NEW APPROACHES FOR THE MASS SPECTROMETRIC DETERMINATION OF TRACE CONCENTRATIONS AND CONGENER GROUP PATTERNS OF CHLORINATED PARAFFINS IN BIOTA

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LIST OF PUBLICATIONS

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PAPER III: New quantification procedure for the analysis of chlorinated paraffins using electron capture negative ionization mass spectrometry.

PAPER IV: Short- and medium-chain chlorinated paraffins in biota from the European Arctic - differences in homologue group patterns.

ABBREVIATIONS

APCI	atmospheric pressure chemical ionisation
BgVV	Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin
CI	chemical ionisation
CID	collision induced dissociation
CH_2Cl_2	dichloromethane
conc.	concentrated
CPs	Chlorinated paraffins (SCCPs: short chain CPs, MCCPs: medium chain CPs, and lCCPs: long chain CPs)
DDD	1,1-dichloro-2,2-*bis*(4-chlorophenyl)-ethane
DDE	1,1-dichloro-2,2-*bis*(4-chlorophenyl)-ethylene
DDT	1,1,1-trichloro-2,2-*bis*(4-chlorophenyl)-ethane
dw	dry weight
ECNI	electron capture negative ionization
EI	electron ionisation
f	female
GC	gas chromatography
HLC	Henry's Law Constant
HPLC	high performance liquid chromatography
HR	high-resolution
HRGC	high-resolution gas chromatography
HRMS	high-resolution mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
LR	low-resolution
LRMS	low-resolution mass spectrometry
lw	lipid weight
m	male
m/z	mass-to-charge ratio
MS	mass spectrometry
MS/MS	tandem mass spectrometry

n.a.	not available
n.d.	not determined
n.s.	not specified
NICI	negative ion chemical ionisation
PCA	principal components analysis
PCBs	polychlorinated biphenyls
POP	persistent organic pollutant
SCGC	short column gas chromatography
S/N	signal-to-noise ratio
SIM	selected ion monitoring
VP	vapour pressure
WS	water solubility
ww	wet weight

SUMMARY

The determination of chlorinated paraffins (CPs) in the environment is important, since CPs are persistent, bioaccumulative and toxic. However, the analysis of these complex mixtures containing thousands of isomers is also a demanding task. CP analysis and especially the quantification of CPs are far from being well established. In this work, new methodologies were developed for the determination of CPs in biota by low-resolution mass spectrometry (LRMS).

The use of expensive high-resolution mass spectrometry was avoided. Therefore, existing clean-up methods were improved to enhance the selectivity as well as the unequivocal identification of CP congener groups. The developed method comprises the following steps: After cold column extraction, lipids were removed by adsorption chromatography on silica gel impregnated with sulfuric acid. Adsorption chromatography on Florisil® allowed the elimination of interfering compounds such as polychlorinated biphenyls (PCBs) and toxaphenes, which interfere the CP analysis by high-resolution gas chromatography (HRGC) coupled to LRMS and employing electron capture negative ionisation (ECNI).

The analysis of complex CP mixtures with short- (SCCPs, $C_{10\text{-}13}$) and medium- (MCCPs, $C_{14\text{-}17}$) chain lengths can be disturbed by mass overlap, if LRMS in the ECNI mode is employed. This is mainly caused by CP congeners with the same nominal mass but five carbon atoms more and two chlorine atoms less and can lead to an overestimation of the total CP concentration. Therefore, a procedure based on a precise check of isotope ratios, retention time ranges and signal shapes was developed to unequivocally identify the most important CP congener groups.

Prior to this work, quantification procedures for CPs were not well established, and systematic errors could not always be avoided. Therefore, a new quantification procedure was developed to overcome the strong dependence of ECNI results on the chlorine content of the standard, and to avoid a tedious and time-consuming selection of the "most similar" reference standard. A linear correlation could be established between the total response factor of a CP mixture and its chlorine content. This allowed the compensation of errors due to differences between the degree of chlorination of CPs in the sample and standard. Quantification errors were considerably reduced and CP pattern matching procedures between standard and sample became unnecessary.

Furthermore, HRGC electron ionisation tandem mass spectrometry (EI-MS/MS) was used for the fast determination of the total CP amount. This method was successfully applied to the determination of total CP concentrations in fish, human milk and eggs and provided a first insight into CP levels.

ECNI-LRMS was employed for the determination of CP levels and congener group patterns in different fishes and seabirds from various regions in Europe (North and Baltic Sea, Central Europe, European Arctic). Results revealed that SCCPs and MCCPs are detectable in fish in the ng/g range. Hence, concentrations are comparable to levels of other persistent organic pollutants (e.g. polychlorinated biphenyls (PCBs), toxaphenes and polybrominated diphenylethers).

SCCP concentrations were between 54 and 1428 ng/g lipid weight (lw), MCCP concentrations varied between <30 and 2448 ng/g lw in cod, dab and flounder from different locations in the North and Baltic Sea.

SCCPs and, for the first time, MCCPs could be detected in biota from the European Arctic. Between 89 and 861 ng/g lw of SCCPs and 107-3717 ng/g lw of MCCPs were detectable in fish and seabirds captured on Bear Island, and between 35 and 139 ng/g lw of SCCPs and 14-96 ng/g lw of MCCPs were found in cod captured close to Iceland and northwest Norway (Lofoten).

Furthermore, CP levels were determined in different fish species from rivers in south Germany (Neckar and Rhine) and north Switzerland (Liechtensteiner Binnenkanal and Necker). The total CP amount was first estimated by EI-MS/MS. Concentrations were between 19 and 256 ng/g wet weight (ww). Furthermore, SCCP and MCCP concentrations were determined for selected samples by ECNI-LRMS. Concentrations were comparable to PCB 138 or PCB 153. Linear correlations were observed between indicator PCBs and SCCPs as well as PCB 180 and MCCPs.

CPs were detectable in human milk as well as in human foodstuff by EI-MS/MS. Total CP concentrations were 2.6-9.6 ng/g ww in human milk and 20-59 ng/g ww in poultry eggs, both from south Germany.

Furthermore, congener and homologue group patterns of technical CP mixtures and standards were investigated and compared to those in brown trout from Central Europe and cod from the Baltic Sea and northwest Europe. SCCP mixtures contained mainly C_{11} and C_{12} congeners (>63%) followed by C_{13} and C_{10}, whereas C_{14} congeners (>45%) followed by C_{15} dominated in MCCPs mixtures. Minor components were C_{16} (<14%) and C_{17}

congeners (<2%). Congener groups with six, seven and eight chlorine atoms were most abundant in all fish samples indicating their specific potential for bioaccumulation.

Similarities and differences in the CP mixtures were further elucidated by principal components analysis (PCA). PCA revealed that differences in CP compositions were mainly caused by their chlorine content. In addition, MCCPs were differentiated by their proportion of C_{14} congeners. Standard SCCP mixtures were similar to technical mixtures of similar chlorine content, whereas MCCP standard mixtures were not, due to their low abundance of $C_{15\text{-}17}$ congeners. CPs in brown trout and cod showed similarities to technical CP mixtures and, hence, no hint for CP biotransformation. However, SCCPs in cod were clustered according to their geographic origin by PCA.

1 INTRODUCTION

1.1 Chlorinated paraffins and other persistent organic pollutants

Technical products consisting of mixtures of polychlorinated compounds like polychlorinated biphenyls (PCBs), bornanes, cyclohexanes or paraffins were widely utilised for technical applications or as pesticides during the last century. In 1966, PCBs were discovered in humans and in the environment for the first time (Jensen, 1966). The detection of further halogenated substances followed.

Today, many of these compounds are classified as persistent organic pollutants (POPs). POPs are defined as chemicals that do not degrade in the environment over long time and are widely distributed geographically. Furthermore, they accumulate in the fatty tissue of living organisms and are toxic to humans and wildlife. To protect human health and the environment from POPs, international treaties like the Stockholm Convention have been stipulated aiming to eliminate or reduce the release of POPs into the environment.

The use of PCBs in new “open” products was banned in Europe already in 1978. Several new products were developed to replace PCBs as plasticisers and flame retardants in PVC, paints, lubricants and sealants. Chlorinated paraffins (CPs) were among them. Their total world production increased to 230 kt/year during this time (Svanberg and Linden, 1979). Currently, CPs are considered as a “new” POP. They have been recently included in the regulatory programme of the European Community and the use of short-chain CPs (SCCPs) is banned for certain applications (European Community, 2000, 2001). However, new replacements such as medium-chain CPs (MCCPs) are already in use.

1.2 Chlorinated paraffins

1.2.1 Classification

CPs (also called polychlorinated *n*-alkanes or PCAs) have the general formula $C_nH_{2n+2-z}Cl_z$ and are complex mixtures containing thousands of different isomers, diastereomers and enantiomers. CPs are subdivided according to their carbon chain length into short chain CPs (C_{10}-C_{13}), medium chain CPs (C_{14}-C_{17}) and long chain CPs (LCCPs, C_{18}-C_{30}). They are produced by radical chlorination of *n*-alkanes, in presence of UV light or heating. The chlorine content of these mixtures varies between 30 and 70%.

1.2.2 Applications

Miscellaneous CP formulations are used in a variety of consumer products and industrial processes due to their wide range of different physical and chemical properties. CPs have been produced since 1930, and the first commercial use was as solvent for Dichloramine T in antiseptic nasal and throat spray (World Health Organization, 1996). Today more than 200 CP formulations are commercially available (Serrone*, et al.*, 1987). Several CP producing companies are located in Europe e.g. in the United Kingdom (Ineos Chlor Ltd., Imperial Chemical Industries), Germany (Leuna Tenside, Hoechst AG, Hüls AG) and Italy (Caffaro) (World Health Organization, 1996). The world-wide production of CPs has been estimated to 300 kt/year in 1993 (Tomy*, et al.*, 1998a). Between 1994 and 1997, the use of SCCPs in Europe decreased by 70% mainly as a result of an agreement for the phase out of SCCPs. By now, less than 15 000 t/year of SCCPs are manufactured in the European Union (European Commission, 2000). In contrast, global usage of MCCPs has increased and surpassed that of SCCPs. MCCPs are partially chosen as replacements for SCCPs. The

total production capacity of MCCPs in the European Union is in the range of 45 000 to 160 000 t/year (Environment Agency United Kingdom, 2002).

The main applications of SCCP and MCCPs are summarised in Table 1.1. CPs are mainly utilised as extreme pressure additives in metal working fluids, and as flame retardants or plasticisers in PVC, rubber, paints, coatings and sealants. In Europe around 70% of all SCCPs are utilised in metal working fluids and around 80% of the MCCPs find application as secondary plasticisers in PVC. The proportion of CPs in the final products is quite high. Products contain between 1 and 20% of CPs, but for special applications even up to 80% can be found. Most of the applied SCCP mixtures have a chlorine content between 50 and 70%, whereas such high-chlorinated MCCP mixtures do not exist. Their chlorine content is usually between 40 and 60%.

Table 1.1: Applications of short- and medium-chain chlorinated paraffins in Europe listed according to their percentage of total use. Information about the chlorine content of the applied CPs and their percentage in the final product is also given (World Health Organization, 1996).

Application / use of SCCPs and MCCPs	Percentage of total use [%]	Chlorine content of the applied CPs [%]	Percentage of CPs in final product [%]
SCCPs			
Metal working fluids	71	49-69	2-10 (up to 80)
Flame retardants in rubber	10	63-71	1-10
Plasticiser/flame retardant in paints and coatings	9	60-65	1-10
Plasticiser/flame retardant in sealants	5	56-65	n.s.
MCCPs			
Secondary plasticiser in PVC	79	ca. 45	10-15
Metal working fluids	9	40-45	5-70
Plasticiser/flame retardant in paints, coatings, sealants	5	50-60	4-20

n.s. not specified

Since their first production, the quality of CPs was improved increasing the purity of the *n*-alkane feedstocks (Muir*, et al.*, 2000). However, the presence of impurities is

unavoidable and CP preparations may therefore contain isoparaffins (ca. 1-2%), aromatic compounds (<0.5%), metals and unreacted *n*-alkanes. Modern commercial products also contain stabilisers (e.g. antimony oxide, epoxides, organotin, usually <0.5%), which are added to inhibit decomposition of CPs by loss of HCl at elevated temperatures (Beratergremium für umweltrelevante Altstoffe (BUA), 1996; European Commission, 2000; World Health Organization, 1996).

1.2.3 Properties

Depending on chain length and chlorine content, chlorinated paraffins are colourless or yellowish, low to highly viscous liquids or glassy to waxy solids. CPs are chemically stable up to 200-300 °C (World Health Organization, 1996).

Information about the environmentally important physico-chemical properties of CPs is not comprehensive due to the complexity of CP mixtures. Differences in chain length, chlorine content and position of the chlorine atoms along the carbon chain were not fully investigated. Data about vapour pressure (VP), water solubility (WS), octanol-water partition coefficient (K_{OW}) and Henry´s Law Constant (HLC) are limited due to the complexity of CP mixtures and to the small number of individual CP congeners available. A selection is summarised in Table 1.2.

Table 1.2: Physico-chemical properties of selected CPs.

Compound	log K_{OW}[a, b]	VP [mPa][a,c]	HLC [Pa m^3 mol^{-1}][a,c]	WS [µg/l][a, d]
$C_{10}H_{18}Cl_4$	5.93	66	14.67	1260
$C_{10}H_{17}Cl_5$	6.04-6.20	4-66	2.62-4.92	678-994
$C_{10}H_{13}Cl_9$	n.a.	0.24	n.a.	n.a.
$C_{11}H_{20}Cl_4$	5.93	10	6.32	575
$C_{11}H_{19}Cl_5$	6.04-6.40	1-2	0.68-1.46	546-962
$C_{11}H_{18}Cl_6$	6.4	0.5-2	n.a.	37
$C_{12}H_{20}Cl_6$	6.40-6.77	n.a.	n.a.	n.a.
$C_{12}H_{18}Cl_8$	7.0	n.a.	n.a.	n.a.
$C_{13}H_{23}Cl_5$	6.61	0.032	4.18	30
$C_{13}H_{21}Cl_7$	7.14	n.a.	n.a.	n.a.
$C_{14}H_{23}Cl_7$	n.a.	$1.1\ 10^{-2}$	0.36	14
$C_{17}H_{32}Cl_4$	n.a.	$4.0\ 10^{-3}$	51.3	$2.9\ 10^{-2}$
$C_{17}H_{27}Cl_9$	n.a.	$1.7\ 10^{-5}$	0.01	$6.6\ 10^{-1}$
$C_{20}H_{38}Cl_4$	n.a.	$4.5\ 10^{-5}$	54.8	n.a.
$C_{20}H_{33}Cl_8$	n.a.	$1.9\ 10^{-7}$	0.02	$5.3\ 10^{-3}$

log K_{OW}: octanol-water partition coefficient, VP: vapour pressure, HLC: Henry's Law Constant, WS: water solubility, n.a.: not available
Data from: [a] (Muir, *et al.*, 2000), [b] (Sijm and Sinnige, 1995), [c] (Drouillard, *et al.*, 1998b), [d] (Drouillard, *et al.*, 1998a)

As shown in Table 1.2, SCCPs have low VPs, partly in the same range as some POPs known to undergo long-range atmospheric transport. VP decreases with increasing carbon chain length and chlorine content (Drouillard, *et al.*, 1998b). MCCPs have lower VPs (<1.1 x 10^{-2} mPa), which makes them less suited for atmospheric transport (Environment Agency United Kingdom, 2002). HLCs for SCCPs are between 0.7-18 Pa m^3/mol, which is similar as for some chlorinated pesticides (e.g. hexachlorocyclohexanes, toxaphenes). It implies transfer from water to air or from moist soil to air (Drouillard, *et al.*, 1998b). HLCs decrease with increasing chlorine contents. However, also the position of chlorine atoms along the carbon chain has significant effects on the physico-chemical properties (Drouillard, *et al.*, 1998a; 1998b).

1.2.4 Sources and environmental distribution

CPs are not known to occur naturally. Losses of CPs during product use and disposal are suspected to be the major source of environmental contamination. CPs may be released into the environment from improperly disposed metal-working fluids, from polymers containing CPs or by leaching from paints and coatings (World Health Organization, 1996). After release to the environment, CPs adsorb strongly to sediments. In water, they are probably transported adsorbed to suspended particles, and in the atmosphere mainly adsorbed to airborne particulates (Moore*, et al.*, 2004; World Health Organization, 1996). The half-lives for CPs in air have been estimated to 0.85-7.2 days, which is long enough for long-range atmospheric transport (World Health Organization, 1996).

CPs and especially high-chlorinated CPs (>60% chlorine) are not readily biodegradable. Limited information is available about bioconcentration factors (BCFs) or bioaccumulation factors. A recent study has suggested that MCCPs may be more easily bioaccumulated than SCCPs because of reduced biotransformation resulting from the longer carbon chain lengths (Fisk*, et al.*, 2000). BCFs of SCCPs are high, up to 7×10^3 for fish and to 1.4×10^5 for mussels. In aquatic organisms CPs are mainly accumulated in liver, fat, bile, intestine and gills. Even though low-chlorinated SCCPs are taken up more easily, body half-lives are longer for SCCPs with high chlorine content and their elimination rate is slower. The retention in fat-rich tissue appears to increase with higher degree of chlorination (World Health Organization, 1996). Moreover, the position of the chlorine atom along the carbon chain length has a significant influence on the bioaccumulation (Environment Agency United Kingdom, 2002; Fisk*, et al.*, 1998).

1.2.5 Toxicology

The toxicity of CP mixtures with different carbon chain length and chlorine content or of single CP isomers has not been sufficiently investigated yet. Thus, only limited information is available. The acute toxicity of CPs of various chain lengths is low (Farrar, 2000; World Health Organization, 1996), but SCCPs showed chronic toxicity to aquatic invertebrates and fish. In repeated dose studies, liver, kidney and thyroid were the primary target organs for the toxicity of CPs. Additionally, liver, thyroid, and kidney carcinomas were observed for SCCPs in rodents (Bucher*, et al.*, 1987; European Commission, 2000; World Health Organization, 1996).

1.2.6 Regulations

As consequence of their physico-chemical and toxicological properties, the decision to discontinue the production of SCCPs was taken in Europe in the late 90ies. Since then, SCCPs 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 Commission, 1995, 2001) and in the list of selected substances for immediate priority action of the Helsinki Commission (HELCOM, 2002). Additionally, the manufacture and use of SCCPs in metal working fluids has been banned in the European Community in 2002 (European Community, 2002). In 2001, SCCPs were also included in the list of priority dangerous substances of the European water framework directive (European Community, 2000, 2001), which requires an extensive monitoring of SCCPs in Europe from 2006 onwards.

1.3 Environmental levels and human exposure

Information about CP levels in the environment is scarce compared to other polychlorinated compounds like PCBs or DDT. Furthermore, a comparison of the existing data is rather difficult and partly impossible, since different analytical methodologies and especially different quantification procedures and standards were applied (Reth*, et al.*, 2005; World Health Organization, 1996; Zencak*, et al.*, 2005).

Despite the difficulties in quantitative analysis, the ubiquitous presence of CPs in the environment is evident from existing data. CPs were found in all compartments of the environment as well as in aquatic and terrestrial food webs of rural and remote areas.

1.3.1 CP levels in Biota

An overview of published CP concentrations in aquatic organisms is shown in Table 1.3. Data are mainly limited to samples from Sweden (1993), Norway (2002), Canada and the USA (1997-1999). SCCP concentrations in various aquatic species were in the order of 100-3700 ng/g lipid weight (lw). Until now, MCCP concentrations have hardly been determined in biota, since SCCPs were considered as more hazardous for the environment. The few reported MCCP concentrations were within the same range as SCCPs. CP concentrations in biota are often comparable to or even higher than those of other POPs (e.g. PCBs, toxaphenes or hexachlorocyclohexanes).

Table 1.3: Overview of SCCP and MCCP levels in aquatic organisms.

Sample	Tissue	Sampling location	Sampling date	CP concentration [ng/g]	Reference
SCCPs					
Yellow perch, cat-fish, zebra mussel	Whole fish or mussel	Detroit River, USA	1997	305-1205 [a]	(Tomy, *et al.*, 1997)
Herring	Muscle	Bothnian Sea	1986	1400 [a]	(Jansson, *et al.*, 1993)
Herring	Muscle	Baltic Proper	1987	1500 [a]	(Jansson, *et al.*, 1993)
Herring	Muscle	Skagerrak	1987	1600 [a]	(Jansson, *et al.*, 1993)
Burbot	Liver	Norway, different locations	2001	226-3700 [a]	(Borgen, *et al.*, 2002)
Trout	Muscle	Norway, different locations	2001	108-1692 [a]	(Borgen, *et al.*, 2002)
Arctic char	Muscle	Bear Island, Norway	2001	592 [a]	(Borgen, *et al.*, 2002)
Silverside, Blue fish	Whole fish	Marmara Sea	1996	326 and 725 [a]	(Coelhan, 1999)
Angler, cod, shark, sole	Fish fillets	Atlantic	1997	311-918 [a]	(Coelhan, 1999)
Beluga	Blubber	St Lawrence River, Canada	1989	370-1360 [b]	(Tomy, *et al.*, 2000)
Walrus	Blubber	Northwest Greenland	1978	360-490 [b]	(Tomy, *et al.*, 2000)
Ringed seal	Blubber	Ellesmere Island, Canada	1994-1995	380-770 [b]	(Tomy, *et al.*, 2000)
MCCPs					
Yellow perch, Catfish	Whole fish	Detroit river, Canada	1995	80, 900 [b]	(Tomy and Stern, 1999)

a: lipid weight, b: wet weight

1.3.2 CPs in air, soil and sediments

Typical CP concentrations in different environmental compartments are shown in Table 1.4. The presence of CPs in air (5-1085 pg/m^3), freshwater particulate matter (65-860 ng/g dry weight), and sediments (1.62-410 ng/g dry weight) underlines the persistence of CPs and their widespread distribution in the environment.

Table 1.4: Overview of SCCP levels in different environmental matrices.

Sample	Sampling location	Sampling date	SCCP concentration	Reference
Air	United Kingdom	1997-1998	5-1085 pg/m^3	(Peters, *et al.*, 2000)
Air	Egbert, Canada	1990	65-924 pg/m^3	(Tomy, 1997)
Freshwater particulate matter	different locations, Germany	n. s.	69-860 ng/g dw	(Maulshagen, *et al.*, 2003)
Freshwater particulate matter	St. Lawrence river, Canada	1999	12-38 ng/l	(Moore, *et al.*, 2004)
Sewage sludge	different locations, United Kingdom	n. s.	7-200 µg/g dw	(Stevens, *et al.*, 2003)
River and pond sediments	different locations, Czech Republic	2001-2002	4-180 ng/g dw	(Stejnarova, *et al.*, 2005)
Lake sediments	Lake Ontario, Canada	1998	<7-410 ng/g dw	(Marvin, *et al.*, 2003)
Sediments	Arctic lakes, Canada	n. s.	1.62-4.52 ng/g dw	(Tomy, *et al.*, 1999a)

dw: dry weight; n. s.: not specified

1.3.3 CPs in human milk and human foodstuff

Data about CP levels in humans are more than scarce although the presence of CPs in human adipose tissue was already reported in 1980 (Campbell and McConnell). Campbell and McConnell detected between <50 to 1500 ng/g of C_{10}-C_{20} CPs in human brain, liver and kidney by thin layer chromatography. Five years later, Schmid and Müller (1985) reported 200 ng/g of MCCPs in human adipose tissue from Switzerland. In 1997, Tomy found a mean SCCP concentration of 12.8 ± 3.2 ng/g lw in human breast milk from three Inuit women living in Canada (Tomy, 1997). Recently, a study about SCCP and MCCP concentrations in the United Kingdom was conducted. Concentrations were between 49 and 820 ng/g lw for SCCPs and 6.2 to 320 ng/g lw for MCCPs (Thomas, *et al.*, 2006). However, it has to be taken into account, that concentrations were partly in the same range as the method blanks.

Investigating human foodstuff, Campbell and McConnell (1980) found 300 ng/g of C_{10-20} CPs in dairy products (n = 13) and 150 ng/g in vegetable oils and derivatives (n = 6). A recently published Japanese study about SCCP concentrations in different food categories revealed that SCCPs were mainly present in the categories fat (150 ng/g lw), fish (17 ng/g lw), shellfish (19 ng/g lw) and meat (7.7 ng/g lw) (Iino*, et al.*, 2005).

1.4 State of the art of CP analysis

Analysis of CPs in environmental samples is far from being well established, and only a small number of laboratories world-wide has currently the necessary expertise . The main reason is the complex composition of CP formulations and hence, of CP patterns present in environmental samples. Complete chromatographic separation of the thousands of CP isomers is currently not possible by any method. Chromatograms of CPs show broad humps of unresolved CP isomers instead of distinct peaks. Moreover, the determination of all congener and homologue groups by mass spectrometry is time-consuming and requires many analyses to include all CP formula groups. Finally, quantification of CPs is extremely difficult, since response factors vary with the degree of chlorination.

The difficulties in the determination of exact CP concentrations were also obvious from the results of the only interlaboratory study on SCCP analysis performed until now (Tomy*, et al.*, 1999b). The applied methods based on high-resolution gas chromatography (HRGC) coupled to high-resolution (HR) or low-resolution (LR) mass spectrometry (MS) using the electron capture negative ion mode (ECNI). The interlaboratory study clearly showed the major limitation of these methods: Quantification of CPs with a chlorine content different from that of the quantification standards led to errors of up to 300%. Later, this problem was extensively described by Coelhan *et al.* (2000) and Zencak *et al.* (2005). Moreover,

HRMS was recommended as detection method to avoid interferences by other polychlorinated compounds or by CPs with similar mass-to-charge ratios. However, this instrumentation is very expensive and therefore hardly available and suitable for routine analysis.

Nevertheless, reliable and affordable analytical methods are needed in near future, since SCCPs have been included in the regulatory programs of the European Community and environmental levels of SCCPs have to be monitored more extensively from 2006 onwards (European Community, 2000, 2001). For this purpose, method improvements and simplifications of the quantification procedures are of top priority. Furthermore, the use of LRMS instead of HRMS is desirable to allow more laboratories to establish CP analysis.

Recently, alternative mass spectrometric methods were reported (Zencak and Oehme, 2004; Zencak*, et al.*, 2003; Zencak*, et al.*, 2004). More detailed information about these approaches and a comparison to the methodologies developed in this work are given in chapter 3.4.

2 AIM OF THE WORK

The aim of this work was to develop and validate a method for the trace analysis of chlorinated paraffins in fish based on low-resolution mass spectrometry and sensitive enough to detect CPs in the ng/g range. The use of expensive HRMS should be avoided, which required the following two tasks:

- Development of a selective clean-up procedure to separate CPs from interfering matrix and other halogenated compounds to avoid an overestimation of CP concentrations in environmental samples.

- Unequivocal identification of CP congener group patterns. This requires the development of identification criteria, which recognise and minimise interferences between CPs with similar mass-to-charge ratios.

Furthermore, a reliable quantification procedure for the determination of CPs had to be developed and evaluated. Methods applied so far could give systematic errors and reported CP concentrations in environmental samples were not comparable, since response factors vary with the degree of chlorination. The aim was to achieve a correct quantification independent of the composition of the CP mixture used as standard.

The developed analytical method should be applied to the determination of so far hardly assessed CP concentrations in marine and freshwater fish from rural and remote areas in Europe. Furthermore, the composition of technical CP mixtures as well as of CPs present in fish should be investigated. Congener group patterns will provide information about

characteristic congener groups and possible transformations of CPs in the environment. Samples from the European Arctic were included to investigate the potential of CPs for long-range transport to remote areas.

In addition, it was planned to evaluate the suitability of a further method for the fast determination of the total CP amount (sum of SCCPs, MCCPs and LCCPs) in fish. The improved sensitivity of this HRGC-EI-MS/MS method should be used to provide information about the presence of CPs in human milk from Germany.

3 RESULTS AND DISCUSSION: METHODOLOGIES

3.1 Analytical methodologies for CP analysis

Extraction and clean-up techniques for the determination of persistent organochlorines (e.g. chlordanes, PCBs and toxaphenes) in environmental samples are also applicable to the analysis of CPs. HRGC-ECNI-MS has become the method of choice due to its high sensitivity and selectivity. However, substantial method adaptations were necessary, since CPs are much more complex than other organochlorines. Hence, the determination of CPs requires a significantly improved selectivity of the clean-up as well as of identification and quantification.

3.2 HRGC-ECNI-MS – applied methods and unsolved problems

Several methods have been suggested for the analysis of CPs in environmental samples, such as HRGC combined with electron capture detection (ECD) (Bergstroem and Jansson, 1998) or electron ionisation (EI) tandem mass spectrometry (MS/MS) (Zencak*, et al.*, 2004) as well as chloride enhanced atmospheric pressure chemical ionisation (APCI) MS (Zencak and Oehme, 2004). However, methods based on ECNI-MS are most often applied.

The use of ECNI-MS was first reported by Gjøs and Gustavsen as promising analytical technique for the analysis of high-chlorinated CPs (Gjøs and Gustavsen, 1982). They discussed the ECNI full scan mass spectra of an industrial SCCP formulation (70% Cl) and an extract of previously exposed fish. They remarked that $[M\text{-}Cl]^-$ ions belong to the major fragments. Later, Tomy *et al.* showed that ECNI leads further to the formation of $[M\text{-}Cl]^-$, $[M\text{-}HCl]^-$ and $[M+Cl]^-$, as well as $[Cl_2]^-$ and $[HCl_2]^-$ ions with different abundances depending on the chlorine content, temperature of the ion source, and injected sample

amount (Tomy, *et al.*, 1998b). The simultaneous formation of several fragment ions implies a high risk of interferences between CP formula and congener groups.

Sensitivity of the full scan method was improved by Coelhan (1999) by applying short column GC-ECNI-LRMS. With this poor chromatographic separation, CPs eluted within seconds as only one peak, which enabled a higher sensitivity. However, this approach does not allow a clear identification and separation of SCCP and MCCPs due to mass overlaps.

Another approach based on HRGC-ECNI-LRMS in the selected ion monitoring (SIM) mode was developed by Jansson *et al.* (1991). Detection of $[Cl_2]^-$ and $[HCl_2]^-$ ions was proposed as alternative to $[M\text{-}Cl]^-$ ions. This approach was also used in combination with ion trap mass spectrometry by Nicholls *et al.* (2001) and later by Castells *et al.* (2004a; 2004b). However, the major drawback of this method is that only the determination of the total CP amount is possible. A differentiation between SCCPs and MCCPs as well as a congener group specific quantification is not possible. Additionally, a very good clean-up is essential to exclude the presence of other chlorinated compounds such as chlordanes and hexachlorocyclohexanes possibly forming $[Cl_2]^-$ and $[HCl_2]^-$ ions (Stemmler and Hites, 1988).

CPs were first determined in environmental samples by Schmid and Müller (1985). They applied HRGC-ECNI-LRMS in the SIM mode selecting the four most abundant $[M\text{-}Cl]^-$ and $[M\text{-}HCl]^-$ ions for the quantification. The today mostly applied method was developed by Tomy *et al.* (1997). HRGC-ECNI-HRMS in SIM mode was used for the measurement of the $[M\text{-}Cl]^-$ ions of the following congener groups: C_{10} ($Cl_{5\text{-}10}$), C_{11} ($Cl_{5\text{-}10}$), C_{12} ($Cl_{6\text{-}10}$) and C_{13} ($Cl_{7\text{-}9}$). Congener group patterns were then generated by correcting the integrated

ion signals of each congener group for isotopes and response factors as described by Tomy *et al.* (1997). Nevertheless, this approach may still introduce errors of up to 300% due to differences between the chlorine content of the CPs in standard and sample (Coelhan*, et al.*, 2000; Zencak*, et al.*, 2005).

3.3 Determination of CPs by HRGC-ECNI-LRMS

In this work, HRGC-ECNI-LRMS in the SIM mode was chosen. MS parameters were modified to favour the formation of $[M\text{-}Cl]^-$ ions for most of the congeners. The detection of the $[M\text{-}Cl]^-$ ions of all congener groups allowed the differentiation between SCCPs and MCCPs. Moreover, it provided the possibility to generate sample specific congener and homologue group patterns. Furthermore, a comparison with recently published ECNI-HRMS is possible.

In contrast to the method based on HRMS, the development of an appropriate clean-up was essential for the elimination of other polychlorinated compounds with similar mass-to-charge ratios (e.g. PCBs and toxaphenes, see chapter 3.3.1.2 and Paper II). Furthermore, interferences between CP congeners with similar masses had to be excluded (chapter 3.3.2 and Paper I). Moreover, a new quantification procedure had to be developed, since all previous approaches based on ECNI-MS were influenced by the chlorine content of the applied standard mixture (chapter 3.3.3 and Paper III).

3.3.1 Sample clean-up

Besides CPs, other polychlorinated compounds are present in biota and may interfere. Therefore, they have to be eliminated by an appropriate clean-up procedure. The selected clean-up procedure is strongly related to the employed detection method. Therefore, a

more careful fractionation of the sample extract is necessary, if a less selective detection is applied.

Friden *et al.* (2004) proposed a clean-up procedure for the ECD detection of CPs in biota consisting of treatment with sulfuric acid, irradiation with UV light (high energy mercury lamp) and high performance gel permeation chromatography (GPC, PL-Gel, 5 µm, 50 Å, 300 mm x 7.5 mm, four columns coupled in series) to achieve a sufficient selectivity. In contrast, a combination of GPC with an unspecific separation on a Florisil® column was reported by Tomy *et al.* (1997) for the analysis of fish samples by HRMS.

3.3.1.1 Developed clean-up procedure

The developed clean-up consisted of three steps. First lipids and polychlorinated compounds were extracted from the dried and homogenised sample with a mixture of *n*-hexane and dichloromethane (1+1, v/v). Then, lipids were removed by column chromatography with silica gel impregnated with sulphuric acid. Finally, CPs were separated from interfering compounds by adsorption chromatography on Florisil®. The developed clean-up procedure is described in detail in Paper II.

3.3.1.2 Method development and optimisation

The suitability of several clean-up techniques was evaluated for CP analysis during the method development. Lipid removal by GPC using a mixture of cyclohexane/ethylacetate (1+1, v/v) also separated CPs from lipids. However, the use of sulphuric acid was preferred, because it allowed the degradation of other possibly interfering compounds (e.g. endosulfane).

