Properties and Analysis of Dioxin-Like Compounds in Marine Samples from Sweden.

by

Kjell Lundgren

Akademisk avhandling

Som med tillstånd av rektorsämbetet vid Umeå Universitet för erhållande av Filosofie Doktorsexamen vid Teknisk-Naturvetenskapliga fakulteten i Umeå, framlägges till offentlig granskning vid Kemiska Institutionen, hörsal KB3B1 i KBC-huset, fredagen den 7 februari, 2003, kl. 10.00.

Fakultetsopponent: Senior research scientist John Jake Ryan, Health Canada, Health Products and Food Branch, Ottawa, Canada.

Front cover:
"Adsorption of planar molecules onto activated carbon graphite"

Environmental chemistry
Department of chemistry
Umeå University
Umeå, Sweden

ISBN 91-7305-366-X

Printed in Sweden by VMC, KBC-huset, Umeå University, Umeå 2003.

Title **Properties and analysis of dioxin-like compounds in marine samples from Sweden.**

Author Kjell Lundgren, Environmental Chemistry, Department of Chemistry, Umeå University, SE-901 87 Umeå, Sweden.

ABSTRACT

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs) have been assigned toxic equivalency factors (TEFs). These compounds are today routinely analysed with sophisticated analytical techniques. In a near future, there might be other dioxin-like compounds such as polychlorinated naphthalenes (PCNs), alkyl-polychlorodibenzofurans (R-PCDFs), and polychlorinated dibenzothiophenes (PCDTs) added to this list of toxic dioxin-like compounds. It is therefore important to have a readiness to analyse these new compounds in environmental samples. In this study, a multi-residue non-destructive analytical method for the analyses of these planar dioxin-like compound classes was developed. The use of HPLC PX-21 carbon column fractionation enabled the separation of interfering PCBs from coplanar PCBs and other planar dioxin-like compounds of interest.

The obtained planar fraction containing the dioxin-like compounds was analysed using high-resolution GC-MS. Levels of PCNs in surface sediments and settling particulate matter in the northern Baltic Sea were determined. The concentrations of PCNs in background surface sediments were approximately 1 ng/g dw and the estimated PCN fluxes were similar to the pre-industrial levels determined in Europe. The PCN congener patterns in the surface sediments suggest that the PCNs deposited in the Baltic Sea originate from similar sources.

Bioaccumulation of PCNs in a benthic food chain (sediment, amphipod, isopod, and four-horned sculpin) from the Gulf of Bothnia was studied. The results indicated that only a few PCN congeners biomagnified. The highest biomagnification factors (BMFs) were found for 2,3,6,7-substituted congeners and those lacking adjacent hydrogen-substituted carbon atoms. The calculated biota to sediment accumulation factors (BSAFs) showed that the tetra- and penta- CNs exhibited BSAF values higher than one, while BSAFs for the more chlorinated PCNs were less than one.

A general difference between the northern and southern parts of the Gulf of Bothnia could be seen in the samples, with the lowest PCN and total PCB concentrations being found in the north and the highest in the south. This gradient is related to distance from the more industrialised and populated regions in the southern parts of Sweden and Finland, and central Europe.

Analysis of R-PCDFs in crustacean samples from the Swedish west coast was performed using HRGC-MS/MS. The ΣR-PCDFs in these samples were present at concentrations up to 10 times higher than the ΣPCDFs. The relatively high concentrations of R-PCDFs in the crab samples demonstrate that these compounds bioaccumulate.

The fate of a pollutant in the environment and the toxicity of a compound are governed by its physicochemical properties. The information found in a data set of properties can predict a compound's mode of action. The following physicochemical properties for 87 PCDFs were measured: ultra-violet-adsorption, relative retention times on two common gas chromatographic stationary phases, and relative mass spectrometric response factors using EI- and NCI- modes.

Key words: Planar dioxin-like compound, polychlorinated dibenzofuran, polychlorinated biphenyl, polychlorinated naphthalene, alkylated polychlorinated dibenzofuran, high-performance liquid chromatography, PX-21 carbon column, biomagnification factor, biota to sediment accumulation factor, fluxes, relative retention time, mass spectrometry, relative response factor, ultra-violet spectroscopy.

ISBN 91-7305-366-X

List of papers

This thesis is based on the following papers, which will be referred to by their Roman numerals I-VII.

I **K. Lundgren, B. van Bavel and M. Tysklind.** Development of a high-performance liquid chromatography carbon column based method for the fractionation of dioxin-like polychlorinated biphenyls.
Journal of Chromatography A 962 (2002) 79-93.

II **K. Lundgren, C. Rappe, and M. Tysklind.** Relative retention times (RRTs) on two common gas chromatographic stationary phases and low-resolution mass spectrometric relative response factors (RRFs) for 87 polychlorinated dibenzofurans.
Submitted to *Journal of Chromatography A* (2002).

III **M. Tysklind, K. Lundgren and C. Rappe.** Ultraviolet absorption characteristics of all tetra- to octachlorinated dibenzofurans.
Chemosphere 27 (1993) 535-546.

IV **K. Lundgren, C. Rappe and H. -R. Buser.** Detection of alkylated polychlorodibenzofurans and alkylated polychlorodibenzo-*p*-dioxins by tandem mass spectrometry for the analysis of crustacean samples.
Chemosphere 23 (1991) 1591-1604.

V **B. van Bavel, C. Näf, P. -A. Bergqvist, D. Broman, K. Lundgren, O. Papakosta, C. Rolff, B. Strandberg, Y. Zebühr, D. Zook, and C. Rappe.** Levels of PCBs in the aquatic environment of the Gulf of Bothnia: Benthic species and sediments.
Marine Pollution Bulletin 32 (1996) 210-218.

VI **K. Lundgren, M. Tysklind, R. Ishaq, D. Broman, and B. van Bavel.** Polychlorinated naphthalene levels, distribution, and biomagnification in a benthic food chain in the Baltic Sea.
Environmental Science and Technology 36 (2002) 5005-5013.

VII **K. Lundgren, M. Tysklind, R. Ishaq, D. Broman, and B. van Bavel.** Flux estimates and sedimentation of polychlorinated naphthalenes in the northern part of the Baltic Sea.
Accepted for publication in *Environmental Pollution* (2002).

List of abbreviations and symbols

AHH	Aryl hydrocarbon hydroxylase
Amino-column	Aminopropylsilica column
BMF	Biomagnification factor
BSAF	Biota to sediment accumulation factor
Di-*ortho* PCB	Di-*ortho* polychlorinated biphenyl
CID	Collision-induced dissociation
DLC	Dioxin-like compound
EI	Electron ionisation
EROD	7-ethoxy resorufin-*O*-deethylase
GC	Gas chromatography
HpCN	Heptachloronaphthalene
HPLC	High-performance liquid chromatography
HRGC	High-resolution gas chromatography
HRMS	High-resolution mass spectrometry
HxCN	Hexachloronaphthalene
LD	Lethal dose
LRMS	Low-resolution mass spectrometry
Mono-*ortho* PCB	Mono-*ortho* polychlorinated biphenyl
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NCI	Negative chemical ionisation
Non-*ortho* PCB	Non-*ortho* polychlorinated biphenyl
OCDD	Octachlorodibenzo-*p*-dioxin
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-*p*-dioxin
PCDF	Polychlorinated dibenzofuran
PCDT	Polychlorinated dibenzothiophene
PCI	Positive chemical ionisation
PCN	Polychlorinated naphthalene
PCXE	Polychlorinated xanthene
PCXO	Polychlorinated xanthone
PeCN	Pentachloronaphthalene
PGC	Porous graphitic carbon
POP	Persistent organic pollutant
PYE	2-(1-pyrenyl)ethyldimethylsilylated silica
QA	Quality assurance
QC	Quality control
QSAR	Quantitative structure-activity relationship
REP	Relative potency

R-PCDF	Alkylated polychlorodibenzofuran
RRF	Relative response factor
RRT	Relative retention time
RSD	Relative standard deviation
RT	Retention time
SAR	Structure-activity relationship
SIR	Selected ion recording
ΣPCN	Tetra- to heptachloronaphthalenes
SPM_1	Settling particulate matter
SPM_2	Semipermeable membrane
SPMD	Semipermeable membrane device
SRM_1	Selected reaction monitoring
SRM_2	Standard reference material
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TCXE	Tetrachloroxanthene
TeCN	Tetrachloronaphthalene
TEF	Toxic equivalency factor
Tetra-*ortho* PCB	Tetra-*ortho* polychlorinated biphenyl
TEQ	Toxic equivalent concentration
Tri-*ortho* PCB	Tri-*ortho* polychlorinated biphenyl
UV	Ultraviolet

Table of contents

Abstract **iii**
List of papers **v**
List of abbreviations and symbols **vi**

1. Introduction **1**
1.1 Compound classes 3
1.1.1 Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) 3
1.1.2 Polychlorinated biphenyls (PCBs) 4
1.1.3 Polychlorinated naphthalenes (PCNs) 6
1.1.4 Alkylated polychlorodibenzofurans (R-PCDFs) 9
1.1.5 Polychlorinated dibenzothiophenes (PCDTs) 11
2. Analysis **13**
2.1 General analytical flow diagram 13
2.2 Cleanup 14
2.3 Fractionation 16
2.4 HRGC-MS analysis 20
2.4.1 Relative retention times (RRTs) and retention indices (RIs) 20
2.4.2 Retention windows 21
2.4.3 Electron ionisation (EI) 23
2.4.4 Chemical ionisation (CI) 24
2.4.5 Tandem mass spectrometry (MS/MS) 25
2.5 Quality assurance and quality control (QA/QC) 27
3. Applications **30**
3.1 PCNs in samples from the Gulf of Bothnia 30
3.1.1 Sampling and a benthic food chain 30
3.1.2 PCN concentrations in surface sediments 32
3.1.3 PCN concentrations in a benthic food chain 35
3.1.4 Biota to sediment accumulation factors (BSAFs) 35
3.1.5 Biomagnification factors (BMFs) 36
3.1.6 Relative potencies (REPs) for PCNs 38
3.2 PCBs in samples from the Gulf of Bothnia 40
3.3 R-PCDFs in biological tissues 41
3.4 UV-spectrometry and relative response factors (RRFs) for PCDFs 41
4. Conclusions and future research **44**
5. Acknowledgements **46**
References **48**
Appendix A-B-C-D **58**
Papers I-VII

1. Introduction

Halogenated aromatic compounds are industrial products or by-products that have been widely dispersed in the environment. Many of these compounds are persistent, toxic, and bioaccumulate in food chains. Some toxic halogenated aromatics have a specific Ah receptor-mediated mechanism, for which structure-activity relationships (SARs) have been derived. The most toxic halogenated aromatic is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD or TCDD) and the toxicities of other individual halogenated aromatics have been determined relative to TCDD. This toxic equivalency factor (TEF) concept is used to determine a toxic equivalent concentration (TEQ) in a sample. A compound may be more or less 'dioxin-like'. In this context dioxin-like refers to compounds that have similarities to TCDD in terms of structure, physicochemical properties, and in the biochemical and toxic responses they elicit. It has been proposed (Ahlborg et al. 1994 and van den Berg et al. 1998) that to include a compound in the TEF scheme it should:

- share certain structural relationships to the PCDD/Fs.
- bind to the Ah receptor.
- elicit Ah receptor-mediated biochemical and toxic responses.
- be persistent and accumulate in the food chain.

Only 17 of the 210 PCDD/Fs have been assigned TEFs and meet the stated criteria. The other PCDD/F congeners have not been assigned TEF-values and are considered to be non-dioxin-like compounds. Non-*ortho* PCBs (four congeners) and mono-*ortho* PCBs (eight congeners) have been assigned TEFs and they are therefore referred to as the dioxin-like PCBs. If the above criteria are used to define and rank dioxin-like compounds then, for example, PCB-126 is more dioxin-like than OCDD. Many laboratories have developed sophisticated cleanup and analytical techniques for the detection of individual 2,3,7,8-chlorine substituted PCDD/Fs and coplanar PCBs in environmental samples, but many other dioxin-like compounds may be added to the list in the future. The lack of analytical and toxicological data excludes the assignment of TEFs to many compounds, which may be potentially dioxin-like. The determination of relative potencies (REPs) for a number of PCNs (Blankenship et al. 2000, Hanberg et al. 1990, and Villeneuve et al. 2000) has shown that eight PCN congeners might be included in the list of compounds with TEFs.

The major part of the work described in this thesis was focused on the cleanup and HRGC-MS analysis of dioxin-like compounds (mono-*ortho* PCBs, non-*ortho* PCBs, PCNs, R-PCDFs, and PCDTs), which might be detected in environmental samples. The dioxin-like compounds were fractionated on a HPLC PX-21 carbon column (Paper I), separated on different GC columns, and analysed using various MS-techniques (Papers II and IV-VII). The analytical procedure (cleanup, fractionation, GC-separation, MS-detection, and quantification) was optimised for the various dioxin-like compounds. Fig. 1 shows a flow diagram of the analytical procedures used, and the stages considered in the various papers included in the thesis (Paper I, development of a fractionation method for the dioxin-like PCBs; Paper II, GC-separation and MS-detection of PCDFs; Paper III, UV characteristics of PCDFs; Paper IV, VI, and VII, analysis of PCNs and R-PCDFs in the planar fraction; Paper V, HRGC-LRMS analysis of PCBs.

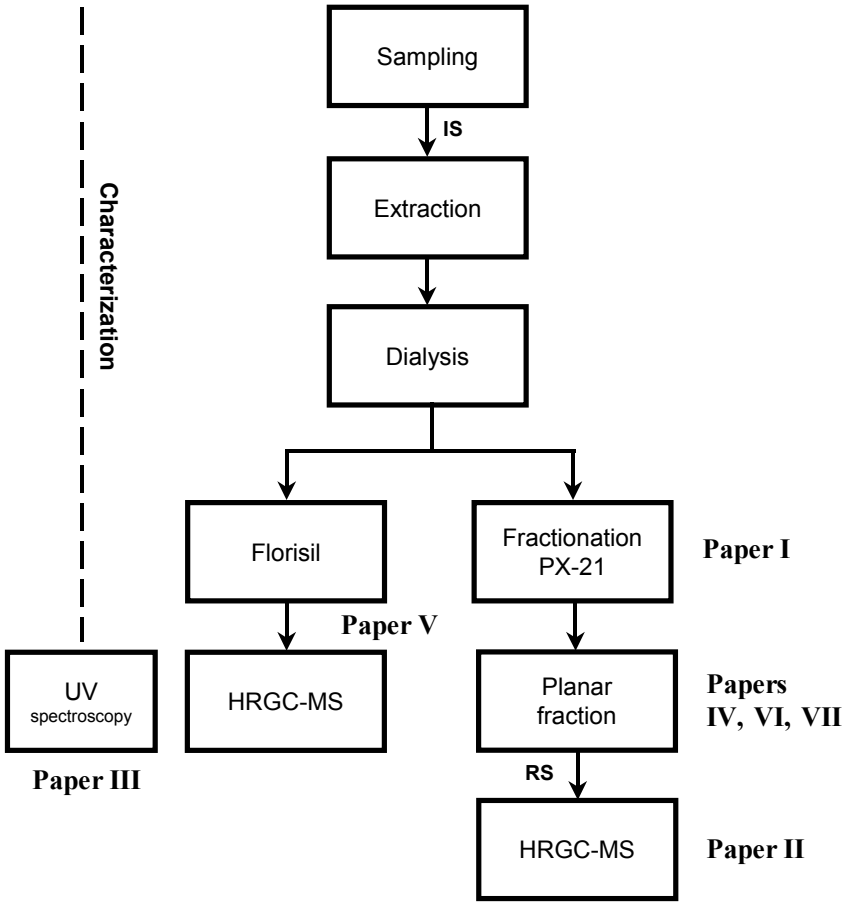
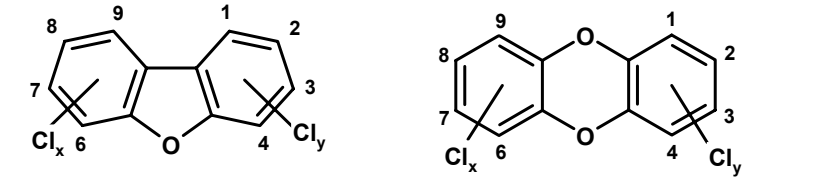


Figure 1. Stages of the analytical procedures considered in Papers I-VII.

1.1 Compound classes

1.1.1 Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs)

The impact of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) on the environment has been a source of great concern since the beginning of the 1970s. The PCDD/Fs are present as trace contaminants in various chemicals and technical mixtures (chlorophenols, PCB formulations, agent orange, etc.), or are produced as unwanted by-products during their manufacture and use. The environmental pollution of PCDD/Fs will therefore occur when these chemicals or technical mixtures are intentionally or accidentally released into the environment. The PCDD/Fs are also produced in a wide variety of industrial and combustion processes (bleaching of pulp, incineration of municipal solid waste, production of iron, steel, and other metals, etc.) and thereby spread over large geographical areas via water and air. These two groups of compounds have a number of common chemical and physical properties (Tysklind 1993), and some of them are known to be extremely toxic. The congener-specific toxicities of these compounds was first reported by Poland and Glover (1973), and it was established that the most toxic PCDD/PCDF congener among all the PCDD/Fs was 2,3,7,8-TCDD. In acute toxicological experiments with guinea pigs (Schwetz et al. 1973), low lethal doses (LD_{50}) of 0.6-2.5 μg/kg bodyweight (orally ingested) were determined for TCDD, showing the extreme toxicity of this congener. A number of accidents involving PCDD/Fs during the late 1960s and 1970s (notably the Yusho accident in Japan, Yu-cheng disease in Taiwan, and an explosion in Seveso, Italy) increased public concern and interest in these pollutants.



