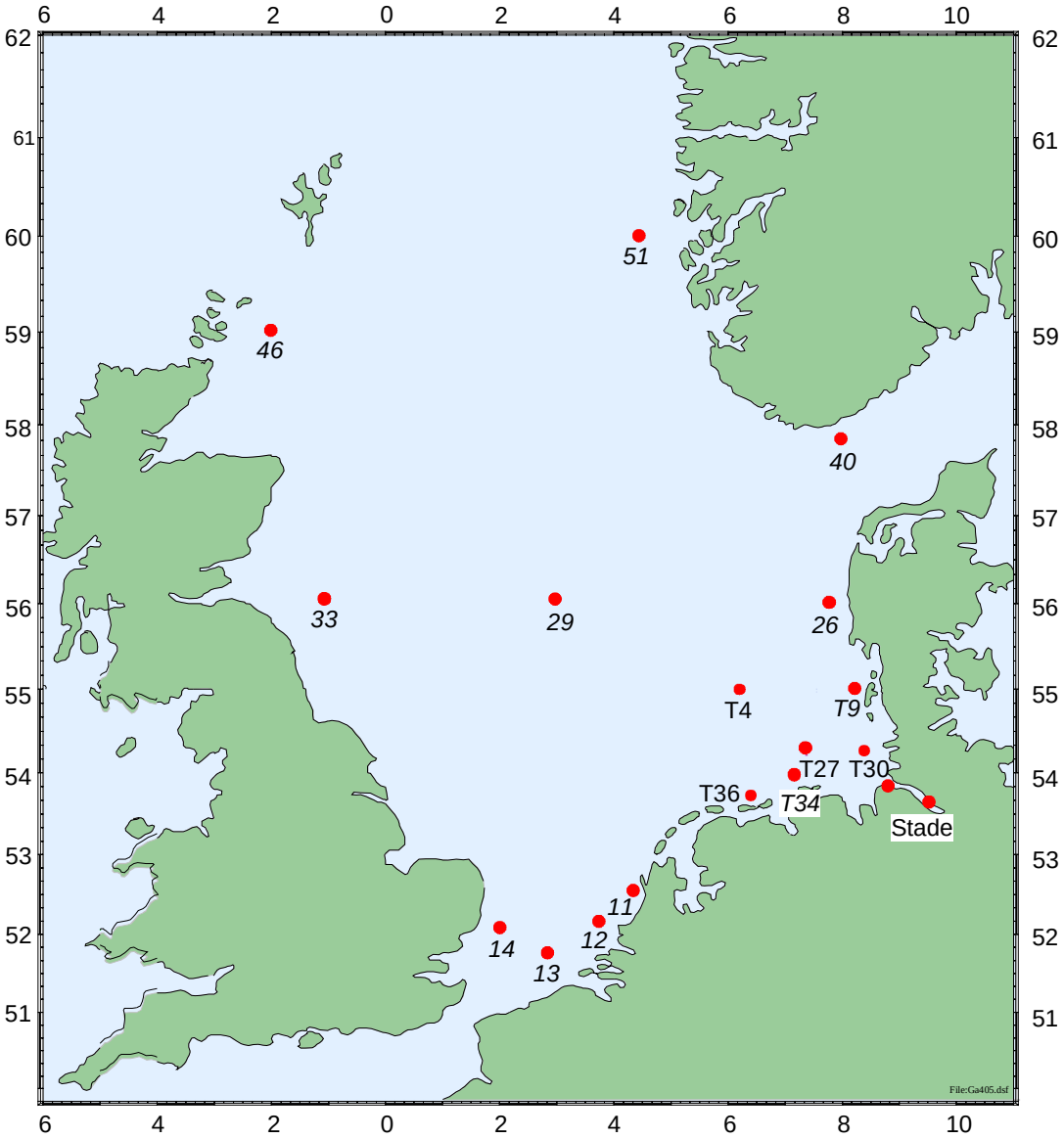


**Figure 2:** Water sampling stations in July/Aug. 2003 (Ga 405).

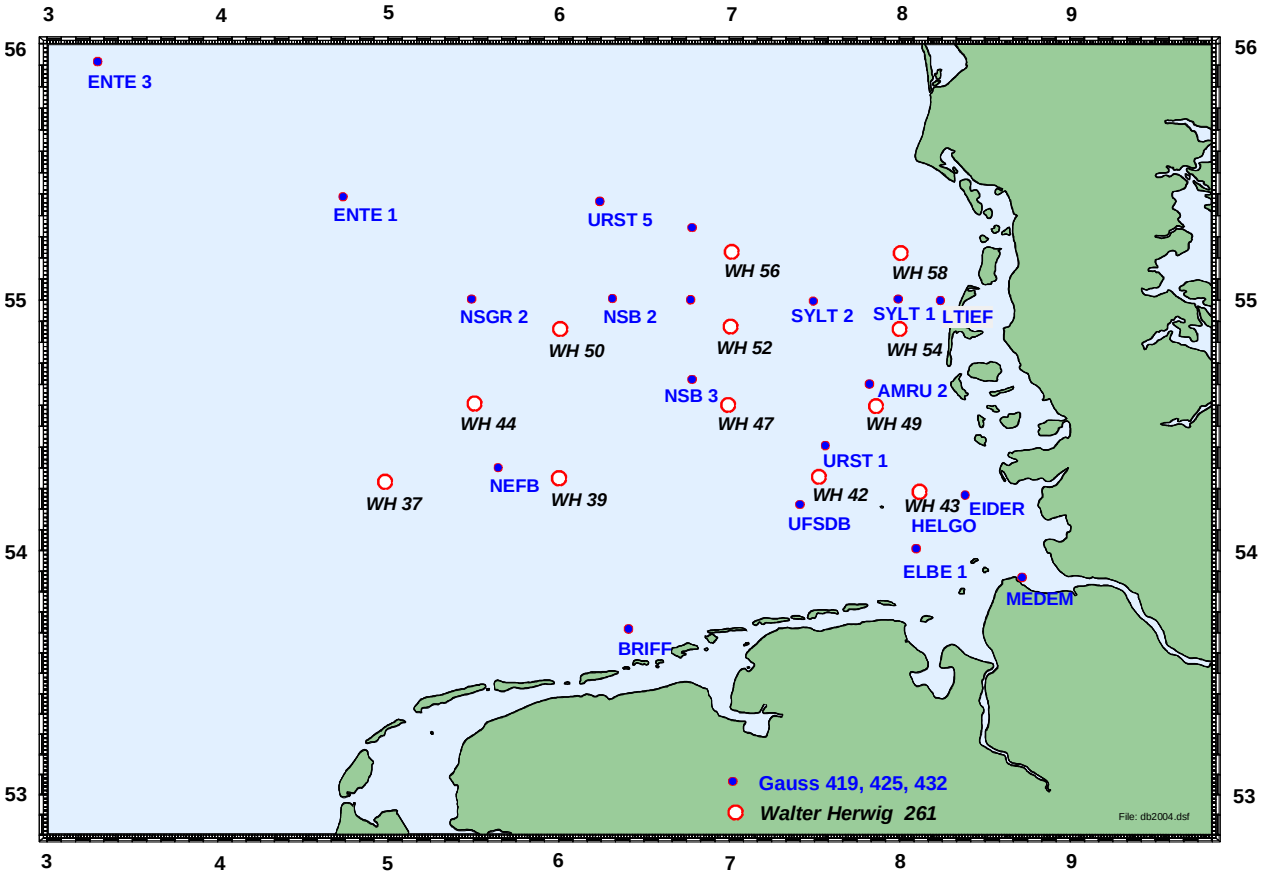


**Figure 3:** Water sampling stations in the German Bight in 2004 and 2005**.**

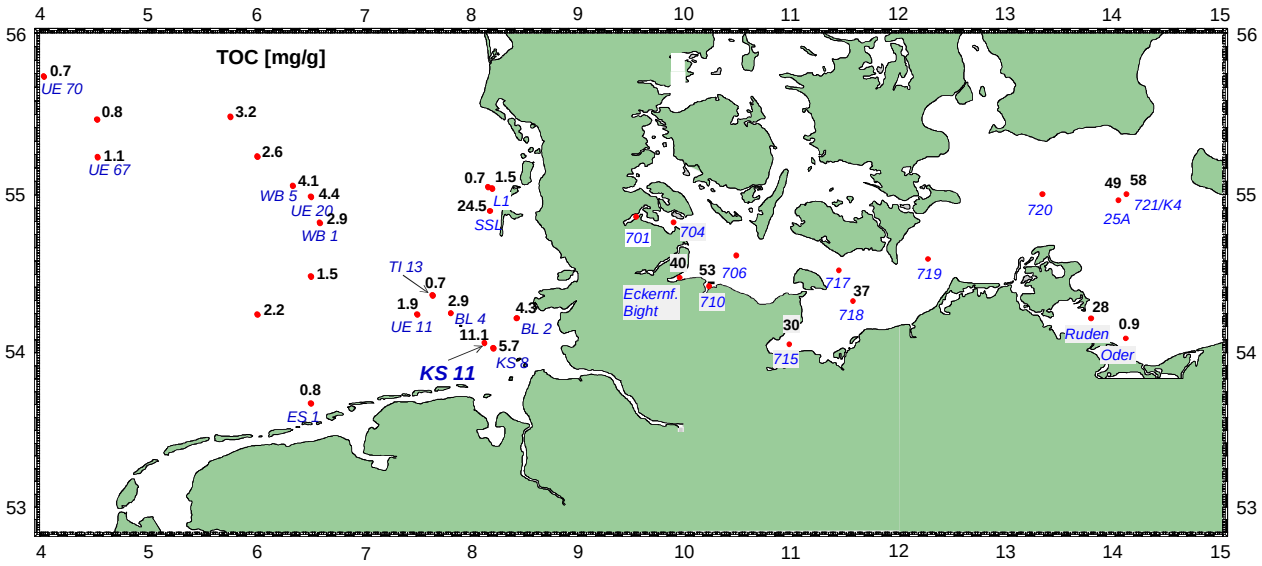


**Figure 4:** Sediment sampling stations in the German Bight and western Baltic Sea as well as water sampling stations in the Baltic Sea. Figures in bold assign the TOC content [mg/g], those in italic station names.

**Table 1 :** Salinity of sea water samples [practical salinity unit] in the North Sea.

| Cruise<br>Station | GA402<br>May 03 | GA405<br>Jul 03 | WH261<br>Feb 04 | GA419<br>May 04 | GA425<br>Jul 04 | GA432<br>Jan 05 |
|---|---|---|---|---|---|---|
| T30/BL2/EIDER | 28.07 | 29.08 | | 29.80 | 29.48 | |
| T9/LTIEF | 29.67 | 30.09 | | 30.74 | 31.03 | |
| T4/NSB2 | 34.39 | | | 34.53 | 34.46 | |
| STADE | < 2 | < 2 | | < 2 | | |
| T27 | 33.42 | | | | | |
| T36 | 31.73 | | | | | |
| T34 | 32.55 | | | | | |
| MEDEM | | | | ca. 15 | | |
| SYLT1 | | | | 31.36 | | 32.21 |
| SYLT2 | | | | 32.71 | 31.92 | |
| ENTE3 | | | | 35.04 | | |
| NEFB | | | | 34.43 | 34.43 | 34.76 |
| UFSDB | | | | 32.58 | 33.11 | |
| AMRU2 | | | | 31.77 | | |
| BRIFF | | | | 32.03 | 32.89 | |
| ELBE1 | | | | | 31.53 | |
| NSB3 | | | | | 33.44 | |
| ENTE1 | | | | | 34.82 | |
| URST1 | | | | | | 32.17 |
| URST5 | | | | | | 34.77 |
| HELG | | | | | | 29.64 |
| 11 | | 33.65 | | | | |
| 12 | | 34.39 | | | | |
| 14 | | 34.57 | | | | |
| 29 | | 35.02 | | | | |
| 33 | | 34.68 | | | | |
| 40 | | 28.22 | | | | |
| 46 | | 35.07 | | | | |
| 51 | | 29.76 | | | | |
| WH/37 | | | 34.5 | | | |
| WH/39 | | | 34.5 | | | |
| WH/42 | | | 32.5 | | | |
| WH/43 | | | 30.0 | | | |
| WH/49 | | | 31.0 | | | |
| WH/47 | | | 33.5 | | | |
| WH/44 | | | 34.5 | | | |
| WH/50 | | | 34.5 | | | |
| WH/52 | | | 33.5 | | | |
| WH/54 | | | 32.0 | | | |
| WH/56 | | | 34.0 | | | |
| WH/58 | | | 32.0 | | | |

**Table 2:** TOC in sediments [mg/g]

| Cruise<br>Station | GA349<br>May 00 | GA402<br>May 03 | GA405<br>Jul 03 | GA419<br>May 04 | Median |
|---|---|---|---|---|---|
| **North Sea** | | | | | |
| KS11 | 14.9 | 11.0 | | 25 | 14.9 |
| KS8 | | 8.2 | | 12.1 | 10.2 |
| WB1 | 1.93 | | | | 1.93 |
| WB5 | | 4.632 | | 5.5 | |
| SSL | | 27.7 | | | 27.7 |
| UE20 | | 3.71 | | 4.8 | 4.26 |
| ES1 | | 0.6 | | | 0.6 |
| UE67 | | 1.22 | | | 1.22 |
| UE70 | | | | 0.5 | 0.5 |
| BL2 | | | | 8.5 | 8.5 |
| BL4 | | | | 1.3 | 1.3 |

| Cruise<br>Station | GA37<br>Aug 01 | GA387<br>Aug 02 | GA421<br>Jun 04 | Median |
|---|---|---|---|---|
| **Baltic Sea** | | | | |
| Eckfbucht | | | 40 | 40 |
| 710 | 54 | 52.8 | 4.8 | 52.8 |
| 25A | | | 48.6 | 48.6 |
| 715 | 29.4 | | 30.8 | 30.1 |
| 718 | 37.8 | | 36.4 | 37.1 |
| Oder | | | 0.9 | 0.9 |
| Ruden | | | 28.1 | 28.1 |
| 721/K4 | | 57.8 | | 57.8 |

## 4.2 Sampling of biota

Fish from the North Sea and the Baltic Sea was sampled by the Institute of Fishery Ecology of the Federal Research Centre for Fisheries (BFA-Fi, Hamburg). Sampling was performed within the framework of the regular annual research cruises for monitoring hazardous substances and bio-effects during late August/September in 2002, 2003, and 2004. The grid of stations covered North Sea areas from the German Bight to the northern Scottish waters as well as the Baltic Sea from the Kiel Bight to the Baltic Proper.

Sampling was performed on a co-operative basis by the Institute of Fishery Ecology. The Institute's available equipment, which included fishery research vessels, and its highly skilled staff with ample experience in fishing and sample preparation ensured:

- the correct catch, identification, and choice of suitable indicator species,
- a correct protocol of catch log data (location, date, time etc.) and biometric data (sex, length, weight, age),
- immediate dissection of liver and fillet sub-samples under controlled conditions avoiding contamination (separated labs on board, clean bench), quick deep-freezing in special frost rooms, and maintaining frozen state during storage and transport.

The cruises were designed for monitoring programmes of the international conventions for the protection of the marine environment such as:

- Coordinated Environmental Monitoring Programme [CEMP] of OSPAR for the North-East Atlantic Ocean
- Cooperative Monitoring in the Baltic Marine Environment [COMBINE] of HELCOM.

Sampling was performed according to the relevant guidelines. Therefore, the results obtained in this project can be fully integrated into existing monitoring programmes and used in assessments. This is particularly important with respect to the occurrence and spatial distribution of substances included in the list of hazardous substances for priority action under the OSPAR and HELCOM hazardous substances strategies. Moreover, concentrations of new substances can be compared to those of substances that have been monitored for many years, such as organochlorine compounds, trace metals, as well as to bio-effect data like the prevalence of fish diseases.

The cruises were carried out by the fishery research vessel FFS Walther Herwig III. The investigation areas for this project were selected from the core areas (N01, N04, N06, N11, P01, P02, BMP, B01, B11) of the Institute's monitoring grid, which has been developed since the early eighties of the last century in order to investigate possible influences of hazardous substances on fish diseases in the areas of past or present pollutant sources and in adjacent areas such as:

- N01 (German Bight): Elbe river, former titanium dioxide dumping site, high shipping traffic
- N04 northern Dogger Bank, high prevalence of fish diseases possibly due to sedimentation of substances originating from industrial areas, rivers, and dumping sites at the English east coast
- N06 industrial region Edinburgh (Firth of Forth)
- Offshore oil and gas production at P01 (Danfield) and P02 (Ekofisk).

Due to variable composition of the catch, it was not always possible to complete the planned sampling programme. Therefore, the spatial coverage of individual years is not comparable. However, dab was caught in the areas N01, N04, N06 and P01 in all three years and was subsequently analysed for brominated flame retardants (PBDEs) at the laboratory of the Federal Environmental Agency (UBA).

An overview of the samples collected for this project is given in Tables 3–5, and the species abbreviations (Rubin-Code) are explained in Table 6. The sampling sites are shown in Figure 5, and detailed logging data of the catches are provided in Table 7. Samples were prepared on board the ship. The laboratories were specially designed to avoid contamination by organic substances (“ceramic” floor coating, walls, benches and cupboards made of sea-water resistant stainless steel, clean bench). Blank controls of analytes at the land-based laboratories had not indicated any contamination of target substances due to sampling and preparation.

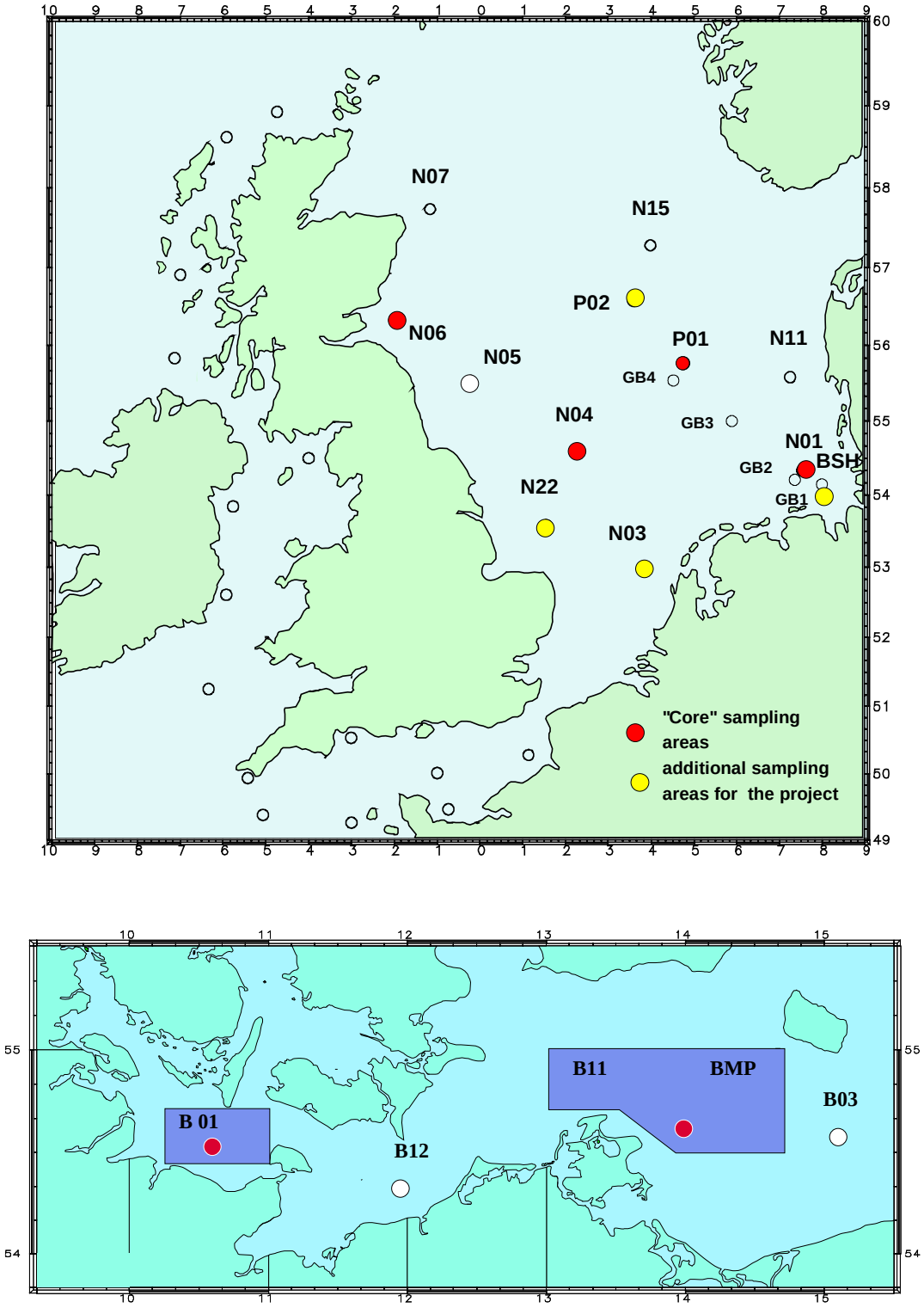


**Figure 5:** Top: Locations of North Sea biota sampling areas of the Institute of Fishery Ecology. Bottom: Sampling area in the Baltic Sea**.**

After dissection, the organ sub-samples (mostly fish livers) were wrapped in pre-cleaned aluminium foil and deep-frozen at -35 °C in the ship's freezing rooms. Insulated containers equipped with cooling elements were used to transport the samples from the ship to the Institute's laboratory, and later to distribute them to the the analysing laboratories.

**Table 3:** Sampling sites of the monitoring campaign WH242 in August/September 2002.

| Year | Area | Species* | Individuals (I) or pooled (P) | No. of samples | Tissue | Analysed by** |
|---|---|---|---|---|---|---|
| 2002 | N01 | LIMA LIM | I | 5 | LI | BSH |
| 2002 | N01 | LIMA LIM | I | 5 | LI | UBA |
| 2002 | N01 | LIMA LIM | I | 5 | LI | UNI BASEL |
| 2002 | N01 | LIMA LIM | P | 9 | LI | UNI BASEL |
| 2002 | N04 | LIMA LIM | I | 5 | LI | BSH |
| 2002 | N04 | LIMA LIM | I | 5 | LI | UBA |
| 2002 | N04 | LIMA LIM | I | 5 | LI | UNI BASEL |
| 2002 | N04 | GADU MOR | I | 5 | LI | BSH |
| 2002 | N04 | GADU MOR | I | 5 | LI | UBA |
| 2002 | N04 | GADU MOR | I | 5 | LI | UNI BASEL |
| 2002 | N06 | LIMA LIM | I | 5 | LI | BSH |
| 2002 | N06 | LIMA LIM | I | 5 | LI | UBA |
| 2002 | N06 | LIMA LIM | I | 5 | LI | UNI BASEL |
| 2002 | P01 | LIMA LIM | I | 5 | LI | BSH |
| 2002 | P01 | LIMA LIM | I | 5 | LI | UBA |
| 2002 | P01 | LIMA LIM | I | 5 | LI | UNI BASEL |
| 2002 | B11 | GADU MOR | I | 5 | LI | BSH |
| 2002 | B11 | GADU MOR | I | 5 | LI | UBA |
| 2002 | B11 | GADU MOR | I | 5 | LI | UNI BASEL |
| 2002 | B11 | PLAT FLE | I | 5 | LI | BSH |
| 2002 | B11 | PLAT FLE | I | 5 | LI | UBA |
| 2002 | B11 | PLAT FLE | I | 5 | LI | UNI BASEL |
| 2002 | B01 | LIMA LIM | I | 5 | LI | BSH |
| 2002 | B01 | LIMA LIM | I | 5 | LI | UBA |
| 2002 | B01 | LIMA LIM | I | 5 | LI | UNI BASEL |
| 2002 | B01 | GADU MOR | I | 5 | LI | BSH |
| 2002 | B01 | GADU MOR | I | 5 | LI | UBA |
| 2002 | B01 | GADU MOR | I | 5 | LI | UNI BASEL |

*Rubin code, see Table 22. **BSH: Bundesamt für Seeschifffahrt und Hydrographie,
UBA: Umweltbundesamt Berlin, Uni Basel: Universität Basel

**Table 4:** Sampling sites of the monitoring campaign WH255 in August/September 2003.

| Year | Area | Species* | Individuals (I) or pooled (P) | No. of samples | Tissue | Analysed by** |
|---|---|---|---|---|---|---|
| 2003 | B01 | GADU MOR | P | 10 | LI | Uni Basel |
| 2003 | B01 | LIMA LIM | P | 18 | LI | Uni Basel |
| 2003 | B01 | GADU MOR | P | 5 | LI | Uni Basel |
| 2003 | B01 | GADU MOR | I | 10 | LI | UBA |
| 2003 | B11 | GADU MOR | I | 11 | LI | UBA |
| 2003 | GB1 | PLAT FLE | P | 10 | LI | Uni Basel |
| 2003 | N04 | LIMA LIM | P | 14 | LI | Uni Basel |
| 2003 | N01 | LIMA LIM | I | 15 | LI | UBA |
| 2003 | N04 | LIMA LIM | I | 10 | LI | UBA |
| 2003 | N06 | LIMA LIM | I | 10 | LI | UBA |
| 2003 | P01 | LIMA LIM | I | 10 | LI | UBA |

*Rubin code, see Table 22. ** UBA: Umweltbundesamt Berlin, Uni Basel: Universität Basel

**Table 5:** Sampling sites of the monitoring campaign WH267 in August/September 2004.

| Year | Area | Species* | Individuals (I) or pooled (P) | No. of samples | Tissue | Analysed by** |
|---|---|---|---|---|---|---|
| 2004 | N01 | LIMA LIM | I | 15 | LI | UBA |
| 2004 | N03 | LIMA LIM | I | 15 | LI | UBA |
| 2004 | N04 | LIMA LIM | I | 15 | LI | UBA |
| 2004 | N06 | LIMA LIM | I | 15 | LI | UBA |
| 2004 | N22 | LIMA LIM | I | 15 | LI | UBA |
| 2004 | P01 | LIMA LIM | I | 15 | LI | UBA |
| 2004 | P02 | LIMA LIM | I | 15 | LI | UBA |
| 2004 | BMP | GADU MOR | P | 10 | LI | Uni Basel |
| 2004 | N02 | CLUP HAR | I | 10 | LI | Uni Basel |
| 2004 | N02 | GADU MOR | I | 10 | LI | Uni Basel |
| 2004 | N05 | MELA AEG | I | 10 | LI | Uni Basel |
| 2004 | N05 | MELA AEG | I | 10 | LI | Uni Basel |
| 2004 | GB1 | PLAT FLE | P | 3 | LI | Uni Basel |
| 2004 | GB1 | PLAT FLE | P | 3 | LI | Uni Basel |
| 2004 | GB1 | PLAT FLE | P | 3 | LI | Uni Basel |
| 2004 | N06 | SCOM SCO | P | 2 | LI | Uni Basel |
| 2004 | N06 | SCOM SCO | P | 2 | LI | Uni Basel |
| 2004 | N06 | SCOM SCO | P | 2 | LI | Uni Basel |
| 2004 | N06 | SCOM SCO | P | 2 | LI | Uni Basel |
| 2004 | N06 | SCOM SCO | P | 2 | LI | Uni Basel |
| 2004 | N06 | SCOM SCO | I | 10 | LI | Uni Basel |
| 2004 | N05 | POLL VIR | I | 1 | LI | Uni Basel |

*Rubin code, see Table 22. ** UBA: Umweltbundesamt Berlin, Uni Basel: Universität Basel

**Table 6:** International Rubin code of fish species abbreviations.

| Abbreviation (Rubin-Code) | Latin | English |
|---|---|---|
| LIMA LIM | Limanda limanda | Dab |
| PLAT FLE | *Platichthys flesus* | Flounder |
| GADU MOR | *Gadus morhua* | Cod |
| CLUP HAR | *Clupea hargenus* | Herring |
| SCOM SCO | *Scomber Scomus* | Mackerel |
| MELA AEG | *Melanogrammus aeglefinus* | Haddock |
| POLL VIR | *Pollachius virens* | Saithe |

**Table 7**: Detailed logging data of the catches.

| | | | Date | | | | | Start position trawl | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cruise** | **Area** | **Haul** | **D** | **M** | **Y** | **h** | **min** | **Latitude [° min N]** | **Longitude [° min]** | **Water depth [m]** |
| WH242 | JMP/N01 | 11 | 24 | 8 | 2002 | 12 | 16 | 54° 17.14 | 7° 30.06 E | 41 |
| WH242 | N04 | 17 | 26 | 8 | 2002 | 4 | 38 | 54° 30.01 | 2° 16.37 E | 20 |
| WH242 | N04 | 20 | 26 | 8 | 2002 | 11 | 41 | 54° 43.26 | 2° 7.92 E | 29 |
| WH242 | N06 | 23 | 27 | 8 | 2002 | 7 | 9 | 56° 18.57 | 2° 4.58 W | 50 |
| WH242 | P01 | 34 | 29 | 8 | 2002 | 9 | 4 | 55° 30.62 | 4° 40.82 E | 36 |
| WH242 | P01 | 35 | 29 | 8 | 2002 | 10 | 51 | 55° 37.25 | 4° 50.14 E | 37 |
| WH242 | B11 | 38 | 31 | 8 | 2002 | 4 | 38 | 54° 47.38 | 13° 6.82 E | 34 |
| WH242 | B11 | 41 | 31 | 8 | 2002 | 12 | 4 | 54° 46.26 | 13° 18.2 E | 41 |
| WH242 | B11 | 42 | 31 | 8 | 2002 | 14 | 6 | 54° 44.30 | 13° 10.63 E | 30 |
| WH242 | B01 | 56 | 3 | 9 | 2002 | 8 | 30 | 54° 31.70 | 10° 39.06 E | 18 |
| WH255 | P01 | 1 | 26 | 8 | 2003 | 5 | 12 | 55° 22.76 | 4° 57.25 E | 46 |
| WH255 | N01 | 16 | 30 | 8 | 2003 | 4 | 37 | 54° 23.77 | 7° 37.82 E | 26 |
| WH255 | N01 | 17 | 30 | 8 | 2003 | 6 | 37 | 54° 20.89 | 7° 29.05 E | 25 |
| WH255 | BSH | 19 | 30 | 8 | 2003 | 10 | 57 | 54° 6.78 | 7° 46.05 E | 43 |
| WH255 | B01 | 21 | 31 | 8 | 2003 | 5 | 22 | 54° 35.46 | 10° 24.35 E | 17 |
| WH255 | B11 | 34 | 3 | 9 | 2003 | 5 | 5 | 54° 44.59 | 13° 9.78 E | 29 |
| WH255 | B11 | 35 | 3 | 9 | 2003 | 6 | 36 | 54° 45.61 | 13° 18.77 E | 41 |
| WH255 | B11 | 36 | 3 | 9 | 2003 | 8 | 30 | 54° 49.67 | 13° 8.81 E | 43 |
| WH255 | B11 | 37 | 3 | 9 | 2003 | 10 | 35 | 54° 49.81 | 13° 17.32 E | 44 |
| WH255 | N06 | 40 | 6 | 9 | 2003 | 5 | 9 | 56° 16.17 | 2° 7.08 W | 52 |
| WH255 | N04 | 49 | 8 | 9 | 2003 | 5 | 5 | 54° 27.23 | 2° 9.35 E | 18 |
| WH255 | N04 | 51 | 8 | 9 | 2003 | 8 | 40 | 54° 38.32 | 2° 16.15 E | 24 |
| WH267 | N03 | 5 | 5 | 9 | 2004 | 7 | 27 | 53° 3.22 | 3° 51.32 E | 23 |
| WH267 | N22 | 9 | 6 | 9 | 2004 | 5 | 8 | 53° 37.03 | 1° 38.76 E | 19 |
| WH267 | N04 | 19 | 8 | 9 | 2004 | 5 | 7 | 54° 43.95 | 2° 24.97 E | 21 |
| WH267 | N06 | 30 | 10 | 9 | 2004 | 7 | 32 | 56° 18.67 | 2° 4.35 W | 50 |
| WH267 | N06 | 32 | 10 | 9 | 2004 | 11 | 34 | 56° 19.15 | 2° 8.46 W | 56 |
| WH267 | P02 | 44 | 13 | 9 | 2004 | 12 | 0 | 56° 40.28 | 3° 11.96 E | 66 |
| WH267 | P01 | 46 | 14 | 9 | 2004 | 5 | 6 | 55° 47.15 | 4° 47.63 E | 39 |
| WH267 | P01 | 47 | 14 | 9 | 2004 | 7 | 33 | 55° 43.24 | 4° 51.58 E | 39 |
| WH267 | P01 | 48 | 14 | 9 | 2004 | 10 | 39 | 55° 40.08 | 4° 55.05 E | 40 |
| WH267 | N01 | 53 | 16 | 9 | 2004 | 5 | 9 | 54° 16.06 | 7° 30.20 E | 40 |
| WH267 | B01 | 78 | 22 | 9 | 2004 | 5 | 9 | 54° 29.69 | 10° 41.18 E | 20 |

# 5 Project 1: Identification of Chlorinated Paraffins and Chlordanes in the North and Baltic Seas

Project number: FKZ 200 25 224/01; Period: 1.05.2002 – 31.10.2005

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## 5.1 Aim of the project

Quantification of PCA in environmental samples is far away from routine analysis. Main reasons are:

- The **very complex composition** present in both technical mixtures and in the environment consisting of ten thousands of compounds, which cannot be resolved by any (chromatographic) method (see also chapter 5.3.1).
- The **presence of many formula and congener groups** requires many analyses to include the majority of them (up to ten per sample).
- **Problems of quantification** due to variable response factors influenced by the degree of chlorination of the PCA present.
- The **recommendation to use high resolution mass spectrometry** as detection method, which is normally not available and very expensive.

Therefore, information about the environmental PCA burden is very limited despite a high annual consumption of up to 300'000 t. Due to the restricted or banned use of sPCA, the application of mPCA has continuously increased and will continue. Already 1994, more mPCA than sPCA were employed (WHO, 1996).

Table 10 in chapter 5.2.1 and table Table 26 in chapter 5.4.2.2 summarise most of the data available at the beginning of this project. In summary, sPCA concentrations in fish and marine mammals were in the order of 100–1700 ng/g lipid. mPCA levels were hardly determined and the few results available are within the same range. However, a direct comparison of data is hampered by the strong influence of the applied reference

standard. Deviations of a factor of 2 and more are the result (see chapter 5.3.4 for details).

PCA concentrations in sediments are even scarcer. The few studies in Canada and Europe (river sediments) showed highly variable sPCA concentrations with a range between a few and several thousands of ng/g dry weight. mPCA levels are hardly available. Table 35 in chapter 5.4.3.3 gives a summary.

The main aim of this part of the project was first to improve and simplify quantification methodology for PCAs and to add at least some information about s-, mPCA and chlordane concentrations in fish from the North and Baltic Sea as well as from the northern North Atlantic. Moreover, levels in sea sediment should be determined. These limited data would then allow to estimate roughly the burden of PCA and to compare it with more "classical" pollutants such as PCB and DDT.

Chlordane was nearly exclusively applied as pesticide in North and Central America. Consequently, many studies exist about concentrations in the environment, in biota and in humans from these regions. Chlordane can be dispersed by atmospheric long-range transport. After volatilisation from soil and water at low latitudes, the pesticide is transported to higher latitudes by air currents. The colder temperatures in the polar regions lead to dry and wet deposition due to re-condensation. Substantial amounts of chlordane have been detected in remote areas such as sub-polar regions (Andersson *et al.*, 1988) and the Arctic (Oehme and Manø, 1984; Bidleman *et al.*, 1989). Several reports are also available about chlordane accumulation in the Arctic marine food-webs (Muir *et al.*, 1988; Hargrave *et al.*, 1992).

Data about chlordane concentrations in the European environment are nearly completely missing, since this pesticide was hardly applied. Consequently, only very few and limited studies have been carried out about levels in marine biota (Marvin *et al.*, 2003; Jansson *et al.*, 1993; Strandberg *et al.*, 1998; Falandysz *et al.*, 2001; Karl *et al.*, 1998; Voorspoels *et al.*, 2004) and in sediments (Strandberg *et al.*, 1998b). Table 31 in chapter 5.4.2.6 gives a summary of these reports. However, levels in the European Arctic are relatively high due to long range transport from North America (cod liver: 0.1–0.2 μg/g

lipid weight, see Table 32; Karlsson, 2000). Therefore, chlordane was included in this study to extend the existing data about levels in European marine fish and sediments. This was possible, because a validated analysis method already existed for biota and therefore only a method adaptation for sediments was necessary.

## 5.2 Survey about properties of PCAs and chlordane

### 5.2.1 Polychlorinated *n*-alkanes (PCAs)

Polychlorinated *n*-alkanes (PCAs, also called chlorinated paraffins or CPs) are complex technical mixtures containing thousands of different isomers, congeners, diastereomers and enantiomers (Muir *et al.*, 2000). They are produced by radical chlorination of *n*-alkanes in presence of UV light or heating. The chlorine content of the products varies between 30 and 70 %. PCAs are divided into short chain PCAs ($C_{10–13}$, sPCAs), medium chain PCAs ($C_{14–17}$, mPCAs) and long chain PCAs ($C_{>17}$, lPCAs) depending on the length of the carbon chain. Since their first large scale usage in 1932 as extreme pressure additives, the purity of PCA products was improved by increasing the purity of the *n*-alkane feedstocks (Muir *et al.*, 2000). Modern commercial products contain additives to inhibit decomposition of PCAs by loss of HCl at elevated temperatures and to increase thermal stability when used as flame retardants (e.g. antimony oxide). Other common stabilizers include epoxides and organotin compounds (GDCh, 1996).

Depending on chain length and chlorine content, PCAs are colourless or yellowish, low to highly viscous liquids or glassy to waxy solids. PCAs are thermally stable up to 200–300 °C. Environmentally important physical-chemical properties of PCAs such as vapour pressure, water solubility and water-octanol distribution are summarised in Table Table 8.

As consequence of the different physical and chemical properties, miscellaneous PCA formulations are used for a wide range of applications. Some examples are: Additives in metal working fluids (short, medium, and long chain PCAs, 50–60 % Cl content), secondary plasticizers in polyvinyl chloride and in other plastics (usually mPCAs, 40–

60 % Cl content) or fire retardants in plastics (short, medium, and long chain PCAs, 50–70 % Cl content).

The world wide production of PCAs has been estimated to be 300 kt/year in 1993 (Muir *et al.*, 2000). It is assumed to be about the same today. For example, the mPCA production capacity in the European Union was estimated to 45'000 to 160'000 t annually in 2002. However, the application of sPCAs decreased from 13'000 to 4'000 t in the European Union during 1994–1998 due to strong regulations (HELCOM, 2002).

**Table 8:** Physical-chemical properties of selected PCAs.

| Compound | Vapour pressure [Pa][a, b] | Water solubility [µg/l][a, b] | log $K_{OW}$[a, c] |
|---|---|---|---|
| $C_{10}H_{18}Cl_4$ | 66 | 1260 | 5.93 |
| $C_{10}H_{17}Cl_5$ | 4–66 | 678–994 | 6.04–6.20 |
| $C_{10}H_{13}Cl_9$ | 0.24 | na | na |
| $C_{11}H_{20}Cl_4$ | 10 | 575 | 5.93 |
| $C_{11}H_{19}Cl_5$ | 1–2 | 546–962 | 6.04–6.40 |
| $C_{11}H_{18}Cl_6$ | 0.5–2 | 37 | 6.4 |
| $C_{12}H_{20}Cl_6$ | na | na | 6.40–6.77 |
| $C_{12}H_{18}Cl_8$ | na | na | 7.0 |
| $C_{13}H_{23}Cl_5$ | 0.032 | 30 | 6.61 |
| $C_{13}H_{21}Cl_7$ | na | na | 7.14 |
| $C_{17}H_{32}Cl_4$ | $4.0\ 10^{-3}$ | $2.9\ 10^{-2}$ | na |
| $C_{17}H_{27}Cl_9$ | $1.7\ 10^{-5}$ | $6.6\ 10^{-1}$ | na |
| $C_{20}H_{38}Cl_4$ | $4.5\ 10^{-5}$ | na | na |
| $C_{20}H_{33}Cl_8$ | $1.9\ 10^{-7}$ | $5.3\ 10^{-3}$ | na |

log $K_{OW}$: octanol-water partition coefficient, na: not available
Data from: [a] Muir *et al.*, 2000, [b] Drouillard *et al.*, 1998, [c] Sijm and Sinnige, 1995

Currently, the manufacture and use of sPCAs is banned in the European Community due to their higher aquatic toxicity compared to mPCA (European Community, 2002; see also Table 9). The average bioconcentration factor for sPCAs was estimated to ca. 1100 and of mPCA to 7300 (OSPAR, 2002).

**Table 9:** Aquatic toxic properties of sPCAs and mPCAs.

| Test system | sPCA | mPCA |
|---|---|---|
| Acute toxicity algae IC50 [mg/l] | 0.043 | >3.2 |
| Acute toxicity daphnia EC50 [mg/l] | 0.3 | 0.059 |
| Acute toxicity fish LC50 [mg/l] | >100 | >10'000 |
| Chronic toxicity daphnia NOEC [mg/l] | 0.005 | 0.01 |
| Chronic toxicity fish NOEC [mg/l] | 0.28 | 0.6 |

Data from: OSPAR (2002)

In Germany, the production of all PCA was stopped in 1998. Moreover, PCA application is prohibited for metal working and leather treatment since summer 2003 (European Community, 2002).

Compared to other chlorinated persistent organic pollutants, limited information is available about the toxicity of PCAs. PCAs have low acute toxicity (Farrar, 2000), but are suspected carcinogens, since liver, thyroid, and kidney carcinomas were observed in mice (Bucher *et al.*, 1997).

Moreover, sPCAs showed chronic toxicity to aquatic biota, whereas mPCAs and lPCAs did not (Thompson, 2000).

sPCAs have been included in the list of substances for priority action of the Convention for the Protection of the Marine Environment of the north-east Atlantic (OSPAR, 2000), in the list of priority dangerous substances of the European water framework directive (European Community, 2001) and in that of selected substances for immediate priority action of the Helsinki Commission (HELCOM, 2002).

Due to their widespread and mainly unrestricted use and due to the properties mentioned above, PCAs are present in aquatic and terrestrial food webs of rural and remote areas. However, information about environmental burden is still very scarce. Typical PCA concentrations in the environment reported prior to this study are summarised in Table 10. Due to the very different physical properties of PCA congeners and homologues, partial fractionation of the original technical composition may occur during phase transition and atmospheric transport.

**Table 10:** Overview of sPCA levels in selected environmental matrices at the start of this project.

| Sample | Sampling location | Sampling date | sPCA concentration | Reference |
|---|---|---|---|---|
| Air | Spitsbergen, Norway | 1999 | 9–57 pg/m$^3$ | Borgen *et al*. 2000 |
| Air | Egbert, Canada | 1990 | 65–924 pg/m$^3$ | Stern and Tomy, 2000 |
| Freshwater biota | different locations, Norway | not specified | 108–3700 ng/g fat | Borgen *et al*. 2001 |
| Freshwater particulate matter | different locations, Germany | not specified | 69–860 ng/g dry weight | Maulshagen *et al.*, 2003 |
| Marine sediments | different locations, Canada | not specified | 4.52–135 ng/g dry weight | Tomy *et al.*, 1999a |

### 5.2.2 Chlordane

The pesticide technical chlordane was first synthesised in 1945 by Kearns *et. al* (1945), and its commercial production started in 1946 by Vesicol Corporation. About 70'000 tons were manufactured between 1960 and 1988 (Dearth and Hites, 1991). Technical chlordane was neither manufactured in Europe nor in Japan (Falandysz *et al.*, 1994), but was commercially available in Germany around 1950. In 1976, the pesticide was removed from the German market. First restrictions on the use of technical chlordane in the United States were decided in 1978 by the US Environmental Protection Agency. In 1983, it was only allowed to be used to control underground termites (WHO, 1984).

Technical chlordane is a contact and stomach pesticide (WHO, 1984) with a broad spectrum. It was mainly used for building protection, ornamental lawns and trees, drainage ditches, but also to protect crops like corn and potatoes (WHO, 1984).

#### 5.2.2.1 Structures

147 compounds could be identified in technical chlordane (Dearth and Hites, 1991). Main constituents are about 24 % *trans*-chlordane, 19 % *cis*-chlordane, 9 % γ-chlordene, 7 % heptachlor, 7 % *trans*-nonachlor, 3 % α-chlordene, 3 % β-chlordene, 1 % chlordane and 19.5 % related compounds including *cis*-nonachlor (Bussart and Schor, 1948). Figure 1 shows the structure of the main chlordane congeners and metabolites.

#### 5.2.2.2 Physical properties

Chlordane is lipophilic and has a high octanol-water partitioning coefficient $K_{OW}$. Some values are given in Table 11. The vapour pressure of technical chlordane is 1.47 hPa at 25 °C. Water solubility of technical chlordane is 32 µg/L for pure water (Johnson-Logan *et al.*, 1992).

**Table 11:** log $K_{OW}$ values of main chlordane compounds (Simpson *et al.*, 1995).

| Compound | log $K_{OW}$ |
|---|---|
| heptachlor | 6.10 |
| trans-chlordane | 6.22 |
| cis-chlordane | 6.10 |
| trans-nonachlor | 6.35 |
| cis-nonachlor | 6.08 |

#### 5.2.2.3 Metabolism

The metabolite oxychlordane can be formed from *cis*- and *trans*-chlordane. Feeding of rats with pure *trans*-chlordane resulted in a larger concentration of oxychlordane in the tissues compared to *cis*-chlordane, which was partly metabolised to more hydrophilic and more easily excretable products (Barnett and Wyman-Dorough, 1974).

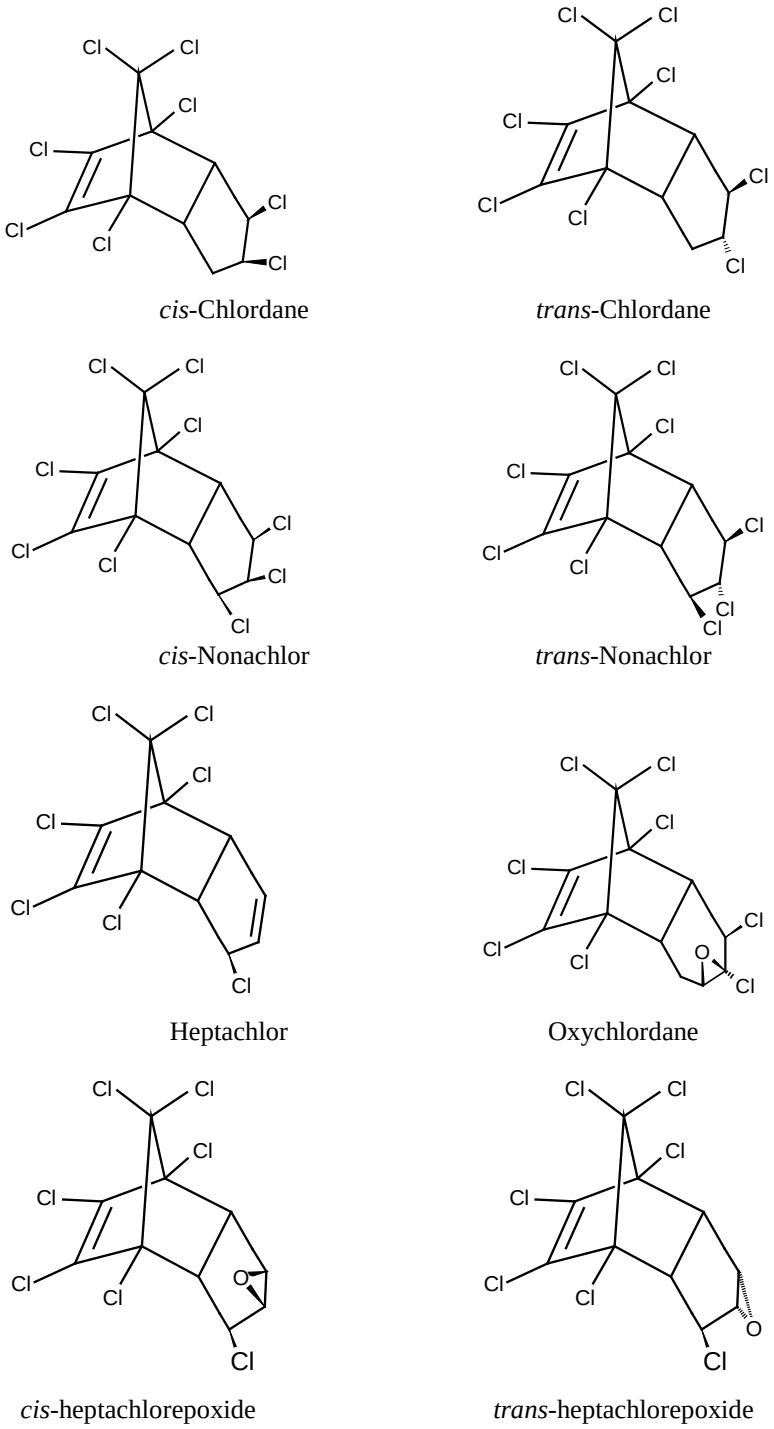


**Figure 6:** Structure of some important chlordane compounds and the main metabolites oxychlordane, *cis*- and *trans*-heptachlorepoxide.

The metabolic pathway of *trans*-nonachlor was also studied in rats. *trans*-Nonachlor degrades via *trans*-chlordane and 1,2-dichlorochlordene to oxychlordane. The same study also revealed that the rate limiting step in the metabolism of *trans*-nonachlor in

humans is the first de-chlorination process to *trans*-chlordane. Furthermore, it was confirmed that the major metabolite of heptachlor is *cis*-heptachlorepoxide (Tashiro and Matsumura, 1978).

Oxychlordane and *cis*-heptachlorepoxide are, unlike other chlordane metabolites, very persistent, lipophilic and accumulate even more effectively than their precursors in biota (Muir *et al.*, 1988; Zhu *et al.*, 1995).

#### 5.2.2.4 Toxicity

Acute toxicity ($LD_{50}$) of chlordane congeners and their metabolites was determined for rats. Pure *cis*- and *trans*-chlordane as well as a 1:1 mixture of the isomers had similar toxicity ($LD_{50}$ = 320–400 mg/kg body weight) while the metabolite oxychlordane was twenty times more toxic (WHO, 1984).

The [$^{3}$H]17-β-estradiol binding to the human estrogenic receptor (hER) plays an important role in estrogenic action. Chlordane enhanced the competitive binding process for other pesticides. Synergistic effects in the order of 150–1600 fold have been found when testing binary mixtures of endosulfane, dieldrin, and toxaphene together with chlordane (Arnold *et al.*, 1996, McLachlan *et al.*, 1997).

## 5.3 Applied methodology

### 5.3.1 State of the art of PCA analysis

The quantification of this extremely complex compound class can be considered as "the challenge and nightmare" in environmental analysis. The reasons are manifold:

- PCAs consist of so many isomers and congeners, that **high resolution gas chromatography cannot resolve** them into single compounds.
- **Electron ionisation** mass spectrometry leads to a **strong fragmentation** of PCAs and, consequently, to **insufficient detection limits**.

- Ions from **different formula groups** including their isotope signals have the **same nominal mass** and **overlapping retention time** ranges and can therefore cause **interferences**, when **low resolution mass spectrometry** is applied.

First methods for PCAs analysis were developed during the 1980ies (Gjøs and Gustavsen, 1982; Schmid *et al.*, 1985). Electron capture negative ion (ECNI) detection was selected due to strongly decreased detection limits caused by restricted molecule fragmentation. This technique is based on the formation of anions due to the high electron affinity of polychlorinated compound by capture of electrons (in thermal equilibrium). Matrix compounds and other interferences have a low electron affinity and do not form anions (Oehme, 1998). Moreover, high resolution mass spectrometry (HRMS) was chosen to suppress disturbances from PCAs themselves as well as from other not completely removed polychlorinated pollutants such as PCB (Tomy *et al.*, 1997). ECNI was still the method of choice when this project was started in 2002 (Froescheis and Ballschmiter, 1998).

- However, **ECNI generates additional problems** influencing the reliability of results (Reth and Oehme, 2004; Reth *et al.*, 2005):
- **Response factors** are **strongly dependent on the number of Cl atoms** and could **vary by one order of magnitude**. Moreover, co-elution of compounds with different degree of chlorination can influence the overall response.
- **Lower chlorinated isomers with <3–4 Cl atoms** have low response factors and could normally **not be detected**.
- Molecular ions $\mathbf{M^{-\cdot}}$ as well as $\mathbf{[M\text{-}Cl]^-}$ and the chlorine adduct $\mathbf{[M+Cl]^+}$ are **formed simultaneously** and increased the risk of interfering mass overlap.
- The **overall degree of chlorination influences the overall response factor** of the PCA mixture present in an environmental sample and required references with similar composition for quantification, which normally are not available.

Nevertheless, the quantification procedures developed of Tomy *et al.* (1997; Tomy and Stern, 1999) allowed to minimise the risk of systematic errors and gave rather reliable results. However, this required HRMS as well as many runs up of the same sample (up to twenty) to detect all possible formula groups (chain lengths from $C_{10–17}$ and number

of Cl from $Cl_{5–11}$). This made PCA analysis very complex, time-consuming and expensive. As a consequence, only a handful of PCA studies existed, and information about the PCA burden is not available from most regions of the world.

### 5.3.2 Challenges of PCA method development

One of the main aims of this project was to make PCAs analysis simpler in order to enable routine analysis. This required to develop alternatives and to overcome the following problems:

- To avoid the use of expensive equipment such as HRMS and, as a consequence:
- **To separate PCAs from all other interfering** (polychlorinated) **contaminants** already during the clean-up step to allow the application of low resolution mass spectrometry (LRMS).
- **To eliminate** or at least minimise **differences in the response factors** between formula groups, congeners and isomers, which would allow:
- To use commercially available **technical mixtures as references for quantification**.
- To allow the **detection of lower chlorinated congeners**.
- To enable the **comparability with ECNI** as the current quantification technique.

### 5.3.3 PCA method development

#### 5.3.3.1 Clean-up and gas chromatography

The first step to establish a simpler PCA method was to collect all PCAs within one fraction without other interfering pollutants (mainly PCB and polychlorinated pesticides such as toxaphene etc.). A carefully optimised fractionation on deactivated Florisil fulfilled this requirement as closely as possible (Reth and Oehme, 2005). Moreover, the analysis of sediments requested a cautious removal of elemental sulphur (e.g. by surface activated copper) and a corresponding method optimisation.

Since PCAs cannot be resolved gas chromatographically even into formula groups, the gas chromatographic separation was carried out with short columns and a quick temperature programme to shorten analysis time and to increase signal height with a corresponding decrease of detection limits. Minimum requirements were maintained such as retention time differentiation between short and medium chain PCAs (Reth and Oehme, 2004).

#### 5.3.3.2 Mass spectrometry

The following mass spectrometric techniques were developed or improved during this project:

- Negative ion chemical ionisation (**NICI**) with a mixture of **$CH_2Cl_2/CH_4$**. Chloride anions are generated from $CH_2Cl_2$.They form exclusively the molecular ion adduct $[M+Cl]^-$ from PCAs (Zencak *et al.*, 2003).
- **EI** combined with **MS/MS** in a ion trap or with a triple quadrupole mass spectrometer by selecting fragments common to all PCAs formula groups and to fragment them further (Zencak *et al.*, 2004).
- **Minimisation and correction** of the influence of the **degree of chlorination** of PCAs in a sample on the overall response factors (Reth *et al.*, 2005).
- **Replacement of HRMS by LRMS** and elimination/minimisation of mass overlap interferences by a careful retention time range selection and isotope ratio control of registered masses (Reth and Oehme, 2004).

In the following, the newly developed and applied mass spectrometric techniques are shortly characterised. Their advantages and disadvantages can be summarised as follows:

**$CH_2Cl_2/CH_4$-NICI:**
**Advantages:**
- Interferences from other polychlorinated compounds (pesticides, PCB) strongly suppressed.
- Response factor of formula groups and between congener groups rather uniform.
- Only $[M+Cl]^-$ ions are formed, interferences by mass overlap minimised.
- Lower chlorinated formula groups ($Cl_3$ and $Cl_4$) detectable.
- Use of LRMS possible.

**Disadvantages:**
- Rather quick contamination of ion source, therefore mainly suited for record of complete formula group patterns and quantification of selected samples with high content of interferences (sediments).

**EI-MS/MS:**
**Advantages:**
- Selection of fragment ions in common to all PCAs formula groups such as $[C_5H_7Cl]^+$ and further fragmentation to e.g. $[C_5H_5]^+$ allows to detect all PCAs simultaneously (sPCAs and mPCAs).
- Systematic errors due to strong influence of degree of chlorination on response factors eliminated or reduced.
- Inexpensive instrumentation such as an ion trap mass spectrometer can be used (costs € 60'000.-)
- Limits of detection sufficient for biota, but can be lowered further (factor of 4–5) using more expensive triple quadrupole mass spectrometry (LRMS). Desirable for sediments of low PCA content.
- Ideal for quick screening and pre-selection of samples for further specific formula and congener group analysis.

**Disadvantages:**
- Only the sum of sPCAs and mPCAs can be determined.
- No differentiation between formula and congener groups possible.

**ECNI-MS with response factor correction**
**Advantages.**
- Systematic errors of quantification due to different degree of chlorination between sample and reference can be compensated. No need for especially adapted quantification references.
- Additional isotope ratio analysis and careful selection of retention time ranges for detection of the different formula groups allow detecting and minimising interferences between sPCAs and mPCAs.
- Number of necessary injections per sample can be reduced.

**Disadvantages:**
- No lower chlorinated formula groups ($Cl_3$ and $Cl_4$) detectable.

#### 5.3.3.3 PCA result comparability, own techniques

Zencak *et al.* (2005) could show that deviations between PCA results obtained by different detection methods do not exceed the precision of a single methodology. Long term precision for spiked samples (five parallels over 4 months) was between 6–13 % depending on the tested method. The long term method precision for naturally exposed fish livers (five parallels over 4 months) includes additionally homogeneity variations of the sampling material. It was for the different techniques at maximum:

**ECNI-LRMS:** ≤12 % (sPCAs), ≤18 % (mPCAs)

**$CH_4$/$CH_2Cl_2$-NICI-LRMS:** ≤28 % (sPCAs), ≤10 % (mPCAs)

**EI-MS/MS:** ≤20 % for s+mPCAs.

- **Main sources of uncertainty** and **systematic errors** are:
- **Homogeneity variations** and **analyte losses** during clean-up.
- **Integration of a signal hump** over a retention time range of several minutes is highly dependent on base-line definition and not well handled by any integration programme.
- **Concentrations of single congener groups** are **often not too much above the quantification limit** (defined at a signal-to-noise ratio of 10:1). Noise fluctuations increase the measuring uncertainty. This is particularly valid for formula and congener group-specific methods.