The applicability of photolysis was evaluated to further improve the selectivity for CPs. Friedman and Lombardo (1975) and later Friden *et al.* (2004) reported the use of irradiation with high intensity UV light. CPs do not undergo photochemical decomposition in contrast to many interfering pesticides and industrial chemicals (e.g. chlorinated aromatic or unsaturated compounds), which are degraded to non-interfering products. The applicability of this technique could be confirmed. However, it excluded the use of $^{13}C_{10}$-*trans*-chlordane as internal standard through the whole procedure. Therefore, photolysis was not included in the clean-up procedure.

Adsorption chromatography is often used for the separation of CPs from interfering compounds. Silica gel (Coelhan, 1999; Rieger and Ballschmiter, 1995), aluminium oxide (Schmid and Müller, 1985; Zitko, 1973) as well as Florisil® (Tomy*, et al.*, 1997) are the most frequently used adsorbents. In this work Florisil® proved to be suitable, since it allowed the separation of CPs from most of the interfering compounds and remaining lipids. On contrary, adsorption chromatography on silica gel did not enable the separation of some other polychlorinated compounds (e.g. toxaphenes). Figure 3.1 shows the elution profile of a mixture of selected halogenated POPs and CPs (PCB 153, toxaphene #44, toxaphene #62, $^{13}C_{10}$-*trans*-chlordane and SCCP standard (55.5% Cl) on a Florisil® column.

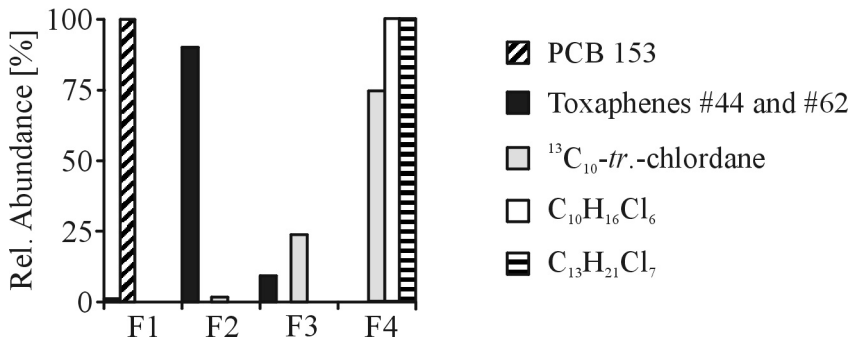

Figure 3.1: Elution profile of a mixture of PCB 153, toxaphene #44, toxaphene #62, $^{13}C_{10}$-*trans*-chlordane and SCCP standard (55.5% Cl) on a Florisil® column (16 g, 1.5% water content; F1: 60 ml of *n*-hexane, F2: 40 ml of *n*-hexane, F3: 20 ml of *n*-hexane and F4: 70 ml of dichloromethane).

Most of the interfering compounds, especially PCBs and toxaphenes, eluted from the column with *n*-hexane, whereas CPs were retained until the solvent was changed to dichloromethane. MCCPs had even higher retention than SCCPs.

The development of the clean-up method included the search for a suitable internal standard, which would allow the compensation of losses during the sample clean-up. Isotope labelled CPs are currently not available, and even if available, their use may be difficult in practice using low-resolution MS. $^{13}C_{10}$-*trans*-chlordane was selected because it behaved most similarly to CPs during the development of the clean-up and fractionation on the Florisil® column was performed so that chlordanes eluted in the CP fraction. This allowed the simultaneous determination of selected chlordanes and the use of $^{13}C_{10}$-*trans*-chlordane as internal standard. Recoveries of the internal standard and CPs were similar (within 10%) after the complete clean-up.

The developed clean-up procedure was very suited for the analysis of fish samples not highly contaminated by other pollutants. Figure 3.2 shows the mass chromatograms of the CP congener groups $C_{12}H_{19}Cl_7$ and $C_{14}H_{23}Cl_7$ in brown trout (Liechtensteiner Binnenkanal,

Switzerland) as well as in a SCCP and MCCP standard. As can be seen, no interferences were present.

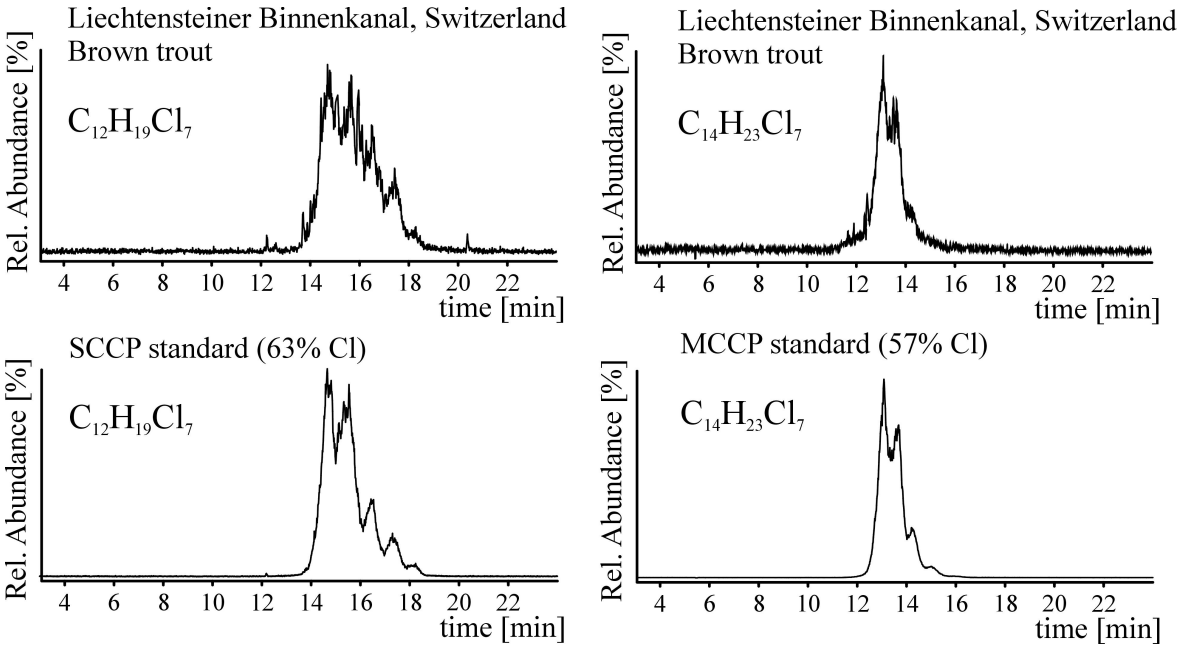

Figure 3.2: Mass chromatograms of the CP congener groups $C_{12}H_{19}Cl_7$ and $C_{14}H_{23}Cl_7$ determined in brown trout (Liechtensteiner Binnenkanal, Switzerland) and in the respective SCCP standard (63% Cl) or MCCP standard (57% Cl) by HRGC-ECNI-LRMS. Different temperature programs were used for the analysis of SCCPs and MCCPs.

3.3.1.3 Method blanks

Method blanks are a further problem of CP analysis, although this is in general hardly addressed in the literature. Due to their properties and widespread use, CPs cause easily laboratory contamination. CPs bind on the active surface of glassware and are difficult to remove by any cleaning process. In previous works limits of detection (LOD) were often not related to the MS performance at a signal-to-noise ratio of 3:1. They were defined as average method blank plus three times the standard deviation indicating that CPs were detectable in the blanks at concentrations relevant for real sample analysis (Thomas*, et al.*, 2006; Tomy and Stern, 1999).

Several measures help to minimise the risk of contamination during clean-up. The use of plastic materials should be completely avoided. A strict separation of glassware used for method development and for samples as well as the usage of solvents of high purity and of only thermally pretreated (600°C) chemicals (sodium sulphate, Florisil®, silica gel) are essential. The blank-free glassware and glass fibre filters can only be obtained after heating them to 450 °C for at least two hours.

The clean-up of the samples presented in this work was performed partly by a second laboratory trained for this purpose. It had to undergo a time-consuming and tedious procedure to lower blanks to an acceptable level. For this reason, measured blank values are reported in chapter 3.5.3 together with determined CP concentrations as well as the laboratory, where the sample clean-up was performed.

3.3.2 Identification

The identification of halogenated compounds is based on GC retention time and presence of specific masses when MS is used for detection. However, these criteria are not always unequivocal if HRGC-ECNI-LRMS is used.

HRGC does not allow the identification of CP congener groups by retention time. The separation of CP mixtures into single congeners is currently not possible by HRGC, due to the extremely high number of different isomers. The mostly applied stationary phase is 5%-phenyl-methylpolysiloxane (DB5-MS, Ultra2, or equivalent). Also when other stationary phases were used (Skopp, 2002), CPs elute as a large hump with defined minima and maxima over a retention time range of several minutes as shown in Figure 3.2.

Recently, an improvement of the HRGC separation of CPs was achieved by comprehensive two dimensional gas chromatography (GCxGC). Korytar *et al.* (2005) applied ECNI combined with a time-of-flight mass spectrometer. The substantially improved (though not yet complete) overall separation allowed a resolution according to the number of chlorine substituents. However, mixtures of SCCPs and MCCPs were still difficult to separate.

High-resolution MS allows an unequivocal identification of congener groups, whereas mass overlaps of different CPs cannot be excluded by LRMS (Tomy*, et al.*, 1997). Especially, high-chlorinated SCCPs coelute with low-chlorinated MCCPs, and form ions with similar nominal masses, which cannot be separated by LRMS (see Paper I).

This work proved that also LRMS can be applied to the identification and separation of SCCPs and MCCPs in environmental samples. However, then knowledge about congener groups likely to interfere and the establishment of identification criteria are essential. A differentiation of interfering CPs can be achieved by retention time range, isotope ratio and peak shape. This approach is presented in detail in Paper I and allowed the use of ECNI-LRMS as real alternative to HRMS. The validity of this procedure was also demonstrated by Zencak *et al.* (2005). A comparison between ECNI-LRMS and ECNI-HRMS showed that comparable results (differences of less than 10%) can be achieved, if the identification criteria mentioned in Paper I are followed.

3.3.3 Quantification

In contrast to methods based on EI-MS (e.g. HRGC-EI-MS/MS), the major drawback of ECNI-MS analysis is the strong dependence of the response factors on the chlorine content

of the studied CPs. CP mixtures contain several hundreds of isomers with different response factors. Their chlorine content can vary between 30 and 70%. Small differences in the chlorine content can easily lead to significant differences in the response factors (Coelhan*, et al.*, 2000; Zencak*, et al.*, 2005). Therefore, quantification is the most difficult task of CP analysis.

Only a few single CP isomers are available as pure compounds and they represent only a very small fraction of all possible isomers. Hence, SCCPs and MCCPs of different chlorine content are usually used as standards (Jansson*, et al.*, 1993; Tomy*, et al.*, 1997). Alternatively, mixtures of CP congeners with a fixed chain length and variable degree of chlorination are applied (Coelhan, 1999).

3.3.3.1 Quantification of CPs - state of the art

Several approaches were suggested for the quantification of CPs. Methods based on electron capture detection (Friden*, et al.*, 2004), EI-MS/MS (Zencak*, et al.*, 2004) or ECNI-MS detection of $[Cl_2]^-$ (Jansson*, et al.*, 1991) allow only the determination of the total CP amount.

Quantification of $[M\text{-}Cl]^-$ ions of different congener groups by ECNI-MS allows the establishment of congener group patterns but is more demanding. Tomy *et al.* (1997) determined all CP congener groups and developed a quantification method, which compensates differences between CP composition of standards and environmental samples. The total CP amount was determined via the average molecular weight calculated from the congener and homologue group pattern of the CPs in the sample and in the standard. Further correction factors were used to compensate for differences between isotope

abundances and between response factors of CPs with different chlorine content. However, own studies revealed that the use of these correction factors had an influence of only 2%.

A comparison between different mass spectrometric methods showed that quantification errors were acceptable for spiked samples, if the same CP mixture was used as spike and as standard. However, in fish samples with compositions different from available CP standard mixtures, ECNI-MS could cause significant errors (Zencak*, et al.*, 2005). Table 3.1 shows the consequences, if CP mixtures of different chlorine content are used as standards. Quantification of a standard mixture with a chlorine content of 55% with standard mixtures of different chlorine content revealed considerable errors and underlined that a standard mixture with a similar chlorine content is required to obtain acceptable results.

Table 3.1: Quantification results in ng and the errors relative to the expected value of 1500 ng for a SCCP mixture of 55% Cl quantified with five SCCP mixtures of various chlorine contents (51, 55, 60, 63, 70%). Quantification was performed according to Tomy *et al.* (1997).

CP mixture	Standard mixtures				
(55%, 1500 ng)	51%	55%	60%	63%	70%
Measured CP amount [ng]	589	1434	2931	5566	7141
Relative error [%]	61	4	95	271	376

Consequently, a comparison of published CP concentrations is difficult, since CP concentrations were determined with different standards and since information is often missing about the chlorine content of the CPs in the samples.

Coelhan *et al*. (2000) reported another approach to avoid systematic errors due to different response factors. Standards for quantification were chosen by comparing the CP mass spectra in samples with those of CP mixtures of defined carbon chain length and different chlorine contents. However, this approach requires a large number of available standards.

Moreover, the interpretation of the mass spectra as well as the search for a suitable standard is time-consuming.

3.3.3.2 Development of a novel quantification procedure

In this work a new quantification procedure was developed to overcome the strong dependence of ECNI results on the chlorine content of the standard, and to avoid a tedious and time-consuming selection of the "most similar" standard (Paper III). Response factors and thus the quantification results were mainly dependant on the chlorine content and less on the carbon chain length. Linear correlation between the total response factor of a CP mixture and its chlorine content could be established. It allowed to compensate differences between the CP chlorine content in the sample and standard. The procedure is described in detail in Paper III. Quantification errors were considerably reduced (<33%, see Table 1 in Paper III) and CP pattern matching procedures between standard and sample became unnecessary.

This procedure was also evaluated using CP mixtures of defined carbon chain length (e.g. only C_{10} congeners) and different chlorine contents as standards. The amount of CPs of each chain length (C_{10}, C_{11}, C_{12} and C_{13}) could be determined in this way. However, the so obtained total amount of SCCPs was not significantly different from that obtained with SCCP mixtures (C_{10}-C_{13}). The requirement of fewer standards and a shorter analysis time led to the selection of SCCP mixtures as proposed in Paper III.

The applicability of the developed quantification procedure to fish analysis was demonstrated in Paper III. Furthermore, this method was successfully applied to the analysis of SCCPs and MCCPs in sediments from the North and Baltic Sea (Hüttig, 2005).

Results obtained with the new quantification procedure were in good agreement with those obtained by a HRGC-EI-MS/MS method (see chapter 4.1.3, Table 4.5).

3.4 Applicability of other new alternative MS techniques

Several new MS approaches have recently been reported for the determination of CPs. Their suitability for the determination of CP concentrations and congener group patterns in fish was also evaluated in this work.

Zencak *et al.* reported a method based on HRGC-NICI-LRMS using a mixture of CH_4/CH_2Cl_2 as reagent gas (Zencak*, et al.*, 2003). $[M+Cl]^-$ ions were almost exclusively formed. This method showed only a very limited influence of the degree of chlorination on response factors, was very selective for CPs and allowed the detection of low-chlorinated CPs (<5 chlorine atoms). CP concentrations as well as the congener group patterns were determined in two pooled samples of North Sea dab. However, the obtained patterns were not comparable to ECNI-MS analysis due to different response factors (Zencak*, et al.*, 2005; 2003). Additionally, the method was not suitable for routine analysis due to a rapid contamination of the ion source.

Recently, HPLC-APCI-MS with chloroform was used for the analysis of CPs in household commodities (Zencak and Oehme, 2004). Similarly to HRGC-CH_2Cl_2-NICI-MS, it leads to the almost exclusive formation of $[M+Cl]^-$ ions. Furthermore, the determination of LCCPs was possible, which is more difficult by GC due to the low volatility of LCCPs. However, all CPs eluted as one unresolved peak, so that a differentiation of SCCPs, MCCPs and LCCPs was not possible with low-resolution MS. Furthermore, response factors varied with the chlorine content as well as with the carbon chain length (Zencak, 2004).

Therefore, HPLC-LRMS analysis of environmental samples, containing SCCPs, MCCPs and LCCPs, was not considered further for the analysis of biota.

HRGC-EI-MS/MS was applied to the fast determination of the total CP amount by Zencak *et al.* (2004). A differentiation of SCCPs, MCCPs and LCCPs is not possible by this technique. However, this method was also used in this work (see chapter 4.1.3), since it is sensitive (LOD of 0.3 ng/µl) and provides a first overview about the total CP concentration within a very short analysis time. Moreover, the retention time range of the CP signal allows a first estimation of the composition of the CPs present in the samples. Figure 3.3 shows the HRGC-EI-MS/MS mass chromatograms of single SCCP, MCCP, and LCCP mixtures as well as of a mixture of them. The egg sample presented in Figure 3.3 contained mainly SCCPs whereas the fish samples (common bream, M12 and M3) had a higher content of MCCPs, which was confirmed by ECNI-MS for sample M3 (see chapter 4.1.3, Table 4.5).

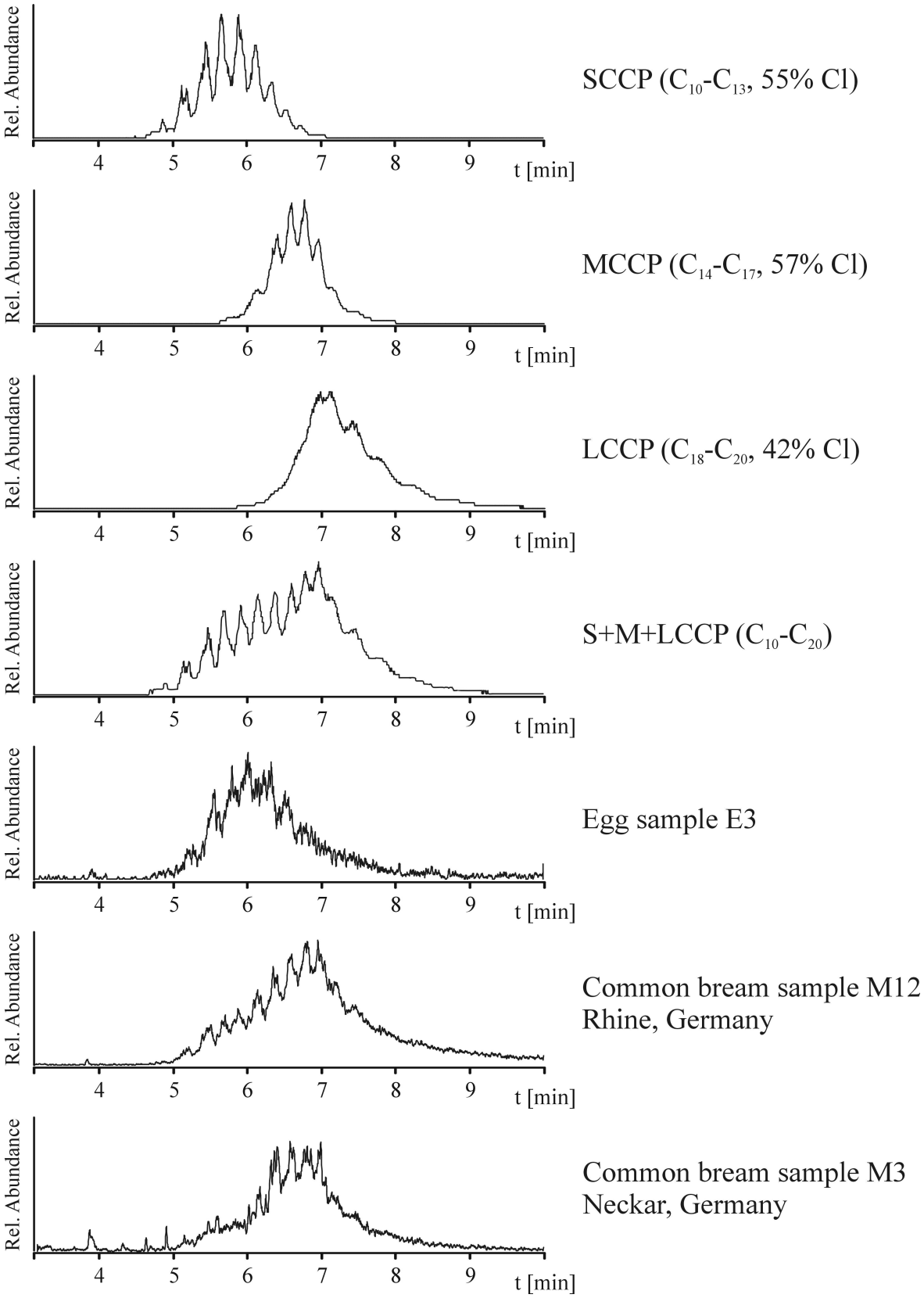

Figure 3.3: Triple quadrupole EI-MS/MS chromatograms (CID of *m/z* 102 → 67) of different CP mixtures (SCCP, 55% Cl; MCCP, 57% Cl; LCCP, 49% Cl and a 1+1+1 mixture of them), two extracts of common bream (M12: Rhine, M3: Neckar) and one egg sample (E3) from Germany.

4 RESULTS AND DISCUSSION: CPs IN BIOTA

4.1 CP levels in marine and freshwater fishes

Fish is a suitable species to evaluate the degree of environmental pollution by halogenated compounds. Fish can accumulate relatively large quantities of halogenated pollutants, since these lipid-soluble chemicals are evenly distributed into the total lipid content. However, many factors have an influence on CP bioaccumulation. Age, size, lipid content and gender play an important role. In contrast to male fish, female fish are often better indicators for recent exposure, since they loose a part of accumulated POPs during reproduction, and show rather constant levels with increasing age. Furthermore, factors such as trophic level, geographic origin and feeding strategies as well as migratory behaviour influence accumulation. Some fish species move over a large area making a clear correlation between the exposure source and found level impossible. Other species (e.g. dab) reside in rather small areas and are therefore more representative for local pollution. In contrast to the variety of fish in the sea, the number of species in rivers or lakes is often limited (even up to one species in some areas). This makes it often more difficult to find a suited freshwater fish of high lipid content as good indicator for pollution (Swedish Environmental Protection Agency, 1998).

In this work CPs were determined in fish from various regions of Europe (North and Baltic Sea, Central Europe and the north of Europe) to obtain a first overview of the degree of contamination. Results are discussed in the following chapters. An investigation of the correlation between the biological parameters, observed congener patterns and levels found was not scope of this study, since the number of samples was too small to draw any significant conclusions.

4.1.1 Fish from the North and Baltic Sea

Former information about concentrations of CPs in fish from the North and Baltic Sea was limited to one single study. CP concentrations of 1.4, 1.5 and 1.6 μg/g lw were detected in three samples of pooled muscle tissue of herring captured close to the Swedish coast (Bothnian Sea, Baltic Proper and Skagerrak) in 1993 (Jansson*, et al.*, 1993). No further data were reported for fish from the Baltic Sea since then.

First data about CP levels in the North Sea and southern Baltic Sea are presented in Paper II and Paper III (Tables 2 and 3). SCCPs and MCCPs were detected in cod (*Gadus morhua*), North Sea dab (*Limanda limanda*) and flounder (*Platychtus flesus*). Fish was captured by the German Federal Research Centre for Fisheries (Hamburg, Germany) in August 2002 and in August 2003 at five locations in the North Sea as well as at two locations in the southern Baltic Sea. Fish of the same size was selected for analysis. The fishes were dissected immediately after their capture and the lipid rich liver was used for analysis. Cod was selected due to the high lipid content of the liver (about 40-60%), its high position in the Baltic Sea food chain and its frequent sampling availability. Cod belongs to the dominant food fish species in the Baltic Sea. North Sea dab and flounder were further selected, since they do not migrate over wide areas. A survey over the analysed fishes from the North and Baltic Sea is given in Table 4.1.

Table 4.1: Summary of analysed fishes from the North and Baltic Sea including capture location and date, species and gender. Samples are arranged according to locations and are identified by a sample number. Maps of the sites are given in Figure 4.1.

Capture location	Coordinates	Species	Capture date	Gender	Sample No.	Size [cm]	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	Cod	01.09.2002	ns	OS6	25	1
B11	54°51´N/14°01´E	Cod	01.09.2002	ns	OS7	25	1
B11	54°51´N/14°01´E	Cod	01.09.2002	ns	OS8	25-26	2
B11	54°51´N/14°01´E	Cod	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	Flounder	31.08.2002	f/m	OS3	28-30	2
B11	54°45´N/13°20´E	Flounder	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	Cod	03.09.2002	ns	OS11	26-27	2
B01	54°40’N/10°28’E	Cod	31.08.2003	ns	OS12	32	1
B01	54°40’N/10°28’E	Cod	31.08.2003	ns	OS13	27	1
B01	54°40’N/10°28’E	Cod	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	Dab	26.08.2002	f	NS2	20-22	5
N04	54°30´N/2°16´E	Dab	08.09.2003	f	NS6	20-24	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	20-24	5
GB1	54°07´N/7°46´E	Flounder	30.08.2003	ns	NS7	31	1

ns: not specified; f: female; m: male; cod (*Gadus morhua*); dab (North Sea dab, *Limanda limanda*); flounder (*Platychtus flesus*)

SCCP and MCCP concentrations determined in these samples are shown in Table 4 of Paper II as well as in Tables 2 and 3 of Paper III. Furthermore, Figure 4.1 shows a map and the SCCP and MCCP concentrations (ng/g lw) determined at various sampling locations in the North and Baltic Sea. SCCP concentrations were between 54 and 1428 ng/g lw, MCCP concentrations varied between <30 and 2448 ng/g lw without taking into account differences in species and locations. High variations in the CP concentrations were observed between different samples and locations.

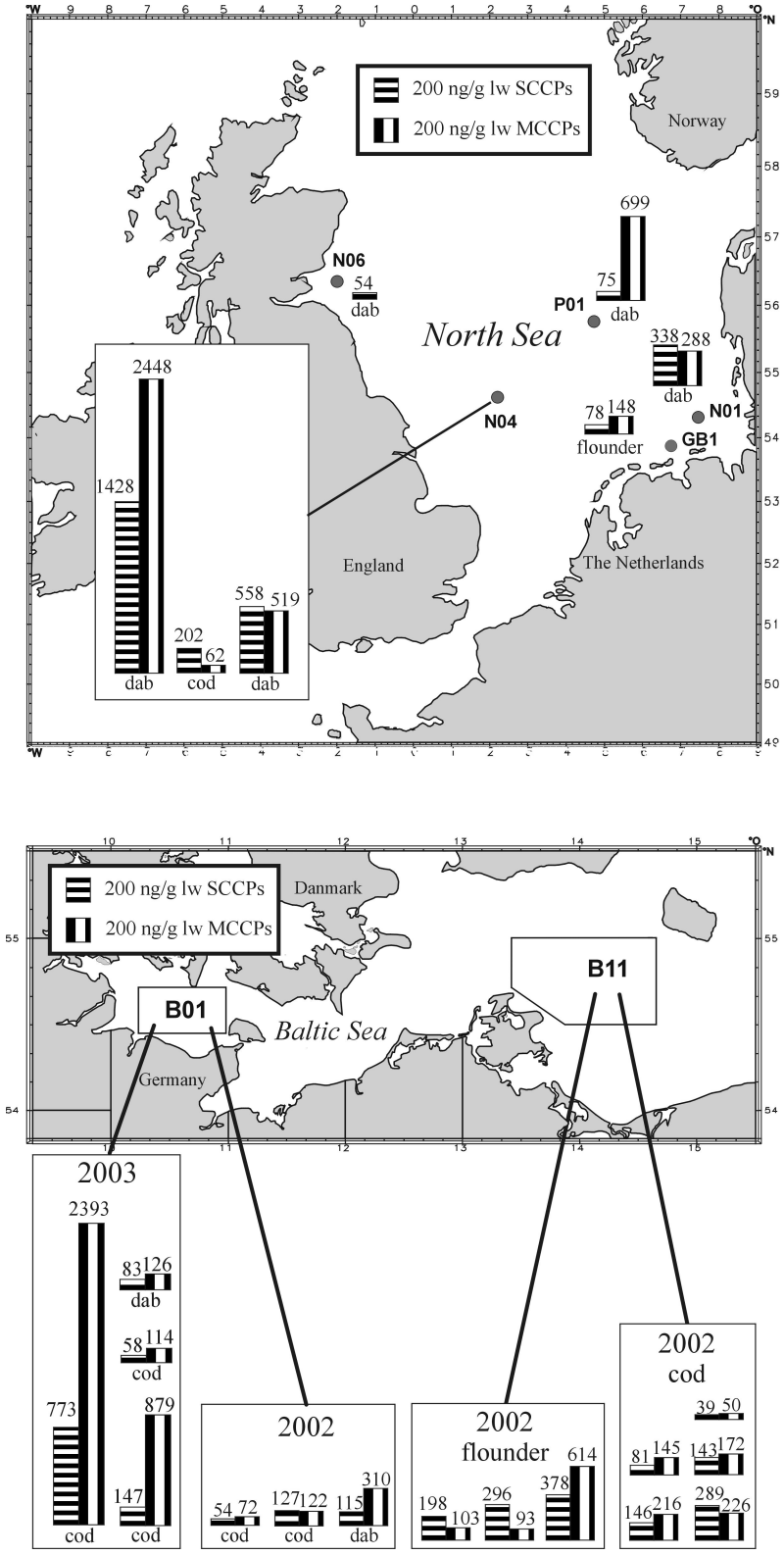

Figure 4.1: SCCP and MCCP concentrations determined by HRGC-ECNI-LRMS in liver from cod, North Sea dab, and flounder from the North and Baltic Sea.

SCCPs and MCCPs are utilised for different applications and are produced in different quantities. Nevertheless, SCCP and MCCP concentrations correlated well for seven cod samples from the Baltic Sea captured in 2002 ($R = 0.82$, $P < 0.05$) as well as for all analysed samples including different fish species from different locations (see Figure 4.2, $R = 0.81$, $P < 0.001$). However, further measurements are essential to confirm these data, since the observed correlation is strongly influenced by the two samples with higher concentrations. Despite the limited number of samples, the here presented data suggest that the bioaccumulation behaviour of SCCPs and MCCPs is similar in fish.

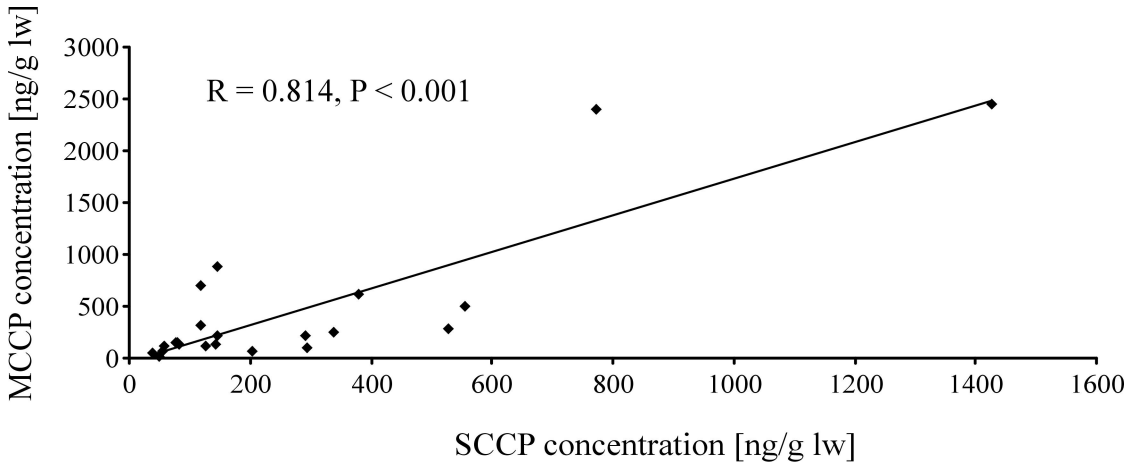

Figure 4.2: Correlation diagram of concentrations (ng/g lw) of SCCPs and MCCPs determined in various fish liver samples from different locations in the North and Baltic Sea.

Additionally, concentrations of chlordane components (*cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor and oxychlordane) were determined in all samples from the North and Baltic Sea by HRGC-ECNI-LRMS as described by Karlsson . Chlordane concentrations were lower than for CPs. Sum concentrations of all analysed chlordanes were between 6.3 and 47 ng/g lw. No significant correlation could be found between single chlordanes and SCCPs or MCCPs.

4.1.2 Fish and seabirds from the Arctic

The presence of CPs in all fish samples from the North and Baltic Sea and similar physicochemical properties as other POPs, undergoing long-range atmospheric transport, supported a study of CP levels in biota from remote areas like the European Arctic. Prior to this work, only one study existed about SCCP levels in marine mammals from the Canadian Arctic. SCCP concentrations varied between 110 and 1360 ng/g ww (Tomy*, et al.*, 2000). MCCP concentrations in biota of the Arctic have never been reported before.

The presence of SCCPs and MCCPs in fish and seabirds from northwest Europe and the Arctic is reported in Paper IV. An overview of the analysed fish and seabird samples is given in Table 4.2. Figure 4.3 shows the capture locations of fish and seabirds as well as the determined SCCP and MCCP concentrations in the liver tissue of each animal.

Table 4.2: Summary of analysed biota from remote areas including capture location and date, species and gender. Samples are arranged according to locations and distinguished by their sample numbers. A map of the site is given in Figure 3.6.