Polychlorinated dibenzofurans PCDFs

Polychlorinated dibenzo-*p*-dioxins PCDDs

Figure 2. General chemical molecular structures and numbering schemes for the PCDFs and PCDDs.

The general molecular structures and numbering schemes for the PCDD/F molecular skeletons are shown in Fig. 2. There are a large number (210) of PCDD and PCDF congeners, since there are numerous chlorination permutations, giving a wide variety of substitution patterns. Only seven of the 75 PCDDs and ten of the 135 PCDFs are routinely analysed. These congeners are substituted with chlorines in all 2,3,7,8 positions and have been assigned toxic equivalency factors. In this thesis these 17 compounds are defined as the dioxins.

1.1.2 Polychlorinated biphenyls (PCBs)

The PCBs comprise a group of 209 structurally different congeners with the empirical formula $C_{12}H_{10-n}Cl_n$ (n = 1-10). There are 10 PCB homologue groups (mono- to deca- CBs) with different numbers of isomers. The general molecular structure and nomenclature of the PCBs is shown in Fig. 3. Today, a numbering system (PCB 1 to PCB 209) developed by Ballschmiter and Zell (1980) are used for the identification of individual PCB congeners.

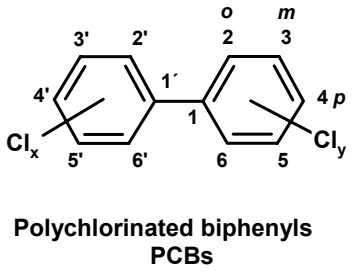


Figure 3. General chemical molecular structure, numbering, and nomenclature of the PCBs (*o, ortho; m, meta;* and *p, para*).

The PCBs are resistant to acids, bases, oxidation, and hydrolysis, so they tend to persist for long periods in the environment. In addition, the PCBs are thermally stable, electrical insulators, and have low flammability (de Vooght and Brinkman 1989). The solubility in water of this group of compounds is low, and they are highly lipophilic. The degree of lipophilicity increases with increasing chlorination (PCB 1, log K_{ow} = 4.46; PCB 209, log K_{ow} = 8.18) (Hawker and Connell 1988), while their solubility in water decreases with increases in chlorination. The PCBs have been detected in various environmental compartments worldwide, and they have been shown to bioaccumulate in food chains (Paper V).

The PCBs can be divided into sub-groups depending on the number of *ortho*-chlorine atoms (chlorine atoms at the 2, 2´, 6, 6´ positions) attached to the

biphenyl ring system. These sub-groups (non-, mono-, di-, tri-, and tetra- *ortho* PCBs; represented by 20, 48, 72, 48 and 21 congeners, respectively) with their respective PCB congeners are listed in appendix A, and the molecular structures of a selection of *ortho*-PCBs are shown in Fig. 4. An important characteristic of the PCBs is their ability to rotate around the phenyl-phenyl (1, 1´) bond. PCB molecules that can rotate freely can attain a planar configuration (coplanar PCBs) similar to the PCDD/Fs. Some of these non- and mono- *ortho* substituted PCBs are therefore referred to as the dioxin-like PCBs, all having chlorine atoms in the *meta*- and *para*- positions. The internal molecular rotation may also be restricted or impossible if the PCB molecule has too many chlorine atoms at *ortho* positions. These poly-*ortho* PCBs can thus only adapt a non-planar configuration.

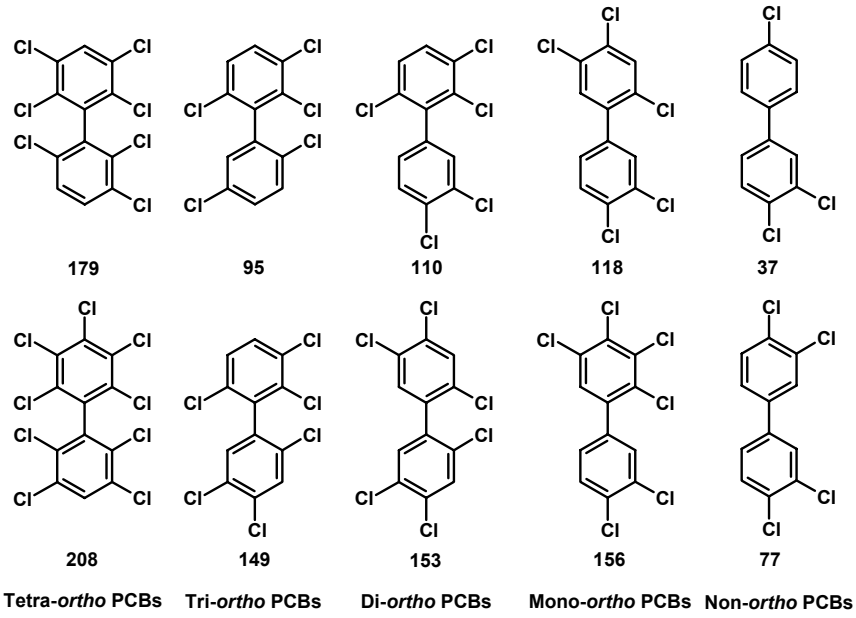


Figure 4. Structural formulae of selected tetra-, tri-, di-, mono-, and non- *ortho* PCBs.

PCBs were first identified in pike, eagle, and human hair samples by Sören Jensen (Jensen 1966), 37 years after their introduction as a commercial product in 1929 by the Monsanto Industrial Chemical Corporation. A range of PCB mixtures (Aroclor, Clophen, Kanechlor, Phenochlor, etc.) containing more or less chlorine were sold during a period of 45 years until the production of this group of compounds was banned in many countries during the mid-1970s. The PCBs were widely used as heat transfer fluids, organic diluents, plasticizers, lubricant inks, fire retardants, paint additives, sealing liquids, immersion oils, adhesives,

dedusting agents, laminating agents, waxes, and as dielectric fluids for capacitors and transformers. The total production of PCBs has been estimated at 1.5 million tonnes (de Vooght and Brinkman 1989).

The dioxin-like and non-dioxin-like effects of PCBs have been reviewed in several publications (Ahlborg et al. 1994, Alcock et al. 1998, Brouwer et al. 1998, and Giesy and Kannan 1998). A wide variety of *in vivo* studies (including acute, short-term, and chronic toxicity) have been performed to examine the potency of dioxin-like PCBs relative to 2,3,7,8-TCDD (Brouwer et al. 1998). In addition, several biodetection systems like Ah receptor-mediated assays (e.g. Ah-immunoassays), *in vitro* bioassays (e.g. EROD or luciferase assays) or immunoassays (EIAs) have been used to test the relative response of dioxin-like compounds. Only four non-*ortho* PCBs and eight mono-*ortho* PCBs have been assigned toxic equivalency factors (TEFs) by the World Health Organisation (van den Berg et al. 1998). The assigned TEFs for these coplanar PCBs range from 0.00001 (PCB 167) to 0.1 (PCB 126).

Contamination by PCBs is still of great concern, due to their continuing atmospheric deposition, formation during combustion processes, and leakage from dumps and landfills, old capacitors and transformers, sealants, and electric equipment (uncontrolled handling of PCB-contaminated waste).

1.1.3. Polychlorinated naphthalenes (PCNs)

Polychlorinated naphthalenes (PCNs) were among the first halogenated hydrocarbons to be released into the marine environment and this group of compounds were first described in 1833 (Laurent 1833). PCNs are ubiquitous environmental contaminants that originate, like PCBs, from technical mixtures (e.g. Halowaxes, Nibren waxes, Seekay waxes, Clonacire waxes, and Cerifal material). These formulations are used in a variety of industrial applications, including cable insulation, wood preservation, engine oil additives, electroplating masking compounds, feedstock for dye production, dye carriers, impregnating agents for condensers and capacitors, and oils for testing refractive indices (Crookes and Howe 1993). The industrial production of PCNs dates back to the beginning of the 20th century and PCNs were most extensively used during the period 1930-1950 (Brinkman and Reymer 1976). The production of technical PCN mixtures has ceased in many countries, but they can still be found in various items, for example, electrical equipment (Weistrand et al. 1992) and

commercial products (Yamashita et al. 2002). Total global production of PCNs has been estimated to amount to 150 000 tonnes (Falandysz 1998).

In addition, PCNs are formed and released into the environment via diverse processes such as municipal waste incineration (Imagawa and Lee 2001), copper ore roasting (Theisen et al. 1993), and chlor-alkali production (Järnberg et al. 1997 and Kannan et al. 1998). These compounds have also been found as impurities in commercial technical PCB formulations (Haglund et al. 1993).
The PCN molecule consists of two fused aromatic rings with 1-8 chlorine atoms substituted in the naphthalene molecule skeleton, so, theoretically, there are 75 possible congeners. The general structure and numbering of the naphthalene molecule is shown in Fig. 5. The molecular structures of PCNs are similar to those of the toxic PCDD/Fs and PCBs.

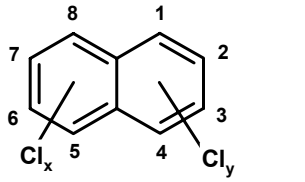


Figure 5. General chemical molecular structure and numbering of the PCNs.

PCNs have physical and chemical properties similar to PCBs. As well as being highly hydrophobic and weather-resistant, they also have high levels of chemical and thermal stability, good electrical insulating properties, and low flammability. Typical physicochemical values for the tetra-CNs include the following: log K_{ow} = 6.19, S_w = 0.004 mg/L (water solubility), and p = 0.048 Pa (vapour pressure at 25 ºC) (Crookes and Howe 1993).

The few studies of the toxicity of PCNs that have been published have shown that the highly chlorinated PCN congeners elicit biochemical responses similar to 2,3,7,8- TCDD. These responses include the induction of hepatic drug-metabolising activities, including the expression of aryl hydrocarbon hydroxylase (AHH) and 7-ethoxyresorufin-*O*-deethylase (EROD) activities (Blankenship et al. 2000, Campbell et al. 1983, Engwall et al. 1994, Hanberg et al. 1990, and Villeneuve et al. 2000). In toxicological tests in which the induction of Ah-receptor responses was monitored, the highly chlorinated PCNs, especially 2,3,6,7-chlorine substituted PCNs, were shown to have a relative potency similar

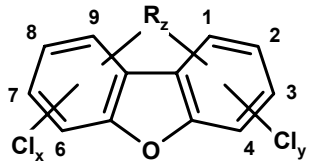
to that of the mono-*ortho* PCBs (van den Berg et al. 1998). This is 3-4 orders of magnitude weaker than 2,3,7,8-TCDD. Seven PCNs have chlorine substituents in all 2,3,6,7-positions: 2,3,6,7-TeCN (PCN 48); 1,2,3,6,7-PeCN (PCN 54); 1,2,3,4,6,7-HxCN (PCN 66); 1,2,3,5,6,7-HxCN (PCN 67); 1,2,3,6,7,8-HxCN (PCN 70); 1,2,3,4,5,6,7-HpCN (PCN 73) and OCN (PCN 75). These congeners are referred to as the 'dioxin-like' PCNs. A recent experiment examined the effects of feeding PCN-contaminated food (containing mixtures of Halowaxes) to juvenile Baltic salmon. The fish showed evidence of hepatotoxicity, dose-dependent induction of EROD activity, adverse effects in their ovaries, and delayed development (Åkerblom et al. 2000). The fact that PCNs are found in the environment at concentrations comparable to, or higher than, those of the mono-*ortho* PCBs and non-*ortho* PCBs, suggests that this group of compounds should be included in environmental monitoring studies. PCN congeners with the highest relative potencies (REPs) should be assigned toxic equivalency factors (TEFs) to enable effective risk assessment.

PCNs tend to accumulate in biota and persist in the environment as a result of their physicochemical properties. Consequently, PCNs have been detected in a wide variety of substrates, organisms and systems, including marine mammals (Helm et al. 2002 and Ishaq et al. 2000), fish (Asplund et al. 1990, Falandysz et al. 1996a, 1996b, 1997b, Järnberg et al. 1993, and Kannan et al. 2000, 2002), birds (Falandysz et al. 1996c, 1997a, 1997c, Järnberg et al. 1993, and Kannan et al. 1999, 2001), soils (Meijer et al. 2001), sediments (Eljarrat et al. 1999, Falandysz et al. 1996a, and Järnberg et al. 1993, 1999), air (Dörr et al. 1996, Harner and Bidleman 1997, and Harner et al. 1998, 2000), and groundwater (Martí et al. 1997). Although PCNs are ubiquitous pollutants, data relating to their concentrations in the environment are rarely reported in comparison, for example, to PCBs and PCDD/Fs.

Historical profiles of PCNs in dated sediment cores have been reported in two recent studies (Gevao et al. 2000 and Yamashita et al. 2000). Both investigations demonstrated that the ΣPCN concentrations in the sediments had decreased since the 1980s. The highest concentrations of PCNs in the sediment cores were found in sediments dated to 1962 in England and 1980 in Japan.

1.1.4. Alkylated polychlorodibenzofurans (R-PCDFs)

The most common chemical process used today to produce pulp, the raw material for paper, is the sulphate process, in which unwanted lignin is removed from wood by reaction with strong alkali and sulfonation. Bleaching with chlorine, chlorine dioxide, or hypochlorite is used for further treatment of the pulps. During this bleaching, a large variety of chlorinated aromatic compounds are formed, including not only polychlorinated dibenzo-*p*-dioxins and dibenzofurans (Swanson 1988), but also alkylated polychlorodibenzofurans (R-PCDFs) (Beck et al. 1989). Historically, this group of compounds was first identified as polychlorinated xanthenes (PCXEs) and xanthones (PCXOs) in sludge samples from seven different pulp and paper mills, sediments, and fish (Kuehl et al. 1987). The contaminants were identified as interfering peaks in mass chromatograms (^{13}C-labeled PCDD/Fs: m/z = 318, 332, and 334) produced during a routine analysis of polychlorinated dibenzo-*p*-dioxins and dibenzofurans using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC-HRMS). A mass resolution of 9000 could not separate the PCXEs and PCXOs from the ^{13}C-PCDD/Fs. The low mass difference (e.g., Δm = 0.022 amu) of the compounds (e.g., ^{13}C-TCDFs and methyl-TCDFs, see appendices B and C) required a mass resolution of at least 15 000 to separate the contaminants by mass. The concentrations of tetrachloroxanthenes (tetra- CXEs) and tetrachloroxanthones (tetra- CXOs) were reported in the above samples. However, there was some doubt regarding the stability of the xanthenes towards oxidative agents like the chlorine and chlorine dioxide used in the bleaching process. In another study (Buser et al. 1989), chlorinated compounds were analysed in sludge and sediments from bleached pulp processes and, contrary to the above identifications, the PCXE- and PCXO- interfering compounds were identified as R-PCDFs. Buser and co-workers concluded that the components were primarily consisted of methyl- polymethyl- and certain other classes of alkyl-PCDFs, and that they can serve as markers for pulp bleaching activities.



Alkylated polychlorinated dibenzofurans
R-PCDFs

Figure 6. General chemical molecular structure and numbering of the R-PCDFs.

The general molecular structural formulae of the R-PCDFs, and their IUPAC numbering, are shown in Fig. 6. One to seven chlorine atoms and alkyl groups can be substituted in the tricyclic dibenzofuran ring system. There are many theoretically possible R-PCDF congeners and a selection of the total numbers of some methyl-polychlorodibenzofurans (methyl-PCDFs) is given in Table 1.

Table 1. The total theoretical number of selected methyl-PCDFs.

	No. of R-PCDFs[a]			
	No. of methyl groups			
No. of Cl-atoms	*1*	*2*	*3*	*4*
1	28	84	140	
2	84	216	280	216
3	140	280		
4	140	216		

[a] Many of these methyl-PCDF congeners were detected in the crustacean samples analysed in Paper IV.

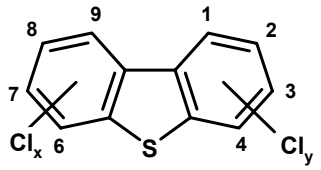
Environmental contamination by R-PCDFs is associated with discharges from pulp and paper mills and as such is related to local contamination around these plants (Buser et al. 1989 and Paasivirta et al. 1989). Consequently, the R-PCDFs have so far only been detected in sludge, sediment, and biological tissue samples from the surroundings of pulp and paper plants. Relatively high concentrations of this group of compounds have been detected in crab hepatopancreas (Paper IV), and trace quantities have been identified in fish (Kuehl et al. 1987). The R-PCDFs seem to bioaccumulate in food chains, but more research is needed to confirm these findings.

Only a limited number of toxicological studies have focused on this group of compounds. Treatment of rats with TCDD, a few congeners of 6-substituted-1,3,8-triCDFs or TCDD plus the 6-substituted-1,3,8-triCDFs has shown that

most of the substituted congeners (6-methyl, ethyl, propyl, *i*-propyl, and *t*-butyl) do not induce AHH and EROD activities (Astroff and Safe 1988). In co-administration studies, the 6-methyl-, ethyl-, propyl-, *i*-propyl-, and *t*-butyl-analogues partially antagonised induction of the monooxygenase enzyme activities by TCDD. The results indicate that the 6-substituted-1,3,8-triCDFs competitively displace TCDD from the Ah receptor and that this interaction may play a role in the mechanism of action of this class of TCDD antagonists. Another study by Shan et al. (2000) reported that the substitution of one chlorine atom of the TCDD molecule with a methyl group resulted in a 100-fold reduction in its REP-value in an *in vitro* luciferase bioassay with mouse hepatoma cells.