Table 12 shows that deviations between different quantification methods are well within 20 % for spiked samples and around this value for real samples with lower levels. $CH_2Cl_2$/$CH_4$-NICI gives often somewhat lower concentrations due to a complete suppression of remaining interferences. This method was mainly applied for the determination of formula and congener group pattern in sediments.

**Table 12:** Comparison of quantification of s- and mPCAs in spiked fish (mackerel) and naturally exposed fish livers using four different mass spectrometric methods (single measurements). Spikes 1 and 2 contained 1500 ng in total (150 ng/g assuming a sample weight of 10 g) of sPCA with 55.5 % Cl content and spike 3 contained 1500 ng each of sPCAs and mPCAs with Cl contents of 55.5 % and 52 %, respectively. The relative deviations from the spiked amount are given in parenthesis.

| | Compound | Expected total (ng) | ECNI-HRMS (ng) | ECNI-LRMS (ng) | $CH_4/CH_2Cl_2$-NICI-LRMS (ng) | EI-MS/MS (ng) |
|---|---|---|---|---|---|---|
| Spike 1 | sPCAs | 1500 | 1720 (14 %) | 1560 (5.7 %) | 1810 (20 %) | 1610 (7 %) |
| Spike 2 | sPCAs | 1500 | 1190 (21 %) | 1610 (7.2 %) | 1300 (13 %) | 1580 (5 %) |
| Spike 3 | sPCAs | 1500 | 1730 (15 %) | 1750 (17 %) | 1290 (14 %) | np |
| | mPCAs | 1500 | 1360 (9 %) | 1510 (1 %) | 1450 (3 %) | np |
| | s+mPCAs | 3000 | 3090 (3 %) | 3260 (9 %) | 2740 (9 %) | 2950 (2 %) |
| | | Mean (ng/g) | | | | |
| Liver 1 | sPCAs | 40 | 57 | 43 | 21 | np |
| | mPCAs | 56 | 52 | 75 | 40 | np |
| | s+mPCAs | 93 | 109 | 118 | 61 | 84 |
| Liver 2 | sPCAs | 21 | 30 | 19 | 23 | np |
| | mPCAs | 24 | 21 | 25 | 25 | np |
| | s+mPCAs | 48 | 51 | 44 | 48 | 59 |
| Liver 3 | sPCAs | 16 | 13 | [na] | 20 | np |
| | mPCAs | 82 | 77 | 76 | 37 | np |
| | s+mPCAs | 94 | 90 | np | 57 | 66 |

na: not analysed
np: instrumentally not possible

#### 5.3.3.4 PCA result comparability, former studies

In 1999, an intercalibration was carried out between seven laboratories using different methods (Tomy *et al.*, 1999b). Deviations between laboratories were minimum a factor of two, and no significant difference between low and high resolution MS methods was observed. This means that the differences between the tested techniques were caused by problems with the applied sample clean-up and quantification procedure and not by mass spectrometric disturbances of LRMS.

Moreover, PCA concentrations should be evaluated with caution, if determined before the 1990ies. No quality assurance or proper method validation data are normally presented. Most of them are indicative at the best.

### 5.3.4 Quantification of PCAs

#### 5.3.4.1 Applied methods

Tomy *et al.* (1997) was the first, who described a detailed quantification method based on ECNI. The concentrations of formula/congener groups and as a sum of total PCA were calculated via the total signal area in the mass chromatograms. A correction of the PCA molecular weight deviation between sample and reference standard was made. Quantification was also based on the internal standard method.

Tomy *et al.* (1997) remarked that the overall response factor is strongly dependent on the degree of chlorination of the PCA. Therefore, it was recommended, that the chlorine content of sample and quantification reference should match as closely as possible to avoid systematic errors. Table 13 shows that the systematic error for ECNI quantification increases dramatically with an increasing deviation between the degree of chlorination of sample and reference. The newly developed techniques CH4/CH2Cl2-NICI-LRMS and EI-MS/MS are much less susceptible to this problem.

**Table 13:** Quantification of three sPCA solutions (overall amount 1500 ng) with variable chlorine content with a sPCAs reference standard of 55 % Cl and 63 % Cl content using ECNI, CH4/CH2Cl2-NICI-LRMS and EI-MS/MS. Relative deviations are given in parenthesis.

| Standard | sPCA 51 % Cl | | sPCA 55 % Cl | | sPCA 63 % Cl | |
|---|---|---|---|---|---|---|
| Sample | sPCA 55 % Cl | sPCA 63 % Cl | sPCA 51 % Cl | sPCA 63 % Cl | sPCA 51 % Cl | sPCA 55 % Cl |
| ECNI-LRMS | 4300 (190 %) | 15600 (940 %) | 522 (65 %) | 5440 (260 %) | 143 (90 %) | 413 (72 %) |
| $CH_4/CH_2Cl_2$-NICI-LRMS | 1770 (18 %) | 1210 (19 %) | 1270 (15 %) | 1030 (31 %) | 1860 (24 %) | 1980 (32 %) |
| EI-MS/MS | 1590 (6 %) | 1450 (3 %) | 1430 (5 %) | 1323 (12 %) | 1700 (14 %) | 1750 (17 %) |

Consequently, it is essential for ECNI quantification to determine the PCA chlorine content in the sample first and to select a corresponding reference standard. Only synthetic reference standards of 51 %, 55 % and 63 % are commercially available, and the use of technical mixtures should be avoided due to possible interferences by additives.

Therefore, this study investigated, if an interpolation was permissible of the dependence of the total response factor on the degree of chlorination (Reth *et al.*, 2005). The total response factor and chlorine content of a PCA mixture are calculated as follows:

1. Determination of total PCA signal area from the mass chromatograms of the congener groups i:

$$\text{Relative total CP area} = \sum_{i} \frac{\text{area}_i(\text{congener group})}{\text{area}_i(\text{ISTD})} \quad (1)$$

The equal amount of internal standard in sample and reference eliminates its quantity.

2. The total response factor is calculated as:

$$\text{Total response factor (PCA mixture)} = \frac{\text{rel. total PCA area (Std.)}}{\text{amount PCA(Std.)}} \quad (2)$$

3. The chlorine content is defined as:

$$\text{Chlorine content (PCA mixt.)} = \sum_{i} \frac{\text{rel. area}(\text{cong. group}_i) \cdot \text{chlorine content}(\text{cong. group}_i)}{\text{rel. total PCA area}} \quad (3)$$

The relation between the total response factor and the chlorine content was established with seven sPCA-mixtures from different manufacturers (degree of chlorination 51–69 %) and nine mPCA (54–58 %) from ICI (UK). A linear correlation was found (see Figure 7). It allowed to determine the total response factor of a PCA-mixture or of the PCA content of a sample after the determination of the chlorine content according to equation (4):

$$\text{Total response factor (sample PCAs)} = a \cdot x + b \quad (4)$$

a: slop; b: intercept; x: determined chlorine content

This total response factor is then used to calculate the total PCA amount of a sample:

$$\text{PCA amount (sample)} = \frac{\text{relative total area (sample)}}{\text{total response factor (calculated for the sample)}} \quad (5)$$

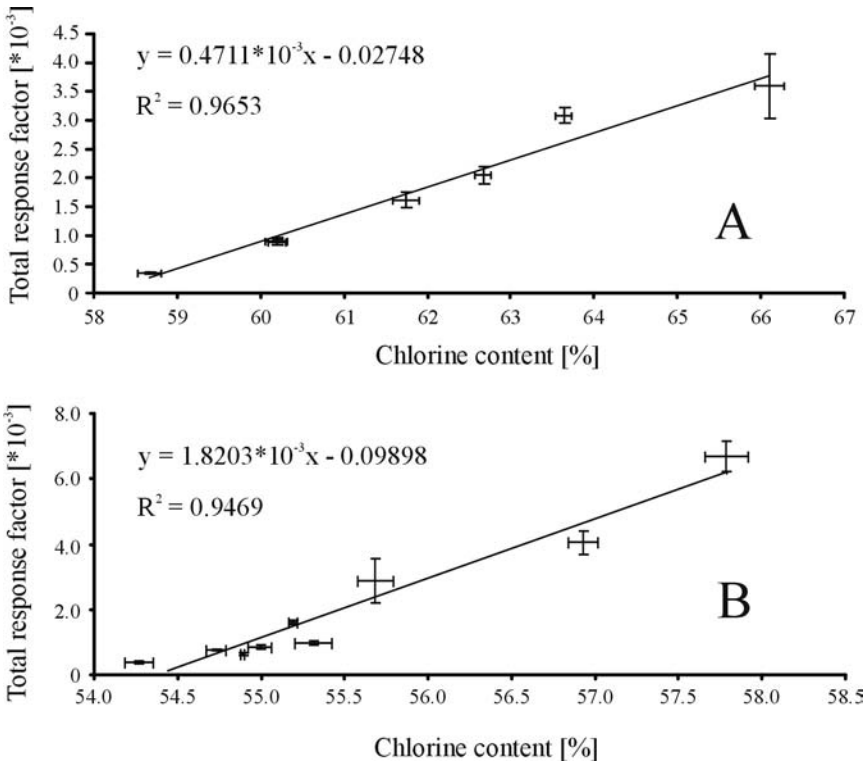


**Figure 7:** Dependence of the total response factor (ECNI) on the degree of chlorination for seven different sPCA mixtures (A, average of five interday measurements) and nine different MCCP mixtures (B: average of three interday measurements). Error bars indicate standard deviations of the determination of the chlorine content in x and of the response factor in y.

Table 14 shows, that the response factor correction works excellent for PCA mixtures being part of the correlation function (maximum 7 % deviation) and well for controls (deviations 8–35 %). As can be seen from Table 15, this procedure reduces dramatically the systematic errors of the method of Tomy *et al.* (1997), if the chlorine content of the reference is not properly selected. It also allows the quantification with the few especially synthesised pure PCA-mixtures. Technical products contain stabilisers and other additives, which might lead to interferences and systematic errors (Muir *et al.*, 2000).

**Table 14:** PCA quantification of different sPCA mixtures (51–69 % Cl) by ECNI with total response factors corrected for the chlorine content. The chlorine contents calculated from ECNI-LRMS measurements are indicated in brackets. The expected amount, the measured amount and the relative error are given. Deviations between expected and found chlorine content are caused by the presence of additives which are not detectable by ECNI.

| sPCA mixture [% chlorine content] | Expected amount [ng] | Measured amount [ng] | Relative deviation [% ] |
|---|---|---|---|
| **Standards used for establishment of linear function ($R^2$ = 0.9730, y = 0.00041x – 0.02391)** | | | |
| 51 (58.7 % Cl) | 1500 | 1553 | 4 |
| 55 (60.2 % Cl) | 1500 | 1572 | 5 |
| 59 (61.7 % Cl) | 1605 | 1602 | 0 |
| 64 (64.5 % Cl) | 1070 | 1030 | 4 |
| 69 (66.1 % Cl) | 1605 | 1724 | 7 |
| **Control standards** | | | |
| 53 (59.8 % Cl) | 1500 | 1379 | 8 |
| 59 (62.7 % Cl) | 1500 | 1690 | 13 |
| 63 (63.6 % Cl) | 1500 | 2030 | 35 |
| Hordalub 80 (56 % Cl | 1500 | 1902 | 27 |

**Table 15:** Comparison of sPCA determination in fish liver by ECNI according to Tomy *et al.* (1997) using three commercial references of different chlorine content (51 %, 55 % and 63 %) and via the total response factor approach. Calculated chlorine contents are given in brackets.

| Sample no. | Quantification according to Tomy et al. [ng/g ww] | | | Total response factor approach[a] |
|---|---|---|---|---|
| | Standard 51 (58.7 % Cl) | Standard 55 (60.3 % Cl) | Standard 63 (64.0 %Cl) | |
| 1 (61.9 % Cl) | 794 | 138 | 43 | 73 |
| 2 (62.9 % Cl) | 1055 | 184 | 58 | 82 |
| | Standard 51 (58.2 % Cl) | Standard 55 (59.9 % Cl) | Standard 63 (63.4 %Cl) | |
| 3 (62.2 % Cl) | 209 | 94 | 21 | 29 |
| 4 (61.7 % Cl) | 358 | 161 | 37 | 57 |
| | Standard 51 (58.6 % Cl) | Standard 55 (60.2 % Cl) | Standard 63 (63.5 %Cl) | |
| 5 (61.1 % Cl) | 168 | 81 | 17 | 34 |
| 6 (62.0 % Cl) | 158 | 76 | 16 | 24 |
| 7 (60.6 % Cl) | 184 | 88 | 18 | 47 |
| 8 (61.8 % Cl) | 2494 | 1197 | 250 | 408 |
| 9 (59.2 % Cl) | 733 | 352 | 73 | 521 |
| 10 (59.9 % Cl) | 56 | 27 | 6 | 21 |

[a] Chlorine content correlation determined with three SCCP mixtures (51 %, 55.5 % and 63 % Cl, $R^2$ = 0.999 for sample 1 and 2, $R^2$ = 0.969 for sample 3 and 4, $R^2$ = 0.964 for sample 5 to 10)

#### 5.3.4.2 Consequences for results, this study

The total response factor correction mode was developed at the end of this project. Therefore, most of the results are quantified by the approach of Tomy *et al.* (1997). The problem about the influence of chlorine content was well known from the beginning. Therefore, the reference standards were selected accordingly, and **deviations between data calculated in either mode do not exceed the general measuring uncertainty** between and within the applied measuring technique.

### 5.3.5 Selection criteria for PCA quantification techniques

Table 49 and Table 50 **in the appendix** give a survey about the applied analysis techniques for each sample. Most samples were analysed in the following sequence:

- **First analysis by EI-MS/MS** to obtain the total PCA content. This methodology has the lowest detection limits.
- **Second analysis by a formula and congener group specific method**. Since the detection limits are higher for this group specific technique, this part could only be carried out for well detectable quantities (ca. 10–20 ng/g for biota and 50 ng/g for sediments).
- Usually **ECNI-MS was applied for biota and $CH_2Cl_2$/$CH_4$-NICI for sediments** due to much more interfering compounds and partly very low concentrations. $CH_2Cl_2$/$CH_4$-NICI is much more time consuming and was only applied, if absolutely necessary.
- Due to **new instrumentation** with considerably lower detection limits and better robustness, the analysis of **sediment was possible with ECNI from 2003–2004**. The analysis of some samples was repeated to compare ECNI and $CH_4$/$CH_2Cl_2$-NICI further.

### 5.3.6 Analysis of chlordane compounds

The analysis of chlordane compounds is well established and mainly carried out by ECNI mass spectrometry with low resolution instruments. Detailed information about state-of-the-art can be found in Karlsson (1999) and Karlsson *et al.* (2000). Usually, the four representatives *cis/trans*-chlordane and *cis/trans*-nonachlor as well as the main metabolites of *cis/trans*-chlordane are quantified. Details about the applied ECNI-methodology are given in appendix 1 in chapter 5.6.9.

Briefly, chlordane analysis contained the following elements:

- The **extraction and clean-up method** was **identical** with those for **PCAs in biota and sediments**.
- All main **chlordane compounds** were present in the **same fraction as PCAs**.
- **Gas chromatographic separation** was carried out on the **same capillary** as PCAs.
- *cis*-Heptachlorepoxide and oxy-chlordane co-eluted, but could be separated by mass spectrometry recording the not disturbed but less abundant masses *m/z* 388 (*cis*-heptachlorepoxide) and *m/z* 424 (oxy-chlordane).
- Some **unusual chlordane representatives such as MC5 and MC7** were also determined (for details, see Karlsson (1999) and Karlsson *et al.* (2000))
- **Quantification** was carried out by **conventional ECNI** according to the validated method described in Karlsson (1999) and Karlsson *et al.* (2000).
- A **method based on EI-MS/MS** was also **developed to check for possible systematic errors** concerning quantification of sediments (for details, see appendix 1, chapter 5.6.10). It had comparable detection limits except for *cis*-heptachlorepoxide, where detectability deteriorated by one order of magnitude.

The same ECNI quantification technique was used on two different instruments for sediment analysis. No significant difference within the measuring uncertainty could be observed. The same was valid between ECNI and EI-MS/MS taken the very low concentrations in the pg/g dw range into account (see Table 16).

**Table 16:** Comparison of quantification of chlordanes [ng/g dw] in sediments by ECNI (two instruments) and EI-MS/MS. The fraction of the Florisil clean-up is assigned, where the compound is present.

| **Sample** | **Method** | **Hepta-chlor** | ***trans*-Chlordane** | ***cis*-Chlordane** | ***trans*-Nonachlor** | ***cis*-Nonachlor** |
|---|---|---|---|---|---|---|
| **Florisil fraction** | | **F1** | **F2** | **F2** | **F1+F2** | **F2** |
| 721 (2004) | EI-MS/MS | 0.054 | 0.153 | 0.116 | 0.13 | 0.039 |
| | ECNI, 1200L | 0.061 | 0.142 | 0.141 | 0.10 | 0.034 |
| | ECNI, HP 5989B | 0.064 | 0.135 | 0.126 | 0.10 | 0.029 |
| | **Average ± STD** | **0.056± 0.005** | **0.143± 0.007** | **0.128± 0.010** | **0.110± 0.014** | **0.034± 0.004** |
| ODER (2004) | EI-MS/MS | 0.056 | 0.066 | 0.083 | 0.12 | 0.021 |
| | ECNI, 1200L | 0.036 | 0.078 | 0.044 | 0.10 | 0.026 |
| | ECNI, HP 5989B | 0.042 | 0.059 | 0.060 | 0.09 | 0.017 |
| | **Average ± STD** | **0.045± 0.008** | **0.068± 0.008** | **0.062± 0.016** | **0.103± 0.012** | **0.021± 0.004** |
| 715 (2004) | EI-MS/MS | <0.4 | 0.047 | 0.027 | 0.60 | 0.023 |
| | ECNI, 1200L | <1.3 | 0.049 | 0.038 | 0.50 | 0.024 |
| | ECNI, HP 5989B | <1.6 | 0.042 | 0.031 | 0.50 | 0.021 |
| | **Average ± STD** | | **0.046± 0.003** | **0.032± 0.005** | **0.53± 0.05** | **0.023± 0.001** |
| RUDEN (2004) | EI-MS/MS | <0.4 | 0.018 | 0.023 | 0.20 | 0.011 |
| | ECNI, 1200L | <1.3 | 0.014 | 0.025 | 0.16 | 0.013 |
| | ECNI, HP 5989B | <1.6 | 0.006 | 0.016 | 0.15 | 0.006 |
| | **Average ± STD** | | **0.013± 0.005** | **0.021± 0.004** | **0.17± 0.02** | **0.010± 0.003** |
| ECKFBU (2004) | EI-MS/MS | <0.4 | 0.047 | 0.033 | 0.17 | 0.032 |
| | ECNI, 1200L | <1.3 | 0.044 | 0.054 | 0.15 | 0.027 |
| | ECNI, HP 5989B | <1.6 | 0.036 | 0.035 | 0.14 | 0.019 |
| | **Average ± STD** | | **0.042± 0.005** | **0.041± 0.009** | **0.15± 0.01** | **0.026± 0.005** |

STD: Standard deviation

### 5.3.7 Method validation and quality control

#### 5.3.7.1 Limits of detection and quantification

Typically, **limits of detection (LOD,** signal-to-noise ratio 3:1**) for s- or mPCAs in fish liver** were about **1 ng/g** and **limits of quantification (LOQ,** signal-to-noise ratio 10:1**)** were ca. **3 ng/g**. **Chlordanes** could be **identified** down to **1-3 pg/g** and **quantified** from ca. **2-10 pg/g** and onwards.

Single **PCA congener groups in sediments** were **detectable down to ca. 5-50 ng/g** depending on their structure. **Limits of quantification were** about **3-5 times higher**. **Total PCAs in sediments** could be quantified with EI-MS/MS **down to 2-8 ng/g**.

**LOQs for chlordanes and oxy-chlordane** in biota and sediments were between **0.5-8 pg/g** for ECNI and EI-MS/MS. In addition, the instrumental limits of detection (LOD) and quantification (LOQ) based on the injected volume are given in Table 17 for PCAs and in Table 18 for chlordanes.

**Table 17:** Instrumental (mass spectrometric) limits of detection (LOD) at a signal-to-noise ratio (S/N) of 3:1 and limits of quantification (LOQ, S/N 10:1) for total sPCA, single sPCA groups, total mPCA and single mPCA groups. Injection volumes were 1-1.5 µl.

| Method | Total sPCA LOD [ng/µl] | Total sPCA LOQ [ng/µl] |
|---|---|---|
| ECNI | ca. 1 | ca. 5 |
| $CH_4/CH_2Cl_2$-NICI | 3 | nd |
| EI-MS/MS (ion trap) | 0.7 | 1-2 |
| EI-MS/MS (triple-quad) | 0.2 | 0.5 |
| **Method** | **Total mPCA LOD [ng/µl]** | **Total mPCA LOQ [ng/µl]** |
| ECNI | ca. 10 | ca. 40 |

nd: not determined

**Table 18:** Instrumental (mass spectrometric) limits of detection at a signal-to-noise ratio (S/N) of 3:1 and limits of quantification (S/N 10:1) for single chlordane compounds in pg/µl. Compared are two measuring techniques (ECNI, EI-MS/MS) as well as two mass spectrometers (HP 5989B and Varian 1200 L). Injection volumes were 1-1.5 µl.

| | Hepta-chlor | *trans*-Chlordane | *cis*-Chlordane | *trans*-Nonachlor | *cis*-Nonachlor | Oxy-chlordane | *trans*-HEP | *cis*-HEP |
|---|---|---|---|---|---|---|---|---|
| **ECNI, HP 5989B** | | | | | | | | |
| LOD | 1.6 | 0.2 | 0.7 | 0.3 | 0.2 | 1.1 | 2.8 | 11 |
| LOQ | 5.3 | 0.5 | 1.6 | 0.9 | 0.4 | 3.8 | 9.4 | 37 |
| **ECNI, 1200L** | | | | | | | | |
| LOD | 1.3 | 0.4 | 1.1 | 0.2 | 0.1 | na | na | na |
| LOQ | 4.4 | 1.2 | 3.6 | 0.7 | 0.4 | na | na | na |
| **EI-MS/MS, 1200L** | | | | | | | | |
| LOD | 0.4 | 0.2 | 0.2 | 0.4 | 0.4 | 0.3 | 2.4 | 96 |
| LOQ | 1.3 | 0.7 | 0.7 | 1.2 | 1.2 | 1.1 | 7.9 | 317 |

na: not analysed; HEP: heptachlorepoxide

### 5.3.7.2 Linearity

#### s- and mPCA:

- **ECNI:** Linearity was very good within two orders of magnitude (1-100 ng/µl). Regression coefficients were always >0.99 (seven measuring points) Figure 3 shows an example of the linear range for a sPCA congener group.
- **$CH_4$/$CH_2Cl_2$-NICI:** Linearity was very good within two orders of magnitude (1-100 ng/µl, $r^2$ >0.998, six measuring points).
- **EI-MS/MS:** Linearity was very good within two orders of magnitude (ion trap: 2-100 ng/µl, $r^2$ >0.998, six measuring points; triple quadrupole: 0.2-100 ng/µl).

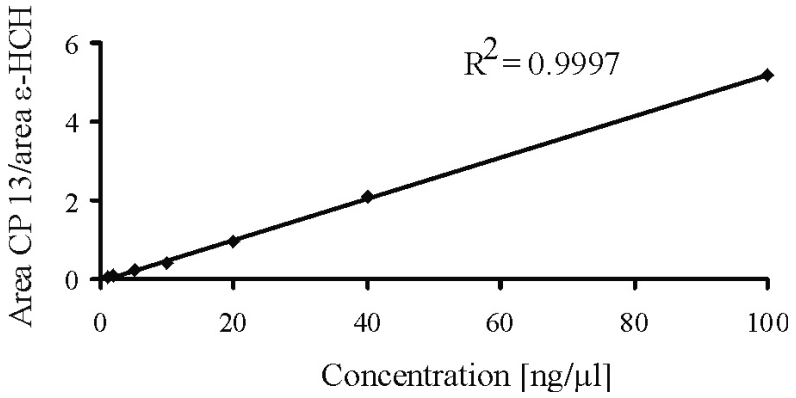


**Figure 8:** Linear range of ECNI for the sPCA congener group $C_{13}Cl_6H_{22}$ from a sPCA mixture with 55.5 % chlorine content. Linearity could be obtained within 1-100 ng/µl. The most abundant isotope signal of the $[M\text{-}HCl]^-$ ion was recorded.

#### Chlordanes:

- ECNI: Linearity was very good within 1-6 pg/µl to ca. 5600 pg/µl ($r^2$ >0.99, 8-10 measuring points).

### 5.3.7.3 Selectivity of clean-up

#### Chloroparaffins

The clean-up step on Florisil allowed separating nearly completely PCA from other interfering compound classes such as PCB or toxaphenes. Moreover, the internal standard $^{13}C_{10}$-*trans*-chlordane was also present in this fraction. Table 19 shows the fractionation behaviour for selected compounds.

**Table 19:** Fractionation of the single PCA congeners $C_{10}H_{16}Cl_6$ and $C_{13}H_{20}Cl_8$ from PCB 153, toxaphene #62 and *trans*-chlordane by Florisil column clean-up. Relative distributions are given (prefraction: 60 ml of *n*-hexane followed by 5 ml of dichloromethane, PCA-fraction: 60 ml of dichloromethane).

| Compound | Distribution [%] | |
|---|---|---|
| | Prefraction | PCA-fraction |
| $C_{10}H_{16}Cl_6$ | 5 | 95 |
| $C_{13}H_{20}Cl_8$ | <1 | 100 |
| PCB 153, HCB, HCH | 100 | <1 |
| Toxaphene #62 | 100 | <1 |
| *trans*-chlordane | 5 | 95 |

#### Chlordanes

The separation behaviour of Florisil for chlordanes was more complex. Table 20 shows the variability between two different batches, which was considerable. Therefore, a large quantity from the same batch was purchased and its elution properties optimised to avoid such a variable elution range. This improved also the recovery of heptachlor to >80 %. Nevertheless, *trans*-nonachlor eluted in about equal parts in the pre- and PCA-fraction and had to be quantified in both. $^{13}C_{10}$-*trans*-chlordane was a suitable internal standard for the PCA-fraction and 4,5-dichlorochlordene (Karlsson *et al.*, 1997) for the pre-fraction. Due to the missing persistence to sulphuric acid, it had to be replaced by ε-HCH for the analysis of biota.

**Table 20:** Fractionation behaviour of the Florisil® clean-up for chlordane compounds carried out on two different batches. Relative recoveries [%] of the total amounts are given. Tetrachloro- (TCN), octachloro-naphthalene (OCN), the chlordane compound MC8 and 4,5-dichlorochlordene were evaluated as internal standards (ISTD).

| Compounds | Column 1 | | Column 2 | |
|---|---|---|---|---|
| | Pre-fraction | PCA-Fraction | Pre-fraction | PCA-Fraction |
| **ISTD** | | | | |
| $^{13}C_{10}$-*trans*-Chlordane | <1 | 87 | <1 | 95 |
| OCN | <1 | 107 | <1 | 106 |
| TCN | 44 | 56 | 25 | 49 |
| MC8 | 5 | 87 | <1 | 109 |
| 4,5-Dichlorochlordene | 97 | 9 | 74 | <1 |
| **Chlordanes** | | | | |
| *trans*-Chlordane | 5 | 86 | <1 | 99 |
| *cis*-Chlordane | <1 | 87 | <1 | 91 |
| Heptachlor | 105 | 5 | 55 | 0 |
| *cis*-Nonachlor | <1 | 89 | 1 | 96 |
| *trans*-Nonachlor | 41 | 47 | 61 | 28 |

### 5.3.7.4 Blank controls

Blanks were always equal to the detection limits due to a very stringent cleaning procedure of glass ware (heating to 450 °C overnight, see experimental in the annex) and many safety measures such as separate glass ware, pretreatment of adsorbents at 600 °C etc. The blank problem is very serious for PCA analysis, but hardly addressed in the literature, which makes many data therein doubtful.

### 5.3.7.5 Recoveries

#### Chloroparaffins

Recoveries of PCA in biota were checked for the whole extraction and clean-up method with spiked muscle tissue from mackerel. As can be seen from Table 21, recoveries were good for PCA and the internal standard.

**Table 21:** Relative recoveries [%] of selected sPCA/mPCA compounds and the internal standard $^{13}C_{10}$-trans-chlordane after stepwise and complete clean-up of spiked sun flower oil or mackerel muscle tissue (spiked amount: 1.5-25 µg of sPCA or 1.5-20 µg of mPCA mixture).

| No. of parallels | Relative recoveries mPCAs [%] | | | |
|---|---|---|---|---|
| | $C_{14}H_{23}Cl_7$ | $C_{15}H_{25}Cl_7$ | $C_{16}H_{27}Cl_7$ | $C_{17}H_{29}Cl_7$ |
| **Extraction** | | | | |
| 1 | 100 | 104 | 95 | 97 |
| **Column chromatography with silica gel/sulphuric acid** | | | | |
| 1 | 100 | 98 | nd | na |
| 2 | 72 | 76 | 79 | 71 |
| 3 | 80 | 99 | 110 | 82 |
| **Adsorption chromatography with Florisil** | | | | |
| 1 | 95 | 98 | 90 | na |
| 2 | 77 | 81 | 76 | na |
| 3 | 71 | 71 | 69 | na |
| **Total clean-up (matrix: sun flower oil)** | | | | |
| 1 | 66 | 68 | 66 | 70 |
| 2 | 89 | 98 | 95 | 92 |
| Total clean-up (matrix: mackerel muscle tissue) | | | | |
| 1 | 57 | 60 | 85 | 83 |

| Experiment | Relative recoveries sPCAs [%] | | |
|---|---|---|---|
| | $C_{11}H_{18}Cl_6$ | $C_{11}H_{17}Cl_7$ | $^{13}C_{10}$-*trans*-chlordane |
| **Total clean-up (matrix: mackerel muscle tissue)** | | | |
| 1 | 101 | 100 | 99 |
| 2 | 99 | 97 | 119 |
| 3 | 84 | 94 | 72 |
| 4 | 89 | 103 | 80 |
| 5 | 76 | 98 | 71 |
| 6 | 67 | 77 | 75 |

na: not analysed; nd: not determined.

Similar recovery results were obtained for the extraction and clean-up procedure of sediments as Table 22 summarises. The recoveries of s- and mPCA as well as the internal standard $^{13}C_{10}$-*trans*-chlordane were well within the acceptation criteria for ultra trace methods. Recoveries were also tested with North Sea sediments containing PCAs levels below the detection limits. 100 ng/g sediment of sPCA (63 % Cl) and 100 ng mPCA (57 % Cl) were added. The recoveries of four parallels were:

| | |
|---|---|
| $^{13}C_{10}$-trans-chlordane: | 78 ± 8 % |
| $C_{11}H_{17}Cl_7$: | 96 ± 7 % |
| $C_{12}H_{19}Cl_7$: | 88 ± 8 % |
| $C_{14}H_{23}Cl_7$: | 74 ± 3 % |

**Table 22:** Relative recoveries [%] of selected sPCA/mPCA compounds and the internal standards $^{13}C_{10}$-*trans*-chlordane, octachloronaphthalene (OCN) and MC8 (a chlordane congener). Extraction as well as a stepwise and complete clean-up of spiked sea sand and sediments were tested (spiked amount 1.5-2.5 µg of sPCA (55.5 % Cl) or 1.5-20 µg of mPCA (52 % Cl) mixture). ECNI was employed for quantification.

| | | | | Recovery in % | | | |
|---|---|---|---|---|---|---|---|
| **No.** | **$^{13}C_{10}$-*trans*-Chlordane** | **$C_{10}H_{16}Cl_6$** | **$C_{11}H_{18}Cl_6$** | **$C_{11}H_{17}Cl_7$** | **$C_{12}H_{20}Cl_6$** | **$C_{12}H_{19}Cl_7$** | **$C_{13}H_{22}Cl_6$** |
| Extraction (matrix: sea sand) | | | | | | | |
| 1[a] | 84 | 101 | 105 | na | 91 | | 116 |
| 2[a] | 78 | 96 | 97 | na | 90 | na | 98 |
| Extraction (matrix: sodium sulphate) | | | | | | | |
| 1 | 69 | na | 97 | 78 | 82 | 78 | na |
| 2 | 64 | na | 100 | 81 | 101 | 85 | na |
| 3 | 74 | 93 | 88 | 84 | 93 | 96 | 97 |
| Adsorption chromatography with Florisil® | | | | | | | |
| 1[b] | 88 | na | 101±9* | 109±11* | 109±12* | 109±12* | na |
| 2 | 75 | na | 82 | 82 | 81 | 86 | na |
| 3 | 88 | na | 90 | 87 | 84 | 96 | na |
| Clean-up (matrix: sea sand) | | | | | | | |
| 1 | 75 | 73 | 81 | na | 75 | 101 | na |
| 2 | 69 | 54 | 63 | na | 60 | 71 | na |
| 3 | 73 | 79 | 78 | na | 86 | 94 | na |
| 4 | 76 | 97 | 89 | na | 101 | 115 | na |

| | Recovery in % | | | | |
|---|---|---|---|---|---|
| | **$^{13}C_{10}$-*trans*-Chlordane** | **$C_{14}H_{24}Cl_6$** | **$C_{15}H_{26}Cl_6$** | **$C_{14}H_{23}Cl_7$** | **$C_{15}H_{25}Cl_7$** |
| Extraction (matrix: sea sand)[a] | | | | | |
| 1 | 75 | 92 | 93 | 96 | 97 |
| 2 | 61 | 87 | 94 | 90 | 98 |
| 3 | 62 | 85 | 89 | 91 | 100 |
| Adsorption chromatography with Florisil® | | | | | |
| 1 | 77 | 103 | 114 | 107 | 117 |
| 2 | 89 | 84 | 92 | 88 | 97 |
| 3 | 86 | 104 | 99 | 104 | 115 |
| Clean-up (matrix: sea sand) | | | | | |
| 1 | 72 | 84 | 86 | 81 | 79 |
| 2 | 83 | 87 | 50 | 82 | 82 |

| | | | | Recovery in % | | | |
|---|---|---|---|---|---|---|---|
| **Nr.** | **$^{13}C_{10}$-*trans*-Chlordane** | **OCN** | **MC8** | **$C_{10}H_{16}Cl_6$** | **$C_{11}H_{17}Cl_7$** | **$C_{14}H_{24}Cl_6$** | **$C_{15}H_{26}Cl_6$** |
| Matrix: North Sea sediment UE 67/GAUSS 349 | | | | | | | |
| 1 | 72 | 85 | - | 0 | 0 | 0 | 0 |
| 2[c] | 77 | 87 | 82 | 120 | 95 | 57 | 71 |
| 3[d] | 72 | 88 | 73 | 84 | 81 | 117 | 126 |
| 4[e] | 67 | 82 | 79 | 120 | 112 | 90 | 114 |
| Matrix: North Sea sediment BL2/GAUSS 387 | | | | | | | |
| 1 | 80 | 74 | 74 | 0 | 0 | 0 | 0 |
| 2[c] | 77 | 93 | 74 | 105 | 102 | 168 | 197 |

a) without copper, b) 5000 ng sPCA with 55,5 % Cl, c) with 500 ng sPCA and 500 ng mPCA, d) with 1000 ng sPCA and 1000 ng mPCA, e) with 1500 ng sPCA and 1500 ng mPCA, * 5 parallel measurements

na: not analysed

As can be seen from Table 22, octachloronaphthalene (OCN) has a yield, which sometimes is more similar to the PCA compounds. Therefore, OCN was used as additional and alternative internal standard in case of a low recovery of $^{13}C_{10}$-*trans*-chlordane.

## Chlordanes

As already discussed before, *trans*-nonachlor cannot be collected completely in the PCA fraction. Tests of the two clean-up steps for biota gave good recoveries of 77-90 % (see Table 23). *trans*-Nonachlor could be recovered to 75 % in the pre- and PCA-fraction. Therefore, both were quantified.

However, *cis*- heptachlorepoxide was non-detectable, and the yield for *trans*-heptachlorepoxide was poor. The reason is degradation by the silica gel coated with sulphuric acid. Since this step is essential for a clean extract, these compounds could not be determined.

Overall chlordane recoveries of the complete extraction and clean-up method were also tested with both sea-sand and air-dried sediment from the site UE 67 (Gauss 332, TOC 0.12 %). Here, clean-up by silica gel coated with sulphuric acid could be omitted for chlordane analysis. Recoveries of the internal standards and the analytes were comparable and in average ≥70 %.

**Table 23:** Relative recoveries [%] of selected chlordane compounds in biota after the two clean-up steps (column chromatography with silica gel/sulphuric acid and adsorption chromatography with Florisil ®). Samples of 10 g were spiked with 10 ng of each compound in 10 µl of cyclohexane.

| Compound | Pre-fraction | PCA-fraction |
|---|---|---|
| **ISTD** | | |
| $^{13}C_{10}$-*trans*-chlordane | 4 | 80 |
| ε-hexachlorocyclohexane | 79 | <1 |
| **Chlordanes** | | |
| *trans*-chlordane | 2 | 77 |
| *cis*-chlordane | 2 | 77 |
| *cis*-nonachlor | 1 | 90 |
| *trans*-nonachlor | 29 | 46 |
| Oxychlordane | <1 | 85 |
| *cis*-heptachlorepoxide | <1 | 24 |
| *trans*-heptachlorepoxide | <1 | <1 |

## 5.4 Results and discussion

### 5.4.1 Formula and congener profiles of technical/synthesised PCAs

Formula and congener profiles were determined by ECNI for all available technical and synthesised PCA mixtures due to the following reasons:

- To determine the mean molecular weight and chlorine content and to **compare** them with the **information from the manufacturer**,
- to investigate **differences in the formula and congener group pattern** between manufacturers, and,
- to enable an **investigation of changes in the formula and congener profiles in biota** and sediments due to enrichment/depletion mechanisms during dispersion, transport and bioaccumulation/metabolisation.

#### 5.4.1.1 sPCA mixtures

Table 24 gives a survey about the determined chlorine content, mean molecular weight and the relative contribution of $C_{10\text{-}13}$ compounds. Figure 4 visualises the differences of the formula group composition between technical and synthesised mixtures for the carbon chain lengths $C_{10\text{-}13}$ and 5-10 Cl-atoms. Similarities and differences can be summarised as follows:

- **$C_{11}$ and $C_{12}$-compounds** contribute ca. **67-82 %** to the total amount.
- **Precision** of the **formula/congener group determination** is about **± 1 %**
- A cluster analysis showed that **average chlorine content and molecular weight** were the **main factors of differentiation** (Hüttig and Oehme, 2005). The mixtures clustered into two groups, one with a Cl content <61 % and a mean molecular mass <400 g/mol and one with >61 % and >400 g/mol. The members of the group with <61 % Cl are marked in Table 24.
- **ECNI overestimated the chlorine content** compared to available supplier information: Range determined 58.5-66.5 %, range specified 49-69 %. The main reason is the too low response of $Cl_{3\text{-}4}$ compounds. Their detection is possible with NICI

using $CH_4/CH_2Cl_2$, where only deviations of 1-2.5 % were observed (Zencak *et al.*, 2003).

- However, **$CH_4/CH_2Cl_2$-NICI contaminates the ion source** rather quickly and was therefore only applied to a few selected biota samples. Figure 5 clearly shows the presence of considerable amounts of $Cl_{3-4}$ compounds (Zencak *et al.*, 2003).
- **Sediments** contained a **rather high background** and partly low concentration, so that determination by ECNI was hampered with the Hewlett Packard mass spectrometer. Therefore, all analysis was carried out by $CH_4/CH_2Cl_2$-NICI until new ECNI instrumentation allowed achieving lower detection limits and higher selectivity in 2003.

**Table 24:** Composition of technical and synthetic PCA mixtures determined by ECNI. Specified and calculated chlorine content [%] are given as well as calculated molecular weights [g/mol] and relative abundances of the $C_{10}$, $C_{11}$, $C_{12}$ and $C_{13}$ congeners. Technical PCA mixture with a chlorine content <61 % are marked with a grey shading.

| No. | Name (company) | Qual. | Chlorine content | | Molecular Mass | Relative Abundance[a] [%] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | spec. | calc. | calc. | $C_{10}$ | $C_{11}$ | $C_{12}$ | $C_{13}$ |
| **A** | $C_{10-13}$, 51 % (Ehrenstorfer) | pure | 51.0 | 59.3 | 385 | 10 | 40 | 39 | 11 |
| **B** | $C_{10-13}$, 55.5 % (Ehrenstorfer) | pure | 55.5 | 60.3-60.8[b] | 394-397 | 10-15 | 41-43 | 32-35 | 9-11 |
| C | $C_{10-13}$, 63 % (Ehrenstorfer) | pure | 63.0 | 63.7 | 427 | 12 | 44 | 31 | 13 |
| **Mixtures of $C_{10-13}$ standards with a Cl-content of 55.5 % and 51 %** | | | | | | | | | |
| **BA_1** | | 4:1 | 54.6 | 60.2 | 393 | 11 | 44 | 36 | 10 |
| **BA_2** | | 3:2 | 53.7 | 60.1 | 392 | 10 | 43 | 37 | 10 |
| **BA_3** | | 2:3 | 52.8 | 59.8 | 390 | 11 | 42 | 37 | 10 |
| **BA_4** | | 1:4 | 51.9 | 59.6 | 389 | 11 | 40 | 39 | 11 |
| **BA_5** | | 1:1 | 53.3 | 60.1 | 392 | 11 | 42 | 36 | 11 |
| **Mixtures of $C_{10-13}$ standards with a Cl-content of 63 % and 51 %** | | | | | | | | | |
| CA_1 | | 4:1 | 60.6 | 63.5 | 426 | 12 | 43 | 33 | 13 |
| CA_2 | | 3:2 | 58.2 | 63.3 | 423 | 13 | 44 | 32 | 12 |
| CA_3 | | 2:3 | 55.8 | 63.0 | 419 | 14 | 43 | 32 | 11 |
| CA_4 | | 1:4 | 53.4 | 62.0 | 410 | 13 | 44 | 32 | 11 |
| CA_5 | | 1:1 | 57.0 | 63.2 | 421 | 15 | 34 | 37 | 13 |
| **Mixtures of $C_{10-13}$ standards with a Cl-content of 63 % and 55.5 %** | | | | | | | | | |
| CB_1 | | 4:1 | 61.5 | 63.4 | 426 | 13 | 39 | 35 | 13 |
| CB_2 | | 3:2 | 60.0 | 63.0 | 421 | 13 | 41 | 33 | 13 |
| CB_3 | | 2:3 | 58.5 | 63.0 | 417 | 15 | 44 | 30 | 11 |
| CB_4 | | 1:4 | 57.0 | 61.9 | 407 | 16 | 42 | 31 | 11 |
| CB_5 | | 1:1 | 59.3 | 63.0 | 418 | 13 | 47 | 28 | 12 |
| **D** | Hordalub 17 (Hoechst) | pure | 49 | 58.5 | 375 | 13 | 44 | 35 | 9 |
| **E** | Hordalub 80 (Hoechst) | pure | 56 | 60.7 | 396 | 13 | 44 | 31 | 11 |
| F | Hordalub 500 (Hoechst) | pure | 62 | 63.9 | 424 | 14 | 53 | 27 | 6 |
| G | Cereclor 60L (ICI) | pure | 59 | 62.0 | 413 | 15 | 30 | 38 | 17 |
| H | Cereclor 70L (ICI) | pure | 69 | 66.5 | 454 | 23 | 33 | 34 | 10 |

[a] Composition: $C_{10}/C_{11}$, 5-10 Cl; $C_{12}$, 6-10 Cl; $C_{13}$, 7-9 Cl as defined by Tomy *et al.*[22]
[b] Five parallels
Qual.: Quality and mixing ratio, pure means no additives, spec.: specified; calc.: calculated

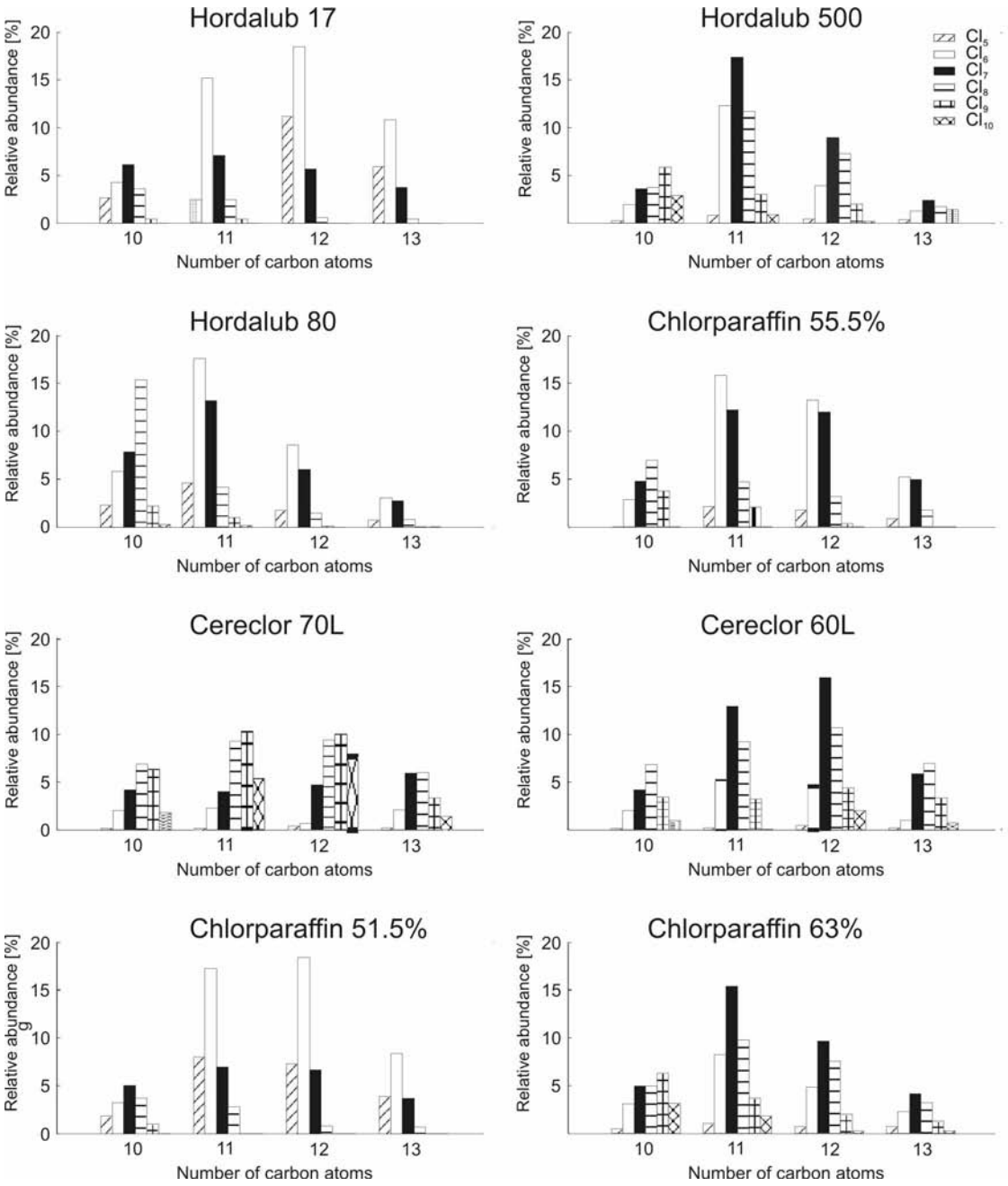


**Figure 9:** ECNI-determination of formula and congener group profiles of selected PCA mixtures of different chlorine contents. $Cl_{3\text{-}4}$ compounds were not detectable. The trade name or the chlorine content of the mixtures are given for each bar graph.

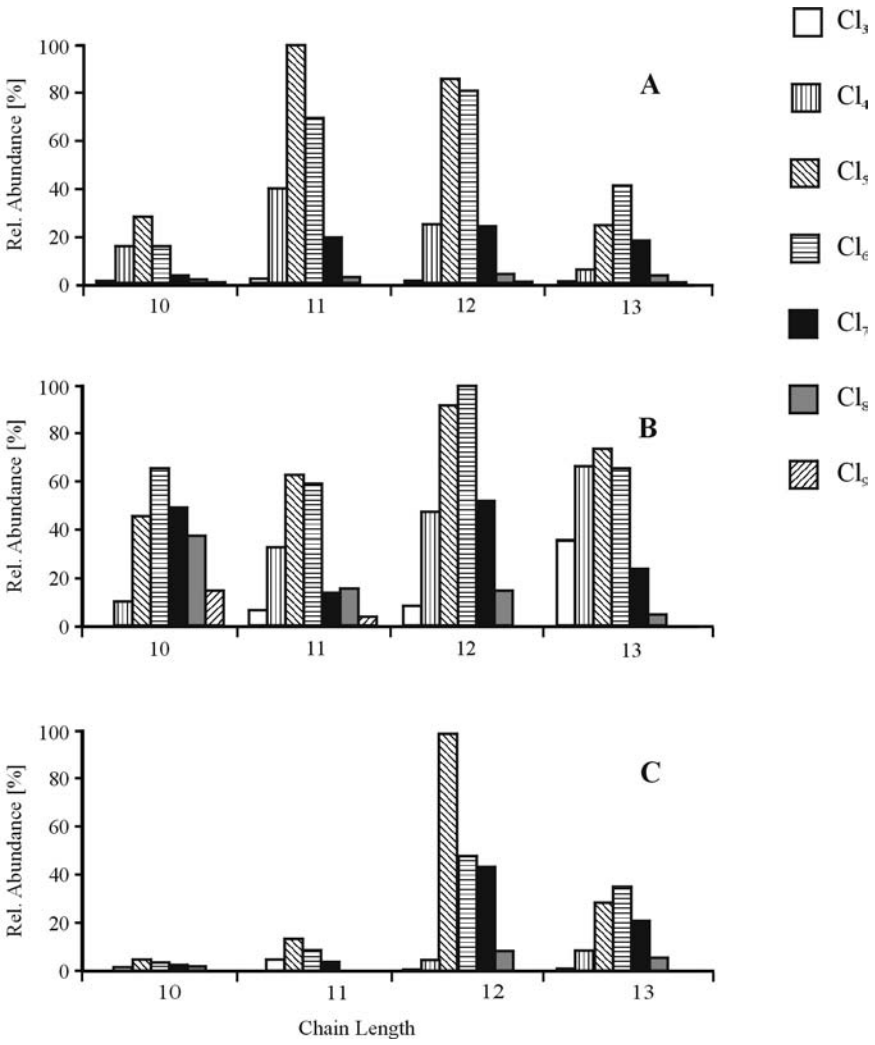


**Figure 10:** $CH_4/CH_2Cl_2$-NICI determination of the formula group patterns of a sPCA mixture (55.5 % chlorine content, A) and of two North Sea dab liver extracts (B, North Sea 54° 15,64 N, 7° 29,79' E, not studied further, C; site NS1, see **Table 24**). $Cl_{3-4}$ compounds are clearly visible.

### 5.4.1.2 mPCA mixtures

mPCA-mixtures were dominated by $C_{14}$-compounds. All other carbon chain lengths were of minor importance as Figure 6 shows.

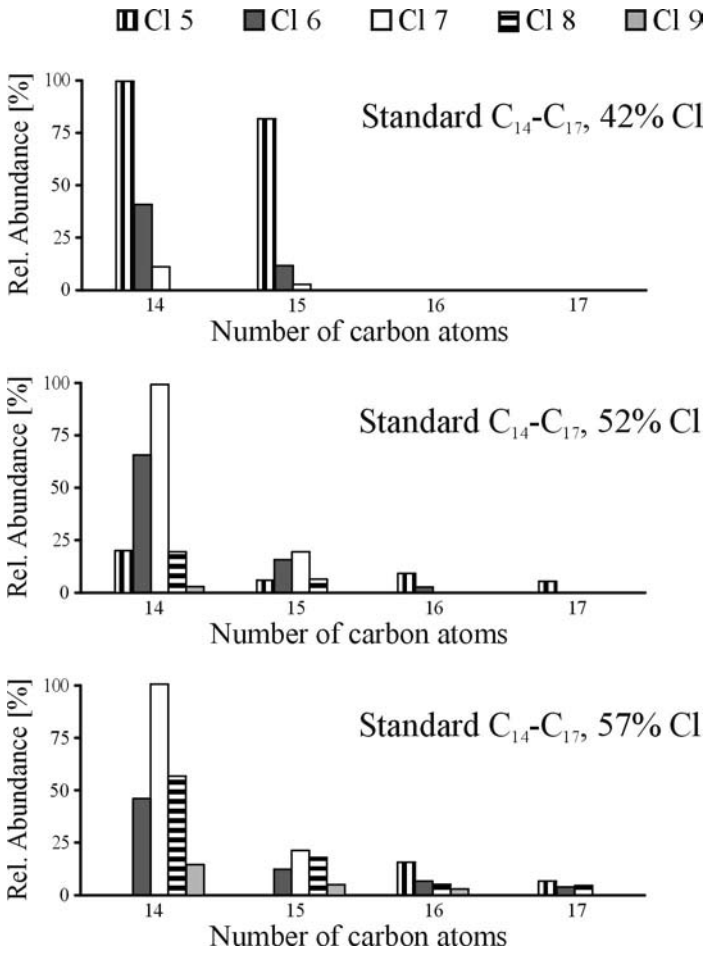


**Figure 11:** $CH_4/CH_2Cl_2$-NICI determination of the formula group patterns $C_{14}$-$C_{17}$ of three mPCA mixtures with differing chlorine content (42, 52 and 57 %). No $Cl_{3\text{-}4}$compounds were present.

## 5.4.2 PCA and chlordanes in biota

### 5.4.2.1 Origin and characterisation of samples

The origin and characterisation of the samples are summarised in Table 25. Sampling and sample selection was carried out as follows:

### Fish livers from the North and Baltic Sea:

- Fish was caught during the **following monitoring expeditions**: Cruise no. 242 of FFS "Walter Hering III" from 22 August to 8 September 2002 and no. 255 from 25 August to 9 September 2004.
- **Five to six sampling sites** were selected in the **North and Baltic Sea** (see Table 25 and Figure 12, Figure 13, Figure 14 for details)
- **Fish livers** were collected from North Sea dab (*Limanda limanda*), flounder (*Platichthys flesus*) and cod (*Gadus morhua*). Totally 60 single samples were obtained.
- **Detection limits of ca. 10 ng/g** (total sPCA) required **minimum 5 g of sample**. This made pooling of up to five liver samples necessary.
- **More details** about sampling is given in the **appendix under sampling details**.

### Cod liver from the northern North Atlantic

- **Caught in 2003** at the north and south coast of **Iceland and the Lofot Islands**.
- **Totally six samples** delivered by the Norwegian Institute for Air Research (Dr. Urs Berger, Tromsø, Norway)

### Fish and sea birds from Bear Island

- Bear Island is known as a **site of elevated concentrations** of polychlorinated compounds (PCBs, DDT, toxaphenes) **due to long range transport** and on-site bioaccumulation (Evenset *et al.*, 2002; Evenseth *et al.*, 2004).
- **Sampling** was carried out in **2001** by Aquaplan-NIVA (Tromsø, Norway, Dr. Guttorm Christensen).
- Two **liver and muscle tissue** samples from Arctic char (*Salvelinus alpinus*) from Lake Ellasjøen, two samples each of liver and muscle tissue from little auk (*Alle alle*), kittiwake (*Rissa tridactyla*), glacous gull (*Larus hyperboreus*), see Table 25 further details and Figure 9 for site description.