Capture location	Coordinates	Species	Capture date	Gender	Sample No.	Size / weight [cm / kg]	Analysed tissue
Lofot Islands	68°08‘N/13°33‘W	Cod	02.02.2004	f	A1	86 / 8.50	L
		Cod	02.02.2004	f	A4	83 / 6.50	L
Northern Iceland	65°74‘N/18°09‘W	Cod	30.09.2003	f	A2	49 / 1.02	L
		Cod	30.09.2003	f	A5	41 / 0.65	L
Southern Iceland	63°28‘N/20°15‘W	Cod	06.11.2003	ns	A3	53 / 1.50	L
		Cod	06.11.2003	f	A6	51 / 1.28	L
Bear Island	74°N/19°E	Arctic char	09.07.2001	f	B1/B3	45 / 0.83	L/M
		Arctic char	09.07.2001	f	B2/B4	47 / 0.85	L/M
		Little auk	08.07.2001	m	C1/C3	12* / 0.17	L/M
		Little auk	08.07.2001	m	C2/C4	12* / 0.17	L/M
		Kittiwake	08.07.2001	m	D1/D3	33* / 0.46	L/M
		Kittiwake	08.07.2001	f	D2/D4	33* / 0.39	L/M
		Glaucous gull	07.07.2001	m	E1/E3	49* / 1.92	L/M
			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

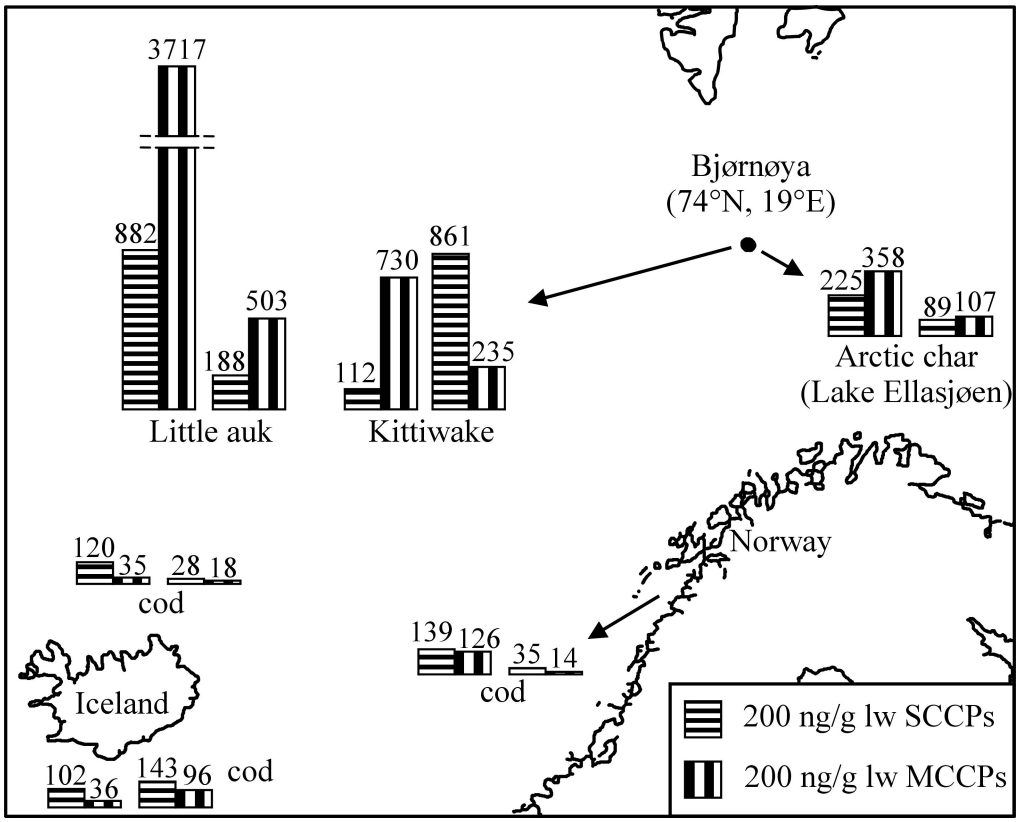

Figure 4.3: SCCP and MCCP concentrations determined in two Arctic char samples and four seabird livers from Bear Island as well as in six cod livers from Iceland and northwest Norway. The analysis was performed by HRGC-ECNI-LRMS.

These results show that SCCPs as well as MCCPs can reach areas far from industrialised regions and can enter in the marine and limnic food chain of the Arctic. CPs were detectable in cod and Arctic char as well as in seabirds. SCCP and especially MCCP concentrations in cod livers from Iceland and northwest Norway were lower than those in cod from the Baltic Sea.

CP concentrations (lw) in samples from the Bear Island were high and comparable with previously reported concentrations of toxaphenes and polybrominated diphenylethers (Evenset*, et al.*, 2005). Studies of Arctic char from Lake Ellasjøen confirmed that this fish is influenced by guano deposition into this lake by the thousands of seabirds living on the surrounding cliffs. Values of $\delta^{15}N$ in Arctic char were 6 - 10‰ higher than for Arctic char

from another lake also situated on Bear Island but hardly influenced by seabirds. This means that the fish from Lake Ellasjøen feed at a higher trophic level, which is also evident from the high POP levels in their tissues (Evenset*, et al.*, 2004; 2005). Highest CP concentrations were found in the seabirds. Future studies of δ^{15}N-values and CP concentrations in different species of the food chain would allow to draw conclusions about the biomagnification behaviour of CPs.

4.1.3 Fish from Central Europe

For the first time CP concentrations were determined in fish from Central Europe. Various fish species captured in the rivers Rhine and Neckar were investigated (see Table 4.3). Muscle tissues of three to four fishes from the same location were pooled for analysis. Since no information about the expected amount of CPs was available, between 5 g (for fishes with a lipid content >10%) and 10 g (for fishes with a lipid content <10%) of muscle tissue were used. The clean-up was performed in the laboratories of the "Chemisches und Veterinäruntersuchungsamt" in Freiburg as developed in this work and described in Paper IV. A screening by HRGC-EI-MS/MS and the determination of the SCCP and MCCP concentrations by HRGC-ECNI-LRMS for selected samples followed in the laboratories of the University of Basel. Information about analysis details is given in Paper IV as well as by Zencak *et al.* (2004).

Additionally, six brown trout samples from two small rivers in the north of Switzerland as well as a pooled sample of lake trout from an alpine lake (Lei da Diavolezza, Switzerland) were investigated for CPs in the laboratories of the University of Basel. Detailed information about the samples is given in Table 4.3. 40 g of each sample were used for analysis.

Table 4.3: Summary of analysed fish samples including capture location and date, species, size and number of pooled fish samples.

Capture location	Species	Capture date	Sample No.	Size [cm]	Pooled fish
Neckar (Ladenburg / Edingen)					
"	White bream[1]	05.05.2003	M1	25-30	4
"	White bream[1]	05.05.2003	M2	31-38	4
"	Common bream[2]	05.05.2003	M3	46-52	3
"	Common bream[2]	05.05.2003	M4	53-55	3
"	Roach[3]	05.05.2003	M5	20-26	3
"	Common eel[4]	05.05.2003	M6	51-60	4
"	Common eel[4]	05.05.2003	M7	63-68	3
Rhine (Altwasser Schmugglermeer / Leopoldshafen)					
"	Common bream[2]	09.-16.05.2003	M12	36-40	4
"	Common bream[2]	09.-16.05.2003	M13	40-41	3
"	Pike[5]	09.-16.05.2003	M14	48-56	4
"	Pike[5]	09.-16.05.2003	M15	57-59	3
"	Pikeperch[6]	09.-16.05.2003	M16	33-36	4
"	Pikeperch[6]	09.-16.05.2003	M17	36-42	3
Lake Constance					
Untersee / Zellersee	Whitefish[7]	06.01.2005	159.6	25-27	5
Untersee / Zellersee	Whitefish[7]	06.01.2005	159.12	23-25	5
Harbour Immenstaad	Carp[8]	19.04.2004	7348.8	17-26	7
Liechtensteiner Binnenkanal					
Balzers	Brown trout[9]	25.04.2002	LBK 1	19-24	11
Schaan	Brown trout[9]	24.04.2002	LBK 2	20-25	11
Triesen	Brown trout[9]	24.04.2002	LBK 3	21-26	11
Necker Achsäge					
”	Brown trout[9]	06.05.2002	N1	28	1
”	Brown trout[9]	06.05.2002	N2	31	1
”	Brown trout[9]	06.05.2002	N3	28	1
Lei da Diavolezza	Lake trout[10]	04.09.2003	LDD	20-23	12

1: *Abramis bjkoerkna*; 2: *Abramis brama*; 3: *Rutilus rutilus*; 4: *Anguilla anguilla*; 5: *Esox lucius*; 6: *Sander lucioperca*; 7: *Coregonus spp.*; 8: *Cyprinus carpio*; 9: *Salmo trutta*; 10: *Salvelinus namaycush*

Quality control was carried out using 0.5 g of CP-free sun-flower oil spiked with 1500 ng of SCCPs and cleaned-up before and after the analysis of a series of samples. The results were always within the acceptance range (average of 1502 ng ± 6%, n = 7). Additionally, whitefish muscle (sample no. 159.12) was divided into six subsamples and each was spiked with 2000 ng of CPs (1000 ng of SCCPs and 1000 ng of MCCPs) and analysed by

HRGC-EI-MS/MS. The results were always within the tolarance (average of 2083 ng ± 5%; after subtraction of the CP amount present in the fish (165 ng): 1918 ng ± 5%). Average recovery was 79% (range of 69-80%) for the internal standard $^{13}C_{10}$-*trans*-chlordane and 73% (range of 70-78%) for CPs.

The influence of matrix on the HRGC-ECNI-LRMS results was investigated with three mackerel muscle samples (S+MCCPs <1 ng/g ww) spiked with CPs (samples 1 and 2 with 1500 ng of SCCPs and sample 3 with 1500 ng of SCCPs and MCCPs each). Relative deviations from expected values were less than 8% for sample 1 and 2. Values were 17% for SCCPs and 1% for MCCPs in sample 3. Interday reproducibility (five measurements over several months) for these samples was <6% for SCCPs and <9% for MCCPs.

Several method blanks were determined in parallel to fish analysis. The average method blank was 6 ng/g ww for sample clean-up in Germany. Therefore, a CP amount ten times higher than the average blank value was chosen as quantification limit. Results below this limit are reported in parenthesis in Table 4.4. Method blanks performed in the laboratories of the University of Basel did not contain detectable CP concentrations (<1 ng/g ww for HRGC-ECNI-LRMS). Therefore, no definition of a limit of quantification (LOQ) was necessary on method blank basis.

The results of the EI-MS/MS screening are listed in Table 4.4. CP concentrations in fish from Germany were between 13 and 256 ng/g ww. Concentrations in fishes with a lipid content of less than 1.2% were not significantly higher than the concentration of the method blank. For these samples a sample size of 10 g of muscle tissue was too low. A quantity of at least 40 g would have allowed to obtain results not influenced by the method blank.

Total CP concentrations were between 32 and 42 ng/g ww in brown trout samples from the Liechtensteiner Binnenkanal and between 19 and 28 ng/g ww for fish from the Necker (Switzerland). 25 ng/g ww were detected in the pooled lake trout sample from the alpine lake “Lei da Diavolezza”. Alpine mountain lakes are contaminated by POPs mainly through atmospheric deposition, since local sources are non-existing or negligible. The presence of CPs in this lake supports the theory that CPs undergo atmospheric transport. Since only one pooled sample (n = 12) was analysed, further studies are necessary to address the extent of CP pollution in Alpine lakes.

Table 4.4: Total CP concentrations (ng/g ww and ng/g lw) in various fish species (muscle tissue) captured in Germany (Neckar, Rhine and Lake Constance) as well as in Switzerland (Liechtensteiner Binnenkanal, Necker and Lei da Diavolezza). HRGC-EI-MS/MS was applied. For samples from Germany (listed above the dashed line) CP concentrations below ten times the average blank value of 6 ng/g are given in parenthesis.

Sample No.	Species	Lipid content [%]	CP concentration [ng/g ww]	CP concentration [ng/g lw]
Neckar				
M1	White bream	4.19	65	1561
M2	White bream	8.49	114	1341
M3	Common bream	12.0	256	2123
M4	Common bream	14.6	196	1345
M5	Roach	1.16	(34)	(2936)
M6	Common eel	25.1	141	565
M7	Common eel	27.4	126	461
Rhine				
M12	Common bream	6.70	125	1860
M13	Common bream	4.02	(55)	(1373)
M14	Pike	0.53	(43)	(8133)
M15	Pike	0.65	(13)	(2019)
M16	Pike perch	0.63	(29)	(4591)
M17	Pike perch	0.62	(16)	(2571)
Lake Constance				
159.6	Whitefish	1.50	(30)	(1993)
159.12	Whitefish	0.70	(14)	(2055)
7348.8	Carp	1.77	(11)	(1733)
Liechtensteiner Binnenkanal				
LBK1	Brown trout	1.6	34	2114
LBK2	Brown trout	1.5	32	2177
LBK3	Brown trout	2.1	42	1993
Necker / Achsäge				
N1	Brown trout	1.6	19	1210
N2	Brown trout	2.5	28	1153
N3	Brown trout	1.8	26	1487
Lei da Diavolezza				
LDD	Lake trout	4.4	25	581

The amount of SCCPs and MCCPs was additionally determined by HRGC-ECNI-LRMS in the samples M1, M3, M5, M6, M14, and M16 from Germany as well as in all samples from Switzerland. CPs were not detected in the method blanks, since this technique is less sensitive than EI-MS/MS (LOD of 1 ng/µl instead of 0.3 ng/µl of SCCP mixture). The SCCP and MCCP concentrations as well as a comparison with HRGC-EI-MS/MS results are listed in Table 4.5.

Concentrations of SCCPs and MCCPs were in the same order of magnitude in all samples. SCCP concentrations were similar or slightly higher than for MCCPs in biota from the Liechtensteiner Binnenkanal. Samples from the Necker showed higher MCCP levels. The two rivers might be influenced by different CP sources. However, MCCP levels were also dominant in trout from the alpine lake.

The results obtained by EI-MS/MS and ECNI-LRMS were generally in good agreement within a factor of two. The sum of SCCPs and MCCPs was 8-47% below the respective total CP concentration determined by EI-MS/MS. The difference could be explained by the presence of congeners with 1-4 chlorine atoms as well as by the presence of long-chain CPs ($C_{>17}$). Low-chlorinated CPs (<5 chlorine atoms) are not detected by ECNI-MS due to their low electron affinity and therefore low response factors. Long-chain CPs can be detected by EI-MS/MS (see chapter 3.4), but were not included in the method set-up for ECNI-LRMS.

Table 4.5: SCCP and MCCP concentrations determined by HRGC-ECNI-MS and total CP concentration obtained by EI-MS/MS in selected fish muscle samples from Germany and Switzerland.

Samples	Lipid content	ECNI-MS concentration [ng/g ww]			EI-MS/MS concentration
	[%]	SCCPs	MCCPs	S+MCCPs	[ng/g ww]
Neckar					
M1 White bream	4.19	35	25	**60**	**65**
M3 Common bream	12.0	108	195	**303**	**256**
M5 Roach	1.16	13	23	**36**	**34**
M6 Common eel	25.1	37	91	**128**	**141**
Rhine					
M14 Pike	0.53	31	19	**50**	**43**
M16 Pike perch	0.63	17	6	**23**	**29**
Liechtensteiner Binnenkanal					
LBK1 Brown trout	1.61	7	11	**18**	**34**
LBK2 Brown trout	1.47	11	9	**20**	**32**
LBK3 Brown trout	2.12	19	14	**33**	**42**
Necker / Achsäge					
N1 Brown trout	1.57	3	7	**10**	**19**
N2 Brown trout	2.46	6	14	**20**	**28**
N3 Brown trout	1.75	5	16	**21**	**26**
Lei da Diavolezza					
LDD Lake trout	4.35	< 1[a]	15	**15**	**25**

ww: wet weight; a: detected, but below the limit of quantification.

Additionally, concentrations of the indicator PCBs were determined for selected samples by the "Chemisches und Veterinäruntersuchungsamt Freiburg" as described in the principles of German official methods (BgVV, 1998). The SCCP and MCCP were comparable to those of PCB 138 and PCB 153 and slightly higher than for other PCB congeners (see Table 4.6).

Table 4.6: Concentrations (ng/g lipid weight) of selected PCBs, SCCPs and MCCPs in different fish muscle samples (M1: white bream, M3: common bream, M5: roach, M6: common eel, M14: pike and M16: pike perch) from the Neckar (Germany).

Sample No.	Concentration [ng/g lw]								
	PCB 28	PCB 52	PCB 101	**PCB 138**	**PCB 153**	PCB 180	PCB 118	**SCCPs**	**MCCPs**
M1	111	207	574	**1259**	**1076**	387	285	**844**	**591**
M3	88	164	388	**1346**	**1014**	554	286	**898**	**1619**
M5	71	102	436	**1307**	**1152**	476	326	**1078**	**1974**
M6	30	115	267	**1191**	**1153**	301	278	**149**	**365**
M14	316	453	897	**2240**	**1927**	761	737	**5851**	**3591**
M16	291	438	696	**1691**	**1427**	537	491	**2705**	**966**

lw: lipid weight

Possible correlations between PCB and CP concentrations were investigated. The following correlations at a significance level of $P < 0.05$ could be found, despite the limited number of samples, and quite different sources, transport mechanisms and metabolisation of PCBs and CPs (see Table 4.7).

Table 4.7: Correlation coefficients (r) between the concentrations of SCCPs, MCCPs and selected PCBs in fish (n = 7) from the Neckar (Germany). Correlations at a significance level of $P < 0.05$ are marked in bold.

Compounds	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	PCB 118
SCCP	**0.9005** **P = 0.014**	**0.8650** **P = 0.026**	**0.9215** **P = 0.009**	**0.9960** **P < 0.001**	**0.9689** **P = 0.001**	**0.8975** **P = 0.015**	**0.9921** **P < 0.001**
MCCP	0.5556 P = 0.252	0.4642 P = 0.354	0.6459 P = 0.166	0.8053 P = 0.088	0.7466 P = 0.053	**0.9063** **P = 0.013**	0.7823 P = 0.066

The concentrations of SCCPs correlated well with all PCB congeners. A high correlation was found between SCCPs and penta- and hexachlorinated biphenyls (PCBs 118, 138 and 153). MCCPs only correlated well with the heptachlorinated biphenyl PCB 180 (see Figure 4.4). Physico-chemical properties (log Kow, vapour pressure, water solubility) of the correlating compounds are in a similar range. The results indicate that SCCPs and PCBs 118, 138 and 153 as well as MCCPs and PCB 180 have similar behaviour in terms of bioaccumulation and persistence.

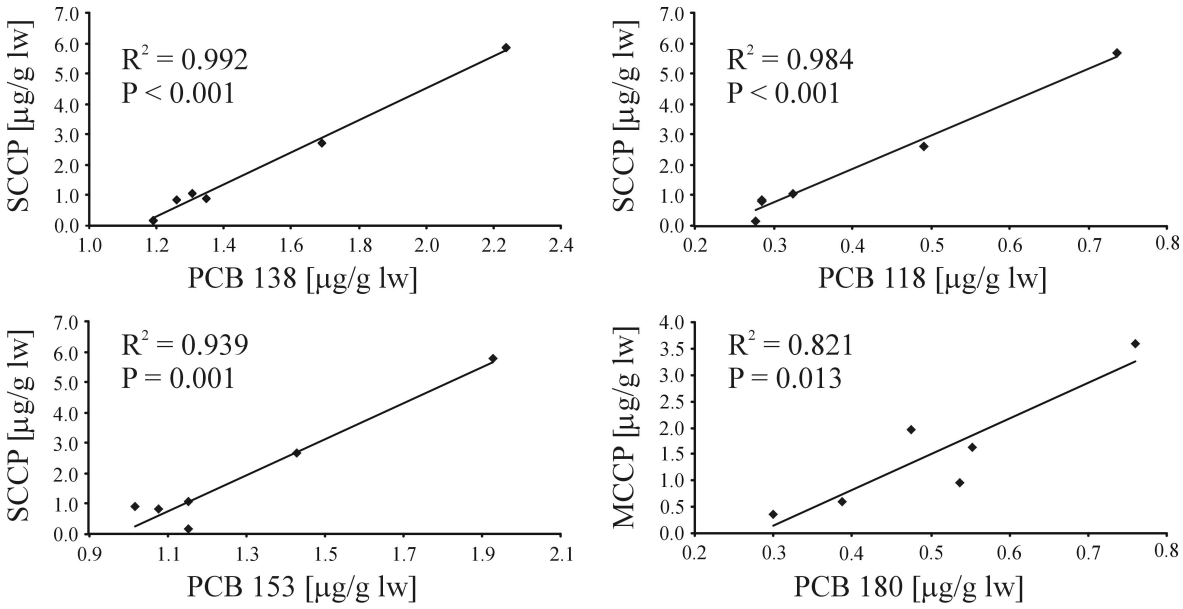

Figure 4.4: Correlation diagrams between concentrations of SCCPs and PCB 138, PCB 118 and PCB 153 as well as between MCCPs and PCB 180 determined in various fish samples from the Neckar (Germany).

4.2 CP levels in humans and human foodstuff

The developed method was applied for the analysis of poultry eggs and human milk samples from south Germany to gain insight into human exposure to CPs. The clean-up was performed in the laboratories of the "Chemisches und Veterinäruntersuchungsamt Freiburg" (Germany) as described in Paper IV. Analysis by HRGC-EI-MS/MS followed in the laboratories of the University of Basel. PCB concentrations were obtained from the

"Chemisches und Veterinäruntersuchungsamt Freiburg" (Germany). The determination of PCBs followed the principles of German official methods (BgVV, 1998).

Human milk samples were extracted as follows: ca. 50 g of human milk were centrifuged (3000 rpm, 4 °C, Varifuge 3.0 R, Heraeus Instruments, Germany) for ten minutes. The lipid fraction on top was separated with a spatula and melted in a water bath at 50 °C. Anhydrous sodium sulphate was added and the extract was stirred until dryness. The extract was covered with *n*-hexane (1-2 cm) and lipids were dissolved by stirring with a glass stirrer. The extract was filtrated with a glass funnel filled with a pre-cleaned piece of cotton wool and sodium sulphate. Lipid extraction was repeated 3 - 4 times. Solvents were evaporated with a speed vac (Laborota 4001 efficient, Heidolph Instruments, Germany, 250-300 mbar). Residues of solvent were evaporated in a preheated sand bath (50 °C) under a gentle stream of nitrogen for 5 - 10 minutes. Lipid removal and adsorption chromatography on Florisil® were performed as described in Paper IV.

4.2.1 CP levels in human milk

Six milk samples were collected in summer 2004 and in January 2005 from volunteering nursing mothers living in Baden-Württemberg (South Germany). Samples were randomly selected and did not represent a specific group of mothers. The age of the donors was 36 years in average (range 29-38 years). Recoveries of the internal standard were between 69 and 102%. Total CP concentrations of the human milk were between 2.6 and 9.6 ng/g ww and are listed in Table 4.8.

Table 4.8: Total CP concentrations (ng/g wet weight and lipid weight) and PCB concentrations in human milk samples from South Germany. Lipid content (%) is also given. Results in brackets did not meet the quality control criterion of ten times higher than the average method blank (0.2 ng/g ww).

Sample No.	Lipid content [%]	Total CP concentration [ng/g ww]	Total CP concentration [ng/g lw]	PCB concentration[a] [ng/g lw]
H1	6.3	3.3	52	153
H2	n.d.	(1.9)	(55)[b]	320
H3	n.d.	9.6	275[b]	251
H4	n.d.	2.6	76[b]	75
H5	2.4	5.2	216	212
H6	3.5	(1.7)	(49)	126

a: Sum of PCBs 28, 52, 101, 153, 138, 180 and 118; b: calculated with an assumed lipid content of 3.5%; n.d. not determined; ww: wet weight; lw: lipid weight.

As already shown in 1980 (Campbell and McConnell) and 1985 (Schmid and Müller), CPs are present in human milk. Currently, CP concentrations are in a similar range as for PCBs taking into account that the sum of the three most characteristic congeners (PCB 153, 138, 180) represents 55-70% of the total PCB content in human milk (Schulte and Malisch, 1984).

4.2.2 CP levels in eggs

The total CP concentration was determined in six pooled samples (n = 10-15) of poultry eggs from south Germany (Baden-Württemberg). The total CP concentrations were between 20 and 59 ng/g ww and are listed in Table 4.9. They were a factor of ten higher than the SCCP concentration (2.0 ng/g ww) determined in egg from Japan (Iino*, et al.*, 2005).

Table 4.9: Total CP concentration in six poultry egg samples determined by HRGC-EI-MS/MS.

Sample No.	Pooled eggs	Lipid content [%]	Total CP concentration [ng/g ww]	PCB concentration[a] [ng/g lw]
E1 (14439.1)	12	9.4	23	173
E2 (20259.1)	10	8.7	24	33
E3 (21451.1)	15	8.2	20	n.d.
E4 (22194.1)	15	9.2	59	n.d.
E5 (22196.1)	15	11.0	25	n.d.
E6 (22199.1)	15	9.2	40	n.d.
Eggs from Japan (Iino*, et al.*, 2005)	n.a.	11.4	2.0 ng/g ww of SCCPs	n.a.

a: Sum of PCBs 138, 153, 180 and 118; n.d.: not determined; n.a. not available; ww: wet weight; lw: lipid weight.

Total CP concentrations were similar to or higher than the sum concentration of PCBs 138, 153, 180 and 118 determined in two of these egg samples. Furthermore, total CP concentrations are comparable to levels of PCBs (10-351 ng/g lw, sum of seven marker PCBs) and DDT compounds (30-5250 ng/g lw, sum of DDT, DDD and DDE) determined in free-range eggs in Belgium in 2005 (van Overmeire*, et al.*, 2005).

4.3 Comparison of congener and homologue group patterns

An advantage of the determination of CPs by HRGC-ECNI-MS is the possibility to obtain information about congener and homologue group patterns and the chlorine content. Partial fractionation of the original technical composition may occur during phase transfer, atmospheric transport or metabolic processes due to the very different physico-chemical properties of CP congeners. Prior to this work only little information about congener and homologue group patterns was published. Isomers of the same formula $C_nH_{2n+2-z}Cl_z$ were summarised and called congener groups. The abundance of each congener group was normalised so that their sum was 100%. The previously published congener group patterns gave information about the composition of technical CPs produced in North America and about CPs detected in the Canadian environment (Marvin*, et al.*, 2003; 2000; Tomy*, et al.*, 1999a). However, such data were missing for CPs manufactured in Europe and for CPs in the European environment.

4.3.1 Congener and homologue group patterns of technical CP mixtures

So far, a comprehensive data set about the composition of technical CP mixtures was not available, although information about the original composition of CPs is essential for the investigation of changes of CP patterns in the environment as well as for analytical purposes. More detailed knowledge about the most representative CP mixtures could improve quantification procedures for CPs. Furthermore, toxicity studies could concentrate on the most abundant congener groups.

For the investigation of the technical CP mixtures different approaches were used in this work. First, congener group patterns were determined by HRGC-ECNI-LRMS and

compared. Then, similarities and differences in these mixtures were further elucidated by principal components analysis (PCA). Finally, the distribution of homologue groups (congener groups with the same number of carbon atoms) was investigated.

4.3.1.1 Congener group patterns of technical SCCPs and MCCPs

The congener composition of five technical SCCP mixtures and seven technical MCCP mixtures was investigated by HRGC-ECNI-LRMS. Moreover, the composition of three commercially available standard SCCP mixtures and three standard MCCP mixtures was determined and compared to that of the technical CP mixtures. CP mixtures and their manufacturers are summarised in Table 4.10.

Table 4.10: Standard and technical SCCP and MCCP mixtures and their manufacturers.

SCCP mixtures	Manufacturer	MCCP mixtures	Manufacturer
Standard mixtures:			
SCCP Standard 51%	a	MCCP Standard 47%	a
SCCP Standard 55%	a	MCCP Standard 52%	a
SCCP Standard 63%	a	MCCP Standard 57%	a
Technical mixtures:			
Hordalub 17	Hoechst (Germany)	Hordaflex SP	Hoechst (Germany)
Hordalub 80	Hoechst (Germany)	Hordalub 80 EM	Hoechst (Germany)
Hordalub 500	Hoechst (Germany)	Cloparin	Caffero (Italy)
Cereclor 60 L	ICI (UK)	Cereclor S52	ICI (UK)
Cereclor 70 L	ICI (UK)	LGC 2	ICI (UK)
		LGC 5	ICI (UK)
		LGC 7	ICI (UK)

a: synthesised by Dr. M. Coelhan (Technical University of Munich) and supplied as analytical standards by Dr. Ehrenstorfer (Germany), 100% purity; ICI: Imperial Chemical Industries

The obtained congener group patterns are shown in Figures 4.5 and 4.6. CP mixtures were arranged according to their chlorine content.

All SCCP mixtures consist mainly of congeners with eleven and twelve carbon atoms. The main difference between the CP mixtures in the left column of Figure 4.5 is caused by their chlorine content. A clear shift of the major congener groups according to the chlorine content could be observed. Congeners with six chlorine atoms were most abundant in the low-chlorinated Hordalub 17 mixture (49% Cl), whereas congeners with eight and nine chlorine atoms belonged to the major congeners in the high-chlorinated Cereclor 70L mixture (69% Cl). The composition of the SCCP mixtures manufactured as analysis standards was consistent with that of the Hordalub mixtures of similar chlorine content.

In contrast, all MCCP mixtures consist mainly of C_{14} congeners (see Figure 4.6). MCCP mixtures of <70% of C_{14} are given in the left column, mixtures of >70% of C_{14} in the right column. Similarly to SCCPs, a clear shift of the major MCCP congener groups related to the chlorine content could be observed. Congeners with six chlorine atoms were most abundant in the low-chlorinated LCG 2 mixture (53.2% Cl), whereas congeners with seven and eight chlorine atoms belonged to the major congeners in the Hordaflex mixture (56.8% Cl).

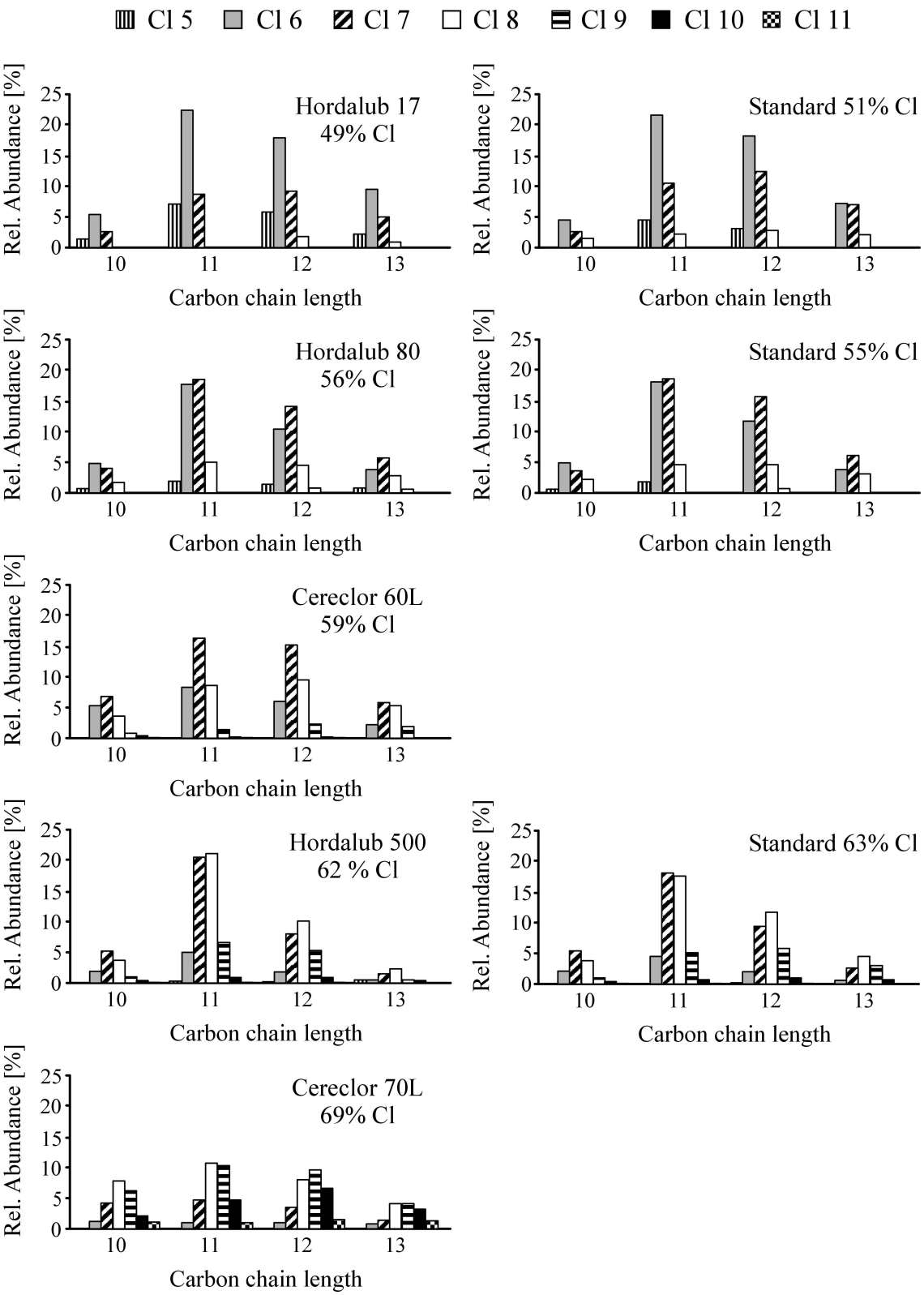

Figure 4.5: Congener group patterns of five different technical SCCP mixtures and three standard SCCP mixtures determined by HRGC-ECNI-LRMS. The technical SCCP mixtures are shown in the left column ranked according to their chlorine content. The standard mixtures are shown in the right column. Mixtures with similar chlorine contents are aligned on the same height.