1.1.5. Polychlorinated dibenzothiophenes (PCDTs)

Polychlorinated dibenzothiophenes (PCDTs) are the sulfur analogues of the PCDFs. Altogether 135 PCDT congeners, from mono- to octa- chlorinated compounds, are possible. The general molecular structure and numbering of the PCDTs is shown in Fig. 7. The PCDTs were initially detected in environmental samples because they interfered at the m/z values used to monitor PCDDs. The differences in the exact molecular masses of PCDTs and PCDDs are as low as 0.018 amu (2 oxygen atoms replaced with one sulfur atom, $2 \times 15.995 - 31.972$ amu). A mass resolution of 12 000 – 26 000 is needed to distinguish between these two classes of substances.



Polychlorinated dibenzothiophenes
PCDTs

Figure 7. General chemical molecular structure and numbering of the PCDTs.

If a MS-instrument is operated with a mass resolution lower than 12 000 and the PCDTs are present in samples, they will interfere in an HRGC-HRMS analysis of PCDDs. Separation of this class of compounds on the selected capillary column is then essential for an accurate analysis. If a polar RT-2330 capillary column is used, the elution windows of the PCDT and PCDD homologues will overlap and cause problems with the identification and quantification of individual PCDT or

PCDD congeners. This problem can be solved using a non-polar DB-5 column, where the PCDT and PCDD homologues are chromatographically separated from each other. The retention times for the PCDTs are normally longer on a GC column than the PCDDs, and the PCDTs can therefore be described as late eluting dioxins.

Pollution by PCDTs originates from various industrial processes. For instance, the PCDTs have been identified in fly ash from municipal solid waste incinerators (Buser et al. 1991); in the effluents of pulp and paper mills (Sinkkonen et al. 1992); in sediments outside a former 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) manufacturing facility (Huntley et al. 1994); and in gas, ash, and slag from a metal reclamation plant (Sinkkonen et al. 1994). The PCDTs seem to bioaccumulate to some extent and tri- to penta- chlorinated PCDTs have been detected in aquatic organisms (crabs and lobsters) from Newark Bay, NJ, USA and Värö, Sweden (Buser and Rappe 1991 and Cai et al. 1994). The compound 2,4,6,8-TCDT has been found to bioaccumulate from contaminated sediments to sandworms, clams, and grass shrimp (Pruell et al. 1993). Finally, 2,3,7,8-TCDT has been found to be inducers of AHH and EROD (Kopponen et al. 1994) indicating that these compounds are dioxin-like.

2. Analysis

2.1 General analytical flow diagram

A multi-residue, non-destructive analytical procedure was developed and used for all the samples (sediment, settling particulate matter, and biota) evaluated in Papers VI and VII. This analytical procedure allowed the analysis of a large number of persistent organic pollutants in addition to the PCBs, PCNs, and R-PCDFs analysed in Papers IV-VII.

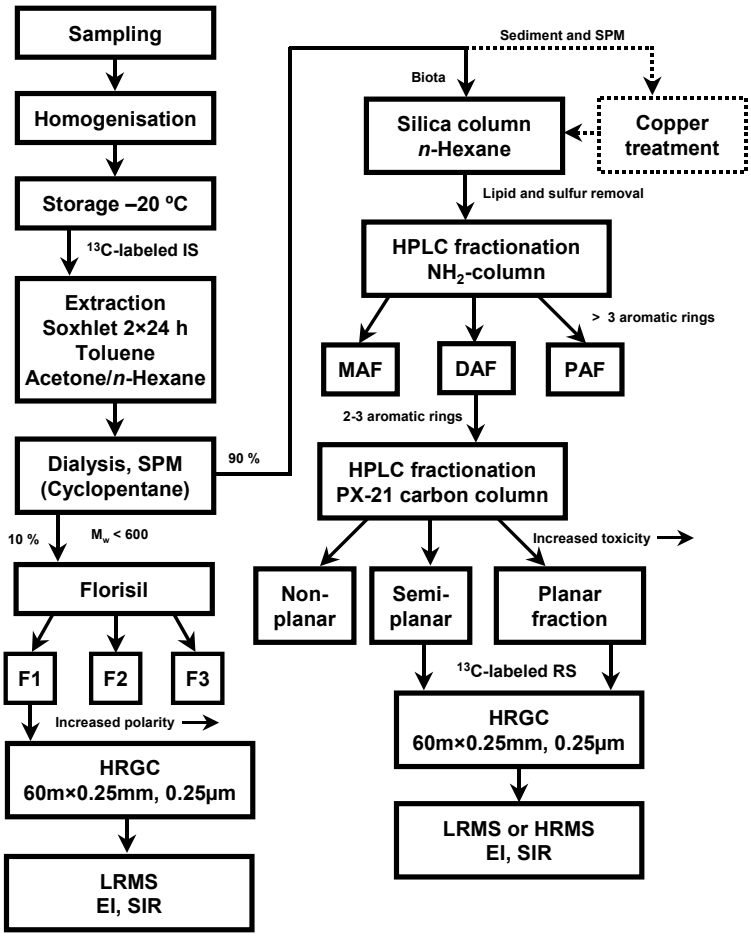


Figure 8. Analytical flow diagram, showing principal cleanup and analysis steps for PCBs, PAHs, planar dioxin-like pollutants (e.g. mono-*ortho* PCBs, non-*ortho* PCBs, PCNs, PCDTs, and R-PCDFs), and PCDD/Fs.

An analytical flow diagram showing the principal cleanup and analysis steps for the different persistent organic pollutants (POPs) is depicted in Fig. 8. The analytical procedure was designed to enable the determination of POPs in various types of biological tissues and sediments, and can be divided into two parts (Bergqvist et al. 1992). The first part is associated with the cleanup and analysis of polycyclic aromatic compounds (PAHs), planar dioxin-like compounds (e.g. mono-*ortho* PCBs, non-*ortho* PCBs, PCNs, PCDTs, and R-PCDFs), and PCDD/Fs. The second part of the analytical procedure is related to the analysis of the bulk of the PCBs and more polar compounds, such as organochlorine pesticides. As can be seen in the figure, the samples were collected, homogenised, and stored before being subjected to a sophisticated, multi-step cleanup procedure. The planar compounds were separated from the sample matrix using a cleanup consisting of two Soxhlet extraction steps, dialysis, a silica column step, and two high-pressure liquid chromatography (HPLC) fractionation steps (using an HPLC amino-column and a PX-21 carbon column). The sample extract was split into two portions after the dialysis step and 10% was used for the analysis of the bulk of the PCBs and pesticides. This portion was fractionated on a Florisil column separating the PCBs from interfering polar compounds. The final analysis of the target compounds was carried out on an HRGC-LRMS or an HRGC-HRMS system.

2.2 Cleanup

All samples from the seas around Sweden (sediment, settling particulate matter, and biological tissues) were homogenised and stored at –20 °C prior to extraction. The cleanup of the crustacean samples (crabs) from Värö and Grebbestad, described in Paper IV, followed a frequently used, destructive PCDD/F protocol developed in the beginning of the 1980s (Smith et al. 1984). A detailed description of this cleanup procedure can be found in paper IV, and will not be further described here. All samples from the Gulf of Bothnia were subjected to the multi-step cleanup depicted in Fig. 8. The homogenate derived from these samples was placed in pre-extracted cellulose thimbles and extracted wet in a Soxhlet apparatus, equipped with a Dean Stark trap for collecting water. The homogenate was extracted with 300 mL of toluene for 24 h followed by 300 mL of an azeotropic mixture of acetone:*n*-hexane (59:41, v/v) for another 24 h. The two extracts were combined and, after evaporation of the solvents, the total organic phase (i.e. lipid weight) for each sample was determined gravimetrically. The prolonged extraction time and the use of hot toluene, a strongly desorbing solvent, followed by the more polar acetone:*n*-hexane mixture released both the

non-polar and polar target compounds from particles to which they were bound (Lamparski and Nestrick 1989). Prior to extraction, ^{13}C-labeled PCBs 77, 118, 126, 153, and 169 were added as internal standards.
Separation according to differences in size between the lipid (organic phase) molecules and the target compounds can be achieved by means of dialysis (Huckins et al. 1990) through a semipermeable membrane (SPM). A membrane pore size of approximately 1 nm allows the small molecules to penetrate the membrane. Sufficiently small molecules then migrate out to the surrounding medium (cyclopentane in this study) and the large molecules (e.g. triglycerides and phospholipids) will remain inside the membrane. It has been shown that 90-99% of the lipids can be separated from the sample extract (Strandberg 1998) without a severe loss of target compounds. The dialysis step of the sample cleanup followed the procedures outlined below.

The organic phase derived from the extraction was dissolved in cyclopentane and transferred to a bottom-sealed semipermeable membrane device (SPMD; 500 mm long, 26 mm wide, and 80 μm thick) mounted inside a dialysis glass funnel. Cleanup was achieved by dialysis through the membrane, using cyclopentane, to reduce the bulk of the lipids. Dialysis through the polymeric film was accomplished by changing the outer cyclopentane (dialysate) after 16, 40 and 64 h (Strandberg et al. 1998). The dialysate fractions, containing about 1-10% of the original lipids, were combined and carefully concentrated to a volume of few millilitres. The sample extract was split into two parts: 90% being used for the analysis of PAHs, PCDDs, PCDFs, and other planar dioxin-like compounds (PCNs, mono-*ortho* PCBs, and non-*ortho* PCBs), and the other 10% being used for the analysis of organochlorine pesticides (HCHs, DDTs, etc.) and the bulk of the PCBs.

The 10% portion was further fractionated on a Florisil column separating the solutes of different polarity into three fractions (Norstom et al. 1988). For this, a glass column was packed with 8 g of methanol-washed Florisil gel (activated at 130 ºC over two days, deactivated with 1.2% water, w/w) and the extract (~1 mL) was eluted with 32 mL of *n*-hexane and 38 mL of 15% (v/v) dichloromethane in *n*-hexane (F1), 56% (v/v) dichloromethane in *n*-hexane (F2), and 66 mL of methanol (F3). The relatively non-polar PCBs were collected in the first fraction (F1) while the different organochlorine pesticides were collected in fractions 1-3 (F1-F3). The fractionation procedure on the Florisil gel column is described in detail in Paper V.

The remaining lipids in the 90% portion from the dialysis step were separated from the extract on a 10% water-deactivated silica column, eluted with *n*-hexane and elemental sulfur was removed by shaking the extract with activated copper if the sample originated from sediment or SPM (Fig. 8).

2.3 Fractionation

After reducing the solvent volume, the extract was fractionated by high-performance liquid chromatography (HPLC) with an aminopropylsilica column (amino-column) using *n*-hexane as the mobile phase (Colmsjö et al. 1987). The HPLC amino-column separates the solutes according to the condensed number of aromatic rings, and it has been shown that the mechanism of separation is mainly due to a charge transfer interaction between the lone pair of electrons on the stationary phase nitrogen and the π-electron cloud of the solute polyaromatics (Snyder and Schunk 1982 and Karelsky et al. 1983). The retention times obtained on the amino-column for toluene and anthracene were used to set the elution windows for the respective fractions. This resulted in the production of three fractions (MAF, the monocyclic aromatic fraction; DAF, the dicyclic aromatic fraction; and PAF, the polycyclic aromatic fraction; see Fig. 8). These fractions contained aliphatic compounds and monocyclic aromatic compounds (MACs), dicyclic aromatic compounds (DACs; e.g. PCNs, PCBs, and PCDD/Fs); and polycyclic aromatic compounds (PACs; e.g. polycyclic aromatic hydrocarbons, PAHs), respectively.

The fraction from the amino-column containing the DACs was then introduced to an HPLC column containing 100 mg PX-21 activated carbon on Lichroprep RP-18 (Paper I). Chromatography using activated carbon separates chlorinated aromatic hydrocarbons on the basis of molecular planarity and to some extent on the degree of chlorination (Stalling et al. 1979 and Huckins et al. 1980). Planar aromatic structures can reach closer to the carbon surface and thus interact more strongly with the carbon surface, resulting in longer retention times for these structures than for non-planar structures. The strength of adsorption will depend upon the interaction between the π-electrons of the aromatic compounds and the π-electrons of the carbon graphite structure.

An important characteristic of the PCBs is their ability to rotate around the phenyl-phenyl bond and attain a planar configuration. The planarity of a PCB congener will depend on the number of *ortho*-chlorine atoms attached onto the biphenyl ring system. The non-*ortho* PCBs can attain a planar configuration, while

the tetra-*ortho* PCBs have restricted rotation and will remain relatively non-planar. The use of active carbon as the stationary phase in chromatography can therefore fractionate the PCBs depending on their number of *ortho*-chlorines, if suitable conditions are applied. The PCBs can be classified as non-*ortho* (20 congeners), mono-*ortho* (48 congeners), di-*ortho* (72 congeners), tri-*ortho* (48 congeners) or tetra-*ortho* (21 congeners), in declining order of planarity. All these PCB congeners are grouped according to their number of *ortho*-chlorine atoms and are listed in appendix A.

The efficiency of PX-21 activated carbon for separating planar compounds from less planar compounds has been well documented since the beginning of the 1980s (Smith et al. 1984) and was therefore chosen as the HPLC stationary phase. Possible alternatives were available, such as 2-(1-pyrenyl)ethyldimethylsilylated silica (PYE) columns (Haglund et al. 1990) or porous graphitic carbon (PGC) columns (Creaser and Al-Haddad 1989). The reason for choosing PX-21 carbon to fractionate the target compounds included its high capacity (and consequently minimal changes in retention times due to matrix effects) and its ability to withstand high pressures.

The HPLC conditions used for optimising of the fractionation of the different *ortho*-PCBs are listed in Table 2 and the results of the first five experiments are shown in appendix D. The HPLC conditions used in experiment 5 were considered to be the best of the tested solvent conditions for the separation of the target compounds. They resulted in a final separation of planar dioxin-like compounds from less planar compounds (e.g. poly-*ortho* substituted PCBs) and other interfering compounds, by gradient elution with a mixture of dichloromethane (DCM, 1%) in *n*-hexane and toluene (0-10%). Planar compounds were back-flushed from the column with 80 mL of pure toluene. The chosen retention windows for tetra- and tri-*ortho*, di-*ortho*, mono-*ortho*, and non-*ortho* PCB fractions were 0-2.5, 2.5-15, 15-40, and 40-70 min, respectively. A tetradecane keeper was added to the fractions containing target compounds prior to evaporation and a recovery standard (^{13}C-labeled PCB 101) was added prior to the final HRGC-MS analysis, which ended the multi-step cleanup procedure.

A sub-sample was used to determine the total organic carbon content (TOC) in the surface sediment and sediment trap samples, using a high-temperature combustion element analyser (Carlo Erba EA 1108). Standard procedures were followed for this, with a combustion temperature of 1030 ºC, using acetanilide for standardization.

Table 2.
HPLC Elution Experiment Conditions.

Exp.	Solvent A	Solvent B	Toluene gradient (%) Time/min (volume/mL)	No. of fractions	Total volume/mL	Total time/min
1	10% DCM in hexane	Toluene	0-10% Toluene 5-40 min (20-160 mL)	17	240	60
2	10% DCM in hexane	Toluene	0-5% Toluene 5-40 min (20-160 mL)	17	240	60
3	5% DCM in hexane	Toluene	0-6% Toluene 7.5-40 min (30-160 mL)	18	320	80
4	1% DCM in hexane	Toluene	0-5% Toluene 7.5-40 min (30-160 mL)	18	320	80
5	1% DCM in hexane	Toluene	0-10% Toluene 7.5-40 min (30-160 mL)	17	280	70
6	Hexane	Toluene	0-10% Toluene 7.5-40 min (30-160 mL)	17	280	70

Flow rate: 4 ml/min, column 1; DCM = Dichloromethane

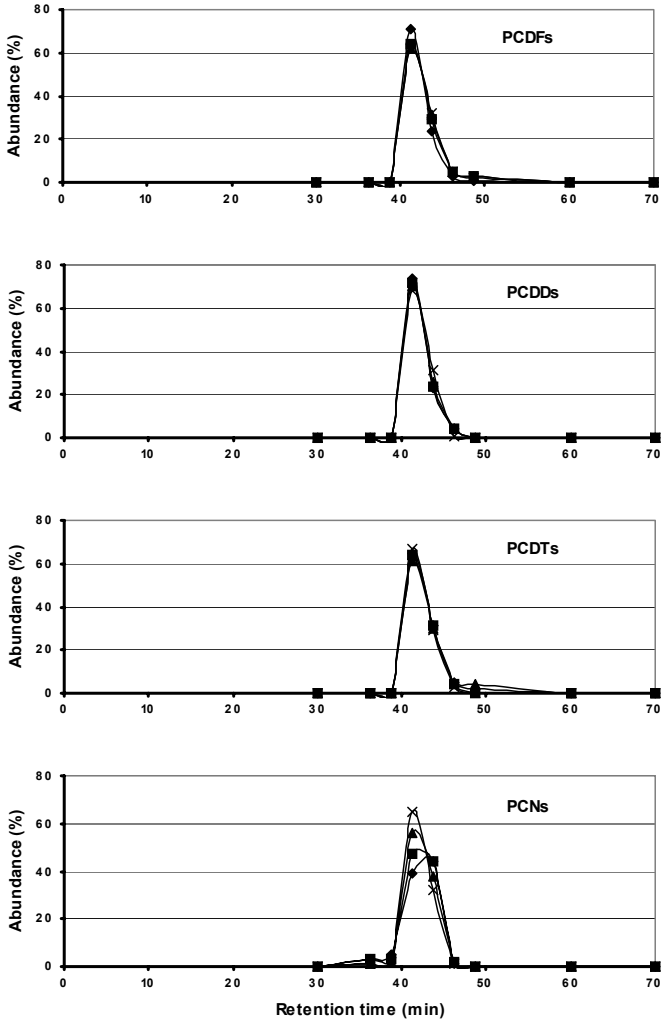


Figure 9. Elution profiles of PCDFs, PCDDs, PCDTs, and PCNs on the 100-mg HPLC PX-21 carbon column. Legends for degree of chlorination: ×, hepta; ▲, hexa; ■, penta; ♦, tetra.