**Table 25:** Survey over analysed biota including capture locations, date, species and gender. Samples are arranged according to locations and not sample numbers. Maps of the sites are given in Figures 7-9.

| Capture location | Coordinates | Species | Capture date | Gender | Sample No. | Size/Weight [cm*/kg] | Pooled livers |
|---|---|---|---|---|---|---|---|
| **B11** | 54°47´N/13°06´E | Cod | 31.08.2002 | ns | **OS1** | 28-31 | 5 |
| **B11** | 54°51´N/14°01´E | " | 01.09.2002 | ns | **OS6** | 25 | 1 |
| **B11** | 54°51´N/14°01´E | " | 01.09.2002 | ns | **OS7** | 25 | 1 |
| **B11** | 54°51´N/14°01´E | " | 01.09.2002 | ns | **OS8** | 25-26 | 2 |
| **B11** | 54°51´N/14°01´E | " | 01.09.2002 | ns | **OS9** | 26 | 1 |
| **B11** | 54°46´N/13°18´E | Flounder | 31.08.2002 | w | **OS2** | 24 | 1 |
| **B11** | 54°44´N/13°10´E | " | 31.08.2002 | f/m | **OS3** | 28-30 | 2 |
| **B11** | 54°45´N/13°20´E | " | 31.08.2002 | f/m | **OS4** | 29-34 | 2 |
| **B01** | 54°31´N/10°39´E | Dab | 03.09.2002 | f | **OS5** | 20-23 | 5 |
| **B01** | 54°31´N/10°39´E | Cod | 03.09.2002 | ns | **OS10** | 24-29 | 3 |
| **B01** | 54°31´N/10°39´E | " | 03.09.2002 | ns | **OS11** | 26-27 | 2 |
| **B01** | 54°40'N/10°28'E | " | 31.08.2003 | ns | **OS12** | 32 | 1 |
| **B01** | 54°40'N/10°28'E | " | 31.08.2003 | ns | **OS13** | 27 | 1 |
| **B01** | 54°40'N/10°28'E | " | 31.08.2003 | ns | **OS15** | 26 | 1 |
| **B01** | 54°40'N/10°28'E | Dab | 31.08.2003 | ns | **OS14** | 22 | 1 |
| **N01** | 54°15´N/7°29´E | Dab | 25.08.2002 | f | **NS1** | 19-22 | 5 |
| **N04** | 54°30´N/2°16´E | " | 26.08.2002 | f | **NS2** | 20-22 | 5 |
| **N04** | 54°30´N/2°16´E | " | 08.09.2003 | ns | **NS6** | | 5 |
| **N04** | 54°43´N/2°07´E | Cod | 26.08.2002 | ns | **NS3** | 22-25 | 5 |
| **N06** | 56°18´N/2°04´W | Dab | 27.08.2002 | f | **NS4** | 20-23 | 5 |
| **P01** | 55°30´N/4°40´E | Dab | 29.08.2002 | f | **NS5** | S | 5 |
| **GB1** | 54°07´N/7°46´E | Flounder | 30.08.2003 | ns | **NS7** | S | 1 |
| **Lofot** | 68°08'N/13°33'W | Cod | 02.02.2004 | f | **A1** | 86/8.50 | 1 |
| **Islands** | | " | 02.02.2004 | f | **A4** | 83/6.50 | 1 |
| **Northern** | 65°74'N/18°09'W | Cod | 30.09.2003 | f | **A2** | 49/1.02 | 1 |
| **Iceland** | | " | 30.09.2003 | f | **A5** | 41/0.65 | 1 |
| **Southern** | 63°28'N/20°15'W | Cod | 06.11.2003 | ns | **A3** | 53/1.50 | 1 |
| **Iceland** | | " | 06.11.2003 | f | **A6** | 51/1.28 | 1 |
| **Bear Island** | 74°N/19°E | Arctic char | 09.07.2001 | f | **B1/B3** | 45/0.83 | L/M |
| | | " | 09.07.2001 | f | **B2/B4** | 47/0.85 | L/M |
| | | Little auk | 08.07.2001 | m | **C1/C3** | 12*/0.173 | L/M |
| | | " | 08.07.2001 | m | **C2/C4** | 12*/0.169 | L/M |
| | | Kittiwake | 08.07.2001 | m | **D1/D3** | 33*/0.458 | L/M |
| | | " | 08.07.2001 | f | **D2/D4** | 33*/0.393 | L/M |
| | | Glaucous | 07.07.2001 | m | **E1/E3** | 49*/1.92 | L/M |
| | | gull | 07.07.2001 | f | **E2/E4** | 44*/1.44 | L/M |

ns.: not specified; f: female; m: male; * For birds: Length of wing span.
L/M: Single samples of liver and muscle tissue

#### 5.4.2.2 PCA concentrations

- **Screening by EI-MS/MS** revealed that **PCA levels** were **well detectable** in all samples and a formula group specific analysis by ECNI possible.
- **Total PCA concentrations** determined by **EI-MS/MS** were **comparable or up to ca. 50 % higher** than those obtained by **ECNI**. The main reason for this deviation is the ability of EI-MS/MS to detect compounds with a lower degree of chlorination as well as those with chain lengths > $C_{17}$.
- **Quantification by ECNI** was either carried out with a **closely matching reference standard** according to Tomy *et al.* (Tomy *et al.*, 1997) or with the **response factor correction mode** (Reth *et al.*, 2005). Both methods give comparable results, if properly optimised. The procedure of Tomy was applied to samples OS1-7 and NS1-5:
- sPCA: Chlorine content 59-62 %, applied quantification standard 60 % Cl.
- mPCA: Chlorine content 55-58 %, applied quantification standard 56 % Cl.
- The chlorination degree of samples and of technical products did not differ significantly (t-test).

Table 47 in the appendix summarises the determined molar mass and chlorine content for all samples from the North and Baltic Sea to enable a comparison with future data.

In order to ensure comparability with other investigations using ECNI quantification, Table 26 summarises both the PCA sum and formula group specific concentrations determined by ECNI mass spectrometry. Table 27 lists the results of most of the very few PCA determinations in fish world-wide for comparison. Only studies after 1990 were included. Earlier PCA determinations suffer normally from a proper quantification procedure and the reported results are indicative at the best.

**Table 26:** PCA concentrations determined by ECNI (sPCAs: $\Sigma C_{10}$-$C_{13}$, mPCAs: $\Sigma C_{14}$-$C_{15}$ and s+mPCAs: $\Sigma C_{10}$-$C_{15}$) and lipid content in biota from the Baltic and North Sea, and from the Northern North Atlantic. Samples are listed in order of capture locations as in **Table 25**.

| Capture location | Sample No. | Species | Lipid content | sPCAs | mPCAs | s+mPCAs | sPCAs | mPCAs | s+mPCAs |
|---|---|---|---|---|---|---|---|---|---|
| | | | [%] | $\Sigma C_{10}$-$C_{13}$ | $\Sigma C_{14}$-$C_{15}$ [ng/g wet weight] | $\Sigma C_{10}$-$C_{15}$ | $\Sigma C_{10}$-$C_{13}$ | $\Sigma C_{14}$-$C_{15}$ [ng/g lipid weight] | $\Sigma C_{10}$-$C_{15}$ |
| **Baltic Sea** | | | | | | | | | |
| B11 | OS1 | Cod | 49 | 143 | 106 | 249 | 289 | 226 | 515 |
| B11 | OS6 | Cod | 49 | 19 | 25 | 44 | 39 | 50 | 89 |
| B11 | OS7 | Cod | 52 | 42 | 75 | 117 | 81 | 145 | 226 |
| B11 | OS8 | Cod | 56 | 73 | 72 | 145 | 143 | 141 | 284 |
| B11 | OS9 | Cod | 56 | 82 | 121 | 203 | 146 | 216 | 363 |
| B11 | OS2 | Flounder | 33 | 127 | 206 | 333 | 378 | 614 | 992 |
| B11 | OS3 | Flounder | 34 | 99 | 31 | 130 | 296 | 93 | 389 |
| B11 | OS4 | Flounder | 33 | 221 | 115 | 336 | 660 | 343 | 1003 |
| B01 | OS5 | Dab | 41 | 48 | 130 | 178 | 115 | 310 | 425 |
| B01 | OS10 | Cod | 57 | 31 | 41 | 72 | 54 | 72 | 126 |
| B01 | OS11 | Cod | 50 | 63 | 60 | 123 | 127 | 122 | 249 |
| B01 | OS12 | Cod | 23 | 34 | 205 | 239 | 147 | 879 | 1028 |
| B01 | OS13 | Cod | 42 | 24 | 47 | 71 | 58 | 114 | 172 |
| B01 | OS15 | Cod | 53 | 408 | 1265 | 1673 | 773 | 2393 | 3166 |
| B01 | OS14 | Dab | 56 | 47 | 71 | 118 | 83 | 126 | 209 |

**Table 26:** Continued**.**

| Capture location | Sample No. | Species | Lipid content [%] | sPCAs $\Sigma C_{10}$-$C_{13}$ | mPCAs $\Sigma C_{14}$-$C_{15}$ | s+mPCAs $\Sigma C_{10}$-$C_{15}$ | sPCAs $\Sigma C_{10}$-$C_{13}$ | mPCAs $\Sigma C_{14}$-$C_{15}$ | s+mPCAs $\Sigma C_{10}$-$C_{15}$ |
|---|---|---|---|---|---|---|---|---|---|
| | | | | [ng/g wet weight] | | | [ng/g lipid weight] | | |
| **North Sea** | | | | | | | | | |
| N01 | NS1 | Dab | 50 | 169 | 123 | 292 | 338 | 288 | 626 |
| N04 | NS2 | " | 52 | 288 | 260 | 548 | 558 | 519 | 1077 |
| N04 | NS6 | " | 37 | 521 | 893* | 1414 | 1428 | 2448 | 3876 |
| N04 | NS3 | Cod | 44 | 90 | 32 | 122 | 202 | 62 | 264 |
| N06 | NS4 | Dab | 54 | 26 | <10 | 26 | 54 | <20 | 54 |
| P01 | NS5 | Dab | 32 | 37 | 221 | 258 | 75 | 699 | 774 |
| GB1 | NS7 | Flounder | 27 | 21 | 41 | 62 | 78 | 148 | 226 |
| Lofot Island | A1 | Cod | 37 | 52 | 47 | 99 | 139 | 126 | 265 |
| " | A4 | " | 49 | 17 | 7 | 24 | 35 | 14 | 49 |
| N Iceland | A2 | " | 46 | 56 | 16 | 72 | 120 | 35 | 155 |
| " | A5 | " | 39 | 11 | 7 | 18 | 28 | 18 | 46 |
| S Iceland | A3 | " | 50 | 52 | 18 | 70 | 102 | 36 | 138 |
| " | A6 | " | 49 | 70 | 47** | 117 | 143 | 96** | 239 |
| Bear Island | B1 (L) | Arctic char | 12 | 27 | 43 | 70 | 225 | 358 | 583 |
| " | B3 (M) | " | 2 | 13 | 47 | 60 | 542 | 1598 | 2500 |
| " | B2 (L) | " | 12 | 11 | 13 | 24 | 89 | 107 | 196 |
| " | B4 (M) | " | 2 | 7 | 10 | 17 | 304 | 435 | 739 |
| Bear Island | C1 (L) | Little auk | 10 | 18 | 48 | 66 | 188 | 503 | 691 |
| " | C3 (M) | " | 5 | 7 | 55 | 62 | 153 | 1197 | 1350 |
| " | C2 (L) | "" | 10 | 88 | 371 | 459 | 882 | 3717 | 4599 |
| " | C4 (M) | | 4 | 16 | 17 | 33 | 427 | 453 | 880 |
| Bear Island | D1 (L) | Kittiwake | 13 | 6 | 39 | 45 | 112 | 730 | 843 |
| " | D3 (M) | " | 10 | 5 | 38 | 43 | 95 | 722 | 817 |
| " | D2 (L) | " | 17 | 44 | 12 | 56 | 861 | 235 | 1096 |
| " | D4 (M) | " | 11 | 5 | 5 | 10 | 41 | 41 | 82 |

* determined with mPCA standard (52 %) due to a low chlorine content; ** no correction of response due to very low chlorine content, calculated according to Tomy *et al.* (1997); L: liver; M: muscle.

**Table 27:** Concentration of PCA in different fish species worldwide. For details, see references.

| Sample type | Location | Country | n[a] | Gender[b] | Tissue | PCA measured | Concentration[c] [ng/µl] | Analytical method | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| White fish | Lake Storvindeln | Sweden | 35 | m+f | Muscle | $C_{10-13}$ 60 % | 1000 (lw) | ECNI-LRMS | 1 |
| Herring | North Sea | - | 1 | ns | Muscle | $C_{10-13}$ | 250 (lw) | " | 2 |
| Herring | Bothnian Sea | Sweden | 100 | m+f | " | $C_{10-13}$ 60 % | 1400 (lw) | " | 1 |
| " | Baltic Proper | " | 60 | m+f | " | " | 1500 (lw) | " | 1 |
| " | Skagerrak | " | 100 | m+f | " | " | 1600 (lw) | " | 1 |
| Carp | Hamilton Harbour | Canada | 3 | ns | Whole | $C_{14-17}$ 52 % | 2630 (ww) | ECNI-HRMS | 3 |
| Yellow perch | Detroit River | USA | 1 | ns | Muscle | $C_{10-13}$ 60-70 % | 1150 (ww) | " | 4 |
| Catfish | " | " | 1 | ns | " | " | 305 (ww) | " | 4 |
| Trout | Remote locations | Norway | 1 (13) | ns | Muscle | $C_{10-13}$ | 110-1700 (lw) | " | 5 |
| Arctic char | Ellasjøen, Bear I. | Norway | 1 | ns | " | " | 592 (lw) | ECNI-HRMS | 5 |
| Arctic char | Velmunden | Norway | 1 | ns | " | " | 500 (lw) | " | 5 |
| Arctic char | Lake Vättern | Sweden | 15 | m+f | " | $C_{10-13}$ 60 % | 570 (lw) | ECNI-LRMS | 1 |
| Burbot | Remote locations | Norway | 1 (6) | ns | Liver | $C_{10-13}$ | 230-3700 (ww) | ECNI-HRMS | 5 |
| Blue fish | Marmara Sea | Turkey | 1 | ns | Whole | " | 725 (lw) | ECNI-LRMS | 2 |
| Cod | Atlantic | - | 1 | ns | " | " | 727 (lw) | " | 2 |
| Angler | Atlantic | - | 1 | ns | " | " | 311 (lw) | " | 2 |
| Carp | East Anglia | UK | ns | ns | Muscle | s+mPCA | 500 (ww) | " | 6 |

[a]: Pooled individuals or number of individuals (in brackets); [b]: M: male, f: female, ns: not specified; [c]: lw: lipid weight, ww: wet weight.
1: Jansson *et al.* (1993), 2: Coelhan (1999); 3: Muir *et al.* (1998); 4: Tomy *et al.* (1997); %: Borgen *et al.* (2001); 6: Nicolls *et al.* (2001).

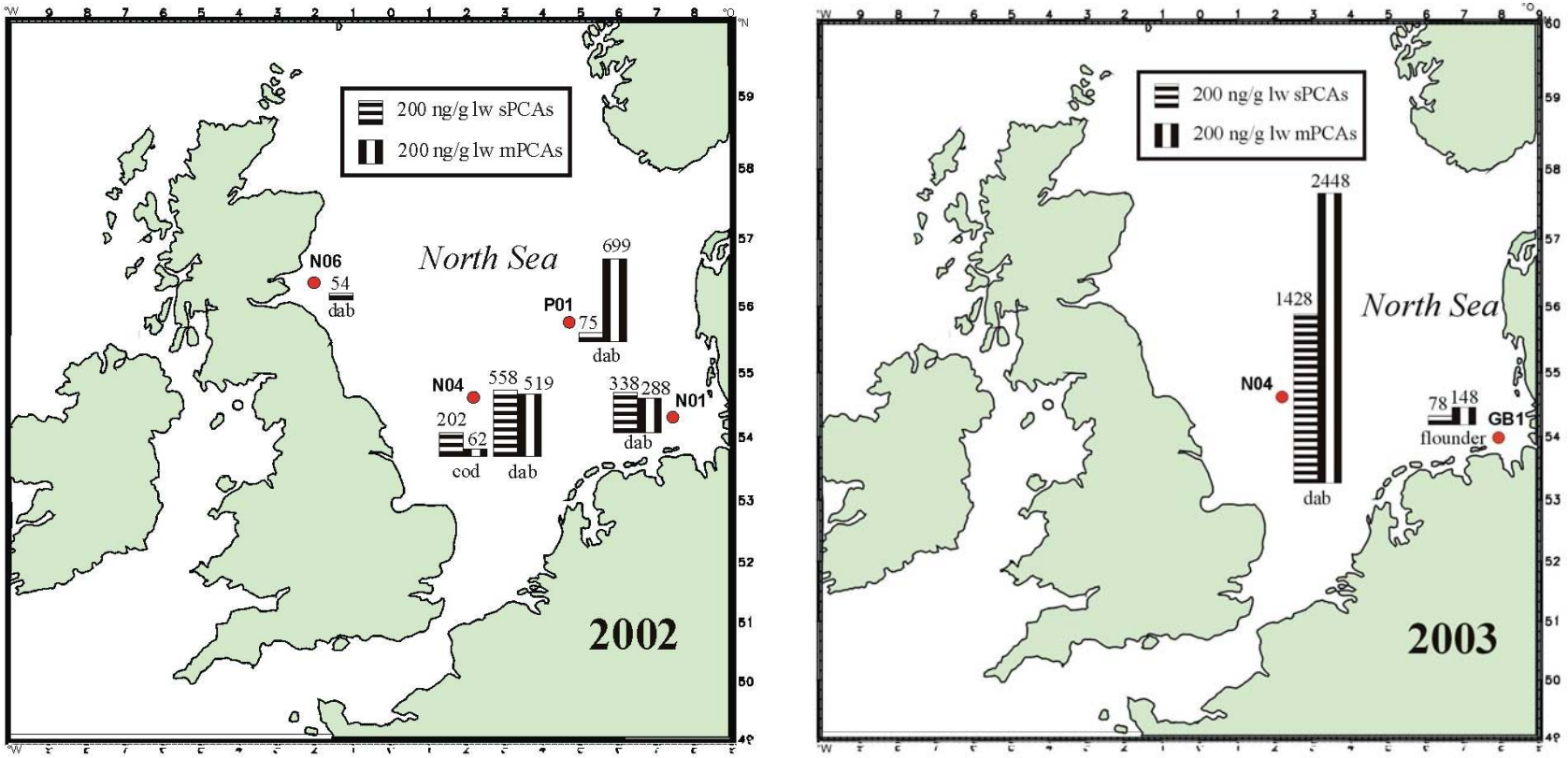


**Figure 12:** Left: Map of the capture locations in the North Sea (for detailed information see **Table 25**) and sPCA and mPCA concentrations (ng/g lipid weight) determined in samples NS1-5 from 2002 (see **Table 26**). Right: Map of the capture locations in the North Sea (for detailed information see Table 18) and sPCA and mPCA concentrations (ng/g lipid weight) determined in samples NS6-7 from 2003 (see **Table 26**).

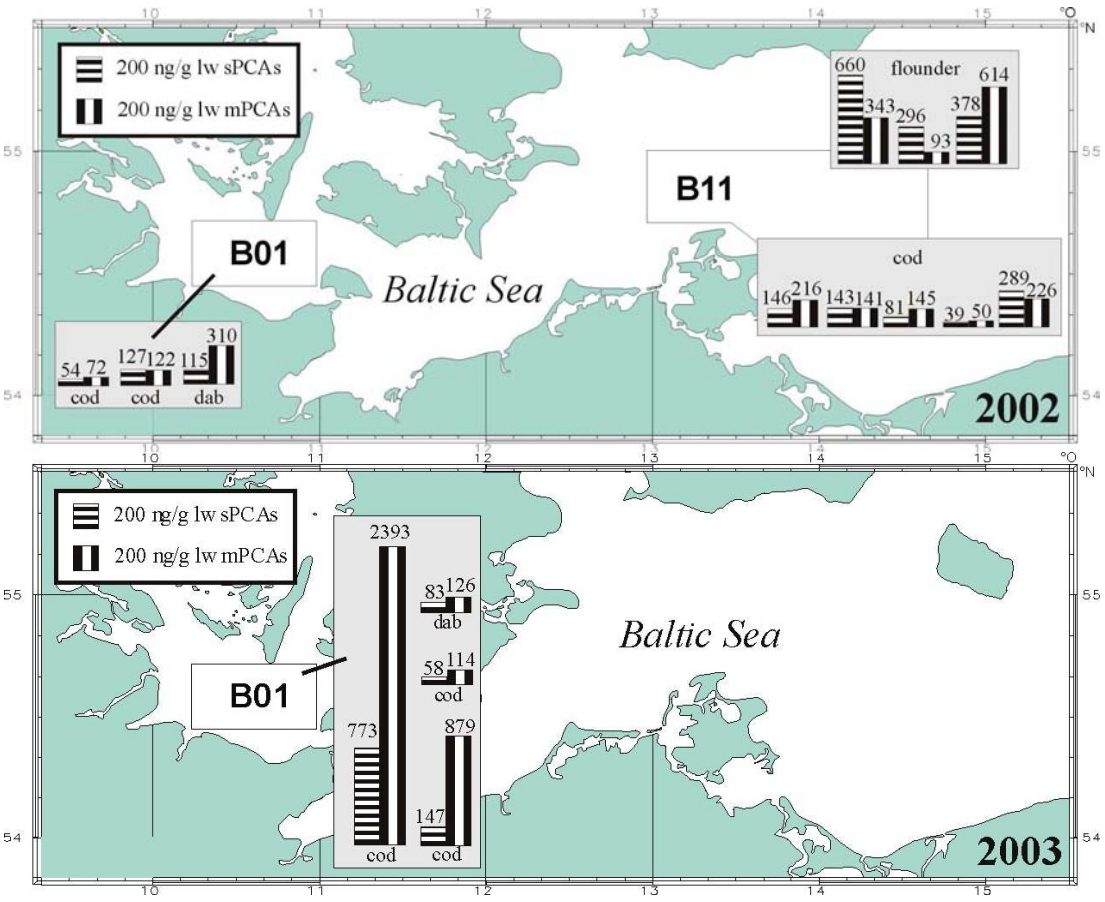


**Figure 13:** Map of the capture locations in the Baltic Sea (for detailed information see **Table 25**) and sPCA and mPCA concentrations (ng/g lipid weight) for 2002 and 2003 (see **Table 26**).

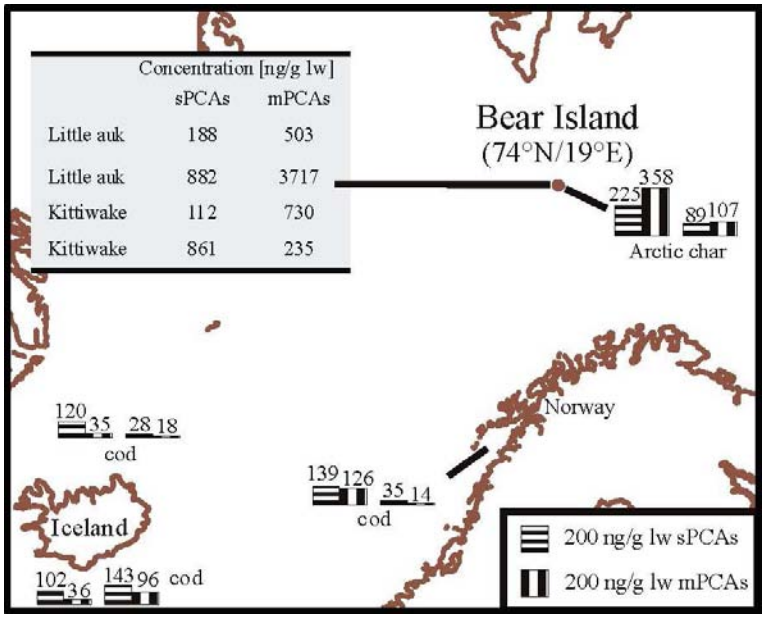


**Figure 14:** Map of the capture locations in the northern North Atlantic and on Bear Island (see Table 25 for details) as well as sPCA and mPCA concentrations (ng/g lipid weight) in the analysed samples (see Table 26). The content in liver is given for the two Arctic char samples.

**Figure 14:**

#### 5.4.2.3 Interpretation of PCA concentrations

**PCA concentrations reported** so far **world-wide** and summarised in Table 27 are **hardly comparable** due to the following reasons:

- **Different quantification** procedures
- **Concentrations** only **expressed on lipid or wet** weight basis
- Analysis of **different tissues and species**.

The concentrations of this study listed in Table 26 are within the same range. Since data from other PCA monitoring programmes are very scarce and due to the reasons given above, no further comparison can be carried out. The findings of the investigation presented here can be summarised as follows:

- **s+mPCA** levels in **fish liver from the North and Baltic Sea** showed **no species-specific** concentration dependence.
- **s+mPCA concentration ranges** were **comparable** for **the North Sea** (54-3880 ng/g lw, mean 985 ng/g lw) and the Baltic Sea (90-3170 ng/g lw, mean 615 ng/g lw).
- The **highest s+mPCA levels** were **far above 1 ppm**, which is remarkable.
- **s+mPCA levels in cod liver** from **remote areas** (Lofot Islands/Iceland) were **considerably lower** (46-265 ng/g lw, mean 149 ng/g lw) than in cod from the North and Baltic Sea (range 62-3170 ng/g lw, mean 622 ng/g lw).
- **Muscle tissue from Arctic char** (200-2500 ng/g lw, mean1005 ng/g lw) from the **background site Bear Island** has **comparable levels as cod liver from the North and Baltic Sea**. Similar concentrations were also reported for PCB and DDT-compounds and toxaphenes (see Evenseth *et al.,* 2004; Evenseth *et al.,* 2005 and Table 28). Main reasons for such an exposure are long range transport and condensation effects, a high precipitation rate around the sampling site Lake Ellasjøen and the breeding sites of thousands of sea birds close by resulting in an input via guano (Evenseth *et al.*, 2004).

- The **analysed birds** had also a **considerable s+mPCA burden**, which was in the **same range as for DDT-compounds, PCB** and toxaphenes (see Table 28). However, no other study of PCA in birds is currently available for comparison.
- **s+mPCA concentrations** expressed in wet weight were **quite similar for liver and muscle from Arctic char** despite a ca. six times lower lipid content of muscle. This is **also valid for the bird samples**, where the lipid content of both tissues was more similar (Table 26).
- The **mPCA concentrations were higher than those for sPCA** in many samples from the Baltic Sea and in birds. No clear picture was observed for the North Sea and northern North Atlantic.

**Table 28:** Comparison of concentrations of s+mPCA, DDT-compounds, PCBs and toxaphenes in biota from the European Arctic. Literature data were given in ng/g ww. Therefore, also s+mPCA levels were expressed similarly.

| Species | Origin and Year | Tissue | Σ DDTs [ng/g ww] | Σ PCBs [ng/g ww] | Toxaphenes [ng/g ww] | s+mPCA, this study |
|---|---|---|---|---|---|---|
| Cod[46] | Iceland 1999 | Liver | 92.1 [a] | 80.1[c] | 81.7[e] | 18-117[f] |
| Arctic char[40] | Bear Island, Lake Ellasjøen 1999 | Muscle | 62.7[b] | 1140[d] | - | 17-70[g] |
| Little auk [40] | Bear Island 1999 | Muscle | 38.9[b] | 208[d] | 8.1[e] | 33-459[g] |
| Kittiwake[40] | Bear Island 1999 | Muscle | 40.2[b] | 736[d] | 18.5[e] | 10-56[g] |

[a]Σ DDTs = p,p‘-DDE, -DDD and DDT, o,p‘-DDT

[b]Σ DDTs = o,p‘- and p,p‘-DDE, -DDD and DDT

[c]Σ PCBs = Sum of 7 congeners (28, 52, 101, 118, 138, 153, 180)

[d]Σ PCBs = Sum of 33 congeners (18, 28, 31, 33, 37, 47, 52, 60, 66, 74, 99, 101, 105, 114, 118, 122, 123, 128, 138, 141, 149, 153, 156, 157, 167, 170, 180, 183, 187, 189, 194, 206, 209)

[e]Toxaphenes = Sum of Toxaphene #26, #50, #62 (according to Parlar)

[f] Lofot Islands and Iceland; [g] Bear Island.

#### 5.4.2.4 PCA formula and congener group profiles

Figure 10 compares the formula and congener group patterns of s- and mPCA for selected samples. **$C_{14}$ compounds** are **dominant** (mean 69 %, range 59-100 %) in the **mPCA-patterns of all samples** as also typical for technical mixtures. No differences between regions were observed.

The picture is **more complex for sPCA-patterns**. **$C_{11}$ and $C_{12}$ congeners** were **most abundant** in the **biota from the Baltic and North Sea**. The distribution resembled those in technical sPCA, but showed a larger variability. To facilitate a comparison of the sPCA patterns, the sums of all congeners of each chain length is given in Figure 11 for **cod from the Baltic Sea** and compared to **technical sPCA mixtures**. The **C-chain patterns** were **quite similar**.

However, **cod livers** from the **northern North Atlantic** showed a **change**. Here, **$C_{10}$ and $C_{12}$ congeners** were **most intense** with exception of one sample from northern Iceland as the C-chain profiles in Figure 12 demonstrate. Moreover, the isomer groups $C_{12}H_{20}Cl_6$ and $C_{12}H_{19}Cl_7$ had a high fraction. $C_{12}H_{19}Cl_7$ contributed about 20 % to the sPCA sum. The change of $C_{10}$ congeners from a minor to a major fraction of sPCA was also observed by Tomy *et al*. (2000) in marine mammals from the Northern North Atlantic such as beluga, walrus and seals.

Table 29 summarises the **relative contribution of $C_{10}$ and $C_{12}$ congeners to total sPCA** as well as the $C_{10}/C_{12}$ ratio for cod liver from the Baltic Sea, the northern North Atlantic and biota liver from bear Island. The **$C_{10}$ fraction and $C_{10}/C_{12}$ ratio increased** to 28.4 % and 0.76, respectively, in **cod liver from the northern North Atlantic** compared to 13.6 % and 0.43 in technical mixtures or 16.8 % and 0.53 in cod liver from the Baltic Sea. A corresponding or even higher increase could **also be observed for biota liver from Bear Island**. The change indicates a fractionation and **enrichment** of the more volatile **$C_{10}$ congeners** during **long range transport** to this remote region.

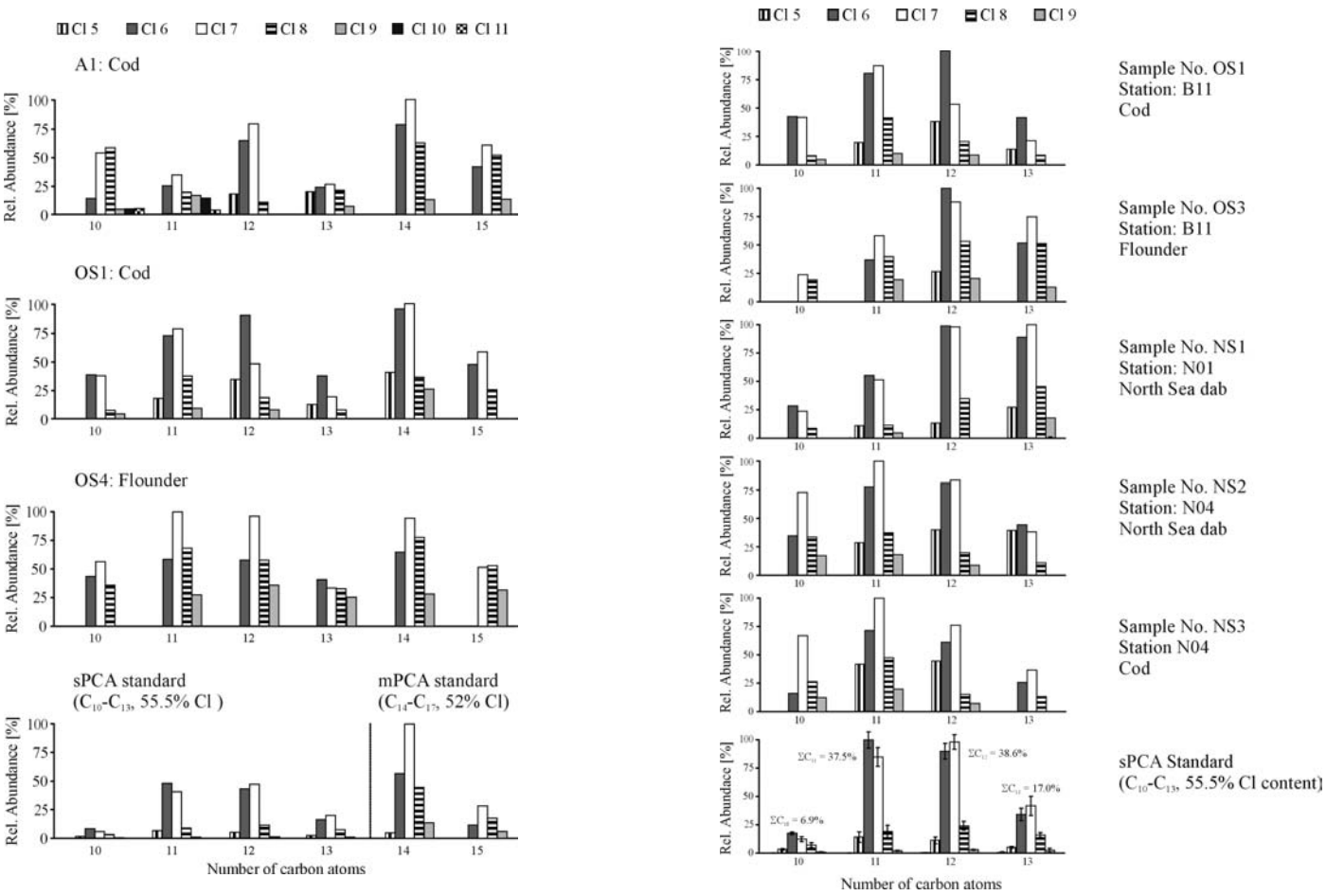


**Figure 15:** Formula group patterns of sPCAs ($C_{10}$-$C_{13}$) in selected samples from the Lofot Island (A1), the Baltic Sea (OS1, OS3, OS4) and the North Sea (NS1-NS3). The patterns for mPCAs ($C_{14}$-$C_{15}$) are quite similar and are therefore only shown for the selected samples to the left. Two standard mixtures are shown for comparison (sPCA: $C_{10}$-$C_{13}$, 55.5 % chlorine content, mPCA: $C_{14}$-$C_{17}$, 52 % chlorine content). Determination was carried out by ECNI.

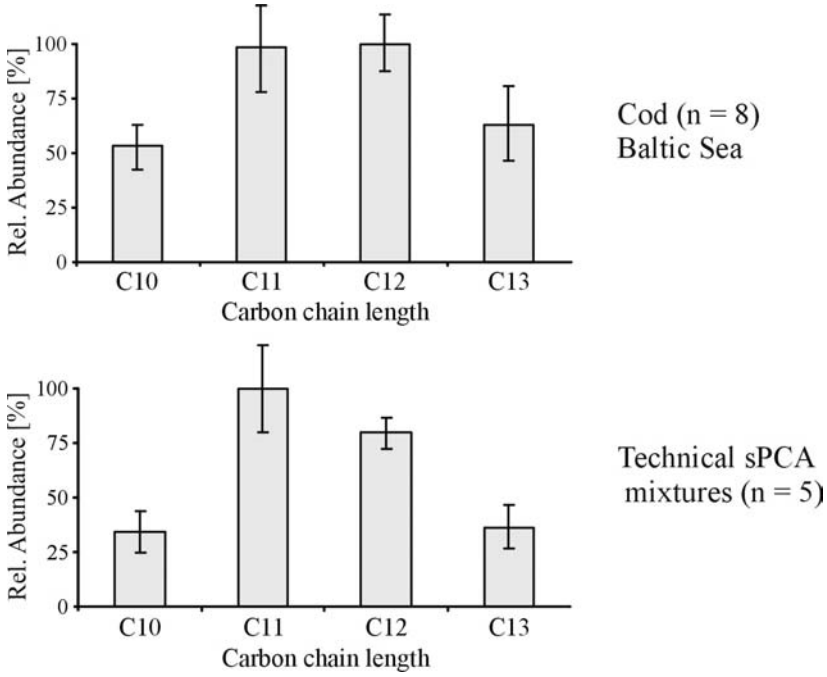


**Figure 16:** Comparison of the averaged C-chain patterns in cod from the Baltic Sea with five technical sPCA mixtures. The bars mark the standard deviations.

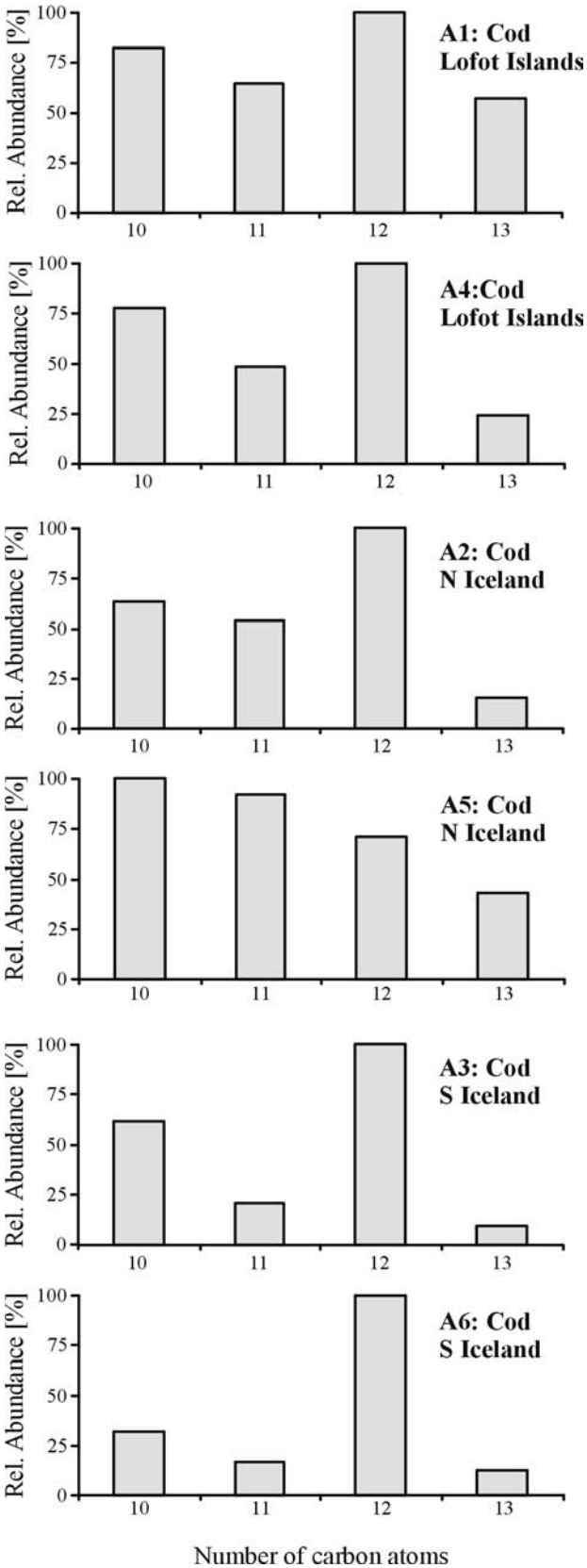


**Figure 17: Comparison of the averaged C-chain patterns of sPCA in selected cod samples from the northern North Atlantic.**

**Table 29:** Relative contribution of $C_{10}$ and $C_{12}$ groups to total sPCA in cod liver from the northern North Atlantic and the Baltic Sea as well as in biota liver from Bear Island. Data are also compared with technical sPCA mixtures. $C_{10}/C_{12}$ ratios are given to illustrate the changes.

| Sample no. | $C_{10}$ fraction [%] | $C_{12}$ fraction [%] | $C_{10}/C_{12}$ ratio |
|---|---|---|---|
| **Northern North Atlantic** | | | |
| A1 | 27.2 | 32.9 | 0.83 |
| A4 | 31.1 | 39.9 | 0.78 |
| A2 | 27.3 | 43.0 | 0.63 |
| A5 | 32.6 | 23.4 | 1.39 |
| A3 | 32.2 | 52.0 | 0.62 |
| A6 | 19.8 | 62.1 | 0.32 |
| **Mean ± STD** | 28.4 ± 4.9 | 42.2 ± 13.8 | 0.76 ± 0.36 |
| **Bear Island** | | | |
| Arctic char B1 | 18.9 | 27.8 | 0.68 |
| Arctic char B2 | 21.4 | 22.2 | 0.96 |
| Little auk C1 | 37.4 | 28.6 | 1.31 |
| Little auk C2 | 19.5 | 29.1 | 0.67 |
| Kittiwake D1 | 23.6 | 18.2 | 1.30 |
| Kittiwake D2 | 27.4 | 28.8 | 0.95 |
| **Baltic Sea** | | | |
| OS1 | 15.0 | 34.5 | 0.43 |
| OS8 | 19.6 | 36.3 | 0.54 |
| OS9 | 22.9 | 33.4 | 0.69 |
| OS10 | 16.3 | 34.0 | 0.48 |
| OS11 | 16.3 | 34.8 | 0.47 |
| OS12 | 14.2 | 29.0 | 0.49 |
| OS13 | 18.0 | 24.9 | 0.72 |
| OS15 | 12.4 | 28.0 | 0.44 |
| **Mean ± STD** | 16.8 ± 3.3 | 31.9 ± 4.0 | 0.53 ± 0.11 |
| **sPCA mixtures** | | | |
| Hordalub 17 | 9.5 | 34.4 | 0.28 |
| Hordalub 80 | 10.5 | 32.0 | 0.33 |
| Hordalub 500 | 12.4 | 27.2 | 0.46 |
| Cereclor 60L | 15.5 | 35.2 | 0.44 |
| Cereclor70L | 20.2 | 30.7 | 0.66 |
| **Mean ± STD** | 13.6 ± 3.9 | 31.9 ± 2.8 | 0.43 ± 0.15 |

STD: Standard deviation

### 5.4.2.5 Chlordane concentrations

Quantification was carried out according to the fully validated method developed by Karlsson *et al.* (1999; Karlsson *et al.*, 2000). A detailed description of the analytical technique is given in appendix 1, chapter 5.6.9. The most abundant chlordane representtatives are *cis/trans*-chlordane (octachloro compounds) and *cis/trans*-nonachlor (nonachlorinated). They were quantified in most samples from the Baltic and North Sea together with the main metabolite oxychlordane formed from *cis/trans*-chlordane. No samples from the northern North Atlantic were analysed, since a more comprehensive study already existed (see Karlsson *et al.*, 2000 and below).

Table 30 summarises the determined concentrations, and Figures 13 and 14 visualise the sum concentrations on lipid basis in sampling site maps of the North and Baltic Sea. Table 31 gives a survey about sum chlordane concentrations in different fish species from the North and Baltic Sea as reported in the literature. So far, only very few European studies included chlordanes, which makes the data base scarce for comparison. Moreover, results are sometimes given as sum concentrations, which eliminate any compound specific differentiation. Furthermore, chlordane compound specific concentrations in cod liver from the Lofot Islands are summarised in Table 32. This is the only study from this region so far.

### 5.4.2.6 Interpretation of chlordane concentrations

The data interpretation can be summarised as follows:

- **Sum concentrations** on **lipid weight basis** (11-47 ng/g lw) were **comparable to other studies of fish** from the Baltic and North Sea (see Table 31).
- **No significant concentration difference** (t-test) was observed **between the Baltic** (Σchlordane 11.3 ± 5.1 ng/g ww) and the **North Sea** (10.4 ± 6.0 ng/g ww) or between the two measuring campaigns.
- However, **compared to cod liver from the Lofot Islands** (Karlsson *et al.*, 2000), the **chlordane compound burden** was **about one order of magnitude lower**. This

is a further confirmation that **chlordane pollution in Europe** is **mainly a problem in cold remote areas** of the Arctic due to long range transport and condensation. The **chlordane concentrations in Arctic cod livers** (Table 32) are **comparable to** those for **PCB** or even higher (see e.g. Table 28).

- ***trans*-Nonachlor contributed most to the sum concentrations** (37 ± 6 %) as typical for most studies (see e.g. Table 31). *trans*-Nonachlor is most persistent of the analysed representatives (Dearth and Hites, 1991b). Moreover, *trans*-nonachlor has a higher Henry constant and is therefore more globally dispersible by atmospheric long range transport (Falandysz *et al.*, 2000).
- ***cis*-Chlordane dominates in fish recently exposed** to technical chlordane (Strandberg *et al.*, 1998). Therefore, any ***trans*-nonaclor/*cis*-chlordane ratio >1** indicate long range transport from **old source areas** (Strandberg *et al.*, 1998). The cod livers of **this study** had a **mean ratio of 1.8 ± 0.6**, which point to old sources and no recent chlordane input.
- Two **further chlordane compounds (MC5 and MC7)** were also **determined**. They are usually not included in chlordane quantification. MC5 has similar concentrations as *trans*-chlordane and is abundant in Arctic regions (Karlsson *et al.*, 2000). **MC5** was **not detectable in any sample** (limit of detection ca.2 pg/g), and **MC7** was **just above the limit of detection** (ca. 1 pg/g).

**Table 30:** Concentrations (ng/g wet weight) of selected chlordanes in fish liver from the North and Baltic Sea collected in 2002 and 2003. Concentrations in brackets are based on lipid weight (lw). Samples OS6 and OS7 could not be analysed, since the complete sample (one liver) had to be used for PCA analysis.

| Capture location | Sample No. | Species | Lipid content [%] | Concentration [ng/g ww and in brackets in ng/g lw] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *trans*-chlordane | *cis*-chlordane | *trans*-nonachlor | *cis*-nonachlor | Oxychlordane | ∑ Chlordanes |
| B11 | OS1 | Cod | 49 | 1,79 | 4,87 | 9,31 | 4,55 | 2,63 | 23,1 (47.1) |
| B11 | OS8 | Cod | 56 | 1,78 | 2,85 | 4,08 | 2,71 | 1,12 | 12,5 (22.3) |
| B11 | OS9 | Cod | 56 | 1,81 | 3,57 | 7,62 | 4,56 | 1,61 | 19,2 (34.3) |
| B11 | OS2 | Flounder | 33 | 0,97 | 2,29 | 5,55 | 0,93 | 0,36 | 10,1 (30.6) |
| B11 | OS3 | Flounder | 34 | 1,46 | 3,51 | 8,01 | 1,10 | 0,42 | 14,5 (42.6) |
| B11 | OS4 | Flounder | 33 | 1,32 | 2,07 | 3,09 | 0,88 | 0,72 | 8,07 (24.5) |
| B01 | OS5 | Dab | 41 | 0,50 | 1,47 | 2,81 | 0,80 | 1,00 | 6,58 (16.0) |
| B01 | OS10 | Cod | 57 | 0,92 | 1,95 | 0,60 | 1,83 | 0,84 | 6,14 (10.8) |
| B01 | OS12 | Cod | 23 | 0,49 | 1,32 | 4,38 | 2,32 | 0,91 | 9,43 (41.0) |
| B01 | OS13 | Cod | 42 | 0,66 | 1,72 | 4,23 | 2,17 | 1,21 | 9,98 (23.8) |
| B01 | OS15 | Cod | 53 | 1,79 | 2,16 | 4,66 | 2,48 | 1,76 | 12,9 (24.3) |
| B01 | OS14 | Dab | 56 | 0,30 | 0,73 | 1,23 | 0,72 | 0,57 | 3,55 (6.34) |
| B01 | OS11 | Cod | 50 | 1,39 | 3,26 | 1,89 | 3,37 | 1,37 | 11,3 (22.6) |
| N01 | NS1 | Dab | 50 | 0,77 | 1,36 | 1,98 | 1,33 | 0,19 | 5,63 (11.3) |
| N04 | NS2 | Dab | 52 | 0,71 | 1,32 | 2,17 | 2,41 | 1,19 | 7,79 (15.0) |
| N04 | NS6 | Dab | 37 | 1,13 | 2,73 | 5,79 | 5,11 | 4,36 | 19,1 (51.6) |
| N04 | NS3 | Cod | 44 | 1,14 | 2,79 | 6,27 | 3,93 | 2,56 | 16,7 (38.0) |
| N06 | NS4 | Dab | 54 | 1,12 | 1,27 | 2,12 | 1,48 | 0,80 | 6,78 (12.6) |
| P01 | NS5 | Dab | 32 | 1,86 | 3,20 | 5,61 | 2,71 | 1,40 | 14,8 (46.3) |
| GB1 | NS7 | Flounder | 27 | 0,12 | 0,26 | 0,53 | 0,53 | 0,42 | 1,85 (6.85) |

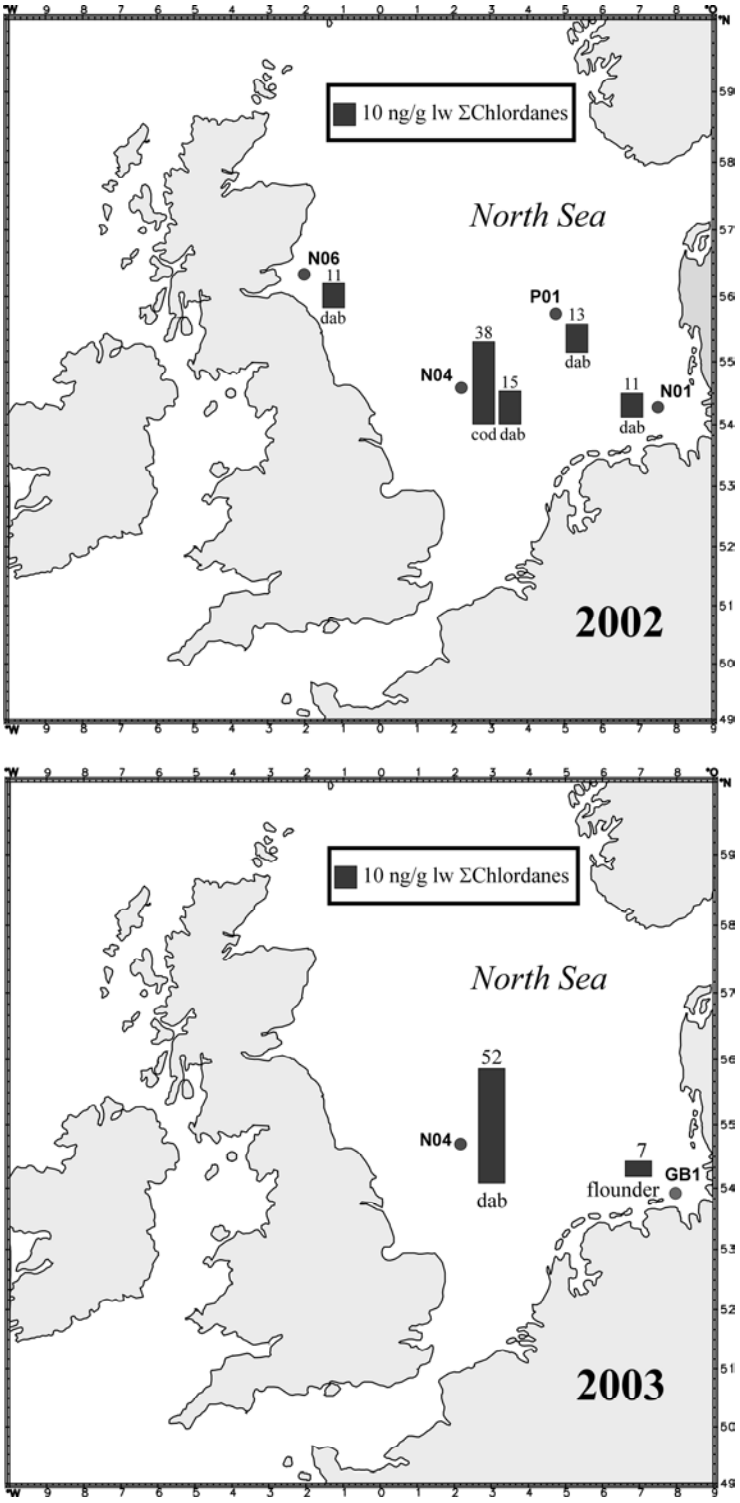


**Figure 18:** Map of capture locations in the North Sea (for detailed information see **Table 25**) and sum concentrations of chlordane compounds (*cis/trans*-nonachlor, *cis/trans*-chlordane and oxychlordane) in fish liver in ng/g lipid weight (see **Table 30**).

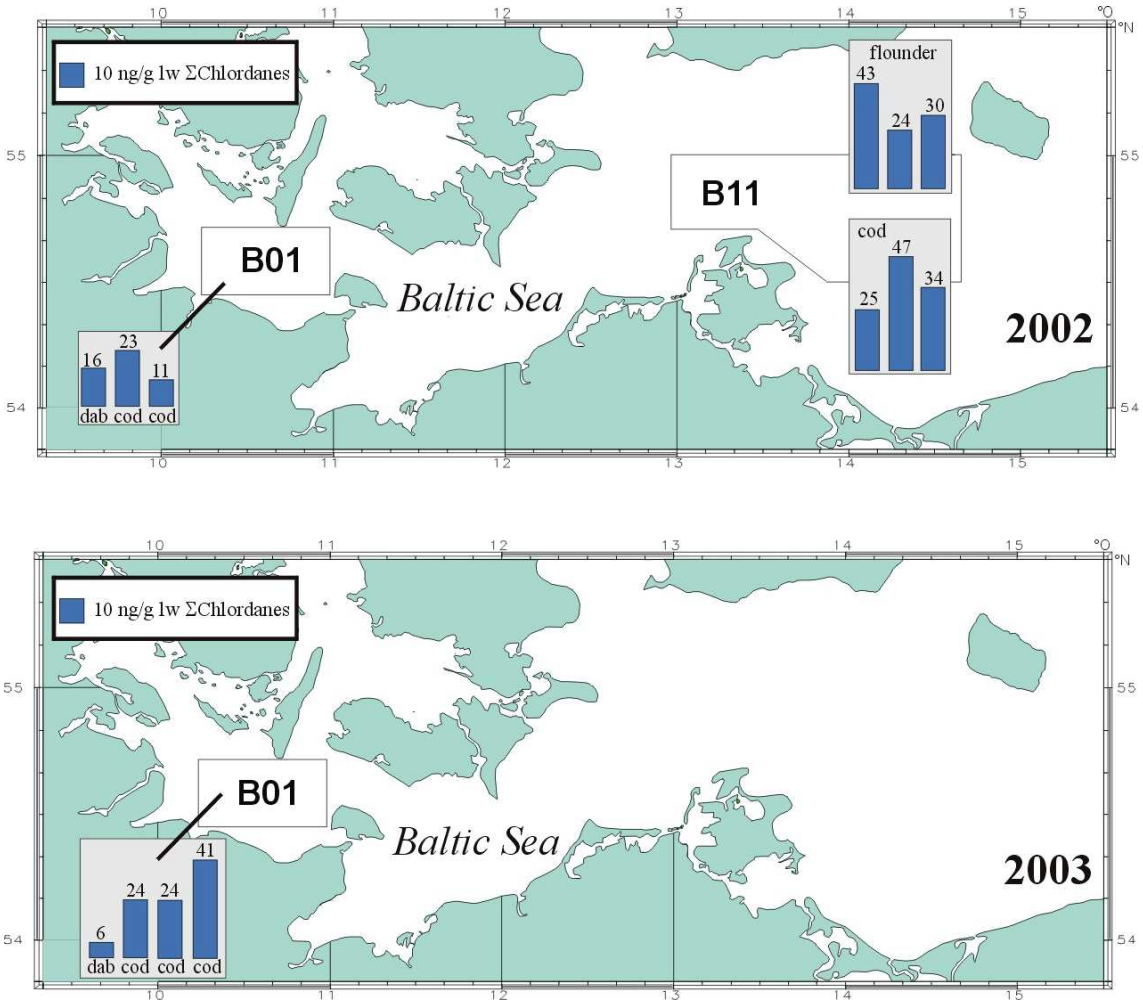


**Figure 19:** Map of capture locations in the Baltic Sea (for detailed information see **Table 25**) and sum concentrations of chlordane compounds (*cis/trans*-nonachlor, *cis/trans*-chlordane and oxychlordane) in fish liver in ng/g lipid weight (see **Table 30**).

**Table 31:** Published concentrations of Σchlordanes (ng/g lipid weight (lw) of *cis/trans*-nonachlor, *cis/trans*-chlordane and oxychlordane) in fish from the North and Baltic Sea.

| Species | Origin | Year | Tissue | Σ Chlordanes [ng/g lw] |
|---|---|---|---|---|
| Herring[b] | Baltic Sea, Gotland | 1978 | Muscle | 600 |
| Herring[c] | Baltic Sea, South-Sweden | 1987 | Muscle | 183 |
| Herring[d] | Baltic Sea, Golf of Gdansk | 1992 | Muscle | 34 |
| Flounder[e] | Baltic Sea, Golf of Gdansk | 1992 | Whole fish | 13 |
| Cod[e] | Baltic Sea, Golf of Gdansk | 1992 | Whole fish | 11 |
| Mackerel[f] | North sea, German Bight | 1993-1996 | Muscle | 5,5 [a] |
| North Sea dab[g] | North Sea, Belgium | 2001 | Liver | 2,5 [a] |
| **This study** | **Baltic Sea (8), North Sea (1)** | **2002-2003** | **Liver** | **11-47** |

a: in ng/g wet weight; b: Marvin *et al.* (2003); c: Jansson *et al.* (1993); d: Strandberg *et al.* (1998); e: Falandysz *et al.* (2001); f: Karl *et al.* (1998); g: Voorspoels *et al.* (2004).

**Table 32:** Average concentrations ng/g (wet weight basis) and standard deviations of chlordane compounds in sixteen livers from male (n=7) and female (n=9) cod from the Lofot Islands caught in 1995 (Karlsson *et al.*, 2000).

| **Compound** | **Concentration (ng/g wet weight)** | | |
|---|---|---|---|
| | **Male** | **Female** | **This Study** |
| *cis*-Chlordane | 53 ± 24 | 48 ± 15 | 2.7 ±1.1 |
| *trans*-Chlordane | 7.7 ± 5.3 | 6.6 ± 6.7 | 1.3 ± 0.5 |
| *cis*-Nonachlor | 36 ± 19 | 46 ± 20 | 3.1 ± 1.0 |
| *trans*-Nonachlor | 88 ± 42 | 108 ± 18 | 4.8 ± 2.7 |
| Oxychlordane | 12 ± 4.2 | 14 ± 7.0 | 1.6 ± 0.7 |

### 5.4.3 PCAs and chlordanes in sediments

#### 5.4.3.1 Origin and characterisation of samples

Sediments were collected during the following monitoring expeditions: FS Gauss no. 371 (August/September 2001), no. 387 (August/September 2002), no. 402 (May/June 2003) as well as no. 419/421 (spring 2004). Table 33 gives an overview of all analysed sediment samples including their TOC content and the applied analytical methods.

Moreover, sediments and suspended particulate matter were obtained from different institutions in order to increase the data base for comparison (see Table 34). These were:

1. Laboratoire d`Etudes ed d`Analyses, Le Havre, France
2. Behörde für Wirtschaft und Arbeit, Hamburg, Germany
3. Landesanstalt für Umweltschutz, Karlsruhe, Germany
4. Akva-plan niva, Tromsø, Norway,
5. BSH, Hamburg, Germany.

#### 5.4.3.2 PCA concentrations

Samples were analysed according to the following procedure:

- All samples were first quantified by EI-MS/MS to obtain the total PCA content.
- In addition, the sPCA and mPCA content was determined in those samples exceeding ca. 50 ng/g dw total PCA. Below this level the quantities were too low for this procedure based on single formula and congener groups. $CH_4$/$CH_2Cl_2$-NICI had to be used. ECNI on the HP mass spectrometer was not sensitive enough.
- New mass spectrometric instrumentation (1200L, Varian, USA) with a significantly improved sensitivity allowed at the end of the project (2004) to determine single congener group profiles. Samples were re-analysed, if a sufficient amount still was

available. The main reason for this re-analysis was the comparability with other data quantified by ECNI (mostly applied world-wide).