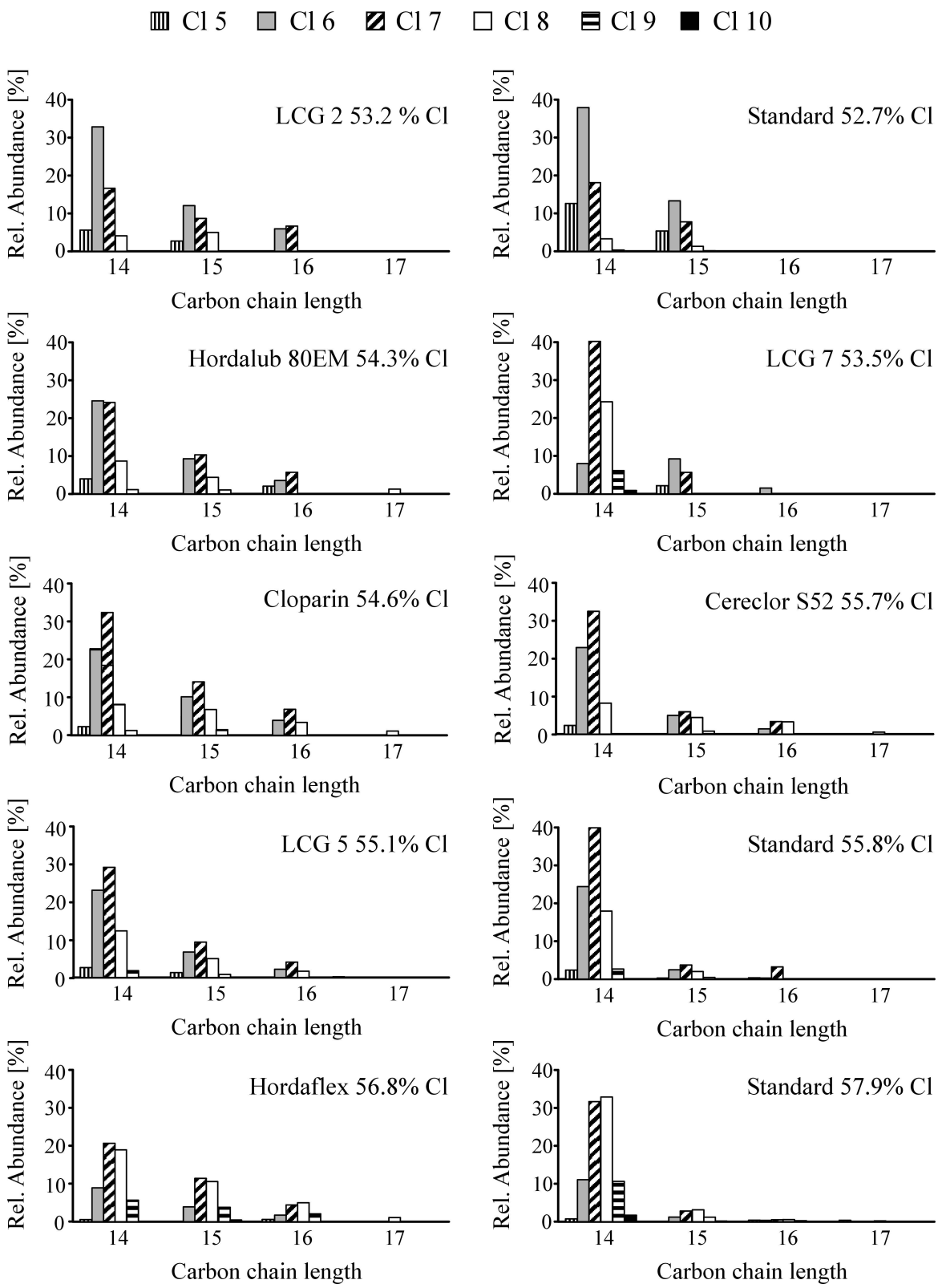

Figure 4.6: Congener group patterns of seven different technical MCCP mixtures and three standard CP mixtures determined by HRGC-ECNI-LRMS. Mixtures with a proportion of C_{14} >70% are arranged in the right column. Additionally, mixtures in each column are ranked according to their chlorine content.

4.3.1.2 Principal components analysis of SCCPs and MCCPs

Similarities and differences in the CP mixtures were further elucidated by principal components analysis (PCA). This technique was often applied in PCB studies to determine characteristic congeners and to elucidate possible PCB sources (Andersen*, et al.*, 2001; Hobbs*, et al.*, 2002; Weis and Muir, 1997). Hüttig and Oehme applied PCA to show similarities in the CP patterns present in sediments (Hüttig and Oehme, 2005). In this work PCA was used to identify significant congener groups in CP mixtures and to evaluate the suitability of SCCP and MCCP standard mixtures for the determination of CPs in fish.

PCA was conducted using the software Statistica (Version 5.5; 1999 Edition, StatSoft, Inc., Tulsa, USA) for multivariate data analysis. The N Variables were the relative abundance of each CP congener group ($C_{10\text{-}13}$ with five to eleven chlorine atoms, N = 28 for SCCPs; $C_{14\text{-}15}$ with five to ten chlorine atoms and C_{16} with five to nine chlorine atoms, C_{17} with eight chlorine atoms, N = 18 for MCCPs). Data were normalized so that the sum of all congener groups was 100%. The loading plot and score plot were obtained after normal-varimax rotation. Loading plots indicate relationships among variables. Score plots give the positions of the samples in the coordinates of the principal components. Similar samples are represented by points located close to each other (Zitko, 1994). The plots of PCA scores and loading factors for the first two principal components (PC) are shown in Figure 4.7 for SCCPs and in Figure 4.8 for MCCPs.

SCCP results revealed that the first and second principal components (PC1 and PC2) accounted for 62.6% and 21.8% of the total variance, respectively. SCCPs congeners were clustered in three groups according to their number of chlorine atoms. PC1 was positively correlated with high-chlorinated SCCPs ($>Cl_8$) and negatively with $C_{11}H_{17}Cl_7$ and

$C_{12}H_{19}Cl_7$ as well as low-chlorinated SCCPs (<Cl_7), indicating that SCCP mixtures were mainly differentiated according to their chlorine content. In the score plot (Figure 4.7, A) CP mixtures with similar chlorine content were located closely, confirming the similarities between the Hordalub mixtures and the standard mixtures of similar chlorine content already observed for the congener group pattern (see Figure 4.5). The carbon chain length has no or only little influence.

Additionally, PCA was applied to seven technical MCCP mixtures as well as to three standard MCCP mixtures (Figure 4.8). PC1 accounted for 41.2% of the total variance and was positively correlated with C_{16} and C_{17} congener groups and high-chlorinated C_{14-15} CPs (>Cl_7) and negatively with low-chlorinated C_{14-15} CPs, indicating also a strong dependence on the chlorine content. In addition, a differentiation between high-chlorinated C_{14} congeners and C_{15-17} congeners was observable, since only C_{14} congeners with seven to ten chlorine atoms had negative loadings on PC 2 (27.5% of the total variance).

MCCP mixtures were ranked along PC1 according to their chlorine content in the score plot (Figure 4.8, A). Additionally, mixtures with a high proportion of C_{14} (>76%, e.g. MCCP standards 55.8% and 57.9%) had low PC2 values. PCA results were different to those of SCCPs. MCCP mixtures consist mainly of congeners with 14 carbon atoms. In contrast to SCCP mixtures, where standard mixtures and technical mixtures of similar chlorine content were plotted closely, the distance between standard MCCP mixtures and technical MCCP mixtures of similar chlorine content was increased and more dependant on the proportion of C_{14} and C_{15} congeners.

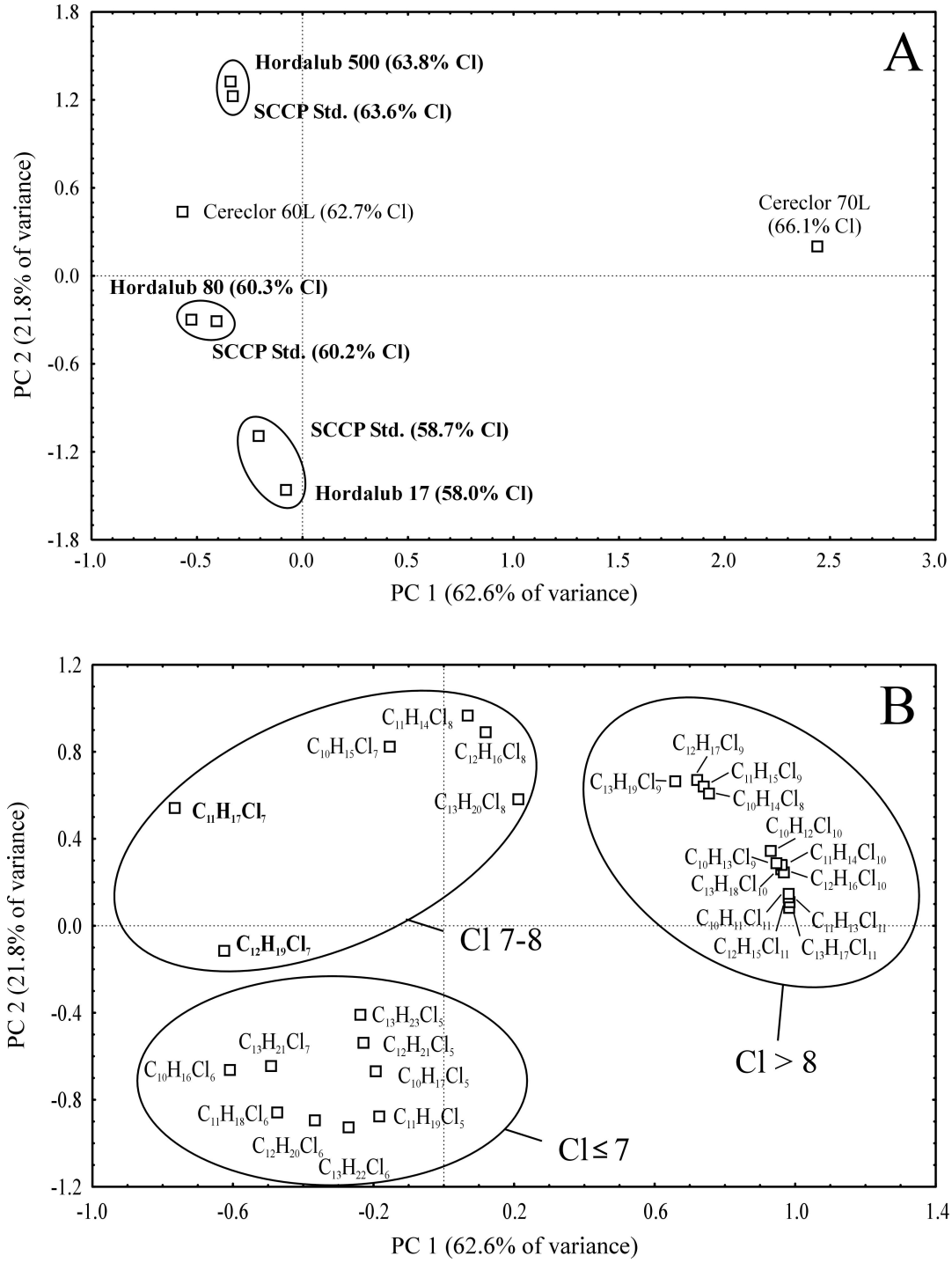

Figure 4.7: Principal components analysis score plot (A) and loading plot (B) of the congener group patterns in five technical SCCP formulations and three SCCP standard mixtures. Chlorine contents were calculated from the ECNI-MS data.

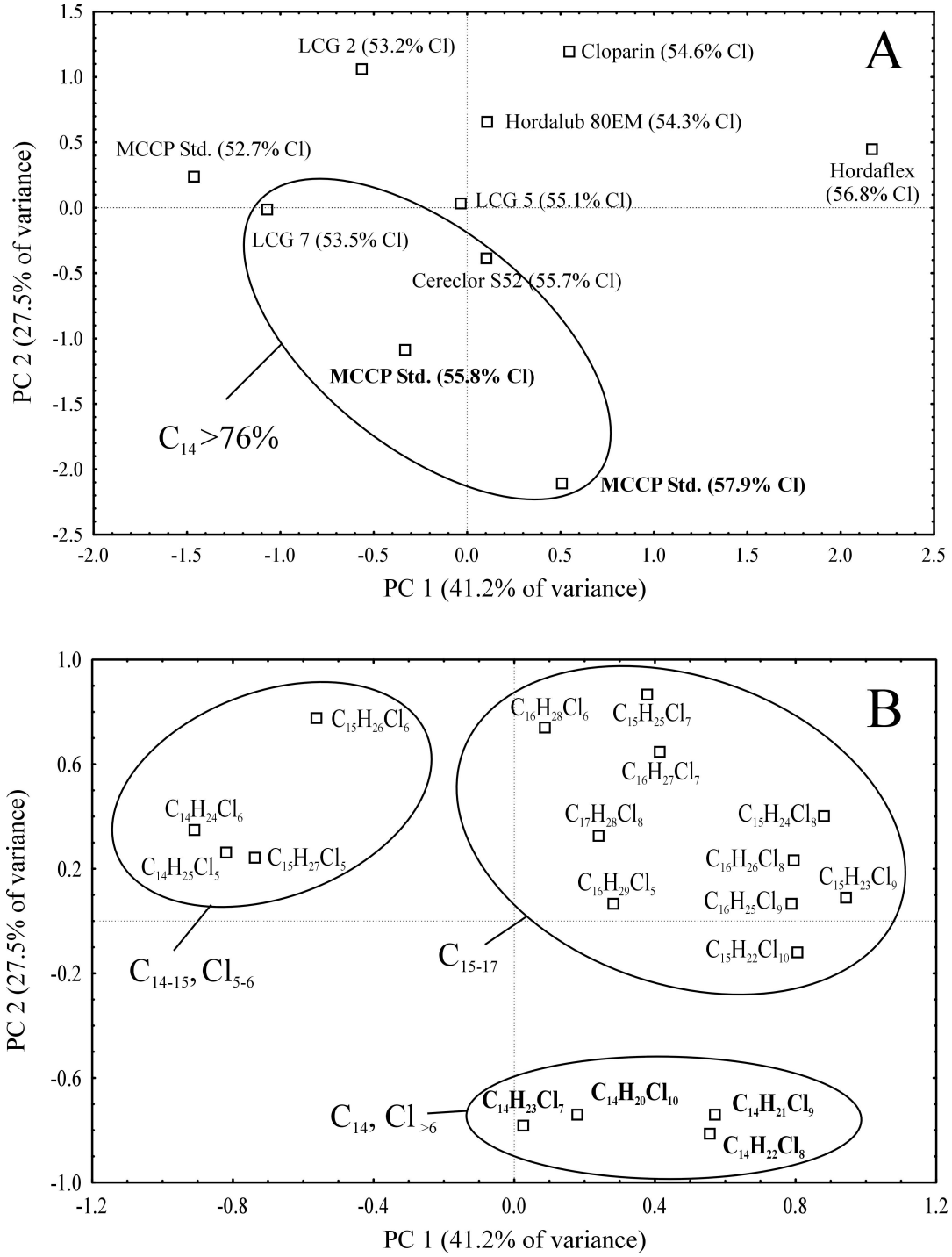

Figure 4.8: Principal components analysis score plot (A) and loading plot (B) of the congener group patterns in seven technical MCCP formulations and three MCCP standard mixtures. Chlorine contents were calculated from the ECNI-MS data.

Quantification of CPs is mainly performed with CP mixtures and not with single isomers. The applied standard CP mixture should be as representative as possible for all CP compositions in real samples. Currently, only three SCCP and three MCCP standard mixtures of 100% purity are available. PCA revealed that the three SCCP standard mixtures were similar to the technical SCCP mixtures and therefore suitable for quantification. MCCP standard mixtures were not positioned closely to the technical MCCP mixtures in the score plot (Figure 4.8, A), since the standard mixtures consisted mainly of C_{14} congeners and the technical MCCP mixtures contained additionally a significant amount of C_{15} congeners. The composition of MCCP standard mixtures should be adapted to that of technical mixtures to improve further quantification results.

The quantification procedure presented in this work (chapter 3.3.3.2 and Paper III) is based on the observation that response factors are mainly dependant on the chlorine content and less on the carbon chain length. PCA confirmed that the differences in the mixtures are mainly caused by the chlorine content.

4.3.1.3 Homologue group patterns of SCCPs and MCCPs

CP mixtures were additionally investigated considering only the influence of the carbon chain length. Changes in the carbon chain length may play an important role, if transformation of CPs in the environment is studied. Congener groups of one chain length were summarised and called homologue groups. The corresponding homologue group patterns as well as the proportion of each homologue group were used to investigate differences between technical CP mixtures and CPs in Arctic biota (see Paper IV, Table 3 and Figure 3). The homologue distribution was rather similar for all technical SCCP mixtures. Homologue groups with eleven and twelve carbon atoms were the major

components of all SCCP mixtures. Additionally, the high amount of C_{10} congeners was remarkable in the Cereclor 70L mixture. The Hordalub 500 mixture was different, since it contained a high proportion of C_{11} (>50%) and less C_{13} compounds. The composition of this mixture is consistent with those of two high-chlorinated SCCP mixtures manufactured in North America (60% C and 70% Cl, Dover Chemicals Corp., Dover, OH and Occidental Chemical Corp. USA), and determined by Tomy *et al.* (1997) using ECNI-MS (see Table 4.11).

Table 4.11: Relative contribution of C_{10}, C_{11}, C_{12} and C_{13} homologue groups in technical Hordalub 500 as well as in two technical SCCP mixtures from North America as reported by Tomy *et al.* (1997).

Technical mixtures	Proportion [%] of			
	C_{10}	C_{11}	C_{12}	C_{13}
Hordalub 500 (62% Cl)	12	54	27	7
PCA 60 (60% Cl)[a]	19	50	29	2.3
PCA 70 (70% Cl)[a]	12	53	32	3.0

a: reported by Tomy *et al.* (1997)

The distribution of homologue groups of technical MCCP mixtures is summarised in Table 4.12. Congeners with 14 carbon atoms were most abundant (proportion of 45-89%) in all MCCP mixtures, followed by C_{15} congeners (proportion of 9-33%) and C_{16} congeners (proportion of <0.5-14%). C_{17} congeners were hardly detectable in the investigated MCCP mixtures (<2%). The MCCP mixtures had a similar composition as two technical MCCPs (mPCA-52, mPCA-53) from the USA characterised by ECNI-HRMS (Tomy*, et al.*, 1997, see Table 4.12).

Table 4.12: Relative contribution of the C_{14}, C_{15}, C_{16} and C_{17} homologue groups in three standard MCCP mixtures and in six technical MCCP mixtures from Europe determined by HRGC-ECNI-MS as well as in two technical MCCP mixtures from North America (taken from Tomy and Stern (1999)).

CP mixtures	Proportion [%] of			
	C_{14}	C_{15}	C_{16}	C_{17}
Standard mixtures:				
MCCP Standard 47%	72	28	< 0.5	< 0.5
MCCP Standard 52%	87	9	4	< 0.5
MCCP Standard 57%	89	9	2	1
Technical mixtures:				
Hordaflex SP	45	30	14	1
Hordalub 80 EM	62	25	11	1
Cereclor S52	76	16	8	1
LGC 2	59	28	13	< 0.5
LGC 5	69	23	8	< 0.5
LGC 7	80	17	2	2
Cloparin	52	33	14	1
mPCA-52[a]	93	< 0.5	7	< 0.5
mPCA-53[a]	54	41	5	< 0.5

a: values taken from Tomy and Stern (1999)

4.3.2 Variations of congener group patterns in fishes

An interesting and currently hardly addressed aspect in CP research is the question, if CPs maintain their composition once released into the environment and accumulated in the fatty tissue of different species. It is well known that the composition of mixtures of polychlorinated compounds can change in the environment and especially in biota. Several studies revealed that differences in metabolisation could lead to an enrichment of certain congeners. For example, mainly toxaphenes #26 (B8-1413), #50 (B9-1679) and #62 (B9-1025) can be found in fish, although the pesticide toxaphene is a mixture of hundreds of components (Vetter and Oehme, 2000). Furthermore, such processes can be enantioselective (Skopp*, et al.*, 2002). Currently, only few congener group patterns of CPs in biota have been published. These data indicate a slight predominance of lower

chlorinated and shorter chained CPs in marine mammals from the Canadian Arctic (Tomy, *et al.*, 2000).

In this work CP congener and homologue group patterns were determined in fish from different locations in Europe to elucidate possible changes in the CP composition. Six brown trout samples from Central Europe (muscle tissue) as well as 14 cod samples (liver tissue) were selected to evaluate changes in CP patterns caused by metabolisation or transport in the environment. In a first round only fish of the same species and similar size were compared, to reduce the number of factors possibly influencing the CP congener group patterns.

4.3.2.1 Congener group patterns in brown trout from Switzerland

Congener group patterns were determined in three pooled samples of brown trout captured in the Liechtensteiner Binnenkanal and in three brown trouts captured in the river Necker in Switzerland and compared to those of technical CP mixtures. A selection of congener group patterns is shown in Figure 4.9. Congeners with eleven and twelve carbon atoms were always most abundant among SCCPs (50-75% of all SCCPs) followed by C_{10} and C_{13} congeners as overall typical for technical SCCP mixtures. MCCP congener group patterns were also similar to those of the technical MCCP mixtures. The proportion of congeners with 14 carbon atoms was between 65-85%.

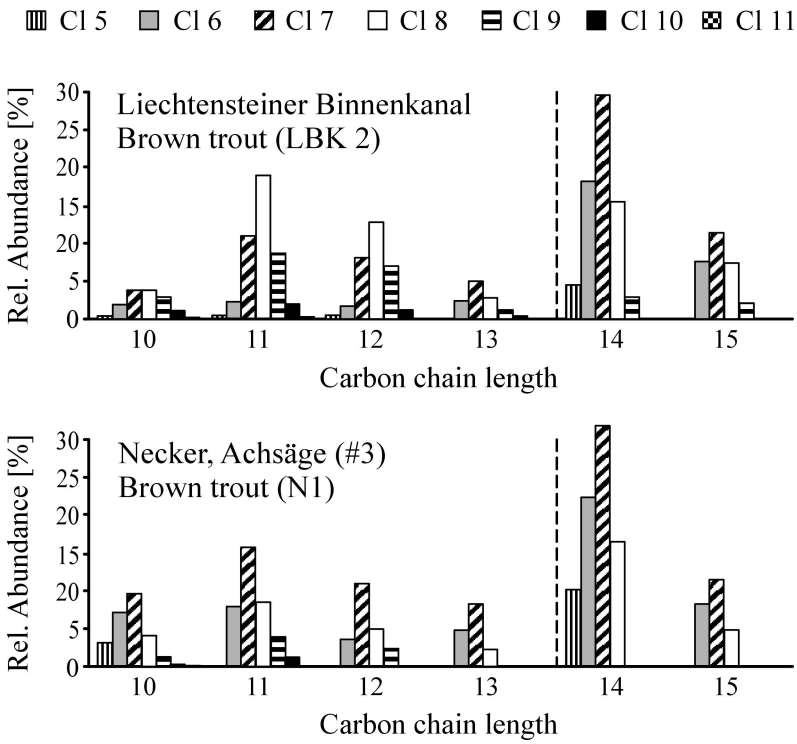

Figure 4.9: Congener group patterns determined by HRGC-ECNI-LRMS in muscle of brown trout from the Liechtensteiner Binnenkanal and the river Necker in Switzerland.

PCA was conducted to confirm the similarity between the CP patterns of brown trout and technical CP mixtures (see Figure 4.10). Results were similar to those of the technical SCCP and MCCP mixtures. Samples were mainly differentiated according to their chlorine content for SCCPs and MCCPs. Furthermore, samples were positioned in the MCCP score plot according to the proportion of C_{14} congener groups.

In the SCCP score plot (Figure 4.10, A) samples had similar loadings as the Hordalub 500, Cereclor 60L and Hordalub 80 mixtures. CP patterns in brown trouts were characterised by congeners with seven and eight chlorine atoms. Chlorine contents of the CPs in the fish samples were between 61 and 64%. This agrees well with the fact that 70% of all SCCPs produced in Europe have chlorine contents of 60-70% (World Health Organization, 1996). However, the fish samples did not contain CPs with more than 65% chlorine, although

high-chlorinated mixtures (e.g. Cereclor 70L) have a higher production volume than low-chlorinated SCCP mixtures.

In the MCCP score plot (Figure 4.10, B) samples were positioned closely to Hordalub 80EM, Cloparin and LCG 5 mixtures confirming that no significant changes in the CP composition are observable in brown trouts. CPs in the fish contained between 71 and 77% of C_{14} congeners. The chlorine contents were between 54.5 and 55.5% and were slightly higher than the chlorine content of most technical MCCP mixtures (ca. 50%).

Additionally, the congener group patterns of six different fish species from the river Neckar in Germany were investigated. Their SCCP and MCCP patterns were also similar to technical SCCP mixtures, although different fish species were analysed. The chlorine contents were between 59.7 to 64.0% for SCCPs and between 55.4 and 57.3% for MCCPs. The CP composition in all fish samples from Central Europe was similar to technical CP mixtures and no significant transformation of CPs could be observed.

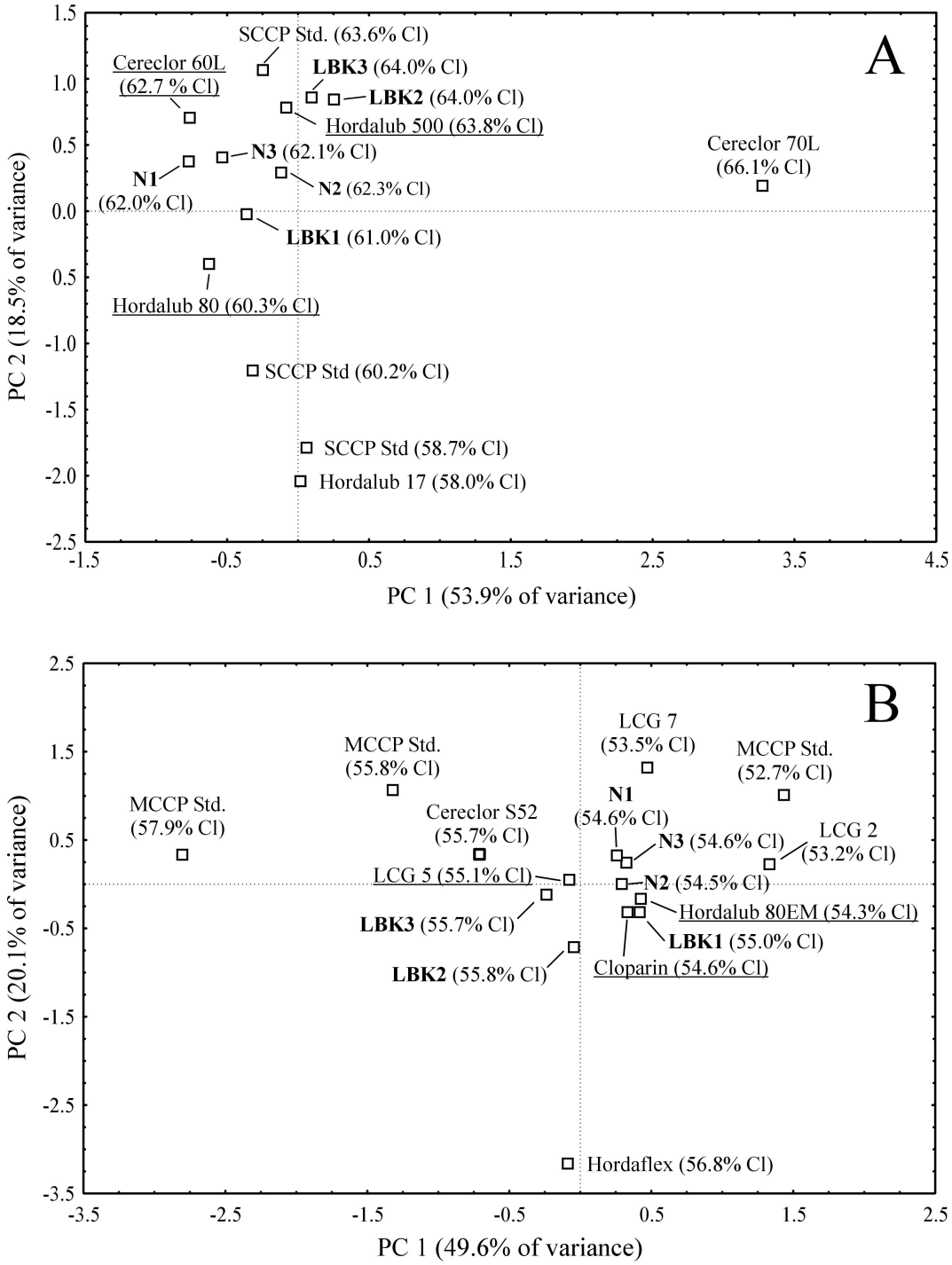

Figure 4.10: Principal components analysis score plots of the SCCP (A) and MCCP (B) congener group patterns in technical CP formulations, CP standard mixtures, and six SCCP and MCCP patterns determined in brown trouts from the Liechtensteiner Binnenkanal (LBK1-3) as well as from the Necker (N1-3). Chlorine contents were calculated from the ECNI-MS data.

4.3.2.2 Congener group patterns in cod from NW Europe and the Baltic Sea

Congener group patterns of cod, dab and flounder from the North and Baltic Sea are discussed in detail in Paper II. MCCP congener group patterns were similar to those of technical MCCP mixtures. Congeners with eleven and twelve carbon atoms were usually most abundant among SCCPs. However, a higher variability was observed in marine fish compared to the freshwater fish from Central Europe.

Remarkable differences in the congener and homologue group patterns were observed in biota from northwest Europe and from the Bear Island located in the European Arctic. A discussion is presented in Paper IV. Congener groups with ten carbon atoms were significantly more abundant than in fish captured closer to industrialised areas. Additionally, mainly high-chlorinated CPs were detected in contrast to data reported for the Canadian Arctic. The higher relative abundance of C_{10} congeners in Arctic samples may be explained by their higher vapour pressures and, thus, their higher tendency to undergo long-range atmospheric transport. However, the additional presence of mainly high-chlorinated congeners does not support this hypothesis, since these congeners have low vapour pressures. Their presence may be explained by their higher persistency and tendency to bioaccumulate. Therefore, the CP patterns in biota from Bear Island may be a result of exposure to atmospheric transported CPs and bioaccumulation processes. A detailed discussion is presented in Paper IV.

PCA was conducted to compare the congener group patterns of the cod liver samples from the Baltic Sea and from northwest Europe with those of technical CP mixtures. PCA plots of the SCCP congener group patterns are shown in Figure 4.11. PC1 and PC2 accounted for 35.1% and 14.3% of the total variance, respectively. Congener groups were clustered in

low- and high-chlorinated SCCPs. Additionally, PC2 was positively correlated with $C_{12}H_{19}Cl_7$, $C_{10}H_{15}Cl_7$ and $C_{10}H_{14}Cl_8$ and negatively with $C_{11}H_{18}Cl_6$, $C_{13}H_{22}Cl_6$ and $C_{13}H_{21}Cl_7$ (see loading plot, Figure 4.11, B).

Cod samples could be distinguished due to their geographic origin in the score plot (Figure 4.11, A). Two groups could be identified additionally to the technical CP mixtures. Cod samples from northwest Europe (A1 to A6) were characterised by C_{10} and C_{12} congeners and were located differently to cod from the Baltic Sea. Cod samples from the Baltic Sea were positioned closely to technical mixtures as well as with negative values for PC2 for samples with a proportion of C_{13} >19% (19 - 28% of C_{13} in samples OS10 to OS13 and OS15).

The SCCP composition in fish captured far away from industrialised areas and possible CP sources was different from technical SCCP mixtures. However, the chlorine contents (59.5 - 62.8%) in the fish were similar to the chlorine content of most technical SCCP mixtures.

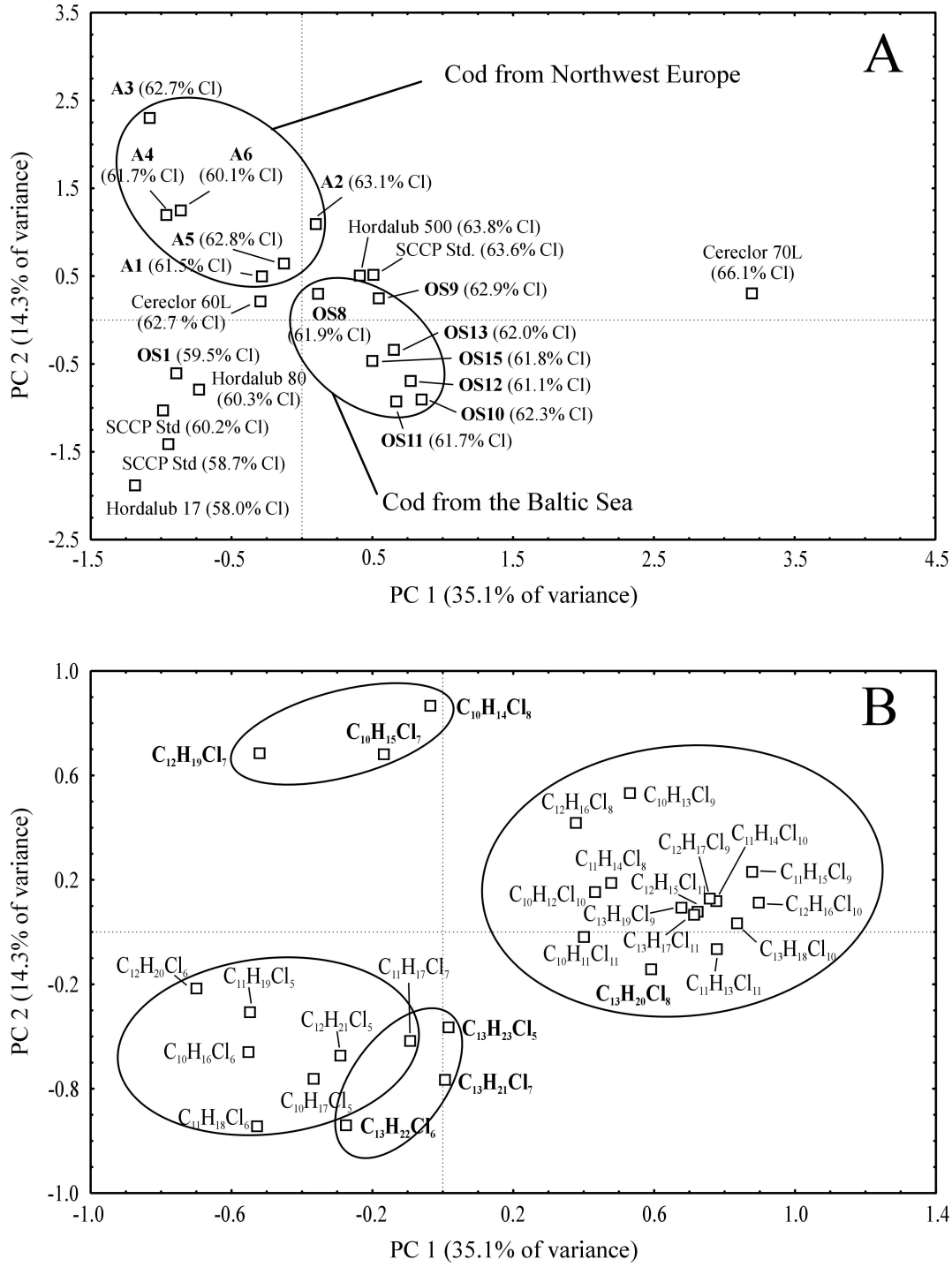

Figure 4.11: Principal components analysis score plot (A) and loading plot (B) for five technical SCCP formulations, three SCCP standard mixtures, 14 SCCP patterns determined in cod from northwest Europe (A1-A6) and from the Baltic Sea (OS1, OS8-OS13 and OS15). Chlorine contents were calculated from the ECNI-MS data.