In addition to the experiments in Table 2, a thorough investigation of the planar fraction was performed. Four mixtures of planar compounds containing PCDFs, PCDDs, PCDTs, and PCNs were injected onto the PX-21 carbon column and fractionated. Nine selected fractions from 30 to 70 min were analysed. Elution profiles of the planar compounds were determined and the results of these elution experiments are shown in Fig. 9. The compounds nearly all eluted from the column after 40 min, which is the cut-off time for the mono-*ortho* PCB

fraction and the point at which the back-flush procedure starts. Only a small portion (< 3%) of the lightly chlorinated PCNs was found in the wrong fraction (Fig. 9).

2.4 HRGC-MS analysis

2.4.1 Relative retention times (RRTs) and retention indices (RIs)

Since the early days of gas chromatography, much effort has been devoted to standardizing methods used to characterize retention data and thus enable the wider use of published results. Retention relative to a single standard eliminates the effects of variations in carrier gas flow rate and film thickness, but it is less reliable than a retention index system based on retention relative to a homologous series of compounds. The most widely used of these systems is the Kováts retention index system (Kováts 1965), which is based on retention of the *n*-alkanes under isothermal conditions. The *n*-alkanes are assigned index values 100 times greater than their respective carbon numbers (100 for methane, 200 for ethane, etc.) at any temperature and on any phase where the system is used.

For mixtures with wide ranges of boiling points, the determination of retention indices under isothermal conditions would be time consuming and unnecessarily restrictive. However, a retention index system equivalent to the Kováts retention index system can be used under temperature-programmed conditions (Hale et al. 1985). The retention time relative to a single standard, and the temperature-programmed retention index system outlined above were used in Papers II and IV, respectively. Relative retention times (RRTs) relative to a single standard (1,3,6,8-TCDF) were determined for all the 87 tetra- to octa- CDFs in Paper II on two common stationary phases (DB-5, 95% methyl-5% phenyl polysiloxane and RT-2330, 90% biscyanopropyl-10% cyanopropylphenyl siloxane) and temperature-programmed retention indices were determined for R-PCDF standards in Paper IV.
The RRTs were calculated from the formula:

$$RRT = \frac{RT_{PCDF\ congener}}{RT_{1,3,6,8-TCDF}}$$

where $RT_{PCDF\ congener}$ is the retention time of the PCDF congener of interest, and $RT_{1,3,6,8\text{-}TCDF}$ is the retention time of the 1,3,6,8-TCDF (the first eluting

PCDD/F), and the temperature-programmed retention indices (RI) were calculated using the following general equation:

$$RI = 100z + 100 \times \left[\frac{T_r(R-PCDF) - T_r(C_z)}{T_r(C_{z+1}) - T_r(C_z)} \right]$$

where T_r(R-PCDF) is the retention time of the R-PCDF of interest and $T_r(C_z)$ and $T_r(C_z+1)$ are the retention times of the normal hydrocarbons bracketing the R-PCDF with carbon numbers z and $z+1$.

2.4.2 Retention windows

Many classes of persistent organic pollutants are globally distributed and can be detected in many compartments of the environment (Wania and Mackay 1996). A sample matrix from an environmental compartment may therefore contain a variety of both known and unknown compounds. GC-MS analysis of a specific class of persistent organic pollutants in a sample may be essential because of the potential presence of interfering compounds that have similar physical and chemical properties (e.g. PCDD/Fs and R-PCDFs, Paper IV) to the target compounds.

The resolving power of an HRGC-HRMS system may be too low for unambiguous analysis of the target compounds since the separating power of the chosen capillary column may be insufficient, and/or the mass resolution required may be too high for the mass spectrometer used. It is therefore important to know the elution windows (the retention time intervals between the first and last eluting isomers of a series of homologues) for as many different compound groups as possible on the particular GC columns used, and the exact masses of the molecular ions and fragment ions of the chemical classes of persistent organic pollutants that may interfere in an analysis.

For these reasons, the elution windows for the PCB, PCN, PCDF, PCDT homologues, and some individual methyl- PCDFs and dimethyl- PCDFs were determined on both a DB-5 and an RT-2330 column. The results of these analyses are shown in Fig. 10. The analysed mixtures were as follows: a PCB window-defining mixture for a DB-5 column, a Halowax 1014 mixture containing PCNs, the 'allmix' described in Paper II with 87 PCDFs, a reaction mixture of PCDTs synthesised via chlorination of dibenzothiophene provided by Hans-Rudolf Buser (Swiss Federal Research station, Wädensvil, Switzerland)

containing nearly all the PCDTs, and a few individual R-PCDF congeners provided by Stephen Safe (Texas A&M University, Department of veterinary physiology, Texas, USA).

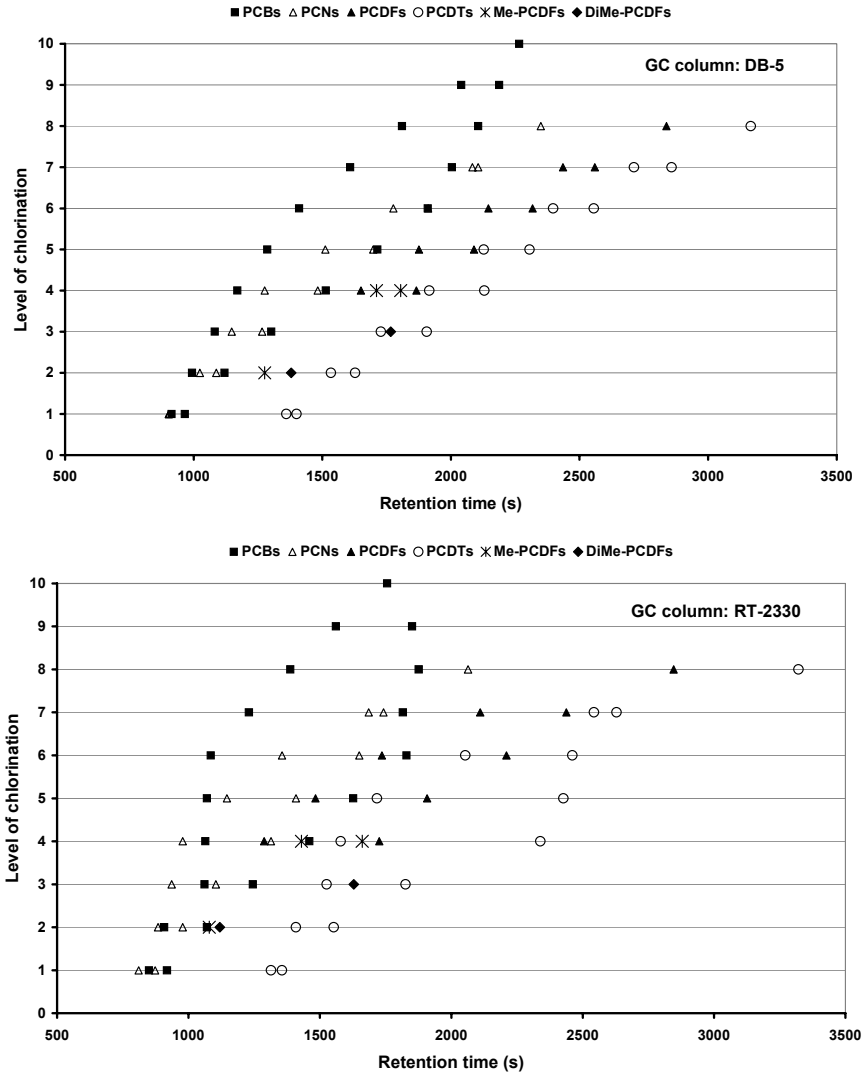


Figure 10. Elution windows for PCBs, PCNs, PCDFs, PCDTs, methyl-PCDFs, and dimethyl-PCDFs on a DB-5 (top) and an RT-2330 (bottom) fused-silica capillary column.

The retention of these compound classes on a non-polar DB-5 column is strongly dependent on the boiling point of the analysed compounds, while the interaction between the molecule and the stationary phase in a polar RT-2330

column is more pronounced. This can be clearly seen in Fig. 10, which shows that the different classes of compounds have more strongly overlapping retention windows when the compound mixtures are analysed on an RT-2330 column. The retention windows obtained for the different classes of compounds were then used for the development of the HRGC-MS analysis of PCBs, PCNs and R-PCDFs described in Papers IV, V, VI, and VII.

2.4.3 Electron ionisation (EI)

The commonest method of ionisation in mass spectrometry is electron ionisation (EI), in which electrons derived from a heated filament are accelerated in an electric field and directed across the ion source of a mass spectrometer interacting with the sample molecules. This ionisation technique works well for many gas-phase molecules, but induces extensive fragmentation. The electron-molecule interaction produces highly reactive, charged species, which would be rapidly quenched or scattered at high pressures and therefore a high vacuum is needed in the instrument. The ion source is kept at a very low pressure of approximately 10^{-5}-10^{-6} Torr using highly efficient vacuum pumps.

The positive or negative ions produced in the ion source are then extracted by a lens system to a mass analyser (e.g., a quadrupole or double focusing magnetic sector analyser) and finally detected. Unit mass resolution can be obtained by a quadrupole filter (LRMS: low-resolution mass spectrometry) and a resolution of 20 000 may be achieved by the use of a magnetic sector analyser (HRGC: high-resolution mass spectrometer). The mass spectra ('fingerprints') obtained are characteristic of the molecule being ionised.

The mass spectrometer can work in several different scan modes (e.g., full-scan, partial scan, and selected ion recording modes). If the full-scan mode is used the mass analyser often monitors the m/z range 50-650, and if a partial scan mode is used an arbitrary mass interval can be analysed. Selected ion recording (SIR) is especially useful for cases (like environmental samples) where the compound or compounds to be quantified are present in complex mixtures, and/or low levels of the compounds are expected. The SIR-mode is more sensitive than the scan modes since the measurement of a chosen single ion (molecular or fragment ion) can be repeated more often during the entire time that the compound resides in the ion source. In addition to that, the level of noise will be reduced. A thousand-fold increase in sensitivity can be attained.

A variety of different MS-methods, ionisation techniques, and scan modes have been used in the investigations presented in the papers included in this thesis. These methods, techniques, and modes are given in Table 3.

Table 3. Mass spectrometric methods, ionisation techniques, and scan modes used in Papers I-II and IV-VII.

	Paper					
	I	*II*	*IV*	*V*	*VI*	*VII*
MS-method	LRMS	LRMS	LRMS	LRMS	HRMS	HRMS
Ionisation	EI	EI, NCI	EI, NCI MS/MS	EI	EI	EI
Mode	Full-scan SIR	SIR	Full-scan SIR SRM	SIR	SIR	SIR

LRMS: low-resolution mass spectrometry; HRMS: high-resolution mass spectrometry; EI: electron ionisation; NCI: electron-capture negative ion chemical ionisation; MS/MS: tandem mass spectrometry; SIR: selected ion recording; SRM: selected reaction monitoring.

2.4.4 Chemical ionisation (CI)

EI leads to fragmentation of the molecular ion, which may result in a relatively low abundance of the molecular ion, or sometimes even prevent its detection. In GC-MS analysis of chlorinated persistent organic pollutants using EI in SIR-mode, the two most abundant ions in the molecular ion chlorine distribution cluster are often monitored, and the frequent fragmentation in EI reduces the sensitivity of the analysis.

A soft ionisation technique, which can increase the relative abundance of the molecular ions, may then be needed to detect the molecular ions. Chemical ionisation (CI) is a soft technique that produces electrons and ions with little excess energy. Thus, this technique yields spectra with less fragmentation in which the molecular species are easily recognized. Consequently, CI is complementary to EI. In CI, ions are produced through collisions between the molecule to be analysed with primary electrons and ions present in the ion source. In chemical ionisation mass spectrometry (CI-MS) a reagent gas is introduced into the ion source and a relatively high pressure is maintained (~1 Torr). Reagent gases such as methane, isobutane, and ammonia are commonly used for this purpose. The reagent gas is ionised by the electron beam to produce thermal electrons and reactant ions, which can then interact with the sample molecules. The ions appearing in the CI mass spectrum of a compound

are due to ion-molecule reactions and electron-capture mechanisms. Since these ion-molecule reactions and electron-capture mechanisms are low in energy compared to the EI process, abundant molecular ions and simple fragmentation patterns are often observed.

Positive (positive chemical ionisation, PCI) and negative (negative chemical ionisation, NCI) ions can be analysed under CI conditions. The simplest CI process, which yields negative ions, is electron-capture. The capture of electrons by sample molecules is a resonance process, which requires electrons of low energy. The electrons are thermalized by inelastic interactions with the reagent gas molecule. Electrons produced in the ionisation of the reagent gas are also thermalized. The electrons can then be captured by the sample molecules entering the ion source. The formation of negative ions by the interaction of electrons and molecules can occur by three mechanisms, formally written as follows:

$e^- + AB \rightarrow AB^-$	associative resonance capture
$e^- + AB \rightarrow A + B^-$	dissociative resonance capture
$AB + e^- \rightarrow A^+ + B^- + e^-$	ion-pair production

Electron-capture NCI is potentially useful for two reasons. The formation of molecular anions under CI conditions provides valuable molecular weight information for qualitative analysis and NCI often shows significantly greater sensitivity for some molecules in comparison with both EI and PCI. NCI is only applicable to molecules that have a positive electron affinity (e.g. halogenated compounds), which applies to many of the persistent organic pollutants. Therefore, NCI is somewhat analogous to electron-capture detection (ECD) as used in gas chromatography. Methane NCI was used in studies described in Paper II, investigating the EI and NCI relative responses for the 87 tetra- to octa-CDFs, and Paper IV, comparing the MS selectivity and sensitivity obtained in EI, NCI, and MS/MS modes for detecting the R-PCDFs (Table 3).

2.4.5 Tandem mass spectrometry (MS/MS)

Tandem mass spectrometry (MS/MS) involves at least two stages of mass analysis, in conjunction with either a dissociation process or a chemical reaction that causes a change in the mass or charge of an ion. In the most common types of MS/MS experiment a first analyser (e.g., a quadrupole filter) is used to isolate a precursor ion (parent ion), which then undergoes fragmentation either

spontaneously or by some form of activation (e.g., collision – induced dissociation, CID) to yield product ions (daughter ions) and neutral fragments.

$$m_p^+ \rightarrow m_f^+ + m_n$$

A second spectrometer analyses the produced product ions in the collision cell.

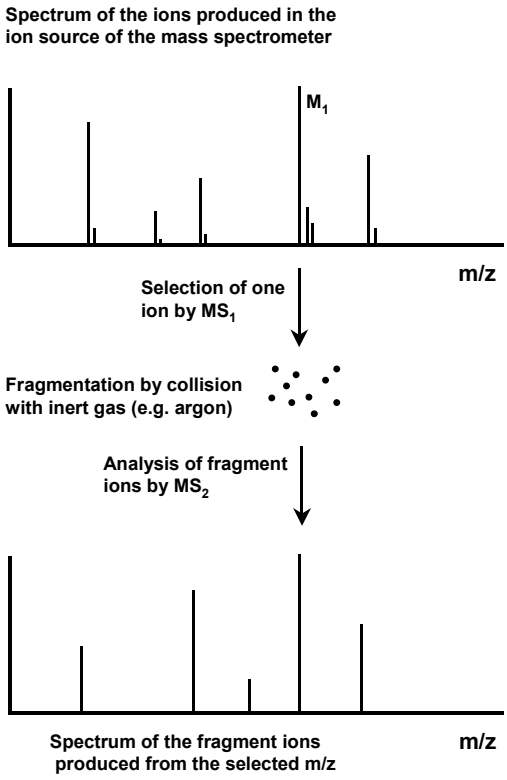


Figure 11. Principle of MS/MS: an ion, M_1, is selected by the first spectrometer, MS_1, fragmented through collision and the fragments are analysed by the second spectrometer, MS_2.

The principle of MS/MS is illustrated in Fig. 11. A common type of instrument that can accomplish this type of MS/MS analysis uses two quadrupoles as analysers. Many MS/MS scan modes can be used, but one of the most common modes for this purpose is selected reaction monitoring (SRM), in which a specific fragmentation reaction is selected and followed. For this scan mode, both the first and second analysers are focused on selected masses (Fig. 11). The method is

analogous to SIR in standard mass spectrometry, but here the ions selected by the first mass analyser are only detected if they produce a given fragment by a selected reaction. The absence of scanning allows one to focus on the precursor and fragment ions over longer periods, increasing the sensitivity, as for selected ion recording, but this sensitivity is now associated with much greater selectivity.

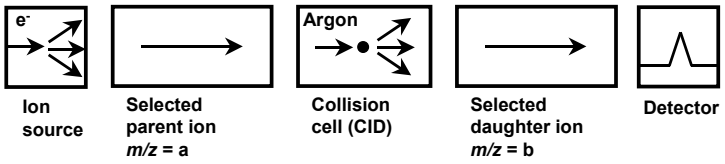


Figure 12. Processes in selected reaction monitoring scan mode.

In the development of the analytical method for the analysing of the R-PCDFs described in Paper IV different MS techniques were compared. One of the investigated analytical methods was MS/MS in selected reaction monitoring scan mode (where the parent ion was M^+ and the daughter ion $M\text{-}Cl^+$). This method was compared with other low-resolution techniques (EI in selected ion recording mode and NCI with methane as buffer gas). The experiments showed that the MS/MS method described above was the most sensitive and selective method for the analysis of R-PCDFs. The method was therefore chosen for the final analysis of R-PCDFs in environmental samples.