#### 5.4.3.3 Interpretation of PCA concentrations

Table 34 summarises concentrations of total PCA and formula-specific results for sPCAs and mPCAs in all sediments. Figure 15 shows a site-specific survey of the total PCA levels. The following conclusions and comparisons could be made:

- **EI-MS/MS allowed detecting PCAs in all sediments** (5-377 ng/g dry weight (dw), see Table 34 for details). Quantification with different reference standards (e.g. a short-chain and medium chain references) agreed well.
- **Sum PCA concentrations** determined by **EI-MS/MS and $CH_4$/$CH_2Cl_2$-NICI** were **comparable within 10-15 %** with a few exceptions.
- **PCA levels in sediments from the Baltic Sea** (45-377 ng/g dw) were generally **higher than in those from the North Sea** (5-355 ng/g dw, ten of sixteen samples below 50 ng/g dw). However, they **were quite equal on TOC basis** (North Sea 2.3-33.1 ng/g TOC, Baltic Sea 2.1-9.4 ng/g TOC)
- PCA concentrations in sediments have only been reported from only very few sites world-wide (see Table 35). As already mentioned for biota, a comparison of levels is hampered by different quantification procedures. Moreover, some locations were contaminated by PCA production or release. Therefore, the published data allow only the remark that total **PCA concentrations (in ng/g dry weight)** in sediments from the **North and Baltic Sea** are about in the **same range** as those other of **not contaminated sites**.
- A limited number of additional sediments were analysed from different regions of Europe to increase the data base for comparison. This was not part of the project study, but the results were also included in Table 35. As can been, **PCA levels** in these **river and sea sediments** are **comparable** with those from the **North and Baltic Sea**. One site at the river Seine estuary had an unusually low TOC content (0.07 %), which resulted in a high PCA burden expressed on TOC basis.

- The **TOC content** is a **good marker for PCA concentrations**. Higher TOC levels indicated usually also a higher PCA burden. This is e.g. demonstrated by the samples from station 710 from 2001 and 2004. The concentration ratio of ca. 8.5 is similar to that of the TOC (2001: 3.1 %, 2004: 0.48 %)
- Since the composition of the TOC can vary substantially due to the former history (e.g. dump sites of sewage sludge indicated with the sample assignment KS), no further interpretation was carried out.
- The **highest PCA concentrations** in the **North Sea** were found at the sites KS11 and KS8 in the Elbe estuary, where **chemical waste and sewage sludge had been dumped**. Correspondingly high levels of hexachlorobenzene, PCBs, DDT and hexachlorocyclohexanes were already reported during the monitoring campaign in 1997 and 1998 (BSH, 2002).
- Concentrations of **mPCA** ($C_{14-16}$, 42-303 ng/g dw) were always **higher than** for **sPCA** ($C_{10-13}$, 18-128 ng/g dw). The **ratio mPCA/sPCA** was between **1.7 to 3.2**. Studies of river and lake sediment from Germany and Switzerland found also 2-10 times higher mPCA concentrations (WHO, 1996). Reason can be the predominant use of mPCAs in Europe (WHO, 1996). Figure 16 summarised the site-specific s- and mPCA levels.
- The average chlorine content of sPCAs in sediments varied between 53-56 % and for mPCAs between 46-50 %. Mean molecular weights were 334-391 g/mol for sPCAs and 365-400 g/mol for mPCAs. Table 48 in appendix 2 summarises the single sample values for future comparison.

**Table 33:** Overview over analysed sediments including sample location, year, total organic carbon (TOC) content and applied methodology. ECNI and $CH_4/CH_2Cl_2$-NICI could not be applied to samples with total PCA concentrations below ca. 50 ng/g dw due to insufficient sensitivity. Determination of formula and congener group profiles by ECNI became only possible with new instrumentation at the end of the project. Samples were re-analysed with ECNI, if a sufficient sample amount still was available (>5 g).

| Sample | Location | Year | Amount [g] | TOC [%] | EI-MS/MS | ECNI* | $CH_4/CH_2Cl_2$ NICI |
|---|---|---|---|---|---|---|---|
| **Baltic Sea** | | | | | | | |
| 710 | 54°25.10´N/10°13.30´E | 2001 | 4 | 3.10 | X | | X |
| | | 2002 | 5 | 5.28 | X | | X |
| | | 2004 | 10 | 0.48 | X | X | X |
| 715 | 54°03.00´N/10°50.90´E | 2001 | 4 | 2.90 | X | | X |
| | | 2004 | 10 | 3.08 | X | X | X |
| 718 | 54°19.00´N/11°33.00´E | 2001 | 4 | 3.80 | X | | X |
| | | 2004 | 10 | ** | X | X | X |
| 721 | 55°00.00´N/14°05.00´E | 2001 | 4 | 6.00 | X | | X |
| | | 2002 | 5 | 5.18 | X | | X |
| | | 2004 | 10 | 4.86 | X | X | X |
| ECKFOE | 54°28.00´N/9°52.00´E | 2004 | 10 | 4.00 | X | X | X |
| ODER | 54°08.00´N/14°10.00´E | 2004 | 10 | ** | X | X | X |
| RUDEN | 54°11.20´N/13°46.00´E | 2004 | 10 | 2.81 | X | X | X |
| **North Sea** | | | | | | | |
| KS 8 | 54°02.00´N/8°12.50´E | 2002 | 30 | 0.34 | X | | |
| | | 2003 | 20 | 0.82 | X | X | X |
| | | 2004 | 10 | 1.21 | X | X | X |
| KS 11 | 54°04.00´N/8°07.50´E | 2002 | 10 | 1.67 | X | | X |
| | | 2003 | 10 | 1.10 | X | X | X |
| | | 2004 | 5 | 2.50 | X | X | X |
| BL 2 | 54°14.00´N/8°22.50´E | 2002 | 10 | 0.57 | X | | |
| BL 4 | 54°30.00´N/7°48.00´E | 2002 | 30 | 0.21 | X | | |
| UE 18 | 54°30.00´N/7°60.00´E | 2002 | 50 | < 0.1 | X | | |
| UE 28 | 54°45.00´N/8°12.00´E | 2002 | 50 | 0.1 | X | | |
| ES 1 | 53°40.50´N/6°30.00´E | 2003 | 20 | 0.06 | X | | |
| Ti 13 | 54°22.50´N/7°38.70´E | 2003 | 20 | 0.07 | X | | |
| UE 15 | 54°30.00´N/6°30.00´E | 2003 | 20 | 0.23 | X | | |
| SSL | 54°54.90´N/8°10.10´E | 2003 | 10 | 2.77 | X | | |
| UE20 | 55°00.00´N/6°30.00´E | 2003 | 20 | 0.37 | X | | |
| L1 | 55°03.00´N/8°12.00´E | 2003 | 20 | 0.13 | X | | |
| WB 5 | 55°04.00´N/6°20.00´E | 2003 | 20 | 0.46 | X | | |
| UE 67 | 55°15.00´N/4°30.00´E | 2003 | 20 | 0.12 | X | | |
| UE 70 | 55°45.00´N/4°00.00´E | 2003 | 20 | 0.07 | X | | |
| WB 1 | 55°50.00´N/6°35.00´E | 2003 | 20 | 0.03 | X | | |
| **Total number** | | | | | **33** | **11** | **18** |

* by 1200L mass spectrometer (Varian, Walnut Creek, USA); ** Data not available

**Table 34:** Total PCA concentrations obtained by EI-MS/MS as well as sPCAs and mPCAs levels determined by $CH_4/CH_2Cl_2$-NICI in sediments from the Baltic Sea. This method covers $Cl_{3-10}$-compounds, while conventional ECNI detects $Cl_{5-10}$ PCA. Concentrations determined by ECNI are given in brackets for some samples to demonstrate that generally lower levels are found with this method.

| Sample site | Total PCA[a] [ng/g dw] | Total PCA[a] [ng/g TOC] | sPCAs[a] $\Sigma C_{10}$-$C_{13}$ | mPCAs[b] $\Sigma C_{14}$-$C_{15}$ | mPCAs[c] $\Sigma C_{14}$-$C_{16}$ | s+mPCAs $\Sigma C_{10}$-$C_{15}$ |
|---|---|---|---|---|---|---|
| | | | [ng/g dw] | | | |
| **Baltic Sea** | | | | | | |
| 710 (2001) | 262 | 8.4 | 98 | 199 | [d] | 297 |
| 710 (2002) | 377 | 7.1 | 128 | 303 | [d] | 431 |
| 710 (2004) | 45 | 9.4 | 13 (15) | 36 (23) | 37 | 49 (38) |
| 715 (2001) | 116 | 4.0 | 48 | 91 | [d] | 139 |
| 715 (2004) | 83 | 2.7 | 35 (22) | 51 (43) | 54 | 86 (65) |
| 718 (2001) | 141 | 3.7 | 21 | 48 | [d] | 69 |
| 718 (2004) | 232 | [b] | 82 (53) | 141(85) | 149 | 223 (138) |
| 721 (2001) | 142 | 2.4 | 105 | 131 | [d] | 236 |
| 721 (2002) | 108 | 2.1 | 91 | 153 | [d] | 244 |
| 721 (2004) | 138 | 2.8 | 44 (27) | 74 (72) | 81 | 118 (99) |
| ECKFBU | 158 | 3.9 | 29 (31) | 70 (39) | 77 | 99 (70) |
| ODER | 75 | [b] | 18 (8) | 42 (22) | 43 | 60 (30) |
| RUDEN | 136 | 4.8 | 25 (26) | 58 (33) | 61 | 83 (59) |

[a] Quantification with sPCA standard of 55.5 % Cl; [b] Quantification with mPCA standard of 57 % Cl; [c] For some samples $C_{16}$ was included to demonstrate that it contribute insignificantly to the total mPCA amount; [d] $C_{16}$ not determinable.

**Table 34 continued:** Total PCA concentrations obtained by EI-MS/MS as well as sPCAs and mPCAs levels determined by CH4/CH2Cl2-NICI in sediments from the North Sea. This method covers Cl3-10-compounds, while conventional ECNI detects Cl5-10 PCA. Concentrations determined by ECNI are given in brackets for some samples to demonstrate that generally lower levels are found with this method.

| Sample site | Total PCA[a] [ng/g dw] | Total PCA[a] [ng/g TOC] | sPCAs[a] $\Sigma C_{10}$-$C_{13}$ | mPCAs[b] $\Sigma C_{14}$-$C_{15}$ | mPCAs $\Sigma C_{14}$-$C_{16}$ | s+mPCAs $\Sigma C_{10}$-$C_{15}$ |
|---|---|---|---|---|---|---|
| | | | [ng/g dw] | | | |
| North Sea | | | | | | |
| KS 8 (2002) | 14 | 4.1 | c | c | c | c |
| KS 8 (2003) | 145 | 18.2 | 30 (27) | 70 (87) | 76 | 100 (114) |
| KS 8 (2004) | 267 | 22.2 | 62 (43) | 145 (48) | 149 | 207 (91) |
| KS 11 (2002) | 112 | 6.7 | 72 | 132 | [b] | 204 |
| KS 11 (2003) | 98 | 8.9 | 18 (13) | 54 (34) | 58 | 72 (47) |
| KS 11 (2004) | 355 | 14.2 | 79 (63) | 250 (149) | 275 | 329 (212) |
| BL 2 | 27 | 4.8 | c | c | c | c |
| BL 4 | 8 | 3.7 | c | c | c | c |
| UE 18 | 5 | 5.2 | c | c | c | c |
| UE 28 | 9 | 9.1 | c | c | c | c |
| ES 1 | 20 | 33.1 | c | c | c | c |
| TI 13 | 15 | 21.4 | c | c | c | c |
| UE 15 | 27 | 11.5 | c | c | c | c |
| SSL | 63 | 2.3 | c | c | c | c |
| UE 20 | 25 | 6.9 | c | c | c | c |
| L 1 | 16 | 12.3 | c | c | c | c |

[a] Quantification with sPCA standard of 55.5 % Cl; [b] Quantification with mPCA standard of 57 % Cl; [c] $C_{16}$ not determinable;
[c] Concentration <50 ng/g dw, too low for group specific quantification.

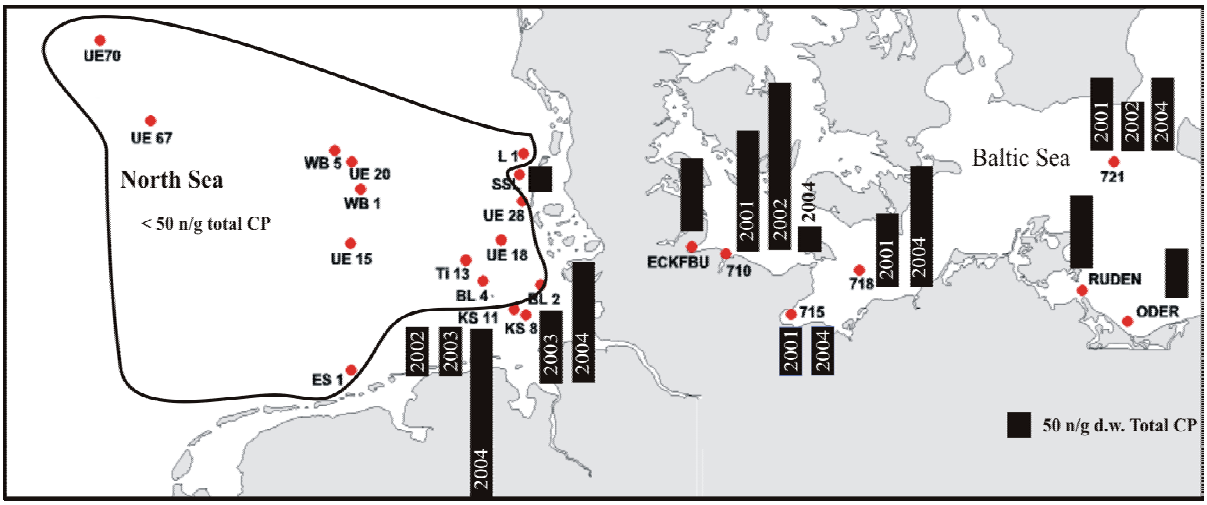


**Figure 20:** -specific survey of total PCA [ng/g] concentrations in sediments from the North and Baltic Sea (2001-2004). Quantification was carried out by EI-MS/MS (see **Table 34** for details).

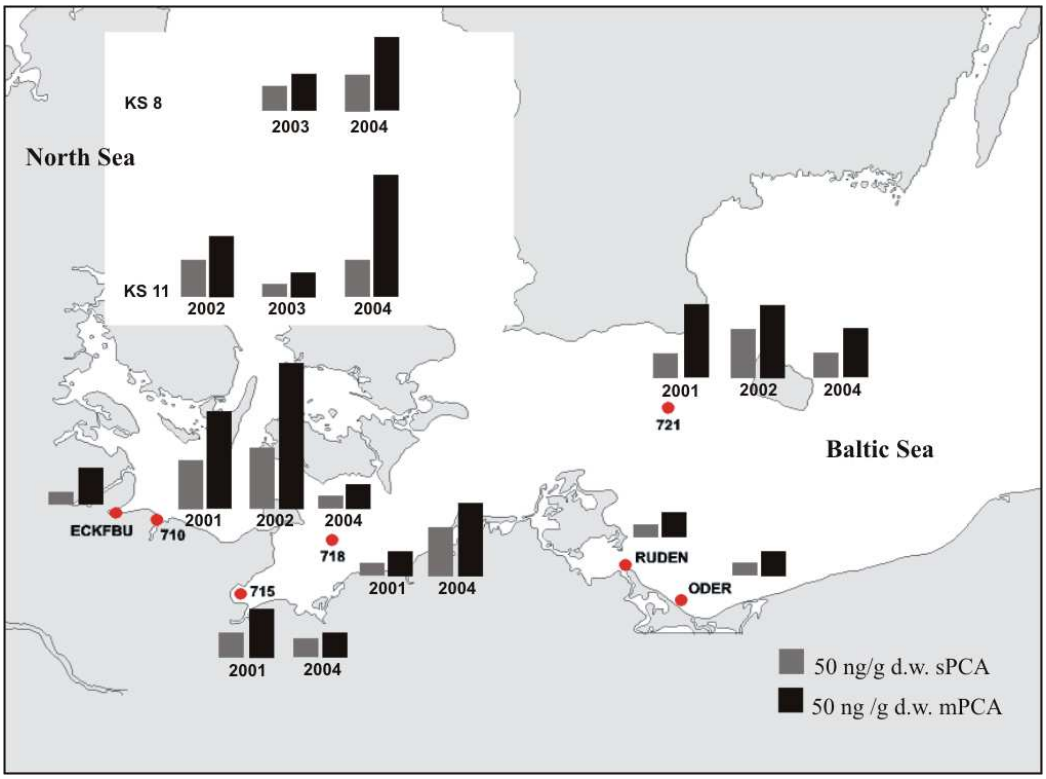


**Figure 21:** Survey over site-specific sPCA and mPCA ($C_{14\text{-}15}$) concentrations in sediments from the North and Baltic Sea in 2001-2004 obtained by $CH_4/CH_2Cl_2$-NICI-MS.

**Table 35:** Literature survey of reported PCA concentrations in sediments world-wide and comparison with additional samples analysed during this study by EI-MS/MS to increase the data base for comparison. All data are given in ng/g dry weight (dw). No TOC data were available for literature data

| Sampling site | Literature | sPCAs[ng/g dw] |
|---|---|---|
| Harbour Hamburg, Germany | 1 | 17 |
| Lake, Canada | 2 | 4.5-147 |
| Lake, Switzerland | 3 | 5 |
| River, USA | 4 | 70 |
| Lake, Canada | 4 | 1.6-257 |
| Harbour, Canada | 5 | 6-290 |
| River Detroit | 1 | 1800 |
| Rivers, Germany | 6 | <5-83 |
| River, Czech Republic | 7 | 5-181 |
| River, Spain | 8 | 250-3'260 |

| Sampling site | Literature | mPCAs[ng/g dw] |
|---|---|---|
| Lake, Canada | 1 | 68 |
| Harbour, Canada | 9 | 290 |
| Rivers, Germany | 6 | <10-370 |
| Sea, Australia | 10 | 1'108-16'403 |

| Sampling site, this study | | TOC [%] | Total PCAs [ng/g dw]* | Total PCAs [µg/g TOC] |
|---|---|---|---|---|
| **River/sea sediments** | | | | |
| Seine estuary 1, 49°27.1´N/0°1.13´E, 03.2004 | | 1.6 | 147 | 6.7 |
| Seine estuary 2, 49°27.6´N/0°3.3´E, 03.2004 | | 0.07 | 84 | 120 |
| Seine estuary 3, 49°28.0´N/0°38.4´E, 03.2004 | | 0.8 | 87 | 10.9 |
| Hamburg harbour, 1, 12.2003 | | 3.1 | 116 | 3.7 |
| Hamburg harbour 2, 02.2004 | | 3.0 | 102 | 3.4 |
| Hamburg harbour 3, 12.2003 | | 2.2 | 154 | 7.0 |
| Tromsø 1, 69°38.6´N/18°57.1´E, 10.2002 | | 1.8 | 103 | 5.7 |
| Tromsø 2, 69°38.6´N/18°57.0´E, 10.2002 | | 2.4 | 71 | 2.9 |
| **Suspended particulate matter** | | | | |
| Elbe, 08.2002 | | 7.9 | 214 | 3.1 |
| North Sea 1, 51°30.0´N/2°40.0´E - 50°0.0´N/2°0.0´E, 07.2002 | | 5.7 | 181 | 3.2 |
| North Sea 2, 54°9.8´N/6°20.9´E, 08.2002 | | 10.7 | 293 | 2.7 |
| Neckar 1 | km 8, 5 August 2002 | 5.7 | 152 | 2.7 |
| | km 8, 2 September 2002 | 3.6 | | |
| | km 8, 25 November 2002 | 5.9 | | |
| | km 8, 16 December 2002 | 5.0 | | |
| Neckar 2 | km 165, 7 August 2002 | 3.4 | 530 | 15.6 |
| Rhine | km 334, 3 August 2002 | 5.2 | 187 | 3.6 |
| | km 334, 4 September 2002 | 1.9 | | |
| | km 334, 18 December 2003 | 4.4 | | |

1: Stern *et al.* (2000); 2: Muir *et al.* (2000b); 3: Schmid and Müller (1985); 4: Tomy *et al.* (1999a); 5: Marvin *et al.* (2003); 6: WHO (1996); Stejnarova *et al.* (2005); 8: Parera *et al.* (2004); 9: Muir *et al.* (2000); 10: Kemmlein *et al.* (2002). *: relative to *trans*-chlordane.

### 5.4.3.4 PCA formula group and congener profiles

Table 36 gives a survey over the relative formula and congener group compositions of the North and Baltic Sea sediments with total PCA concentrations > 50 ng/g dw. They were determined with $CH_4/CH_2Cl_2$-NICI, which is able to determine compounds with 3-10 Cl atoms. Moreover, these results were partly checked by ECNI-MS on a new instrument (Varian 1200L), which had a strongly improved sensitivity. The formula and congener group composition could be confirmed within the measuring uncertainty of the methodology (see chapter 5.3.3.3) but without the $Cl_3$-$Cl_4$-compounds. In addition, the relative formula and congener group fractions were also determined for river sediments and suspended particulate matter. The results are summarised in Table 37. The findings can be summarised as follows:

- PCAs with **$C_{13}$ and $C_{14}$ chain** and **4 to 6 Cl atoms** were the **main components** in the **marine sediments** with exception of $C_{10}H_{14}Cl_8$ for station 718 and $C_{12}H_{20}Cl_6$ for 710 (2001).
- **$C_{17}$ chains** were **not detected** in any sediment.
- **sPCAs in marine sediments** consisted to **50-87 % of $C_{12}$- and $C_{13}$-compounds** and **mPCAs between 56-81 % of $C_{14}$-constituents**. The content of $C_{16}$ chains length was maximum 12 % in mPCAs.

Differences were found between marine and river sediments as well as suspended particulate matter (SPM).

- The **chlorine content of sPCAs** was **lower in marine sediments** (51-59 %) than in river sediments/SPM (58-63 %). Main compounds were $C_{11}$ and $C_{12}$ chains with 7-8 Cl. However, the **chlorine content of mPCAs** and their composition was **comparable**.
- The **fraction of $C_{11}$ compounds** was somewhat **lower in marine sediments** (19-34 %) than in river sediments/SPM (24-43 %). Also sediments from Czech rivers contained a higher amount of $C_{11}$ congeners (Steinarova *et al.*, 2005). Moreover, a $C_{11}$ amount around 40 % was reported for SPM from German rivers and creeks (Maulshagen *et al.*, 2003).

Formula and congener group profiles are scarce in the literature. Moreover, one has to be cautious with comparisons due to different applied methods (see chapter 5.3.3.4).$C_{12}$ and $C_{13}$ chains dominated in sediments from Australia (Kemmlein *et al.*, 2002) and Lake Ontario (42-81 %, ECBR (2000)). Three of four sediments from Australia showed also higher $C_{15}$ than $C_{14}$ fractions and even $C_{16-17}$ were detectable (Kemmlein *et al.*, 2002). Higher $C_{13}$ fractions were reported from sites influenced by local sources (Marvin *et al.*, 2003). However, Štejnarova *et al.* (2005) found sediments dominated by $C_{11}$ congeners.

Formula and congener profiles from selected sediments and from technical mixtures were also compared by principal component analysis, a chemometric procedure. It allows to identify similarities and changes due to e.g. degradation between the PCA composition in technical products and real samples. The following technical PCA mixtures were used as reference:

- **sPCAs:** Mixture with 55,5 % Cl content (Dr. Ehrenstorfer, Germany); Hordalub 17, 80 and 500 (Hoechst, Germany).
- **mPCAs:** Mixture with 57 % Cl (Dr. Ehrenstorfer, Germany); Hordaflex SP, Hordalub 80EM, Chlorparaffin 40fl and 45fl (Hoechst, Germany); Cloparin 50 (Caffaro, Italy).

Figure 17 summarises the comparison of technical product and sediment compositions on the basis of the two principal components, which explain 78 % of the total variance for sPCA and 89 % for mPCA. The following conclusions could be drawn for sPCA:

- The technical products **Hordalub 80** and the **sPCA mixture 55.5 % Cl** of Dr. Ehrenstorfer had a **very similar composition**, which deviated from other commercial PCAs and sediments (Group 1)
- **Most of the sediments** formed a **separate cluster** (group 2), which had a certain resemblance with the technical product Hordalub 17 despite ist lower chlorine content of 51 %. Smaller differences within the group can be explained by the varying dominance of $C_{11}$ to $C_{13}$ congeners (see also Table 36).

- **Hordalub 500 did not fit into any group** due to a high content of $C_{11}$ (47 %) and sample 721 due to a $C_{13}$ fraction of 78 %.

The picture was much **more complex for mPCA**:

- A **certain similarity between Hordalub 80EM, Cloparin 50** and **most** of the selected **sediments** could be observed. Since only two main congener groups are present in mPCA ($C_{14\text{-}15}$), changes in their concentration ratio will directly influence the position in the principal components plot.
- The technical products **Chlorparaffin 45fl, Hordaflex SP** and the **mixture from Ehrenstorfer** deviated **much from sediments** due to significantly different $C_{14}/C_{15}$ ratios.

The similarity of the PCA patterns of some technical products with those in sediments might be an indication of ist major application. However, it cannot be excluded that fractionation, accumulation and degradation processes in sediments might result in a pattern similar to certain technical mixtures. Controlled degradation studies of technical PCA in activated sewage sludge could answer this question at least partially.

Moreover, the similarities and differences in the principal component plots do not mean, that a technical mixture is better suited as reference standard for quantification. Response factors are also influenced by the chlorine content within each congener group, which was not included in this comparison.

**Table 36:** Relative abundances obtained by $CH_4/CH_2Cl_2$-NICI-MS of sPCA- and mPCA congener groups in selected sediments from the North and Baltic Sea and in technical PCA mixtures. No determination was possible for samples with a total PCA level <50 ng/g dw (see **Table 34** and 5.4.3.1 for explanation).

| | Relative Abundance [%] | | | | | | | Main component | |
|---|---|---|---|---|---|---|---|---|---|
| | $C_{10}$ | $C_{11}$ | $C_{12}$ | $C_{13}$ | $C_{14}$ | $C_{15}$ | $C_{16}$ | sPCA | mPCA |
| | | | sPCA | | | mPCA | | | |
| **Baltic Sea** | | | | | | | | | |
| 710 (2001) | 6 | 10 | 45 | 40 | 65 | 35 | * | $C_{12}Cl_6$ | $C_{14}Cl_5$ |
| 710 (2002) | 8 | 25 | 28 | 38 | 78 | 22 | * | $C_{13}Cl_4$ | $C_{14}Cl_4$ |
| 710 (2004) | 15 | 20 | 25 | 39 | 66 | 28 | 6 | $C_{13}Cl_4$ | $C_{14}Cl_{4/5}$ |
| 715 (2001) | 8 | 11 | 39 | 42 | 59 | 41 | * | $C_{13}Cl_4$ | $C_{14}Cl_5$ |
| 715 (2004) | 13 | 20 | 28 | 39 | 61 | 30 | 9 | $C_{13}Cl_4$ | $C_{14}Cl_4$ |
| 718 (2001) | 33 | 16 | 23 | 27 | 70 | 30 | * | $C_{10}Cl_8$ | $C_{14}Cl_4$ |
| 718 (2004) | 13 | 21 | 29 | 38 | 61 | 30 | 9 | $C_{13}Cl_4$ | $C_{14}Cl_4$ |
| 721 (2001) | 6 | 7 | 9 | 78 | 62 | 38 | * | $C_{13}Cl_7$ | $C_{14}Cl_6$ |
| 721 (2002) | 7 | 35 | 27 | 31 | 70 | 30 | * | $C_{13}Cl_4$ | $C_{14}Cl_4$ |
| 721 | 13 | 19 | 27 | 42 | 57 | 32 | 12 | $C_{13}Cl_4$ | $C_{14}Cl_4$ |
| ECKFBU | 0 | 23 | 32 | 45 | 56 | 32 | 12 | $C_{13}Cl_4$ | $C_{14}Cl_4$ |
| ODER | 15 | 22 | 28 | 36 | 65 | 28 | 7 | $C_{13}Cl_4$ | $C_{14}Cl_4$ |
| RUDEN | 12 | 21 | 28 | 38 | 58 | 33 | 10 | $C_{13}Cl_4$ | $C_{14}Cl_5$ |
| **North Sea** | | | | | | | | | |
| KS8 (2003) | 8 | 17 | 28 | 47 | 57 | 32 | 11 | $C_{13}Cl_4$ | $C_{14}Cl_4$ |
| KS 8 (2004) | 16 | 23 | 28 | 36 | 65 | 28 | 7 | $C_{13}Cl_4$ | $C_{14}Cl_5$ |
| KS 11 (2002) | 10 | 25 | 30 | 34 | 81 | 19 | * | $C_{13}Cl_4$ | $C_{14}Cl_4$ |
| KS 11 (2003) | 14 | 22 | 25 | 40 | 62 | 28 | 10 | $C_{13}Cl_4$ | $C_{14}Cl_5$ |
| KS 11 (2004) | 0 | 20 | 32 | 48 | 59 | 28 | 12 | $C_{13}Cl_5$ | $C_{14}Cl_{4/5}$ |
| **Technical mixtures** | | | | | | | | | |
| Hordaflex SP | | | | | 53 | 48 | * | | $C_{14}Cl_6$ |
| Cloparin 50 | | | | | 69 | 31 | * | | $C_{14}Cl_6$ |
| Hordalub 80 EM | | | | | 55 | 44 | * | | $C_{14}Cl_5$ |
| Chlorparaffin 40fl | | | | | 67 | 33 | * | | $C_{14}Cl_4$ |
| Chlorparaffin 45fl | | | | | 31 | 69 | * | | $C_{15}Cl_5$ |
| mPCA 57.0 % Cl[a] | | | | | 80±4.2 | 20±4.1 | * | | $C_{14}Cl_6$ |
| Hordalub 17 | 7 | 34 | 34 | 25 | | | * | $C_{12}Cl_5$ | |
| Hordalub 80 | 9 | 36 | 34 | 19 | | | * | $C_{11}Cl_5$ | |
| Hordalub 500 | 9 | 47 | 30 | 11 | | | * | $C_{12}Cl_6$ | |
| sPCA 55.5 % Cl[a] | 9±0.3 | 39±2.3 | 33±2.2 | 19±0.5 | | | * | $C_{11}Cl_5$ | |

* $C_{16}$ not determined; [a] five parallels.

**Table 37:** Relative abundance of s- and mPCA formula and congener groups ($C_{10\text{-}15}Cl_{5\text{-}10}$), calculated molecular weight, chlorine content and main components in sediments and suspended matter from rivers. Determination was carried out by HRGC-ECNI-LRMS.

| | **Molecular weight** | | **% Cl** | | **Relative Abundance** | | | | | | **Main component** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **sPCA** | **mPCA** | **sPCA** | **mPCA** | **$C_{10}$** | **$C_{11}$** | **$C_{12}$** | **$C_{13}$** | **$C_{14}$** | **$C_{15}$** | **sPCA** | **mPCA** |
| **River sediments** | | | | | | | | | | | | |
| Seine 1 | 414 | 451 | 61 | 56 | 9 | 36 | 31 | 25 | 74 | 26 | $C_{11}Cl_7$ | $C_{14}Cl_7$ |
| Seine 2 | 381 | 445 | 58 | 56 | 12 | 36 | 21 | 30 | 61 | 39 | $C_{11}Cl_7$ | $C_{14}Cl_7$ |
| Seine 3 | 419 | 448 | 61 | 56 | 12 | 26 | 31 | 30 | 71 | 29 | $C_{11}Cl_7$ | $C_{14}Cl_7$ |
| Hamburg 1 | 447 | 442 | 65 | 55 | 9 | 45 | 31 | 16 | 59 | 41 | $C_{11}Cl_8$ | $C_{14}Cl_7$ |
| Hamburg 2 | 440 | 435 | 64 | 55 | 5 | 32 | 43 | 20 | 61 | 39 | $C_{12}Cl_8$ | $C_{14}Cl_6$ |
| Hamburg 3 | 414 | 441 | 64 | 55 | 39 | 28 | 23 | 10 | 59 | 41 | $C_{10}Cl_7$ | $C_{14}Cl_7$ |
| Tromsø 1 | 424 | 446 | 63 | 53 | 9 | 38 | 33 | 20 | 66 | 34 | $C_{11}Cl_7$ | $C_{14}Cl_7$ |
| Tromsø 2 | 429 | 453 | 62 | 57 | 10 | 24 | 37 | 30 | 60 | 40 | $C_{12}Cl_8$ | $C_{14}Cl_7$ |
| **Suspended particulate matter** | | | | | | | | | | | | |
| Elbe | 421 | 437 | 63 | 55 | 19 | 42 | 29 | 10 | 54 | 46 | $C_{11}Cl_7$ | $C_{15}Cl_6$ |
| Neckar 1, km 8 | | | | | | | | | | | | |
| 5.8.2002 | 426 | 465 | 63 | 58 | 12 | 36 | 32 | 21 | 69 | 31 | $C_{11}Cl_7$ | $C_{14}Cl_8$ |
| 2.9.2002 | 423 | 460 | 63 | 57 | 12 | 39 | 32 | 17 | 67 | 33 | $C_{11}Cl_7$ | $C_{14}Cl_8$ |
| 25.11.2002 | 424 | 458 | 63 | 57 | 21 | 35 | 28 | 16 | 79 | 21 | $C_{11}Cl_7$ | $C_{14}Cl_{7/8}$ |
| 16.12.2002 | 433 | 465 | 63 | 58 | 10 | 34 | 32 | 23 | 74 | 26 | $C_{12}Cl_8$ | $C_{14}Cl_{7/8}$ |
| Rhine, km 334 | | | | | | | | | | | | |
| 6.8.2002 | 429 | 455 | 63 | 57 | 11 | 30 | 36 | 24 | 65 | 35 | $C_{12}Cl_7$ | $C_{14}Cl_7$ |
| 4.9.2002 | 421 | 460 | 62 | 57 | 10 | 29 | 34 | 27 | 60 | 40 | C12Cl7 | C14Cl7 |
| 18.12.2003 | 429 | 452 | 63 | 57 | 12 | 33 | 36 | 20 | 71 | 29 | $C_{12}Cl_8$ | $C_{14}Cl_7$ |

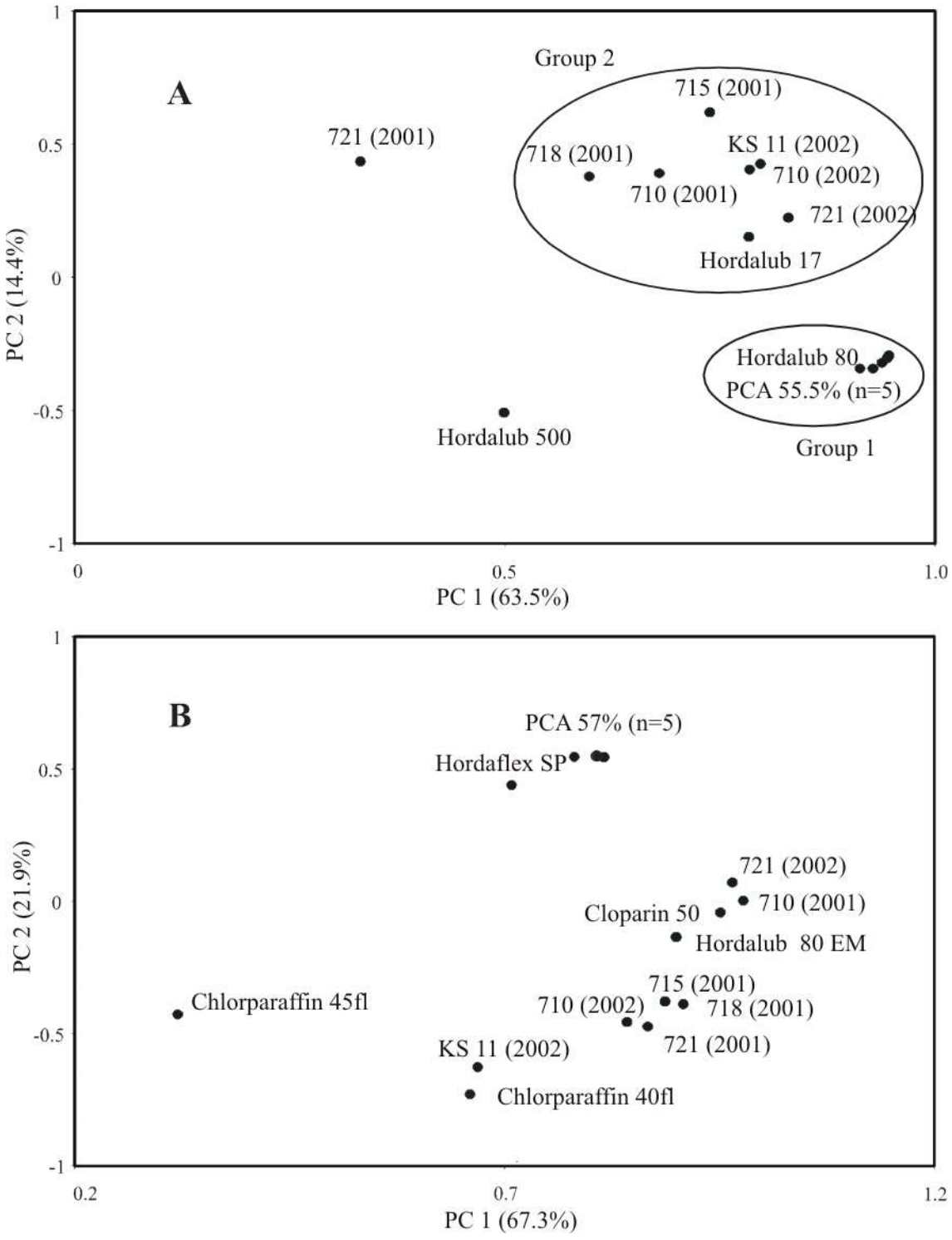


**Figure 22:** Principal component plots of the factor analysis of the congener group composition of sPCA (A) and mPCA (B) in technical mixtures from European producers and sediments from the North and Baltic Sea ( years 2001-2002). Results were obtained by $CH_4/CH_2Cl_2$-NICI-MS. Two groups with related patterns are marked for sPCAs.

#### 5.4.3.5 Chlordane concentrations in sediments

Table 38 shows the results for the chlordane representatives *cis/trans*-nonachlor and *cis/trans*-chlordane. Moreover, heptachlor was determined, but was only present in two samples. Figure 18 visualises the levels at most of the sampling points. Quantification was carried out by ECNI-MS on the Hewlett-Packard system. For quality control, some samples from 2003 and 2004 were analysed by ECNI on two mass spectrometers (Varian 1200L and Hewlett-Packard MS Engine) as well as by EI-MS/MS with an ion trap MS. In general, results were well comparable within 10 % with exceptions of up to 20 % mainly for the EI-MS/MS technique.

The following conclusion can be drawn:

- ***trans*-Nonachlor was most dominant** in the majority of sediments. This was not the case for literature data. However, a further control of the reference standards did not reveal any systematic error, and similar results were obtained for all three measuring techniques. Therefore, the results presented here are considered correct.
- ***cis/trans*-Nonachlor concentrations** were about **ten times higher in the Baltic Sea** (0.12-0.46 ng/g dw) than in the North Sea (0.001-0.34 ng/g dw). No reason can be given.
- ***cis/trans*-Chlordane** levels were partly higher in the North Sea than in the Baltic Sea, when compared on a wet weight basis. It was vice versa expressed on TOC content.
- **Concentrations** of ***cis*-and of *trans*-chlordane** were **comparable**.
- **Very little information** about **chlordane concentrations** in sediments is available **in the literature**. Overall, **chlordane concentrations in the sediments** analysed in this project were within the same order of magnitude **as those published**.
- Strandberg *et al.* (1998b) found in sediments from the northern Baltic Sea somewhat higher concentrations of *trans*-nonachlor (0.006-0.13 ng/g dw) compared to cis-nonachlor (0.03-0.05 ng/g dw) and of cis-chlordane (0.09-0.19 ng/g dw) relative to *trans*-chlordane. Heptachlor was not detectable.

- Sum levels of the four isomers were 0.02-0.2 ng/g dw in sediments from New Zealand (Simpson *et al.*, 1996). Heptachlor was non-detectable. Mekong sediments from Vietnam contained 0.02-0.34 ng/g dw of *trans*-nonachlor, < 0.02-0.14 ng/g dw of *trans*-chlordane and 0.02 to 0.36 ng/g dw of *cis*-chlordane (Hung *et al.*, 2004).

**Table 38:** Concentrations of chlordanes [ng/g dry weight respective in ng/g TOC] for sediments from the North and Baltic Sea. The internal standard 4,5-DCCD was not added to the samples of 2002. Therefore, *trans*-nonachlor, which elutes in the fractions F1 and F2 was not quantifiable.

| Sample | *trans*-Nonachlor ng/g dw (ng/g TOC) | *cis*-Nonachlor ng/g dw (ng/g TOC) | *trans*-Chlordane ng/g dw (ng/g TOC) | *cis*-Chlordane ng/g dw (ng/g TOC) | Heptachlor ng/g dw (ng/g TOC) |
|---|---|---|---|---|---|
| **Baltic Sea** | | | | | |
| 710 (2001) | 0.35 (14) | 0.02 (<1) | 0.04 (1) | 0.03 (1) | <0.030 |
| 710 (2002) | na | 0.01 (<1) | 0.04 (1) | 0.07 (1) | <0.030 |
| 710 (2004) | 0.12 (25) | 0.002 (<1) | 0.006 (1) | 0.008 (2) | <0.030 |
| 715 (2001) | 0.14 (4) | 0.02 (<1) | 0.05 (2) | 0.04 (1) | <0.030 |
| 715 (2004) | 0.42 (14) | 0.021 (1) | 0.042 (1) | 0.031 (1) | <0.030 |
| 718 (2001) | 0.20 (7) | 0.02 (<1) | 0.06 (2) | 0.03 (1) | <0.030 |
| 718 (2004) | 0.30 [a] | 0.019 | 0.047 | 0.041 | <0.030 |
| 721 (2002) | na | 0.03 (<1) | 0.06 (1) | 0.06 (1) | <0.030 |
| 721 (2004) | 0.30 (8) | 0.029 (1) | 0.135 (3) | 0.126 (3) | 0.064 (1) |
| ECKFBU (2004) | 0.44 (11) | 0.019 (<1) | 0.036 (1) | 0.035 (1) | <0.030 |
| ODER (2004) | 0.36 [a] | 0.017 | 0.059 | 0.060 | 0.042 |
| RUDEN (2004) | 0.33 (12) | 0.006 (<1) | 0.006 (<1) | 0.016 (1) | <0.030 |
| **North Sea** | | | | | |
| KS 8 (2002) | na | < 0.002 | 0.002 (1) | 0.007 (2) | <0.030 |
| KS8 (2003) | 0.30 (36) | 0.008 (1) | 0.016 (2) | 0.019 (2) | <0.030 |
| KS8 (2004) | 0.34 (34) | 0.006 (<1) | 0.021 (2) | 0.018 (2) | <0.030 |
| KS 11 (2002) | na | 0.007 (<1) | 0.04 (2) | 0.08 (5) | <0.030 |
| KS11 (2003) | 0.28 (26) | 0.005 (<1) | 0.013 (1) | 0.010 (1) | <0.030 |
| KS11 (2004) | na | 0.012 (<1) | 0.027 (1) | 0.035 (1) | <0.030 |
| BL 2 (2002) | na | 0.004 (<1) | 0.008 (1) | 0.01 (2) | <0.030 |
| BL 4 (2002) | na | < 0.002 | 0.001 (1) | < 0.007 | <0.030 |
| UE 18 (2002) | na | < 0.002 | < 0.002 | < 0.007 | <0.030 |
| UE 28 (2002) | na | < 0.002 | < 0.002 | < 0.007 | <0.030 |
| ES 1 (2003) | 0.01 (22) | < 0.002 | 0.01 (16) | 0.01 (16) | <0.030 |
| TI 13 (2003) | 0.006 (9) | < 0.002 | 0.01 (14) | 0.008 (11) | <0.030 |
| UE 15 (2003) | 0.006 (2) | < 0.002 | 0.03 (11) | 0.01 (4) | <0.030 |
| UE20 (2003) | 0.01 (3) | 0.002 (1) | 0.04 (10) | 0.01 (4) | <0.030 |
| L1 (2003) | 0.06 (48) | < 0.002 | 0.005 (4) | < 0.007 | <0.030 |
| WB 5 (2003) | 0.03 (6) | < 0.002 | 0.006 (1) | 0.01 (2) | <0.030 |
| UE 67 (2003) | 0.001 (6) | < 0.002 | 0.11 (87) | 0.03 (22) | <0.030 |
| UE 70 (2003) | 0.001 (10) | < 0.002 | 0.10 (142) | < 0.007 | <0.030 |

na: not analysed; [a] TOC not available

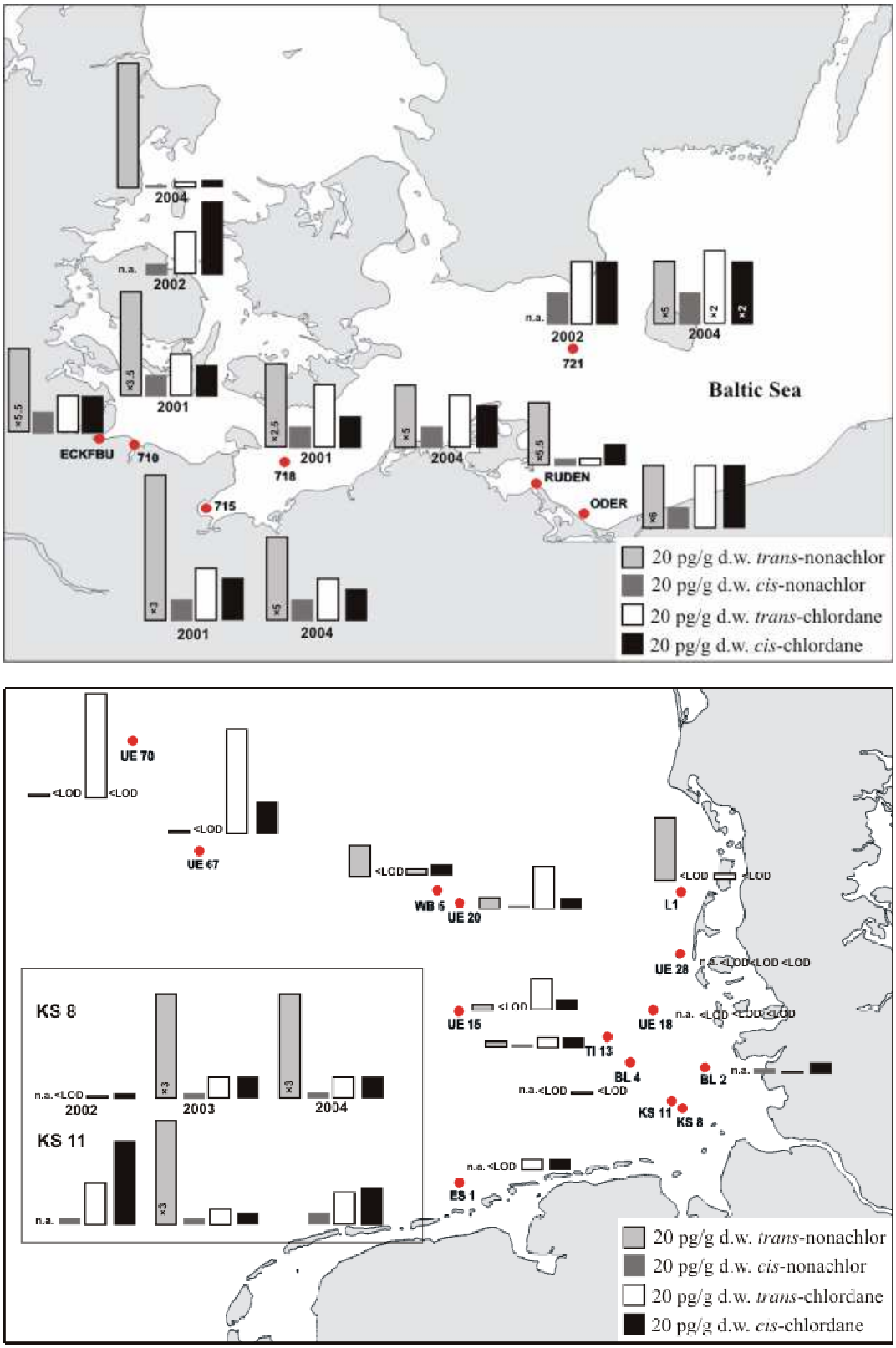


**Figure 23:** Concentrations of chlordanes in sediments from the Baltic and North Sea (ECNI-MS) at selected sampling sites in 2001-2004.

### 5.4.4 PCAs in water

Only two sea water samples were analysed due to the expected very low concentrations. Samples of 100 l were taken at the stations T9a (55°0,00`N/8°15,0`O, Lister Tief) und Cuxhaven (53°52,0`N/8°44,5`O) by the Federal Maritime and Hydrographic Agency in Hamburg (Germany, Bundesamt für Seeschifffahrt und Hydrographie (BSH)) in September 2002.

100 l of water were extracted at the BSH with 1000 ml of n-pentane. Prefractionation was carried out on silica gel. Fraction 1 was eluted with dichloromethane/n-hexane 1+1 (v/v) and fraction 2 with acetone.

No PCAs could be detected by EI-MS/MS at a detection limit of 0.4 ng/l water, which corresponds to an absolute detection limit of 0.2 ng for the most abundant fragmentation. The determination of PCAs in surface water in Germany in 1987 and 1994 showed a remarked decrease of concentrations (with the reservation concerning the reliability of data given in chapter 5.3.3.4) as Table 39 shows (WHO, 1996). 50-190 ng/l were detected in 1994 compared to several thousands of ng/l in 1987. However, no information about blank controls are presented, which is a very critical factor.

Since then, a further decrease in the PCA-concentrations in surface water can be assumed due to the partly ban of the use of sPCA. Moreover, a further dilution takes place in the Sea. Therefore, despite the currently very low detection limit of 0.4 ng/l no PCA was found sea water.

**Table 39:** Concentrations of sPCAs und mPCAs in surface water in Germany (WHO, 1996).

| River, Location | 1987 [ng/l] | | 1994 [ng/l] | |
|---|---|---|---|---|
| | sPCAs | $C_{14-18}$ | sPCAs | $C_{14-17}$ |
| Lech near Augsburg | | | 50 | <50 |
| Lech near Gersthofen, upstream PCA manufacturer | 500 | 4'500 | 80 | 90 |
| Lech near Langweid, downstream of PCA manufacturer | 600 | 4'000 | 100 | 190 |
| Lech near Rain | | | 120 | 170 |
| Donau near Marxheim, upstream concluence of river Lech | 1'200 | 4'000 | 60 | <60 |
| Donau near Marxheim, upstream concluence of river Lech | 1'200 | 20'000 | 60 | 70 |

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## 5.6 Appendix 1: Methodology

### 5.6.1 Employed PCA reference standards

PCA reference mixtures could only be purchased from Ehrenstorfer (Augsburg, Germany). Further references were supplied from the state laboratory of Basel-Stadt, from a manufacturer (Caffaro, Italien) and from LCG (Teddington, UK). Table 40 summarises the available reference mixtures and their composition.

**Table 40:** Composition and source of the sPCA and mPCA reference mixtures employed for quantification or for composition characterisation.

| Name | Company | Chain length | Chlorine content |
|---|---|---|---|
| **sPCA mixtures** | | | |
| Hordalub 17 | Hoechst | $C_{10}$-$C_{13}$ | 49 % |
| Hordalub 80 | Hoechst | $C_{10}$-$C_{13}$ | 56 % |
| Hordalub 500 | Hoechst | $C_{10}$-$C_{13}$ | 62 % |
| Cereclor 60 L | ICI | $C_{10}$-$C_{13}$ | 59 % |
| Cereclor 70 L | ICI | $C_{10}$-$C_{13}$ | 69 % |
| **Standard solutions** | | | |
| Chloroparaffin | Ehrenstorfer | $C_{10}$-$C_{13}$ | 51.5 % |
| Chloroparaffin | Ehrenstorfer | $C_{10}$-$C_{13}$ | 63 % |
| Chloroparaffin | Ehrenstorfer | $C_{10}$-$C_{13}$ | 55.5 % |
| **mPCA mixtures** | | | |
| Cloparin 50 | Caffaro | $C_{14}$-$C_{17}$ | 51 % |
| Cloparin 51 PL | Caffaro | ? | 51 % |
| Cloparol 49 ST | Caffaro | ? | 49 % |
| Chlorparaffin 40 fl. | Hoechst | $C_{14}$-$C_{17}$ | 41 % |
| Chlorparaffin 45 fl. | Hoechst | $C_{14}$-$C_{17}$ | 45 % |
| Hordalub 80 EM | Hoechst | $C_{14}$-$C_{17}$ | 49 % |
| Hordaflex SP | Hoechst | $C_{14}$-$C_{17}$ | 56 % |

| | | | |
|---|---|---|---|
| Cereclor | ICI | $C_{14}$-$C_{17}$ | 52 % |
| Cereclor | ICI | $C_{14}$-$C_{17}$ | 53 % [a] |
| Cereclor | ICI | $C_{14}$-$C_{17}$ | 53 % [a] |
| Cereclor | ICI | $C_{14}$-$C_{17}$ | 53 % [a] |
| Cereclor | ICI | $C_{14}$-$C_{17}$ | 54 % [a] |
| Cereclor | ICI | $C_{14}$-$C_{17}$ | 55 % [a] |
| Cereclor | ICI | $C_{14}$-$C_{17}$ | 55 % [a] |
| mPCAs | Ehrenstorfer | $C_{14}$-$C_{17}$ | 47 % |
| mPCAs | Ehrenstorfer | $C_{14}$-$C_{17}$ | 52 % |
| mPCAs | Ehrenstorfer | $C_{14}$-$C_{17}$ | 57 % |

[a] calculated chlorine content based on ECNI results, no data available from manufacturer.
ICI: Imperial Chemical Industries (England)

### 5.6.2 Reference standards for chlordane

The reference compounds listed in Table 41 were used for method development, validation and quantification of chlordanes.

**Table 41:** Manufacturer and concentrations chlordane solutions used for quantification. The purity was >99 % except for MC8 and 4,5-dichlorchlordene.

| Compound | Manufacturer | Concentration |
|---|---|---|
| *trans*-Chlordane | Promochem | 8.08 ng/µl* |
| *cis*-Chlordane | Promochem | 4.98 ng/µl* |
| *trans*-Nonachlor | Promochem | 43.18 ng/µl* |
| *cis*-Nonachlor | Promochem | 44.32 ng/µl* |
| *cis*-Heptachlorepoxide | Ehrenstorfer | 10 ng/µl* |
| *trans*-Heptachlorepoxide | Ehrenstorfer | 10 ng/µl* |
| Heptachlor | Promochem | 188.4 ng/µl* |
| Oxychlordane | Ehrenstorfer | 10 ng/µl* |
| $^{13}C_{10}$-*trans*-Chlordane | Cambridge Isotope Laboratories | 100 ng/µl** |
| MC8 | Karlsson (2000) | 1 ng/ µl* |
| 4,5-Dichlorochlorden | [29] | 1 ng/ µl* |
| Octachloronaphthalene | Ehrenstorfer | 10 ng/µl* |
| Tetrachloronaphthalene | Ehrenstorfer | 10 ng/µl* |

* in cyclohexane, ** in *n*-nonane

### 5.6.3 Clean-up procedure for fish (PCAs and chlordanes)

Up to 7 g of the pooled fish liver samples were mixed with the tenfold quantity of water-free sodium sulphate (dried at 600 °C overnight) in a mortar. The free-flowing powder was transferred to a glass column (length 30 cm, ID 2.0 cm). 10 ng of the internal standards $^{13}C_{10}$-*trans*-chlordane dissolved in 10 µl of cyclohexane were added to the top of the column. Elution was carried out by drop wise addition of 250 ml of *n*-hexane/dichloromethane (1+1, v/v). The eluate was completely evaporated to dryness with a Turbo Vap (Zymark, Hopkinton, USA).

Lipid removal was carried out with a glass column (length 30.0 cm, ID 2.0 cm) filled from bottom to top as follows: 1 g of water-free sodium sulphate, 40 g of a mixture of silica gel/conc. sulphuric acid (44 % (weight) of conc. $H_2SO_4$) and 1 g of water-free sodium sulphate again. The system was conditioned with 60 ml of *n*-hexane/dichloromethane (1+1, v/v). A pressure of 96.5 hPa was applied with a membrane pump (Elite 801, Hagen, Köln, Germany, flow 1.3 l/min). The sample was transferred to the top of the column and eluted under pressure with totally 130 ml of *n*-hexane/dichloromethane (1 + 1, v/v). The solution was concentrated to ca. 150 µl with a Turbo Vap, 10 ml of *n*-hexane were added twice followed by a volume reduction to ca. 200 µl.

Interfering compounds were removed by column chromatography on a glass column (length 30 cm, ID 1.5 cm) filled with (bottom to top): 1 g of water-free sodium sulphate, 16 g of Florisil® (deactivated with 1.5 % water, 60-100 mesh, Fluka, Buchs, Switzerland) and 1 g of water-free sodium sulphate. Conditioning was carried out with 30 ml of *n*-hexane. After transfer of the sample, the pre-fraction was eluted with 60 ml of *n*-hexane followed by 7 ml of dichloromethane. The fraction containing PCA was eluted with 60 ml of dichloromethane and concentrated to ca. 100 µl with a Turbo Vap. 20 ml of cyclohexane were added and the volume reduced again to 100 µl. The sample was ready for quantification after addition of 10 ng of the recovery standard ε-HCH (dissolved in 10 µl of cyclohexane).

### 5.6.4 Clean-up procedure for sediments (PCAs and chlordanes)

Sediments were dried at room temperature for 10 days (open-air, fume hood) and sieved with 2 mm mesh size. Depending on the TOC content, 2-50 g sediment were taken, and 10 µl of a solution containing $^{13}C_{10}$-*trans*-chlordane and octachloronaphthalene in cyclohexane (concentration of 1 ng/µl each) were added as internal standards. The dried sediment was filled into a pre-cleaned Soxhlet thimble (heated to 300 °C for 6 h, made from glass fibres, 30 mm diameter, 100 mm length, Schleicher&Schüll, Germany). Elemental sulphur was removed with activated copper powder (230 mesh, Merck, Germany; activated with concentrated nitric acid) during the Soxhlet extraction. Soxhlet

extraction was carried out overnight with 200 ml of *n*-hexane/dichloromethane (1+1, v/v). The extract was concentrated to ca. 200 µl with a Turbo Vap (Zymark, USA), 10 ml of *n*-hexane were added twice followed by a volume reduction to ca. 200 µl.