In contrast to the SCCP patterns, no differences could be observed between MCCP patterns in samples from the Baltic Sea and northwest Europe (see Figure 4.12). PCA results were similar to those obtained for brown trout (chapter 4.3.2.1) and for technical MCCP mixtures (chapter 4.3.1.2). Samples were differentiated according to their chlorine content (PC1) and their proportion of C_{14} congeners (PC2). Except for sample A4 (11% of C_{15}), the proportion of C_{15} was between 18 - 46% (average of 35%) for all samples, thus similar to technical MCCP mixtures (21 - 43% of C_{15}, average of 32%).

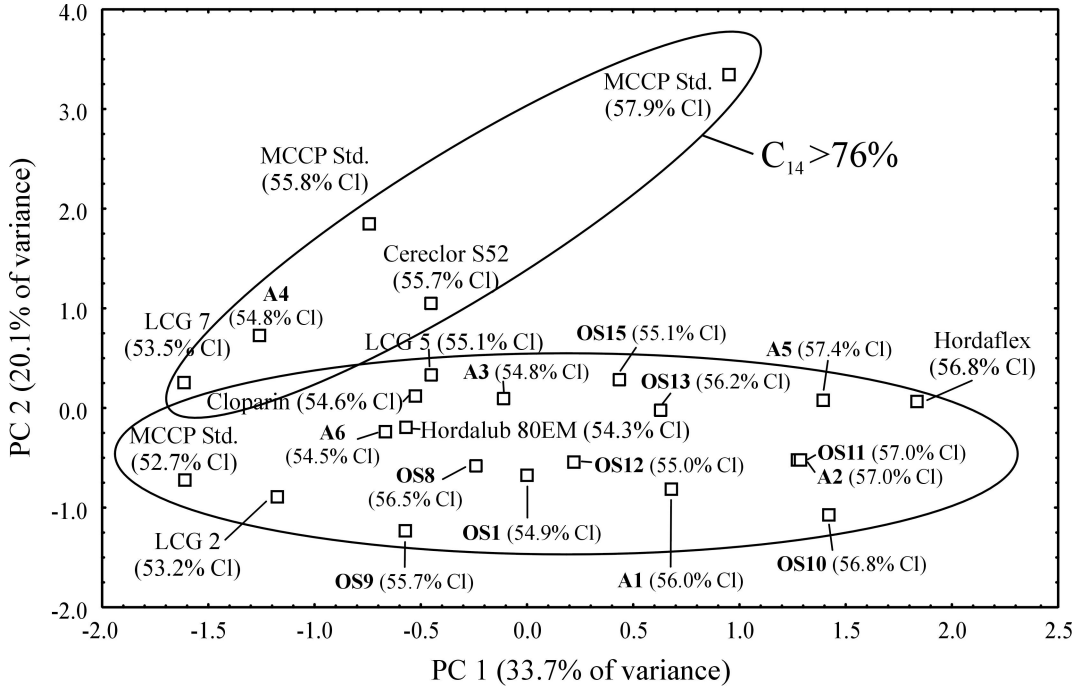

Figure 4.12: Principal components analysis score plot for seven technical MCCP formulations, three MCCP standard mixtures, 14 MCCP patterns determined in cod from northwest Europe (A1-A6) and from the Baltic Sea (OS1, OS8-OS13 and OS15). Chlorine contents were calculated from the ECNI-MS data.

Additionally, the congener group patterns of nine samples of dab and flounder from the North and Baltic Sea were investigated by PCA. Their SCCP and MCCP patterns were similar to technical CP mixtures, although different fish species were analysed. In this work congener group patterns of ten different fish species were determined. The PCA

results obtained for nine fish species were similar, not indicating any species specific biotransformation of CPs. Most variable results were observed for cod samples, however, these may be caused by geographical variation.

5 CONCLUSIONS AND OUTLOOK

The methodologies developed in this work allow the difficult determination of CPs in biota and, hence, enable the accomplishment of further studies concerning CP distribution in the environment.

Major problems of CP analysis were solved. Prior to this work only limited information about CP levels in Europe was available. In addition, published concentrations were hardly comparable. The new quantification allows a more precise determination of CP levels and, for the first time, a comparison of CP concentrations determined in different laboratories independently of the used standard mixture. Furthermore, the use of LRMS instead of expensive HRMS makes CP analysis affordable for many laboratories.

Studies about toxicology, environmental distribution, and fate of CPs are in an early stage due to the difficulties of CP analysis. The developed method proved to be suitable for the determination of CP congener group patterns in fish. Similarities and differences can be further investigated by principal components analysis. Hence, this offers new possibilities to elucidate changes of CP compositions in the environment, during biomagnification processes, and by long-range transport of CPs.

The emerging technique of GCxGC coupled to ECNI-MS may improve CP separation significantly in future opening new interesting aspects of CP analysis. Currently, GCxGC results are obtained by monitoring *m/z* 70-73, which correspond to the nonspecific $[Cl_2]^-$ and $[HCl_2]^-$ fragment ions. The choice of more specific ions would enhance selectivity further and identify characteristic isomers, which could be candidates for future toxicity

and bioaccumulation studies. The quantification approach described in this work should also be applicable for GCxGC-ECNI-MS opening new horizons in CP analysis.

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Paper I:

Limitations and possibilities of low resolution mass spectrometry in the electron capture negative ionization mode for the analysis of short and medium chain chlorinated paraffins.

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PAPER IN FOREFRONT

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Limitations of low resolution mass spectrometry in the electron capture negative ionization mode for the analysis of short- and medium-chain chlorinated paraffins

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Abstract The analysis of complex mixtures of chlorinated paraffins (CPs) with short (SCCPs, C_{10}–C_{13}) and medium (MCCPs, C_{14}–C_{17}) chain lengths can be disturbed by mass overlap, if low resolution mass spectrometry (LRMS) in the electron capture negative ionization mode is employed. This is caused by CP congeners with the same nominal mass, but with five carbon atoms more and two chlorine atoms less; for example $C_{11}H_{17}{}^{37}Cl^{35}Cl_6$ (*m/z* 395.9) and $C_{16}H_{29}{}^{35}Cl_5$ (*m/z* 396.1). This can lead to an overestimation of congener group quantity and/or of total CP concentration. The magnitude of this interference was studied by evaluating the change after mixing a SCCP standard and a MCCP standard 1+1 (S+MCCP mixture) and comparing it to the single standards. A quantification of the less abundant C_{16} and C_{17} congeners present in the MCCP standard was not possible due to interference from the major C_{11} and C_{12} congeners in the SCCPs. Also, signals for SCCPs (C_{10}–C_{12}) with nine and ten chlorine atoms were mimicked by MCCPs (C_{15}–C_{17}) with seven and eight chlorine atoms (for instance $C_{10}H_{12}Cl_{10}$ by $C_{15}H_{24}Cl_8$). A similar observation was made for signals from C_{15}–C_{17} CPs with four and five chlorine atoms resulting from SCCPs (C_{10}–C_{12}) with six and seven chlorine atoms (such as $C_{15}H_{28}Cl_4$ by $C_{10}H_{16}Cl_6$) in the S+MCCP mixture. It could be shown that the quantification of the most abundant congeners (C_{11}–C_{14}) is not affected by any interference. The determination of C_{10} and C_{15} congeners is partly disturbed, but this can be detected by investigating isotope ratios, retention time ranges and the shapes of the CP signals. Also, lower chlorinated compounds forming $[M+Cl]^-$ as the most abundant ion instead of $[M\text{-}Cl]^-$ are especially sensitive to systematic errors caused by superposition of ions of different composition and the same nominal mass.

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Introduction

Commercially produced chlorinated paraffins (CPs) are classified according to their carbon chain length into short chain CPs (SCCPs, C_{10}–C_{13}), medium chain CPs (MCCPs, C_{14}–C_{17}) and long chain CPs (LCCPs, >C_{17}). The chlorine content of these mixtures can vary from 30–70% depending on their application [1]. Technical CPs are mainly used as extreme pressure additives in lubricants and cutting oils as well as plasticizers and fire retardants. They have also found application as replacements for other persistent polychlorinated chemicals, such as polychlorinated biphenyls [2]. CPs are classified as persistent and non-biodegradable, and they accumulate in the food chain [3]. Though the global production of SCCPs has been reduced since the early 1980s [2], the overall annual production is still in the range of 380,000 tons [4]. Global redistribution by long-range atmospheric transport is suggested as the reason for the ubiquitous occurrence of CPs in the environment, including in remote areas such as the Canadian Arctic [5]. SCCPs are of particular interest due to the high amounts released into the environment, and due to them having the highest toxicity of all CP products [2].

The quantification of CPs is a demanding task. Although the levels of CPs were determined in environmental samples in the 1980s [6], as yet only very limited information has been published about the levels and fate of short- and medium-chain CPs in the environment [1]. This is mainly due to the extreme complexity of CP mixtures containing thousands of different isomers, enantiomers and diasteriomers. Currently, no gas chromatographic technique is able to separate CPs partly or completely into single isomers [7].

CP analysis is mainly carried out by high resolution gas chromatography (HRGC) coupled to high resolution (HR) mass spectrometry (MS) in the electron capture neg-

ative ionization (ECNI) mode. This is a very selective detection method, which eliminates interferences by other polychlorinated pollutants and by CPs with the same nominal mass [7]. However, this detection method is not available at many laboratories and is too costly for routine analysis.

Therefore, low resolution (LR) MS is also used for the quantification of CPs. An international intercomparison of methods for SCCP analysis showed that LRMS and HRMS can give comparable quantitative results [8]. Indeed, published levels of CPs in biota were similar when quantified by LRMS and HRMS [7, 9]. Nevertheless, the use of LRMS instead of HRMS increases the risk of interference, which has to be controlled and eliminated, if possible. An improved sample clean-up, removing other interfering polychlorinated compounds, is one possibility [10]. However, disturbances might also occur between molecular ions and fragments of CPs with the same nominal mass, if mixtures of SCCPs and MCCPs are present. This can result in an overestimation of the total PCA concentration.

This work investigates the risk from systematic errors due to mass interferences between SCCPs and MCCPs when using LRMS in the ECNI mode. Ways to minimize disturbances will be discussed in detail too, such as the careful selection of congener masses and retention time ranges, or by checking signal shapes and isotope ratios. It will be demonstrated that a simultaneous quantification of SCCPs and MCCPs and their differentiation in environmental samples is also possible by LRMS.

Experimental

Chemicals and solvents

Cyclohexane for pesticide residue analysis was purchased from Scharlau (Barcelona, Spain). Technical SCCPs ($C_{10–13}$, 55.5% chlorine, 100 ng/µl, solution in cyclohexane) and technical MCCPs ($C_{14–17}$, 52% chlorine, 100 ng/µl, solution in cyclohexane) as well as ε-hexachlorocyclohexane (ε-HCH, 10 ng/µl, solution in cyclohexane, purity 99.9%) were obtained from Ehrenstorfer (Augsburg, Germany). Solutions for analysis contained 25 ng/µl of the respective CP mixture and 50 pg/µl of ε-HCH.

Instrumentation

Chromatographic separations were carried out on an HP 5890II (Hewlett Packard, Palo Alto, USA) gas chromatograph equipped with a split/splitless injector and a fused silica capillary column (15 m, 0.25 mm i.d.) coated with a 0.25 µm-thick film of DB-5MS (crosslinked 5%-phenyl-95%-methylpolysiloxane, J&W Scientific, Folsom, USA). Sample volumes of 2 µl were injected in the splitless mode (splitless time 2 min) at an injector temperature of 275 °C. Helium (99.999%, Carbagas, Basel, Switzerland) was used as carrier gas at a column inlet pressure of 68.9 kPa (10 psi). The temperature program was: 100 °C, isothermal for 2 min, then 10 °C/min to 260 °C, isothermal for 10 min.

An HP 5989B mass spectrometer (Hewlett Packard, Palo Alto, USA) was employed in the electron capture negative ionization (ECNI) mode using methane (99.995%, Carbagas, Basel, Switzerland) as reagent gas at a pressure of 1.0–1.6 mbar (0.8–1.2 Torr). The mass spectrometer was tuned to optimal performance using perfluorotributylamine at *m/z* 283, 414 and 452. The electron energy was set to 100 eV. The ion source temperature was 200 °C, the quadrupole temperature 100 °C, and the transfer line temperature 280 °C. Compounds were detected in the selected ion monitoring (SIM) mode at a dwell time of 100 ms per ion using the two most abundant $[M\text{-}Cl]^-$ isotope ions of each CP congener (see Table 1) and *m/z* 254.9 for the internal standard ε-HCH.

Results and discussion

Studies of the composition of CP mixtures and congener-specific analyses of environmental samples are scarce due to the complexity of CP mixtures. HRMS at a resolution of 12,000 is often used to exclude interferences from CP fragments with the same nominal mass as the $[M\text{-}Cl]^-$ ions or from other organic pollutants not removed by the applied clean-up procedure [7]. As shown below, LRMS (~1000 resolving power) requires more detailed knowledge about possible interferences to enable the correct determination of CP compositions.

Congeners with similar nominal masses

Technical CPs contain thousands of isomers with the general elemental composition $C_nH_{2n+2-x}Cl_x$, resulting in some overlap of the chlorine isotope pattern of different CP congeners. Table 1 summarizes the mass-to-charge ratios of the two most abundant isotope signals of each congener normally used for quantification and identification of CPs [7]. It demonstrates that congeners with five carbon atoms more and two chlorine atoms less have a nearly identical nominal mass-to-charge ratio, which cannot be resolved by LRMS.

Influence of mass overlap on CP composition

A SCCP standard, a MCCP standard and a 1+1 mixture of both were used to study the influence of mass overlap on composition, at first ignoring correct isotope ratios and retention time ranges. The observed homologue and congener patterns of all three are given in Fig. 1. The SCCP mixture contained mainly congeners with C_{11} and C_{12} chains (relative contribution of ΣC_{11} 33%, of ΣC_{12} 38%). C_{10} and C_{13} congeners represented 8 and 21%, respectively. The MCCP mixture consisted mainly of C_{14} congeners (ΣC_{14} 75%, ΣC_{15} 21%, ΣC_{16} 2%, ΣC_{17} 2%).

Figure 1A shows that the presence of C_{15} and especially C_{16}, as well as C_{17} congeners in the SCCP mixture, is mimicked by masses with similar mass-to-charge ratios originating from C_{10}, C_{11} and C_{12} congeners with correspondingly five carbon atoms less and two chlorine atoms more (see Table 1 for details). C_{16} and C_{17} congeners cannot be quantified by LRMS, if C_{11} and C_{12} are major components in an environmental sample. Furthermore, a small amount of C_9 (2%) and C_{14} (1.5%) congeners were detected in the SCCP mixture.

On the other hand, C_{16} and C_{17} congeners are minor components in the MCCP mixture (see Fig. 1B) and do not therefore affect the quantification of the higher chlori-

Table 1 Mass-to-charge ratios of the $[M\text{-}Cl]^-$ ions (abbreviated to X in the table) of the two most abundant isotopes of SCCP and MCCP congeners used for quantification and identification

Short chain CPs			Medium chain CPs		
CP congener	Most abundant isotope (100%)	Second abundant isotope	CP congener	Most abundant isotope (100%)	Second abundant isotope
$C_9H_{14}Cl_6$	298.9 (X+2)	300.9 (X+4, 64%)	$C_{14}H_{26}Cl_4$	299.1 (X)	301.1 (X+2, 96%)
$C_9H_{13}Cl_7$	332.9 (X+2)	334.9 (X+4, 80%)	$C_{14}H_{25}Cl_5$	335.1 (X+2)	333.1 (X, 78%)
$C_9H_{12}Cl_8$	366.9 (X+2)	368.9 (X+4, 96%)	$C_{14}H_{24}Cl_6$	369.0 (X+2)	371.0 (X+4, 64%)
$C_9H_{11}Cl_9$	402.8 (X+4)	400.8 (X+2, 89%)	$C_{14}H_{23}Cl_7$	403.0 (X+2)	405.0 (X+4, 80%)
			$C_{14}H_{22}Cl_8$	436.9 (X+2)	438.9 (X+4, 96%)
			$C_{14}H_{21}Cl_9$	472.9 (X+4)	470.9 (X+2, 89%)
			$C_{14}H_{20}Cl_{10}$	506.9 (X+4)	504.9 (X+2, 78%)
$C_{10}H_{18}Cl_4$	243.1 (X)	245.1 (X+2, 96%)			
$C_{10}H_{17}Cl_5$	279.0 (X+2)	277.0 (X, 78%)			
$C_{10}H_{16}Cl_6$	312.9 (X+2)	314.9 (X+4, 64%) 64%	$C_{15}H_{28}Cl_4$	313.1 (X)	315.1 (X+2, 96%)
$C_{10}H_{15}Cl_7$	346.9 (X+2)	348.9 (X+4, 80%)	$C_{15}H_{27}Cl_5$	349.1 (X+2)	347.1 (X, 78%)
$C_{10}H_{14}Cl_8$	380.9 (X+2)	382.9 (X+4, 96%)	$C_{15}H_{26}Cl_6$	383.0 (X+2)	385.0 (X+4, 64%)
$C_{10}H_{13}Cl_9$	416.8 (X+4)	414.8 (X+2, 89%)	$C_{15}H_{25}Cl_7$	417.0 (X+2)	419.0 (X+4, 80%)
$C_{10}H_{12}Cl_{10}$	450.8 (X+4)	448.8 (X+2, 78%)	$C_{15}H_{24}Cl_8$	451.0 (X+2)	453.0 (X+4, 96%)
			$C_{15}H_{23}Cl_9$	486.9 (X+4)	484.9 (X+2, 89%)
			$C_{15}H_{22}Cl_{10}$	520.9 (X+4)	518.9 (X+2, 78%)
$C_{11}H_{20}Cl_4$	257.1(X)	259.1 (X+2, 96%)			
$C_{11}H_{19}Cl_5$	293.0 (X+2)	291.0 (X, 78%)			
$C_{11}H_{18}Cl_6$	327.0 (X+2)	329.0 (X+4, 64%)	$C_{16}H_{30}Cl_4$	327.1 (X)	329.1 (X+2, 96%)
$C_{11}H_{17}Cl_7$	360.9 (X+2)	362.9 (X+4, 80%)	$C_{16}H_{29}Cl_5$	363.1 (X+2)	361.1 (X, 78%)
$C_{11}H_{16}Cl_8$	394.9 (X+2)	396.9 (X+4, 96%)	$C_{16}H_{28}Cl_6$	397.0 (X+2)	399.0 (X+4, 64%)
$C_{11}H_{15}Cl_9$	430.9 (X+4)	428.9 (X+2, 89%)	$C_{16}H_{27}Cl_7$	431.0 (X+2)	433.0 (X+4, 80%)
$C_{11}H_{14}Cl_{10}$	464.8 (X+4)	462.8 (X+2, 78%)	$C_{16}H_{26}Cl_8$	465.0 (X+2)	467.0 (X+4, 96%)
			$C_{16}H_{25}Cl_9$	500.9 (X+4)	498.9 (X+2, 89%)
			$C_{16}H_{24}Cl_{10}$	534.9 (X+4)	532.9 (X+2, 78%)
$C_{12}H_{22}Cl_4$	271.1 (X)	273.1 (X+2, 96%)			
$C_{12}H_{21}Cl_5$	307.0 (X+2)	305.1 (X, 78%)			
$C_{12}H_{20}Cl_6$	341.0 (X+2)	343.0 (X+4, 64%)	$C_{17}H_{32}Cl_4$	341.1 (X)	343.1 (X+2, 96%)
$C_{12}H_{19}Cl_7$	374.9 (X+2)	376.9 (X+4, 80%)	$C_{17}H_{31}Cl_5$	377.1 (X+2)	375.1 (X, 78%)
$C_{12}H_{18}Cl_8$	408.9 (X+2)	410.9 (X+4, 96%)	$C_{17}H_{30}Cl_6$	411.1 (X+2)	413.1 (X+4, 64%)
$C_{12}H_{17}Cl_9$	444.9 (X+4)	442.9 (X+2, 89%)	$C_{17}H_{29}Cl_7$	445.0 (X+2)	447.0 (X+4, 80%)
$C_{12}H_{16}Cl_{10}$	478.8 (X+4)	476.8 (X+2, 78%)	$C_{17}H_{28}Cl_8$	479.0 (X+2)	481.0 (X+4, 96%)
			$C_{17}H_{27}Cl_9$	514.9 (X+4)	512.9 (X+2, 89%)
			$C_{17}H_{26}Cl_{10}$	548.9 (X+4)	546.9 (X+2, 78%)
$C_{13}H_{24}Cl_4$	285.1 (X)	287.1 (X+2, 96%)			
$C_{13}H_{23}Cl_5$	321.1 (X+2)	319.1 (X, 78%)			
$C_{13}H_{22}Cl_6$	355.0 (X+2)	357.0 (X+4, 64%)			
$C_{13}H_{21}Cl_7$	389.0 (X+2)	391.0 (X+4, 80%)			
$C_{13}H_{20}Cl_8$	422.9 (X+2)	424.9 (X+4, 96%)			
$C_{13}H_{19}Cl_9$	458.9 (X+4)	456.9 (X+2, 89%)			
$C_{13}H_{18}Cl_{10}$	492.9 (X+4)	490.9 (X+2, 78%)			

nated C_{11} and C_{12} congeners with corresponding mass. C_{16} and C_{17} congeners contribute less than 4% to the overall quantity of C_{11} and C_{12} congeners present in the 1+1 mixture. This is in agreement with published C_{16}+C_{17} contents of ≤7% in technical CPs and environmental samples [11]. Therefore, the systematic error by interference for C_{11} and C_{12} congeners is comparable to the quantification uncertainty. Trace amounts (<2%) of C_{13} congeners were present in the MCCP mixture. The C_9 (11%) and C_{10}(7%) congeners apparently present were mimicked by C_{14} and C_{15} congeners with similar mass-to-charge ratios (see Table 1). C_9 congeners are usually not determined in environmental samples, whereas the amount of C_{10} congeners could be overestimated.

Figure 1C shows the resulting change of pattern for a 1+1 mixture of SCCPs and MCCPs due to the interferences described above. The presence of C_{11} and C_{12} congeners led to a 3–25-fold overestimation of the concentrations of C_{16} and C_{17} congeners. The C_{15}–C_{17} congeners with four and five chlorine atoms (25%) apparently present were mimicked by the respective short chain congeners (C_{10}–C_{12}) with six and seven chlorine atoms (such as $C_{15}H_{28}Cl_4$ and $C_{10}H_{16}Cl_6$). In addition, traces (<1%) of C_{10}–C_{12} congeners with nine and ten chlorine atoms were

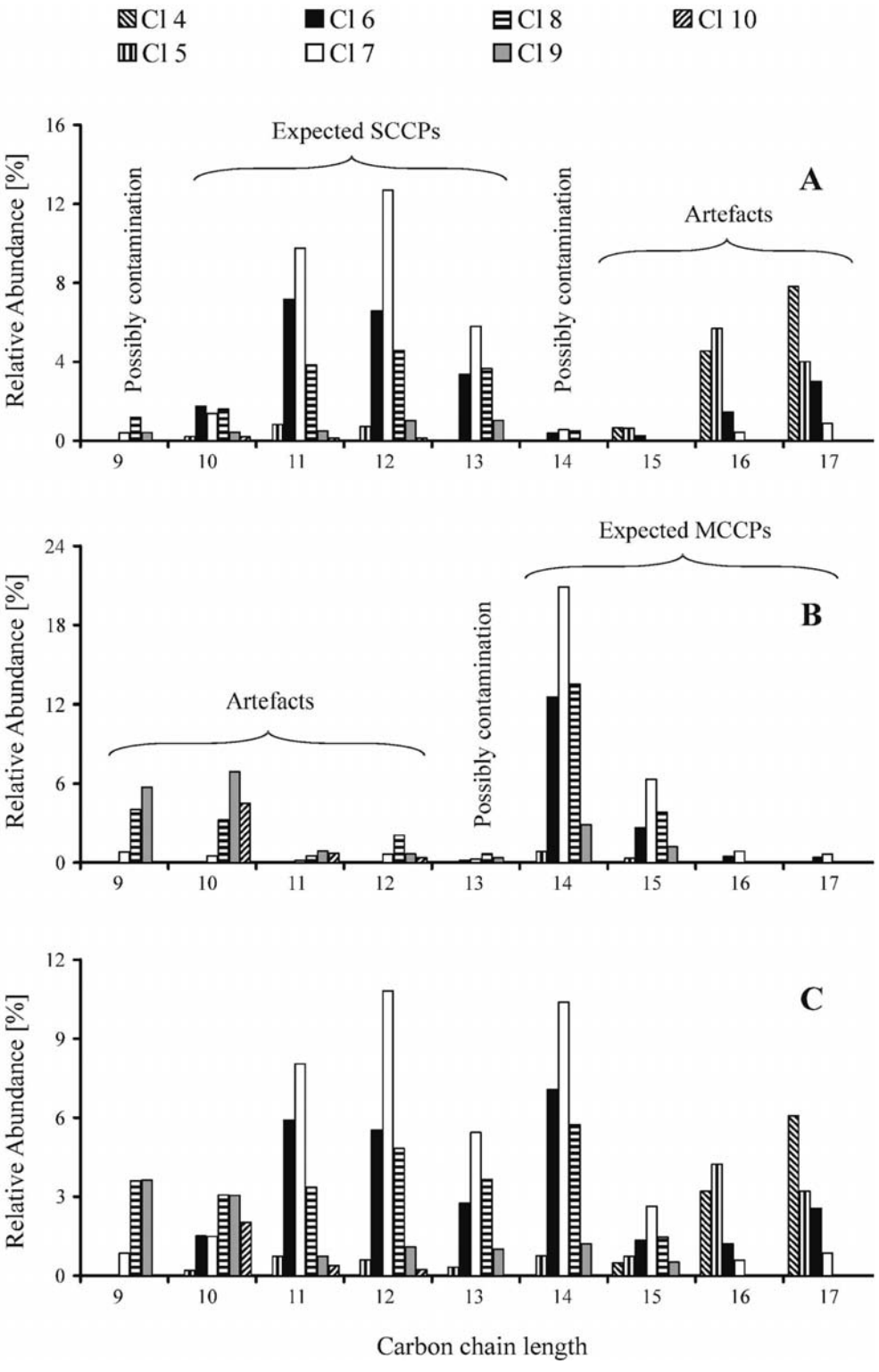

Fig. 1 Homologue and congener pattern (C_9–C_{17}) of a SCCP standard (**A**), a MCCP standard (**B**) and a mixture (1+1) of a SCCP and MCCP standard (**C**), as determined by HRGC-ECNI-LRMS

mainly simulated by the respective medium chain congeners (C_{15}–C_{17}) with seven and eight chlorine atoms (for example $C_{10}H_{12}Cl_{10}$ and $C_{15}H_{24}Cl_8$). Multiple HCl elimination, as reported by Coelhan [9], can also lead to corresponding interferences, but was not observed in this work, possibly due to other ion source conditions.

Identification of interferences by isotope ratios

One way to detect possible interferences due to co-eluting CPs is to control the chlorine isotope ratio. Here, the signal area of the most abundant $[M\text{-}Cl]^-$ isotope was divided by that of the second most abundant $[M\text{-}Cl]^-$ isotope, and this ratio was employed. A significant deviation (>0.10)

Table 2 Selected isotope ratios (higher mass divided by lower mass) of the signal area of the two most abundant isotopes of the $[M\text{-}Cl]^-$ ions (see Table 1) determined in a SCCP standard, a MCCP standard and in a 1+1 mixture. Allocations of origin are given for the latter. Isotope ratios have a precision of 0.10

CP congener	Isotope ratios			Possible origin of the signal in the S+MCCP mixture
	SCCP	MCCP	S+MCCP	
$C_{10}H_{18}Cl_4$	–	–	–	–
$C_{10}H_{17}Cl_5$	1.00	–	1.18	SCCP
$C_{10}H_{16}Cl_6$	1.60	–	1.58	SCCP
$C_{10}H_{15}Cl_7$	1.45	2.07	1.39	SCCP
$C_{10}H_{14}Cl_8$	2.65	1.30	1.55	[a]
$C_{10}H_{13}Cl_9$	0.34	1.59	1.12	[a]
$C_{10}H_{12}Cl_{10}$	0.63	1.85	1.64	[a]
$C_{11}H_{20}Cl_4$	–	–	–	–
$C_{11}H_{19}Cl_5$	0.96	–	0.90	SCCP
$C_{11}H_{18}Cl_6$	1.63	–	1.59	SCCP
$C_{11}H_{17}Cl_7$	1.28	0.41	1.26	SCCP
$C_{11}H_{16}Cl_8$	1.20	1.00	1.23	SCCP
$C_{11}H_{15}Cl_9$	0.48	1.48	0.67	[a]
$C_{11}H_{14}Cl_{10}$	0.44	2.12	0.89	[a]
$C_{12}H_{22}Cl_4$	–	–	–	–
$C_{12}H_{21}Cl_5$	0.87	–	0.85	SCCP
$C_{12}H_{20}Cl_6$	1.64	–	1.64	SCCP
$C_{12}H_{19}Cl_7$	1.27	1.62	1.31	SCCP
$C_{12}H_{18}Cl_8$	1.07	4.54	1.23	SCCP
$C_{12}H_{17}Cl_9$	1.01	0.26	0.57	[a]
$C_{12}H_{16}Cl_{10}$	0.96	0.35	0.43	MCCP
$C_{13}H_{24}Cl_4$	–	–	–	–
$C_{13}H_{23}Cl_5$	0.85	–	0.86	SCCP
$C_{13}H_{22}Cl_6$	1.52	1.82	1.47	SCCP
$C_{13}H_{21}Cl_7$	1.30	1.47	1.31	SCCP
$C_{13}H_{20}Cl_8$	1.02	3.16	1.10	SCCP
$C_{13}H_{19}Cl_9$	1.08	0.23	0.84	[a]
$C_{14}H_{26}Cl_4$	–	–	–	–
$C_{14}H_{25}Cl_5$	–	0.75	0.78	MCCP
$C_{14}H_{24}Cl_6$	0.54	1.74	1.56	MCCP
$C_{14}H_{23}Cl_7$	1.19	1.30	1.29	MCCP
$C_{14}H_{22}Cl_8$	0.63	1.07	1.06	MCCP
$C_{14}H_{21}Cl_9$	–	1.09	1.13	MCCP
$C_{15}H_{28}Cl_4$	1.56	–	1.52	SCCP
$C_{15}H_{27}Cl_5$	0.74	0.91	0.75	SCCP
$C_{15}H_{26}Cl_6$	0.60	1.87	1.32	[a]
$C_{15}H_{25}Cl_7$	–	1.33	1.27	MCCP
$C_{15}H_{24}Cl_8$	–	1.07	1.09	MCCP
$C_{15}H_{23}Cl_9$	–	1.11	1.23	MCCP
$C_{16}H_{30}Cl_4$	1.69	–	1.60	SCCP
$C_{16}H_{29}Cl_5$	0.59	–	0.60	SCCP
$C_{17}H_{32}Cl_4$	1.63	–	1.68	SCCP
$C_{17}H_{31}Cl_5$	0.76	–	0.74	SCCP

[a] Identification was not possible

should be observable as long as the number of chlorine atoms in the overlapping mass signal is different. Isotope ratios of the respective congeners groups were determined in the SCCP and the MCCP standard as well as in their 1+1 mixture. Isotope ratios and their origin or eventual disturbance are listed in Table 2.

The isotope ratios allowed to identify the origin of the CP signal for the following CPs: C_{10} with 5–7 chlorine atoms, C_{11}, C_{12} and C_{13} with 5–8 chlorine atoms, C_{14} with 5–9 chlorine atoms and C_{15} with 7–9 chlorine atoms (see Table 2). A disturbance was observed in eight of overall 43 isotope ratios.

As mentioned before, masses similar to medium chain congeners (C_{15}–C_{17}) with four and five chlorine atoms can originate from the respective short chain congeners (C_{10}–C_{12}) with six and seven chlorine atoms. This was also confirmed by the isotope ratios of these congeners. They were identical for $C_{15}H_{28}Cl_4$, $C_{16}H_{30}Cl_4$, $C_{17}H_{32}Cl_4$ and $C_{15}H_{27}Cl_5$, $C_{16}H_{29}Cl_5$ and $C_{17}H_{31}Cl_5$ in the 1+1 mixture and in the SCCP standard (see Table 2).

The two most abundant $[M\text{-}Cl]^-$ isotopes of higher chlorinated short chain congeners (7–9 chlorine atoms) overlap with those from compounds with two carbon atoms more and one chlorine atom less (for instance $C_{12}H_{17}{}^{37}Cl_2{}^{35}Cl_7$ by $C_{14}H_{22}{}^{37}Cl_5{}^{35}Cl_3$, see Table 3). Tomy et al. [7] also remarked upon this. The isotope ratios of short chain congeners (C_{10}–C_{13}) with nine or ten chlorine atoms were in the 1+1 mixture between those of the SCCP and MCCP standard and did not allow any allocation.

Control of retention times

CP congeners cannot be identified by retention time due to insufficient separation by HRGC. However, the retention time range of congener groups varies sufficiently to differentiate between SCCPs and MCCPs in many cases. Combining this information with the isotope ratios helps to determine whether a group of congeners in a sample originates from SCCPs, MCCPs or from both.

The mass chromatograms of the most abundant isotope signals of the elemental compositions of $C_{10}H_{14}Cl_8$ and $C_{15}H_{26}Cl_6$ are shown in Fig. 2 for a SCCP and a MCCP standard, and a mixture of both. These elemental compositions have the same nominal mass but different isotope ratios. Moreover, retention time ranges and overall signal shapes deviate. $C_{10}H_{14}Cl_8$ congeners eluted between 14–18 min for the SCCP mixture, and $C_{15}H_{26}Cl_6$ congeners between 16–20 min for the MCCP mixture. The shapes of the chromatographic signals were influenced by both the selected mass and the origin (s or m) of the CPs. In the S+MCCP mixture, the shape and the retention time range of the respective chromatographic signals, as well as the isotope ratios, deviated from those of the single CP standards due to the interferences between $C_{10}H_{14}Cl_8$ and $C_{15}H_{26}Cl_6$. For quantification, a selection of a retention time window for the main elution range of a congener group would allow us to improve selectivity somewhat, although at the risk of introducing a systematic error due to the cut-off of minor humps.