2.5 Quality assurance and quality control (QA/QC)

Quality assurance and quality control criteria are important to ensure that analytical results are reported correctly. It is therefore crucial to set out these criteria before starting an analysis. The following internal quality control criteria were applied to the analysis of the persistent organic compounds in this project, to guarantee reliable analytical results:

- GC retention times should be within ± 2 s compared to the internal and external standards used.
- The recovery of a ^{13}C-labeled internal standard should be within 50-110%.
- If a HRMS instrument was used, it had to operate with a resolution equal to or greater than 8000 ($R \geq 8000$).

- The isotope ratios of the two molecular ions of the ion chlorine distribution cluster analysed in SIR-mode should be within ± 10% of the theoretical values.
- The signal to noise ratio should be equal to or greater than 3 ($S/N \geq 3$).
- Procedure blanks should be run concurrently with each batch of 10 samples.

In addition to these criteria, the PCN analysis was subjected to external quality control in an inter-laboratory calibration study (Harner and Kucklick 2002). The inter-laboratory study was initiated to investigate the consistency in reported concentrations of PCNs and this particular exercise was designed to check the quantification step of a PCN analysis. Nine participating labs from seven countries quantified individual homologue groups, ΣPCN, (the sum of 2- to 8-chlorinated homologues) and selected congeners in two test solutions derived from Halowax 1014. The means of the reported ΣPCN values were within less than 15% of the known concentrations of the two test solutions (high and low PCN concentrations) and the relative standard deviation among laboratories was 11%. The results of the inter-laboratory study showed that the participating laboratories, including our laboratory, were able to quantify the PCNs with high accuracy and precision.

The method validation process for the analysis of PCBs on the HPLC 100-mg PX-21 carbon column described in Paper I consisted of a multitude of tests. The method was compared with results from an inter-laboratory study involving 19 laboratories analysing mono-*ortho* PCBs and non-*ortho* PCBs in a herring oil sample (de Vooght et al. 1994). The validation of the method also included the following exercises:

- The analysis of PCBs in a cod liver oil sample (standard reference material, SRM 1588) and a marine sediment sample (SRM 1941).
- Triplicate analyses of mono-*ortho* and non-*ortho* PCBs in a homogenous sediment sample and a pooled amphipod sample.
- Recovery experiments analysing di-*ortho*, mono-*ortho,* and non-*ortho* PCBs in the fractions obtained from the carbon column.
- Analyses of PCBs and PCNs in spiked (two levels: 5 and 25 times the expected concentrations of the target compounds) herring oil and settling particulate matter (SPM) samples.

The outcome of these rigorous validation exercises showed that the HPLC carbon column method could pass the quality control criteria and provides an excellent method for detecting the investigated compounds.

3. Applications

3.1 PCNs in samples from the Gulf of Bothnia

3.1.1 Sampling and a benthic food chain

Between autumn 1991 and autumn 1993 surface sediments, amphipods (*Monoporeia affinis*), isopods (*Saduria entomon*), and four-horned sculpins (*Oncocottus quadricornis*) were collected at five different coastal locations in the Gulf of Bothnia. All came from sea-floor areas characterized by sediment accumulation (Figure 13). The sampling locations, from north to south, were as follows: Harufjärden (HF), Umeå (UM), Hornslandet (HL), Gävlebukten (GB), and Simpnäs (SN). The samples were taken from the second accumulation depression from the coastline in order to establish background levels. In addition, sediment traps for collecting settling particulate matter (SPM) were positioned near the sea bottom at two offshore stations (Bothnian Bay: F9 and Bothnian Sea: SR5) and two coastal stations (HF and SN, Fig.13) in the Gulf of Bothnia.

Figure 13. Sampling locations in the seas around Sweden. Abbreviations: HF, Harufjärden; UM, Umeå; HL, Hornslandet; GB, Gävlebukten; and SN, Simpnäs.

The benthic food chain studied consists of species living in or close to sediment accumulation areas. Amphipods and isopods are sediment-dwelling crustaceans. Amphipods feed on material from the sediment and serve as a food source for isopods, which are also carrion feeders. Four-horned sculpins are bottom-dwelling fish that feed on both isopods and amphipods. The sculpins are sedentary, and are, therefore, good indicators of environmental pollution within the region they inhabit. The investigated benthic food chain is illustrated in Fig. 14.

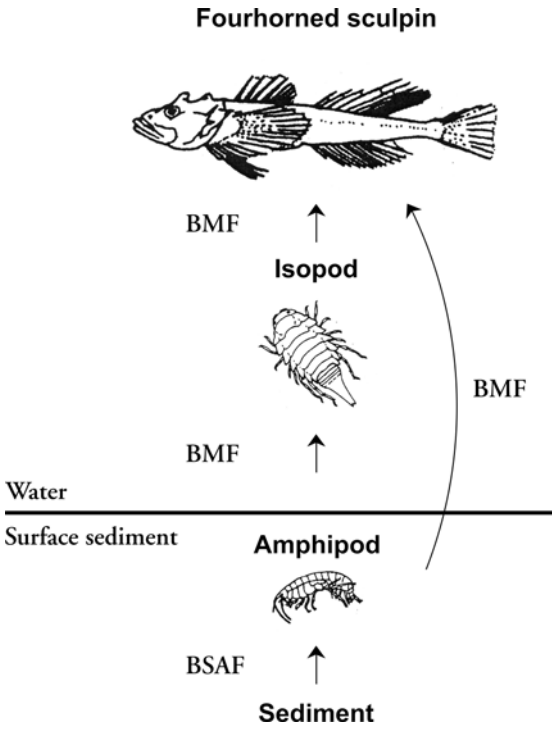


Figure 14. The benthic food chain (amphipod, isopod, and four-horned sculpin) investigated in the Gulf of Bothnia. BMF, biomagnification factor; BSAF, biota to sediment accumulation factor.

3.1.2 PCN concentrations in surface sediments

The analyses of the surface sediment samples show that the average concentrations of total PCNs were lowest in the Bothnian Bay (locations HF and UM) and highest in the Bothnian Sea (locations HL, GB, and SN). The concentrations found in the two areas were 0.088-0.33 and 0.48-1.9 ng/g dw, respectively. These results indicate that there is a higher level of deposition of PCNs in the southern part of the Gulf of Bothnia and this gradient is probably related to distance from the more industrialised and populated regions in the southern parts of Sweden and Finland, and central Europe. Similar background surface sediment PCN levels (0.27 to 2.5 ng/g dw) have been determined previously near these locations (Järnberg et al. 1999). PCN homologue profiles and congener patterns for surface sediments collected in the Baltic Sea (Paper VI, Järnberg et al. 1999 and Falandysz et al. 1996a) are shown in Figs. 15 and 16. The PCN profiles in the sediments varied between locations, but the PCN congener patterns were similar (Figs 15 and 16). The similarity of the patterns suggests that the PCNs deposited in the Baltic Sea originate from similar sources and may reflect a long-range atmospheric transport of PCNs throughout the Baltic Sea.

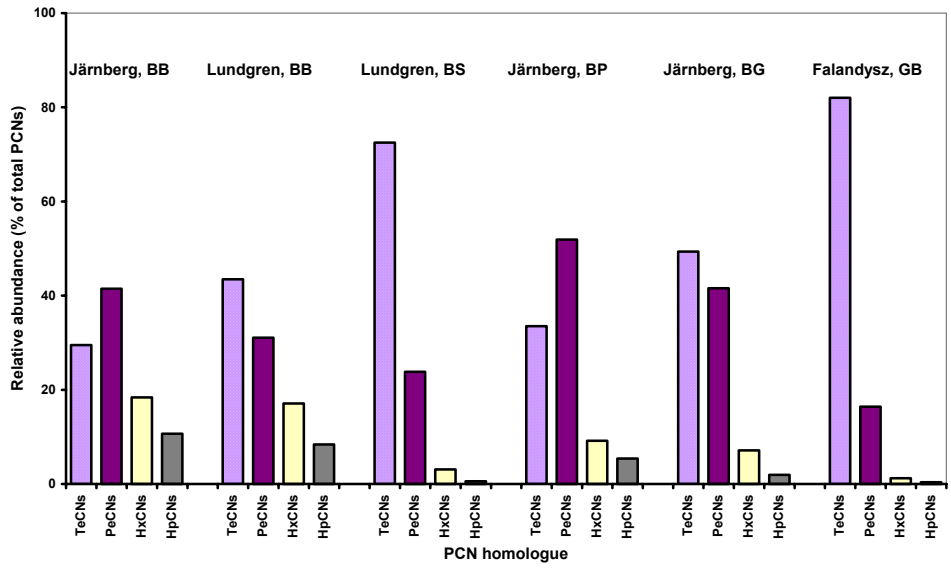


Figure 15. PCN homologue profiles in sediments from the Baltic Sea. Data from Järnberg et al. 1999 and Falandysz et al. 1996a. (BB, Bothnian Bay; BS, Bothnian Sea; BP, Baltic Proper; BG, Gulf of Gotland; GB, Gulf of Gdańsk).

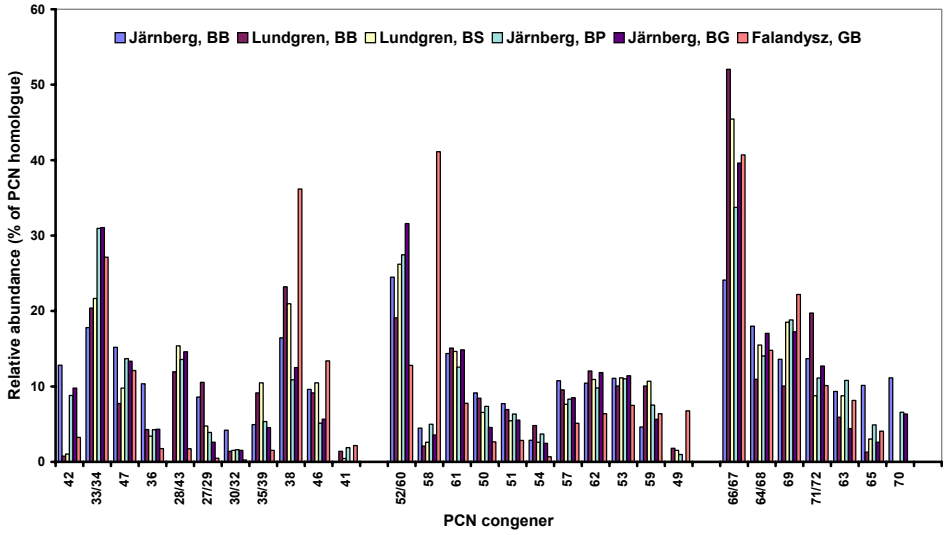


Figure 16. PCN patterns in sediments from the Baltic Sea. Data from Järnberg et al. 1999 and Falandysz et al. 1996a. (BB, Bothnian Bay; BS, Bothnian Sea; BP, Baltic Proper; BG, Gulf of Gotland; GB, Gulf of Gdańsk).

Quantifiable concentrations of PCNs have been found in other surface sediments from different parts of the world. In Europe, detected background concentrations in surface sediments have been found in the range of 0.5-7.6 ng/g dw in the Baltic Sea (Falandysz et al. 1996a and Järnberg et al. 1999). A sample from the Swedish west coast (Kattegat) contained 0.6 ng/g dw (Järnberg et al. 1999). Sediment samples from Swedish, English, and Finnish lakes contained 0.1-1.3 ng/g dw (Järnberg et al. 1999), 2.8 ng/g dw (Gevao et al. 2000), and 0.5-3.5 ng/g dw (Koistinen et al. 1990), respectively. ΣPCN concentrations in the Venice and Orbetello lagoons in Italy ranged from 0.03-1.5 ng/g dw (Eljarrat et al. 1999). Across Europe, in surface sediments, a relatively constant background concentration of PCNs (~1 ng/g dw) is seen.

Furthermore, in North America, the recorded background sediment concentrations are of the same order of magnitude as those in Europe. Lake Michigan sediments contained 0.3-0.8 ng/g dw (Kannan et al. 2000) and sediments from the Detroit river contained 1.1-8.5 ng/g dw according to studies by Kannan et al. (2000) and Furlong et al. (1988), respectively. However, elevated PCN levels have been found near local sources: for instance 19 600 ng/g dw in marsh sediments near a former chlor-alkali plant in Georgia, U.S.A. (Kannan et al. 1998), and 61 000 ng/g dw in the Trenton channel of the Detroit river

(Furlong et al. 1988). In Japan, the concentration of PCNs in the surface sediments of Tokyo Bay was found to be 1.8 ng/g dw in a study by Yamashita et al. (2000). This PCN concentration is, again, similar to background concentrations found elsewhere in sediments from the northern hemisphere.

The analysis of PCNs in the settling particulate matter (SPM) from the sediment traps enabled PCN fluxes in the Gulf of Bothnia to be determined. The values thus obtained were relatively low, ranging from 0.49 to 0.93 $\mu g\, m^{-2}\, y^{-1}$. Higher ΣPCN fluxes were measured in the Bothnian Sea (0.93 $\mu g\, m^{-2}\, y^{-1}$, SN and 0.86 $\mu g\, m^{-2}\, y^{-1}$, SR5) compared to the Bothnian Bay (0.58 $\mu g\, m^{-2}\, y^{-1}$, HF and 0.49 $\mu g\, m^{-2}\, y^{-1}$, F9). The ΣPCN fluxes near the coasts were elevated in both basins, but they were of the same order of magnitude as the pre-industrial levels (0.4-0.6 $\mu g\, m^{-2}\, y^{-1}$) determined in a sediment core from Esthwaite Water, a lake in a semi-rural location in northwest England (Gevao et al. 2000). The estimated ΣPCN flux in the top layer of the core (dated 1995) was 2.7 $\mu g\, m^{-2}\, y^{-1}$. ΣPCN fluxes calculated from this sediment core peaked in the late 1950s to mid-1960s (11.5 $\mu g\, m^{-2}\, y^{-1}$). In a sediment core collected in Tokyo Bay, Japan, the estimated flux of ΣPCNs was 9.1 $\mu g\, m^{-2}\, y^{-1}$ (Yamashita et al. 2000), and the level had peaked during the 1980s (22 $\mu g\, m^{-2}\, y^{-1}$), 20 years later than the ΣPCN fluxes in England.

The SPM sample contained a greater proportion of the lower chlorinated homologues (TeCNs and PeCNs) compared to the surface sediment sample. This indicates that the higher chlorinated congeners (HxCNs and HpCNs) may have a greater tendency to be adsorbed onto particles (Harner et al. 1998), and thus a greater probability of being retained in the sediments. The higher chlorinated naphthalenes are also more lipophilic ($\log K_{ow}$: 6.19, TeCNs; 6.87, PeCNs; 7.58, HxCNs; 8.3, HpCNs; Crookes and Howe 1993) and will partition to a higher extent into the lipid phase of particles than the lower chlorinated naphthalenes. In addition, the relatively high water solubility (TeCNs between 4.0 and 8.3 μg/L; OCN 0.08 μg/L) of the lower chlorinated congeners favours their mobility in the water column (Crookes and Howe 1993). Rough estimates of the total deposition of PCNs in sediments in the Bothnian Bay and Bothnian Sea, based on the ΣPCN fluxes and the total surface areas of the basins, suggests approximately 20 $kg\, y^{-1}$ were deposited in the Bothnian Bay and 71 $kg\, y^{-1}$ in the Bothnian Sea during the period of sampling, around 1992.

3.1.3 PCN concentrations in a benthic food chain

The total average concentration of PCNs in the lipids decreased from the bottom to the top of the benthic food chain ($C_{\Sigma PCNs,amphipod} > C_{\Sigma PCNs,isopod} > C_{\Sigma PCNs,sculpin}$). The average levels in amphipods were between 10 and 69 ng/g lw, compared to 3.9-16 ng/g lw in the isopods. The ΣPCN levels in the four-horned sculpins (0.54-1.5 ng/g lw) were very similar in all the samples analysed. Generally, naphthalenes with low levels of chlorination were found in the food chain base and higher chlorinated homologues accumulated toward the top of the chain. The amphipod samples showed no major deviation in homologue distribution from the sediments in which they lived. This may be due to the limited capacity of these organisms to excrete, eliminate and/or metabolize the PCNs. In general, the PCN pattern shifts from a sediment-like pattern (sediment, amphipod) to a non-sediment-like pattern (isopod, sculpin) when moving from the bottom to the top of the food chain (Paper VI). The differences in patterns might reflect congener-specific patterns of rapid excretion, intestinal absorption, and metabolic transformations in the species studied.

3.1.4 Biota to sediment accumulation factors (BSAFs)

Biota to sediment accumulation factors (BSAFs) have been advocated as simple measures for predicting the bioaccumulation of sediment-associated neutral organic contaminants by or in faunal invertebrates. The basic assumption of this concept is that contaminants partition between tissue lipids and the total organic carbon (TOC) in the exposed sediment. BSAFs (expressed in units of g lipid/g TOC) are calculated by the following equation:

$$BSAF = \frac{C_t}{C_s}$$

where

C_t = tissue contaminant concentration (ng/g lw)
C_s = sediment contaminant concentration (ng/g TOC)

In this study, PCN-BSAFs were calculated for the amphipods in relation to the sediments they lived in (Fig. 14; Paper VI). The calculated average BSAF values for the PCNs, in decreasing order, were as follows: TeCNs 2.9 (1.4 - 4.1) > PeCNs 1.4 (0.87 - 1.9) > HxCNs 0.88 (0.69 - 1.4), and HpCNs 0.90. The BSAF values for the isomers within each homologue group were similar (i.e. were

within narrow intervals) and in general they were higher for lower chlorinated PCN congeners than for higher chlorinated congeners. The BSAF values reflect the total uptake of PCNs in the amphipods. This can take place in the gut, through the skin and via the gills. The results suggest the amphipods assimilate the lower chlorinated PCNs more efficiently. The transport medium for all the uptake routes is probably water. PCN congeners that are more soluble in water (TeCNs between 4.0 and 8.3 μg/dm^3; OCN 0.08 μg/dm^3)(Crookes and Howe 1993), are therefore more readily available, and seem to have higher levels of accumulation.