Sample clean-up was carried out on a glass column (length 30 cm, ID 1.3 cm) filled from bottom to top with: 1 g of water-free sodium sulphate, 16 g of Florisil® (60-100 mesh, Fluka, Buchs, Switzerland, deactivated with 1.5 % of water) and 1 g of water-free sodium sulphate again.

Conditioning was carried out with 30 ml of *n*-hexane. After transfer of the sample, the pre-fraction was eluted with 60 ml of *n*-hexane followed by 5 ml of dichloromethane. The fraction containing CP was eluted with 55 ml of dichloromethane and concentrated to ca. 200 µl with a Turbo Vap. 10 ml of cyclohexane were added twice and the volume reduced between to 100 µl. The sample was ready for quantification after volume reduction to 100 µl and addition of 10 ng of the recovery standard ε-HCH (dissolved in 10 µl of cyclohexane).

Sediments with a higher TOC-content (> 1%) and Baltic Sea sediments in general required a further clean-up step directly after extraction. A column filled with silica gel/conc. sulphuric acid was employed analogue to biota to remove matrix compounds and a yellow to greenish colour. Only 20 g of the mixture silica gel/conc. sulphuric acid (44 % weight) were used and eluted with 70 ml of *n*-hexane/dichloromethane (1+1, v/v).

### 5.6.5 Quantification of PCAs by ECNI-LRMS

Gas chromatographic separations were carried out on an HP 5890II gas chromatograph (Hewlett Packard, Palo Alto, USA) equipped with an Hewlett Packard 7673 auto sampler. A fused silica capillary was employed of 15 m length and 0.25 mm ID coated with a 0.25 µm film of DB5-MS (5 % phenyl-95 %-methylsiloxane, J&W Scientific, Folsom, USA). The injected volume was 1.5 µl in the splitless mode (2 min splitless time). The injector temperature was 275 °C. He was used as carrier gas (99,999 % purity, Carbagas, Switzerland) at a head pressure of 68.9 kPa (10 psi). The following

temperature programme was employed: 100 °C isothermal for 2 min, with 15 °C/min to 280 °C, isothermal for 8 min or alternatively 100 °C isothermal for 2 min, with 10 °C/min to 260 °C, isothermal for 10 min.

An HP 5989B mass spectrometer was applied in the ECNI mode (Hewlett Packard, Palo Alto, USA). Regular performance optimisation was carried out with perfluoro-tributylamine selecting the mass fragments *m/z* 283.0, 414.0 and 453.0. The temperature of the transfer line was 280 °C, of the ion source 200 °C and of the quadrupole 100 °C. Methane was used as reagent gas (99,995 %, Carbagas, Switzerland) at a pressure of 1-1.6 hPa (0.9-1.1 Torr). Table 42 and Table 43 summarise the selected masses in the selected ion monitoring mode. The most abundant chlorine isotope signal was selected for quantification and the second one for confirmation.

Formula and congener patterns were determined on a CP-3800 (Varian, Walnut Creek, USA) gas chromatograph. A fused silica capillary was employed of 15 m length and 0.25 mm ID coated with a 0.25 µm film of DB5-MS (5 %-phenyl-95 %-methylsiloxane, J&W Scientific, Folsom, USA). The injected volume was 2.0 µl in the splitless mode (3 min splitless time) using an autosampler (Combi Pal autosampler, CTC Analytics, Switzerland). The injector temperature was 275 °C. He was used as carrier gas (99,999 % purity, Carbagas, Switzerland) at a head pressure of 68.9 kPa (10 psi). The following temperature programme was employed: 100 C isothermal for 2 min, with 10 °C/min to 260 °C, isothermal for 10 min.

**Table 42:** Mass-to-charge ratios of the $[M\text{-}Cl]^-$ ions (abbreviated as X in the table) of the two most abundant isotope signals of sPCA and mPCA congeners used for quantification and identification in the ECNI-MS mode.

| Short chain CPs | | | Medium chain CPs | | |
|---|---|---|---|---|---|
| **sPCA congener** | **Most abundant isotope (100 %)** | **Second abundant isotope** | **mPCA congener** | **Most abundant isotope (100 %)** | **Second abundant isotope** |
| $C_{10}H_{18}Cl_4$ | 243.1 (X) | 245.1 (X+2, 96 %) | $C_{14}H_{26}Cl_4$ | 299.1 (X) | 301.1 (X+2, 96 %) |
| $C_{10}H_{17}Cl_5$ | 279.0 (X+2) | 277.0 (X, 78 %) | $C_{14}H_{25}Cl_5$ | 335.1 (X+2) | 333.1 (X, 78 %) |
| $C_{10}H_{16}Cl_6$ | 312.9 (X+2) | 314.9 (X+4, 64 %) 64 % | $C_{14}H_{24}Cl_6$ | 369.0 (X+2) | 371.0 (X+4, 64 %) |
| $C_{10}H_{15}Cl_7$ | 346.9 (X+2) | 348.9 (X+4, 80 %) | $C_{14}H_{23}Cl_7$ | 403.0 (X+2) | 405.0 (X+4, 80 %) |
| $C_{10}H_{14}Cl_8$ | 380.9 (X+2) | 382.9 (X+4, 96 %) | $C_{14}H_{22}Cl_8$ | 436.9 (X+2) | 438.9 (X+4, 96 %) |
| $C_{10}H_{13}Cl_9$ | 416.8 (X+4) | 414.8 (X+2, 89 %) | $C_{14}H_{21}Cl_9$ | 472.9 (X+4) | 470.9 (X+2, 89 %) |
| $C_{10}H_{12}Cl_{10}$ | 450.8 (X+4) | 448.8 (X+2, 78 %) | $C_{14}H_{20}Cl_{10}$ | 506.9 (X+4) | 504.9 (X+2, 78 %) |
| | | | | | |
| $C_{11}H_{20}Cl_4$ | 257.1(X) | 259.1 (X+2, 96 %) | $C_{15}H_{28}Cl_4$ | 313.1 (X) | 315.1 (X+2, 96 %) |
| $C_{11}H_{19}Cl_5$ | 293.0 (X+2) | 291.0 (X, 78 %) | $C_{15}H_{27}Cl_5$ | 349.1 (X+2) | 347.1 (X, 78 %) |
| $C_{11}H_{18}Cl_6$ | 327.0 (X+2) | 329.0 (X+4, 64 %) | $C_{15}H_{26}Cl_6$ | 383.0 (X+2) | 385.0 (X+4, 64 %) |
| $C_{11}H_{17}Cl_7$ | 360.9 (X+2) | 362.9 (X+4, 80 %) | $C_{15}H_{25}Cl_7$ | 417.0 (X+2) | 419.0 (X+4, 80 %) |
| $C_{11}H_{16}Cl_8$ | 394.9 (X+2) | 396.9 (X+4, 96 %) | $C_{15}H_{24}Cl_8$ | 451.0 (X+2) | 453.0 (X+4, 96 %) |
| $C_{11}H_{15}Cl_9$ | 430.9 (X+4) | 428.9 (X+2, 89 %) | $C_{15}H_{23}Cl_9$ | 486.9 (X+4) | 484.9 (X+2, 89 %) |
| $C_{11}H_{14}Cl_{10}$ | 464.8 (X+4) | 462.8 (X+2, 78 %) | $C_{15}H_{22}Cl_{10}$ | 520.9 (X+4) | 518.9 (X+2, 78 %) |
| | | | | | |
| $C_{12}H_{22}Cl_4$ | 271.1 (X) | 273.1 (X+2, 96 %) | $C_{16}H_{30}Cl_4$ | 327.1 (X) | 329.1 (X+2, 96 %) |
| $C_{12}H_{21}Cl_5$ | 307.0 (X+2) | 305.1 (X, 78 %) | $C_{16}H_{29}Cl_5$ | 363.1 (X+2) | 361.1 (X, 78 %) |
| $C_{12}H_{20}Cl_6$ | 341.0 (X+2) | 343.0 (X+4, 64 %) | $C_{16}H_{28}Cl_6$ | 397.0 (X+2) | 399.0 (X+4, 64 %) |
| $C_{12}H_{19}Cl_7$ | 374.9 (X+2) | 376.9 (X+4, 80 %) | $C_{16}H_{27}Cl_7$ | 431.0 (X+2) | 433.0 (X+4, 80 %) |
| $C_{12}H_{18}Cl_8$ | 408.9 (X+2) | 410.9 (X+4, 96 %) | $C_{16}H_{26}Cl_8$ | 465.0 (X+2) | 467.0 (X+4, 96 %) |
| $C_{12}H_{17}Cl_9$ | 444.9 (X+4) | 442.9 (X+2, 89 %) | $C_{16}H_{25}Cl_9$ | 500.9 (X+4) | 498.9 (X+2, 89 %) |
| $C_{12}H_{16}Cl_{10}$ | 478.8 (X+4) | 476.8 (X+2, 78 %) | $C_{16}H_{24}Cl_{10}$ | 534.9 (X+4) | 532.9 (X+2, 78 %) |
| | | | | | |
| $C_{13}H_{24}Cl_4$ | 285.1 (X) | 287.1 (X+2, 96 %) | $C_{17}H_{32}Cl_4$ | 341.1 (X) | 343.1 (X+2, 96 %) |
| $C_{13}H_{23}Cl_5$ | 321.1 (X+2) | 319.1 (X, 78 %) | $C_{17}H_{31}Cl_5$ | 377.1 (X+2) | 375.1 (X, 78 %) |
| $C_{13}H_{22}Cl_6$ | 355.0 (X+2) | 357.0 (X+4, 64 %) | $C_{17}H_{30}Cl_6$ | 411.1 (X+2) | 413.1 (X+4, 64 %) |
| $C_{13}H_{21}Cl_7$ | 389.0 (X+2) | 391.0 (X+4, 80 %) | $C_{17}H_{29}Cl_7$ | 445.0 (X+2) | 447.0 (X+4, 80 %) |
| $C_{13}H_{20}Cl_8$ | 422.9 (X+2) | 424.9 (X+4, 96 %) | $C_{17}H_{28}Cl_8$ | 479.0 (X+2) | 481.0 (X+4, 96 %) |
| $C_{13}H_{19}Cl_9$ | 458.9 (X+4) | 456.9 (X+2, 89 %) | $C_{17}H_{27}Cl_9$ | 514.9 (X+4) | 512.9 (X+2, 89 %) |
| $C_{13}H_{18}Cl_{10}$ | 492.9 (X+4) | 490.9 (X+2, 78 %) | $C_{17}H_{26}Cl_{10}$ | 548.9 (X+4) | 546.9 (X+2, 78 %) |

A triple quadrupole mass spectrometer was applied in the ECNI mode (1200L, Varian, Walnut Creek, USA). Regular performance optimisation of both quadrupoles was carried out. The temperature of the transfer line was 280 °C, of the ion source 200 °C and of the manifold 40 °C. Methane was used as reagent gas (99,995 %, Carbagas, Switzerland) at a pressure of 7.3 mbar (5.5 Torr). The dwell time was 0.25 ms per ion group. Table 42 and Table 43 summarised the selected masses in the selected ion monitoring mode.

**Table 43:** Mass-to-charge ratios of ion selected for the internal standards ε-HCH and $^{13}C_{10}$-*trans*-chlordane as well as for other polychlorinated compounds included during method development. The ECNI-MS mode was applied.

| Compound | Ion | *m/z* | *m/z* most abundant isotope |
|---|---|---|---|
| α-HCH | $[M-Cl]^-$ | 253 | 255 |
| ε-HCH | $[M-Cl]^-$ | 253 | 255 |
| $^{13}C_{10}$-*trans*-Chlordane | $[M]^-$ | 416 | 420 |
| *cis*-Chlordane | $[M]^-$ | 406 | 410 |
| *cis*-Nonachlor | $[M]^-$ | 440 | 444 |
| Heptachlor | $[M-2Cl]^-$ | 299 | 302 |
| Octachloronaphthalene | $[M-2Cl]^-$ | 332 | 334 |
| p,p'-DDT | $[M+H-3Cl]^-$ | 248 | 248 |
| PCB 153 | $[M]^-$ | 358 | 360 |
| Toxaphene #44 | $[M-Cl]^-$ | 373 | 377 |
| Toxaphene #62 | $[M-Cl]^-$ | 373 | 377 |
| *trans*-Chlordane | $[M]^-$ | 406 | 410 |
| *trans*-Nonachlor | $[M]^-$ | 440 | 444 |

### 5.6.6 Quantification of PCAs by ECNI-HRMS

A comparison between ECNI-HRMS and ECNI-LRMS combined with the newly developed clean-up was carried out to ensure, that the latter has the necessary selectivity for PCA. The ECNI-HRMS measurements were carried out at the Norwegian Institute for Air Research (Kjeller, Norway). Results obtained by LRMS and HRMS were well comparable (see chapter 5.3.3.3).

Gas chromatographic separations were carried out with an HP 5890II gas chromatograph (Hewlett Packard, Palo Alto, USA) equipped with a split/splitless injector and a capillary of 25 m length and 0.20 mm ID coated with a 0.33 µm film of HP-1 (100 % dimethylpolysiloxane, Hewlett Packard, Palo Alto, USA). A sample volume of 1 µl was injected in the splitless mode (splitless time 2 min). The injector temperature was 260 °C. He (99,999 %) was used as carrier gas at an inlet pressure of 138 kPa. The temperature programme was as follows: 150 °C for 2 min, then with 7 °C/min to 260 °C, isothermal for 8 min and finally with 10 °C/min to 280 °C, isothermal for 13 min.

A VG Autospec (Micromass, Manchester, UK) mass spectrometer was employed in the ECNI-mode. Argon was applied as reagent gas at an ion source pressure of $2 \cdot 10^{-5}$ mbar. The mass resolution was 12'000 at an acceleration voltage of 6 kV. The ion source temperature was set to 170 °C, the filament current to 0.3-1 mA and the electron energy to 25-40 eV. Perfluorokerosene was used for mass calibration. The most abundant isotope signals of the $[M\text{-}Cl]^-$ ions were recorded in the selected ion mode for PCAs, and $[M]^-$ or $[M\text{-}4Cl\text{-}2H]^-$ ions for $^{13}C_{10}$-*trans*-chlordane. The dwell time per ion was 50 ms.

### 5.6.7 Group pattern determination of PCAs by $CH_4/CH_2Cl_2$-NICI

Gas chromatographic separations was carried out on an HP 5890II gas chromatograph (Hewlett Packard, Palo Alto, USA), equipped with a Hewlett Packard 7673 auto sampler. A fused silica capillary was employed of 15 m length and 0.25 mm ID coated with a 0.25 µm film of DB5-MS (5%-phenyl-95%-methylsiloxane, J&W Scientific, Folsom, USA). The injected volume was 1.5 µl in the splitless mode (2 min splitless time). The injector temperature was 275 °C. He was used as carrier gas (99,999 % purity, Carbagas, Switzerland) at a head pressure of 68,9 kPa (10 psi). The following temperature programme was employed: 100 °C isothermal for 2 min, with 10 °C/min to 260 °C, isothermal for 10 min.

An HP 5989B mass spectrometer was applied in the ECNI mode (Hewlett Packard, Palo Alto, USA). Regular performance optimisation was carried out with perfluorotributylamine selecting the mass fragments *m/z* 283.0, 414.0 and 453.0. The temperature of the transfer line was 270 °C, of the ion source 200 °C and of the quadrupole 100 °C. Dichloromethane (99.8 %, Scharlau, Barcelona, Spain)was mixed with methane at a pressure ratio of 1+4 and a total reagent gas pressure of 200 Pa (1,5 Torr) using a modified reagent gas inlet (Zencak *et al.*, 2003). The most abundant isotope masses of the $[M+Cl]^-$ adducts and of the internal standard $^{13}C_{10}$-*trans*-Chlordan were recorded in the selective ion mode (Zencak *et al.*, 2003).

### 5.6.8 Quantification of PCAs by EI-MS/MS

Gas chromatographic separations were carried out on a Star 3400 CX gas chromatograph (Varian, Wallnut Creek, USA) using a capillary of 5 m length and 0.25 mm ID coated with 0.25 µm of DB35-MS (35 % phenyl-methylpolysiloxane, J&W Scientific, Folsom, USA). A restriction capillary of 9 cm length and 0.05 mm ID was connected to the outlet to reduce the flow into the ion trap mass spectrometer. The injector temperature was 250 °C. Helium (99,999 %, Carbagas, Switzerland) was used as carrier gas at a pressure of 68.9 kPa. The temperature programme was as follows: 100 °C for 1 min, then with 50 °C/min to 300 °C, isothermal for 4 min. Splitless injections (splitless time 1.5 min) of 2.5 µl sample volume were carried with a Varian 8200 autosampler.

A Saturn 2000 (Varian, Wallnut Creek, USA) ion trap mass spectrometer was employed. The following EI-MS/MS conditions were applied: Electron energy 70 eV, filament current 70 µA, scan range *m/z* 50-300, scan time 0.3 s, maximum ionisation time 25 ms, isolation time 5 ms and excitation time 20 ms. The selected ions to be fragmented as well as further parameters are given in Table 44. The precursor ion *m/z* 383 $[M-Cl]^+$ and the product ion *m/z* 276 $[M-4Cl]^+$ were selected for the internal standard $^{13}C_{10}$-*trans*-chlordane (excitation amplitude 0.85 V and excitation storage level *m/z* 169,0).

Additionally, a Varian triple quadrupole mass spectrometer (Wallnut Creek, USA) was used. Gas chromatographic separations were carried out on a CP-3800 gas chromatograph (Varian, Wallnut Creek, USA) applying a capillary of 15 m length and 0.25 mm ID coated with a film of 0.25 µm DB5-MS (5 % phenyl-methylpolysiloxane, J&W Scientific, Folsom, USA). An injector temperature of 275 °C and Helium as carrier gas (flow 2 ml/min) were used. The temperature programme was as follows: 100 °C for 1 min, then with 50 °C/min to 300 °C, isothermal for 4 min. Splitless injections (splitless time 1.5 min) of 2.5 µl sample volume were carried with a Pal autosampler (CTC Analytics, Zwingen, Switzerland).

The following EI-MS/MS conditions were applied: Electron energy 70 eV, filament current 300 µA, resolution of quadrupole 1 at 0.8 u and of quadrupole 3 at 1.2 u, Ar as collision gas at a pressure of 0.12-0.15 Pa (0.9-1.1 mTorr). The selected ions to be fragmented as well as further parameters are given in Table 44. The precursor ion *m/z* 383 $[M\text{-}Cl]^+$ and the product ion *m/z* 276 $[M\text{-}4Cl]^+$ were selected for the internal standard $^{13}C_{10}$-*trans*-chlordane (collision energy -21 V).

**Table 44:** Selected precursor and product ions for the determination of the total PCA content by EI-MS/MS with an ion trap and triple quadrupole mass spectrometer. Important instrument parameters and the masses for the internal standard $^{13}C_{10}$-*trans*-chlordane are also given.

| | | Ion trap | | Triple quadrupole |
|---|---|---|---|---|
| **Precursor ion (*m/z*)** | **Product ion (*m/z*)** | **Excitation amplitude (V)** | **Excitation "storage level" (*m/z*)** | **Collision energy (V)** |
| 91 $[C_7H_7]^+$ | 53 $[C_4H_5]^+$ | 2,0 | 39,9 | -16,0 |
| 102 $[C_5H_7Cl]^+$ | 65 $[C_5H_5]^+$ | 0,6 | 44,7 | -10,0 |
| 102 $[C_5H_7Cl]^+$ | 67 $[C_5H_7]^+$ | * | * | -18,0 |
| 383 $[M\text{-}Cl]^+$ | 276 $[M\text{-}4Cl]^+$ | 0,85 | 169,0 | -21,0 |

*not detectable in the ion trap.

### 5.6.9 Quantification of chlordanes by ECNI-LRMS

Gas chromatographic separations were carried out on an HP 5890II gas chromatograph (Hewlett Packard, Palo Alto, USA), equipped with a Hewlett Packard 7673 auto sampler. A fused silica capillary was employed of 15 m length and 0.25 mm ID coated

with a 025 µm film of DB5-MS (5% phenyl-95 %-methylsiloxane, J&W Scientific, Folsom, USA). The injected volume was 1.5 µl in the splitless mode (2 min splitless time). The injector temperature was 240 °C. He was used as carrier gas (99,999 % purity, Carbagas, Switzerland) at a head pressure of 68,9 kPa (10 psi). The following temperature programme was employed: 90 °C isothermal for 2 min, with 30 °C/min to 150 °C, then 4 °C/min to 250 °C, 2 min isothermal.

Also here an HP 5989B mass spectrometer was applied in the ECNI mode (Hewlett Packard, Palo Alto, USA). The temperature of the transfer line was 250 °C, of the ion source 200 °C and of the quadrupole 100 °C. Methane was used as reagent gas (99,995 %, Carbagas, Switzerland) at a pressure of 1-1.6 hPa (0.9-1.1 Torr).

Table 45 summarises the selected masses for chlordanes in the selected ion monitoring mode. The most abundant chlorine isotope signal was selected for quantification and the second one for confirmation. The metabolites oxy-chlordane, *trans*- and *cis*-hepta-chlorepoxide could not be completely separated on the selected capillary. *cis*-Hepta-chlorepoxide and oxy-chlordane co-eluted in front of *trans*-heptachlorepoxide. Quan-tification of cis-heptachlorepoxide was therefore carried out with the not disturbed but less abundant mass *m/z* 388 and of oxy-chlordane with *m/z* 424. The presence of the chlordanes MC5 and MC7 was confirmed by their retention relative to *cis*-chlordane (MC5: 0,982; MC7: 1,021, (Karlsson, 1999; Karlsson *et al.*, 2000)).

**Table 45:** Mass-to-charge ratios of ions of single chlordane compounds used for quantification and identification in the ECNI-MS mode.

| Compound | Quantification ion *m/z* | Identification ion *m/z* |
|---|---|---|
| *trans*-Chlordane | 410 $[M+4]^-$ | 408 $[M+2]^-$ |
| *cis*-Chlordane | 410 $[M+4]^-$ | 408 $[M+2]^-$ |
| *trans*-Nonachlor | 444 $[M+4]^-$ | 442 $[M+2]^-$ |
| *cis*-Nonachlor | 444 $[M+4]^-$ | 442 $[M+2]^-$ |
| MC5 | 410 $[M+4]^-$ | 408 $[M+2]^-$ |
| MC7 | 410 $[M+4]^-$ | 408 $[M+2]^-$ |
| MC8 | 410 $[M+4]^-$ | 408 $[M+2]^-$ |
| Oxy-chlordane | 354 $[M+6-2Cl-2H]^-$ | 352 $[M+4-2Cl-2H]^-$ |
| Oxy-chlordane | 424 $[M+2]^-$ | 422 $[M]^-$ |
| 4,5-Dichlorochlordene | 302 [M] | 299 [M-2Cl-HCl] |
| Heptachlor | 302 $[M+2-2Cl]^-$ | 300 $[M-2Cl]^-$ |
| *trans*-Heptachlorepoxide | 354 $[M+2-Cl+H]^-$ | 352 $[M-Cl+H]^-$ |
| *cis*-Heptachlorepoxide | 390 $[M+4]^-$ | 388 $[M+2]^-$ |

Alternatively, a CP 3800 gas chromatograph (Varian, Walnut Creek, USA) was employed for detection combined with a triple quadrupole mass spectrometer (1200L, Varian, Walnut Creek, USA). The injected volume was 2 µl in the splitless mode (2.5 min splitless time, Combi Pal autosampler, CTC Analytics, Switzerland). The injector temperature was 240 °C. He was used as carrier gas (99,999 % purity, Carbagas, Switzerland) at a head pressure of 68.9 kPa (10 psi). The following temperature programme was employed: 90 °C isothermal for 2 min, with 30 °C/min to 150 °C, then 4 °C/min to 210 °C and finally with 20 °C/min to 260 °C (5 min isothermal). Methane was used as reagent gas (99,995 %, Carbagas, Switzerland) at a pressure of 7.3 mbar (5.5 Torr). The dwell time was 0.5 s per ion group.

### 5.6.10 Quantification of chlordanes by EI-MS/MS

The standard ECNI quantification was checked by an additionally developed EI-MS/MS method. Gas chromatographic separations were carried out on a CP-3800 gas

chromatograph (Varian, Wallnut Creek, USA) applying a capillary of 15 m length and 0.25 mm ID coated with a film of 0.25 µm DB5-MS (5 % phenyl-methylpolysiloxane, J&W Scientific, Folsom, USA). An injector temperature of 240 °C and Helium as carrier gas (flow 2 ml/min, quality 5.0, Carbagas, Switzerland) were used. The temperature programme was as follows: 90 °C for 2 min, then with 15 °C/min to 260 °C, isothermal for 2 min. Splitless injections (splitless time 2 min) of 2.0 µl sample volume were carried with a Pal autosampler (CTC Analytics, Zwingen, Switzerland).

The following EI-MS/MS conditions were applied: Electron energy 70 eV, filament current 150 µA, resolution of quadrupole 1 at 0.8 u and of quadrupole 3 at 1.2 u, Ar as collision gas at a pressure of 0.12-0.15 Pa (0.9-1.1 mTorr). The selected ions to be fragmented as well as further parameters are given in Table 46. The precursor ion *m/z* 383 $[M\text{-}Cl]^+$ and the product ion *m/z* 276 $[M\text{-}4Cl]^+$ were selected for the internal standard $^{13}C_{10}$-*trans*-chlordane (collision energy -26 V). The dwell time was 50 ms per ion.

**Table 46:** Selected precursor and product ions for the determination of selected chlordanes by EI-MS/MS with a triple quadrupole mass spectrometer. Important instrument parameters and the masses for the internal standard $^{13}C_{10}$-*trans*-chlordane are also given.

| **Compound** | **Precursor ion (*m/z*)** | **Product ion (*m/z*)** | **Collision energy (V)** |
|---|---|---|---|
| Oxychlordane | 185 | 121 | -17,5 |
| *trans*-heptachlorepoxide | 217 | 181 | -22,5 |
| Heptachlor, 4,5-DCCD | 272 | 237 | -22,5 |
| *cis*- heptachlorepoxide | 353 $[M\text{-}Cl]^+$ | 265 | -25,0 |
| *trans/cis*-Chlordane | 373 $[M\text{-}Cl]^+$ | 266 $[M\text{-}4Cl]^+$ | -25,0 |
| $^{13}C_{10}$-*trans*-Chlordane | 383 $[M\text{-}Cl]^+$ | 276 $[M\text{-}4Cl]^+$ | -26,0 |
| *trans/cis*-Nonachlor | 409 $[M\text{-}Cl]^+$ | 300 $[M\text{-}4Cl]^+$ | -25,0 |

## 5.7 Appendix 2: Average molar mass and Cl content of sample PCAs

Table 47 summarises the average molar mass and chlorine content of PCAs present in the biota OS1-OS15 and NS1-NS7 to enable a comparison with future data. Table 48 gives the same survey for sediment samples.

**Table 47:** Average molar mass and average chlorine content of PCAs present in fish liver from the North and Baltic Sea and of the reference standards used for quantification according to Tomy *et al.* (1997). ECNI was applied for determination.

| | | | sPCAs | | mPCAs | |
|---|---|---|---|---|---|---|
| Capture location | Sample No. | Species | Average molar mass [g/mol] | Calculated Cl content [%] | Average molar mass [g/mol] | Calculated Cl content [%] |
| B11 | OS1 | Cod | 388 | 60 | 425 | 55 |
| B11 | OS6 | Cod | 413 | 61 | 468 | 58 |
| B11 | OS7 | Cod | 414 | 62 | 450 | 56 |
| B11 | OS8 | Cod | 413 | 62 | 452 | 57 |
| B11 | OS9 | Cod | 422 | 63 | 447 | 56 |
| B11 | OS2 | Flounder | 420 | 61 | 431 | 56 |
| B11 | OS3 | Flounder | 412 | 60 | 453 | 58 |
| B11 | OS4 | Flounder | 412 | 62 | 447 | 57 |
| B01 | OS10 | Cod | 426 | 62 | 456 | 57 |
| B01 | OS11 | Cod | 419 | 62 | 456 | 57 |
| B01 | OS12 | Cod | 413 | 61 | 440 | 55 |
| B01 | OS13 | Cod | 415 | 62 | 447 | 56 |
| B01 | OS15 | Cod | 415 | 62 | 439 | 55 |
| B01 | OS5 | Dab | 382 | 62 | 411 | 53 |
| B01 | OS14 | Dab | 414 | 61 | 454 | 57 |
| | | | | | | |
| N01 | NS1 | Dab | 397 | 59 | 443 | 57 |
| N04 | NS2 | Dab | 392 | 60 | 433 | 56 |
| N04 | NS3 | Cod | 394 | 61 | 445 | 57 |
| N06 | NS4 | Dab | 374 | 61 | - | - |
| P01 | NS5 | Dab | 394 | 61 | 436 | 56 |
| N04 | NS6 | Dab | 392 | 59 | 426 | 54 |
| GB1 | NS7 | Flounder | 405 | 60 | 447 | 56 |
| sPCA standard for quantification | | | $398 \pm 7$ * | $60 \pm 1$* | | |
| mPCA standard for quantification | | | | | $436 \pm 2$** | $56 \pm 1$** |

* n = 7, ** n = 4

**Table 48:** $CH_4/CH_2Cl_2$-NICI-MS determination of average molar mass and average chlorine content of PCAs present in sediments from the Baltic and North Sea and of the reference standards used for quantification (see Tomy *et al.* (1997)). This procedure required sediments with levels >50 ng/g dw.

| Sample | | sPCAs | | mPCAs* | |
|---|---|---|---|---|---|
| | | calculated molecular weight [g/mol] | calculated chlorine content [%] | calculated molecular weight [g/mol] | calculated chlorine content [%] |
| **2001**[a] | | | | | |
| 710 | Baltic Sea | 379 | 55 | 394 | 49 |
| 715 | Baltic Sea | 361 | 53 | 387 | 48 |
| 718 | Baltic Sea | 358 | 56 | 381 | 48 |
| 721 | Baltic Sea | 391 | 55 | 391 | 49 |
| **2002**[a] | | | | | |
| 710 | Baltic Sea | 358 | 54 | 373 | 47 |
| 721 | Baltic Sea | 364 | 55 | 378 | 47 |
| KS 11 | North Sea | 348 | 53 | 365 | 46 |
| **2003**[b] | | | | | |
| KS 8 | North Sea | 345 | 53 | 400 | 50 |
| KS 11 | North Sea | 341 | 53 | 381 | 47 |
| **2004**[b] | | | | | |
| 710 | Baltic Sea | 337 | 53 | 391 | 49 |
| 715 | Baltic Sea | 341 | 53 | 384 | 48 |
| 718 | Baltic Sea | 338 | 53 | 377 | 46 |
| 721 | Baltic Sea | 344 | 53 | 398 | 49 |
| ECKFBU | Baltic Sea | 350 | 53 | 395 | 49 |
| ODER | Baltic Sea | 337 | 53 | 373 | 46 |
| RUDEN | Baltic Sea | 342 | 53 | 382 | 47 |
| KS 8 | North Sea | 334 | 53 | 366 | 45 |
| KS 11 | North Sea | 358 | 53 | 392 | 48 |

*molecular weight and chlorine content calculated on basis of the $C_{14\text{-}15}Cl_{4\text{-}10}$

## 5.8 Appendix 3: Overview analysed samples and applied techniques

Table 49 and Table 50 summarises all available information about samples such as sampling site, sampling date, species, gender, pooled individuals and applied measuring techniques for both screening, final quantification as well as determination of formula and congeners group patterns.

**Table 49:** Survey over analysed fish samples (cod: *Gadus morhua*, flounder: *Platychtus flesus*, North Sea dab: *Limanda limanda*) from the North and Baltic Sea. EI-MS/MS was applied for screening and ECNI-MS for quantification of PCAs and chlordanes. Only sample NS1 was analysed by $CH_4$/$CH_2Cl_2$-NICI as part of method development.

| Capture location | Coordinates | Species | Capture date | Gender | Sample No. | No. of livers | Total PCAs (EI-MS/MS) | s+mPCAs (ECNI-MS) | chlordanes (ECNI-MS) |
|---|---|---|---|---|---|---|---|---|---|
| B11 | 54°47´N/13°06´E | Cod | 31.08.2002 | ni | OS1 | 5 | X | X | X |
| B11 | 54°51´N/14°01´E | Cod | 01.09.2002 | ni | OS6 | 1 | X | X | |
| B11 | 54°51´N/14°01´E | Cod | 01.09.2002 | ni | OS7 | 1 | X | X | |
| B11 | 54°51´N/14°01´E | Cod | 01.09.2002 | ni | OS8 | 2 | X | X | X |
| B11 | 54°51´N/14°01´E | Cod | 01.09.2002 | ni | OS9 | 1 | X | X | X |
| B11 | 54°46´N/13°18´E | Flounder | 31.08.2002 | f | OS2 | 1 | X | X | X |
| B11 | 54°44´N/13°10´E | Flounder | 31.08.2002 | f/m | OS3 | 2 | X | X | X |
| B11 | 54°45´N/13°20´E | Flounder | 31.08.2002 | f/m | OS4 | 2 | X | X | X |
| B01 | 54°31´N/10°39´E | Dab | 03.09.2002 | f | OS5 | 5 | X | X | X |
| B01 | 54°31´N/10°39´E | Cod | 03.09.2002 | ni | OS10 | 3 | | X | X |
| B01 | 54°31´N/10°39´E | Cod | 03.09.2002 | ni | OS11 | 2 | | X | X |
| B01 | 54°40’N/10°28’E | Cod | 31.08.2003 | ni | OS12 | 1 | | X | X |
| B01 | 54°40’N/10°28’E | Cod | 31.08.2003 | ni | OS13 | 1 | | X | X |
| B01 | 54°40’N/10°28’E | Cod | 31.08.2003 | ni | OS15 | 1 | | X | X |
| B01 | 54°40’N/10°28’E | Dab | 31.08.2003 | ni | OS14 | 1 | | X | X |
| N01 | 54°15´N/7°29´E | Dab | 25.08.2002 | f | NS1 | 5 | X | X | X |
| N04 | 54°30´N/2°16´E | Dab | 26.08.2002 | f | NS2 | 5 | X | X | X |
| N04 | 54°30´N/2°16´E | Dab | 08.09.2003 | ni | NS6 | 5 | | X | X |
| N04 | 54°43´N/2°07´E | Cod | 26.08.2002 | ni | NS3 | 5 | X | X | X |
| N06 | 56°18´N/2°04´W | Dab | 27.08.2002 | f | NS4 | 5 | X | X | X |
| P01 | 55°30´N/4°40´E | Dab | 29.08.2002 | f | NS5 | 5 | X | X | X |
| GB1 | 54°07´N/7°46´E | Flounder | 30.08.2003 | ni | NS7 | 1 | | X | X |

f: female, m: male, ni: not identified

**Table 50:** Survey over analysed biota from the northern North Atlantic and Bear Island. Sampling sites: Cod (*Gadus morhua*) from NW Europe, Arctic char (*Salvelinus alpinus*) and seabirds (little auk: *Alle alle*, kittiwake: *Rissa tridactyla*, glaucous gull: *Larus hyperboreus*) from the Arctic Bear Island (Lake Ellasjøen). Information is also given about the applied methods for the determination of PCAs.

| Origin | Coordinates | Species | Tissue | Capture date | Gender | Size [mm] | Weight [g] | Sample No. | Total PCAs (EI-MS/MS) | s+mPCAs (ECNI-MS) |
|---|---|---|---|---|---|---|---|---|---|---|
| Lofot Islands | 68°08‘N/13°33‘W | Cod | Liver | 02.02.04 | f | 860 | 8500 | A1 | X | X |
| Lofot Islands | 68°08‘N/13°33‘W | Cod | Liver | 02.02.04 | f | 830 | 6500 | A4 | X | X |
| N Iceland | 65°74‘N/18°09‘W | Cod | Liver | 30.09.03 | f | 490 | 1019 | A2 | X | X |
| N Iceland | 65°74‘N/18°09‘W | Cod | Liver | 30.09.03 | f | 410 | 653 | A5 | X | X |
| S Iceland | 63°28‘N/20°15‘W | Cod | Liver | 06.11.03 | ni | 530 | 1490 | A3 | X | X |
| S Iceland | 63°28‘N/20°15‘W | Cod | Liver | 06.11.03 | f | 514 | 1275 | A6 | X | X |
| Bear Island | 74°N/19°E | Arctic char | Liver | 09.07.01 | f | 446 | 831 | B1 | X | X |
| | | | Muscle | | | | | B3 | X | X |
| Bear Island | 74°N/19°E | Arctic char | Liver | 09.07.01 | f | 465 | 850 | B2 | X | X |
| | | | Muscle | | | | | B4 | X | X |
| Bear Island | 74°N/19°E | Little auk | Liver | 08.07.01 | m | 121[a] | 173 | C1 | X | X |
| | | | Muscle | | | | | C3 | X | X |
| Bear Island | 74°N/19°E | Little auk | Liver | 08.07.01 | m | 122[a] | 169 | C2 | X | X |
| | | | Muscle | | | | | C4 | X | X |
| Bear Island | 74°N/19°E | Kittiwake | Liver | 08.07.01 | m | 330[a] | 458 | D1 | X | X |
| | | | Muscle | | | | | D3 | X | X |
| Bear Island | 74°N/19°E | Kittiwake | Liver | 08.07.01 | f | 326[a] | 393 | D2 | X | X |
| | | | Muscle | | | | | D4 | X | X |
| Bear Island | 74°N/19°E | Glaucous | Liver | 07.07.01 | m | 490[a] | 1921 | E1 | X | |
| | | | Muscle | | | | | E3 | X | |
| Bear Island | 74°N/19°E | Glaucous | Liver | 07.07.01 | f | 439[a] | 1441 | E2 | X | |
| | | | Muscle | | | | | E4 | X | |

ni: not identified; f: male; m: male; [a:] length of wing span

## 5.9 Appendix 4: Publications and conference contributions

Most recent publications and presentations are given first:

Reth M., Ciric A., Christensen G.N., Heimstad E.S. and Oehme M. “Short- and medium-chain chlorinated paraffins in biota from the European Arctic - differences in homologue group patterns” *Sci. Total Environ.* **2005**, submitted.

Reth M., Kypke K., Schächtele J. and Oehme M. “Chlorinated paraffins in human milk from Germany analyzed by HRGC-EI-MS/MS” *Organohalogen Compd.* **2005**, 67, 1671-1673.

Reth M., Zencak Z. and Oehme M. “New quantification procedure for the analysis of chlorinated paraffins using electron capture negative ionization mass spectrometry” *J. Chromatogr. A* **2005**, 1081, 225-231.

Zencak Z., Borgen A.R., Reth M. and Oehme M. “Evaluation of four mass spectrometric methods for the analysis of polychlorinated *n*-alkanes” J. *Chromatogr. A.* **2005**, 1067, 295-301.

Reth M., Zencak Z. and Oehme M. “First study of congener group patterns and concentrations of short- and medium-chain chlorinated paraffins in fish from the North and Baltic Sea” *Chemosphere* **2005**, 58, 847-854.

Reth M., Ciric A., Evenset A., Heimstad E.S. and Oehme M. “Chlorinated paraffins in fishes and seabirds from northwest Europe and the Arctic island Bjørnøya” *Organohalogen Compd.* **2005**, 67, 1671-1673.

Hüttig, J. and Oehme, M. “Congener group patterns of short and medium chain chloro-paraffins in marine sediments” *Organohalogen Compd.* **2005**, 67, 2041-2043

Hüttig, J. and Oehme, M. “Presence of chlorinated paraffins in sediments from the North and Baltic Sea” *Arch. Environ. Contam. Toxicol.* **2005**, in press.

Hüttig, J. and Oehme, M. “Multivariate cluster analysis as a versatile tool for the quality assessment of short chain chloroparaffin quantification in environmental samples” *J. Environ. Monit.* **2005**, 7, 319-324.

Ciric, A. “Bestimmung von kurz- und mittelkettigen polychlorierten *n*-Alkanen in arktischen Fisch- und Seevogelproben“ (Diploma Thesis) **2004**, University of Basel, Switzerland.

Zencak, Z. and Oehme, M. Chloride “Enhanced Atmospheric Pressure Chemical Ionization Mass Spectrometry of Polychlorinated n-Alkanes” *Rapid Commun. Mass Spectrom.* **2004**, 18, 2235-2240.

Hüttig, J.; Zencak, Z. and Oehme, M. “Levels of Chlorinated Paraffins in North and Baltic Sea Sediments” *Organohalogen Compd*. **2004**, 66, 1344-1349.

Reth, M.; Zencak, Z. and Oehme, M. “A new quantification procedure for the analysis of chlorinated paraffins using electron capture negative ionization mass spectrometry” *Organohalogen Compd*. **2004**, 66, 501-509.

Zencak, Z. and Oehme, M. “Analysis of chlorinated paraffins by chloride enhanced APCI-MS” *Organohalogen Compd*. **2004**, 66, 310-314.

Hüttig, J. and Oehme, M. “Determination of Short- and Medium-Chain Chlorinated Paraffins in North Sea” Conference DETECTA 04, 10-11 June, **2004**, Basel, Switzerland.

Reth, M. and Oehme, M. “Analysis of Short Chain Polychlorinated *n*-Alkanes in Biota” Conference DETECTA 04, 10-11 June, **2004**, Basel, Switzerland.

Zencak, Z. and Oehme, M. “Dichloromethane as NICI reagent gas for the selective detection of polychlorinated *n*-alkanes” Conference DETECTA 04, 10-11 June, **2004**, Basel, Switzerland.

Hüttig, J. and Oehme, M. “Determination of Short- and Medium-Chain Chlorinated Paraffins in North Sea” SETAC Europe 14th Annual Meeting, 18-22 April, **2004**, Prague, Czech Republic.

Reth, M. and Oehme, M. “Limitations of low resolution mass spectrometry in the electron capture negative ionization mode for the analysis of short- and medium-chain chlorinated paraffins” *Anal. Bioanal. Chem.* **2004**, 378, 1741-1747.

Zencak, Z.; Reth, M. and Oehme, M. “Determination of Total Polychlorinated *n*-Alkane Concentration in Biota by Electron Ionization-MS/MS” *Anal. Chem.* **2004**, 76, 1957-1962.

Hüttig, J. and Oehme, M. "Analysis of Short Chain Polychlorinated Paraffins in North Sea Sediments” Annual meeting of the Swiss Chemical Society, 9 October, **2003**, Lausanne, Switzerland.

Zencak, Z. and Oehme, M. "Quantification of polychlorinated n-Alkanes by ion trap EI-MS/MS” Annual meeting of the Swiss Chemical Society, 9 October, **2003**, Lausanne, Switzerland.

Reth, M. and Oehme, M. "Analysis of Short Chain Polychlorinated *n*-Alkanes in Biota” Annual meeting of the Swiss Chemical Society, 9 October, **2003**, Lausanne, Switzerland.

Zencak, Z.; Reth, M. and Oehme, M. “Dichloromethane-Enhanced Negative Ion Chemical Ionization of Polychlorinated n-Alkanes” Anal. Chem. **2003**, 75, 2487-2492.

Reth, M.; Zencak, Z. and Oehme, M. “Analysis of Short-Chain Polychlorinated n-Alkanes in Fish Samples by HRGC-NICI-LRMS” Organohalogen Compd. **2003**, 60, 444-447.

Zencak, Z. and Oehme, M. “Dichloromethane as NICI reagent gas for the selective detection of polychlorinated n-alkanes” Organohalogen Compd. **2003**, 60, 488-491.

Zencak, Z. and Oehme, M. “Fast ion trap EI-MS/MS detection of polychlorinated n-alkanes in biota samples” Organohalogen Compd. **2003**, 60, 440-443.

Reth, M. and Oehme, M. “Determination of Polychlorinated n-Alkanes in Biota Samples - Challenges and Problems” 3rd Workshop on Analytical Artefacts in Environmental Analysis and Related Areas (Artefacta), 16-17 June, **2003**, Munich, Germany.

Zencak, Z. and Oehme, M. “Dichloromethane as Reagent Gas for the selective Detection of Polychlorinated n-Alkanes by Negative Ion Chemical Ionization” Annual meeting of the Swiss Chemical Society for mass Spectrometry (SGMS), 14-15 November, **2002**, Beatenberg, Switzerland

Reth, M.; Zencak, Z. and Oehme, M. “Determination of Short-Chain Polychlorinated Paraffins in Fish Samples by HRGC-NICI-LRMS”, „r+d“ Conference, 15-18 October, **2002**, Basel, Switzerland.

Zencak, Z. and Oehme, M. “Dichloromethane Enhanced Negative Ion Chemical Ionization of Polychlorinated n-Alkanes”, r+d“ Conference, 15-18 October, **2002**, Basel, Switzerland.

Reth, M. “Entwicklung eines Aufarbeitungsverfahrens zur Bestimmung von kurzkettigen polychlorierten *n*-Alkanen in fetthaltigen Proben“ (Diploma Thesis) **2002**, University of Basel, Switzerland.

# 6 Project 2: Identification of Organic Compounds in the North and Baltic Seas

Project number: FKZ 200 25 224/02; Period: 3.12.2002 – 31.10.2005
Dr. Norbert Theobald, Dr. Sieglinde Weigelt-Krenz and Dr. Anne-Christina Baaß
Bundesamt für Seeschifffahrt und Hydrographie, Bernhard-Nocht-Str. 78, D-20359 Hamburg

## 6.1 Aim of the Project

The aim of the project „Identification of Organic Pollutants in the North and Baltic Seas“ is to identify and quantify toxic organic substances in the marine environment of the North and Baltic Seas for which environmental data are insufficient or not available at all. The selection of compounds is based on the lists of substances identified for priority action under the OSPAR and HELCOM Conventions and the European Commission’s Water Framework Directive. While implementing the European Water Framework Directive (EU WFD 2455/2001EU), it had been realised that no information was available on the occurrence of some of the compounds of the 33 priority pollutants listed in Annex X of the EU WFD. In particular, information on environmental concentrations in the coastal and marine waters was lacking. The sub-project carried out by Bundesamt für Seeschifffahrt und Hydrographie (BSH) was aimed at monitoring the concentrations of the following four compounds of the WFD list:

- Chlorpyrifos (-ethyl and –methyl)
- Endosulfan (I and II)
- Pentachlorophenol
- Trifluralin

In addition,

- Dicofol and
- Trichlorpyridinol

were added to the list of compounds to be surveyed at the beginning of the project; both are not listed by the WFD. Trichlorpyridinol is a potentially stable degradation product

of Chlorpyrifos. Dicofol is included in two lists of the OSPAR Convention – the reference list of substances agreed at the 3rd North Sea Conference (e.g. Annex 1D to the Hague Declaration and the list of Potential Endocrine Disruptors – Part B).

In addition, dicofol, endosulfan, PCP and Trifluralin are in the OSPAR list of substances for priority action. For all, "Background documents" have been published by the OSPAR Commission. Endosulfan, PCP and trifluralin are in the list of substances identified as of concern by HELCOM (1992)

In order to obtain reliable monitoring data, analytical methods had to be developed and validated for ultratrace analysis with limits of detection in the low pg/L range for water, and in the ng/kg range for sediment and biota samples.

Because of the intermediate polarity of the target compounds, all three compartments – water, sediment and biota – had to be investigated in order to obtain a comprehensive overview of the occurrence of these pesticides in the marine environment.

To achieve that goal, representative sampling of water and sediment had to be carried out by the BSH in the North Sea and Baltic Sea. Samples were taken during routine monitoring surveys of the Bund/Länder-Messprogramm (BLMP). Biota (fish) sampling was carried out by Dr. M. Haarich during monitoring surveys of the Bundesforschungsanstalt für Fischerei (BfA-Fi) .

## 6.2 Survey of Properties of the Target Compounds

### 6.2.1 Chlorpyrifos-ethyl and –methyl

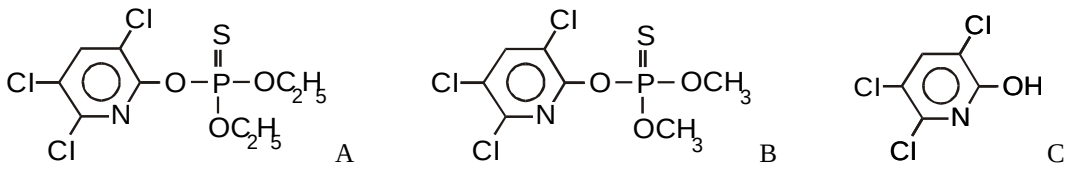



**Figure 24:** Structures of Chlorpyrifos-ethyl (A), –methyl (B) and their main metabolite 3,5,6-Trichloro-2-pyridinol (TCPy) (C)

**Chlorpyrifos-ethyl** (Figure 24) is one of the most widely used insecticides of about 100 manmade organophosphate insecticides. The substance is marketed under the trade names Dursban and Lorsban (pan-uk, 2005). It is used as a broad-spectrum insecticide for the treatment of grain, cotton and vegetable crops (Extoxnet Information About Chlorpyrifos, 1993). The compound was introduced in 1965 (Hayes, 1990) and is produced mainly by Dow AgroSciences (Indianapolis, USA). European manufacturers are Frunol (Unna, Germany) and Point Enterprises (Switzerland). About 1000 t is used annually in Europe (pan-uk, 2005).

Key physico-chemical properties determining the environmental behaviour of chlorpyrifos-ethyl and –methyl are listed in Table 51. The log $K_{ow}$ value of 4.7 – 5.3 indicates an affinity of chlorpyrifos-ethyl to accumulate in sediments and tissues of marine organisms.

**Table 51** Physico-chemical properties of chlorpyrifos-ethyl and –methyl.

| | **Chlorpyrifos-ethyl** | **Chlorpyrifos-methyl** | **TCP** |
|---|---|---|---|
| CAS number | 2921-88-2 | 5598-13-0 | 6515-38-4 |
| Log $K_{ow}$ | 4.7 (23 °C)-5.27 [a, e] | 4.3[e] | 1.8[d] |
| Log $K_{OC}$ [L/kg] | 3.6-4.5 [a, b] | | |
| Solubility in water [mg/L] | 2 (25°C) [b] | 4[f] | 220[d] |
| Vapour pressure [Pa] | $1.87x10^{-3}$ (25°C) [a, b] | 4.2x10-5 (25°C) [f] | $2.68x10^{-5}$ (25°C)[d] |
| Atmospheric OH rate constant [$cm^3$/molecule s] | $9.17x10^{-11}$ (25°C) [c] | | |
| Biodegradation | >month [b] | | |

Log Kow: n-octanol-water partition coefficient, log KOC: soil-water partition coefficient
Data from: [a](Extoxnet Information about Chlorpyrifos, 1993), [b](Barrett et al., 2000), [c](www.chemindustry.de), [d](Watson, 2002), [e](Noble, 1993), [f](www.inchem.org)

Chlorpyrifos-ethyl exhibits a high acute toxicity for mammals via inhalation or ingestion routes ($LD_{50}$ value = 135 mg/kg bodyweight rat (Römpp, 1995)). For fish, aquatic invertebrates and other marine organisms, chlorpyrifos is highly toxic: cholinesterase inhibition was observed in acute toxicity tests of fish exposed to very low concentrations. The following publications give an overview of the effects on aquatic organisms: (Extoxnet Information about Chlorpyrifos, 1993), (datasheet on

chlorpyrifos) and (Barrett et al., 2000). An overview of the lowest values of prolonged acute toxicity of chlorpyrifos-ethyl for aquatic organisms is shown in Table 52. Up to now, no data is available on the chronic toxicity of chlorpyrifos to organisms in the marine environment which are exposed to low concentrations for several years.

**Table 52:** Survey of lowest value of prolonged toxicity of chlorpyrifos-ethyl to aquatic organisms.

| Aquatic organism | Species | Value/period [d] | Conc. [µg/L] |
|---|---|---|---|
| Algae | *Scenedemus subspicatus* | NOEC/4 | 27.5 |
| Crustacean | *Mysidopsis bahia* | NOEC/35 | 0.005 |
| Fish | *Pimephales promelas* | NOEC/30 | 0.012 |

Data from: (Data sheet on chlorpyrifos-ethyl)

Environmental concentrations of chlorpyrifos-ethyl in different environmental samples are shown in Table 53. Concentrations on the order of of 0.4-0.9 ng/L have been found in rain water (TNO rapport, 2002). A detailed estimation of chlorpyrifos-ethyl deposition in surface and ground water is provided in a drinking water assessment by the environmental fate and effects division (Barrett, 1998). A study by the National Oceanic and Atmospheric Administration (Johnson et al., 1999) yields data on chlorpyrifos-ethyl concentrations in marine sediments and marine biota (Table 53).

**Table 53:** Overview of chlorpyrifos-ethyl concentrations in water samples, sediments, and biota.

| Sample | Sampling location | Sampling date | Concentration [ng/L]/[µg/kg dry wt] | Limit of detection [µg/kg] |
|---|---|---|---|---|
| Rain water | Netherlands | 2000/2001 | 0.9/0.4[a] | - |
| Surface water | US | 1995 | 130 [c] | 4 |
| Ground water | US | 1998 | 36 [c] | 4 |
| Marine sediment | US | 1994-1997 | 0.84-5.68 [d] | 0.25 |
| Mussel tissue | US | 1994-1997 | 4.18-52.92 [d] | 0.25 |

Data from: [a](TNO rapport 2002), [c](Barrett, 1998), [d](Johnson et al., 1999)

The major route of dissipation appears to be a slow aerobic and anaerobic degradation and photolytic degradation in soil to 3,5,6-trichloro-2-pyridinol (TCPy)(Figure 24) as

the main metabolite (Barrett et al. 2000; Simon, 2001; Yücel et al., 1999; Watson, 2002).

Precautions and restrictions are imposed by EPA in order to reduce potential hazards (Extoxnet Information about Chlorpyrifos, 1993).

**Chlorpyrifos-methyl** has the same skeletal structure as chlorpyrifos-ethyl but the two ethyl groups are replaced by two methyl groups ( Figure 24). The compound is used to protect stored grain against insect infestation, which includes weevils, beetles, and moths. It was registered after 1984 and is manufactured and marketed by Dow under the trade name Reldan® (Bangs, 2000). Chlorpyrifos-methyl shows a moderate acute toxicity for mammals ($LD_{50}$-value = 3000 mg/kg bodyweight rat (Römpp, 1995)).

### 6.2.2 Dicofol

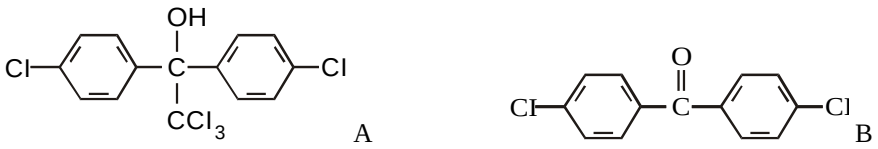



**Figure 25:** Structures of dicofol (A) and its main metabolite 4,4'-dichlorobenzophenone (B)

**Dicofol** – marketed under the trade name Kelthane® – is a member of the group of organochloride insecticides (Figure 25). There is only a single manufacturer of Dicofol in Europe, based in north-east Spain. The annual total production at this plant is 1500 t. 290 t of this volume is used in Western Europe as an acaricide for the treatment of fruit, vegetables, and crops (Background Document on Dicofol, 2002). Important physico-chemical properties of dicofol are listed in Table 54.

**Table 54:** Physico-chemical properties of dicofol.

| **Parameter** | |
|---|---|
| CAS number | 115-32-2 |
| Log Kow | 5.0[a], 3.54[b] |
| Solubility in water [mg/L] | 1.2 (24°C)[c] |
| Biodegradation | > months[a] |
| Vapour pressure [Pa] | $1.6x10^{-6}$ [a] |
| Half-life in water | pH 7: 4 d, pH 9: 0.02 d |
| Half-life in soil | pH 7: 30-35 d |
| Atmospheric half-life [d] | 3.1 d [a] |

Data from: [a](Lerche et al., 2002), [b](www.inchem.org), [c](OSPAR Background Document on dicofol)

The high log $K_{ow}$ value of 3.5 – 5.0 (see Table 54) indicates an affinity of dicofol to accumulate in sediments and biological tissues. The acute oral toxicity of dicofol is 690 mg/kg bodyweight rat ($LD_{50}$-value) (Römpp, 1995). It has been classified by the World Health Organisation as a Class III "slightly hazardous" pesticide (pan-uk, 1999). Within the framework of this study, dicofol and its degradation product 4,4'-dichlorobenzophenone (Figure 25) were screened for teratogenic effects by Kamman (Kammann, 2004) (see Chapter 6.4.6.2): effects were found for dicofol at concentrations of 2-10 mg/L; no effects were observed for dichlorobenzophenone.

Table 55 gives an overview of acute dicofol toxicity to organisms in the marine environment. No data are available on chronic toxicity following exposure to low concentrations for several years, particularly with respect to marine aquatic organisms.

**Table 55:** Survey of acute / chronic toxicity of dicofol to aquatic organisms.

| **Aquatic organism** | **Value/period [d]** | **Conc. [µg/L]** |
|---|---|---|
| Algae | LC50/acute | 73.0 |
| Crustacean | LC50/acute | 80.0 |
| Mollusc | LC50/acute | 15.0 |
| Fish | LC50/300 | 4.5 |

Data from: (OSPAR Background document on dicofol)

Research and monitoring studies providing data on dicofol concentrations in water and sediment samples are listed in the Background Document on Dicofol from OSPAR (see Table 56). The document provides information on the accumulation in biota, which is reported to be between 8050 and 25000 (Background Document on Dicofol).

No information is currently available on the distribution of dicofol in the marine environment.

**Table 56:** Overview of dicofol concentrations in the environment.

| Sample | Sampling location | Sampling date | Concentration [ng/L; µg/kg] | Limit of detection |
|---|---|---|---|---|
| River water | US | 1996 | 2.5 [a] | - |
| Sediment | US | 1996 | 23.7 [a] | - |
| River water | Greece | 1996 | <0.1 [a] | - |
| Sediment | Greece | 1996 | 2.2[a] | - |
| Drinking water | US | - | >LOD, <LOQ [b] | - |
| Groundwater | US | 1982 | 0.2-1.8[b] | - |

Data from: [a](OSPAR Background document on Dicofol), [b](Chemical fact sheet www.speclab.com)

In Europe, dicofol is used mainly in Mediterranean countries, e.g. Portugal, Spain, France, Italy, and Greece. In Belgium and the UK, it is registered and used in minor quantities of 200 and 1002 kg/a, respectively. In Germany, Denmark, the Netherlands, Sweden, Norway, Finland, and Switzerland, dicofol is not registered at present.