Formation of $[M+Cl]^-$

The ECNI mass spectra of CPs contain the three main ions $[M\text{-}HCl]^-$, $[M\text{-}Cl]^-$ and $[M+Cl]^-$ with a structure-de-

Table 3 Mass-to-charge ratios of $[M\text{-}Cl]^-$ ions (assigned as X in the table) of the most abundant isotopes of higher chlorinated short chain congeners, and the corresponding overlap with X+8 and X+10 isotopes of CP isomers with two carbons more and one chlorine less

Short chain CPs			Overlapping CP congener		
CP congener	Most abundant isotope (100%)	Second abundant isotope	CP congener	X+8 isotope	X+10 isotope
$C_{10}H_{15}Cl_7$	346.9 (X+2)	348.9 (X+4, 80%)	$C_{12}H_{20}Cl_6$	347.0 (3.3%)	349.0 (0.2%)
$C_{10}H_{14}Cl_8$	380.9 (X+2)	382.9 (X+4, 96%)	$C_{12}H_{19}Cl_7$	380.9 (8.2%)	382.9 (1.0%)
$C_{10}H_{13}Cl_9$	416.8 (X+4)	414.8 (X+2, 89%)	$C_{12}H_{18}Cl_8$	414.9 (16%)	416.9 (3.1%)
$C_{11}H_{17}Cl_7$	360.9 (X+2)	362.9 (X+4, 80%)	$C_{13}H_{22}Cl_6$	361.0 (3.3%)	363.0 (0.2%)
$C_{11}H_{16}Cl_8$	394.9 (X+2)	396.9 (X+4, 96%)	$C_{13}H_{21}Cl_7$	395.0 (8.2%)	397.0 (1.0%)
$C_{11}H_{15}Cl_9$	430.9 (X+4)	428.9 (X+2, 89%)	$C_{13}H_{20}Cl_8$	428.9 (16%)	430.9 (3.1%)
$C_{12}H_{19}Cl_7$	374.9 (X+2)	376.9 (X+4, 80%)	$C_{14}H_{24}Cl_6$	375.0 (3.3%)	377.0 (0.2%)
$C_{12}H_{18}Cl_8$	408.9 (X+2)	410.9 (X+4, 96%)	$C_{14}H_{23}Cl_7$	409.0 (8.2%)	411.0 (1.0%)
$C_{12}H_{17}Cl_9$	444.9 (X+4)	442.9 (X+2, 89%)	$C_{14}H_{22}Cl_8$	442.9 (16%)	444.9 (3.1%)
$C_{13}H_{21}Cl_7$	389.0 (X+2)	391.0 (X+4, 80%)	$C_{15}H_{26}Cl_6$	389.0 (3.3%)	391.0 (0.2%)
$C_{13}H_{20}Cl_8$	422.9 (X+2)	424.9 (X+4, 96%)	$C_{15}H_{25}Cl_7$	423.0 (8.2%)	425.0 (1.0%)
$C_{13}H_{19}Cl_9$	458.9 (X+4)	456.9 (X+2, 89%)	$C_{15}H_{24}Cl_8$	456.9 (16%)	458.9 (3.1%)

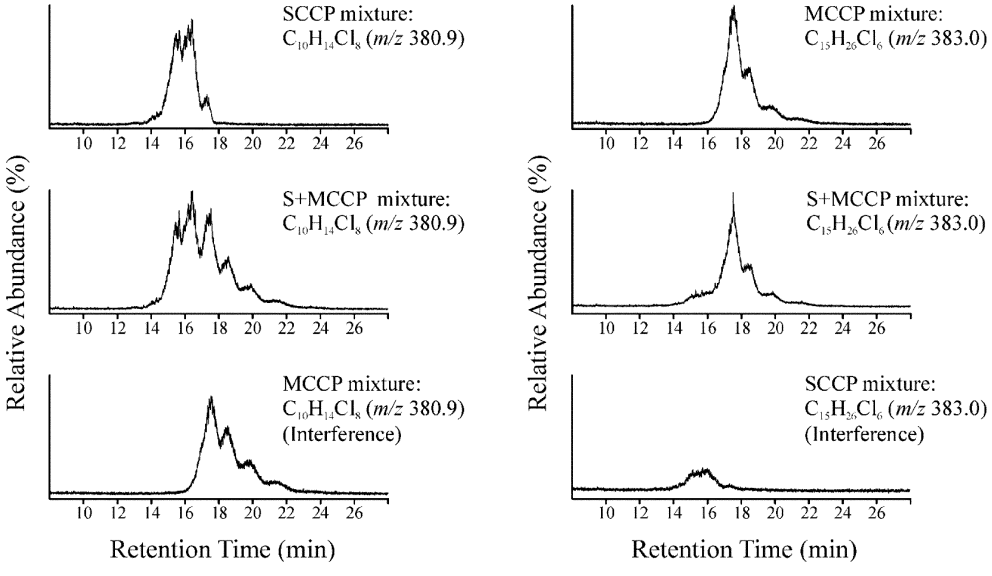

Fig. 2 ECNI-mass chromatograms of the $[M\text{-}Cl]^-$ ions of $C_{10}H_{14}Cl_8$(*m/z* 380.9) and of $C_{15}H_{26}Cl_6$(*m/z* 383.0) for a SCCP standard, a MCCP standard and a 1+1 mixture of both, respectively

pendent relative abundance. Tomy et al. [12] and later Zencak et al. [13] showed that the relative abundance of the adduct ion is much higher for lower chlorinated congeners (4–5 chlorine atoms) than for higher chlorinated ones. The $[M+Cl]^-$ ion was the base ion for the investigated single tetrachlorodecanes, but it decreased to about 60% for the pentachlorodecane and 10% for the hexachlorodecane [13].

As can be seen from Table 4, the $[M+Cl]^-$ ions from SCCP congeners with 4–6 chlorine atoms can also cause mass overlap. $[M\text{-}Cl]^-$ anions from MCCP congeners with five carbon atoms more and the same number of chlorine atoms may also disturb SCCPs.

In CP mixtures with a low chlorine content (<50%) and therefore more congeners with four chlorine atoms, the resulting superposition of $[M\text{-}Cl]^-$ ions of SCCPs with $[M+Cl]^-$ ions of SCCPs with two chlorine atoms less could lead to an overestimation of congeners with five and six chlorine atoms. The latter can also mimic the presence of MCCP congeners with five carbon atoms more and the same number of chlorine atoms. Such congeners are normally just detectable in MCCP mixtures with a chlorine content of <43%. Fortunately, the $[M+Cl]^-$ anions formed from MCCPs with low chlorine content (<50%) do not disturb the quantification of SCCPs. Zencak et al. [13] described a method to eliminate interferences between $[M+Cl]^-$ and $[M\text{-}Cl]^-$ ions by applying a mixture of CH_4/CH_2Cl_2 as reagent gas. This enhanced the formation of $[M+Cl]^-$ ions and suppressed the formation of $[M\text{-}Cl]^-$ ions for all congeners.

Linearity and detection limits

Linearity was investigated for two major congener groups ($C_{11}H_{18}Cl_6$,$C_{12}H_{20}Cl_6$) in the SCCP standard (55.5% chlorine content) and for two groups ($C_{14}H_{23}Cl_7$, $C_{15}H_{25}Cl_7$) in the MCCP standard (52% chlorine content). A good linearity, comparable to HRMS [7, 11], was achieved for LRMS between 1–100 ng of technical CPs for SCCPs and MCCPs (R^2>0.993, seven measuring points). The limit of

Table 4 Mass-to-charge ratios of $[M+Cl]^-$ ions of the most abundant isotopes of lower chlorinated short chain congeners, and the corresponding overlaps with the second most abundant isotope signals from $[M-Cl]^-$ ions of short chain congeners with the same number of carbons and two chlorines more. Interference with the $[M-Cl]^-$ ions of the most abundant isotope signals of MCCP congeners is also shown

[a] Second most abundant isotope

Short chain CPs		Short chain CPs		Medium chain CPs	
CP congener	$[M+Cl]^-$ (M+Cl+2)	CP congener	$[M-Cl]^-$ (M-Cl+4)	CP congener	$[M-Cl]^-$ (M-Cl+2)
$C_{10}H_{18}Cl_4$	315.0	$C_{10}H_{16}Cl_6$	314.9 (64%)	$C_{15}H_{28}Cl_4$	315.1 (96%)[a]
$C_{10}H_{17}Cl_5$	348.9	$C_{10}H_{15}Cl_7$	348.9 (80%)	$C_{15}H_{27}Cl_5$	349.1
$C_{10}H_{16}Cl_6$	382.9	$C_{10}H_{14}Cl_8$	382.9 (96%)	$C_{15}H_{26}Cl_6$	383.0
$C_{11}H_{20}Cl_4$	329.0	$C_{11}H_{18}Cl_6$	329.0 (64%)	$C_{16}H_{30}Cl_4$	329.1 (96%)[a]
$C_{11}H_{19}Cl_5$	362.9	$C_{11}H_{17}Cl_7$	362.9 (80%)	$C_{16}H_{29}Cl_5$	363.1
$C_{11}H_{18}Cl_6$	396.9	$C_{11}H_{16}Cl_8$	396.9 (96%)	$C_{16}H_{28}Cl_6$	397.0
$C_{12}H_{22}Cl_4$	343.0	$C_{12}H_{20}Cl_6$	343.0 (64%)	$C_{17}H_{32}Cl_4$	343.1 (96%)[a]
$C_{12}H_{21}Cl_5$	377.0	$C_{12}H_{19}Cl_7$	376.9 (80%)	$C_{17}H_{31}Cl_5$	377.1
$C_{12}H_{20}Cl_6$	410.9	$C_{12}H_{18}Cl_8$	410.9 (96%)	$C_{17}H_{30}Cl_6$	411.1

detection (LOD) for the two major components $C_{11}H_{18}Cl_6$ and $C_{12}H_{20}Cl_6$ of the SCCP mixture was 1 ng/µl of technical CPs at a signal-to-noise ratio of 3:1, and the limit of quantification (LOQ) was 2 ng/µl at a signal-to-noise ratio of 10:1. The LODs for the two major components $C_{14}H_{23}Cl_7$ and $C_{15}H_{25}Cl_7$ in the MCCP mixture were 0.5 and 1 ng/µl of technical MCCPs and the LOQs were 2.5 and 5 ng/µl, respectively. HRMS provides a better sensitivity. Analytical detection limits for major components of SCCPs and MCCPs were between ~60 pg and ~200 pg at a signal-to-noise ratio of 4:1 [7, 11]. However, the sensitivity of LRMS is well-suited for the analysis of CPs in the environment [10].

Conclusions

There is no doubt that using LRMS instead of HRMS will increase the risk of systematic errors due to mass interferences between different CP congeners, as discussed earlier. Nevertheless, the quantification of major congener groups is not affected by any interference (C_{10} with 5–7 chlorine atoms, C_{11}, C_{12} and C_{13} with 5–8 chlorine atoms, C_{14} with 5–9 chlorine atoms and C_{15} with 7–9 chlorine atoms). However, the determination of $C_{10}H_{14}Cl_8$ and $C_{15}H_{26}Cl_6$ congeners can be disturbed if both are present in a sample. An evaluation of isotope ratios, retention time ranges and HRGC signal shapes allows us to detect such problems and to avoid a corresponding overestimation of their concentrations. However, this does not work for the quantification of C_{16} and C_{17} congeners, and will lead to values that are too high, if high levels of C_{11} and C_{12} congeners are present. Furthermore, the presence of SCCPs with nine and ten chlorine atoms can be mimicked by MCCPs, and vice versa for MCCPs with four and five chlorine atoms by SCCPs. The sensitivity of LRMS is lower than for HRMS, but still appropriate for the determination of CPs in environmental samples. Also, despite the discussed interferences, the differentiation and simultaneous quantification of major SCCPs and MCCPs is still possible by LRMS.

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Paper II:

First study of congener group patterns and concentrations of short- and medium-chain chlorinated paraffins in fish from the North and Baltic Sea.

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First study of congener group patterns and concentrations of short- and medium-chain chlorinated paraffins in fish from the North and Baltic Sea

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Abstract

This study presents the first investigation of concentrations and congener group patterns of short- (SCCPs) and medium-chain chlorinated paraffins (MCCPs) in fish from the North and Baltic Sea. North Sea dab, cod and flounder were studied. High resolution gas chromatography (HRGC) coupled to low resolution mass spectrometry (LRMS) in the electron capture negative ionization mode (ECNI) was employed. Good linearity ($R^2 > 0.993$, 7 measuring points) was achieved between 1 and 100 ng/g of CP mixtures for SCCPs and MCCPs. The limits of detection were 0.5–1 ng/μl of CP mixture for the major congener groups of SCCPs and MCCPs. A clean-up comprising fat extraction, adsorption chromatography on silicagel impregnated with concentrated sulphuric acid and adsorption chromatography on Florisil was employed to avoid interferences from other polychlorinated compounds. Recoveries of CPs in spiked samples ranged between 80% and 100%. Accuracy was controlled with spiked samples and deviated not more than 10% from the expected values. Quantification was performed with standards of an average chlorine content as close as possible to that of the samples (SCCPs: 59–62%, MCCPs: 53–58%). SCCP concentrations ranged between 19 and 286 ng/g liver wet weight (ww), MCCP concentrations were comparable with a range of 25–260 ng/g ww. Congener group patterns were also determined and discussed. In samples from the Baltic Sea the SCCP congener pattern was similar to that of commercial SCCP mixtures or C_{13} congeners were most abundant. In samples from the North Sea a higher relative abundance of C_{10} congeners was observed.

Keywords: CPs; Polychlorinated *n*-alkanes; Clean-up; LRMS; Congener group pattern

1. Introduction

Commercial chlorinated paraffins (CPs) are complex mixtures of polychlorinated *n*-alkanes with a chlorination degree between 30% and 70% by weight. These mixtures are subdivided according to their carbon chain length into short chain (SCCP, C_{10}–C_{13}), medium chain (MCCP, C_{14}–C_{17}) and long chain ($C_{>17}$) CP products (Muir et al., 2000). Currently, over 200 commercial CP formulations are in use for a wide range of industrial applications (Alcock et al., 1999). CPs, especially SCCPs, are mainly utilized as extreme pressure additives in metal working fluids, as plasticisers and as flame retardants.

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Moreover, they are also applied to a minor degree as additives in rubbers, paints, coatings and sealant/adhesives, as well as in processing of leather and textiles (OSPAR Commission, 2001). MCCPs are more frequently employed as secondary plasticisers for polyvinyl chloride and other polymers (Muir et al., 2000). Since the introduction of CPs in the 1930s, the annual CP production worldwide increased to more than 300000 tons (Rossberg et al., 2003). Due to the phase out of many persistent organic pollutants (POPs), CPs have become the most frequently employed group of high molecular weight chlorinated hydrocarbons (Muir et al., 2000).

Of all the CP mixtures, SCCPs are of particular interest, because they have the greatest potential for environmental release (higher vapor pressure and higher water solubility) and the highest toxicity (Tomy et al., 1998a). They are classified as very toxic to aquatic organisms and may cause long term adverse effects in the aquatic environment (OSPAR Commission, 2001). Carcinogenicity in rats and mice was also observed (OSPAR Commission, 2001). SCCPs are furthermore classified as persistent, and their high octanol–water partition coefficient ($\log K_{ow}$ 4.4–8, depending on the degree of chlorination) implies a high potential for bioaccumulation (Muir et al., 2000). They are ubiquitously present in the environment including remote areas like the Canadian Arctic (Tomy et al., 1999, 2000). Therefore, a majority of the countries joining the "Convention for the Protection of the Marine Environment of the Northeast Atlantic" ("OSPAR Convention") voted in favour of a prohibition of releases to the sea, which implies a ban of their manufacture and use (Farrar, 2000). Moreover, the European Union included SCCPs in the list of *priority hazardous substances* (European Community, 2001) and decided an elimination of discharges, emissions and losses of SCCPs within the Water Framework Directive 2000/60/EC for the protection of the aquatic environment (European Community, 2000). Although the production of SCCPs has been voluntarily reduced from 13000 tons in 1994 to 4000 tons in 1998 by the European industry, it is assumed that the quantity of imported SCCPs is still huge (OSPAR Commission, 2001). Despite their unfavourable properties and their wide application, very limited knowledge is available about environmental concentrations, metabolic pathways and toxicokinetics of CP congeners compared to other organochlorine compounds such as PCBs or toxaphenes (Muir et al., 2000). Most of the few reported environmental concentrations have been measured in North America and information about CP concentration in biota from Europe concentrates on a few data from Norwegian fresh water lakes (Borgen et al., 2002). Therefore, a monitoring project was established by the German Federal Environmental Agency to support international decision makers such as PARCOM (Paris Commission of the "Convention for the Prevention of Marine Pollution from Landbased Sources") and OSPAR.

Currently, only a small number of laboratories analyse CPs in environmental samples worldwide (UNECE, 2003). The complex composition of the technical mixtures containing thousands of isomers, enantiomers and diasterioisomers is probably the main reason (Tomy et al., 1997). No gas chromatographic technique is able to separate CPs partly or completely into single isomers. High resolution gas chromatography (HRGC) allows to distinguish several humps of coeluting compounds (Froescheis and Ballschmiter, 1998). Currently, HRGC coupled to high resolution mass spectrometry (HRMS) and electron capture negative ionization (ECNI) is the standard method for CP determination (Muir et al., 2000). Quantification is carried out with CP mixtures of different degree of chlorination, since defined reference standards are not available (Tomy and Stern, 1999; Coelhan et al., 2000). HRMS shows low detection limits and high selectivity, which enables the complete suppression of interferences from other polychlorinated pollutants and from some CPs themselves (Tomy et al., 1997). However, this instrumentation is not available at many laboratories. Therefore, there is a need for a less complex detection method allowing an extensive monitoring of CPs.

The aim of this work was to establish the first data about CPs in fish from the North and Baltic Sea. Similarities and differences in observed concentrations and congener group patterns should also be studied. Since the overall detection limits for CPs are much higher than for other polychlorinated pollutants, higher sample amounts are necessary requiring pooling and a careful selection of fish individuals to allow a comparison. This makes sampling rather demanding. Moreover, the robustness of a simple clean-up technique combined with HRGC-ECNI low resolution mass spectrometry (LRMS) was investigated as an alternative. Despite its sufficient selectivity and sensitivity, the risk of interferences is higher than for HRMS. It was evaluated, if the improved clean-up technique as well as a precise control of retention times and isotope ratios of the CPs could compensate for this. The latter procedure and the risk of disturbances by mass overlap were described in detail elsewhere (Reth and Oehme, 2004). Additionally, it has already been shown that both HRMS and LRMS can give comparable results (Zencak et al., in press, 2004).

2. Experimental

2.1. Chemicals and solvents

Solvents of pesticide residue analysis quality were obtained from Scharlau (Barcelona, Spain). Technical

SCCPs (C_{10-13}, 55.5% chlorine, 100 ng/μl, solution in cyclohexane) and technical MCCPs (C_{14-17}, 52% chlorine, 100 ng/μl, solution in cyclohexane) as well as ε-hexachlorocyclohexane (ε-HCH, 10 ng/μl, solution in cyclohexane, purity 99.9%) were obtained from Ehrenstorfer (Augsburg, Germany). $^{13}C_{10}$-*trans*-chlordane (100 ng/μl, solution in nonane, purity 99%) was purchased from Cambridge Isotope Laboratories (Andover, USA). Florisil®PR (60–100 mesh) and sodium sulphate (Pestanal®) were obtained from Fluka (Buchs, Switzerland), and silica gel (200–400 mesh, 0.035–0.070 mm) from CU Chemie Uetikon AG (Uetikon, Switzerland). All three chemicals were dried overnight at 600 °C and kept afterwards for another 6 h at 130 °C before usage. Sixteen grams of dried Florisil®PR were deactivated with 240 μl of bidistilled water and shaken for 2 h before usage. A silica gel/sulphuric acid (44%) mixture was made by adding 10 ml of concentrated sulphuric acid to 41.6 g of dried silica gel. This mixture was shaken for 6 h before usage.

2.2. Fish samples

Despite the difficulties of collecting fish samples offshore and the small size of some fish livers, which extended the requirement for pooling, a total of 12 samples from 42 individuals captured at six locations in the North Sea and Baltic Sea within 10 days between August and September 2002 could be obtained from the Federal Research Centre for Fisheries (Hamburg, Germany). Liver samples of North Sea dab (*Limanda limanda*), cod (*Gadus morhua*) and flounder (*Platichthys flesus*) were partly pooled from two to five individuals (see Table 1) taking size and gender into consideration. A map of the capture locations is shown in Fig. 1. All samples were dissected immediately after their capture.

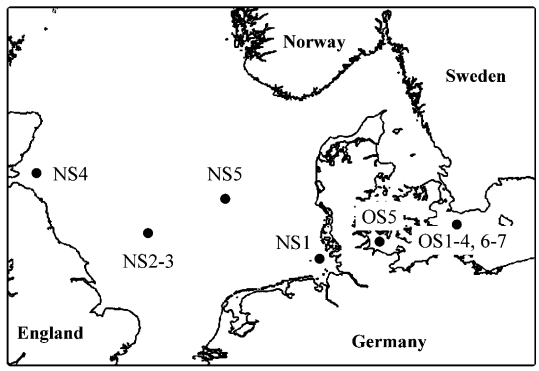

Fig. 1. Map of the capture locations of the samples NS1 to NS5 and OS1 to OS7 (for details see Table 1).

Great care was taken to avoid contact with plastic materials and other possible contamination sources. After transport by an air carrier on dry ice, they were stored at −24 °C until analysis.

2.3. Sample clean-up

The liver samples were homogenized with a 10-fold amount of water free sodium sulphate. The mixture was dry-packed into a glass column (30 cm long, 2.0 cm i.d.) and a 1 cm layer of water free sodium sulphate was placed on top. Ten nanograms of $^{13}C_{10}$-*trans*-chlordane (internal standard) in 10 μl of cyclohexane were added onto the water free sodium sulphate prior to column extraction of lipids with 250 ml of *n*-hexane/dichloromethane (1 + 1, v/v). Afterwards solvents were removed with a Turbo Vap 500 (Zymark, Hutchinson, USA). For lipid removal a glass column (30 cm long, 2.0 cm i.d.) filled with 1 g of water free sodium sulphate, 40 g of a silica gel/sulphuric acid (44%)

Table 1
Details about the analysed fish samples (species, sex, capture location, capture date, number of pooled livers, and length of the fishes)

Sample no.	Species	Sex	Capture location	Date	Pooled livers	Length [cm]
			Baltic Sea			
OS1	Cod (*Gadus morhua*)	*	54°47′ N/13°06′ E	31.08.02	5	28–31
OS2	Flounder (*Platichthys flesus*)	m	54°46′ N/13°18′ E	31.08.02	1	24
OS3	Flounder (*Platichthys flesus*)	m	54°44′ N/13°10′ E	31.08.02	2	28–30
OS4	Flounder (*Platichthys flesus*)	f	54°45′ N/13°20′ E	31.08.02	2	29–34
OS5	North Sea dab (*Limanda limanda*)	f	54°31′ N/10°39′ E	03.09.02	5	20–23
OS6	Cod (*Gadus morhua*)	*	54°51′ N/14°01′ E	01.09.02	1	25
OS7	Cod (*Gadus morhua*)	*	54°51′ N/14°01′ E	01.09.02	1	25
			North Sea			
NS1	North Sea dab (*Limanda limanda*)	f	54°15′ N/7°29′ E	25.08.02	5	19–22
NS2	North Sea dab (*Limanda limanda*)	f	54°30′ N/2°16′ E	26.08.02	5	20–22
NS3	Cod (*Gadus morhua*)	*	54°43′ N/2°07′ E	26.08.02	5	22–25
NS4	North Sea dab (*Limanda limanda*)	f	56°18′ N/2°04′ W	27.08.02	5	20–23
NS5	North Sea dab (*Limanda limanda*)	f	55°30′ N/4°40′ E	29.08.02	5	20–24

f: female, m: male, *: not determined.

mixture and 1 g of water free sodium sulphate was conditioned with 50 ml of *n*-hexane/dichloromethane (1 + 1, v/v). The fat extract was transferred to the column and eluted with 130 ml of *n*-hexane/dichloromethane (1 + 1, v/v) under an air pressure of 96.5 hPa generated by a diaphragm pump. The eluate was evaporated to 0.2 ml with a Turbo Vap 500, diluted and reduced twice to 0.2 ml after adding 10 ml of *n*-hexane. The extract was transferred to a Florisil® column (20 cm long, 1.5 cm i.d.) packed with 1 g of water free sodium sulphate, 16 g of Florisil® (deactivated with 1.5% of water) and 1 g of water free sodium sulphate. The column was conditioned with 20 ml of *n*-hexane and the sample eluted with 60 ml of *n*-hexane and 7 ml of dichloromethane (prefraction, containing PCBs and toxaphenes) and 60 ml of dichloromethane (CP-fraction). The CP-fraction was concentrated to 0.2 ml, 20 ml of cyclohexane were added and finally the volume reduced to 100 µl with a Turbo Vap 500. Ten nanograms of ε-HCH in 10 µl of cyclohexane were added as recovery standard to the sample extract before further analysis by HRGC-ECNI-LRMS.

For the determination of recovery or accuracy 1.5 µg of SCCPs and/or 5 µg of MCCPs in 150 µl of cyclohexane were spiked to 10 g of homogenized mackerel muscle or to 5 g of fish oil, which proved to be free of CPs. The sample clean-up was then performed as described above. In order to minimize the risk of contamination only glassware heated to 450 °C for 3 h as well as heat-treated sodium sulfate, silica gel and Florisil® was used for the CP analysis. Washing procedures for glassware including rinsing with detergent (Deconex, VWR International, Dietikon, Switzerland) and heating to 270 °C turned out to be insufficient for an elimination of CPs. Method blanks consisting of 20 g of sodium sulfate (preheated at 600 °C for 6 h) were extracted and analyzed in the same manner as the samples.

2.4. Instrumentation

Chromatographic separations were carried out on an HP 5890II (Hewlett Packard, Palo Alto, USA) gas chromatograph equipped with a split/splitless injector and a fused silica capillary column (15 m, 0.25 mm i.d.) coated with a 0.25 µm thick film of DB5-MS (5% phenyl-methylpolysiloxane, J&W Scientific, Folsom, USA). Sample volumes of 1.5 µl were injected in the splitless mode (2 min) at an injector temperature of 275 °C. Helium (99.999%, Carbagas, Basel, Switzerland) was used as carrier gas at a column inlet pressure of 68.9 kPa (10 psi). The temperature program was as follows: 100 °C, isothermal for 2 min, then 10 °C/min to 280 °C, isothermal for 8 min.

An HP 5989B (Hewlett Packard, Palo Alto, USA) mass spectrometer was employed in the ECNI mode using methane (99.995%, Carbagas, Basel, Switzerland) as reagent gas at a pressure of 120 Pa (0.9 Torr). The mass spectrometer was tuned to optimal performance using perfluorotributylamine at *m/z* 283, 414 and 452. The electron energy was 100 eV. The ion source temperature was 200 °C, the quadrupole temperature 100 °C and the transfer line temperature 280 °C.

2.5. Identification and quantification

Under ECNI conditions, CPs form mainly the $[M-Cl]^-$, $[M-HCl]^-$ and $[M+Cl]^-$ ions (Tomy et al., 1998b; Zencak et al., 2003). According to Tomy et al. (1997) the $[M-Cl]^-$ ion of each congener group was selected for quantification. A dwell time of 100 ms per ion was used in the selected ion monitoring (SIM) mode. Congeners with 10–15 carbon atoms and 5–9 chlorine atoms were analyzed. The mass-to-charge ratios used for their quantification and identification are published elsewhere (Reth and Oehme, 2004). The most abundant isotope was used for quantification, the second abundant isotope for identification of possible interferences from CPs themselves or from interfering compounds. Congener groups were identified by the retention time, by the signal shape and by the correct isotope ratio (Reth and Oehme, 2004). For the internal standard the most abundant isotope of the $[M]^-$ ion (*m/z* 419.8) was recorded, for the recovery standard ε-HCH the most abundant isotope of the $[M\text{-}Cl]^-$ ion (*m/z* 254.9). The quantification procedure applied was similar to that described previously by Tomy et al. (1997) with two exceptions. Theoretical correction factors for different isotope abundances as well as for different response factors were not taken into account (see Results and discussion).

3. Results and discussion

3.1. Clean-up efficiency

For CP analysis HRMS at a resolution of 12 000 is often used to exclude interferences from other organic pollutants not removed by the applied clean-up procedure (Tomy et al., 1997). The use of LRMS (unit resolution) requires a more efficient clean-up to eliminate other interfering chlorinated contaminants with similar mass-to-charge ratios and retention time range such as toxaphenes, PCBs and chlordanes. Due to the hundreds of CP isomers present, HRGC-signals are usually very broad and have elution time ranges of up to 4 min. Therefore, HRGC allowed only to separate CPs from those contaminants, which are more volatile like hexachlorobenzene or hexachlorocyclohexanes. Other interfering compounds had to be removed by adsorption chromatography on Florisil and silica gel impregnated with concentrated sulphuric acid. Table 2 summarises the distribution of selected organochlorines just after the Florisil® column. PCB 153 and PCB 197, Toxaphene

Table 2
Distribution [%] of PCB 153 and 197, toxaphene #44 and #62, *o*,*p*′-DDT and α-HCH between the pre- and CP-fraction of the adsorption chromatography on Florisil® (Prefraction: 60 ml of *n*-hexane and 7 ml of dichloromethane, CP-fraction: 60 ml of dichloromethane)

Compound	Distribution [%]	
	Prefraction	CP-fraction
$C_{10}H_{16}Cl_6$	5	95
$C_{13}H_{20}Cl_8$	0	100
PCB 153	100	0
PCB 197	100	0
Toxaphene #44	100	0
Toxaphene #62	99	1
o,*p*′-DDT	100	0
α-HCH	68	32

#44 and #62 as well as *o*,*p*′-DDT eluted in the prefraction and were therefore completely separated from CPs. Thirty-two percent of α-HCH were still in the CP-fraction, but could be removed later by GC.

3.2. Linearity and detection limits

Linearity was investigated for two major congener groups ($C_{11}H_{18}Cl_6$, $C_{12}H_{20}Cl_6$) of the SCCP standard (55.5% chlorine content) and for two groups ($C_{14}H_{23}Cl_7$, $C_{15}H_{25}Cl_7$) of the MCCP standard (52% chlorine content). A good linearity, comparable to HRMS (Tomy et al., 1997; Tomy and Stern, 1999) and EI-MS/MS (Zencak et al., 2004), was achieved for LRMS between 1 and 100 ng of technical CPs for SCCPs and MCCPs ($R^2 > 0.993$, 7 measuring points). The limit of detection (LOD) for the two major congener groups $C_{11}H_{18}Cl_6$ and $C_{12}H_{20}Cl_6$ of the SCCP standard was 1 ng/μl of technical CPs at a signal-to-noise ratio of 3:1, and the limit of quantification (LOQ) was 2 ng/μl at a signal-to-noise ratio of 10:1. The LODs for the two major congener groups $C_{14}H_{23}Cl_7$ and $C_{15}H_{25}Cl_7$ in the MCCP standard were 0.5 and 1 ng/μl of technical MCCPs and the LOQs were 1.5 and 3 ng/μl, respectively. However, HRMS and EI-MS/MS provide a better sensitivity. HRMS detection limits for major components of SCCPs and MCCPs were between ~60 pg and ~200 pg at a signal-to-noise ratio of 4:1 (Tomy et al., 1997; Tomy and Stern, 1999). EI-MS/MS allowed the detection of 100–200 pg (Zencak et al., 2004). Nevertheless, the sensitivity of LRMS is well suited for the analysis of CPs in the environment.

3.3. Quantification

Quantification was performed similarly to the quantification procedure described by Tomy et al. (1997) calculating the total CP amount over the average molecular weight of the sample and of the standard. For sample NS3 (286 ng/g SCCPs) calculations were performed with and without theoretical correction factors for different isotope abundances as well as for different response factors as described by Tomy et al. (1997). The difference in the SCCP concentration was 1% (283 ng/g), since these two corrections compensated each other. Correcting just the different isotope abundances of the congeners increased the SCCP amount by 1.7% (291 ng/g), whereas the correction of response factors decreases the SCCP amount by 2.4% (279 ng/g). Since no significant differences were observed the quantification procedure was kept as simple as possible.

3.4. Quality control

Recoveries of SCCPs, MCCPs and of the internal standard $^{13}C_{10}$-*trans*-chlordane in spiked mackerel muscle tissue and fish oil (1.5–5 μg CP mixture, $n = 5$) were within 80–100%. Accuracy was controlled with spiked samples and was within 10% of the expected values. Method blanks consisting of sodium sulfate were equal to the detection limits.

3.5. Selection of reference standards

To minimize systematic errors of the quantification due to a deviating chlorine content between sample and quantification reference (Coelhan et al., 2000), standards were selected with an average molecular weight and chlorine content as similar as possible (see Table 3). A SCCP standard with 55.5% chlorine and a MCCP standard with 52% chlorine content were best suited. The measured chlorine content of the CP standards was 8% higher than the chlorine content specified by the manufacturer. This is caused by the strong dependence of the degree of chlorination on the ECNI response factors. Congeners with 3 and 4 chlorine atoms cannot be detected by ECNI-MS, whereas the amount of congeners with 7 and more chlorine atoms may be overestimated. Coelhan (2002) reported that the estimation of the chlorine content of CP mixtures by ECNI-MS led to systematic deviation for chlorine contents <55% due to the low response factors for low chlorinated CPs. Zencak et al. (2003) and later Moore et al. (2004) showed that this problem can be minimized by using chemical ionization techniques. These show more uniform response factors, but are not well suitable for routine analysis.