BSAFs have also been calculated for 13 PCBs (tri- to deca- PCBs) in another study (Boese et al. 1996) where the investigated organisms were deposit-feeding clams (*Macoma nasuta*). The clam-box experiments in the cited study recorded PCB-BSAFs (0.13-3.35) similar to the above PCN-BSAFs. The BSAFs determined for the PCBs decreased with increasing chlorination, as also seen for the PCNs in the amphipod study. The assimilation of the PCBs and PCNs from the sediments to the lipids in the clams and amphipods seems to follow a similar mechanism. In contrast, in another study (Magnusson 2001), the echinoderms *Amphiura chiajei* and *A. filiformis* showed BSAFs for PCB homologues that increased with increasing degree of chlorination. The differing BSAF homologue profiles in the species may reflect congener-specific or homologue-specific patterns of rapid excretion, intestinal absorption, and metabolic transformations in the species studied.

3.1.5 Biomagnification factors (BMFs)

Only a few studies of biomagnification of PCNs in food chains have been reported in the scientific literature (Falandysz and Rappe 1996b, Falandysz et al. 1997c, Falandysz et al. 1997b, and Tysklind et al. 1998). The following parts of the food web have been studied: a pelagic food chain (plankton, herring, and harbor porpoise) (Falandysz and Rappe 1996b), black cormorant in relation to fish (Falandysz et al. 1997c), fish in relation to mussel (Falandysz et al. 1997b), and salmon in relation to its food (Tysklind et al. 1998). Our investigation of the food chain transfer of PCNs within a bentic food chain comprising three species has added further insight into the bioaccumulation and biomagnification of these compounds. In general, low BMFs (BMF = $C_{\text{Fourhorned sculpin, lw}}/C_{\text{Amphipod, lw}}$) were found in the benthic food chain studied, again indicating that many congeners are excreted, eliminated, and/or metabolised at the higher trophic levels. The average BMFs for PCN homologues were in the range of 0.040–0.65. HxCNs accumulate more than the other homologues. Five HxCN isomers (PCNs 66/67, 69, and

71/72) dominated the BMF pattern (Fig. 16, top). The BMF pattern obtained previously for two components of a benthic food chain (flounder in relation to mussel; Fig. 16, bottom) resembled the pattern described in Paper VI.

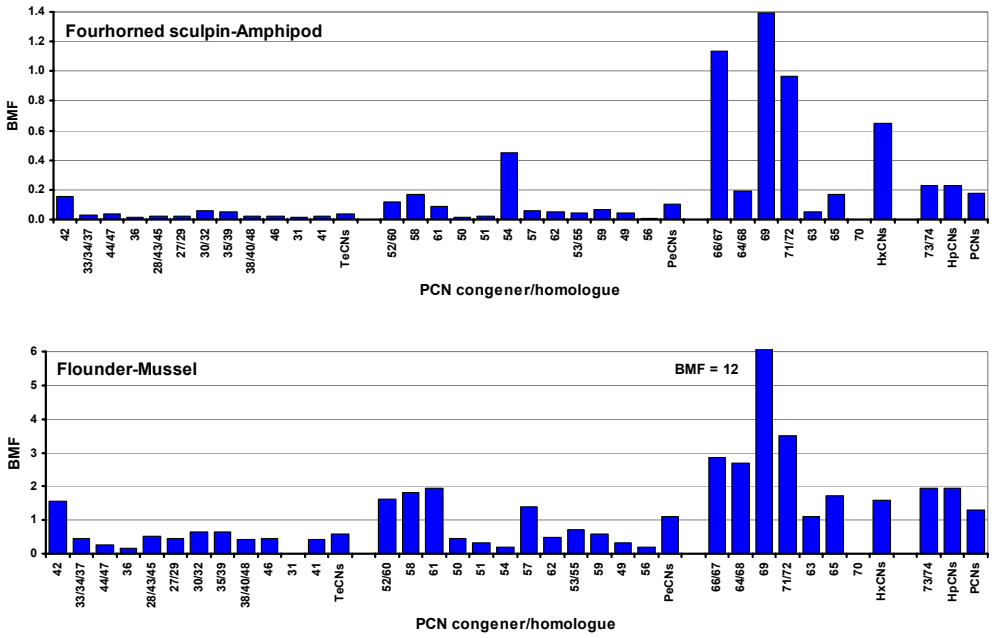


Figure 17. BMFs for individual PCNs calculated in two different food chains, four-horned sculpin related to amphipod (top; Paper VI) and flounder related to mussel (bottom; Falandysz et al. 1997b).

The BMF values for most of the TeCNs were low (BMF ≤ 0.056). Only PCN 42 has a relatively high BMF (0.15). PCNs 44, 45, and 48 (three isomers which lack adjacent carbon atoms substituted with hydrogen substituents) unfortunately coelute with other TeCN isomers on the DB-5 type column making it impossible, here, to evaluate the biomagnification of these isomers. There is a need to separate all the PCN congeners on either a single GC column or a dual GC column system for this type of investigation.

On the basis of their BMF values, PeCN isomers (BMFs = 0.010-0.45) can be divided into three categories. PCN 54 has the highest BMF value (0.45). This is the only 2,3,6,7-chlorine substituted PeCN isomer and is structurally similar to 2,3,7,8-TCDD. PCNs 52, 60, 58, and 61 are PeCN isomers have intermediate

BMF values (0.087-0.17). This group of PCN congeners has no adjacent carbon atoms that lack chlorine substituents. Low BMF values (0.010-0.069) were found for the remaining PeCN isomers, which all have adjacent carbon atoms with hydrogen substituents. PCN 55 belongs to the intermediate group, but coelutes with PCN 53 on a non-polar DB-5 type capillary GC column. The low BMF value indicates that the eluting peak may mainly consist of PCN 53. In addition, separation of these two isomers on an Rt-βDEXcst capillary GC column has shown that PCN 55 is not present in Halowax 1014 (Helm et al. 1999).

Of all the PCN congeners analysed, only PCN 66/67 (BMF = 1.1), and PCN 69 (BMF =1.4) biomagnified. In the flounder-mussel study, the BMFs reported for the HxCNs were one order of magnitude higher (Fig. 17). In our study, as well as in the flounder-mussel study, the lowest BMF values among the HxCN isomers were observed for PCNs 63 and 65. These isomers are the only HxCNs that have two adjacent carbon atoms substituted with hydrogen.

PCNs that either completely lack or have only a few chlorine atoms are reportedly metabolised via arene oxides, and it has been suggested that they form both hydroxylated and mercapturic acid pathway metabolites. Metabolism via arene oxides may be the reason why PCN members with adjacent carbon atoms substituted with hydrogen tend to have the lowest BMF values (Chu et al. 1977). The PCN congeners with the highest BMFs (e.g. many of the 2,3,6,7-chlorine substituted PCNs) in our study were also the most potent in previous toxicological studies (Hanberg et al. 1990, Villeneuve et al. 2000, Blankenship et al. 2000, and Hanberg et al. 1991). Unfortunately, only 22 of all the 75 PCN congeners have been tested for dioxin-like toxicity. Quantitative structure-activity relationships (QSARs) have been established to model BMFs for the PCNs (Tysklind et al. 1998). In this QSAR study, several of the 16 PCN congeners with the highest biomagnification potentials were 2,3,6,7-substituted PCN congeners (PCNs 48, 66, 67, 70, and 73).

3.1.6 Relative potencies (REPs) for PCNs

The toxic equivalency factor (TEF) concept is based on the effects induced and toxic responses to a compound, or compounds, relative to a reference substance. In this case, a TEF value indicates an order of magnitude estimate of the toxicity of a compound relative to TCDD. Consensus TEF values have been derived using careful scientific judgement after considering all available scientific data (van den Berg et al. 1998). However, when the potency of a compound relative to

TCDD has been obtained in a single *in vivo* or *in vitro* study, it will be referred to as a relative potency (REP) value. TEF values used for coplanar PCBs (non-*ortho* and mono-*ortho* PCBs) are listed in Table 4. Historically, di-*ortho* PCBs (Safe 1990) have also been assigned TEFs, but they have been later excluded from this list (Table 4, WHO-TEFs). In Table 4, REPs obtained for PCNs (Blankenship et al. 2000, Hanberg et al. 1990, and Villeneuve et al. 2000) are indicated for comparison. As seen in Table 4, some of the PCNs have REPs similar to the TEFs of the mono-*ortho* PCBs. These are 3-4 orders of magnitude less potent than TCDD.

Table 4. Assigned toxic equivalent factors (TEFs) for dioxin-like PCBs and reported relative potencies (REPs) for PCNs.

PCB	TEF			PCN	REP[d]
	Ahlborg[a]	Safe[b]	WHO[c]		
Non-*ortho* PCB				**DiCN**	
77	0.0005	0.01	0.0001	4	0.000000035
81			0.0001	5	0.00000002
126	0.1	0.1	0.1	**TeCN**	
169	0.01	0.05	0.01	40	0.000017
				PeCN	
Mono-*ortho* PCB				54	0.00017
105	0.0001	0.001	0.0001	56	0.000046
114	0.0005	0.001	0.0005	57	0.0000016
118	0.0001	0.001	0.0001	**HxCN**	
123	0.0001	0.001	0.0001	63	0.002
156	0.001	0.001	0.0005	64	0.00002
157	0.001	0.001	0.0005	66	0.004
167	0	0.001	0.00001	67	0.001
189	0	0.001	0.0001	68	0.002
				69	0.002
Di-*ortho* PCB				70	0.0021
All congeners	0	0.00002	0	71	0.000007
				HpCN	
2,3,7,8-TCDD	1	1	1	73	0.003

[a] Ahlborg et al. 1989 and 1994 [b] Safe 1990 [c] van den Berg et al. 1998 [d] Blankenship et al. 2000, Hanberg et al. 1990, and Villeneuve et al. 2000.

If TEFs are used, the toxicity of a mixture of compounds present in biological tissue can be expressed as a single value, the toxic equivalent concentration (TEQ). TEQ concentrations in samples are calculated using the following equation:

$$TEQ = \Sigma_{n1}\left[PCDD_i \times TEF_i\right] + \Sigma_{n2}\left[PCDF_i \times TEF_i\right] + \Sigma_{n3}\left[PCB_i \times TEF_i\right]$$

TEQ values can be used as relative measures between different abiotic samples, e.g., sediment and soil, to prioritise remedial actions. The PCN-REPs listed in Table 4 have been used to calculate the PCN-TEQs in the surface sediment and SPM samples. As detailed in Paper VII, the PCN-TEQ accounted for 2.5% of the PCDD/F-TEQ in the deposited sediments in the Gulf of Bothnia.

3.2 PCBs in samples from the Gulf of Bothnia

The collection of samples (surface sediments, amphipods, isopods, and four-horned sculpins) at the coastal locations in the Gulf of Bothnia, together with the following multi-residue, non-destructive analytical procedure, allowed the analysis of a large number of persistent organic pollutants in addition to the PCNs. The concentrations of 68 identified PCB congeners in the collected samples were reported in Paper V. The sample extracts were fractionated on a Florisil column and the PCBs were detected in fraction one (F1, Fig. 8). Among the analysed PCBs, there were four dioxin-like PCBs (mono-*ortho* PCBs 105, 118, 123, and 156: the PCBs marked in bold in appendix A). A general difference between the northern and southern parts of the Gulf of Bothnia could be seen in surface sediments, amphipods and isopods, with the lowest PCB concentrations being found in the north (location UM, Fig. 13) and the highest concentrations in the south (location SN). The total PCB concentrations in the samples were as follows: surface sediments, 2.0-14 ng/g dw; amphipods, 380-960 ng/g lw; and isopods 710-2400 ng/g lw. However, the most northern sampling location in the Bothnian Bay (HF), displayed intermediate PCB concentrations (sediments, 8.4 ng/g dw; amphipods, 690 ng/g lw; isopods, 2100 ng/g lw) compared to the rest of the sampling locations. A local PCB source in the region may be partially responsible for the relatively high levels of PCBs found there. Similar total PCB concentrations (5-10 ng/g dw) in surface sediments collected in the Gulf of Bothnia near Finland have been reported by Perttilä and Haahti (1986). The north-south trend of PCB concentrations was not so clear for the four-horned sculpin samples (530-2200 ng/g lw). Amounts of specific congeners found in surface and core sediments from the Baltic proper (Nylund et al. 1992 and Kjeller and Rappe 1994) correspond well with amounts found at the most southern location in the Bothnian Sea (SN). The PCB congener pattern differed between

the Bothnian Bay and Bothnian Sea, indicating that the PCB contamination may originate from different sources for each investigated sea basin.

3.3 R-PCDFs in biological tissues

The development of an HRGC-MS/MS technique for the detection of R-PCDFs is described in Paper IV. The method was compared with various MS-techniques and found to be a selective and sensitive technique for the analysis of R-PCDFs. The MS/MS-instrument was operated in SRM-mode, extracting the molecular ions M^+ of the R-PCDFs in the first MS and detecting the daughter ions $(M\text{-}Cl)^+$ in the second MS. This MS/MS-method was used for the analysis of R-PCDFs in biological tissues. Two crab hepatopancreas samples from Värö and Grebbestad situated on the Swedish west coast (Fig. 13) were collected in order to investigate the possible influence from a pulp and paper plant (Värö) and analysed using HRGC-MS/MS. The R-PCDFs in these samples (ΣR-PCDFs) were present at levels up to 10 times higher (Värö crab, 15.5 ng/g lw; Grebbestad crab, 22.1 ng/g lw) than the ΣPCDF levels. The R-PCDF congeners detected in the samples were substituted with 1-4 chlorine atoms and 1-4 methyl groups. The relatively high concentrations of R-PCDFs in the crab samples demonstrate that these dioxin-like compounds can bioaccumulate in crustacean species. However, the sample closest to the pulp and paper plant showed unexpectedly the lowest concentrations. Kuehl et al. (1987) detected trace quantities of TCXE isomers in fish. If these identified TCXE isomers were R-PCDFs, as proposed by Buser et al. (1989), it would show that the R-PCDFs can bioaccumulate in species higher up in the food chain. However, to our knowledge, no data on biomagnification of this group of compounds is available in the scientific literature.

3.4 UV-spectroscopy and relative response factors (RRFs) for PCDFs

The UV-absorption spectra presented in Paper III show that every specific PCDF congener has a unique spectral "fingerprint". The shapes of the spectra can be used to identify structures with similar properties, and can provide a continuous scale to express chemical similarities and differences. For example, the spectra for the 2,3,7,8-substituted PCDF congeners all have a characteristic shape. This has also been shown for the dioxin-like PCBs in another study (Andersson et al. 1997). The UV-spectra for 2,3,7,8-TCDF and PCB 77 ('dioxin-like' compounds) are compared with spectra for 1,4,6,9-TCDF and PCB 54 ('non-dioxin-like' compounds) in Fig. 18 (note that the wavelength scales are different for the TCDFs and PCBs). These compounds represent the extremes

(compounds with or without four lateral substituted chlorine atoms) of the respective homologue classes.

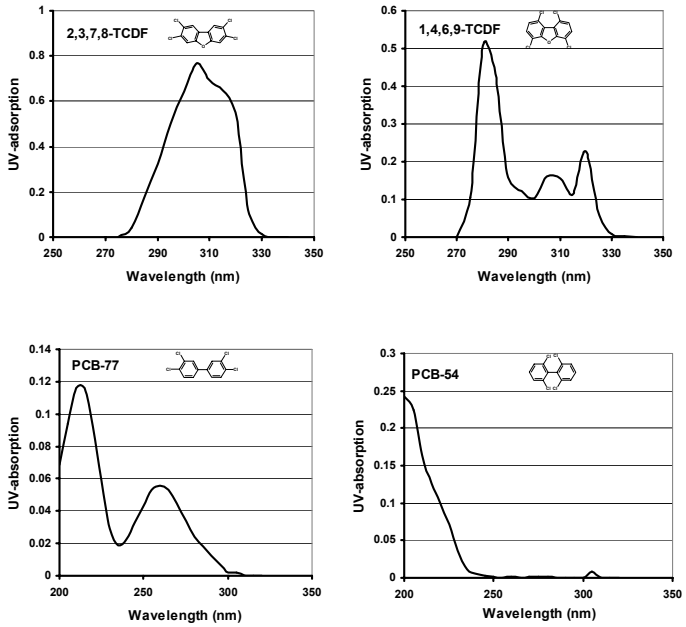


Figure 18. UV-spectra for 'dioxin-like' compounds (2,3,7,8-TCDF and PCB 77) and 'non-dioxin-like' compounds (1,4,6,9-TCDF and PCB 54).

2,3,7,8-TCDF has a symmetric UV-spectrum with a high intensity band at 305.6 nm and a second band at 317.4 nm. These bands are absent in the 1,4,6,9-TCDF UV-spectrum (Fig. 18). The characteristic UV-spectra for a 'dioxin-like' PCB (chosen representative: PCB 77) exhibits a second distinct maximum at approximately 250 nm. This absorption maximum is absent for the 'non-dioxin-like' PCBs (e.g., PCB 54). The digitalized UV-spectra of the individual PCDF congeners have been used as physicochemical descriptor variables in multivariate quantitative structure-activity relationships (QSARs) for modelling toxicity (Tysklind et al. 1993). In the cited study, the UV spectral data were found to be the most important parameters to describe the relationship between chemical properties and toxicity. Spectral data such as UV-absorption parameters are strongly correlated with fundamental chemical properties of series of halogenated aromatic compounds, and can therefore be used to identify dioxin-like compounds among large groups of isomers or congeners.