### 6.2.3 Endosulfan I and II

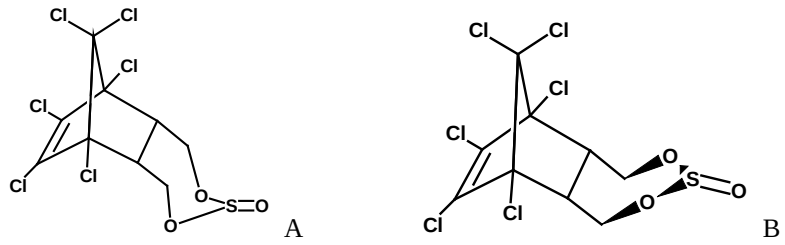



**Figure 26:** Structures of endosulfan I or α (A) and endosulfan II or β (B).

**Endosulfan** (trade names: e. g. Cyclodan and Endosol) is used as a contact insecticide for a wide variety of insects and mites. Endosulfan is a sulphite ester of a chlorinated cyclodienediol (Figure 26). The technical mixture contains two parts of endosulfan I- and one part of endosulfan II isomer. It is used mainly in temperate, subtropic, and tropic climatic zones. In Europe, it has been registered since 1956 (Schadstoffberatung Tübingen, 2001). World-wide production in 1992 was about 5000 to 10000 t. One of the major manufacturers is Bayer CropScience. 469 t of the compound was used in Europe in 1999, mainly in southern Europe (OSPAR Background Document on Endosulfan, 2002; BCMAF, 1999).

**Table 57:** Physico-chemical properties of endosulfan.

| | **Endosulfan I** | **Endosulfan II** | **Technical mixture (2:1)** |
|---|---|---|---|
| CAS number | 959-98-8 | 33213-65-9 | 115-29-7 |
| Log Kow | 4.74 (22°C; pH 5)[a] | 4.79 (22°C; pH 5)[a] | 3.8[b] |
| Log $K_{OC}$ [L/kg] | 3.48-5.30 [a] | See endosulfan I [a] | - |
| Solubility in water [mg/L] | 0.41 [a], 0.15 (22°C) | 0.23 [a], 0.06 (22°C) | 0.33 (20°C) |
| Biodegradation | - | - | >month [b] |
| Vapour pressure [Pa] | $1.9x10^{-3}$(25°C) [a] | $9.2x10^{-3}$(25°C) [a] | 3.6x10-5 [b] |
| Atmospheric half-life [d] | - | - | 1.3 [b] |

Data from: [a](Background Document on Endosulfan, 2002), [b](Lerche et al., 2002).

The German Gefahrstoffverordnung classifies endosulfan I and II as „toxic“ ($LD_{50}$ value: 80 mg/kg bodyweight rat (Römpp, 1995)). Endosulfan has a very high acute

toxicity to some aquatic organisms (see Table 58). Nothing is presently known about its chronic toxicity following several years' exposure to low concentrations, particularly with respect to marine aquatic organisms.

**Table 58:** Survey of acute / chronic toxicity of endosulfan to aquatic organisms.

| **Aquatic organism** | **Species** | **Value/period [d]** | **Conc. [µg/L]** |
|---|---|---|---|
| Algae | *Chlorella vulgaris* | NOEC/5 | 10.0 [a] |
| Crustacean | *Mysidopsis bahia* | LC50/acute | 0.04 [b] |
| Fish | *Oncorhynchus mykiss* | NOEC/28 | 0.001 [a] |

Data from: [a] (Datasheet on Endosulfan), [b](OSPAR Background document on Endosulfan)

The following studies provide an overview of the distribution of endosulfan and/or the long-time water quality trends in Europe and the US: (Serdar and Miller, 2002), (Muschal and Cooper), (WHO, 1994), (Johnson et al., 1999), (Background Document on Endosulfan). Endosulfan has been found in Arctic air and in the Bering Sea, i.e. it is a currently used pesticide which is widely distributed in the atmosphere and sea of the polar environment. Typical concentrations are shown in Table 59. Some samples from regions where endosulfan consumption is high (e. g. South Africa, US) were found to have extremely high endosulfan concentrations, with $LC_{50}$ values for fish and crustaceans clearly exceeded. No information is presently available on endosulfan concentrations in the marine environment of northern Europe.

**Table 59:** Overview of endosulfan concentrations in the environment.

| Sample | Sampling location | Sampling date | Concentration [ng/L] or [µg/kg dry wt] | Limit of detection [ng/L] or [µg/kg] |
|---|---|---|---|---|
| Creek water | US | 1993 | 31 (I) [a] 13 (II) [a] | 5 (I) [a] 5 (II) [a] |
| River water | US | 1991-1997 | 0.002-0.2[b] | 0.002[b] |
| Surface water | Canada | 1980 | 11[c] | - |
| Surface water | South Africa | 1998 | 830-3160[d] | 100[d] |
| Mussel tissue | US | 1994-1997 | 1.6-7.9 (I) [e] | 0.68 (I) |
| | | | 1.5-6.3 (II) [e] | 0.88 (II) |
| River sediment | US | 1977 | 4780 [c] | - |

Data from: [a](Serdar and Miller, 2002), [b](Muschal and Cooper), [c](WHO, 1994), [d](Dalvie et al., 2003), [e](Johnson et al., 1999)

According to the background paper on endosulfan published by the OSPAR Commission (OSPAR, 2002), endosulfan is used mainly in southern Europe (1999: 469.3 t/a), while the countries bordering the North Sea and Baltic Sea consumed only 38.1 t/a in 1999. In most north European countries, use of endosulfan was phased out in the mid-1990s. Only Belgium, France, and Switzerland reported consumption figures for 1999.

### 6.2.4 Pentachlorophenol

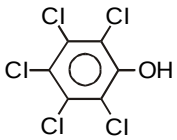



**Figure 27:** Structure of Pentachlorophenol

Figure 27 shows the structure of pentachlorophenol (PCP). PCP production was banned in Europe in the early eighties. Nevertheless, 100 t of imported PCP was used as an algicide and bactericide in the European Union's timber and textile industry in 1997 (Eurochlor, 1997).

**Table 60:** Physico-chemical properties of PCP.

| **Parameter** | |
|---|---|
| CAS number | 87-86-5 |
| Log Kow | 5.1 [a] |
| Log $K_{OC}$ [L/kg] | 5.46 [c] |
| Solubility in water [mg/L] | 20[b] |
| Biodegradation | months [a] |
| Vapour pressure [Pa] | $7.0x10^{-3}$ [a] |
| Atmospheric half-life [d] | 19 [a] |

Data from: [a](Lerche et al. 2002), [b](Gestis Stoffdatenbank); [c](Data sheet on PCP)

The acute toxicity of PCP corresponds to the classification "toxic" according to the German Gefahrstoffverordnung ($LD_{50}$ value =56 mg/kg bodyweight rat [Sigma-Aldrich, 2003)). Table 61 shows the toxicities of PCP to aquatic organisms. No information is available on the chronic toxicity to marine aquatic organisms following exposure to low concentrations for several years.

**Table 61:** Survey of acute / chronic toxicity of PCP to aquatic organisms.

| **Aquatic organism** | **Species** | **Value/period [d]** | **conc. [µg/L]** |
|---|---|---|---|
| Algae | *Selenastrum capricornutum* | NOEC/4 | 5.0 |
| Crustacean | *Daphnia magna* | NOEC/21 | 1.8 |
| Fish | *Salmo gairdneri* | NOEC/28 | 1.8 |

Data from: (Data sheet on PCP)

In the past twenty years, several monitoring surveys for PCP have been carried out in the North Sea. Typical concentrations in water and sediment samples are shown in Table 62. All of these studies show considerable contamination by PCP. However, most data are quite old and are not suitable for a current evaluation.

**Table 62:** Overview of PCP concentrations in the environment.

| Sample | Sampling location | Sampling date | Concentration [ng/L]/[µg/kg dry wt] | Limit of detection [ng/L]/[µg/kg] |
|---|---|---|---|---|
| Sea water | North Sea; estuaries | 1983-1997 | 6-100 [a] | - |
| Sea water | German Bight | 1988 | 0.1 – 6.4[d] | |
| Marine sediment | North Sea; estuaries | 1991-1994 | 26.5(max. value)[a] | <10 |
| River water | Rhine | 1999 | 30 [b] | - |
| River sediment | Netherlands | 1980 | 0.2-1.5 [c] | 0.1 |

Data from: [a](Eurochlor, 1997); [b](RIWA, 2002); [C](Wegman, 1983), [d](Hühnerfuß et al., 1990)

### 6.2.5 Trifluralin

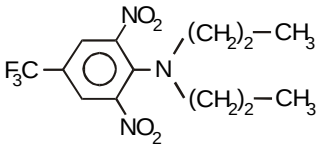



**Figure 28:** Structure of Trifluralin.

**Trifluralin** (Figure 28) is a dinitroaniline derivative which is used as a selective herbicide. It has a low water solubility and a high affinity for soil. The substance is marketed under different trade names (e. g. TREFLAN (Trifluralin 480 g/L)) for pre-sowing treatment of grasses and dicotyledonous weeds (Extoxnet Information on Trifluralin, 1996; Dow AgroScience, 2005). It was first registered in the US in 1963 (Pesticide News, 2001). In the European Union, trifluralin is manufactured at a single production facility (Manerbio, northern Italy). The production volume is about 6000 t per year. and consumption in the European Union is 3200 t (OSPAR Background Document on trifluralin, 2004). Trifluralin is subject to rapid photodegradation in air but is persistent in sediment. Under aerobic as well as anaerobic conditions, several metabolites of trifluralin have been observed, which points to its degradability in the environment.

**Table 63:** Physico-chemical properties of trifluralin.

| **Parameter** | |
|---|---|
| CAS number | 1582-09-8 |
| Log $K_{OW}$ | 5.3 [a] |
| Log $K_{OC}$ [L/kg] | 3.8-4.1 [a] |
| Solubility in water [mg/L] | 0.19 [a] |
| Biodegradation | months [b] |
| Vapour pressure [Pa] | $9.5x10^{-3}$ [a] |
| Atmospheric half-life [d] | 0.22 [a] |

Data from: [a] (OSPAR Background Document on Trifluralin, 2004); [b](Lerche et al. 2002)

Acute toxicity for mammals is relatively low ($LD_{50}$-value = 10000 mg/kg bodyweight rat (Römpp, 1995)). In contrast, the acute ecotoxicity effects on maritime unicellular algae are high (Walsh, 1972). NOEC values for different types of aquatic organisms vary on the order of 1-100 µg/L (see Table 64). No information is presently available on the chronic toxicity of trifluralin to organisms in the marine environment.

**Table 64:** Survey of acute / chronic toxicity of trifluralin to aquatic organisms.

| **Aquatic organism** | **Species** | **Value/period [d]** | **Conc. [µg/L]** |
|---|---|---|---|
| algae | *Chlorococcum sp.* | EC50/- | 2.5 [b] |
| crustacean | *Daphnia magna* | NOEC/64 | 2.4 [a] |
| fish | *Pimephales promelas* | NOEC/35 spinal cord deformation | 0.3 [a] |

Data from: [a](Data sheet on Trifluralin); [b](Walsh, 1972)

Unlike data on trifluralin concentrations in surface, ground and drinking water, information about levels in sea water is scarce. Analyses of samples from the North and Baltic Seas in 1997-1999 showed trifluralin concentrations of <0.02-0.05 ng/L (North Sea) and <0.02-0.06 ng/L (Baltic Sea) (Meeresumweltdatenbank (MUDAB)). The limit of quantification was 0.02 ng/L. The distribution of trifluralin is relatively uniform, with samples from the open sea occasionally having slightly higher concentrations than those from the coastal waters. In contrast to the low concentrations found in the North and Baltic Seas, concentrations in the North Pacific Ocean were 100 times higher. In 1993, Chernyak found a trifluralin concentration of 1.15 ng/L in that area (limit of

quantification: 0.01 ng/L) (Chernyak et al., 1996). Although trifluralin is subjected to rapid atmospheric and photochemical degradation, the authors attributed the observed trifluralin concentrations to long-range atmospheric transport. No data on trifluralin concentrations in sediments from the North and Baltic Seas are available.

Trifluralin is a licensed product, which is used as a herbicide in most European countries. Approximately 3200 t is used annually in the EU, the most important users being France (1600 t/a) and the UK (657 t/a) (OSPAR background document, 2004). In 1995, 100 to 200 t of the product was used in Germany.

## 6.3 Applied Methodology

### 6.3.1 State of the art of analysis

As the target compounds have been commercially available for a long time, several analytical methods for their determination are described in literature. Most methods are based on GC procedures with different detectors, such as ECD, NPD and MS. MS has been used both with electron impact ionisation (EI) and negative chemical ionisation (NCI). A selected list of relevant recent publications has been compiled in Table 65.

**Table 65:** Overview of detection methods for the target compounds

| Compound | Detection method | Matrix | LOD | Source |
|---|---|---|---|---|
| Chlorpyrifos | MS (EI) | sediment | 1 µg/kg dw | (Yim et al., 2002) |
| | MS (NCI) | water | 1-10 µg/L | (Liapis et al., 2000) |
| Dicofol | MS (NCI) | water | 1-10 µg/L | (Liapis et al., 2000) |
| Endosulfan I | ECD | water | 0.0185 µg/L | (Lipidoki, 2003) |
| and II | MS (NCI) | water | 1-10 µg/L | (Liapis et al., 2000) |
| PCP | ECD | sediment | 0.1 µg/kg | (Wegman et al., 1983) |
| Trifluralin | ECD | sediment | 1 µg/kg | (Lee et al., 1983) |
| | MS (NCI) | water | 1-10 µg/L | (Liapis et al., 2000) |

Liapis (Liapis et al., 2000) described the simultaneous determination of chlorpyrifos-ethyl and –methyl, dicofol, endosulfan I and II, and trifluralin in water samples after

solid phase extraction (SPE). The detection was done by GC-MS in EI and NCI mode. This procedure was considered a suitable basis for the method used in the present study.

Although the analysis of dicofol by GC techniques has been described in several publications, there are indications that this may be problematic.

PCP and TCPy are too polar for a direct and sensitive GC analysis. Because of the high polarity of the PCP hydroxyl group, gaschromatographic separation of the underivatized substance is problematic (strong tailing; losses by adsorption in the injector), rendering a reproducible interpretation of the peaks, and thus quantification, impossible. Therefore, PCPy is generally analysed after derivatisation. However, this makes the analyses labour-intensive and more susceptible to errors. Alternatively, methods using HPLC techniques which do not require a derivatisation step generally have a lower sensitivity and selectivity. Both approaches were tested in this survey.

### 6.3.2 Method development

The limits of detection in published methods generally were much too high for this study. Considering the low concentrations at which organic pollutants are present in marine matrices, the planned limits of quantification (LOQs) were 30 pg/L for water, 50 ng/kg dw for sediment, and 0.5 µg/kg ww for biota samples (project proposal). In the course of the project, these targets were lowered further because observed concentrations in the North Sea and Baltic Sea very often were below these limits.

Chlorpyrifos (-ethyl, -methyl), endosulfan (I, II), and trifluralin were analysed by a commonly used procedure, as planned.

Dicofol proved to be fairly unstable under various conditions and, therefore, had to be treated and analysed separately.

Because of the common phenolic substructure of PCP and TCPy, the methodology for PCP and TCPy was developed in a common procedure but separate from the other substances.

The extraction principles were similar for all compounds. While solid phase extraction (SPE) with a polymer resin was used for water samples, microwave assisted extraction (MAE) was used for sediment and biota samples (6.3.2.1 and 6.6.3 to 6.6.5).

#### 6.3.2.1 Methods for Chlorpyrifos, Endosulfan and Trifluralin

### GC-MS analysis

A common GC-MS analysis method is used for all three matrices (water, sediment and biota). Experimental details are provided in the appendix under 6.2.

GC analysis using a standard non-polar capillary column was unproblematic and yielded baseline separation of all compounds. MS analysis was performed both in EI and NCI mode. The parameters for GC and MS are summarised in Table 82 (chapter 6.6.2).

Deuterated analogues of trifluralin, chlorpyrifos and endosulfan I were used as internal standards for quantification.

As has been demonstrated in the chapter on validation (6.3.3), the NCI method was more sensitive with all compounds, and was consequently used to analyse all samples. Apart from the inherently high sensitivity of the NCI method for pure standard solutions, it was found to have additional advantages in real samples with matrix underground. Due to its high selectivity, all MS traces showed very clean baselines with little chemical noise. Examples of this are shown in Figure 29.

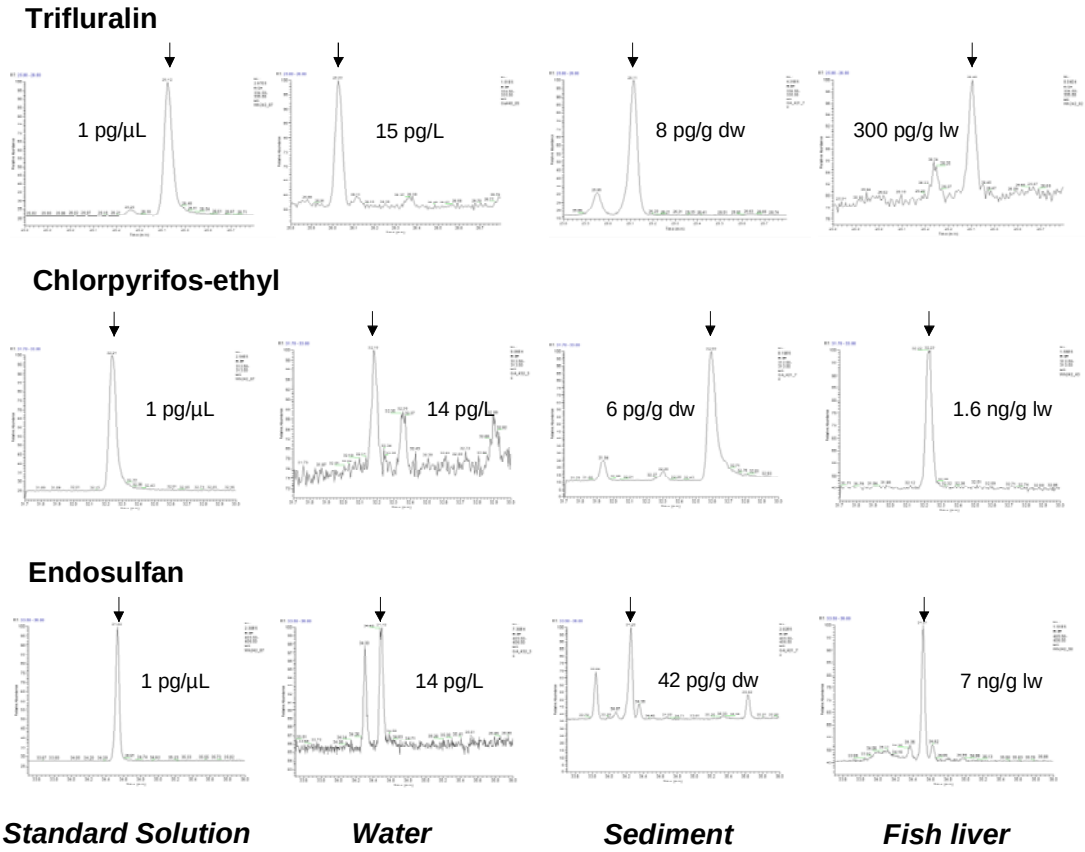



**Figure 29:** Typical GC-NCI-MS traces of trifluralin, chlorpyrifos-ethyl, and endosulfan I for standard solutions and real samples (Retention times may differ because analysis occurred over a long time period)

## Analytical procedure for water samples

The principles of the analytical procedure for sea water samples are summarised in Figure 30; experimental details are described in the appendix (6.6.3).

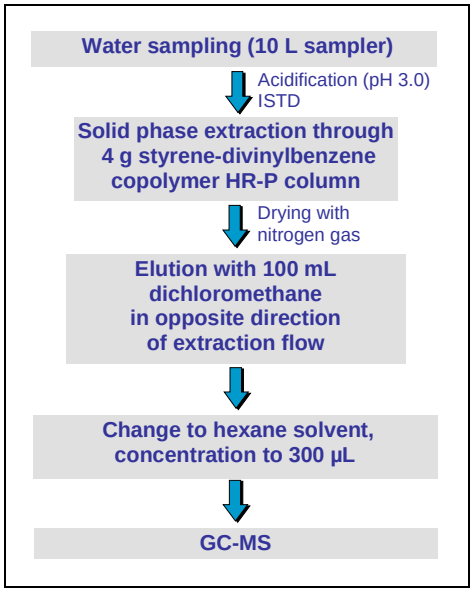



**Figure 30:** Overall scheme for the analysis of sea water for chlorpyrifos, endosulfan, and trifluralin

As shown under „Validation“ (6.3.3), the procedure yields reliable results and a sensitivity which is better than required in the project plan. Owing to the clean base line of the chromatograms, sensitivity can be further increased by injecting larger volumes into the GC-MS. This was demonstrated for water extracts by increasing the injection volume from 2 µl to 10 µl, which yielded an increase in sensitivity by a factor of about 4.

## Analytical procedure for sediment samples

The optimised method for the analysis of sediment samples consists of microwave assisted extraction (MAE) and a silica gel clean-up as described in Appendix 6.6.4. The scheme in Figure 31 summarises the procedure.

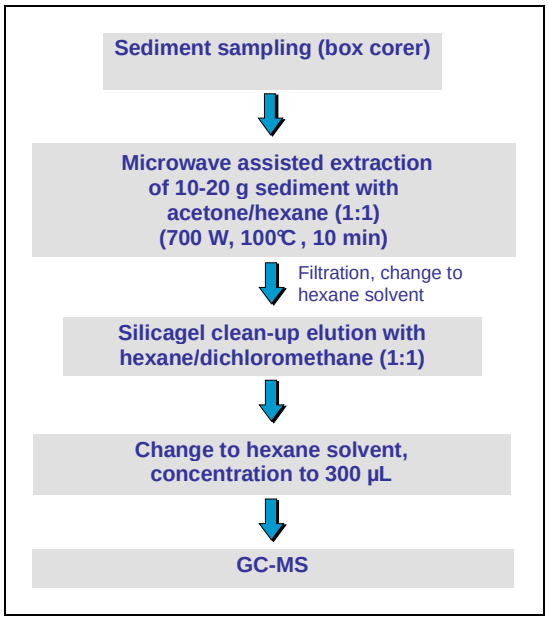



**Figure 31:** Overall scheme for the analysis of sediments for chlorpyrifos, endosulfan, and trifluralin

### Analytical procedure for biota samples

After optimisation of the method, sample preparation was carried out via MAE, gel permeation chromatography (GPC), and neutral aluminium oxide clean-up. The procedure is summarised in Figure 32, experimental details are presented in Appendix 6.6.5.

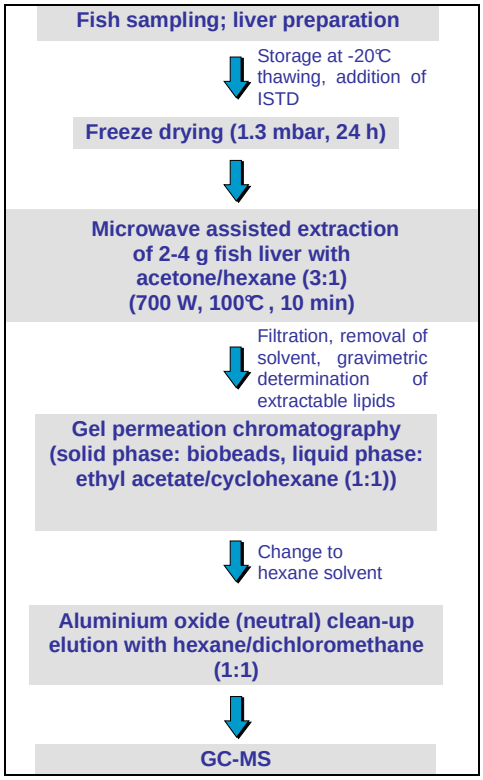



**Figure 32:** Overall scheme for the analysis of biota samples for chlorpyrifos, endosulfan, and trifluralin

### 6.3.2.2 Methods for Dicofol

As dicofol was found to be thermally unstable during gaschromatographic separation, the compound had to be injected “on column” at low temperature. Mass spectrometric detection of dicofol was carried out in EI mode. An HPLC-MS/MS method proved to be unsuitable for dicofol because the yield of ions was too low in the ESI mode. Experimental details are presented in Appendix 6.7.

#### 6.3.2.3 Methods for Pentachlorophenol and Trichloropyridinol

PCP and TCPy are susceptible to adsorption in the GC system because of their polar phenolic substructure. Direct GC analysis was only possible at high concentrations (>1ng). Therefore, two alternative approaches were applied during the study.

First, an HPLC-MS method was developed in order to overcome thermal stress during the analysis. This was successful in principle. A reversed phase HPLC gradient separation yielded well-defined, non-tailing peaks both for PCP and TCPy, and ESI MS showed M-1 ions in the negative ionisation mode with a good ion yield (Figure 52, Appendix 6.6.6.1). Unfortunately, it was not possible to fragment the pseudo-molecular ion; the high selectivity of MS-MS thus could not be used, but only the less selective single-stage MS in SIM mode. Consequently, this method was applicable only to water samples, where matrix disturbances are much less pronounced than in sediment or biota samples. But even with water samples only a moderate sensitivity could be obtained.

The second approach used a derivatisation step with heptafluorobutyric acid anhydrate (HFBAA) to form the HFBA esters and a GC-MS analysis in the NCI mode. This technique was used for sediments and biota. Only PCP could be derivatised and analysed by this method.

### HPLC-MS method for water samples

The principles of the analytical procedure for sea water samples are summarised in Figure 33; experimental details are provided in the Annex (6.6.6).

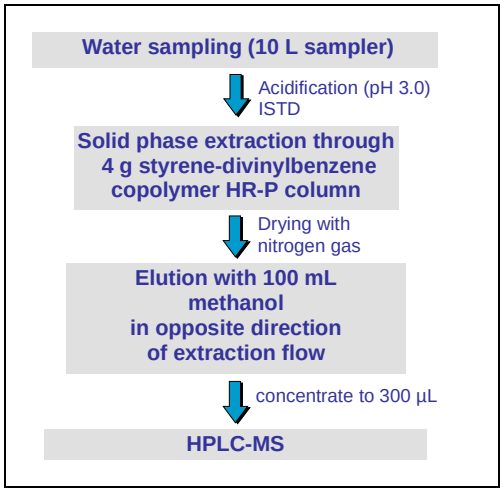



**Figure 33:** Overall scheme for the analysis of sea water for PCP and TCPy

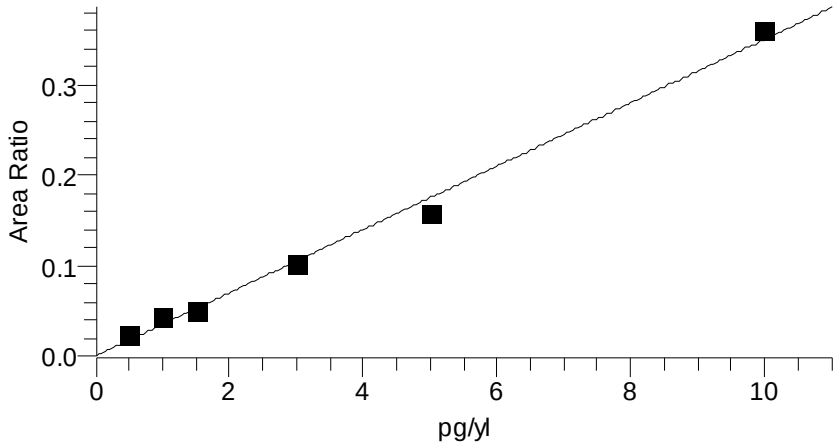
## 6.3.3 Method validation and quality control

### 6.3.3.1 Linearity

Figure 34 shows the calibration curves for the GC-NCI-MS analysis of chlorpyrifos, endosulfan, and trifluralin. They all show good linearity in the low concentration range, which applies to nearly all marine samples of this study.

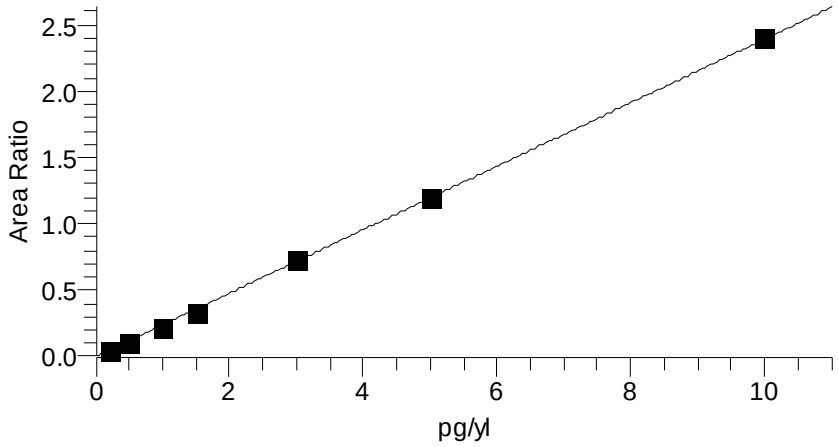
Chlorpyrifos-methyl

Y = 0.00101086+0.0349017*X  R^2 = 0.9934  W: Equal



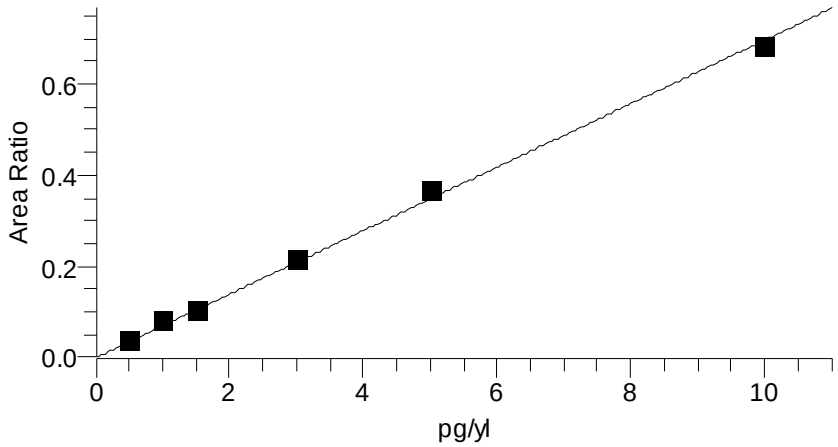

Chlorpyrifos-ethyl

Y = 0.239272*X  R^2 = 0.9996  W: Equal




Endosulfan_I

Y = 0.0696181*X  R^2 = 0.9975  W: Equal



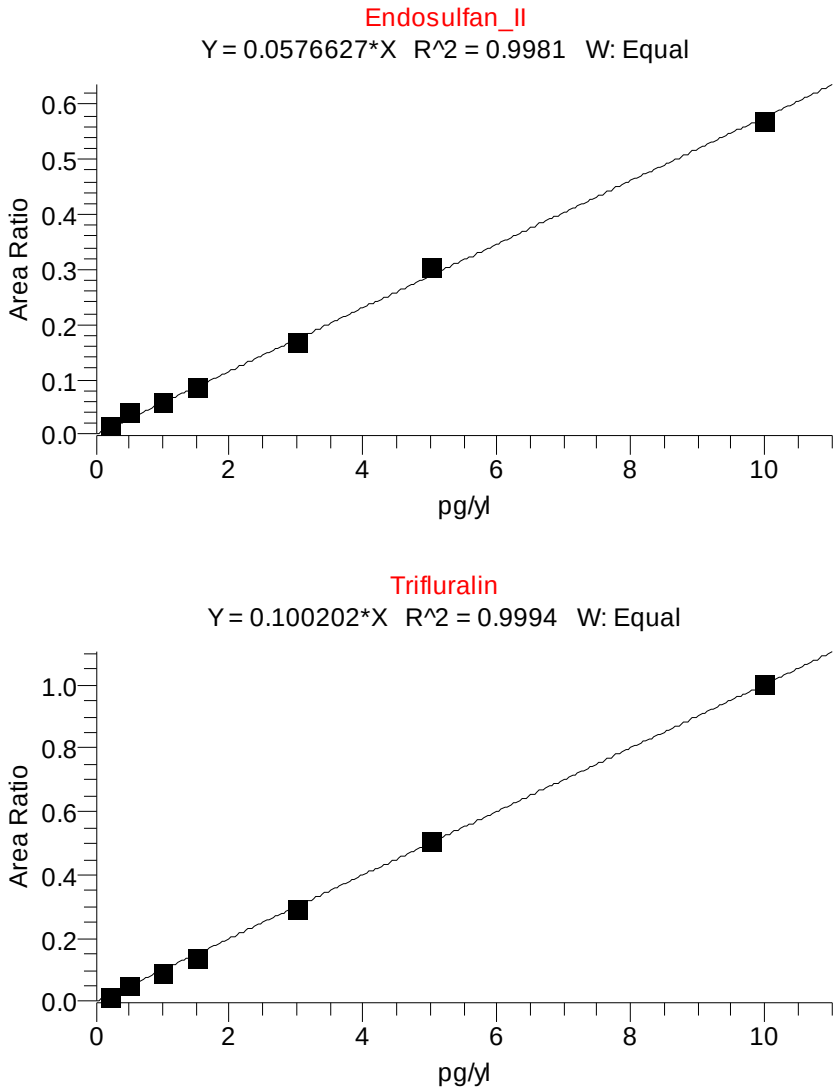



**Figure 34:** Calibration curves of chlorpyrifos, endosulfan, and trifluralin

### 6.3.3.2 Blank values

#### Water samples

Chlorpyrifos-ethyl and –methyl, trifluralin, and endosulfan II showed no blank values above the limits of detection (LOD). In the internal standard (ISTD) $D_4$-endosulfan I traces of non-deuterated endosulfan I were found. Thus a blank value is caused in high concentrated solutions of ISTD (e. g. 4 ng/L). In lower concentrated ISTD solutions, (e. g. 0.4 ng/L) the amount of endosulfan I is below the limit of detection whereby the blank value is deleted.

**Table 66:** Blank values in water samples

| | Trifluralin | Chlorpyrifos-methyl | Chlorpyrifos-ethyl | Endosulfan I | Endosulfan II |
|---|---|---|---|---|---|
| SPE system[a] | <LOD | <LOD | <LOD | <LOQ | <LOD |
| Sea water[b] | <LOD | <LOD | <LOD | <LOD | <LOD |
| Internal Standard[a] (0.4 ng/L water) | <LOD | <LOD | <LOD | <LOD | <LOD |

[a]: Average of 4 measurements; [b]: Average of 2 measurements

#### Sediment samples

No problems were encountered with blank values in sediment samples.

**Table 67:** Blank values in sediment samples

| | Trifluralin | Chlorpyrifos-methyl | Chlorpyrifos-ethyl | Endosulfan I | Endosulfan II |
|---|---|---|---|---|---|
| Blank value of the system[a] | <LOD | <LOD | <LOD | <LOD | <LOD |
| Silica sand[b] | <LOD | <LOD | <LOD | <LOD | <LOD |
| Internal Standard[b] (0.1 µg/kg dw) | <LOD | <LOD | <LOD | <LOD | <LOD |

[a]: Average of 2 measurements containing blank values from solvents, rotary evaporator, silica gel column, filtration unit; [b]: Average of 3 measurements

### Biota samples

No blank problems were observed with biota samples and the work-up procedure.

**Table 68:** Blank values in biota samples

| | Trifluralin | Chlorpyrifos -methyl | Chlorpyrifos -ethyl | Endosulfan I | Endosulfan II |
|---|---|---|---|---|---|
| Blank value of the system[a] | <LOD | <LOD | <LOD | <LOD | <LOD |
| Internal Standard[b] (1.3 µg/kg dw Fish liver matrix) | <LOD | <LOD | <LOD | <LOD | <LOD |

[a]: Average of 2 measurements containing blank values from solvents, rotary evaporator, columns (GPC, aluminium oxide, silica gel), filtration unit; [b]: Average of 3 measurements

## 6.3.3.3 Recoveries

### Water samples

Table 69 shows the relative recoveries of the analytes (average 5 spike experiments with a concentration of 4 ng/L) in sea water samples. At a range of 72 to 120 %, they may be considered good.

**Table 69:** Relative recoveries of the analytes in sea water samples

| Analyte | Recovery [%] |
|---|---|
| Chlorpyrifos-ethyl | 85 |
| Chlorpyrifos-methyl | 90 |
| Endosulfan I | 85 |
| Endosulfan II | 90 |
| Trifluralin | 72 |
| Dicofol | |
| PCP | 105 |
| TCPy | 120 |

In Table 70. the absolute recoveries of the analytes during sample work-up are summarised for the sea water analysis. As expected, no significant losses are observed.

**Table 70:** Absolute recoveries of the analytes; addition of ISTD after extraction and elution [%] in sea water samples

| Analyte | Recovery [%] |
|---|---|
| Chlorpyrifos-ethyl | 94 |
| Chlorpyrifos-methyl | 109 |
| Endosulfan I | 88 |
| Endosulfan II | 99 |
| Trifluralin | 81 |

## Sediment samples

Relative and absolute recoveries of the analytes in sediment samples are shown in Table 71 and Table 72, respectively. Both relative and absolute recoveries are sufficiently high. The standard deviations are satisfactory.

**Table 71:** Relative recoveries of the analytes (spike experiments with a concentration of 0.2 µg/kg (one experiment) and 4 µg/kg (average of 5 experiments) in sediment samples

| Analyte | Recovery [%] 0.2 µg/kg | Recovery [%] (M±STD) 4 µg/kg |
|---|---|---|
| Chlorpyrifos-ethyl | 118 | 85±12 |
| Chlorpyrifos-methyl | 63 | 86±12 |
| Endosulfan I | 82 | 93±3 |
| Endosulfan II | 111 | 87±11 |
| Trifluralin | 87 | 94±9 |

**Table 72:** Absolute recoveries [%] of the ISTD referred to $D_6$-Chlorpyrifos-methyl as recovery standard (7 experiments; concentration of ISTD 0.2 µg/kg) in sediment samples; addition of the recovery standard (0.004 ng/µL) after extraction and elution

| Analyte | Recovery [%] (M±STD) |
|---|---|
| $D_{10}$-Chlorpyrifos-ethyl | 74±11 |
| $D_4$-Endosulfan I | 80±11 |
| $D_{14}$-Trifluralin | 74±10 |

## Biota samples

Relative and absolute recoveries of the analytes in fish liver show a wider range and larger variation (see Table 73 and Table 74) than the two other matrices. This is caused by interfering effects of matrix during the analysis and problems observed with the GPC clean-up column. The absolute recovery of $D_{14}$-Trifluralin is low because separation from the lipids via GPC is difficult and, therefore, a large amount of trifluralin is lost during separation. Quantification nevertheless is reliable because the relative recovery of trifluralin – referred to $D_{14}$-Trifluralin – is good (Table 73), and both have a chemically identical behaviour.

**Table 73:** Relative recoveries [%] of the analytes in biota samples *

| **Analyte** | ***Lima lim* spike: 3.2 µg/kg lipid** | ***Lima lim* spike: 211 µg/kg lipid** | ***Gadu mor* spike: 44 µg/kg lipid** | ***Gadu mor* spike: 3 µg/kg lipid** | ***Plat fle* spike: 0.8 µg/kg lipid** |
|---|---|---|---|---|---|
| Chlorpyrifos-ethyl | 143 | 105 | 109 | 117 | 132 |
| Chlorpyrifos-methyl | 87 | 114 | 80 | 68 | 80 |
| Endosulfan I | 105 | 90 | 61 | 111 | 104 |
| Endosulfan II | 147 | (500) | 57 | 145 | 127 |
| Trifluralin | 129 | 106 | 111 | 110 | 100 |

* *Lima lim: Limanda limanda*, dab; *Gadu mor: Gadua morhua*, cod
*Plat fle:Platichtys flesus*, flounder

**Table 74:** Absolute recoveries [%] of the ISTD referred to $D_6$-Chlorpyrifos-methyl as recovery standard in biota samples; addition of the recovery standard (0.004 ng/µL) after extraction and clean-up.

| **Analyte** | **Recovery [%] Lima lim (2 experiments) (M±STD)** | **Recovery [%] *Gadu mor* (one experiment)** | **Recovery [%] *Plat fle* (one experiment)** |
|---|---|---|---|
| $D_{10}$-Chlorpyrifos-ethyl | 79±3 | 58 | 20 |
| $D_4$-Endosulfan I | 65±5 | 68 | 27 |
| $D_{14}$-Trifluralin | 20±22 | 60 | 54 |

#### 6.3.3.4 Limits of quantification

Table 75 to Table 77 show the limits of quantification (LOQ) of the analytes in the different matrices. LOQ are calculated for a signal-to-noise ratio (S/N) of 9; the limits of detection (LOD) are calculated for an S/N ratio of 3.

**Table 75:** Limits of quantification of the analytes [pg/L] in sea water samples

| Analyte | Limit of Quantification [pg/L] |
|---|---|
| Chlorpyrifos-ethyl | 10 |
| Chlorpyrifos-methyl | 10 |
| Endosulfan I | 20 |
| Endosulfan II | 20 |
| Trifluralin | 7 |
| Dicofol | 1000 |
| PCP | 200 |
| TCPy | 400 |

As shown in Table 76, the LOQs for sediments were improved during the project by increasing the amount of sediment sample and increasing the polarity of the extraction solvent. In this way, different samples may have partly different LOQs.

**Table 76:** Comparison of LOQs of the analytes [ng/g dw] in sediment samples for different sample amounts (20 and 40 g of sediment) and different solvents (Solvent: A: acetone/hexane 50/50; B: acetone/ hexane 75/25, MAE: 10 min, 100°C, 700 W)

| Analyte | LOQ (20 g sediment) [ng/g] A[a] | LOQ (40 g sediment) [ng/g] A[b] | LOQ (40 g sediment) [ng/g] B[c] |
|---|---|---|---|
| Trifluralin | 0.005 | 0.003 | 0.0006 |
| Chlorpyrifos-methyl | 0.034 | 0.025 | 0.023 |
| Chlorpyrifos-ethyl | 0.017 | 0.009 | 0.007 |
| Endosulfan I | 0.024 | 0.010 | 0.011 |
| Endosulfan II | 0.061 | 0.030 | 0.014 |

[a]GA371, GA387, GA402 and GA405; [b]GA419; [c]GA421.

The LOQs for biota vary depending on the particular fish species (Table 77) and are due primarily to different amounts of lipid and matrix background levels in the three fish species studied.

**Table 77:** Limits of quantification (LOQ=threefold LOD) /detection (LOD=threefold peak-to-peak noise) of the analytes [µg/kg lipid] in fish liver samples.

| Analyte | LOQ [µg/kg lw] *Lima limanda* | LOQ [µg/kg lw] *Gadua morhua* | LOQ [µg/kg lw] *Platichtys flesus* |
|---|---|---|---|
| Chlorpyrifos-ethyl | 0.6 | 3.4 | 0.6 |
| Chlorpyrifos-methyl | 3.6 | 3.0 | 0.6 |
| Endosulfan I | 0.4 | 4.8 | 2.1 |
| Endosulfan II | 1.1 | 6.9 | 1.1 |
| Trifluralin | 0.1 | 2.1 | 0.6 |

#### 6.3.3.5 Laboratory Performance Study

In 2004, the PCP method involving HPLC-MS analysis was checked through participation in a QUASIMEME laboratory performance study for PCP in sea water. The result showed excellent agreement with the assigned value (Z score: -0.42), which underlines the accuracy of the method developed.

## 6.4 Results and Discussion

### 6.4.1 Chlorpyrifos

The analytical method that has been developed and validated (6.3.3) is characterised by its high sensitivity, a prerequisite to the determination of pollutants in marine matrices. Table 78 summarises the LODs and LOQs achieved in the different matrices. The values obtained are on the order of the concentrations of classical pollutants like HCH, PCBs, DDT-metabolites or PAH which are presently observed in the open marine environment.

**Table 78:** Limits of detection (LOD) at a signal-to-noise ratio (S/N) of 3:1 and limits of quantification (LOQ, S/N 9:1) for Chlorpyrifos

| | LOD | LOQ | Sample amount |
|---|---|---|---|
| **Water** | | | |
| Chlorpyrifos-ethyl | 3 pg/L | 10 pg/L | 10 L |
| Chlorpyrifos-methyl | 3 pg/L | 10 pg/L | 10 L |

| | LOD | LOQ | Sample amount |
|---|---|---|---|
| **Sediment** | | | |
| Chlorpyrifos-ethyl | 3 ng/kg | 7 ng/kg | 40 g |
| Chlorpyrifos-methyl | 8 ng/kg | 25 ng/kg | 40 g |
| **Biota** | | | |
| Chlorpyrifos-ethyl | 0.2 µg/kg | 0.6 µg/kg | 3 g |
| Chlorpyrifos-methyl | 1.2 µg/kg | 3.6 µg/kg | 3 g |

#### 6.4.1.1 Water

Chlorpyrifos was determined in sea water samples of the North Sea and Baltic Sea which had been collected during 7 cruises in 2003 to 2005 (Table 85 and Table 86, Appendix 6.6.8)

Chlorpyrifos-methyl was below the LOD in nearly all samples.

Chlorpyrifos-ethyl was found in most samples at concentrations of < 10 to 110 pg/L with a median of 26 pg/L. More than 95 % of the values were above the LOD. The geographical distribution of concentrations is shown in Figure 35 and Figure 36.

In the German Bight, clear and steady gradients were not found in all surveys. For example, a gradient extending from the coasts to the open sea was observed in May 2003, whereas the distribution in May and July 2004 was found to be quite uniform. Although slightly higher concentrations occurred in the river Elbe at Stade (85 to 180 pg/L), indicating an input of chlorpyrifos-ethyl to the German Bight, this had no major effect on the distribution pattern in the marine waters. Other sources will have to be considered as an explanation for the “background level” of 10 to 30 pg/L in the southern North Sea and the higher levels found sporadically at the western border of the German Bight. The cause may be either atmospheric deposition or inputs from the river Rhine/Schelde or the UK.

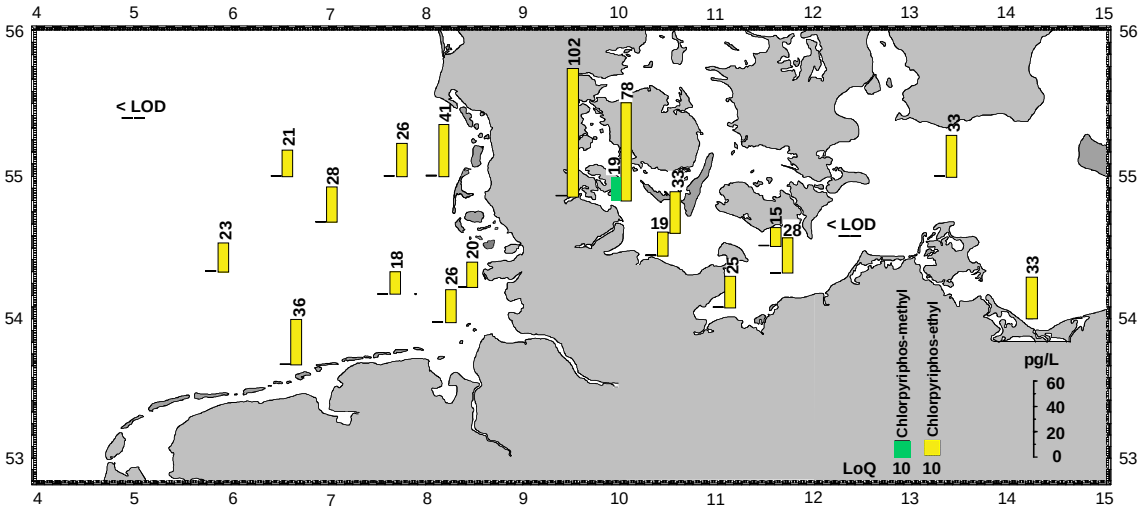



**Figure 35:** Chlorpyrifos concentrations [pg/L] in surface water (5 m) in the North Sea (July 2004) and Baltic Sea (June 2004)

The findings of the survey in July 2003, which covered the entire North Sea, are in line with those of the German Bight survey. Concentrations in the southern North Sea were significantly higher than in the northern part.

Concentrations in the western Baltic Sea were similar to those in the German Bight (Figure 35). The values at most stations ranged from 19 to 33 pg/L. However, concentrations of 78 and 102 pg/L were found in the Flensburg Fjord. These elevated levels are attributable to the slow water exchange in this bight and intensive agricultural activities in the area.

In 2004, some samples were taken in the Greenland Sea – along the 75° latitude – in order to investigate possible long-range transports and determine background concentrations. However, no chlorpyrifos above the LOD was found in these samples.

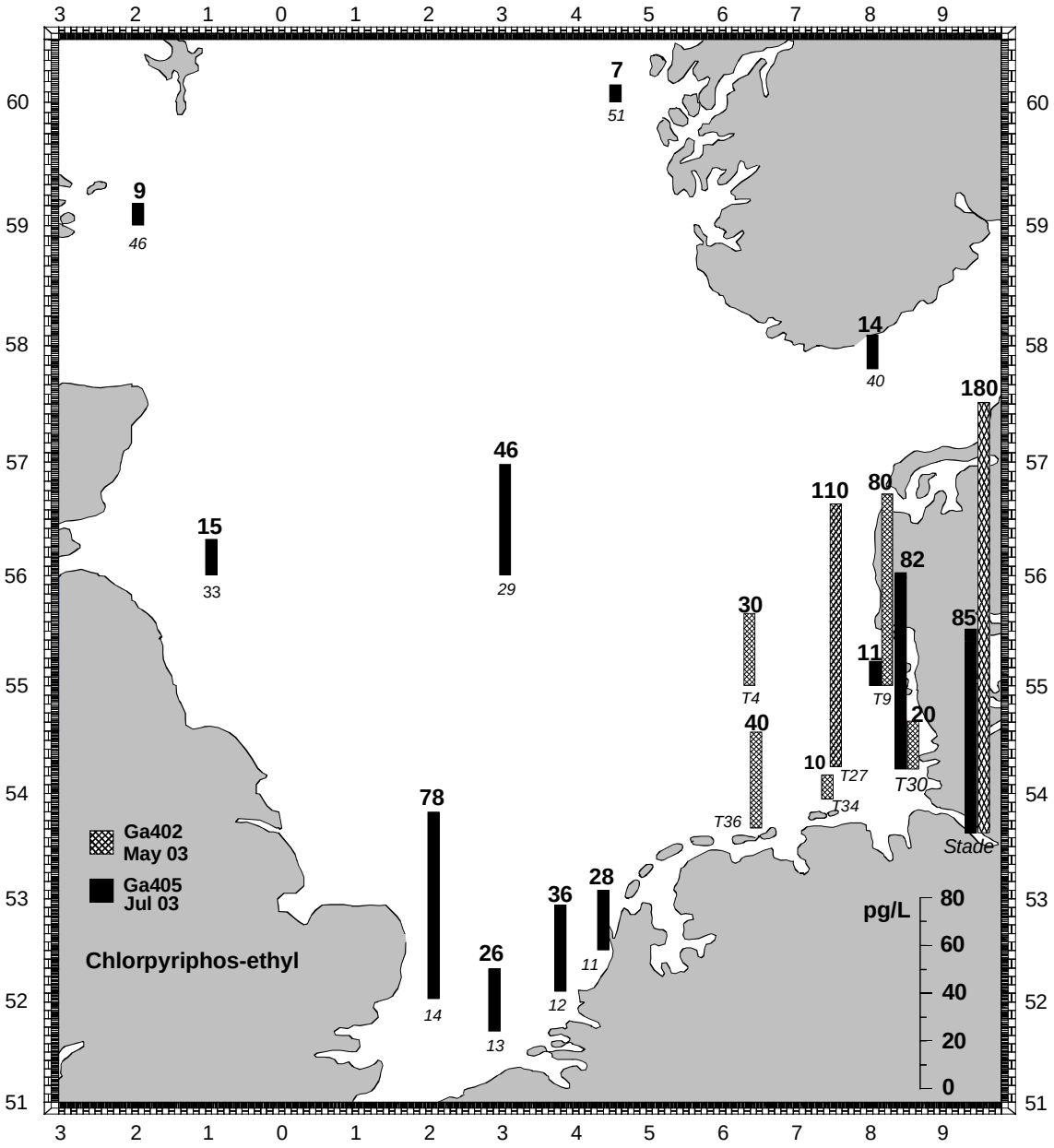



**Figure 36:** Chlorpyrifos-ethyl concentrations [pg/L] in surface water (5 m) in 2003

No seasonal influence on chlorpyrifos levels was observed in the investigations. During two cruises in February 2004 and January 2005, median concentrations of 39 and 13 pg/L, respectively, were found.

### 6.4.1.2 Sediment

Chlorpyrifos was determined in 30 surface sediment samples from the North Sea and Baltic Sea which had been collected during 6 cruises in 2003 to 2005 (Table 87,

Appendix 6.6.8) Concentrations of chlorpyrifos-methyl were below the LOD in all sediment samples from the German Bight and western Baltic Sea.

Chlorpyrifos-ethyl was found at concentrations from < 10 to 33 ng/kg dw with a median of 9 ng/kg dw. About 43 % of the determinations were < LOD. This rendered the interpretation of data more difficult.

The geographical distribution of the insecticide in the North Sea and Baltic Sea is shown in Figure 37. In the German Bight, the substance was determined only at stations characterised by higher silt levels and TOC values above 3 mg/g. Remarkably, values as low as 5 to 14 ng/kg dw were even found at KS 11, the station which normally has the highest contamination level. The limited amount of data does not, however, allow a statistically valid interpretation of the geographical distribution in terms of spatial gradients or input structures.

Concentrations in the western Baltic Sea were even lower than in the German Bight.

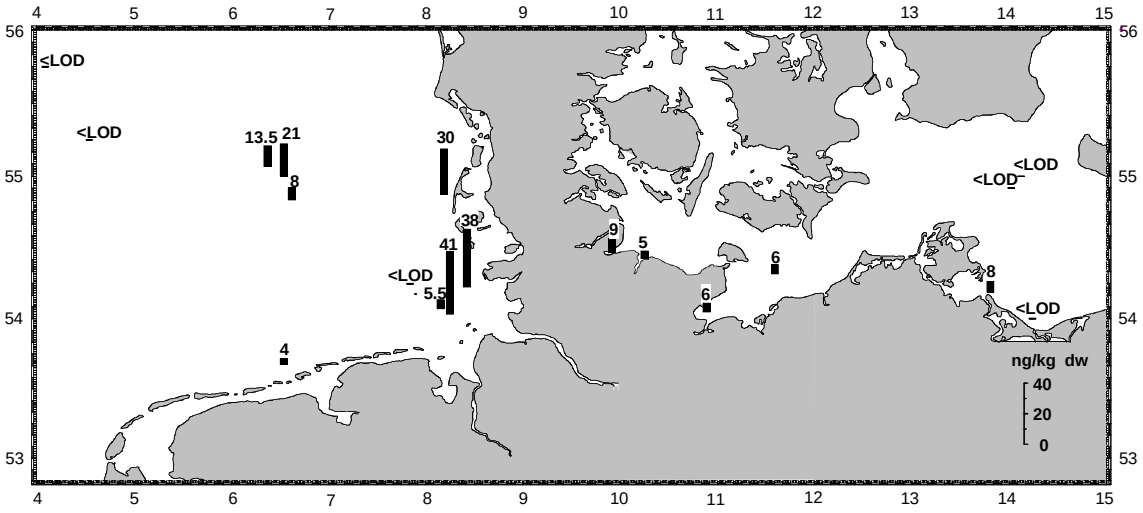



**Figure 37 :** Chlorpyrifos-ethyl concentrations [ng/kg] in surface sediments (0-2 cm)

The estimated “enrichment” of chlorpyrifos-ethyl in sediment as compared to the water phase is less than 1000. This means that sediment is not a major sink for this compound and will not be a good monitoring matrix.

Concentrations in sediment are low in comparison with classical pollutants. At KS 11, for example, HCH isomers ranged from 0.01 to 0.2 µg/kg. The more lipophilic DDD and CB153 ranged from 1 to 5 µg/kg dw. PAHs like, e.g., BaP showed sediment concentrations of 40 to 240 µg/kg dw.

#### 6.4.1.3 Biota

25 liver samples of three different fish species (dab (*Limanda limanda*), cod *(Gadua morhua*) and flounder (*Platichtys flesus*)) were examined, which had been collected during two cruises in the North Sea and western Baltic Sea in 2001 and 2002. (Table 88, Appendix 6.6.8)

Chlorpyrifos-methyl was below the LOD in most samples.

Chlorpyrifos-ethyl in most samples was found at concentrations from 0.1 to 3.4 µg/kg wet weight (0.3 to 13 µg/kg lipid weight), with a median of 0.6 µg/kg ww (2.77 µg/kg lw). The geographical distribution of the samples and their concentrations are shown in Figure 38. Variability of the data may be considered normal. Because of the limited data set presently available it is not possible to evaluate the results under the aspect of regional patterns, species differences, or time variability. Therefore, it is not possible presently to comment on the significance of the higher values found in the western Baltic Sea.

The estimated “enrichment” of chlorpyrifos-ethyl in biota compared to the water phase is approximately $10^4$ to $10^5$.

The chlorpyrifos values observed are on the same order as classical pollutants like HCH and HCB; they are, however, below the levels of the very lipophilic DDT and PCB group. In 2000, typical HCH concentrations in the German Bight ranged from 0.2 to 0.6 µg/kg ww; HCB had a median of 0.84 µg/kg ww, and the sum of DDTs had a median of 3.7 µg/kg ww (BSH, 2005).