3.6. CP concentrations in fish liver samples

SCCPs could be detected in all analysed fish liver samples. MCCPs were also present except in sample NS4. Concentrations and lipid contents are summarized in Table 4. The SCCP concentrations varied between 19 and 286 ng/g (wet weight). The MCCP quantities

Table 3
Measured average molecular weight and chlorine content of the SCCPs and MCCPs in the analysed samples and standards used for quantification

Sample no.	Species	SCCPs		MCCPs	
		Average molecular weight [g/mol]	Calculated chlorine content [%]	Average molecular weight [g/mol]	Calculated chlorine content [%]
OS1	Cod	388	60	425	55
OS2	Flounder	420	61	431	56
OS3	Flounder	412	60	453	58
OS4	Flounder	412	62	447	57
OS5	North Sea dab	382	62	411	53
OS6	Cod	413	61	468	58
OS7	Cod	414	62	450	56
NS1	North Sea dab	397	59	443	57
NS2	North Sea dab	392	60	433	56
NS3	Cod	394	61	445	57
NS4	North Sea dab	374	61	–	–
NS5	North Sea dab	394	61	436	56
	SCCP standard	398 ± 7*	60 ± 1*		
	MCCP standard			436 ± 2**	56 ± 1**

* $n = 7$.
** $n = 4$.

Table 4
CP concentrations and lipid content of the analysed samples (SCCPs: $\sum C_{10}$–C_{13}, MCCPs: $\sum C_{14}$–C_{15}, ng/g wet weight)

Sample no.	Species	Lipid content [%]	SCCPs	MCCPs
			$\sum C_{10}$–C_{13} [ng/g wet weight]	$\sum C_{14}$–C_{15} [ng/g wet weight]
	Baltic Sea			
OS1	Cod	49	143	106
OS2	Flounder	33	127	206
OS3	Flounder	34	99	31
OS4	Flounder	33	221	115
OS5	North Sea dab	41	48	130
OS6	Cod	49	19	25
OS7	Cod	52	42	75
	North Sea			
NS1	North Sea dab	50	169	123
NS2	North Sea dab	52	286	260
NS3	Cod	44	90	32
NS4	North Sea dab	54	26	<10
NS5	North Sea dab	32	37	221

(25–260 ng/g wet weight) were in a similar range (see Table 4). These results were in good agreement with the few CP concentrations determined worldwide in fish (SCCPs: 100–1700 ng/g lipid weight (Muir et al., 2000; Borgen et al., 2001); MCCPs (C_{14}–C_{17}): 68–904 ng/g wet weight (Tomy and Stern, 1999).

3.7. Congener group pattern

Currently, only few congener group patterns of CPs have been published. In the analyzed samples the MCCP congener group patterns were similar to that of the MCCP mixture (C_{14}: 78%). Congeners with 14 carbon atoms (>60%) were more abundant than C_{15} congeners (see Fig. 2). However, the observed homologue and congener patterns of SCCPs differed from sample to sample (see Fig. 3). Congeners with 11 and 12 carbon atoms were usually most abundant in the samples as also typical for commercial SCCP mixtures.

NS4 and NS5 contained low SCCP concentrations, which did not allow to determine the CP pattern properly. Sample NS1 was caught much closer to the estuary of the Elbe and had a higher relative concentration of C_{13} congeners (see Fig. 3). However, the samples NS2

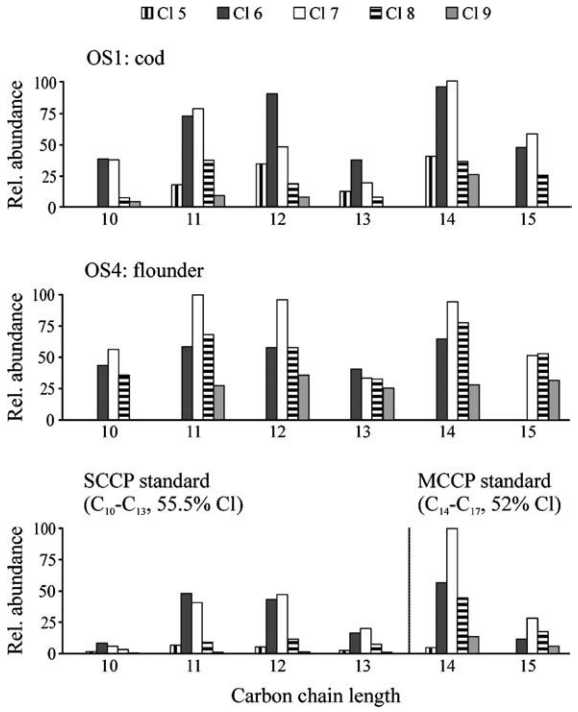

Fig. 2. Congener pattern (C_{10}–C_{15}) of sample OS1, OS4 (see Table 1), of the SCCP standard (C_{10}–C_{13}, 55.5% chlorine) and MCCP standard (C_{14}–C_{15}, 52% chlorine) used for quantification.

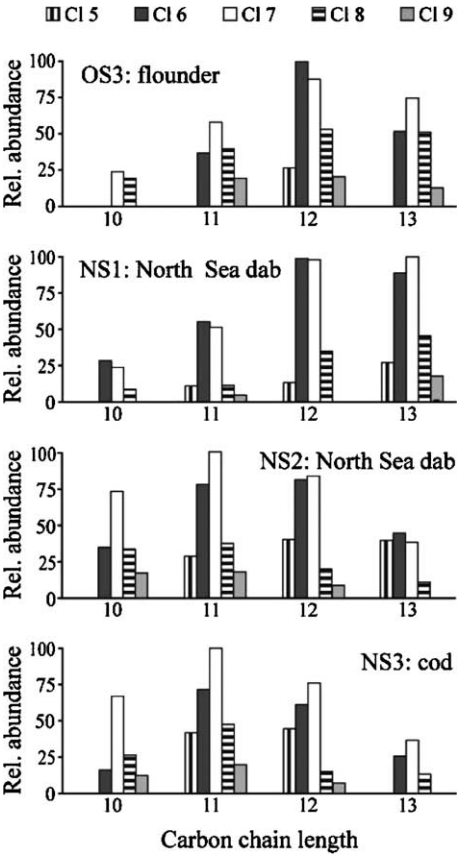

Fig. 3. Congener pattern (C_{10}–C_{13}) of sample OS3, NS1, NS2 and NS3 (see Table 1).

and NS3 further away in the North Sea, showed a higher relative amount of C_{10} congeners compared to the other samples and the standard mixture. Tomy et al. (2000) observed a predominance of the shorter chain congeners (C_{10} and C_{11}) in marine mammals from the Canadian Arctic and suggested that their higher relative abundance was due to long range atmospheric transport, since C_{10} and C_{11} congeners have higher vapor pressures (Drouillard et al., 1998). Samples from urbanised and industrialized areas showed a higher relative abundance of less volatile congeners and a pattern closer to commercial CP mixtures (Tomy et al., 2000). Similar observations were made by Marvin et al. (2003) for sediments from Lake Ontario. They reported of a higher relative abundance of C_{13} congeners in sediments potentially influenced by local industrial sources of SCCPs.

Only two sample locations in the Baltic Sea were studied. OS1 to OS7 showed a SCCP composition, which was quite similar to that of commercial CP mixtures. For OS5 to OS7 it was difficult to obtain a proper CP pattern due to low SCCP concentrations. OS1 and OS4 to OS7 had similar relative C_{10} and C_{13} abundances, whereas OS2 and OS3 showed a higher relative abundance of C_{13} compared to C_{10} congeners (see Fig. 3).

A comparison of congener patterns in biota is difficult, since there are many parameters influencing the concentration ratio between congener groups. Examples are environmental processes including transport and distribution as well as changes during bioaccumulation or metabolization. Such a comparison requires a large number of data and was outside the scope of this work, which intended to demonstrate the applicability of the developed method and to obtain some indications about the variability of CP patterns and CP concentrations.

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Paper III:

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New quantification procedure for the analysis of chlorinated paraffins using electron capture negative ionization mass spectrometry

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Abstract

An improved quantification procedure for the analysis of chlorinated paraffins (CPs) is presented based on electron capture negative ionization mass spectrometry. It compensates differences in response factors between reference CP mixtures and the CP pattern present in environmental samples. The use of a CP standard with a matching degree of chlorination is no longer necessary. It could be shown that the response factors of C_{10}-, C_{11}-, C_{12}- and C_{13}-CP mixtures of both 50 and 60% chlorine content were only slightly influenced by the carbon chain length. A linear correlation ($R^2 = 0.965$) between the total response factor of a CP mixture and its chlorine content was obtained for seven short chain chlorinated paraffin mixtures (SCCP, C_{10}–C_{13}) with different composition and chlorine content (51–69%). Maximum single deviations were <7% for this reference set. It allowed to determine the correct total response factor of the CP composition present in a sample. The deviations were not more than 7–33% for five independent SCCP control samples compared to up to 373% for the conventional procedure. The procedure was tested by quantifying the SCCP and MCCP levels in 10 fish liver samples. The proposed method allowed to compensate the influence of the degree of chlorination of the applied reference standard on the total response factor.

Keywords: Polychlorinated *n*-alkanes; PCAs; CPs; Quantification; ECNI mass spectrometry

1. Introduction

The environmental analysis of polychlorinated compounds applied as technical mixtures such as polychlorinated biphenyls, toxaphenes or chlorinated paraffins (CPs) is very demanding due to their complex composition, changes in the congener patterns in the environment and the lack of suitable reference standards. The presence of thousands of different isomers, enantiomers and diastereomers in CP mixtures makes them the real challenge of all.

CPs contain polychlorinated *n*-alkanes and are subdivided according to their carbon chain length into short chain CPs (SCCPs, C_{10}–C_{13}), medium chain CPs (MCCPs, C_{14}–C_{17}) and long chain CPs (LCCPs, $C_{>17}$). Furthermore, the degree of chlorination can vary between 30 and 70% depending on the field of application [1].

Currently, worldwide only a few laboratories analyse CPs [2], though these compounds are classified as persistent, bioaccumulate and are toxic to aquatic organisms [3]. The demonstrated presence of CPs in air, sediments, fish and marine mammals underlines the necessity for a more permanent monitoring [4–7]. CPs are listed in the priority substance list of the European water framework directive. Consequently, environmental levels of CPs should be monitored in Europe in 2006, which will require reliable analytical methods and quantification procedures [8,9]. The current standard analysis technique is high-resolution gas chromatography (HRGC) combined with high-(HRMS) or low-resolution mass spectrometry (LRMS) in the electron capture negative ion (ECNI) mode [4,10,11]. Quantification is usually performed with technical or synthetically adapted CP mixtures as well as CP compositions of defined carbon

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chain lengths [4,10,12]. Congener groups with 5–10 chlorine atoms are usually quantified in environmental samples [4,10].

The high selectivity and sensitivity of ECNI-MS is well documented [13]. However, its main limitation is the strong dependence of the response factors of congeners on their degree of chlorination [14]. The use of well-defined single references compounds is not possible, since environmental samples still contain thousands of congeners of variable composition, which cannot be separated by HRGC. Therefore, only quantification of single congener groups with many unresolved isomers is possible [10].

The quantification procedure described by Tomy et al. is mainly applied [10]. However, the results are strongly influenced by the degree of chlorination of the selected technical standard [15]. Moreover, technical mixtures can contain interfering additives such as stabilizers [10]. Even mixtures from tailored synthesis do not match well with the composition of CPs in environmental samples leading to differences in response factors [4]. Tomy et al. and Coelhan et al. already reported that quantification differences of 100% and more are possible, if the chlorine content of the standard mixture is changed or does not fit to the sample [10,12]. Later, Zencak et al. quantified three SCCP mixtures with different degree of chlorination against each other and showed that errors of 65–940% can occur, when ECNI-MS is applied [15]. The quantified amount was lower, if the employed standard had a higher chlorine content and vice versa. Congeners with a higher degree of chlorination have higher response factors due to a higher electron affinity [13]. Consequently, quantification has to be carried out with a standard composition similar to that of the sample. Coelhan et al. tried to compensate this with mixtures of homologues of different chlorine contents and by selecting standards for each chain length with mass spectra as similar as possible to those of the respective homologue groups in the sample [12]. However, this also requires a large number of standard mixtures and the interpretation of the data is time-consuming. Moreover, it may be difficult to define “similar”.

Recently, three new mass spectrometric methods have been developed to minimize the quantification problem. The first one is based on electron ionization tandem mass spectrometry (EI-MS/MS) and allows a fast determination of the total CP amount, but cannot distinguish between SCCPs, MCCPs and LCCPs [16]. The second one applies negative ion chemical ionization (NICI) MS with CH_4/CH_2Cl_2 as reagent gas. This leads to equal response factors for congeners of different degree of chlorination and enables the precise determination of CP congener patterns [14]. However, it is not suitable for a high sample throughput due to a rapid contamination of the ion source [14]. The third technique uses metastable atom bombardment combined with high-resolution MS [17]. A recently published comparison showed that ECNI-LRMS, ECNI-HRMS, EI-MS/MS and NICI-MS give comparable results for standards and spiked samples. However, a difference in the CP chlorine content between sample and standard can result in deviations of >100% for ECNI-MS due to changed response factors [15].

Therefore, this study developed a compensation technique, which minimized the influence of the degree of chlorination on the response factors of different CP mixtures, when ECNI is employed. On this basis a procedure was developed, which allows quantification even when the chlorine content of reference standard and sample does not match well. Details of the approach and its performance are described and discussed.

2. Experimental

2.1. Standards

Three tailor-made SCCP mixtures ($C_{10–13}$, 51, 55.5 and 63% chlorine, 100 ng/μl, solutions in cyclohexane) and four MCCP mixtures ($C_{14–17}$, 52 and 57% chlorine, 100 ng/μl, solutions in cyclohexane) were obtained from Ehrenstorfer (Augsburg, Germany). Additionally, a 1 + 1 solution was prepared from the SCCP mixtures with 51 and 55.5% (53% Cl) as well as from the SCCP mixtures with 55.5 and 63% (59% Cl). The SCCP mixture Hordalub 80 (56% Cl) from Hoechst (Frankfurt, Germany) was diluted to 100 ng/μl in cyclohexane. The SCCP mixtures Cereclor 60 L (59% Cl) and Cerechlor 70 L (69% Cl), both from Imperial Chemical Industries (ICI, London, UK), were diluted to 107 ng/μl in cyclohexane. A 1 + 1 mixture of them (64% Cl) was used as linearity control. Four pure MCCP mixtures from ICI and of different chlorine content were diluted to 100 ng/μl in cyclohexane. The MCCP mixtures Hordalub 80 EM (49% Cl) and Hordaflex SP (56% Cl) from Hoechst (Frankfurt, Germany) and the MCCP mixture Cloparin 50 from Caffaro (Cesano Maderno, Italy) were diluted to 100 ng/μl in cyclohexane. Synthesized C_{10}-, C_{11}-, C_{12}- and C_{13}-CP mixtures of both 50% and 60% chlorine content (20 μg/μl, solution in cyclohexane) were provided by Dr. Mehmet Coelhan from the Technical University of Munich (Germany) and diluted to 100 ng/μl in cyclohexane. [$^{13}C_{10}$] *trans*-Chlordane (100 ng/μl, solution in nonane, purity 99%) was purchased from Cambridge Isotope Labs. (Andover, USA) and employed as internal standard (ISTD). ε-Hexachlorocyclohexane (ε-HCH) was obtained from Ehrenstorfer and used as recovery standard. Reference solutions for quantification of the fish liver samples contained 1500 ng of CPs, 10 ng of ε-HCH and 10 ng of [$^{13}C_{10}$] *trans*-chlordane in ca. 150 μl of cyclohexane.

2.2. Chemicals and solvents

Cyclohexane, dichloromethane and *n*-hexane for pesticide residue analysis were obtained from Scharlau (Barcelona, Spain). Florisil PR (60–100 mesh) and sodium sulphate (Pestanal grade) were purchased from Fluka (Buchs, Switzerland), and silica gel (200–400 mesh, 0.035–0.070 mm) from

CU Chemie Uetikon (Uetikon, Switzerland). All three chemicals were dried overnight at 600 °C and kept for another 6 h at 130 °C before usage.

2.3. Fish samples and clean-up

Seven cod samples (G*adus morhua*) and a dab sample (*Limanda limanda*) were collected at two different locations in the Baltic Sea (54°33.36N/10°42.13E and 54°51.76N/14°01.51E) by the Federal Research Centre for Fisheries (Hamburg, Germany) in August 2002 and August 2003. Furthermore, a flunder (*Platychtus flesus*) and five North Sea dabs (*Limanda limanda*) were caught in the North Sea in August 2003. Single livers as well as one pooled liver sample ($n = 5$) were analysed. The clean-up method is published elsewhere and therefore only briefly described [4]. 2–8 g of fish liver was homogenized with a 10-fold excess of anhydrous sodium sulphate. Ten nanograms of [$^{13}C_{10}$] *trans*-chlordane (internal standard) in 10 μl of cyclohexane was added and the sample was extracted with 250 ml of *n*-hexane/CH_2Cl_2 (1 + 1, v/v) in a glass column. After concentration, lipids were removed by column chromatography on 40 g of silica gel impregnated with 44% (w/w) of conc. H_2SO_4. The lipid-free sample was eluted with 120 ml of *n*-hexane/CH_2Cl_2 (1 + 1, v/v). A further fractionation was carried out on 16 g of Florisil (1.5% water content) with 85 ml of *n*-hexane (fraction 1), 5 ml of CH_2Cl_2 (fraction 2) and 60 ml of CH_2Cl_2 (fraction 3). The last fraction contained all CPs. Ten nanograms of ε-HCH in 10 μl of cyclohexane was added as a recovery standard to the concentrated CP fraction before analysis. This clean-up allows elimination of the interferences caused by other chlorinated compounds (e.g. toxaphenes) so that ECNI-LRMS can be applied successfully [4].

2.4. Instrumentation

Chromatographic separations were performed on an HP 5890II (Hewlett-Packard, Palo Alto, CA, USA) gas chromatograph equipped with a split/splitless injector and a fused silica capillary column (15 m, 0.25 mm i.d.) coated with a 0.25 μm thick film of DB5-MS (5% phenylmethylpolysiloxane, J&W Scientific, Folsom, CA, USA). Sample volumes of 1.5 μl were injected in the splitless mode (2 min) at an injector temperature of 275 °C. Helium (99.999%, Carbagas, Basel, Switzerland) was used as carrier gas at a column inlet pressure of 68.9 kPa (10 psi). The temperature programme was as follows: 100 °C, isothermal for 2 min, then 15 °C/min to 280 °C and isothermal for 8 min.

An HP 5989B (Hewlett-Packard) mass spectrometer was employed in the ECNI mode using methane (99.995%, Carbagas, Basel, Switzerland) as reagent gas at a pressure of 127 Pa (0.95 Torr). The electron energy was 100 eV. The ion source temperature was kept at 200 °C, the quadrupole temperature at 100 °C and the transfer line temperature at 280 °C. The most abundant isotopes of the $[M - Cl]^-$ ions of CPs and of the $[M]^-$ ion of [$^{13}C_{10}$] *trans*-chlordane were detected in the selected ion monitoring (SIM) mode with dwell times of 75 ms for each ion as described in detail elsewhere [18].

3. Results and discussion

3.1. Influence of the chlorine content

CP homologues with different chlorine contents were compared to investigate the influence of the chlorine content on the response factor. The total response factors of the CP mixtures were calculated as follows. First, the relative total CP area is needed (see Eq. (1)):

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

where "*i*" assigns the CP congener group. The amount of internal standard in the sample and in the standard solutions was the same in this study and could therefore be eliminated from the equations. The total response factor can then be expressed as:

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

The total response factors of C_{10}-, C_{11}-, C_{12}- and C_{13}-CPs with two different degrees of chlorination are shown in Fig. 1. They increased by a factor of 2.5–5.7 from 50 to 60% Cl.

3.2. Influence of the carbon chain length

Total response factors were compared to different SCCP homologues with the same chlorine content but different chain lengths. Data were normalized to the C_{10} mixtures and are given in Fig. 2. The total response factors were hardly influenced for CPs with a degree of chlorination of 50%.

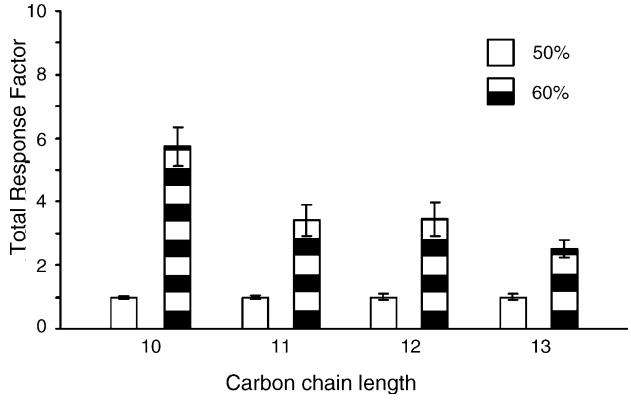

Fig. 1. Total response factors (average of three measurements) of C_{10}-, C_{11}-, C_{12}- and C_{13}-CPs determined by HRGC-ECNI-LRMS. Results were normalized to the mixtures with a chlorine content of 50%. Error bars indicate the standard deviation.

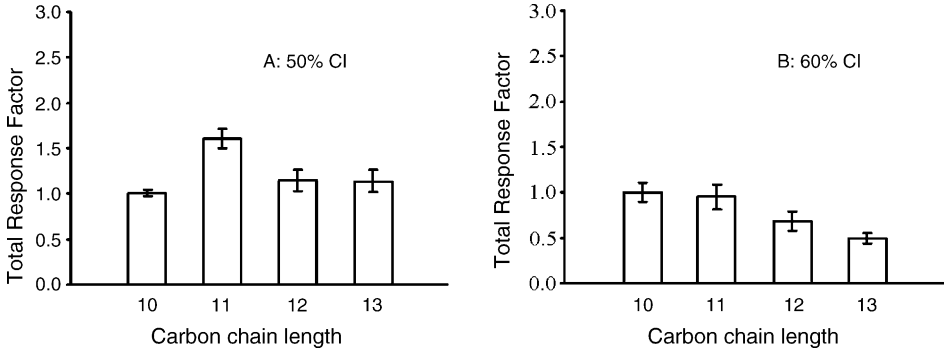

Fig. 2. Total response factors (average of three measurements) of SCCP homologues with a degree of chlorination of 50% (A) and 60% (B) determined by HRGC-ECNI-LRMS. Results were normalized to the mixtures of C_{10}-homologues. Error bars indicate the standard deviation.

They decreased slightly with increasing the chain length for 60% Cl. However, compared to the chlorine content, the differences were much lower (factor of 0.5–1.6).

3.3. Total response factors of CP mixtures

The correlation between response factor and chlorine content was investigated using SCCP mixtures. The chlorine content of a CP mixture can be calculated as shown in Eq. (3).

$$\text{Chlorine content (CP mixt.)} = \sum_i \frac{\text{rel. area (cong. group } i\text{)chlorine content (cong. group } i\text{)}}{\text{rel. total CP area}} \quad (3)$$

The relative area is the area of the congener group divided by the area of the internal standard ([$^{13}C_{10}$] *trans*-chlordane). The calculation of the chlorine content is similar to that of the average molar mass as described by Tomy et al. [10].

If determined by ECNI the chlorine content of low chlorinated CP mixtures is systematically too high, since congeners with 3 and 4 chlorine atoms are not detected and the relative abundances of higher chlorinated congeners are overestimated due to much higher response factors. Therefore, the chlorine content determined in this way always differs from the value specified by the manufacturer of the CP mixture [14]. In contrast to low chlorinated CPs, the chlorine content of highly chlorinated CPs (>65%) is slightly underestimated.

Already Tomy et al. assumed that the signal area of a congener group is proportional to the number of chlorine atoms as well as to its molar concentration. Therefore, they proposed to divide the signal area of each congener group by the number of their chlorine atoms as a quantification correction [10]. This relation was investigated in detail with seven SCCP mixtures of different composition and chlorine content (51–69%). A reasonably linear correlation was found between chlorine content and total response factor (see Fig. 3A). Slopes and intercepts deviated not more than 12% for five repetitions on five different days. Correlation coefficients (R^2) were always >0.9. The same was found for MCCPs as shown in Fig. 3B. Slopes and intercepts deviated not more than 8% for three repetitions on three different days. Correlation coefficients (R^2) were always >0.8. As can be seen in Fig. 3, a slight variation of the measured chlorine content can lead to a considerable variation in the response factor. Therefore, an accurate determination of the chlorine content is very important.

The chlorine content of technical SCCP mixtures can vary between 30 and 70%. However, the working range of ECNI-MS methods is limited. In mixtures with a chlorine content lower than 50%, many congener groups are not detected since they contain congeners with 1–4 chlorine atoms. Therefore, a mixture of a chlorine content of 51% was set as lowest point of the linearity. A mixture of 69% was chosen to cover the whole range up to highly chlorinated mixtures, although CPs with such high chlorine contents could not be detected in environmental samples. As can be seen in Fig. 3A, the

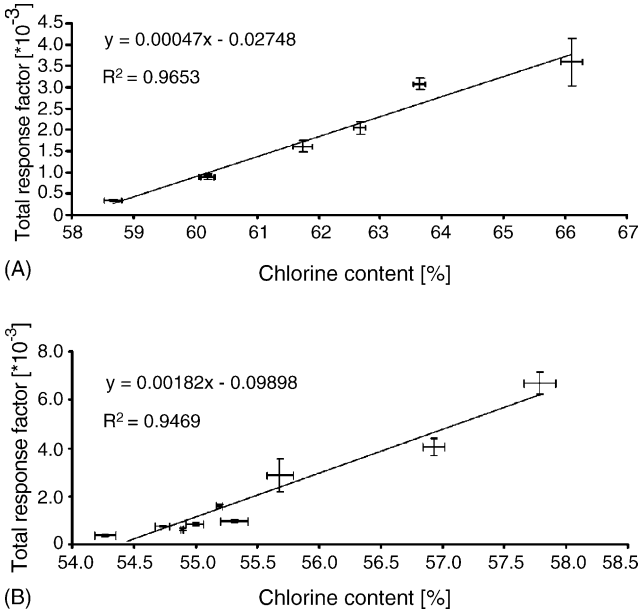

Fig. 3. Dependence of the total response factor on the degree of chlorination for seven different SCCP mixtures ((A) 51–69% chlorine, average of five interday measurements) and nine different MCCP mixtures (B) average of three interday measurements). Error bars indicate the standard deviation.

Table 1
CP quantification of different SCCP mixtures (51–69% Cl) based on chlorine content corrected total response factors: the expected amount, the measured amount and the relative error are given

SCCP mixture (% chlorine content)	Expected amount (ng)	Measured amount (ng)	Relative error (%)
Standards used for establishment of linear function ($R^2 = 0.9730$, $y = 0.00041x - 0.02391$)			
51 (58.7% Cl)	1500	1600	7
55[a] (60.2% Cl)	1500	1600	7
59 (61.7% Cl)	1600	1600	0
64 (64.5% Cl)	1100	1000	7
69 (66.1% Cl)	1600	1700	6
Control standards			
55[a] (60.2% Cl)	1500	1600	7
53 (59.8% Cl)	1500	1400	7
59 (62.7% Cl)	1500	1700	13
63 (63.6% Cl)	1500	2000	33
Hordalub 80 (56% Cl) (60.3% Cl)	1500	1900	27

The chlorine contents calculated from the ECNI-LRMS measurements are given in brackets.
[a] The CP mixture with a chlorine content of 55% was analyzed twice.

response factor varied more for this highly chlorinated mixture. The ionization might be influenced by different chemical and physical properties of highly chlorinated mixtures which have for example two chlorine atoms bound to one carbon atom (e.g. $C_{10}H_{11}Cl_{11}$).

3.4. Quantification approach

The total response factor of the CPs in the sample can be determined from the chlorine content using the linear correlation:

$$\text{Total response factor (CPs in the sample)} = ax + b \quad (4)$$

where a is the slope of the linear regression, x the chlorine content calculated from the ECNI analysis and b the axis intercept. Once, the total response factor is determined, the total CP amount in the sample can be calculated as follows:

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

Compared to the conventional quantification procedure, this modified quantification procedure is independent from the chlorine content of the CP standard and requires only the establishment of the correlation by determining the total response factors for a set of CP standards prior to the analysis of a series of samples.

3.5. Quantification of different SCCP mixtures

The procedure was first checked with different SCCP mixtures. Five mixtures were used to establish the linear function, and another five SCCP mixtures of different chlorine content were quantified as controls. The expected and determined amounts and deviations are listed in Table 1.

Deviations were <7% for CP mixtures being part of the correction function. Control samples with chlorine contents of 53, 55, 59 and 63% showed relative errors between 7 and 33%. This is one order of magnitude less than the systematic errors observed by Coelhan et al. [12] and Zencak et al. [15] (65–940%) and acceptable for a quantification of such complex mixtures with thousands of isomers. The conventional quantification of the SCCP mixture of 55% based on the single members of the linear function led to errors of 61, 7, 93, 113 and 373%, respectively.

3.6. Quantification of biota

Ten fish liver samples from the North and Baltic Sea were quantified by both the conventional [10] and the modified quantification procedure described above. The composition of CPs in fish can be quite different. This can be seen from the chlorine content or the average molar mass as demonstrated by other research groups [10,11]. In general, the average molar mass increases with higher chlorine content. The chlorine contents of SCCPs varied between 59 and 62% (388–422 g/mol) in fish from the North and Baltic Sea [4]. Borgen et al. reported average molar masses from 378 to 456 g/mol in freshwater fishes from different locations in Norway (chlorine contents were not specified) [11].

First, the chlorine contents of the SCCPs in the fish samples were determined by HRGC-ECNI-LRMS. The degree of chlorination varied between 59.2–62.9% (392–426 g/mol). For routine analysis of the samples only three SCCP standard were used for the establishment of the linear function (51, 55.5 and 63%, according to the manufacturer). The limited number of standard mixtures was chosen to reduce the overall time of the nevertheless long analysis procedure and to avoid the use of technical CP mixtures with additives. Therefore, the only three commercially available CP standards of 100% purity were used. Their measured chlorine contents were 58.5, 60.1 and 63.6% (389, 403, 437 g/mol, average of three measurements, relative standard deviations of less than 1%). The deviation to the manufacturer declaration is due to the reasons given before. The chlorine contents of the samples

Table 2
SCCP concentrations (ng/g wet weight) of 10 fish liver samples obtained with the described method and based on three commercial SCCP standards with different chlorine content (51, 55.5 and 63%)

	Quantification			
	According to Tomy et al. [10]			Via total response factor[a]
	Standard 51 (58.7% Cl)	Standard 55 (60.3% Cl)	Standard 63 (64.0% Cl)	
Sample 1 (61.9% Cl)	790	140	43	73
Sample 2 (62.9% Cl)	1060	180	58	82
	Standard 51 (58.2% Cl)	Standard 55 (59.9% Cl)	Standard 63 (63.4% Cl)	
Sample 3 (62.2% Cl)	210	94	21	29
Sample 4 (61.7% Cl)	360	160	37	57
	Standard 51 (58.6% Cl)	Standard 55 (60.2% Cl)	Standard 63 (63.5% Cl)	
Sample 5 (61.1% Cl)	170	81	17	34
Sample 6 (62.0% Cl)	160	76	16	24
Sample 7 (60.6% Cl)	180	88	18	47
Sample 8 (61.8% Cl)	2500	1200	250	410
Sample 9 (59.2% Cl)	730	350	73	520
Sample 10 (59.9% Cl)	56	27	6	21

The chlorine contents calculated from the ECNI-LRMS measurements are given in brackets.
[a] Linearity determined with three SCCP mixtures (51, 55.5 and 63% Cl, $R^2 = 0.999$ for samples 1 and 2, $R^2 = 0.969$ for sample 3 and 4, $R^2 = 0.964$ for samples 5–10).

were between those of the standards and none of them would therefore be ideal.

The results obtained by the correction mode and the conventional quantification procedure are listed in Table 2. Considerable differences were found, when the standards 55 and 63 were used (for example sample 8: 1200 and 250 ng/g). The corrected results were mainly between the results of the standards 55 and 63 (Table 3).

The correction was also applied to the quantification of MCCPs. The degree of chlorination of the sample CPs varied between 53.5 and 57.0% (426–456 g/mol). Only two suitable MCCP standard mixtures could be found (52 and 57%, according to the manufacturer). Their measured chlorine contents were 55.4 and 57.8% (442 g/mol, 460 g/mol, average of three measurements, relative standard deviations of 2 and 1.5%). The interday reproducibility of MCCPs was not as good as that for SCCPs. Especially low chlorinated MCCPs, such as $C_{15}H_{26}Cl_6$ had a retention time range of six minutes, which increases the possibilities of integration errors. The calculated chlorine contents of the MCCPs in samples 1, 3 and 4 were coincidentally close to one of the MCCP standards. Therefore, both calculation methods gave similar results. Sample 9 contained a high amount of lower chlorinated MCCPs. Therefore, its chlorine content was far below that of the standard mixtures, and it had to be excluded from quantification. The calculated chlorine

Table 3
MCCP concentrations (ng/g wet weight) of 10 fish liver samples obtained with the described method and based on two commercial MCCP standards with 52 and 57% chlorine content

	Quantification		
	According to Tomy et al. [10]		Via total response factor
	Standard 52 (56.3% Cl)	Standard 57 (58.0%)	
Sample 1 (56.5% Cl)	74	64	72
Sample 2 (55.7% Cl)	110	92	120
	Standard 52 (55.4% Cl)	Standard 57 (57.3%)	
Sample 3 (56.8% Cl)	62	36	39
Sample 4 (57.0% Cl)	97	56	58
	Standard 52 (55.4% Cl)	Standard 57 (57.3%)	
Sample 5 (55.0% Cl)	480	33	210
Sample 6 (56.2% Cl)	320	22	47
Sample 7 (56.7% Cl)	630	44	71
Sample 8 (55.1% Cl)	3120	220	1270
Sample 9 (53.5% Cl)	890	63	n.d.
Sample 10 (56.0% Cl)	240	17	40

The chlorine contents calculated from the ECNI-LRMS measurements are given in brackets. n.d. not determined.

content of sample 2 was slightly below that of the available standards. Consequently, the procedure by Tomy et al. led to an underestimation of the MCCP concentration.