The determination of RRTs for the 87 PCDFs in studies described in Paper II using two stationary GC phases (DB-5 and RT-2330) enabled PCDF-RRFs to be calculated. A comparison between the EI-RRFs and NCI-RRFs showed that the mass spectrometric NCI-responses varied to a higher degree than the EI-responses. This difference between the EI- and NCI- responses was highest for the TCDFs. For this group of compounds the ratio between the highest and lowest RRF was 26 in the NCI-mode, but only 2.3 in the EI-mode. The large variation in NCI-RRFs (0.073-1.91) for the TCDF molecular ions may be explained by differences in their mass spectrometric fragmentation patterns and/or differences in the TCDF molecules' ability to capture electrons.

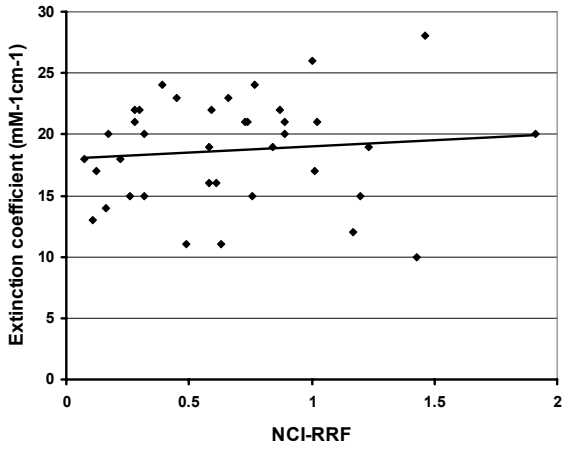


Figure 19. NCI-RRFs for all the TCDFs plotted against the molecular extinction coefficients of the first absorption maximum in their respective UV-spectra.

The molecular absorption of UV-radiation ('photon-capture' capacity) and electron-capture capacity may be correlated to each other. In Fig. 19, the NCI-RRFs for all the TCDFs are plotted against the molecular extinction coefficients of the first absorption maximum in their respective UV spectra. There seems to be no linear correlation between the calculated NCI-RRFs and the molecular extinction coefficients (ε_{max1}, Paper III). The results of this exercise show that different mechanisms govern the level of molecular UV-absorption and the electron-capture capacity of these compounds.

4. Conclusions and future research

The analytical methodology presented in this study has been found to be satisfactory for studying dioxin-like compounds (e.g. non-*ortho* PCBs, mono-*ortho* PCBs and PCNs) in a wide range of environmental samples (sediments, SPMs and biological tissues). HPLC PX-21 carbon column fractionation enabled the separation of interfering PCBs from coplanar PCBs and other planar dioxin-like compounds of interest (e.g. PCNs, PCDTs and R-PCDFs). The final analysis by HRGC-LRMS (for the bulk of the PCBs and mono-*ortho* PCBs), HRGC-MS/MS (R-PCDFs), and HRGC-HRMS (for various compounds, including non-*ortho* PCBs and PCNs) allowed the identification and detection of ppt concentrations of the dioxin-like compounds.

The measured PCN concentrations in the surface sediments collected in the Gulf of Bothnia (0.09-2.8 ng/g dw) were of the same order of magnitude as European, American, and Asian background concentrations. The levels were higher in the southern Bothnian Sea compared to the northern Bothnian Bay. The calculated ΣPCN fluxes were also higher in the Bothnian Sea (0.9 μg m^{-2} y^{-1}) compared to the Bothnian Bay (0.5-0.6 μg m^{-2} y^{-1}). This gradient is probably related to distance from the more industrialised and populated regions in the southern parts of Sweden and Finland, and central Europe. Further, the fluxes were slightly elevated near the coasts and of the same order of magnitude as pre-industrial ΣPCN fluxes determined in England. The PCN homologue distribution changed from dominance by TeCNs in samples from the open sea to a more even distribution of the homologues in samples collected near the coast, due to differences in the physicochemical properties of these homologues. The PCN congener patterns were similar in marine sediments throughout the Baltic Sea, indicating that the deposition of PCNs may originate from similar sources. The total annual sedimentation of PCNs in the two basins of the Gulf of Bothnia was calculated to be 91 kg y^{-1} (0.03 kg y^{-1} PCN-TEQs). The deposition of PCNs and PCDD/Fs (93 kg y^{-1}) in the Gulf of Bothnia were similar and PCN-TEQ accounted for 2.5% of the PCDD/F-TEQ in the deposited sediments.

Bioaccumulation and biomagnification of PCNs were studied in a three-species marine benthic food chain consisting of amphipods, isopods, and four-horned sculpins. Tetra- and penta- CNs exhibited BSAF values greater than one, while BSAFs for the higher chlorinated PCNs were lower than one. This suggests that amphipods assimilate the lower chlorinated PCNs more efficiently than the

higher chlorinated homologues. A decrease in ΣPCN concentrations from the lowest to the highest trophic level was demonstrated (amphipods, 10-69 ng/g lw; isopods, 3.9-16 ng/g lw; four-horned sculpins, 0.54-1.5 ng/g lw) and the calculated BMFs for each PCN congener indicate that a few congeners biomagnified significantly. The highest BMFs (0.09-1.4) were found for 2,3,6,7-substituted congeners, and those lacking adjacent hydrogen-substituted carbon atoms. Among all the PCN congeners analysed, only PCNs 66/67 and PCN 69 biomagnified.

Analysis of R-PCDFs in crustacean samples from Värö and Grebbestad was performed using HRGC-MS/MS. The ΣR-PCDFs in these samples were present at concentrations up to 10 times higher than the ΣPCDFs. The relatively high concentrations of R-PCDFs in the crab samples demonstrated that these compounds bioaccumulate.

In order to improve the analysis of PCNs, there is a need for a quantification method involving the use of ^{13}C-labeled PCNs and many individual native PCN standards. Recently a number of ^{13}C-labeled PCNs have been synthesised and these individual ^{13}C-labeled PCNs can today be purchased from a commercial supplier. Therefore, it is now possible to develop such an isotope dilution method with ^{13}C-labeled internal standards (using, for instance, one ^{13}C-labeled PCN congener for each PCN homologue). The use of ^{13}C-labeled PCNs will result in a proper analysis of the PCNs with a better quality control. True PCN recoveries can for example be calculated.

The elution order of individual native PCNs on different GC stationary phases needs to be elucidated and investigated using all 75 PCNs, since the elution order of some PCNs on the DB-5 capillary column reportedly differs between research groups (Järnberg 1997 and Takasuga et al. 1994). By tradition, the separation of PCNs has been performed on these DB-5 type GC capillary columns with relatively low separation efficiencies. If other GC stationary phases with higher separation efficiencies (e.g. RT-2330) are used, fewer PCN congeners will co-elute. For example, separation of the PCNs on an Rt-βDEXcst capillary GC column can completely separate the penta- and hexa- CNs (Helm et al. 1999). A development of this congener-specific analysis is essential in, for example, bioaccumulation studies where biomagnification factors for individual PCNs have to be calculated. Co-elution of PCN congeners in these types of analyses may make the results unusable for the purposes of the study, and interpretation of the data obtained difficult or impossible.

5. Acknowledgements

Det är många personer som bidragit på olika sätt, så att den här avhandlingen kunnat färdigställas. Först och främst vill jag tacka mina handledare Christoffer Rappe, Bert van Bavel och Mats Tysklind som stöttat mig under alla år som doktorand. Ett speciellt tack går till Bert som lurade upp mig till Storuman och som i bastun övertalade mig att börja med andra halvlek, efter sju års paus i arbetet.

Ett stort tack går också till Hans-Rudolf Buser som med sin stora kunskap visade hur en mass spektrometer kan användas. Järnvägshotellet ”Du Lac” i Wädenswil och den schweiziska gästfriheten är minnen som alltid kommer att finnas kvar.

Många arbetskamrater som är kvar på miljökemi, men även de som numera arbetar på andra arbetsplatser, har under åren hjälpt till med olika saker (provupparbetning, analyser och artiklar) så att avhandlingarbetet har kunnat fortskrida och avslutas. Ni, gamla och unga miljökemister, ska alla ha ett stort tack och lycka till med era fortsatta förehavanden.

Det finns några personer som hjälpt till lite extra.
Mats Tysklind och Bert van Bavel, som med mig under åren som gått fört ändlösa diskussioner om forskning samt små och stora vardagsbekymmer. Peter Haglund, HPLC gurun som löst alla HPLC problem med glans. Bo Strandberg, uppreningsspecialist som oförtrutet tagit fram nya fraktioner för analys. Per-Anders Bergqvist, som förhandlat fram projektmedel. Douglas Zook, som överfört nödvändiga språkkunskaper. Maria Hjelt, som hjälpt till med det laborativa arbetet och den tidskrävande miljögiftskvantifieringen.

Samarbetet med institutet för tillämpad miljöforskning (ITM), Stockholms Universitet, har betytt mycket under resans gång och det har varit trevligt att träffa så många duktiga miljökemister från huvudstaden. Följande personer har varit inblandade på något sätt i ”Bottniska viken året” projektet: Cecilia Bandh, Dag Broman, Rasha Ishaq, Carina Näf, Orania Papacosta, Harald Pettersen, Carl Rolff och Yngve Zebühr. Tack alla för ett gott samarbete. Rasha Ishaq som följt med på resan hela vägen, och som dessutom bjudit på en oförglömlig vecka i Barcelona, ska ha en extra eloge för sitt helhjärtade engagemang.

Statens naturvårdsverk, Kempe stiftelsen, samt Kempefonderna har ekonomiskt stöttat delprojekten i avhandlingen. Tack för att ni bidragit med lönemedel samt pengar till nödvändiga konferensresor, som varit ovärdeliga kunskapskällor.

Under åren har det blivit många oförglömliga minnen från olika resor i tjänsten t.ex. när Gunilla Lindström försökte lära mig hur man öppnar champagneflaskor på tågen genom Rhendalen samt att man bör ta sjösjukepiller när man ska ut på valsafari på Stilla Havet. Tack alla ni som följde med och gjorde resorna intressanta och roliga.

Ett stort tack ska Björn Sigurdsson ha som med stor datorskicklighet och entusiasm hjälpt till med färdigställandet av figurer. Med sitt arbete har han förhöjt kvaliten på avhandlingen betydligt.

John Blackwell får ett speciellt tack för sin språkgranskning av artiklar och avhandling samt den proffsiga attityd han visat, att alltid ställa upp när det har behövts.

Avslutningsvis vill jag tacka min familj, Kerstin, Kristina, Karin och Björn som fått utstå mycket under alla år som jag varit ”frånvarande”. Utan ert stöd hade ingenting blivit gjort.

References

Ahlborg U. Nordic risk assessment of PCDDs and PCDFs.
Chemosphere 19 (1989) 603-608.

Ahlborg U., Becking G. C., Birnbaum L. S., Brouwer A., Derks H. J. G. M., Feeley M., Golor G., Hanberg A., Larsen J. C., Liem A. K. D., Safe S. H., Schlatter C., Wærn F., Younes M., and Yrjänheikki E. Toxic equivalency factors for dioxin-like PCBs.
Chemosphere 28 (1994) 1049-1067.

Alcock R., Behnisch P., Jones K., and Hagenmaier H. Dioxin-like PCBs in the environment – human exposure and the significance of sources.
Chemosphere 28 (1998) 1457-1472.

Andersson P. L., Haglund P., and Tysklind M. Ultraviolet absorption spectra of all 209 polychlorinated biphenyls evaluated by principal component analysis.
Fresenius Z. Anal. Chem. 357 (1997) 1088-1092.

Asplund L., Grafström A. -K., Haglund P., Jansson B., Järnberg U., Mace D., Strandell M., and de Wit C. Analysis of non-*ortho* polychlorinated biphenyls and polychlorinated naphthalenes in Swedish dioxin survey samples.
Chemosphere 20 (1990) 1481-1488.

Astroff B. and Safe S. Comparative antiestrogenic activities of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 6-methyl-1,3,8-trichlorodibenzofuran in the female rat.
Toxicol. Appl. Toxicol. 95 (1988) 435-443.

Ballschmiter K. and Zell M. Analysis of polychlorinated biphenyls (PCBs) by glass capillary gas chromatography. Composition of technical Aroclor- and Clophen PCB mixtures.
Fresenius Z. Anal. Chem. 302 (1980) 20-31.

Beck H., Droβ A., Eckhart K., Mathar W., and Wittkowski R. PCDDs, PCDFs, and related compounds in paper products.
Chemosphere 19 (1989) 655-660.

Bergqvist P. -A., Bandh C., Broman D., Ishaq R., Lundgren K., Näf C., Pettersen H., Rappe C., Rolff C., Strandberg B., Zebühr Y., and Zook D. R. Multi-residue analytical method including planar PCB, dioxins and other organic contaminants for marine samples.
Organohalogen Compd. 9 (1992) 17-20.

Blankenship A.L., Kannan K., Villalobos S.A., Villeneuve D.L., Falandysz J., Imagawa T., Jakobsson E., and Giesy J. P. Relative potencies of individual polychlorinated naphthalenes and halowax mixtures to induce Ah receptor-mediated responses.
Environ. Sci. Technol. 34 (2000) 3153-3158.

Boese B. L., Lee H., Specht D. T., Pelletier J., and Randall R. Evaluation of PCB and hexachlorobenzene biota-sediment accumulation factors based on ingested sediment in a deposit-feeding clam.
Environ. Toxicol. Chem. 15 (1996) 1584-1589.

Brinkman U. A. Th. and Reymer H. G. M. Polychlorinated naphthalenes.
J. Chromatogr. 127 (1976) 203-243.

Brouwer A., Ahlborg U. G., van Leeuwen F. X. R., and Feeley M. M. Report of the WHO working group on the assessment of health risks for human infants from exposure to PCDDs, PCDFs, and PCBs.
Chemosphere 37 (1998) 1627-1643.

Buser H. -R., Kjeller L. -O., Swanson S. E., and Rappe C. Methyl- polymethyl-, and alkylpolychlorodibenzofurans identified in pulp mill sludge and sediments.
Environ. Sci. Technol. 23 (1989) 1130-1137.

Buser H. -R., Dolezal I. S., Wolfensberger M., and Rappe C. Polychlorodibenzothiophenes, the sulfur analogues of the polychlorodibenzofurans identified in incineration samples.
Environ. Sci. Technol. 25 (1991) 1637-1643.

Buser H. -R. and Rappe C. Determination of polychlorodibenzothiophenes, the sulfur analogues of the polychlorodibenzofurans, using various gas chromatographic/mass spectrometric techniques.
Environ. Sci. Technol. 63 (1991) 1210-1217.

Cai Z., Giblin D. E., Ramanujam V. M. S., Gross M. L., and Cristini A.
Mass-profile monitoring in trace analysis: Identification of polychlorodibenzothiophenes in crab tissues collected from the Newark/Raritan bay system.
Environ. Sci. Technol. 28 (1994) 1535-1538.

Campbell M. A., Bandiera S., Robertson L., Parkinson A., and Safe S. Hepta-, hexa-, tetra- and dichloronaphthalene congeners as inducers of hepatic microsomal drug-metabolizing enzymes.
Toxicology 26 (1983) 193-205.

Chu I., Villeneuve D.C., Secours V., and Viau A. Metabolism of chloronaphthalenes.
J. Agric. Food Chem. 25 (1977) 881-883.

Colmsjö A. L., Zebühr Y., and Östman C. E. Group separation of PCDDs, PCDFs, PACs and aliphatic compounds on an amino bonded stationary phase for HPLC.
Chromatographia 24 (1987) 541-544.

Creaser C. S. and Al-Haddad A. Fractionation of polychlorinated biphenyls, polychlorinated dibenzo-*p*-dioxins, and polychlorinated dibenzofurans on porous graphitic carbon.
Chemosphere 25 (1992) 1981-2008.

Crookes M. J. and Howe P. D. Environmental hazard assessment: Halogenated naphthalenes. Report TSD/13, Toxic Substances Division, Directorate for air, Climate and Toxic Substances, Department of the Environment, London, UK (1993).

De Vooght P. and Brinkman U. A. T. Production, properties, and usage of polychlorinated biphenyls. In Kimbrough R. D. and Jensen A. A. (eds), Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. Elsevier Science Publishers, Amsterdam, The Netherlands, (1989) 3-45.

De Vooght P., Haglund P., Reutergård L. B., de Wit C., and Waern F. Fishing for quality in environmental analysis. Interlaboratory study on non- and mono-*ortho* chlorinated biphenyls.
Anal. Chem. 66 (1994) 305A-311A.

Dörr G., Hippelein M., and Hutzinger O. Baseline contamination assessment for a new resource recovery facility in Germany. Analysis and seasonal/regional variability of ambient air concentrations of polychlorinated naphthalenes (PCN).
Chemosphere 33 (1996) 1563-1568.

Eljarrat E., Caixach J., Jiménez B., González M.J., and Rivera J. Polychlorinated naphthalenes in sediments from the Venice and Orbetello lagoons, Italy.
Chemosphere 38 (1999) 1901-1912.

Engwall M., Brunström B., and Jakobsson E. Ethoxyresorufin-*O*-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH)-inducing potency and lethality of chlorinated naphthalenes in chicken (*Gallus domesticus*) and eider duck (*Somatera mollissima*) embryos.
Arch. Toxicol. 68 (1994) 37-42.

Falandysz J. Polychlorinated naphthalenes: an environmental update.
Environ. Pollut. 101 (1998) 77-90.

Falandysz J., Strandberg L., Bergqvist P. -A., Kulp S. -E., Strandberg B., and Rappe C. Polychlorinated naphthalenes in sediment and biota from the Gdańsk basin, Baltic Sea.
Environ. Sci. Technol. 30 (1996a) 3266-3274.

Falandysz J. and Rappe C. Spatial distribution in plankton and bioaccumulation features of polychlorinated naphthalenes in a pelagic food chain in southern part of the Baltic Proper.
Environ. Sci. Technol. 30 (1996b) 3362-3370.