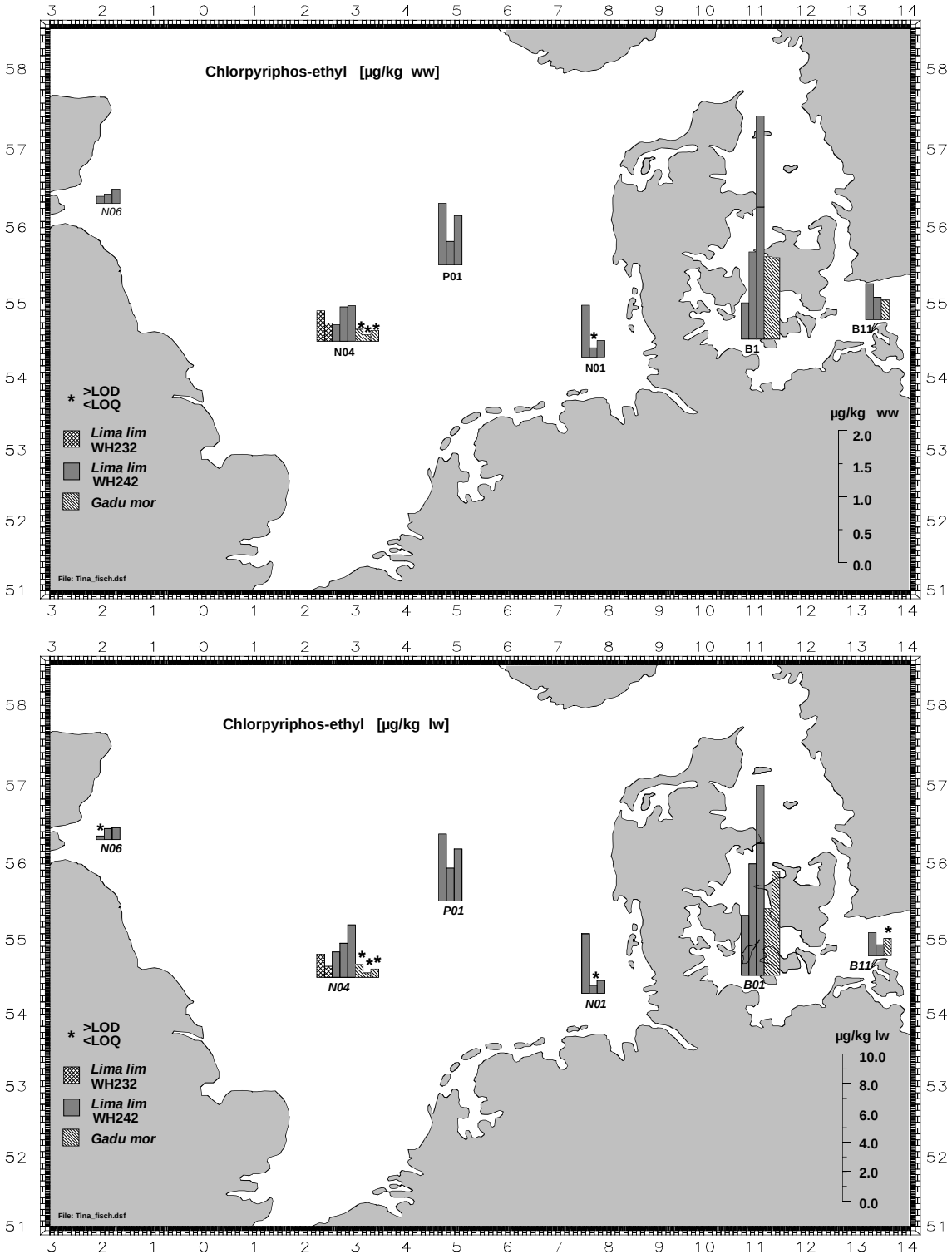



**Figure 38:** Concentrations of chlorpyrifos-ethyl in fish liver; upper fig. in µg/kg wet weight, lower fig. in µg/kg lipid

### 6.4.1.4 Discussion

This is the first time that chlorpyrifos has been detected in the marine environment, including concentrations above the LOQ. Although samples from the marine environment of the North Sea and Baltic Sea contained no chlorpyrifos-methyl, the homologue ester chlorpyrifos-ethyl was identified in most water and biota samples from those areas. Positive findings were less frequent in sediment samples. The concentrations found in water and sediments were very low, with a median of 26 pg/L in water samples, and 9 ng/kg dw in sediments. Considerable bioaccumulation was observed in fish liver. The concentrations found in the different matrices are shown in Figure 39.

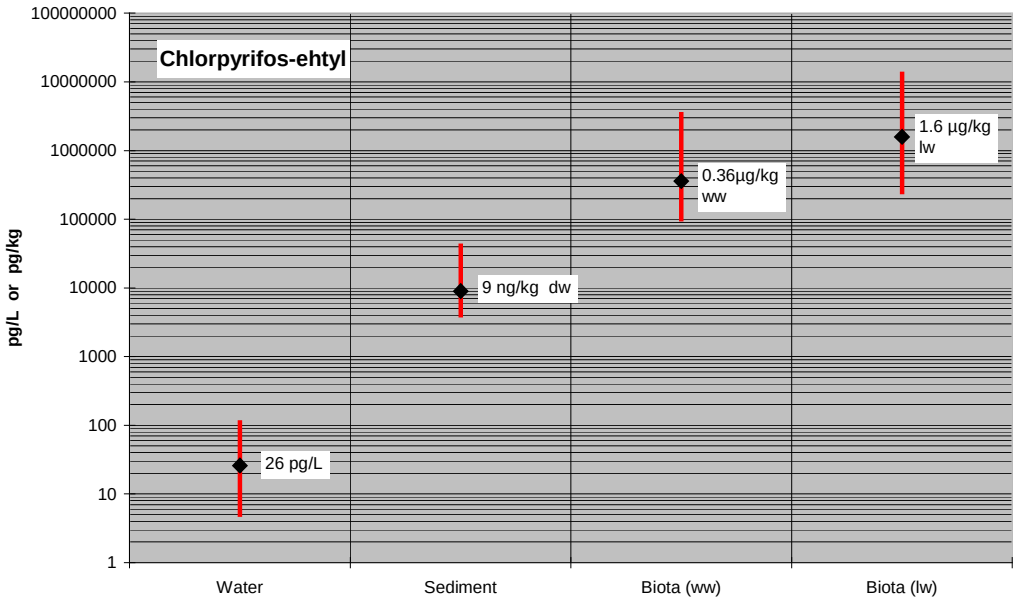



**Figure 39:** Summary of chlorpyrifos-ethyl concentrations in different marine matrices; min – max range and median values

Based on these data, the enrichment from water to sediment is calculated at about 350, that from water to biota (fish liver) at about 14000 (wet weight) and 62000 (lipid weight). The relatively low enrichment is in accordance with the moderate log $K_{OW}$ value of 3.6 – 4.5.

The distribution pattern and low concentrations observed in water from the North Sea and Baltic Sea is best explained by a general low-level background burden with a few

minor local sources. Concentrations in the river Elbe – generally the most important source of pollutant input to the German Bight - are relatively low, and thus also the influence on chlorpyrifos contamination is low.

A comparison of these findings with literature data is difficult because LOD in most studies are much higher (>ng/L) than in this survey, and chlorpyrifos is mostly reported to be below the LOD.

It was found in several studies (e.g. Dabrowski et al., 2002) that concentrations of chlorpyrifos in the lower ng/L range for water and low µg/kg range for sediments occurred in river runoff following application of the pesticide. These studies were carried out mainly in areas with intensive use of the insecticide – in the US, China, and southern Europe.

Atmospheric transport and deposition is well documented. Apart from atmospheric transport after spraying applications, volatilisation and escape to the atmosphere has been suggested (Nhan et al., 2002). Strong evidence for the importance of atmospheric long-range transport is provided by Gabarino et al. (2002), who observed high concentrations of chlorpyrifos (70 – 80 ng/L) in arctic snow in Alaska.

The fate of chlorpyrifos in the marine environment was investigated by Nhan et al. (2002) and Kale et al. (1999) in $^{14}$C-chlorpyrifos experiments in microcosms. Considerable degradation was observed in the marine environment, with TCPy the main degradation product. Although rapid sorption onto sediment was observed, only 1-2 % of the initial amount of $^{14}$C-chlorpyrifos was detected in sediment by the end of the experiment. Accumulation in fauna and flora reached maxima of 5.8 and 2.2 %, respectively. The balance of radioactivity suggests that the main loss of the insecticide from the system occurred through volatilisation and escape to the air. The persistence half-life in the microcosms was calculated at 5 days. Similar half-lifes of 3.5 and 20 days in pond water are reported by Racke (1992).

Sorption to sediments did not lead to significant enrichment in the sediments investigated. Therefore, sediments should not be the primary monitoring matrix. This is

somewhat contradictory to the well-described high affinity of chlorpyrifos to soil (Wauchope et al., 1992, Dabrowski et al., 2002, Racke, 1992).

Concentrations in sea water were found to be well below the levels of acute toxicity reported for aquatic organisms, which range from 3 to 806 µg/L (U.S. Environmental Protection Agency, 1986). However, Owen et al. (2002) reported inhibition of hemolymph acetylcholinesterase activity in a tropical scallop with chlorpyrifos levels as low as 0.1 to 10 ng/L, which is closer to the observed concentrations.

Compared to the EQS value of 30 ng/L, as proposed for evaluating inland and transitional waters for the WFD, the observed sea water concentrations for chlorpyrifos are quite low.

Considering the short half-life and volatility of the substance and the low levels of local sources, it is remarkable that chlorpyrifos has been detected in the marine environment of the North and Baltic Seas at all. In water, concentrations of chlorpyrifos-ethyl are higher than those of the classical lipophilic pollutants such as HCB, DDT, PCB or PAHs, but below HCH concentrations. In sediments and biota, concentrations are below the levels of classical pollutants.

Unfortunately, it has not been possible to obtain detailed production or consumption data for Germany or Europe. An evaluation of the findings regarding impacts on the marine environment is difficult as long as figures on chlorpyrifos consumption in the states bordering the North Sea and Baltic Sea are not available for comparison with substance levels found in the sea. According to pan-uk (2005), in Europe about 1000 t is used annually. Thus, chlorpyrifos does not play the same important role in Europe as it does in the US, where an estimated 10000 t is used annually (EPA at www.epa.gov/oppsrrd1/op/chlorpyrifos/summary.htm).

As a consequence of the positive findings, the BSH will determine chlorpyrifos on a voluntary basis during the next two years in order to support the results of this study. However, because of the low absolute concentrations it is not considered necessary to include chlorpyrifos as a routine parameter in the standard monitoring program.

### 6.4.2 Trichloropyridinol (TCPy)

#### 6.4.2.1 Water

The analytical method which has been developed for the determination of trichloropyridinol is characterised by a moderate sensitivity, making it suitable for first screening in sea water. An LOD of 0.1 ng/L and LOQ is 0.4 ng/L has been achieved. A full validation of the method was not performed because no positive findings were obtained with the screened samples.

Trichlorpyridinol was analysed in sea water samples from the North Sea which were taken during 3 cruises in 2003 to 2004. TCPy was below 0.1 ng/L (LOD) in all samples.

#### 6.4.2.2 Discussion

TCPy is the principal metabolite of chlorpyrifos, but it also is a metabolite of the herbicide triclopyr (Petty et al., 2001).

The half-lives of TCPy in water range from 4 to 8.8 days, in sediments from 3.8 to 13.3 days (Petty et al., 2001). According to a US EPA review (1989), it adsorbs weakly to soil particles and appears to be moderately mobile and persistent in soils.

Considering the low concentrations of chlorpyrifos observed and the moderate environmental persistence reported for TCPy, it is no surprise that no TCPy was found in the sea water samples analysed. In view of these findings, it was decided that no project priority should be given to a further improvement of the method and lowering of the LOQ to detect ultra-trace levels of TCPy or to the development of a sensitive analytical procedure for sediment and biota samples.

### 6.4.3 Endosulfan

The analytical method which has been developed and validated (3.3) is characterised by its high sensitivity, which is necessary for the determination of pollutants in marine matrices. Table 79 summarises the LODs and LOQs achieved in the different matrices.

**Table 79:** Limits of detection (LOD) at a signal-to-noise ratio (S/N) of 3:1 and limits of quantification (LOQ, S/N 9:1) for endosulfan

| | LOD | LOQ | Sample amount |
|---|---|---|---|
| **Water** | | | |
| Endosulfan I | 7 pg/L | 20 pg/L | 10 L |
| Endosulfan II | 7 pg/L | 20 pg/L | 10 L |
| **Sediment** | | | |
| Endosulfan I | 4 ng/kg | 11 ng/kg | 40 g |
| Endosulfan II | 4 ng/kg | 14 ng/kg | 40 g |
| **Biota** | | | |
| Endosulfan I | 0.1 | 0.4 µg/kg | 3 g |
| Endosulfan II | 0.4 | 1.1 µg/kg | 3 g |

The values obtained are comparable to those of classical pollutants like HCH, PCBs, DDT-metabolites or PAH, which are presently observed in the open marine environment.

#### 6.4.3.1 Water

Endosulfan was determined in sea water samples from the North Sea and Baltic Sea which had been collected during 7 cruises in 2003 to 2005 (Table 89 to Table 91, Appendix 6.6.8).

Endosulfan II was below the LOQ in nearly all samples. Endosulfan I was found in most samples at concentrations of < 7 to 50 pg/L with a median of 25 pg/L. About one third of the data was below the LOQ. The geographical distribution of the concentrations is shown in Figure 40 and Figure 41.

In the German Bight, concentrations were within a fairly narrow range of <10 to 50 pg/L; no clear gradients were observed in any of the surveys. Although slightly higher concentrations were found occasionally in the river Elbe at Stade (May 2004: 50 pg/L), indicating endosulfan input to the German Bight via this river, this had no appreciable influence on endosulfan distribution in the marine waters. Other sources must be considered to explain the “background level” of 10 to 20 pg/L in the southern North Sea

and the German Bight. This may be either atmospheric deposition or inputs from the rivers Rhine/Schelde or the UK.

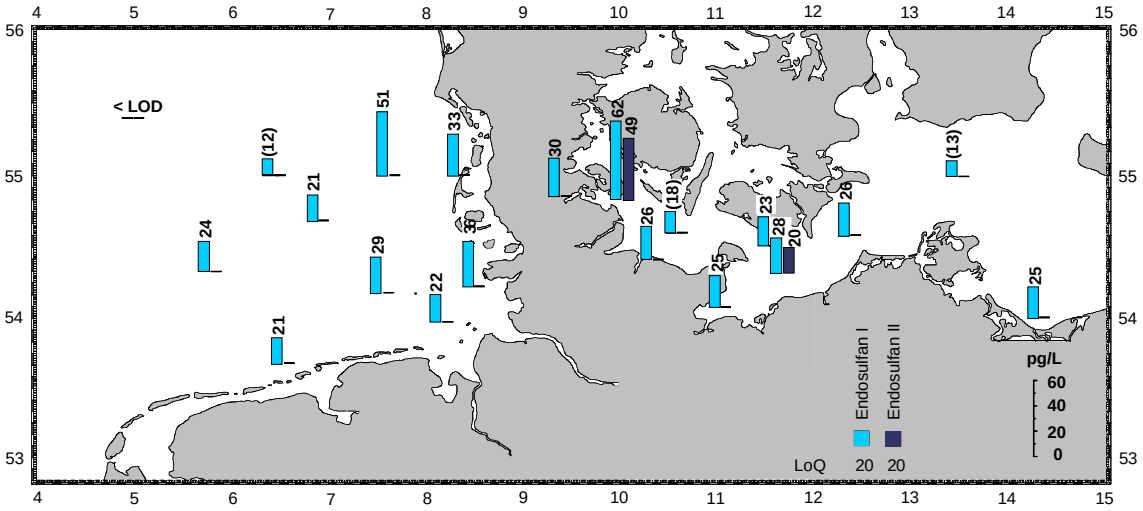



**Figure 40:** Endosulfan concentrations [pg/L] in surface water (5 m) in the North Sea (July 2004) and Baltic Sea (June 2004)

In the western Baltic Sea, concentrations were in a similar range as in the German Bight. Mainly endosulfan I was detected. Values at most stations ranged from 20 to 30 pg/L. However, a concentration of 62 pg/L was measured in the Flensburg Fjord. This elevated level can be explained by slow water exchange in this bight, and possibly by agricultural activities in the area.

The results of the survey in July 2003, which covered the entire North Sea, were similar to those of the German Bight survey. Concentrations in the southern and central North Sea tend to be higher than in the northern North Sea.

In 2004, a few samples were taken in the Greenland Sea – along the 75° latitude – in order to investigate possible long-range transports and determine background concentrations. No endosulfan above the LOD was detected in these samples. However, the LOD was considered too high for such remote areas.

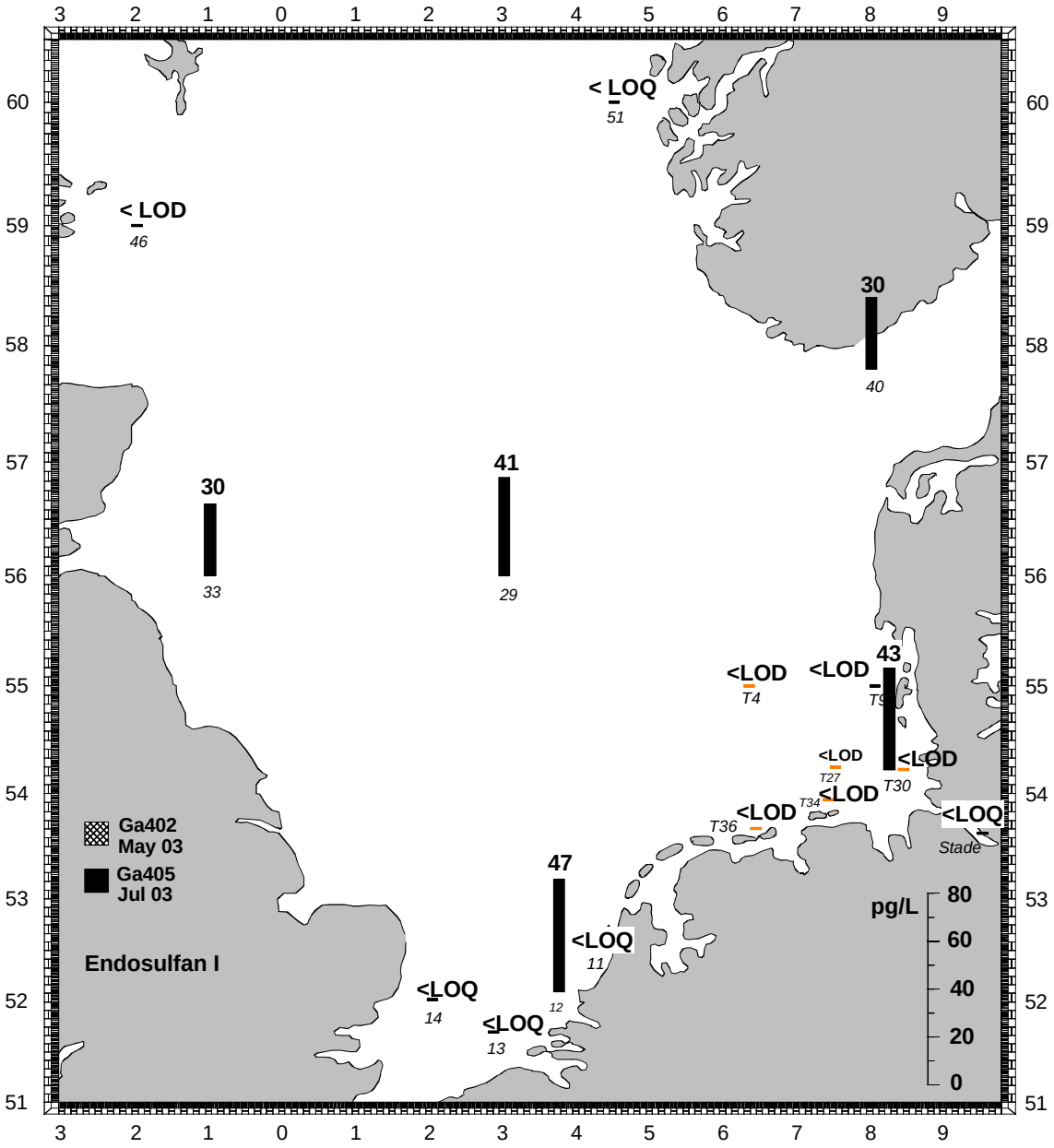



**Figure 41:** Endosulfan I concentrations [pg/L] in surface water (5 m) in 2003

### 6.4.3.2 Sediment

Endosulfan was determined in 30 surface sediment samples from the North Sea and Baltic Sea which had been collected during 6 cruises in 2003 to 2005 (Table 42 and Table 43, Appendix 6.8). While concentrations of Endosulfan II were below the LOD in

all sediment samples from the German Bight, it was found at some stations in the western Baltic Sea.

Endosulfan I was determined at concentrations between <10 and 200 ng/kg dw with a median of 20.5 ng/kg. As about 60 % of the determinations were < LOD, the data can only be interpreted to a limited extent.

The spatial distribution in the German Bight and western Baltic Sea is shown in Figure 42. In the German Bight, Endosulfan I was only detected at stations having higher silt levels and TOC values above 3 mg/g. Surprisingly, even at station KS 11 which usually has the highest contamination levels, the measured values were 21 ng/kg dw or less. The limited data set does not allow a statistically relevant interpretation of the geographical distribution with respect to spatial gradients or input structures.

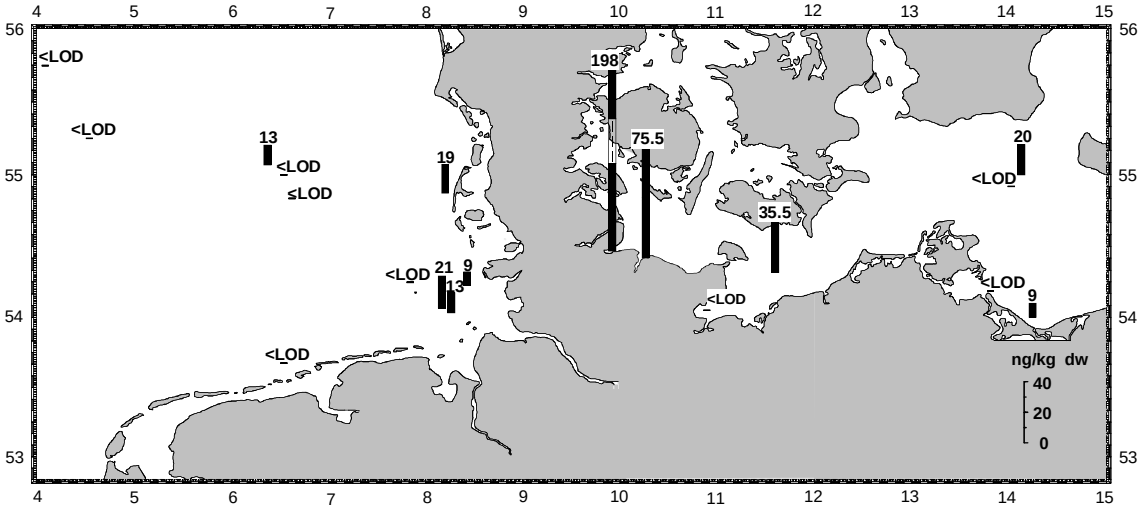



**Figure 42:** Endosulfan I concentrations [ng/kg dw] in surface sediments (0-2 cm)

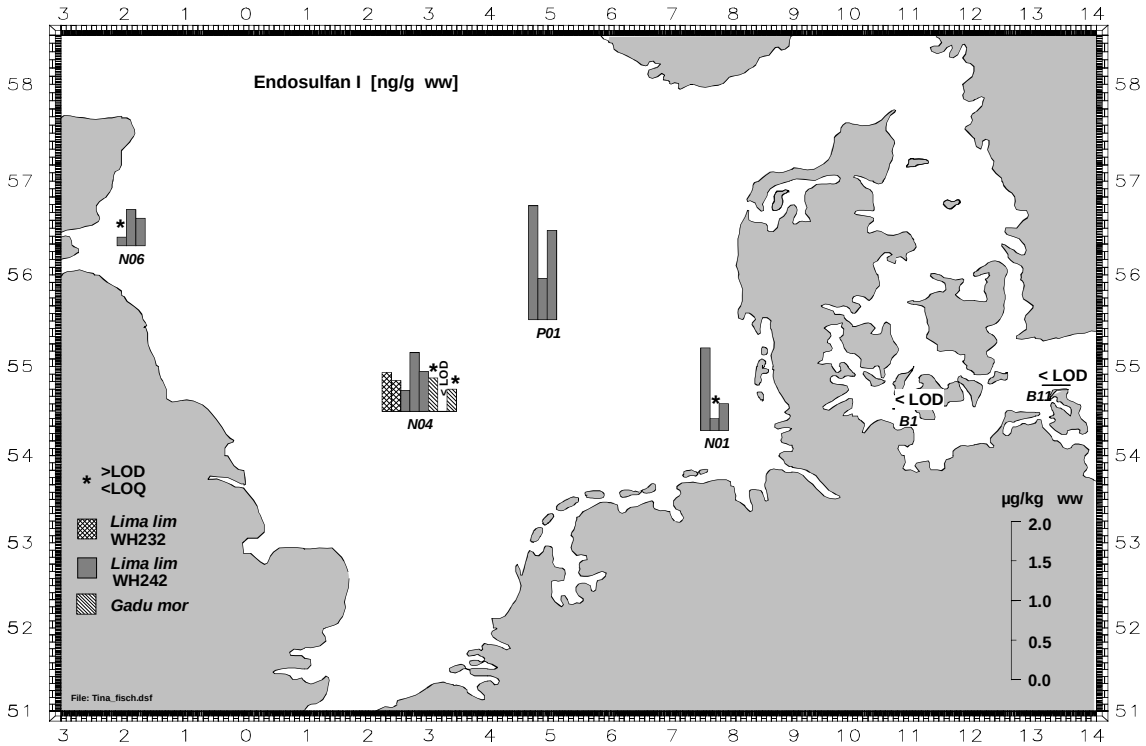
In the western Baltic Sea, concentrations were higher than in the German Bight, and there were more locations with positive findings. Whether this is due to the generally higher TOC values in the Baltic or to local/regional input sources cannot be ascertained on the basis of the limited data available. However, it is remarkable that in the vicinity of stations having elevated endosulfan concentrations in water (4.3.1), endosulfan levels

in sediments were rather high as well (station in the Eckernförde Bight). Here, even endosulfan II was detected.

### 6.4.3.3 Biota

25 liver samples from three different fish species (Dab (*Limanda limanda*), cod *(Gadus morhua*) and flounder (*Platichtys flesus*)), which had been collected during two cruises in the North Sea and western Baltic Sea in 2001 and 2002, were analysed. (see Table 94, Appendix 6.6.8)

Endosulfan I was found in most samples in the range of 0.1 to 1.4 µg/kg ww (0.26 to 7 µg/kg lw) with a median of 0.44 µg/kg ww (1.9 µg/kg lw). The geographical distribution of the samples and their concentrations are shown in Figure 43. The high variability of the data may be considered normal. All samples from the Baltic Sea were below the LOD. Because of the limited data set presently available, it is difficult to interpret the results with respect to possible regional patterns, species differences or time variability. The concentrations of endosulfan II were always lower than those of endosulfan I, and often below the LOD.



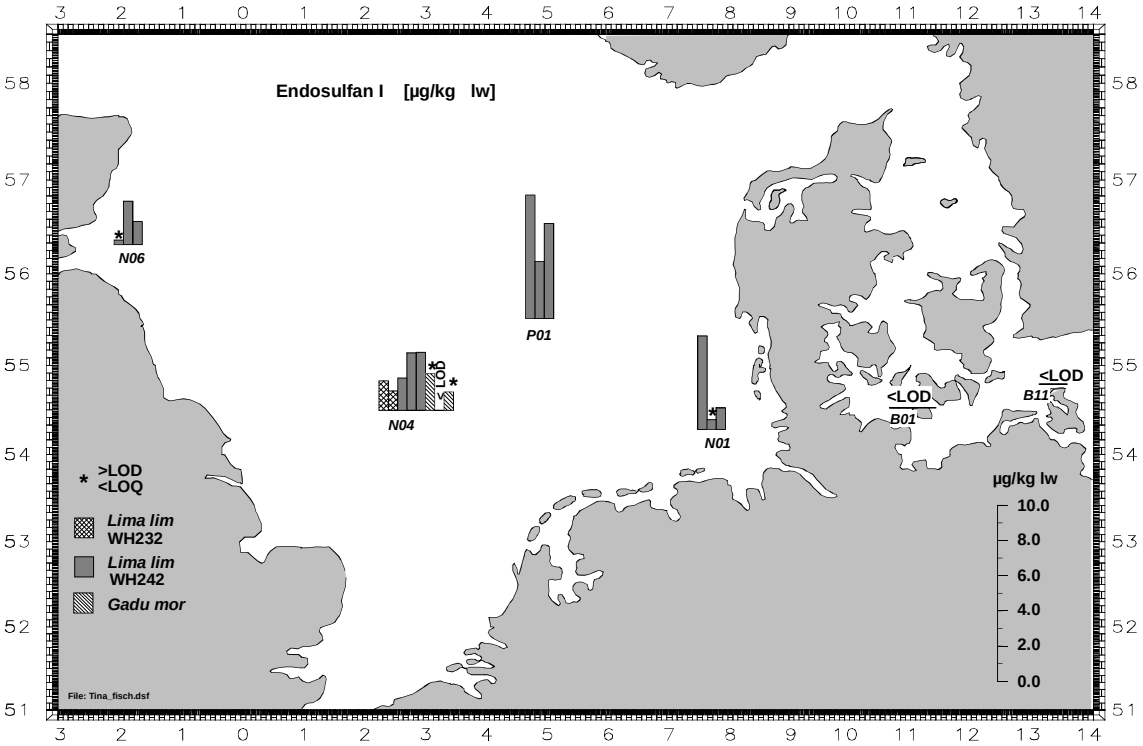



**Figure 43:** Concentrations of endosulfan I in fish liver; upper fig. in µg/kg wet weight, lower fig. in µg/kg lipid weight

### 6.4.3.4 Discussion

This is the first time that endosulfan was detected in the North Sea and Baltic Sea, and concentrations above the LOQ were found. While endosulfan II levels were generally lower than endosulfan I levels and often were not detectable in marine samples from the North Sea and Baltic Sea, the isomer endosulfan I was identified in many water samples and some sediment and biota samples. However, concentrations were very low, with a median of 25 pg/L in water samples and 20.5 ng/kg dw in sediments. Median concentrations of 0.44 µg/kg ww and 1.9 µg/kg lipid were found in fish liver.

Comparisons with literature data are difficult because the LODs in most studies were far higher (>ng/L) than in this survey and endosulfan is mostly reported to be below LOD.

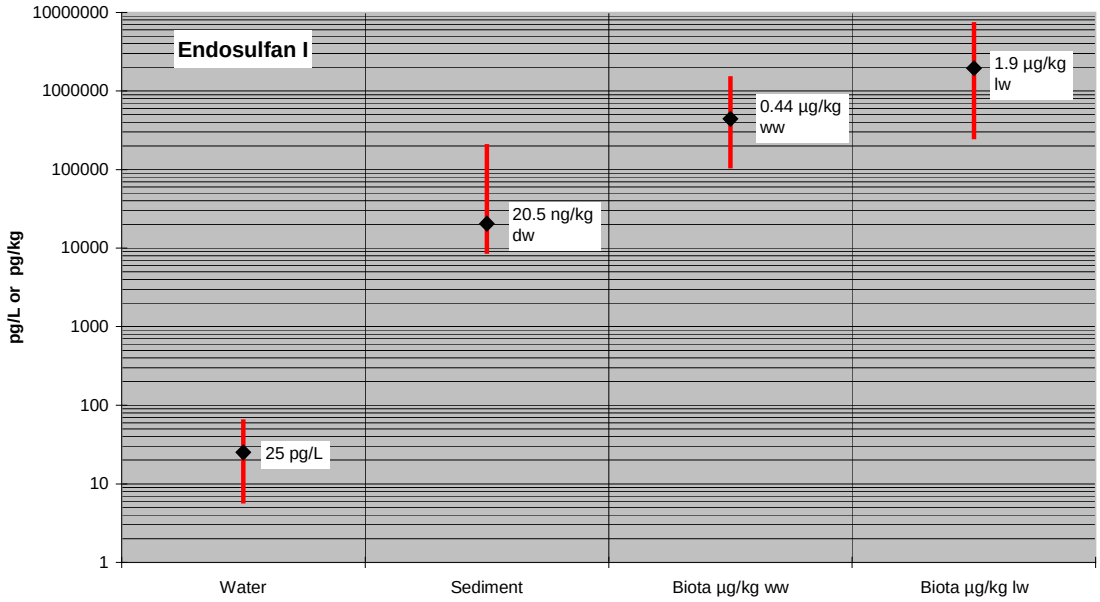



**Figure 44:** Summary of endosulfan I concentrations in different marine matrices; min – max range and median values

The observed low concentrations and distribution patterns are best explained by a general low-level background burden with minor local sources.

Concentrations in the river Elbe – generally the most important source of pollutant input to the German Bight - are low, often below the LOQ. Therefore, a distinct gradient was not found in the German Bight.

Considering the low concentrations of local sources, it is remarkable that endosulfan was detected at all in the North Sea and Baltic Sea. In water, endosulfan concentrations were well above those of the classical lipophilic pollutants like HCB, DDT, PCB or PAHs but below HCH concentrations.

Atmospheric transport and deposition is well documented (Carrera et al, 2002; Chernyak et al, 1996). Quaghebeur et al. ( 2004) reported endosulfan in rain water in Belgium in the range of 1 to 224 ng/L; in the time from 1997 to 2001, they observed a clear downward trend in concentrations.

Sorption to sediments does not lead to a significant enrichment in the sediments investigated. The estimated “enrichment” of endosulfan in sediment as compared to the water phase is about 820 (Figure 44). This means that sediment is not a major sink for this compound and will not be a good monitoring matrix. The low values are remarkable but can be explained by the intermediate polarity (log $K_{OW}$ 3.8 – 4.7) and a relatively rapid degradation (e.g. Helm et al., 2002 : half-life of endosulfan I : 0.046 – 0.14 y).

Compared to the classical pollutants, concentrations in sediment are low. At KS 11, for example, HCH isomer concentrations range from 10 to 200 ng/kg. Levels of the more lipophilic DDD and CB153 range from 1000 to 5000 ng/kg. PAH like, e.g., BaP shows sediment concentrations of 40 to 240 µg/kg (BSH, 2005).

The bioaccumulation potential of endosulfan becomes apparent when comparing concentrations in the three compartments investigated (Figure 44). The estimated “enrichment” of endosulfan in biota as compared to the water phase is about 17600 based on wet weight, and 76000 based on lipid weight.

The observed endosulfan levels are comparable to those of classical pollutants like HCH and HCB, but are below the concentrations of the more lipophilic DDT and PCB group. Typical HCH concentrations in the German Bight ranged from 0.2 to 0.6 µg/kg ww in 2000; HCB had a median of 0.84 µg/kg ww, and the sum of DDTs had a median of 3.7 µg/kg ww (BSH, 2005).

The results from the North and Baltic Seas are well explained by known endosulfan use in Europe. According to the background paper on endosulfan issued by the OSPAR Commission (OSPAR, 2002), endosulfan is used mainly in the south of Europe (1999: 469.3 t/a), while in 1999 only 38.1 t/a was used in the countries bordering the North Sea and Baltic Sea. In most north European countries, endosulfan has not been used any more since the mid-1990s. Only Belgium, France, and Switzerland reported applications in 1999.

Measured concentrations in sea water were well below the level of acute toxicity reported for aquatic organisms; the lowest NOEC was reported to be 1 ng/L (see Chapter 6.2.3). The observed sea water concentrations of endosulfan are about ten times lower than the WFD EQS of 0.5 ng/L.

In view of the positive findings, the BSH will determine endosulfan levels on a voluntary basis during the next two years in order to support the results of this study. However, because of the low absolute concentrations, it is recommended not to include endosulfan as a routine parameter in the standard monitoring program.

### 6.4.4 Trifluralin

The analytical method which has been developed and validated (3.2 and 3.3) is characterised by a very high sensitivity, which is a prerequisite to the determination of pollutants in marine matrices. Table 80 summarises the LODs and LOQs achieved in the different matrices.

**Table 80:** Limits of detection (LOD) at a signal-to-noise ratio (S/N) of 3:1 and limits of quantification (LOQ, S/N 10:1) for Trifluralin

| | **LOD** | **LOQ** | **Sample amount** |
|---|---|---|---|
| Water | 2 pg/L | 7 pg/L | 10 L |
| Sediment | 0.4 ng/kg dw | 1 ng/kg dw | 40 g |
| Biota | 0.03 µg/kg lw | 0.1 µg/kg lw | 3 g |

The values obtained are on the order of the levels of classical pollutants like HCH, PCBs, DDT-metabolites or PAH presently observed in the open marine environment.

#### 6.4.4.1 Water

Trifluralin was determined in sea water samples from the North Sea and Baltic Sea which had been collected during 7 cruises in 2003 to 2005 (Table 95 and Table 96, Appendix 6.8).

Trifluralin was found in most samples at concentrations from < 7 to 500 pg/L. A strong seasonal variation was observed, with a maximum in winter.

In summer, very low concentrations in a range of <5 to 100 pg/L with a median of 15 pg/L were observed in the German Bight and North Sea. The geographical distribution of the concentrations is shown in Figure 45 to Figure 47. In the German Bight, most concentrations were within a fairly narrow range from 10 to 30 pg/L. No clear and steady gradients were found during the surveys. A rather even distribution slightly above the LOQ was generally found in the southern part of the North Sea. One cause of this distribution is the absence of significant local inputs, e.g. by the river Elbe. Trifluralin levels in the river Elbe at Stade were only found to be slightly higher (30 to 81 pg/L); this input to the German Bight has a major impact on the distribution in marine waters. Other sources must be considered to explain the "background level" of 10 to 20 pg/L in the southern North Sea and the occasionally higher levels at the western border of the German Bight. This may either be due to atmospheric deposition or inputs from the rivers Rhine/Schelde or the UK.

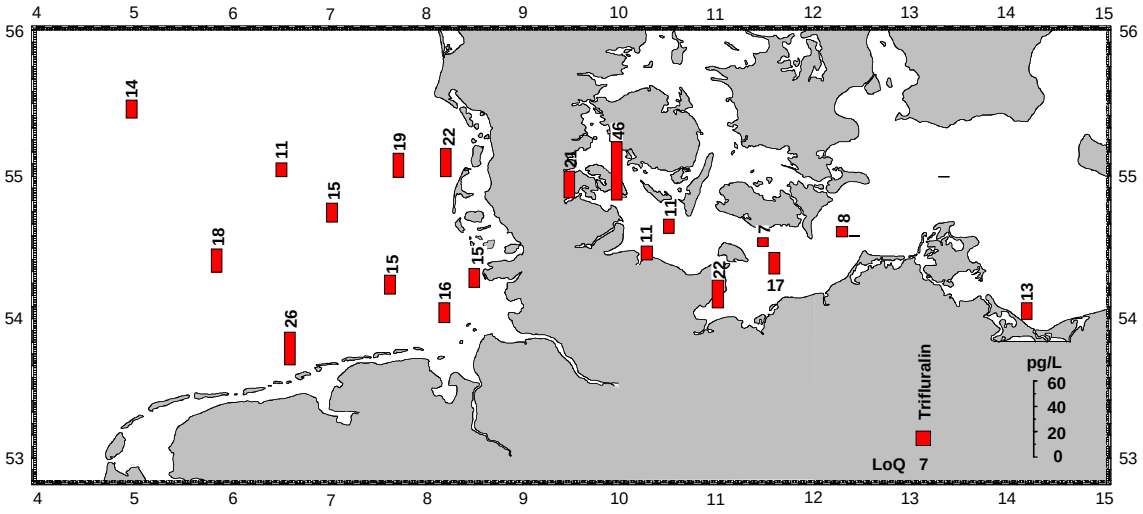



**Figure 45:** Trifluralin concentrations [pg/L] in surface water (5 m) in the North Sea (July 2004) and Baltic Sea (June 2004)

The results of the survey in July 2003, which covered the entire North Sea, were in line with findings in the German Bight. Concentrations in the southern North Sea were significantly higher than in the northern part, where hardly any trifluralin was found.

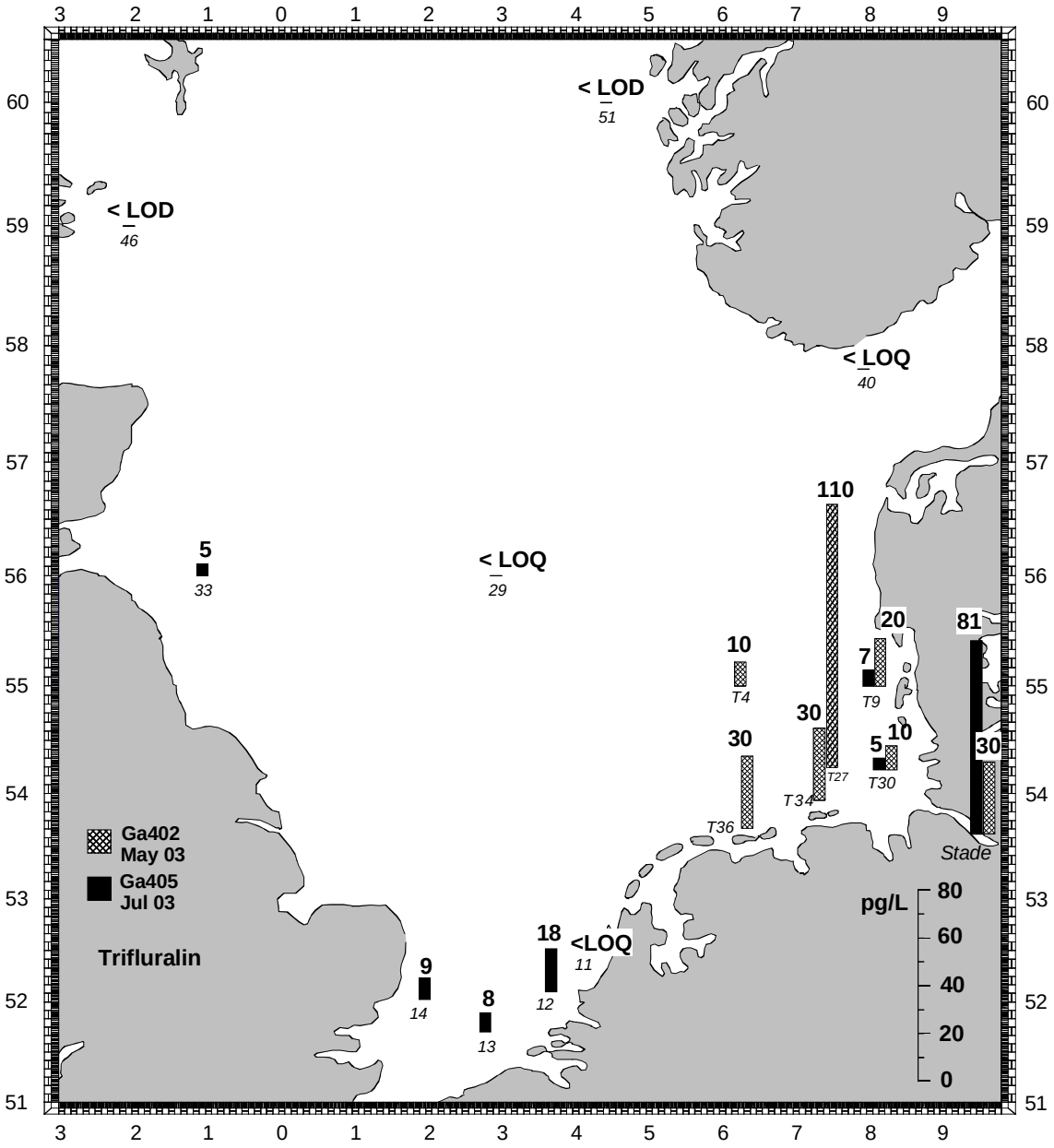



**Figure 46:** Trifluralin concentrations [pg/L] in surface water (5 m) in 2003

In 2004, some samples were taken in the Greenland Sea to check for possible long-range transports and investigate background concentrations. No trifluralin above the LOD was detected in these samples.

In winter, concentrations in the German Bight were an order of magnitude higher than in summer, and were in the range from 100 to 580 pg/L with a median of 180 pg/L. The

geographical distribution (Figure 47) shows the highest concentrations not in the coastal area along the Elbe plume but at the western edge of the German Bight.

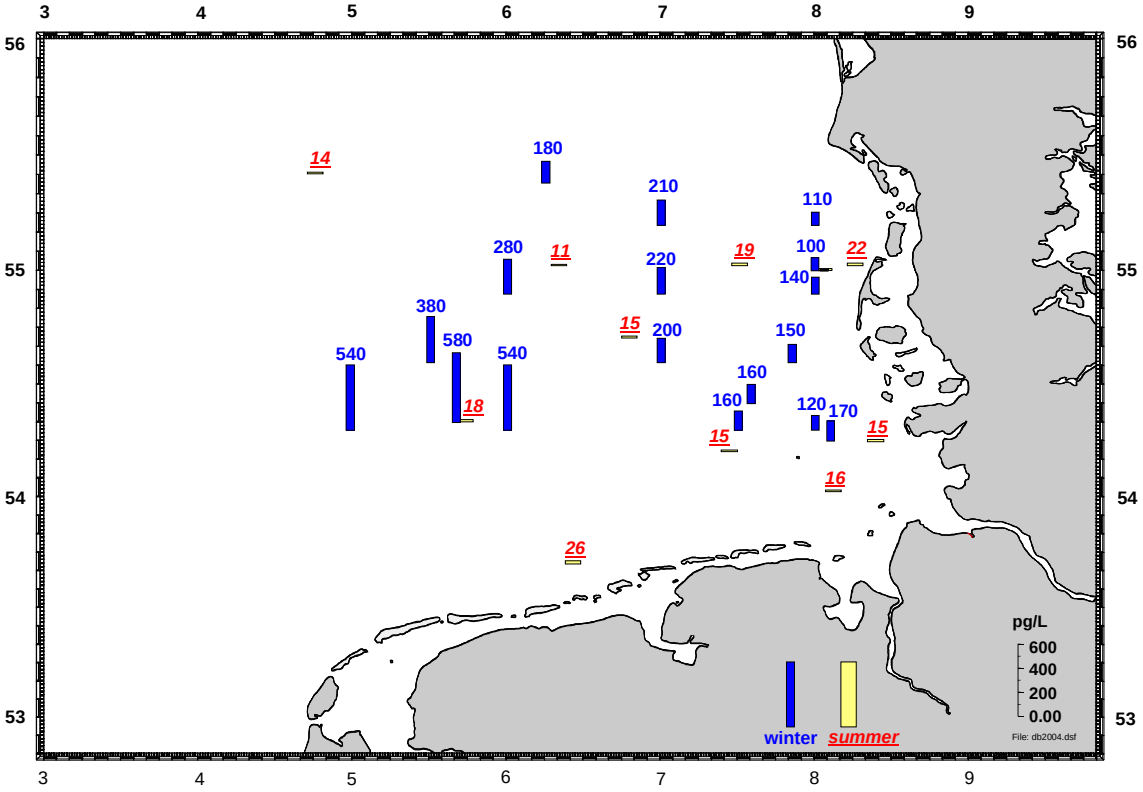



**Figure 47:** Trifluralin concentrations [pg/L] in surface water (5 m) in summer and winter

In the western Baltic Sea, summer concentrations resembled those in the German Bight. The values at most stations ranged from 7 to 46 pg/L. The highest concentrations were observed in the Lübeck Bight and Flensburg Fjord, where concentrations of 21 and 46 pg/L, respectively, were found. These elevated levels can be explained by slow water exchange in this bight and high agricultural activities in the area.

### 6.4.4.2 Sediment

Trifluralin was identified in 30 surface sediment samples from the North Sea and Baltic Sea which were collected during 6 cruises in 2003 to 2005 (Table 97, Appendix 6.8). The geographical distribution is shown in Figure 48. Concentrations were very low in all sediment samples from the German Bight and western Baltic Sea. They varied

between < 1 and 22 ng/kg dw with a median of 10 ng/kg. Because of the very low LOD, trifluralin was detected in 83 % of the samples. Remarkably, even station KS 11, which normally has the highest pollution levels, showed low values of only 5 to 15 ng/kg dw. Values in the central German Bight were slightly higher. This is in line with the distribution that has been observed occasionally in the water phase. However, the data do not yet allow a statistically valid interpretation of the geographical distribution with respect to spatial gradients or input structures.

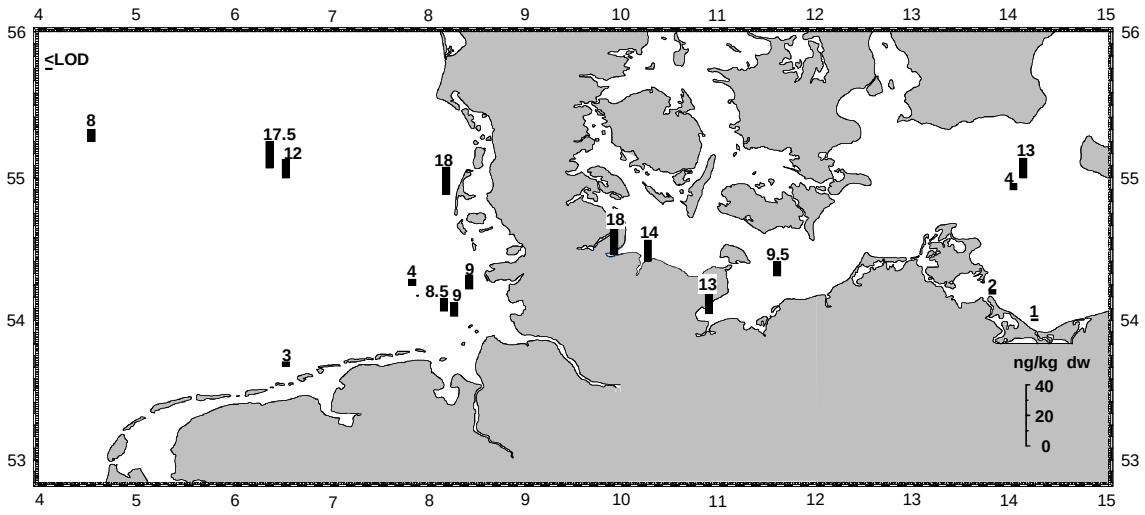



**Figure 48:** Trifluralin concentrations [ng/kg dw] in surface sediments (0-2 cm), single or median values according to Table 97

In the western Baltic Sea, concentrations are on the same order as in the German Bight, with positive findings at a larger number of stations because TOC values are generally higher at these stations than in the German Bight, where sandy sediments are more common.

### 6.4.4.3 Biota

25 liver samples from three different fish species (dab (*Limanda limanda*), cod *(Gadus morhua*) and flounder (*Platichtys flesus*)) collected during two cruises in the North Sea and western Baltic Sea in 2001 and 2002 were investigated. (Table 98, Appendix 6.8) Trifluralin was found in most samples in the range from 0.1 to 1.74 µg/kg ww (0.28 to 8.86 µg/kg lw), with a median of 0.42 µg/kg ww (1.7 µg/kg lw). The geographical distribution of the samples and its concentrations are shown in Figure 49.

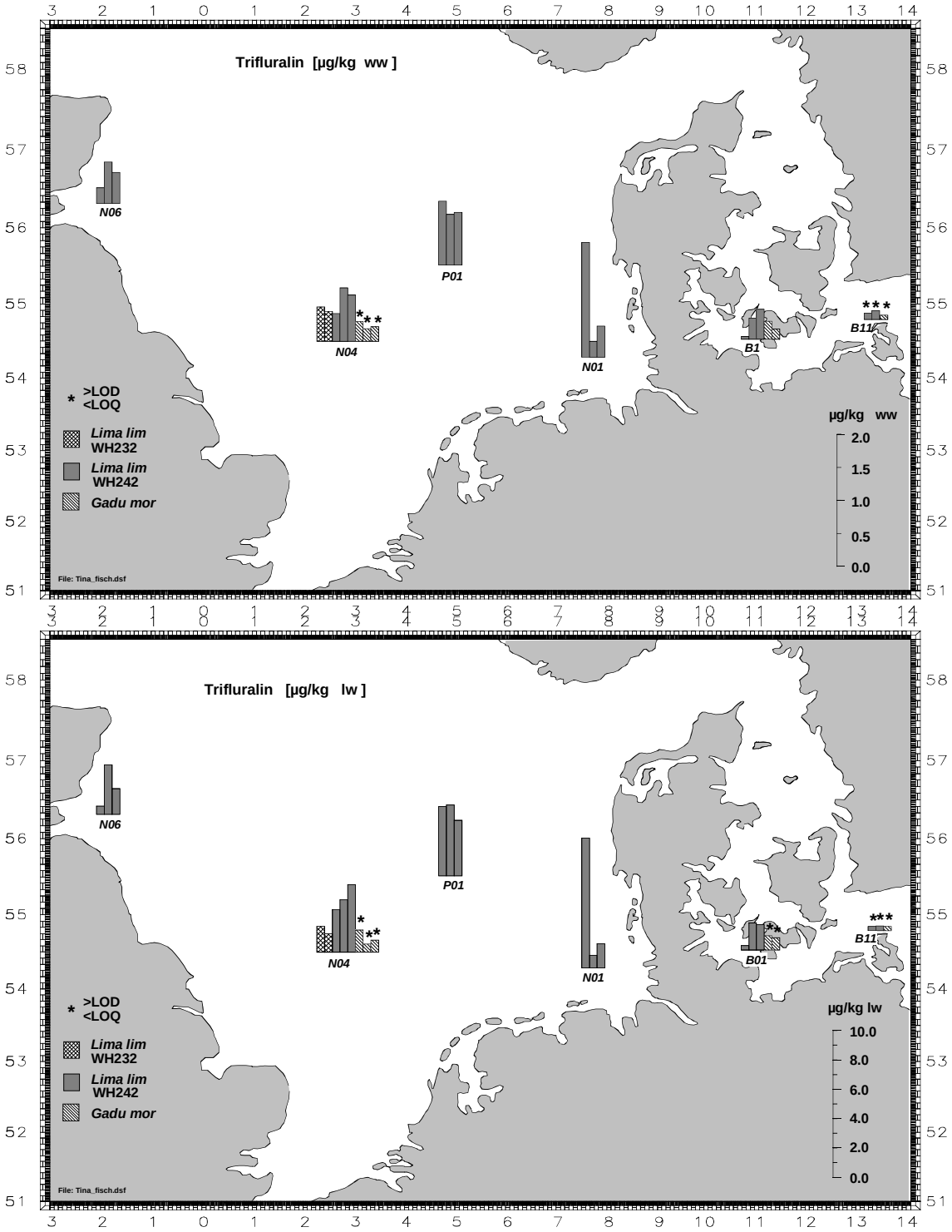



**Figure 49:** Trifluralin concentrations in fish liver; upper Fig. in µg/kg wet weight, lower Fig. in µg/kg lipid weight

Data variability was found to be in the normal range. Samples from the Baltic Sea had lower levels than North Sea samples. However, the limited data set currently available

does not allow an evaluation of the results with respect to regional patterns, species differences, or time variability.

#### 6.4.4.4 Discussion

As trifluralin analyses were made with a very low LOQ, the substance was detectable in most samples of water, sediment and fish liver from the North Sea and Baltic Sea. Concentrations were very low in summer, with a median of 15 pg/L in water samples, 10 ng/kg dw in sediments, and 0.42 µ/kg ww in fish liver samples. In winter, water concentrations were about 10 times higher, which is due to preferred use of the herbicide as a preseed agent in winter.

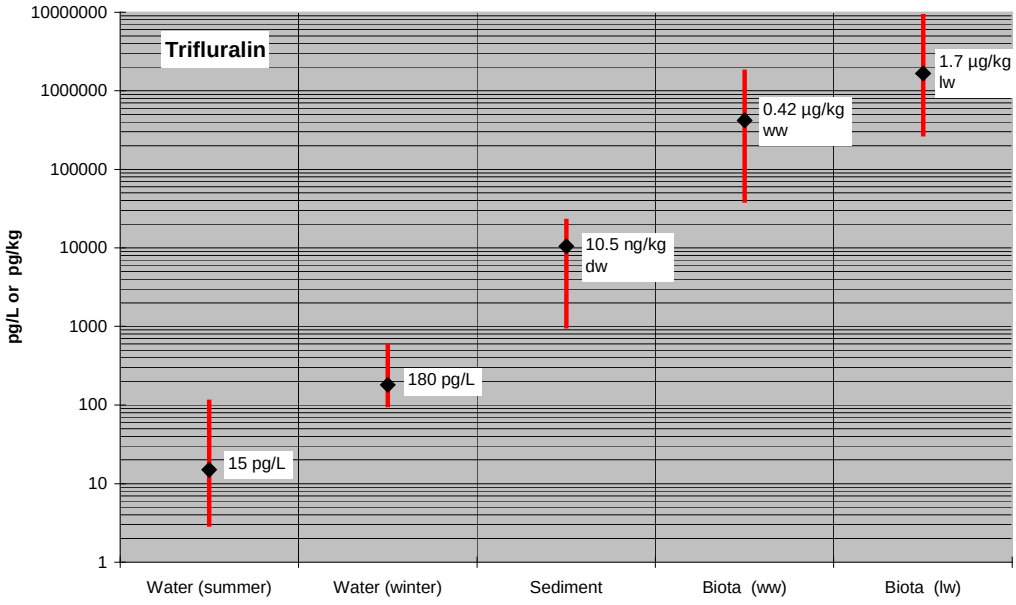



**Figure 50:** Summary of trifluralin concentrations in different marine matrices; min – max range and median values

Comparisons with literature data are difficult because LODs in most studies are far higher (>ng/L) than in the present survey, and trifluralin is mostly found to be below the LOD. The concentrations reported in this survey compare well with data from the same laboratory (BSH) which, however, had been obtained by a different analysis technique and with a higher LOD (liquid/liquid extraction and analysis by GC-MS in EI mode). Concentrations of 30 pg/L or lower had been obtained in those analyses.

These findings allow the conclusion that trifluralin is stable enough to be present in the marine environment of the North Sea and Baltic Sea. Concentrations are highest during and after the main application season in winter. The 10 times lower concentrations in summer indicate a moderately fast degradation of trifluralin in the (marine) environment. The distribution pattern is best explained by a low, diffuse contamination level (e.g. by atmospheric deposition) with minor local input sources.

Concentrations in the river Elbe - generally the most important input source of pollutants to the German Bight - are relatively low, which means that this source of trifluralin contamination is less important.

In summer, the trifluralin levels in water exceed those of the classical lipophilic pollutants, e.g. HCB, DDT, or PCB, but are lower than HCH levels. Concentrations in winter, however, are higher than the HCH levels. Compared to other herbicides – e.g. atrazine, diuron or isoproturon – trifluralin concentrations are lower by a factor of about 10 (BSH, 2005).