4. Conclusions

The determination of the CP chlorine content requires the quantification of all CP congener groups in a sample. Moreover, only a limited number of CP standard mixtures with different composition and different chlorine content are commercially available. However, it is important to use a standard mixture with chlorine content similar to that of the sample to avoid systematic errors of 100% or more. The presented quantification procedure makes benefit from the linear relation between response factors and chlorine contents and allows a quantification of SCCPs and MCCPs independent from the chlorine content of the used standard mixtures. The correction mode was only tested on quadrupole MS. Therefore, it is important to check it also on other types of mass spectrometers.

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Paper IV:

Short- and medium-chain chlorinated paraffins in biota from the European Arctic - differences in homologue group patterns.

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Short- and medium-chain chlorinated paraffins in biota from the European Arctic — differences in homologue group patterns

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Abstract

Congener and homologue group patterns of chlorinated paraffins (CPs) in biota can be influenced by different processes, but these are not well studied yet. Short- (SCCPs) and medium-chain chlorinated paraffins (MCCPs) were quantified in liver from Arctic char and seabirds (little auk and kittiwake) collected at Bear Island (European Arctic) as well as in cod from Iceland and Norway. CP concentrations were between 5 and 88 ng/g wet weight (ww) for SCCPs and between 5 and 55 ng/g ww for MCCPs with one exception of 370 ng/g measured in a liver sample from little auk. The SCCP homologue group patterns were compared with those of technical mixtures and of SCCPs present in cod liver from the Baltic Sea. The latter showed a more common SCCP homologue distribution (sum of C_{11} and C_{12} >60%) in contrast to cod liver from the Northwest of Europe, which had a high abundance of C_{10} and C_{12} congeners. Seabirds from Bear Island contained an equally distributed SCCP homologue group pattern. In Arctic char, the SCCP distribution was closer to technical products, but with a high proportion (average of 18.9%) of C_{10} congeners. A comparison of C_{10}/C_{12} ratios confirmed the higher abundance of C_{10} congeners in samples from higher latitudes. For the first time, MCCPs could be detected in Arctic samples. The average proportion of C_{14} congeners was 65.8%. The C_{14}/C_{15} abundance ratio was similar to technical mixtures. High-chlorinated CPs ($Cl_{>7}$) were also detectable. The average chlorine content of the SCCPs was 61.9% (59.0–63.3%), and that of the MCCPs 55.8% (54.5–57.4%).

Keywords: Polychlorinated *n*-alkanes; Chlorinated paraffins; Congener pattern; Homologue distribution; Arctic; Biota

1. Introduction

Chlorinated paraffins (CPs) are complex mixtures of polychlorinated *n*-alkanes containing thousands of different isomers, enantiomers and diastereomers. They are subdivided according to their carbon chain length into short chain CPs (SCCPs, C_{10}–C_{13}), medium chain CPs (MCCPs, C_{14}–C_{17}) and long chain CPs (LCCPs, $C_{>17}$). The degree of chlorination can vary between 30% and 70% depending on the field of application. CPs are utilized as additives in metal working fluids, as flame retardants and as plasticizers. Furthermore, they can be found as additives in paints, coatings and sealants (Tomy et al., 1998). The production is estimated to be 300 kt/year worldwide (Rossberg et al., 2003).

CPs are of concern due to their environmental and toxicological properties. They are classified as persistent,

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bioaccumulative, and are toxic to aquatic organisms (Tomy et al., 1998). Therefore, SCCPs were listed in the priority substance list of the European Water Framework Directive, which requires an extensive monitoring of SCCPs in the European Union from 2006 on (European Community, 2000, 2001). The presence of CPs in sediments, fish, marine mammals, and in air underlines the necessity for a more intensive monitoring in the environment including remote areas (Tomy et al., 2000; Marvin et al., 2003; Reth et al., 2005a). Information about environmental levels is currently scarce compared to other persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and toxaphenes. One reason for this is the time-consuming and complex analysis of CPs.

Currently, only a few laboratories analyse CPs worldwide (UNECE expert group on POPs, 2003). During the past years, different analytical approaches have been developed based on high resolution gas chromatography (HRGC) combined with different mass spectrometric techniques. Some allow the determination of the total amount of CPs (e.g. by electron ionization tandem mass spectrometry or by electron capture negative ionization (ECNI) mass spectrometry detecting nonspecific ions like $[HCl_2]^-$) (Jansson et al., 1991; Nicholls et al., 2001; Castells et al., 2004a,b; Zencak et al., 2004). However, it is also desirable to distinguish different congener and homologue groups to gain more information about the composition of CPs in the environment. Different techniques are available such as metastable atom bombardment (MAB) mass spectrometry (Moore et al., 2004), negative ion chemical ionization mass spectrometry using dichloromethane as reagent gas (Zencak et al., 2003) and ECNI in combination with high- or low-resolution mass spectrometry (Tomy et al., 1997; Marvin et al., 2003; Reth et al., 2005a). In this work, ECNI combined with low-resolution mass spectrometry (LRMS) was employed, since ECNI-MS is the mostly applied technique at the moment. Therefore, this should allow comparisons with other published data obtained by ECNI-MS. A comparison with congener and homologue group patterns obtained by other MS methodologies should be avoided due to different response factors and, therefore, slightly different patterns.

The prediction of environmental dispersion is hardly possible, since CP mixtures contain thousands of isomers with large differences in their physico-chemical properties. Vapor pressures (VPs) of S+MCCPs range between $1.7*10^{-8}$ and 0.5 Pa and octanol–water partition coefficients (log K_{ow}'s) between 5.06 and 8.96. Estimated Henry's law constants (HLCs) of individual S+MCCP congeners were reported to be between 0.003 and 54.82 Pa m^3 mol^{-1} (Tomy et al., 1998). Modelling of the environmental behaviour of such complex mixtures is demanding and requires much more data than currently available (Tomy et al., 1998).

The presence of POPs (e.g. PCBs, DDT and toxaphenes) in the Arctic is well documented (AMAP, 2004). A small island in the central Barents Sea, Bjørnøya (Bear Island, 74°N, 19°E), has attracted special attention. Although 500 km away from any known point source, high concentrations of POPs have been found in seabirds, as well as in samples from one lake, Lake Ellasjøen, on the island. Lake Ellasjøen is contaminated due to two reasons. The lake area is mountainous and receives a lot of precipitation and thus more airborne contaminants than other parts of the island. Moreover, large seabird colonies use the lake as resting area and input of guano from these birds causes an increase in POP levels (Evenset et al., 2004). Values of $\delta^{15}N$ in Arctic char were 6–10‰ higher than for Arctic char from another lake also situated on Bear Island but hardly influenced by seabirds. This means that the fish from Lake Ellasjøen feed at a higher trophic level, which is also evident from the high POP levels in their tissues (Evenset et al., 2004, 2005).

CPs have partially similar chemical and physical properties as PCBs. Therefore, long-range atmospheric transport is possible as demonstrated by Tomy et al. (1999). SCCPs were detected in sediment and air samples as well as in marine mammals from the Canadian Arctic (Rossberg et al., 2003). Lower chlorinated and shorter chained CPs were most abundant as expected by the higher Henry's Law constants. However, data from the European Arctic are nearly absent. Borgen et al. (2002b,a) found 592 ng/g (lipid weight, lw) of high-chlorinated SCCPs (average molar mass of 453 g/mol) in one sample (muscle) of Arctic char from Bear Island and between 1.8 and 10.6 ng/m^3 of SCCPs (average molar mass of 400 g/mol) in ambient air from Bear Island. To the best of our knowledge, no further data are available.

In 2002, the German Federal Environmental Agency initiated a monitoring study to obtain information about the so far unknown CP levels in fish and sediments from the North and Baltic Sea. Since CPs were found in nearly all fish samples (Reth et al., 2005a), the project was extended to fish and seabirds from northwest Europe and the Arctic in a second round. S+MCCP concentrations of this part are presented here. Special attention was focused on the investigation of potential changes in the pattern of congener and homologue groups. Patterns of technical CP mixtures and cod from the Baltic Sea were compared with patterns of samples

from the European Arctic to study the latitudinal influence.

2. Methods and materials

2.1. Standards

Three tailor-made SCCP mixtures ($C_{10–13}$, 51%, 55.5% and 63% chlorine, 100 ng/μl, solutions in cyclohexane, 100% purity) and two MCCP mixtures ($C_{14–17}$, 52% and 57% chlorine, 100 ng/μl, solutions in cyclohexane, 100% purity) were obtained from Ehrenstorfer (Augsburg, Germany) and used for quantification. The SCCP mixtures Hordalub 17 (49% Cl), Hordalub 80 (56% Cl) and Hordalub 500 (62% Cl), from Hoechst (Frankfurt, Germany) were diluted to 100 ng/μl in cyclohexane. The SCCP mixtures Cereclor 60 L (59% Cl) and Cerechlor 70 L (69% Cl), both from Imperial Chemical Industries (ICI, London, England), were diluted to 107 ng/μl in cyclohexane. The MCCP mixture Cloparin® 50 from Caffaro (Cesano Maderno, Italy) and the MCCP mixtures Hordalub 80 EM (49% Cl) and Hordaflex SP (56% Cl) from Hoechst (Frankfurt, Germany) were diluted to 100 ng/μl in cyclohexane. Eight pure MCCP mixtures from ICI (London, England) with different chlorine content were diluted to 100 ng/μl in cyclohexane. $^{13}C_{10}$-*trans*-chlordane (100 ng/μl, solution in nonane, purity 99%) was purchased from Cambridge Isotope Laboratories (Andover, USA) and employed as internal standard (ISTD). ε-Hexachlorocyclohexane (ε-HCH) was obtained from Ehrenstorfer (Augsburg, Germany) and used as recovery standard. Reference solutions for quantification contained 1500 ng of CPs, 10 ng of ε-HCH and 10 ng of $^{13}C_{10}$-*trans*-chlordane in ca. 150 μl of cyclohexane.

2.2. Chemicals and solvents

Cyclohexane, dichloromethane and *n*-hexane for pesticide residue analysis were obtained from Scharlau (Barcelona, Spain). Concentrated sulfuric acid was purchased from J.T. Baker (Deventer, The Netherlands). Florisil®PR (60–100 mesh) and sodium sulfate (Pestanal®) were obtained from Fluka (Buchs, Switzerland), and silica gel (200–400 mesh, 0.035–0.070 mm) from CU Chemie Uetikon AG (Uetikon, Switzerland). All three chemicals were dried overnight at 600 °C and kept for another 6 h at 130 °C before usage.

2.3. Fish and seabird samples

Cod samples (*Gadus morhua*) from Northern Europe were obtained from the Norwegian Institute for Air Research in Tromsø (see Table 1 for details). Two fishes were caught close to the Northern Norwegian coast (sample nos. A1 and A4, Lofoten, 68°08′N/13°33′W), two south of Iceland (sample nos. A3 and A6, Vestmannaeyjar,

Table 1
Details about the analyzed samples: cod (*Gadus morhua*) from Iceland (65°74′N/18°09′W and 63°28′N/20°15′W) and Norway (68°08′N/13°33′W); Arctic char (*Salvelinus alpinus*), little auk (*Alle alle*) and kittiwake (*Rissa tridactyla*) from Bear Island

Sample no.	Species	Tissue	Sex	Age	Length (mm)	Weight (g)	Sampling date
A1	Cod	Liver	f	n.d.	860	8500	02.02.2004
A4	Cod	Liver	f	n.d.	830	6500	02.02.2004
A2	Cod	Liver	f	n.d.	490	1019	30.09.2003
A5	Cod	Liver	f	n.d.	410	653	30.09.2003
A3	Cod	Liver	n.d.	n.d.	530	1490	06.11.2003
A6	Cod	Liver	f	n.d.	514	1275	06.11.2003
B1	Arctic char	Liver	f	14	446	831	09.07.2001
B3		Muscle					
B2	Arctic char	Liver	f	14	465	850	09.07.2001
B4		Muscle					
C1	Little auk	Liver	m	n.d.	121[a]	173	08.07.2001
C3		Muscle					
C2	Little auk	Liver	m	n.d.	122[a]	169	08.07.2001
C4		Muscle					
D1	Kittiwake	Liver	m	n.d.	330[a]	458	08.07.2001
D3		Muscle					
D2	Kittiwake	Liver	f	n.d.	326[a]	393	08.07.2001
D4		Muscle					

Information about analyzed tissue, sampling date, sex, age, weight and length or wing length is given.
[a] Wing length in mm; f: female m: male; n.d. not determined.

63°28′N/20°15′W) and two north of Iceland (sample nos. A2 and A5, Akureyri, 65°74′N/18°09′W).

Samples from Bear Island were provided by Akvaplan-niva in Tromsø. Two Arctic chars (*Salvelinus alpinus*) were caught in Lake Ellasjøen. Two little auks (*Alle alle*) and two kittiwakes (*Rissa tridactyla*) were shot at Bjørnøya (74°N, 19°E) in July 2001. Liver and muscle tissue were analysed. More detailed information is given in Table 1.

Cod samples (*Gadus morhua*) were collected at two different locations in the Baltic Sea (54°33.36N/10°42.13E and 54°51.76N/14°01.51E) by the Federal Research Centre for Fisheries (Hamburg, Germany) in August 2002 and August 2003. The length of the fish was between 240 and 320 mm. Between one and five livers from each location were pooled to achieve a sufficient detection limit. Eight cod liver samples were analysed.

2.4. Extraction and clean-up

The clean-up method is published elsewhere and therefore only briefly described (Reth et al., 2005a). 2–5 g of fish liver were homogenized with a tenfold excess of anhydrous sodium sulfate. 10 ng of $^{13}C_{10}$-*trans*-chlordane (internal standard) in 10 µl of cyclohexane was added and the sample extracted with 250 ml of *n*-hexane/CH_2Cl_2 (1+1, v/v) in a glass column. Lipid content was determined gravimetrically before lipids were removed by column chromatography on 40 g of silica gel impregnated with 44% (weight) of conc. sulfuric acid. The lipid-free sample was eluted with 120 ml of *n*-hexane/CH_2Cl_2 (1+1, v/v). A further fractionation was carried out on 16 g of Florisil® (1.5% water content) with 85 ml of *n*-hexane (fraction 1), 5 ml of CH_2Cl_2 (fraction 2) and 65 ml of CH_2Cl_2 (fraction 3) to eliminate interferences by other polychlorinated compounds. The last fraction contained all CPs. 10 ng of ε-HCH in 10 µl of cyclohexane was added as recovery standard to the concentrated CP fraction before analysis.

2.5. Instrumentation

Chromatographic separations were performed on an HP 5890II (Hewlett Packard, Palo Alto, USA) gas chromatograph equipped with a split/splitless injector and a fused silica capillary column (15 m, 0.25 mm i.d.) coated with a 0.25 µm thick film of DB5-MS (5% phenylmethylpolysiloxane, J&W Scientific, Folsom, USA). Sample volumes of 1.5 µl were injected in the splitless mode (2 min) at an injector temperature of 275 °C. Helium (99.999%, Carbagas, Basel, Switzerland) was used as carrier gas at a column inlet pressure of 68.9 kPa (10 psi). The temperature programme was as follows: 100 °C, isothermal for 2 min, then 15 °C/min to 280 °C and isothermal for 8 min.

An HP 5989B (Hewlett Packard, Palo Alto, USA) mass spectrometer was employed in the ECNI mode using methane (99.995%, Carbagas, Basel, Switzerland) as reagent gas at a pressure of 120 Pa (0.9 Torr). The electron energy was 100 eV. The ion source temperature was kept at 200 °C, the quadrupole temperature at 100 °C and the transfer line temperature at 280 °C. The two most abundant isotopes of the $[M\text{-}Cl]^-$ ions of CPs, the $[M]^-$ ion (*m/z* 419.8) of $^{13}C_{10}$-*trans*-chlordane, and the most abundant isotope of the $[M\text{-}Cl]^-$ ion (*m/z* 254.9) of the recovery standard ε-HCH were detected in the selected ion monitoring (SIM) mode with dwell times of 75 ms for each ion as described in detail elsewhere (Reth and Oehme, 2004). Congeners with 10 to 15 carbon atoms and 5 to 10 chlorine atoms were analysed.

2.6. Identification and quantification

As described previously congener groups were identified by the retention time range, the signal shape and the correct isotope ratio (Reth and Oehme, 2004). The most abundant isotope signal was used for quantification and the second one for identification of possible interferences (Reth et al., 2005a). The mass-to-charge ratios used for quantification and identification were previously published (Reth and Oehme, 2004). Quantification was performed according to Reth et al. (2005b), which allows an accurate quantification even if the degree of chlorination of the CPs in the sample is different from the chlorine content of the standard. Three SCCP standards (51%, 55%, and 63% Cl) and two MCCP standards (52% and 57% Cl) from Ehrenstorfer were used for this purpose.

2.7. Quality control

Glassware heated to 450 °C for 3 h as well as heat-treated sodium sulfate, silica gel and Florisil® was used to minimize the risk of contamination. Method blanks consisted of 20 g of sodium sulfate (preheated at 600 °C for 6 h). A good linearity ($R^2 > 0.993$, 7 measuring points) was achieved between 1 and 100 ng of technical SCCPs and MCCPs (detection of the most abundant congener groups). The limits of detection (LODs) were between 0.5 and 1 ng/µl of SCCP and MCCP mixtures (55.5% and 52% chlorine content, detection of the most abundant congener groups) at a signal-to-noise ratio of

3:1, and the limits of quantification (LOQs) were between 1.5 and 3 ng/μl at a signal-to-noise ratio of 10:1. Method blanks were below detection limits. The relative distribution of the homologue groups in the standard mixtures was determined five times and the relative standard deviation was less than 10%. More method validation details are given elsewhere (Reth et al., 2005a; Zencak et al., 2005).

3. Results and discussion

3.1. CP concentrations in Arctic fish and seabirds

SCCPs and MCCPs were detected in all samples, and their concentrations are listed in Table 2. SCCP concentrations in the cod samples ranged from 11 to 70 ng/g wet weight (ww, median of 52 ng/g ww). MCCP concentrations were between 7 and 47 ng/g ww (median of 17 ng/g ww). The range of SCCPs and MCCPs in the cod samples was comparable with previously detected levels of PCB 153 (31–122 ng/g ww, median of 71 ng/g ww) and polybrominated diphenylether (PBDE) 47 (7–32 ng/g ww, median of 14.5 ng/g ww) in the same fishes (Heimstad and Herzke, 2004). Moreover, samples A2 and A6 showed the highest SCCP levels as well as the highest PCB 153 levels with 122 ng/g ww and 118 ng/g ww, respectively. The CP concentrations obtained in this study were lower than those previously reported for fishes from the North- and Baltic Sea (Reth et al., 2005a,b).

In the Arctic char samples from Lake Ellasjøen on Bear Island SCCP and MCCP levels were comparable and ranged between 7 and 27 ng/g ww for SCCPs and between 10 and 47 ng/g ww for MCCPs. SCCP concentrations were similar or slightly higher than the SCCP concentration (7.7 ng/g ww) found by Borgen et al. (2001) in an Arctic char from the same location. Moreover, the range of SCCPs as well as MCCPs was comparable with previously detected sum concentrations of PBDEs and toxaphenes in Arctic char from Bear Island captured also in July 2001 (Evenset et al., 2005). The average sum of PBDEs (PBDE 33, 47, 71, 99, 100, 119 and 153) was 17.5±7.36 ng/g ww and the average sum of toxaphenes (#26, #40 and #50) was 20.9±14.2 ng/g ww.

In the seabirds, concentrations ranged between 5 and 88 ng/g ww for SCCPs and between 5 and 55 ng/g ww

Table 2
SCCP and MCCP concentrations (ng/g wet and lipid weight) determined by HRGC-ECNI-LRMS in fish and seabirds from Northern Europe

Sample no.	Lipid (%)	SCCP concentration (ng/g ww)	SCCP concentration (ng/g lw)	SCCP concentration (ng/g lw)	MCCP concentration (ng/g lw)
Cod (L)					
A1	37	52	47	140	130
A4	49	17	7	35	14
A2	47	56	16	120	35
A5	39	11	7	28	18
A3	51	52	18	100	36
A6	49	70	47	140	96
Arctic char					
B1 (L)	12	27	43	230	360
B3 (M)	2	13	47	540	1600
B2 (L)	12	11	13	89	110
B4 (M)	2	7	10	300	440
Little auk					
C1 (L)	10	18	48	190	500
C3 (M)	5	7	55	150	1200
C2 (L)	10	88	370	880	3700
C4 (M)	4	16	17	430	450
Kittiwake					
D1 (L)	5	6	39	110	730
D3 (M)	5	5	38	95	720
D2 (L)	6	44	12	860	240
D4 (M)	12	5	5	41	41

The lipid content (%) is also listed.
L: liver; M: muscle; ww: wet weight; lw: lipid weight.

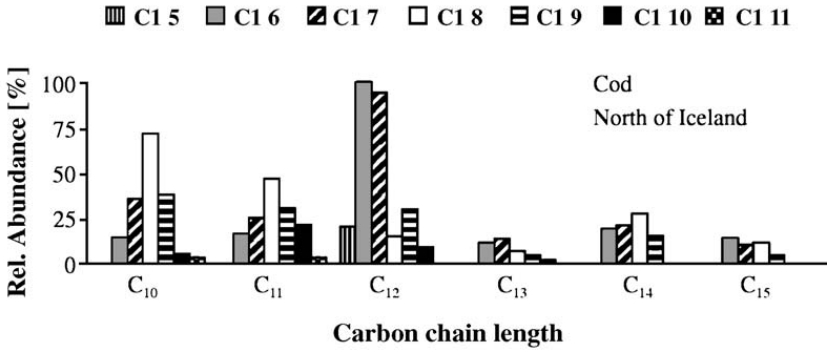

Fig. 1. Congener group pattern of the cod liver sample A2 from the northern coast of Iceland (Akureyri) determined by HRGC-ECNI-LRMS.

for MCCPs with one exception of 370 ng/g ww measured in a liver sample from little auk. CPs were detected in both muscle and liver of each species from Bear Island at variable concentrations without any clear trend. S+MCCP lipid weight data were higher for samples from Bear Island than for the cod samples from northwest Europe. SCCP concentrations calculated on lipid weight basis were comparable with SCCP levels reported for marine mammal blubber from the Canadian Arctic (Tomy et al., 2000).

3.2. Congener and homologue group patterns: Northwest Norway, Iceland and Bear Island

The congener groups C_{10} with seven and eight chlorine atoms and especially C_{12} with six and seven chlorine atoms were most abundant in all cod samples from northwest Norway and Iceland. Fig. 1 shows such a congener group pattern for the cod liver sample A2 captured in the north of Iceland.

All congener groups within one chain length were summarised and presented as a homologue group pattern for better comparison. Fig. 2 shows the homologue group patterns of the cod liver samples A1 to A6. C_{10} and C_{12} homologues were most abundant. Especially, the homologue group patterns in samples from the same location were similar except for sample A5 showing an increased abundance of C_{10} and C_{11} homologues.

A different distribution of the homologue groups was observed for Arctic char from Bear Island (see Fig. 2). Homologues with 11 and 12 carbon atoms were dominant. Compared to technical CP mixtures (see Table 3), the proportion of congeners with ten carbon atoms was high (average of 18.9%). The abundance of C_{10} homologues was higher than that of C_{13} homologues. Moreover, the rather equal abundance of SCCP

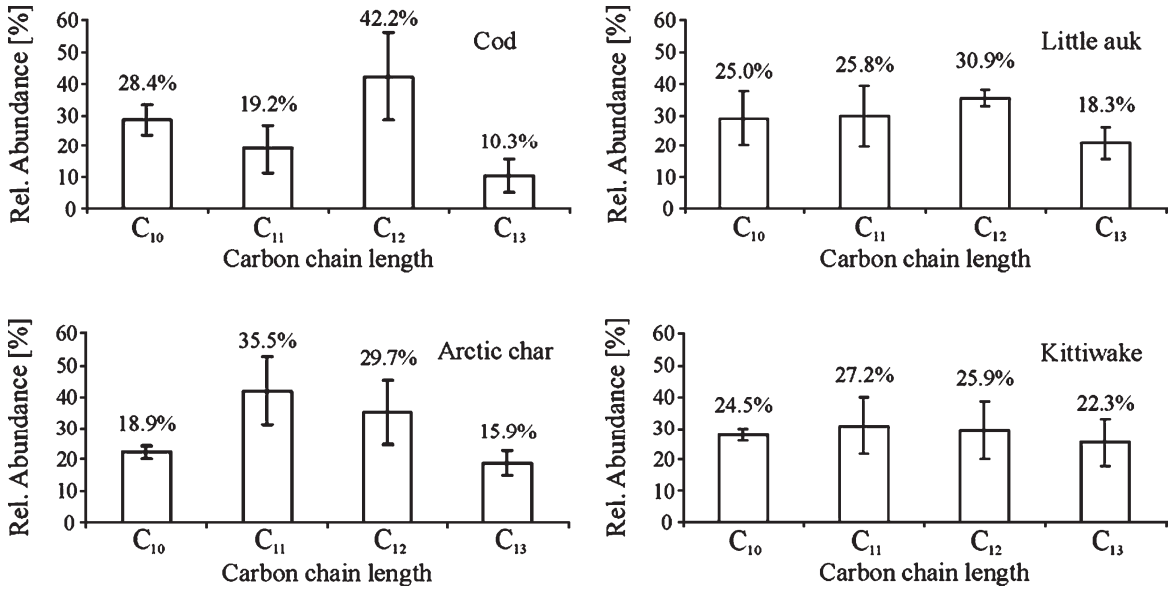

Fig. 2. Average homologue group patterns of six cod liver samples from Northern Europe, of two Arctic chars and two seabirds (little auk and kittiwake) captured on Bear Island. The homologue group patterns of the samples from Bear Island are the average of muscle and liver tissue of two individuals. Standard deviations are assigned.

Table 3
Relative contribution of the C_{10}, C_{11}, C_{12} and C_{13} homologue groups in technical SCCPs as well as in synthetic SCCP standard mixtures

CP mixtures	Proportion (%) of			
	C_{10}	C_{11}	C_{12}	C_{13}
Technical mixtures				
Hordalub 17 (49% Cl)	9.5	38.2	34.4	17.9
Hordalub 80 (56% Cl)	10.4	43.8	32.0	13.8
Hordalub 500 (62% Cl)	12.4	53.5	27.2	6.9
Cereclor 60 (59% Cl)	15.5	32.3	35.2	17.0
Cereclor 70 (69% Cl)	20.2	32.3	30.7	16.8
Mean±S.D.	13.6±3.9	40.0±8.0	31.9±2.8	14.5±4.0
Standard mixtures				
SCCP Standard (51% Cl)	8.1	38.3	36.4	17.2
SCCP Standard (55% Cl)	9.8	42.3	34.1	13.8
SCCP Standard (63% Cl)	12.8	44.6	29.4	13.2

S.D.: standard deviation.

homologue groups in the seabirds was surprising (see Fig. 2). The abundance of C_{10} and C_{13} homologues was higher than in technical SCCP mixtures. The distribution of the homologue groups in the liver and in the muscle of the same species was similar for the samples B1/B3, C1/C3 and D1/D3, but in the other three samples slight differences were observable.

3.3. Homologue group patterns: industrial and standard mixtures, cod from the Baltic Sea

Five different technical SCCP mixtures of two European manufacturers were analysed and the proportion of each homologue group was determined (see Table 3). The homologue groups C_{11} and C_{12} were dominating in all mixtures (sum>63%). The composition of the three synthesised standard mixtures was well comparable with that of the technical Hordalub mixtures (see Table 3).

In the eight pooled cod samples from the Baltic Sea, the C_{11} and C_{12} homologue groups were also most abundant. The SCCP homologue group pattern within samples varied less than for the Arctic. Moreover, the average homologue group pattern was more similar to technical CP mixtures (see Fig. 3). An explanation could be the shorter distance to source regions and the correspondingly reduced possibility for environmental transformations.

3.4. Comparison of homologue group ratios

The relative abundance of C_{10} homologues was significantly higher in samples from Northern Europe (average of 24.7%) compared to the technical CP mixtures ($P<0.05$). Only the high-chlorinated Cereclor 70 (69% Cl) showed a higher proportion of C_{10} congener groups. For a better comparison, the abundance ratio between C_{10} and C_{12} homologues was calculated (see Fig. 4). C_{12} was selected, since its relative quantity was quite similar in all samples (32.7 ±8.97%). The C_{10}/C_{12} homologue ratio was higher in biota from the north of Europe compared to that in cod from the Baltic Sea and to the C_{10}/C_{12} homologue ratio determined in technical SCCP mixtures. This might be due to an enrichment of more volatile CP congeners in the Arctic. In contrast, the ratio of the two main MCCPs homologues C_{14} and C_{15} was rather similar for all samples (see Fig. 4). Exceptions were one cod sample from the Lofoten with more than 89% of C_{14} and one Arctic char with only C_{14} detectable. For all other samples including technical mixtures, the relative abundance of C_{14} congeners was between 55% and 82% (65.8±6.16%).

3.5. Chlorine contents

A clear dominance of more volatile, lower chlorinated CPs indicating fractionation during (atmospheric) transport was not observed in any Arctic sample. The chlorine content of the CPs was calculated from the congener group patterns according to Reth et al. (2005b). This allows a comparison of the samples, even though a slight overestimation of the real chlorine content (by ca. 1–3%) cannot be avoided by ECNI-MS. Similar chlorine contents were observed for all samples. The average

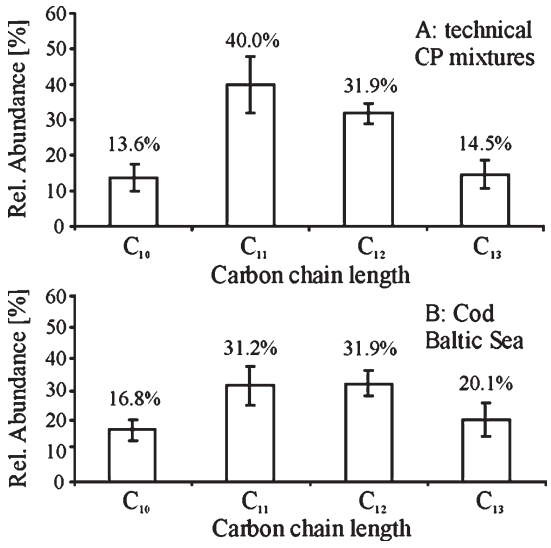

Fig. 3. Average SCCP homologue group patterns of five technical SCCP mixtures (A) and of eight cod liver samples from the Baltic Sea (B). Standard deviations are assigned.

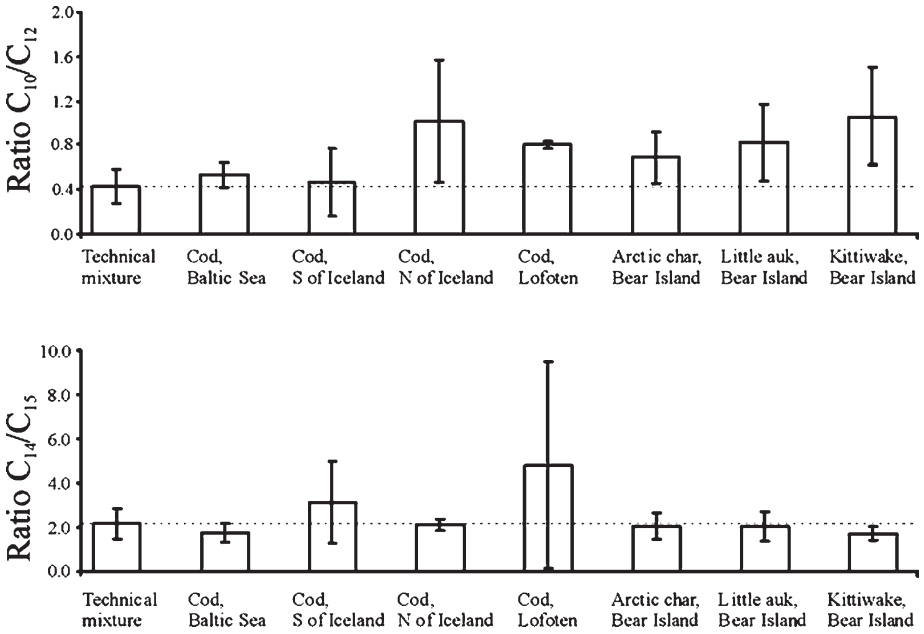

Fig. 4. Means of the ratios of the homologue groups C_{10}/C_{12} and C_{14}/C_{15} determined in technical SCCPs and MCCPs and the studied biota. Standard deviations are assigned.

calculated chlorine content of the SCCPs was 61.9% (59.0–63.3%), and that of the MCCPs 55.8% (54.5–57.4%). The chlorine content in muscle and liver from the same animal was rather similar for samples B1–4, C1 and 3 and D1 and 3 (deviation ≤ 0.2%).

The chlorine contents were surprisingly high considering that the samples were from remote areas. Drouillard et al. (1998) showed that shorter chained and lower chlorinated congeners are the most volatile components of technical CPs. However, a study of the dietary accumulation of CPs by juvenile rainbow trout found that lower chlorinated and shorter chained CPs are more susceptible to biotransformation. Moreover, predicted half-lives and biomagnification factors in fish are higher for high-chlorinated SCCPs (Fisk et al., 1998). More information about influence of transport, bioaccumulation and biomagnification on the CP composition in the Arctic with much larger and well selected number of samples is needed for further conclusions.

4. Conclusions

As other POPs, also chlorinated paraffins are present in biota from the European Arctic. Considerable differences can be found between the CP homologue group patterns of fish caught close to CP sources and biota from remote areas. Therefore, a consequent analysis with methods quantifying single congener groups is essential to achieve a better understanding of environmental transformation of CPs. A higher abundance of C_{10} congeners was observed for the samples from the remote areas. However, also high-chlorinated SCCPs as well as MCCPs were present. The CP patterns in the Arctic organisms might be a result of exposure to CPs transported via the atmosphere as well as via birds and the corresponding bioaccumulation and biomagnification processes. Therefore, further studies should be carried out with samples of a single species with a representative age and gender distribution as well as well-defined food habits to obtain more information about homologue specific accumulation/degradation processes.

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Eidesstattliche Erklärung

Ich erkläre, dass ich die Dissertation „New approaches for the mass spectrometric determination of trace concentrations and congener group patterns of chlorinated paraffins in biota" selbständig nur mit der darin angegebenen Hilfe verfasst und bei keiner anderen Universität und bei keiner anderen Fakultät der Universität Basel eingereicht habe.

Basel, 13. Dezember 2005

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