Atmospheric transport and deposition is well documented. Apart from atmospheric transport following spraying applications, also volatilisation and escape to the atmosphere has been suggested as a transport path (Alegria et al. 1999, Rice et al. 1997, Waite et al 1995).

Sorption to sediment had not led to significant enrichment in the sediments investigated. The estimated “enrichment” of trifluralin in sediment as compared to the water phase is about 700. Sediment thus is no major sink for this herbicide. The low values are remarkable considering the relatively high log $K_{OW}$ of 5.3 and the reported persistence in soil. (OSPAR, Background document on trifluralin, 2004). The limited accumulation may be due to rather rapid (photo)degradation.

Compared to classical pollutants, concentrations of trifluralin in sediment are low. At the station KS 11, for example, HCH isomers range between 10 and 200 ng/kg. The more lipophilic DDD and CB153 range from 1000 to 5000 ng/kg. PAH, e.g. BaP, have levels of 40 to 240 µg/kg in sediment.

The bioaccumulation potential of trifluralin becomes apparent when comparing concentrations in the three compartments investigated (Figure 50). The enrichment factor calculated for the median between water and fish liver is 28000 based on wet weight, and 113000 based on lipid weight.

The observed trifluralin values are comparable to those of HCH and HCB but lower than the concentrations of DDT-metabolites and PCBs. Typical HCH concentrations in the German Bight ranged from 0.2 to 0.6 µg/kg ww in 2000; HCB had a median of 0.84 µg/kg ww, and the sum of DDTs had a median of 3.7 µg/kg ww (BSH, 2005).

Trifluralin is a licensed product used as a herbicide in most European countries (see chapter 6.2.5). Approximately 3200 t is used annually in the EU, the largest users being France (1600 t/a) and the UK (657 t/a) (OSPAR Background document, 2004). In 1995, consumption in Germany was between 100 and 200 t. This is a plausible explanation for the presence of trifluralin in the North Sea and Baltic Sea, with water concentrations peaking in winter.

The concentrations observed in sea water are well below the level of acute toxicity reported for aquatic organisms, which is in the low µg/L range (chapter 2.5). Compared to the WFD EQS value of 30 ng/L, the trifluralin concentrations detected in sea water are considerably lower.

In view of the positive findings, the BSH will determine trifluralin on a voluntary basis during the next two years in order to support the results of this study. Especially the seasonal variation and geographical distribution will be investigated in more detail. Thereafter, it will be decided whether trifluralin should be included as a routine parameter in the standard monitoring program.

### 6.4.5 Pentachlorophenol (PCP)

An HPLC-MS method was developed to analyse water samples for PCP (chapter 3.3), which proved to be simple and had a sensitivity that was sufficient for first screening. An LOQ of 0.2 ng/L was achieved for a 10 L water sample. However, this method was

not selective enough for sediment and biota samples because of the higher matrix underground of these marine matrices. Therefore, a GC-NCI-MS method with a derivatisation step was developed. This method had a good sensitivity and, in principle, was also suitable for water samples. However, due to lack of time and technical problems the procedure could not be applied to environmental samples within this project. For the same reason, validation of the method had not been completed with all matrices by the end of the project.

#### 6.4.5.1 Water

Pentachlorophenol was determined in sea water samples from the North Sea and Baltic Sea which had been collected during 4 cruises in 2003 to 2004.

Pentachlorophenol was detected in the river Elbe and at some coastal stations in the German Bight. At most stations in the open North Sea and Baltic Sea it was below the LOQ of 0.2 ng/L. The river Elbe clearly continues to be an input source for PCP, at concentrations ranging from 0.86 to 1.4 ng/L.

In 2004, some samples were taken in the Greenland Sea in order to investigate possible long range transports and determine background concentrations. No PCP above the LOD was detected in these samples.

In the western Baltic Sea, samples were taken in 2004 but the analysis could not be completed in the course of the project.

#### 6.4.5.2 Sediment and Biota

The planned analysis of sediment and biota samples could not be completed due to technical problems with the microwave extractor, the GPC pre-separation, and time constraints. In a preliminary test analysis, no PCP was detected.

#### 6.4.5.3 Discussion

Current PCP levels in the German Bight are far below those of 1988, which ranged from 0.1 to 6.4 ng/L (Hühnerfuss et al. 1990). Low levels (0.1 ng/L) were observed in the

outer German Bight, which are comparable to present values. However, concentrations in the coastal areas were much higher than today, which proves that pollutant loads in rivers (especially the Elbe) have decreased considerably during the past years.

Whether other input sources to the North Sea are also relevant presently cannot be derived from the data because most values were below the detection limit; no spatial distribution pattern could thus be generated for the North Sea. However, high mean PCP concentrations of 6 to 100 ng/L, which had been found in estuarine regions in 1983 – 1997 [Eurochlor, 1997] are no longer observed.

Nevertheless, the river Elbe still is an input source of PCP to the North Sea.

In the river Rhine, PCP has been detected occasionally in the past 5 years. The maximum value, found at Lobith, was 130 ng/L, and the mean value was 30 ng/L (RIWA, 2004). In general, values were below the LOD of 10 ng/L, though.

Detailed data on manufacturing and use of PCP and derivatives have been summarised in the OSPAR "Background document on PCP" (OSPAR, 2001). Nevertheless, it is difficult to derive thereof detailed input data. Today the predominant use of PCP containing chemicals is in the treatment of wood. In 1996 almost 90 % of the total EU consumption of PCP was in France, Portugal and Spain (total of ca. 20 – 400 t).

Measured concentrations in sea water are well below the level of toxicity reported for aquatic organisms; NOEC are reported in the low µg/L range (s. Chapter 2.4). The observed concentrations of PCP are well below the WFD EQS of 200 ng/L.

Based on these positive findings, the BSH will determine pentachlorophenol on a voluntary basis during the next two years in order to support the results of this study. The method should be improved for that purpose, and the LOQ should be lowered to 0.05 ng/L. However, because of the low absolute concentrations, it is not considered necessary to include pentachlorophenol as a routine parameter in the standard monitoring program.

### 6.4.6 Dicofol

The analysis of dicofol turned out to be quite problematic because of its thermal and chemical instability. It was difficult to control the GC-MS response even with the use of GC injectors, which are generally considered optimal for thermolabile compounds (on column and PTV injectors). The degradation led to dichlorobenzophenone (DBP). It is not unlikely that many of the methods described in literature in fact detected DBP formed in-situ during GC analysis.

Unfortunately, dicofol was found to have a poor ESI-MS sensitivity in HPLC-MS analysis. The approach of using HPLC-MS to reduce thermal stress during the analysis thus was not successful.

Nevertheless, it was possible to develop a GC-MS method having a moderate sensitivity, which was suitable for first screening of sea water. The LODs and LOQs achieved were 0.3 and 1 ng/L, respectively. (The sensitivity is considered "moderate" in comparison with the LOD that is normally used for marine matrices at the BSH. Compared to literature data it may be considered good). A full validation of the method was not performed because no positive findings were obtained in sample screening.

#### 6.4.6.1 Water

Dicofol was determined in sea water samples from the North Sea which had been collected during 2 cruises in 2003 and 2004 [1]. Dicofol was below the LOD ( 0.3 ng/L) in all samples.

Because of the instability of dicofol observed during the development of the analytical method, it was considered useful to investigate the stability of the pesticide in natural waters. For that purpose, sea water was spiked with dicofol and its degradation was observed for four weeks. Figure 51 shows the temporal trend of dicofol and of its

[1] Altogether, samples were taken during 4 cruises. However, samples from the first cruise had been eluted with acetone, which later was found to enhance degradation of dicofol to DBP – with a half-life of 45 min. at room temperature! Samples from the 4th cruise were not analysed because of the negative findings for the two other cruises.

degradation product DBP. Rapid degradation of dicofol is observed at room temperature, with a half-life of 15 hours. DBP is formed at the same rate and is evidently more stable in the water phase.

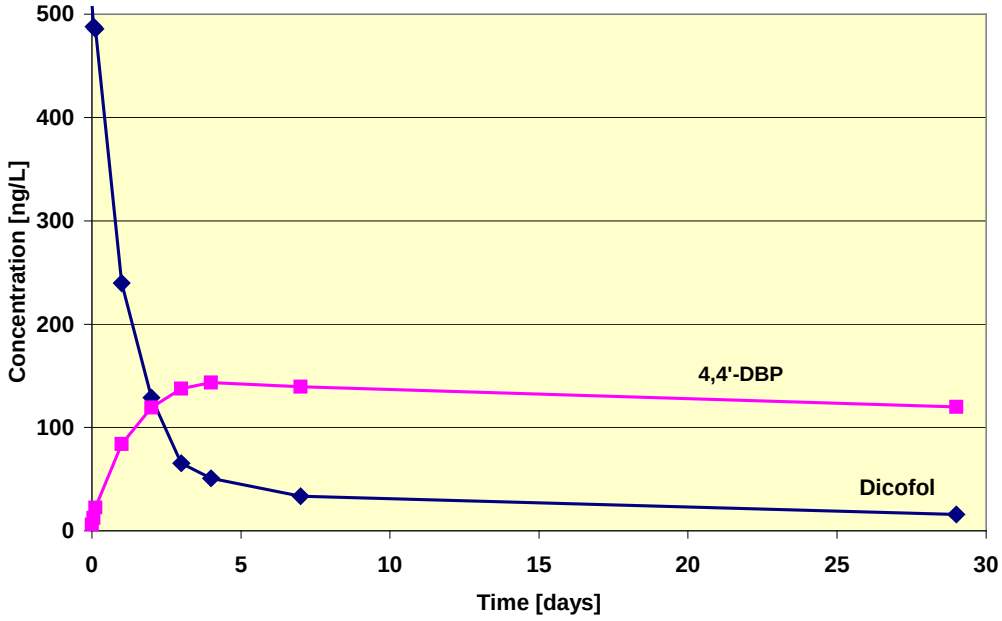



**Figure 51:** Degradation of dicofol in sea water at room temperature

Therefore, DCB was analysed in selected water samples from the river Elbe and the German Bight and was in fact detected at concentrations of <0.2 to 3.8 ng/L; maximum concentrations were found in the river Elbe, at Stade.

### 6.4.6.2 Toxicity screening of dicofol and dichlorobenzophenone

Dicofol and dichlorobenzophenone were tested for teratogenic effects in a fish egg test (DIN 3815-6T6) (expanded to include teratogenic effects)) in order to estimate their toxicity for marine organisms. The tests were performed at Bundesforschungsanstalt für Fischerei, Hamburg, by U. Kammann (2004).

In the tests, the influence of the substances on the embryonic development of zebra danio (*Danio rerio*) was determined (Vobach, 2002). No effects on fish egg development were observed with the metabolite 4,4'-dichlorbenzophenone at

concentrations up to 100 mg/L. In contrast, Dicofol was found to have a significant influence on embryonic development at concentrations of 2-10 mg/L (Table 81).

**Table 81:** Influence of dicofol on the embryonic development of *Danio rerio*; number of eggs 60, limit of significance 10 % (Kammann, BFA, 2004)

| Effect | EC50 [mg/L] |
|---|---|
| Lethal | 11 |
| Non-lethal | 2.1 |
| Variances on vertebral column | 7.3 |
| Formation of oedema | 5.3 |
| Coagulation | 11 |

Endpoints of the investigation were coagulation and lethal variances in the embryo (somits, caudal ablation, spontaneous motion, cardioplegia) as well as non-lethal variances (vertebral column, oedema, pigments, pigments of eyes, disposition of eyes).

A literature survey yielded no indication of any other toxicity risk of DCB; it is not considered toxic.

### 6.4.6.3 Discussion

Dicofol was not detectable in sea water from the North Sea and Baltic Sea (LOD: 0.3 ng/L). Degradation experiments in sea water showed a half-life of less than a day. Therefore, no further investigations in sediments or biota were carried out. Also LOD lowering to the lower pg/L range was not considered to be of priority interest in the project.

Walsh and Hites (1979) showed that the degradation of dicofol in water depends on the pH and increases under neutral and basic pH conditions. As sea water has a basic pH value between 8 and 8.5, this is supported by the quick degradation observed in sea water and the fact that up to now no occurrence of dicofol in marine waters has been reported in literature.

In the river Elbe – generally the most important source of pollutant input to the German Bight – no dicofol was detected but its degradation product DCB was present at 3.8

ng/L. When interpreting this result it has to be considered that DCB is not a specific indicator of dicofol, because other compounds, e.g. DDT, also degrade to DCB. Due to this lack of specificity and its non-toxicity, the determination of DCB was not continued.

The LOD for dicofol is well below the toxic level for aquatic organisms, which is in the range of µg/L (Table 55, chapt. 2.2)

In Germany, dicofol has been prohibited since 1995. It is not registered in most North Sea and Baltic Sea countries - Denmark, the Netherlands, Sweden, Norway, and Finland – and in Switzerland. Therefore, the lack of positive findings in the North Sea and Baltic Sea is not unexpected. The main use of dicofol in Europe (ca. 290 t) is reported for Spain (100-150 t), France (14 t), Portugal (4.8 t), and the UK (1 t). Dicofol therefore may be considered to be of lower priority concern for the North Sea and Baltic Sea (unless inputs of Russia, Poland or the Baltic states are of importance). The detection limits in this study were not low enough to detect inputs resulting from atmospheric deposition of long-range transports.

There remains an uncertainty because no data on the occurrence of dicofol in sediments and biota could be gained. However, in view of the low stability of dicofol in sea water, it is considered unlikely that elevated concentrations will be found in these matrices.

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## 6.6 Appendix

### 6.6.1 Reagents and solvents

The reagents and solvents were of the highest purity available. The following solvents were used: acetone (Merck, SupraSolv®), dichloromethane, *n*-hexane (Baker, Ultra resi-analyzed) methanol (Baker, Hplc-analyzed). Deionized water was obtained from an ultra-filtration system (Millipore milli-Q academic A10, 18.2 MΩcm).

All glassware was washed twice with acetone, dried, and then washed twice with *n*-hexane. After that the glassware was heated at 180°C for 5 h.

### 6.6.2 GC-MS method for the determination of Chlorpyrifos, Endosulfan and Trifluralin

All analyses were carried out with a *Trace GC* capillary gas chromatograph coupled to a *Trace MS* mass spectrometer (both Thermo Finnigan). Analyses were conducted with the following parameters:

| | |
|---|---|
| **Column:** | ZB-5 from Phenomenex (5 % Phenyl-, 95 % dimethylpolysiloxan) 30 m x 0.25 i. d. x 0.25 µm; 1 m precolumn ZB-5 |
| **Carrier gas:** | Helium 1.5 mL/min |
| **Oven temperature:** | 40 °C, 1 min isotherm; linear temperature gradient (5.5 °C/min to 320 °C; then 10 min isotherm) |
| **Injector:** | PTV splitless (Inlet temperature: 50 °C; split flow: 30 ml/Min; splitless time: 1 Min; injection phases: injection: 40 kPa, 50 °C, 50 mL/Min , 0.01 Min; evaporation: 140 kPa, 14.5 °C/s, 60 °C, 0.5 Min; transfer: 210 kPa, 14.5 °C/Min, 280 °C, 10 Min) |
| **Interface temperature:** | 280 °C |
| **Detection mode:** | NCI |
| **Reagent gas:** | Methane |
| **Source temperature:** | 130 °C (NCI) |
| **Emission current:** | 150 µA |
| **Dwell time per mass:** | 50 ms |

Table 82 shows the relevant MS parameters for the target compounds and their internal standards (ISTD).

**Table 82:** Parameters of the target substances: molecular weight (MW), retention time (RT; GC conditions see above) and masses used for analysis in SIM mode (m/z)

| Target-substances/ Internal standards | MW | RT | GC-MS (m/z) | |
|---|---|---|---|---|
| | | | EI-Modus | NCI-Modus |
| $D_{14}$-Trifluralin | 349 | 24.77 | 267, 315 | 349, 319 |
| Trifluralin | 335 | 24.93 | 264, 306 | 335, 305 |
| Chlorpyriphos-methyl | 321 | 28.83 | 288, 286 | 212, 141 |
| $D_{10}$-Chlorpyriphos-ethyl | 359 | 30.46 | 326, 324 | 322 |
| Chlorpyriphos-ethyl | 349 | 30.59 | 315, 199 | 313 |
| $D_4$-Endosulfan I | 408 | 32.75 | 237, 272 | 376, 410 |
| Endosulfan I | 404 | 32.82 | 241, 265 | 406, 372 |
| Endosulfan II | 404 | 34.73 | 241, 265 | 406, 370 |

### 6.6.3 Procedure for the analysis of Chlorpyrifos, Endosulfan and Trifluralin in water

Water samples were taken at 5 m water depth using a 10 L glass bowl. Prior to solid phase extraction (SPE), the samples were acidified to pH 2.5-3.0 (25 % hydrochloric acid, p. a.). $D_{14}$-Trifluralin, $D_{10}$-Chlorpyrifos-ethyl, and $D_4$-Endosulfan I (Dr. Ehrens-dorfer GmbH) were added as internal standards (e.g. 0.4 ng/L). The seawater was pumped through a column filled with 2 g Chromafix HR-P resin (Macherey and Nagel, Düren) in a custom made extraction system. The loaded columns were dried and stored in the dark at 8°C until elution. All target substances were eluted with 100 ml dichloromethane in the opposite direction of sampling. After solvent change to hexane, the extracts were concentrated to 300 µl and analysed by GC-MS (see 6.6.2).

### 6.6.4 Procedure for the analysis of Chlorpyrifos, Endosulfan and Trifluralin in sediment

The sediments were dried at room temperature for 72 h and ground (centrifugal force ball mill (Retsch) 15 min, 50 rmp). Before microwave assisted extraction (MAE), $D_{14}$-Trifluralin, $D_{10}$-Chlorpyrifos-ethyl and $D_4$-Endosulfan I were added as internal standards (0.1 µg/kg) to 40 g sediment. The sediment was mixed with 0.5 g activated copper powder to remove sulphur. Hot extraction was accomplished in a microwave system (µPrep-A, MLS, 10 min; 100°C) using acetone/n-hexane (75/25 v/v) as solvent. During extraction the sediment was stirred. After cooling, the solution was separated from the sediment by filtration (filtration unit 30 mL from sartorius; glass microfibre filter (whatman, 25 mm GF/A; heated at 500°C for 3 h)). The extraction was carried out twice. Both extracts were combined and the solvent was changed to n-hexane. The extracts were cleaned up on silica gel (silica gel 60, Merck, Darmstadt; heated for 2 h at 150°C, deactivated with 6 % w/w water). Elution was done with *n*-hexane/dichloromethane (50/50 v/v) and pure dichloromethane. The solvent of both fractions was changed to *n*-hexane, and $D_6$-chlorpyrifos-methyl was added as recovery standard (0.1 µg/kg). The first fraction generally contained all target compounds. It was concentrated to 300 µl and analysed by GC-MS (see 6.6.2).

### 6.6.5 Procedure for the analysis of Chlorpyrifos, Endosulfan and Trifluralin in fish liver

After preparation, the liver samples were stored at -20°C. The tissue was thawed at room temperature before analysis. All liver tissue (ca. 1-4 g ww) was used for sample preparation. Before freeze drying at 1.3 mbar for 48 h (Steris, Lyovac GT2) $D_{14}$-Trifluralin, $D_{10}$-Chlorpyrifos-ethyl and $D_4$-Endosulfan I were added as internal standards (1.3 µg/kg ww). Hot extraction was accomplished in a microwave oven (10 min; 100°C) using acetone/*n*-hexane (75/25 v/v) as solvent. During the extraction the samples were stirred. After cooling the solution was separated from the tissue by filtration (filtration unit 30 mL from sartorius; glass microfibre filter (whatman, 25 mm GF/A; heated at 500°C for 3 h)). The extraction was carried out twice. Both extracts were combined and the solvent removed in a smooth nitrogen stream and the lipid

content (extractable with *n*-hexane/acetone) was determined gravimetrically. To remove lipids from the sample, the extracts were cleaned by gel permeation chromatography (GPC; Biobeads, column: r = 2 cm; h = 14 cm; p = 2 bar, flow = 4 mL/min). After solvent change to hexane, the extracts were cleaned up on neutral aluminium oxide (Merck, Darmstadt; heated for 5 h at 500°C, desactivated with 19 % w/w water). Elution was done with hexane/dichloromethane (50/50 v/v) and pure dichloromethane. The solvent of both fractions was changed to *n*-hexane, and $D_6$-Chlorpyrifos-methyl was added as recovery standard. The first fraction contained all target compounds. It was concentrated to 300 µl and analysed by GC-MS (see 6.6.2).

### 6.6.6 Procedure for the analysis of Pentachlorophenol and Trichloropyridinol in water

Sea water samples were taken using a 10 L sampler (glass bowl) and were acidified with 25 % hydrochloric acid (p. a.) to pH 2.5-3.0. $^{13}$C-pentachlorophenol was added as internal standard. The sample was pumped through a column filled with 2 g Chromafix HR-P resin (Macherey and Nagel) in a custom made extraction system. After drying in a gentle nitrogen stream, the loaded columns were stored in the dark at 8°C until elution. PCP and TCPy were eluted with methanol (100 mL). The solvent volume was reduced to 300 µL and the extract analysed by HPLC-MS.

#### 6.6.6.1 HPLC-MS analysis

A combination of an Agilent 1100 HPLC system and a SCIEX API 2000 triple quadropole MS was used for HPLC-MS analysis; the parameters were as follows:

| | |
|---|---|
| **Column:** | Synergi 4 µ Hydro-RP (Phenomenex; C18 phase with polar endcapping) 75 x 2.00 mm |
| **HPLC system:** | Agilent 1100 |

**HPLC-Gradient Program:**

| Runtime [min] | Flow rate [µL/min] | A [%] | B [%] |
|---|---|---|---|
| -15 | 170 | 85 | 15 |
| 0.1 | 170 | 85 | 15 |
| 4.0 | 170 | 70 | 30 |
| 16.0 | 220 | 50 | 50 |
| 25.0 | 220 | 30 | 70 |
| 39.0 | 220 | 5 | 95 |
| 48 | 220 | 5 | 95 |

**Eluent A:** Water, 10 mM ammonium acetate, 10 mM acetic acid

**Eluent B:** Methanol, 10 mM ammonium acetate, 10 mM acetic acid

**Oven:** 23 °C

**MS system:** API 2000 (Applied Biosystems), ESI source, negative mode, MRM

Table 83 summarises the MS parameters for the target compounds and their internal standard (ISTD) labelled with $^{13}C$.

**Table 83:** Parameters of the target substances: molecular weight (MW), retention time (RT; LC-MS conditions see above) and masses used for analysis in SIM mode (m/z)

| Substance | MW [g/mol] | RT [min] | Quantifier [[m/z] | Qualifier [m/z} |
|---|---|---|---|---|
| Pentachlorophenol | 264 | 33.6 | 264.8 | 266.8 |
| $^{13}C$-Pentachlorophenol | 270 | 33.5 | 270.8 | 272.8 |
| TCPy | 197 | 23.9 | 195.9 | 197.9 |

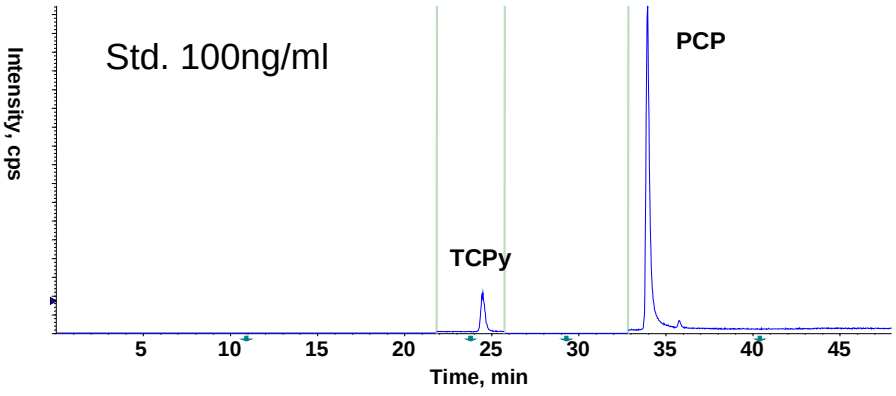


**Figure 52:** HPLC-MS Chromatogram (SIM) of a standard solution of TCPy and PCP

### 6.6.7 Procedure for the analysis of Dicofol in water

Extraction see chapter 6.6.3.

#### 6.6.7.1 GC-EI method for Dicofol

Because of its low thermal stability, dicofol could not be analysed by the NCI method described in chapter 6.2. Dicofol was analysed using an "on-column" injector. The analyses were carried out with a capillary gas chromatograph coupled to a *MD800* mass spectrometer (Thermo Electron). Separations were conducted as follows:

| | |
|---|---|
| **Column:** | HP-5 (Cross-linked 5 % Phenyl ME Polysiloxane) 30 m x 0.25 i. d. x 0.5 µm |
| **Pre-column:** | HP-retention gap (uncoated, deactivated), ID 0.53 µm ZB-5 |
| **Carrier gas:** | Helium 1.5 mL/min |
| **Oven:** | 50 °C/ 0.1 min isotherm; linear temperature gradient (10 °C/min to 300 °C); 10 min isotherm) |
| **Injector:** | on-column injection |
| **Detector:** | MS from Thermo Electron MD800, EI source |
| **Source temperature:** | 200°C (EI) |
| **Interface temperature:** | 250°C |

Table 84 shows the GC-MS parameters for Dicofol.

**Table 84:** Parameters of Dicofol: molecular weight (MW), retention time (RT; GC conditions see above) and masses used for analysis in SIM mode (m/z)

| Substance | MW [g/mol] | RT [min] | m/z |
|---|---|---|---|
| Dicofol | 368 | 20.65 | 253, 251, 139 |

### 6.6.8 Tables

**Table 85:** Chlorpyrifos-ethyl concentrations in sea water [ng/L] in the North Sea

| Cruise Station | GA402 May 03 | GA405 Jul 03 | WH261 Feb 04 | GA419 May 04 | GA425 Jul 04 | GA432 Jan 05 |
|---|---|---|---|---|---|---|
| T30/BL2/EIDER | 0.02 | 0.082 | | 0.013 | 0.02 | |
| T9/LTIEF | 0.08 | 0.011 | | 0.007 | 0.041 | |
| T4/NSB2 | 0.03 | | | 0.022 | 0.021 | |
| STADE | 0.18 | 0.085 | | 0.129 | | |
| T27 | 0.11 | | | | | |
| T36 | 0.04 | | | | | |
| T34 | 0.01 | | | | | |
| MEDEM | | | | 0.047 | | |
| NSGR2 | | | | 0.02 | | |
| SYLT1 | | | | 0.013 | | 0.005 |
| SYLT2 | | | | 0.018 | 0.026 | |
| ENTE3 | | | | 0.011 | | |
| NEFB | | | | 0.046 | 0.023 | 0.017 |
| UFSDB | | | | 0.04 | 0.018 | |
| AMRU2 | | | | 0.018 | | |
| BRIFF | | | | 0.018 | 0.036 | |
| ELBE1 | | | | | 0.026 | |
| NSB3 | | | | | 0.028 | |
| ENTE1 | | | | | <LOD | |
| URST1 | | | | | | 0.013 |
| URST5 | | | | | | 0.013 |
| HELG | | | | | | 0.008 |
| 11 | | 0.028 | | | | |
| 12 | | 0.036 | | | | |
| 13 | | 0.026 | | | | |
| 14 | | 0.078 | | | | |
| 29 | | 0.046 | | | | |
| 33 | | 0.015 | | | | |
| 40 | | 0.014 | | | | |
| 46 | | 0.009 | | | | |
| 51 | | 0.007 | | | | |
| WH/37 | | | 0.052 | | | |
| WH/39 | | | 0.035 | | | |
| WH/42 | | | 0.031 | | | |
| WH/43 | | | 0.057 | | | |
| WH/49 | | | 0.032 | | | |
| WH/47 | | | 0.07 | | | |
| WH/44 | | | 0.102 | | | |
| WH/50 | | | 0.043 | | | |

| Cruise Station | GA402 May 03 | GA405 Jul 03 | WH261 Feb 04 | GA419 May 04 | GA425 Jul 04 | GA432 Jan 05 |
|---|---|---|---|---|---|---|
| WH/52 | | | 0.097 | | | |
| WH/54 | | | 0.027 | | | |
| WH/56 | | | 0.026 | | | |
| WH/58 | | | 0.013 | | | |
| **Min** | 0.01 | 0.007 | 0.013 | 0.007 | 0.018 | 0.005 |
| **Max *** | 0.11 | 0.082 | 0.102 | 0.047 | 0.041 | 0.017 |
| **Median *** | 0.035 | 0.027 | 0.039 | 0.018 | 0.026 | 0.013 |
| **Mean *** | 0.05 | 0.04 | 0.05 | 0.02 | 0.03 | 0.01 |

* Values from Stade (Elbe) are not taken into account

**Table 86:** Chlorpyrifos-ethyl concentrations in sea water [ng/L] in the Baltic Sea

| Cruise Station | GA421 Jun 04 |
|---|---|
| 710 | 0.019 |
| 704 | 0.078 |
| 701 | 0.102 |
| 706 | 0.033 |
| 717 | 0.015 |
| 715 | 0.025 |
| 718 | 0.028 |
| 719 | <LOD |
| 720 | 0.033 |
| ODER | 0.033 |
| **Min** | 0.015 |
| **Max** | 0.102 |
| **Median** | 0.033 |
| **Mean** | 0.04 |

**Table 87:** Chlorpyrifos-ethyl concentrations in sediments [µg/kg dw]

| Cruise | GA349 | GA402 | GA405 | GA419 | Median |
|---|---|---|---|---|---|
| Station | May 00 | May 03 | Jul 03 | May 04 | |
| North Sea | | | | | |
| KS11 | 0.006 | 0.005 | <LOD | 0.014 | 0.006 |
| KS8 | | <LOD | | 0.041 | 0.041 |
| WB1 | 0.008 | | | | 0.008 |
| WB5 | | 0.012 | <LOD | 0.014 | 0.013 |
| SSL | | 0.03 | | | 0.03 |
| UE20 | | <LOD | | 0.021 | 0.021 |
| ES1 | | 0.004 | | | 0.004 |
| UE67 | | <LOD | | | <LOD |
| UE70 | | | | <LOD | <LOD |
| BL2 | | | | 0.038 | 0.038 |
| BL4 | | | | <LOD | <LOD |
| Min | 0.006 | 0.004 | | 0.014 | 0.004 |
| Max | 0.008 | 0.03 | | 0.041 | 0.041 |
| Median | 0.007 | 0.0085 | | 0.021 | 0.017 |
| Mean | 0.01 | 0.01 | | 0.03 | 0.02 |

| **Cruise** | **GA371** | **GA387** | **GA421** | |
|---|---|---|---|---|
| **Station** | **Aug 01** | **Aug 02** | **Jun 04** | **Median** |
| **Baltic Sea** | | | | |
| Eckfbucht | | | 0.009 | 0.009 |
| 710 | 0.006 | <LOD | 0.004 | 0.005 |
| 25A | | | <LOD | <LOD |
| 715 | <LOD | | 0.006 | 0.006 |
| 718 | <LOD | | 0.006 | 0.006 |
| Oder | | | <LOD | <LOD |
| Ruden | | | 0.008 | 0.008 |
| 721/K4 | | <LOD | | <LOD |
| **Min** | 0.006 | | 0.004 | 0.005 |
| **Max** | 0.006 | | 0.009 | 0.009 |
| **Median** | 0.006 | | 0.006 | 0.006 |
| **Mean** | 0.01 | | 0.01 | 0.01 |

**Table 88:** Chlorpyrifos concentrations [µg/kg] in fish liver samples

| | | Chlorpyrifos-ethyl | | Chlorpyrifos-methyl | | |
|---|---|---|---|---|---|---|
| **Station** | ***Species*** | **µg/kg ww** | **µg/kg lw** | **µg/kg ww** | **µg/kg lw** | **cruise** |
| NO4/31 | *Lima lim* | 0.46 | 1.58 | 0.39 | 1.35 | WH232 |
| NO4/32 | *Lima lim* | 0.27 | 0.76 | <LOD | <LOD | WH232 |
| NO4/25 | *Lima lim* | 0.25 | 1.72 | <LOD | <LOD | WH242 |
| NO4/29 | *Lima lim* | 0.52 | 2.31 | <LOD | <LOD | WH242 |
| NO4/38 | *Lima lim* | 0.54 | 3.58 | <LOD | <LOD | WH242 |
| NO1/02 | *Lima lim* | 0.79 | 4.05 | <LOD | <LOD | WH242 |
| NO1/17 | *Lima lim* | 0.14 | 0.52 | 0.15 | 0.56 | WH242 |
| NO1/20 | *Lima lim* | 0.25 | 0.88 | 0.3 | 1.06 | WH242 |
| NO6/56 | *Lima lim* | 0.11 | 0.25 | <LOD | <LOD | WH242 |
| NO6/59 | *Lima lim* | 0.14 | 0.75 | <LOD | <LOD | WH242 |
| NO6/64 | *Lima lim* | 0.22 | 0.83 | <LOD | <LOD | WH242 |
| PO1/72 | *Lima lim* | 0.94 | 4.59 | 0.67 | 3.26 | WH242 |
| PO1/76 | *Lima lim* | 0.36 | 2.27 | <LOD | <LOD | WH242 |
| PO1/78 | *Lima lim* | 0.75 | 3.56 | 0.56 | 2.68 | WH242 |
| NO4/41 | *Gadu mor* | 0.18 | 0.87 | <LOD | <LOD | WH242 |
| NO4/44 | *Gadu mor* | 0.1 | 0.3 | <LOD | <LOD | WH242 |
| NO4/54 | *Gadu mor* | 0.16 | 0.58 | <LOD | <LOD | WH242 |
| B1/113 | *Lima lim* | 0.55 | 4.07 | <LOD | <LOD | WH242 |
| B1/116 | *Lima lim* | 1.32 | 7.6 | <LOD | <LOD | WH242 |
| B1/120 | *Lima lim* | 3.38 | 12.96 | <LOD | <LOD | WH242 |
| B1/135 | *Gadu mor* | 1.25 | 4.54 | <LOD | <LOD | WH242 |
| B1/136 | *Gadu mor* | 1.23 | 7.07 | <LOD | <LOD | WH242 |
| B11/105 | *Gadu mor* | 0.3 | 1.17 | <LOD | <LOD | WH242 |
| B11/93 | *Plat fle* | 0.55 | 1.58 | <LOD | <LOD | WH242 |
| B11/94 | *Plat fle* | 0.34 | 0.76 | <LOD | <LOD | WH242 |
| **Min** | | 0.1 | 0.25 | <LOD | <LOD | |
| **Max** | | 3.38 | 12.96 | <LOQ | <LOQ | |
| **Median** | | 0.36 | 1.58 | | | |
| **Mean** | | 0.60 | 2.77 | | | |

**Table 89:** Endosulfan I concentrations in sea water [ng/L] in the North Sea

| **Cruise** | **GA402** | **GA405** | **WH261** | **GA419** | **GA425** | **GA432** |
|---|---|---|---|---|---|---|
| **Station** | **May 03** | **Jul 03** | **Feb 04** | **May 04** | **Jul 04** | **Jan 05** |
| T30/BL2/EIDER | <LOD | 0.043 | | 0.006 | 0.036 | |
| T9/LTIEF/St.25 | <LOD | <LOQ | | 0.019 | 0.033 | |
| T4/NSB2 | <LOD | | | 0.018 | 0.012 | |
| STADE | <LOD | <LOQ | | 0.049 | | |
| T27 | <LOD | | | | | |
| T36 | <LOD | | | | | |
| T34 | <LOD | | | | | |
| MEDEM | | | | 0.017 | | |
| NSGR2 | | | | 0.025 | | |
| SYLT1 | | | | n.a. | | 0.013 |
| SYLT2 | | | | n.a. | 0.051 | |
| ENTE3 | | | | 0.018 | | |
| NEFB | | | | 0.016 | 0.024 | 0.012 |
| UFSDB | | | | 0.017 | 0.029 | |
| AMRU2 | | | | 0.024 | | |
| BRIFF | | | | 0.024 | 0.021 | |
| ELBE1 | | | | | 0.022 | |
| NSB3 | | | | | 0.021 | |
| ENTE1 | | | | | <LOD | |
| URST1 | | | | | | 0.007 |
| URST5 | | | | | | 0.013 |
| HELG | | | | | | 0.014 |
| 11 | | <LOQ | | | | |
| 12 | | 0.047 | | | | |
| 13 | | LOQ | | | | |
| 14 | | <LOD | | | | |
| 29 | | 0.041 | | | | |
| 33 | | 0.03 | | | | |
| 40 | | 0.03 | | | | |
| 46 | | <LOD | | | | |
| 51 | | <LOQ | | | | |
| WH/37 | | | <LOQ | | | |
| WH/39 | | | <LOQ | | | |
| WH/42 | | | <LOQ | | | |
| WH/43 | | | 0.031 | | | |
| WH/49 | | | <LOQ | | | |
| WH/47 | | | 0.041 | | | |
| WH/44 | | | 0.034 | | | |
| WH/50 | | | 0.045 | | | |
| WH/52 | | | 0.051 | | | |

| **Cruise** | **GA402** | **GA405** | **WH261** | **GA419** | **GA425** | **GA432** |
|---|---|---|---|---|---|---|
| **Station** | **May 03** | **Jul 03** | **Feb 04** | **May 04** | **Jul 04** | **Jan 05** |
| WH/54 | | | 0.032 | | | |
| WH/56 | | | <LOQ | | | |
| WH/58 | | | <LOQ | | | |
| **Min** | | 0.03 | 0.031 | 0.006 | 0.012 | 0.007 |
| **Max *** | | 0.047 | 0.051 | 0.025 | 0.051 | 0.014 |
| **Median *** | | 0.041 | 0.0375 | 0.018 | 0.024 | 0.013 |
| **Mean *** | | 0.04 | 0.04 | 0.02 | 0.03 | 0.01 |

* Values from Stade (Elbe) are not taken into account

**Table 90:** Endosulfan II concentrations in sea water [ng/L] in the North Sea

| **Cruise**<br>**Station** | **GA402**<br>**May 03** | **GA405**<br>**Jul 03** | **WH261**<br>**Feb 04** | **GA419**<br>**May 04** | **GA425**<br>**Jul 04** | **GA432**<br>**Jan 05** |
|---|---|---|---|---|---|---|
| T30/BL2/EIDER | <LOD. | 0.034 | | 0.007 | <LOD | |
| T9/LTIEF/St.25 | <LOD. | <LOQ | | 0.006 | <LOD | |
| T4/NSB2 | <LOD. | | | <LOD | <LOD | |
| STADE | <LOQ | 0.03 | | 0.028 | | |
| T27 | 0.15 | | | | | |
| T36 | <LOD. | | | | | |
| T34 | <LOQ | | | | | |
| MEDEM | | | | 0.009 | | |
| NSGR2 | | | | <LOD | | |
| SYLT1 | | | | 0.013 | | <LOD |
| SYLT2 | | | | 0.009 | <LOD | |
| ENTE3 | | | | <LOD | | |
| NEFB | | | | 0.017 | <LOD | <LOD |
| UFSDB | | | | 0.018 | <LOD | |
| AMRU2 | | | | <LOD | | |
| BRIFF | | | | <LOD | <LOD | |
| ELBE1 | | | | | <LOD | |
| NSB3 | | | | | <LOD | |
| ENTE1 | | | | | <LOD | |
| URST1 | | | | | | <LOD |
| URST5 | | | | | | <LOD |
| HELG | | | | | | <LOD |
| 11 | | <LOD. | | | | |
| 12 | | <LOQ | | | | |
| 13 | | <LOQ | | | | |
| 14 | | <LOQ | | | | |
| 29 | | <LOQ | | | | |
| 33 | | 0.037 | | | | |
| 40 | | <LOQ | | | | |
| 46 | | <LOD. | | | | |
| 51 | | <LOQ | | | | |
| WH/37 | | | <LOQ | | | |
| WH/39 | | | <LOQ | | | |
| WH/42 | | | <LOQ | | | |
| WH/43 | | | <LOQ | | | |
| WH/49 | | | <LOQ | | | |
| WH/47 | | | LOQ | | | |
| WH/44 | | | 0.042 | | | |
| WH/50 | | | 0.037 | | | |
| WH/52 | | | 0.061 | | | |
| WH/54 | | | <LOQ | | | |
| WH/56 | | | <LOQ | | | |
| WH/58 | | | <LOQ | | | |

| Cruise<br>Station | GA402<br>May 03 | GA405<br>Jul 03 | WH261<br>Feb 04 | GA419<br>May 04 | GA425<br>Jul 04 | GA432<br>Jan 05 |
|---|---|---|---|---|---|---|
| **Min** | 0.15 | 0.03 | 0.037 | 0.006 | 0 | 0 |
| **Max *** | 0.15 | 0.037 | 0.061 | 0.018 | 0 | 0 |
| **Median *** | 0.15 | 0.034 | 0.042 | 0.011 | | |
| **Mean *** | 0.15 | 0.03 | 0.05 | 0.01 | | |

Values from Stade (Elbe) are not taken into account

**Table 91:** Endosulfan concentrations in sea water [ng/L] in the Baltic Sea

| **Cruise** | **GA421** | |
|---|---|---|
| **Station** | **Jun 04** | |
| | **Endosulfan I** | **Endosulfan II** |
| 710 | 0.026 | 0.008 |
| 704 | 0.062 | 0.049 |
| 701 | 0.03 | 0.009 |
| 706 | 0.018 | <LOD |
| 717 | 0.023 | 0.004 |
| 715 | 0.025 | 0.009 |
| 718 | 0.028 | 0.02 |
| 719 | 0.026 | 0.01 |
| 720 | 0.013 | 0.007 |
| ODER | 0.025 | 0.01 |
| **Min** | 0.013 | < LOD |
| **Max** | 0.062 | |
| **Median** | 0.0255 | |
| **Mean** | 0.03 | |

**Table 92:** Endosulfan I concentrations in sediments [µg/kg dw]

| **Cruise** | **GA349** | **GA402** | **GA405** | **GA419** | **Median** |
|---|---|---|---|---|---|
| **Station** | **May 00** | **May 03** | **Jul 03** | **May 04** | |
| **North Sea** | | | | | |
| KS11 | <LOD | <LOD | <LOD | 0.021 | 0.021 |
| KS8 | | <LOD | | 0.013 | 0.013 |
| WB1 | <LOD | | | | <LOD |
| WB5 | | 0.013 | <LOD | <LOD | 0.013 |
| SSL | | 0.019 | | | 0.019 |
| UE20 | | <LOD | | <LOD | <LOD |
| ES1 | | <LOD | | | <LOD |
| UE67 | | <LOD | | | <LOD |
| UE70 | | | | <LOD | <LOD |
| BL2 | | | | 0.009 | 0.009 |
| BL4 | | | | <LOD | <LOD |
| **Min** | 0 | 0.013 | 0 | 0.009 | 0.009 |
| **Max** | 0 | 0.019 | 0 | 0.021 | 0.021 |
| **Median** | | 0.016 | | 0.013 | 0.013 |
| **Mean** | | 0.016 | | 0.014 | 0.015 |

| **Cruise** | **GA371 (ISIS)** | **GA387** | **GA421** | |
|---|---|---|---|---|
| **Station** | **Aug 01** | **Aug 02** | **Jun 04** | **Median** |
| **Baltic Sea** | | | | |
| Eckfbucht | | | 0.198 | 0.198 |
| 710 | 0.076 | 0.075 | <LOD | 0.0755 |
| 25A | | | <LOD | <LOD |
| 715 | <LOD | | <LOD | <LOD |
| 718 | 0.029 | | 0.042 | 0.0355 |
| Oder | | | 0.009 | 0.009 |
| Ruden | | | <LOD | <LOD |
| 721/K4 | | 0.02 | | 0.02 |
| **Min** | 0.029 | 0.02 | 0.009 | 0.009 |
| **Max** | 0.076 | 0.075 | 0.198 | 0.198 |
| **Median** | 0.0525 | 0.0475 | 0.042 | 0.0355 |
| **Mean** | 0.0525 | 0.0475 | 0.083 | 0.0676 |

**Table 93:** Endosulfan II concentrations in sediments [µg/kg dw] of the Baltic Sea (concentrations in the North Sea were all < LOD)

| **Fahrt**<br>**Station** | **GA371**<br>**Aug 01** | **GA387**<br>**Aug 02** | **GA421**<br>**Jun 04** |
|---|---|---|---|
| Eckfbucht | | | 0.086 |
| 710 | <LOD | <LOD | <LOD |
| 25A | | | <LOD |
| 715 | <LOD | | <LOD |
| 718 | <LOD | | 0.011 |
| Oder | | | <LOD |
| Ruden | | | <LOD |
| 721/K4 | | <LOD | |
| **Min** | 0 | 0 | 0.011 |
| **Max** | 0 | 0 | 0.086 |
| **Median** | | | 0.0485 |
| **Mean** | | | 0.0485 |

**Table 94:** Endosulfan concentrations in fish liver [µg/kg]

| Station | *species* | ng/g ww Endosulfan I | ng/g fat | ng/g ww Endosulfan II | ng/g fat | % fat | cruise |
|---|---|---|---|---|---|---|---|
| NO4/31 | *Lima lim* | 0.49 | 1.68 | 0.24 | 0.82 | 29.2 | WH232 |
| NO4/32 | *Lima lim* | 0.39 | 1.09 | 0.26 | 0.72 | 36 | WH232 |
| NO1/02 | *Lima lim* | 1.04 | 5.3 | 0.51 | 2.57 | 19.6 | WH242 |
| NO1/17 | *Lima lim* | 0.15 | 0.56 | <LOD | <LOD | 27.1 | WH242 |
| NO1/20 | *Lima lim* | 0.34 | 1.22 | 0.17 | 0.59 | 28.1 | WH242 |
| NO4/25 | *Lima lim* | 0.26 | 1.82 | 0.13 | 0.89 | 14.5 | WH242 |
| NO4/29 | *Lima lim* | 0.74 | 3.26 | 0.3 | 1.34 | 22.7 | WH242 |
| NO4/38 | *Lima lim* | 0.5 | 3.3 | <LOD | <LOD | 15.2 | WH242 |
| NO6/56 | *Lima lim* | 0.11 | 0.26 | <LOD | <LOD | 41.6 | WH242 |
| NO6/59 | *Lima lim* | 0.46 | 2.47 | 0.24 | 1.29 | 18.6 | WH242 |
| NO6/64 | *Lima lim* | 0.35 | 1.32 | <LOD | <LOD | 26.5 | WH242 |
| PO1/72 | *Lima lim* | 1.44 | 7.01 | 0.7 | 3.43 | 20.5 | WH242 |
| PO1/76 | *Lima lim* | 0.52 | 3.25 | 0.34 | 2.12 | 16.4 | WH242 |
| PO1/78 | *Lima lim* | 1.13 | 5.4 | 0.51 | 2.43 | 21 | WH242 |
| NO4/41 | *Gadu mor* | 0.42 | 2.07 | 0.11 | 0.53 | 20.3 | WH242 |
| NO4/44 | *Gadu mor* | <LOD | <LOD | <LOD | <LOD | 34.5 | WH242 |
| NO4/54 | *Gadu mor* | 0.28 | 1.04 | 0.07 | 0.24 | 27 | WH242 |
| B1/113 | *Lima lim* | <LOD | <LOD | <LOD | <LOD | 13.4 | WH242 |
| B1/116 | *Lima lim* | <LOD | <LOD | <LOD | <LOD | 17.3 | WH242 |
| B1/120 | *Lima lim* | <LOD | <LOD | <LOD | <LOD | 26.1 | WH242 |
| B1/135 | *Gadu mor* | <LOD | <LOD | <LOD | <LOD | 27.5 | WH242 |
| B1/136 | *Gadu mor* | <LOD | <LOD | <LOD | <LOD | 17.4 | WH242 |
| B11/105 | *Gadu mor* | <LOD | <LOD | <LOD | <LOD | 25.4 | WH242 |
| B11/93 | *Plat fle* | <LOD | <LOD | <LOD | <LOD | 34.5 | WH242 |
| B11/94 | *Plat fle* | <LOD | <LOD | <LOD | <LOD | 45.4 | WH242 |
| **Min** | | 0.11 | 0.26 | 0.07 | 0.24 | 13.4 | |
| **Max** | | 1.44 | 7.01 | 0.7 | 3.43 | 45.4 | |
| **Median** | | 0.44 | 1.945 | 0.25 | 1.09 | 25.4 | |
| **Mean** | | 0.54 | 2.57 | 0.30 | 1.41 | 25.03 | |

**Table 95:** Trifluralin concentrations in sea water [ng/L] in the North Sea

| Cruise<br>Station | GA402<br>May 03 | GA405<br>Jul 03 | WH261<br>Feb 04 | GA419<br>May 04 | GA425<br>Jul 04 | GA432<br>Jan 05 |
|---|---|---|---|---|---|---|
| T30/BL2/EIDER | 0.01 | 0.005 | | 0.011 | 0.015 | |
| T9/LTIEF/St.25 | 0.02 | 0.007 | | 0.009 | 0.022 | |
| T4/NSB2 | 0.01 | | | 0.016 | 0.011 | |
| STADE | 0.03 | 0.081 | | 0.056 | | |
| T27 | 0.11 | | | | | |
| T36 | 0.03 | | | | | |
| T34 | 0.03 | | | | | |

| Cruise<br>Station | GA402<br>May 03 | GA405<br>Jul 03 | WH261<br>Feb 04 | GA419<br>May 04 | GA425<br>Jul 04 | GA432<br>Jan 05 |
|---|---|---|---|---|---|---|
| MEDEM | | | | 0.044 | | |
| NSGR2 | | | | 0.008 | | |
| SYLT1 | | | | 0.016 | | 0.1 |
| SYLT2 | | | | 0.017 | 0.019 | |
| ENTE3 | | | | 0.003 | | |
| NEFB | | | | 0.05 | 0.018 | 0.58 |
| UFSDB | | | | 0.029 | 0.015 | |
| AMRU2 | | | | 0.022 | | |
| BRIFF | | | | 0.007 | 0.026 | |
| ELBE1 | | | | | 0.016 | |
| NSB3 | | | | | 0.015 | |
| ENTE1 | | | | | 0.014 | |
| URST1 | | | | | | 0.16 |
| URST5 | | | | | | 0.18 |
| HELG | | | | | | 0.17 |
| 11 | | <LOQ | | | | |
| 12 | | 0.018 | | | | |
| 13 | | 0.008 | | | | |
| 14 | | 0.009 | | | | |
| 29 | | <LOQ | | | | |
| 33 | | 0.005 | | | | |
| 40 | | <LOQ | | | | |
| 46 | | <LOD. | | | | |
| 51 | | <LOD. | | | | |
| WH/37 | | | 0.541 | | | |
| WH/39 | | | 0.541 | | | |
| WH/42 | | | 0.155 | | | |
| WH/43 | | | 0.121 | | | |
| WH/49 | | | 0.146 | | | |
| WH/47 | | | 0.201 | | | |
| WH/44 | | | 0.381 | | | |
| WH/50 | | | 0.286 | | | |
| WH/52 | | | 0.221 | | | |
| WH/54 | | | 0.141 | | | |
| WH/56 | | | 0.212 | | | |
| WH/58 | | | 0.113 | | | |
| **Min** | 0.01 | 0.005 | 0.113 | 0.003 | 0.011 | 0.1 |
| **Max *** | 0.11 | 0.018 | 0.541 | 0.05 | 0.026 | 0.58 |
| **Median *** | 0.025 | 0.008 | 0.2065 | 0.016 | 0.0155 | 0.17 |
| **Mean *** | 0.04 | 0.02 | 0.25 | 0.02 | 0.02 | 0.24 |

* Values from Stade (Elbe) are not taken into account

**Table 96:** Trifluralin concentrations in sea water [ng/L] in the Baltic Sea

| **Cruise** | **GA421** |
|---|---|
| **Station** | **Jun 04** |
| 710 | 0.011 |
| 704 | 0.046 |
| 701 | 0.021 |
| 706 | 0.011 |
| 717 | 0.007 |
| 715 | 0.022 |
| 718 | 0.017 |
| 719 | 0.008 |
| 720 | 0.006 |
| ODER | 0.013 |
| **Min** | 0.006 |
| **Max** | 0.046 |
| **Median** | 0.012 |
| **Mean** | 0.02 |

**Table 97:** Trifluralin concentrations in sediment [µg/kg dw]

| **Cruise** | **GA349** | **GA402** | **GA405** | **GA419** | **Median** |
|---|---|---|---|---|---|
| **Station** | **May 00** | **May 03** | **Jul 03** | **May 04** | |
| **North Sea** | | | | | |
| KS11 | <LOD | 0.008 | 0.005 | 0.015 | 0.008 |
| KS8 | | 0.009 | | 0.014 | 0.012 |
| WB1 | <LOD | | | | |
| WB5 | | 0.014 | <LOD | 0.021 | 0.018 |
| SSL | | 0.018 | | | 0.018 |
| UE20 | | 0.009 | | 0.016 | 0.013 |
| ES1 | | 0.003 | | | 0.003 |
| UE67 | | 0.008 | | | 0.008 |
| UE70 | | | | <LOD | <LOD |
| BL2 | | | | 0.009 | 0.009 |
| BL4 | | | | 0.004 | 0.004 |
| **Min** | | 0.003 | 0.005 | 0.004 | 0.003 |
| **Max** | | 0.018 | 0.005 | 0.021 | 0.018 |
| **Median** | | 0.009 | 0.005 | 0.0145 | 0.009 |
| **Mean** | | 0.01 | 0.01 | 0.01 | 0.010 |

| **Cruise** | **GA371 (ISIS)** | **GA387** | **GA421** | | |
|---|---|---|---|---|---|
| **Station** | **Aug 01** | **Aug 02** | **Jun 04** | | **Median** |
| **Baltic Sea** | | | | | |
| Eckfbucht | | | 0.018 | | 0.018 |
| 710 | 0.014 | 0.016 | 0.001 | | 0.014 |
| 25A | | | 0.004 | | 0.004 |
| 715 | 0.012 | | 0.014 | | 0.013 |
| 718 | 0.011 | | 0.008 | | 0.010 |
| Oder | | | 0.001 | | 0.001 |
| Ruden | | | 0.002 | | 0.002 |
| 721/K4 | | 0.013 | | | 0.013 |
| **Min** | 0.011 | 0.013 | 0.001 | | 0.001 |
| **Max** | 0.014 | 0.016 | 0.018 | | 0.018 |
| **Median** | 0.012 | 0.015 | 0.004 | | 0.011 |
| **Mean** | 0.012 | 0.015 | 0.007 | | 0.009 |

**Table 98:** Trifluralin concentrations in fish liver [µg/kg]

| Station | *Species* | ng/g ww | ng/g fat | % lipid | Cruise |
|---|---|---|---|---|---|
| NO4/31 | *Lima lim* | 0.52 | 1.76 | 29.2 | WH232 |
| NO4/32 | *Lima lim* | 0.45 | 1.25 | 36 | WH232 |
| NO1/02 | *Lima lim* | 1.74 | 8.86 | 19.6 | WH242 |
| NO1/17 | *Lima lim* | 0.24 | 0.88 | 27.1 | WH242 |
| NO1/20 | *Lima lim* | 0.47 | 1.66 | 28.1 | WH242 |
| NO4/25 | *Lima lim* | 0.42 | 2.9 | 14.5 | WH242 |
| NO4/29 | *Lima lim* | 0.81 | 3.56 | 22.7 | WH242 |
| NO4/38 | *Lima lim* | 0.7 | 4.59 | 15.2 | WH242 |
| NO6/56 | *Lima lim* | 0.24 | 0.58 | 41.6 | WH242 |
| NO6/59 | *Lima lim* | 0.63 | 3.38 | 18.6 | WH242 |
| NO6/64 | *Lima lim* | 0.47 | 1.76 | 26.5 | WH242 |
| PO1/72 | *Lima lim* | 0.97 | 4.73 | 20.5 | WH242 |
| PO1/76 | *Lima lim* | 0.77 | 4.83 | 16.4 | WH242 |
| PO1/78 | *Lima lim* | 0.8 | 3.8 | 21 | WH242 |
| NO4/41 | *Gadu mor* | 0.3 | 1.46 | 20.3 | WH242 |
| NO4/44 | *Gadu mor* | 0.19 | 0.54 | 34.5 | WH242 |
| NO4/54 | *Gadu mor* | 0.22 | 0.81 | 27 | WH242 |
| B1/113 | *Lima lim* | 0.04 | 0.3 | 13.4 | WH242 |
| B1/116 | *Lima lim* | 0.32 | 1.84 | 17.3 | WH242 |
| B1/120 | *Lima lim* | 0.45 | 1.73 | 26.1 | WH242 |
| B1/135 | *Gadu mor* | 0.27 | 0.97 | 27.5 | WH242 |
| B1/136 | *Gadu mor* | 0.15 | 0.83 | 17.4 | WH242 |
| B11/105 | *Gadu mor* | 0.07 | 0.28 | 25.4 | WH242 |
| B11/93 | *Plat fle* | 0.1 | 0.28 | 34.5 | WH242 |
| B11/94 | *Plat fle* | 0.14 | 0.31 | 45.4 | WH242 |
| **Min** | | 0.04 | 0.28 | 13.4 | |
| **Max** | | 1.74 | 8.86 | 45.4 | |
| **Median** | | 0.42 | 1.66 | 25.4 | |
| **Mean** | | 0.46 | 2.16 | 25.03 | |

## 6.7 Publications and conference contributions of project results

Gerwinski W., Theobald N. and Weigelt S. “Überblick über das Vorkommen von WRR-Substanzen im Wasser der Nord- und Ostsee“ 2. Bund/Länder-Messprogramm Nord- und Baltis Sea Erfahrungsaustausch, **2004**, Vilm

Theobald N., Caliebe C., Gerwinski W., Weigelt S. “Messen wir noch die richtigen Schadstoffe? – Vorkommen und Bedeutung neuer und klassischer organischer Schadstoffe in Nord- und Baltis Sea“, Meeresumwelt-Symposium, **2004**, Hamburg

Baaß A.-C., Weigelt-Krenz S., Theobald N. “Water Framework Directive Priority Chemicals in the Marine Environment“ CEEC Workshop: From Lakes to Oceans, **2005**, Zürich

Theobald N., Baaß A.-C., Jerzycki-Brandes K., Weigelt-Krenz S. “Organohalogen Pesticides in the North and Baltic Sea” Dioxin 2005, **2005**, Toronto

# 7 List of Tables and Figures