Falandysz J., Strandberg L., Kulp S. -E., Strandberg B., Bergqvist P. -A., and Rappe C. Congener-specific analysis of chloronaphthalenes in white-tailed sea eagles *Haliaeetus albicilla* breeding in Poland.
Chemosphere 33 (1996c) 51-69.

Falandysz J. and Rappe C. Specific pattern of tetrachloronaphthalenes in black cormorant.
Chemosphere 35 (1997a) 1737-1746.

Falandysz J., Strandberg L., Strandberg B., Bergqvist P. -A, and Rappe C. Spatial distribution and bioaccumulation of polychlorinated naphthalenes (PCNs) in mussel and fish from the Gulf of Gdańsk.
Sci. Total Environ. 203 (1997b) 93-104.

Falandysz J., Strandberg B., Strandberg L., Bergqvist P. -A., and Rappe C. Concentrations and biomagnification of polychlorinated naphthalenes in black cormorants *Phalacrocorax carbo sinensis* from the Gulf of Gdańsk, Baltic Sea.
Sci. Total Environ. 204 (1997c) 97-106.

Furlong E. T., Carter D. S., and Hites R. A. Organic contaminants in sediments from the Trenton channel of the Detroit river, Michigan.
J. Great Lakes Res. 14 (1988) 489-501.

Gevao B., Harner T., and Jones K. C. Sedimentary record of polychlorinated naphthalene concentrations and deposition fluxes in a dated lake core.
Environ. Sci. Technol. 34 (2000) 33-38.

Giesy J. P. and Kannan K. Dioxin-like and non-dioxin-like toxic effects of polychlorinated biphenyls (PCBs): Implications for risk assessment.
Crit. Rev. Toxicol. 28 (1998) 511-569.

Haglund P., Asplund L., Järnberg U., and Jansson B. Isolation of toxic polychlorinated biphenyls by electron donor-acceptor high-performance liquid chromatography on a 2-(1-pyrenyl)ethyldimethylsilylated silica column.
J. Chromatogr. 507 (1990) 389-398.

Haglund P., Jakobsson E., Asplund L., Athanasiadou M., and Bergman Å. Determination of polychlorinated naphthalenes in polychlorinated biphenyl products via capillary gas chromatography-mass spectrometry after separation by gel permeation chromatography.
J. Chromatogr. 634 (1993) 79-86.

Hale M. D., Hileman F. D., Mazer T., Shell T. L., Noble R. W., and Brooks J. Mathematical modelling of temperature programmed capillary gas chromatographic retention indexes for polychlorinated dibenzofurans.
Anal. Chem. 57 (1985) 640-648.

Hanberg A., Wærn F., Asplund L., Haglund P., and Safe S. Swedish dioxin survey: Determination of 2,3,7,8-TCDD toxic equivalent factors for some polychlorinated biphenyls and naphthalenes using biological tests.
Chemosphere 20 (1990) 1161-1164.

Hanberg A., Ståhlberg M., Georgellis A., de Wit C., and Ahlborg U. G. Swedish dioxin survey: Evaluation of the H-4-II E bioassay for screening environmental samples for dioxin-like enzyme induction.
Pharmacol. Toxicol. 69 (1991) 442-449.

Harner T. and Bidleman T. F. Polychlorinated naphthalenes in urban air.
Atmos. Environ. 31/32 (1997) 4009-4016.

Harner T., Kylin H., Bidleman T. F., Halsall C., Strachan W. M. J., Barrie L. A., and Fellin P. Polychlorinated naphthalenes and coplanar polychlorinated biphenyls in arctic air.
Environ. Sci. Technol. 32 (1998) 3257-3265.

Harner T., Lee R. G. M., and Jones K. C. Polychlorinated naphthalenes in the atmosphere of the United Kingdom.
Environ. Sci. Technol. 34 (2000) 3137-3142.

Harner T. and Kucklick J. Intercalibration study for the polychlorinated naphthalenes (PCNs): Phase 1 results.
Organohalogen Compd. 58 (2002) 93-96.

Hawker D. W. and Connell D. W. Octanol-Water partition coefficients of polychlorinated biphenyl congeners.
Environ. Sci. Technol. 22 (1988) 382-387.

Helm P., Jantunen L. M. M., Bidleman T. F., and Dorman F. Complete separation of isomeric penta- and hexachloronaphthalenes by capillary gas chromatography.
J. High Resol. Chromatogr. 22 (1999) 639-643.

Helm P. A., Bidleman T. F., Stern G. A., and Koczanski K. Polychlorinated naphthalenes and coplanar polychlorinated biphenyls in beluga whale (*Delphinapterus leucas*) and ringed seal (*Phoca hispida*) from the eastern Canadian arctic.
Environ. Pollut. 119 (2002) 69-78.

Huckins J. N., Stalling D. L., and Petty J. D. Carbon-foam chromatographic separation of non-*o-o´*-chlorine substituted PCBs from aroclor mixtures.
J. Assoc. Off. Anal. Chem. 63 (1980) 750-755.

Huckins J. N., Tubergen M. W., Lebo J. A., Gale R. W., and Schwartz T. R. Polymeric film dialysis in organic solvent media for cleanup of organic contaminants.
J. Assoc. Off. Anal. Chem. 73 (1990) 290-293.

Huntley S. L., Wenning R. J., Paustenbach D. J., Wong A. S., and Luksemburg W. J. Potential sources of polychlorinated dibenzothiophenes in the Passaic river, New Jersey.
Chemosphere 29 (1994) 257-272.

Imagawa T. and Lee C. W. Correlation of polychlorinated naphthalenes with polychlorinated dibenzofurans formed from waste incineration.
Chemosphere 44 (2001) 1511-1520.

Ishaq R., Karlson K., and Näf C. Tissue distribution of polychlorinated naphthalenes (PCNs) and non-*ortho* chlorinated biphenyls (non-*ortho* CBs) in harbour porpoises (*Phocoena phocoena*) from Swedish waters.
Chemosphere 41 (2000) 1913-1925.

Jensen S. Report of a new chemical hazard.
New Sci. 32 (1966) 612-612.

Järnberg U., Asplund L., de Wit C., Grafström A. -K., Haglund P., Jansson B., Lexén K., Strandell M., Olsson M., and Jonsson B. Polychlorinated biphenyls and polychlorinated naphthalenes in Swedish sediment and biota: Levels, patterns, and time trends.
Environ. Sci. Technol. 27 (1993) 1364-1374.

Järnberg U., Asplund L., de Wit C., Egebäck A. -L., Wideqvist U., and Jakobsson E. Distribution of polychlorinated naphthalene congeners in environmental and source-related samples.
Arch. Environ. Contam. Toxicol. 32 (1997) 232-245.

Järnberg U. Analytical methods for studying polychlorinated naphthalene congener profiles and levels in the environment.
Ph.D. Thesis, (1997) Stockholm University, Sweden, ISBN 91-7153-658-2.

Järnberg U., Asplund L., Egebäck A. -L., Jansson B., Unger M., and Wideqvist U. Polychlorinated naphthalene congener profiles in background sediments compared to a degraded Halowax 1014 technical mixture.
Environ. Sci. Technol. 33 (1999) 1-6.

Kannan K., Imagawa T., Blankenship A. L., and Giesy J. P. Isomer-specific analysis and toxic evaluation of polychlorinated naphthalenes in soil, sediment, and biota collected near the site of a former chlor-alkali plant.
Environ. Sci. Technol. 32 (1998) 2507-2514.

Kannan K., Kawano M., Kashima Y., Matsui M., and Giesy J. P. Extractable organohalogens (EOX) in sediment and biota collected at an estuarine marsh near a former chloralkali facility.
Environ. Sci. Technol. 33 (1999) 1004-1008.

Kannan K., Imagawa T., Yamashita N., Miyazaki A., and Giesy J. P. Polychlorinated naphthalenes in sediment, fishes and fish-eating waterbirds from Michigan waters of the Great Lakes.
Organohalogen Compd. 47 (2000) 13-16.

Kannan K., Hilscherova K., Imagawa T., Yamashita N., Williams L. L., and Giesy J. P. Polychlorinated naphthalenes , -biphenyls, -dibenzo-*p*-dioxins, and dibenzofurans in double-crested cormorants and herring gulls from Michigan waters of the great lakes.
Environ. Sci. Technol. 35 (2001) 411-447.

Kannan K., Corsolini S., Imagawa T., Focardi S., and Giesy J. P. Polychlorinated –naphthalenes, -biphenyls, -dibenzo-*p*-dioxins –dibenzofurans and *p,p´*-DDE in bluefin tuna, swordfish, cormorants and barn swallows from Italy.
Ambio 31 (2002) 207-211.

Karelsky D., Shelly D. C., and Warner I. M. A study of polynuclear aromatic hydrocarbons on an amino bonded phase liquid chromatographic column in the normal and reversed phase.
J. Liq. Chrom. 6 (1983) 471-495.

Kjeller L. -O. and Rappe C. Time trends in levels, patterns and profiles for polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls in a sediment core from the Baltic proper.
Environ. Sci. Technol. 29 (1995) 346-355.

Koistinen J., Paasivirta J., and Särkkä J. Organic chlorine compounds in lake sediments. IV. Dioxins, Furans and related chloroaromatic compounds.
Chemosphere 21 (1990) 1371-1379.

Kopponen P., Sinkkonen S., Poso A., Gynther J., and Kärenlampi S. Sulfur analogs of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and diphenyl ethers as inducers of CYP1A1 in mouse hapatoma-cell culture and structure-activity-relationships.
Environ. Toxicol. Chem. 13 (1994) 1543-1548.

Kováts E. Gas chromatographic characterization of organic substances in the retention index system.
Adv. Chromatogr. 1 (1965) 229-247.

Kuehl D. W., Butterworth B. C., DeVita W. M., and Sauer C. P. Environmental contamination by polychlorinated dibenzo-*p*-dioxins and dibenzofurans associated with pulp and paper mill discharge.
Biomed. Environ. Mass Spectrom. 14 (1987) 443-447.

Lamparski L. L. and Nestrick T. J. Novel extraction device for the determination of chlorinated dibenzo-*p*-dioxins (CDDs) and dibenzofurans (CDFs) in matrices containing water.
Chemosphere 19 (1989) 27-31.

Laurent M. A. Sur les chlorures de naphtaline.
Ann. Chim. Phys. 52 (1833) 275-285.

Magnusson K. Fate and effect of organic pollutants in marine fauna and sediments.
Ph.D. Thesis, (2001) Göteborg University, Sweden, ISBN 91-628-4870-4.

Martí I. and Ventura F. Polychlorinated naphthalenes in groundwater samples from the Llobregat aquifer (Spain).
J. Chromatogr. A 786 (1997) 135-144.

Meijer S. N., Harner T., Helm P. A., Halsall C. J., Johnston A. E., and Jones K. C. Polychlorinated naphthalenes in U.K. soils: Time trends, markers of source, and equilibrium status.
Environ. Sci. Technol. 35 (2001) 4205-4213.

Norstrom R. J., Simon M., Muir D. C. G., and Schweinsburg R. E. Organochlorine contaminants in arctic marine food chains: identification, geographical distribution and temporal trends in polar bears.
Environ. Sci. Technol. 22 (1988) 1063-1071.

Nylund K., Asplund L., Jansson B., Jonsson P., Litzén K., and Sellström U. Analysis of some polychlorinated pollutants in sediment and sewage sludge.
Chemosphere 24 (1992) 1721-1730.

Paasivirta J., Mäntykoski K., Koistinen J., Kuokkanen T., Mannila E., and Rissanen K. Structure analyses of planar polyaromatic compounds in environment.
Chemosphere 19 (1989) 149-154.

Perttilä M. and Haahti H. Chlorinated hydrocarbons in the water and sediments of the sea areas around Finland.
National Board of Waters Publications 68 (1986) 197-200.

Poland A. and Glover E. Chlorinated dibenzo-*p*-dioxin: potent inducers of delta-aminolevulinic acid synthetase and aryl hydrocarbon hydroxylase: II. A study of the structure-activity relationship.
Mol. Pharmacol. Toxicol. 9 (1973) 736-747.

Pruell R. J., Rubenstein N. I., Taplin B. K., LiVolski J. A., and Bowen R. D. Accumulation of polychlorinated organic contaminants from sediment by three benthic marine species.
Arch. Environ. Contam. Toxicol. 24 (1993) 290-297.

Safe S. Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs).
Crit. Rev. Toxicol. 21 (1990) 51-88.

Schwetz B. A., Norris J. M., Sparschu G. L., Rowe V. K., Gehring P. J., Emerson J. L., and Gehring C. G. Toxicology of chlorinated dibenzo-*p*-dioxins.
Environ. Health Perspect. 5 (1973) 87-99.

Shan G., Sanborn J. I. R., Gilman S. D., Nagy S., Gee S. J., Stoutamire D. W., Mercer R., Jones A. D., Stanker L. H., Denison M. S., and Hammock B. D. Surrogates for dioxin analyses: analytical, ELISA and toxicological aspects.
Organohalogen Compd. 45 (2000) 188-191.

Sinkkonen S., Paasivirta J., Koistinen J., Lahtiperä M., and Lammi R. Polychlorinated dibenzothiophenes in bleached pulp mill effluents.
Chemosphere 24 (1992) 1755-1763.

Sinkkonen S., Vattulainen A., Aittola J. -P., Paasivirta J., Tarhanen J., and Lahtiperä M. Metal reclamation produces sulphur analogues of toxic dioxins and furans.
Chemosphere 28 (1994) 1279-1288.

Smith L. M., Stalling D. L., and Johnson J. L. Determination of part-per-trillion levels of polychlorinated dibenzofurans and dioxins in environmental samples.
Anal. Chem. 56 (1984) 1830-1842.

Snyder L. R. and Schunk T. C. Retention mechanism and the role of the mobile phase in normal-phase separation on amino-bonded-phase columns.
Anal. Chem. 54 (1982) 1764-1772.

Stalling D. L., Huckins J. N., Petty J. D., Johnson J. L. and Sanders H. O. An expanded approach to the study and measurement of PCBs and selected planar halogenated aromatic environmental pollutants.
Ann. NY Acad. Sci. 320 (1979) 48-59.

Strandberg B. The use of semipermeable membrane devices in studies of concentrations, distribution and fate of organochlorine compounds in the environment.
Ph.D. thesis, (1998) Institute of Environmental Chemistry, Umeå University, Sweden, ISBN 91-7191-523-0.

Strandberg B., Bergqvist P. -A., and Rappe C. Dialysis with semipermeable membranes as an efficient lipid removal method in the analysis of bioaccumulative chemicals.
Anal. Chem. 70 (1998) 526-533.

Swanson S. E. Dioxins in the bleach plant. A study of the occurrence and formation of polychlorinated dibenzofurans and dibenzo-*p*-dioxins in the chlorine bleaching of wood pulp.
Ph.D. thesis, (1988) Environmental Chemistry, Umeå University, Sweden, ISBN 91-7174-381-2.

Takasuga T., Inoue T., Ohi E., and Ireland P. Development of an all congener specific, HRGC/HRMS analytical method for polychlorinated naphthalenes in environmental samples.
Organohalogen compd. 19 (1994) 177-182.

Theisen J., Maulshagen A., and Fuchs J. Organic and inorganic substances in the copper slag "Kieselrot".
Chemosphere 26 (1993) 881-896.

Tysklind M. Multivariate chemical characterization and modelling of polychlorinated dioxins and dibenzofurans.
Ph.D. Thesis, (1993) Umeå University, Sweden, ISBN 91-7174-771-0.

Tysklind M., Lundgren K., Rappe C., Eriksson L., Jonsson J., and Sjöström M. Multivariate quantitative structure-activity relationships for polychlorinated dibenzo-*p*-dioxins and dibenzofurans.
Environ. Toxicol. Chem. 12 (1993) 659-672.

Tysklind M., Nyström M., Åkerblom N., Andersson P. L., van Bavel B., and Norrgren L. Determination and modelling of biomagnification factors for polychlorinated naphthalenes (PCNs) in Salmon (*Salmon salar*).
Organohalogen Compd. 39 (1998) 13-16.

Van den Berg M., Birnbaum L., Bosveld A. T. C., Brunström B., Cook P., Feeley M., Giesy J. P., Hanberg A., Hasegawa R., Kennedy S. W., Kubiak T., Larsen J. C., van Leeuwen F. X. R., Liem A. K. D., Nolt C., Peterson R. E., Poellinger L., Safe S., Schrenk D., Tillit D., Tysklind M., Younes M., Wærn F., and Zacharewski T. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife.
Environ. Health Perspect. 106 (1998) 775-792.

Villeneuve D. L., Kannan K., Khim J. S., Falandysz J., Nikiforov V. A., Blankenship A. L., and Giesy J. P. Relative potencies of individual polychlorinated naphthalenes to induce dioxin-like responses in fish and mammalian *in vitro* bioassays.
Arch. Environ. Contam. Toxicol. 39 (2000) 273-281.

Wania F. and Mackay D. Tracking the distribution of persistent organic pollutants.
Environ. Sci. Technol. 30 (1996) 390A-396A.

Weistrand C., Lundén Å., and Norén K. Leakage of polychlorinated biphenyls and naphthalenes from electronic equipment in a laboratory.
Chemosphere 24 (1992) 1197-1206.

Yamashita N., Kannan K., Imagawa T., Villeneuve D. L., Hashimoto S., Miyazaki A., and Giesy J. P. Vertical profile of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, naphthalenes, biphenyls, polycyclic aromatic hydrocarbons, and alkylphenols in a sediment core from Tokyo Bay, Japan.
Environ. Sci. Technol. 34 (2000) 3560-3567.

Yamashita N., Taniyasu S., Hanari N., and Horii Y. Polychlorinated naphthalenes contaminations in some commercial products in Japan.
Organohalogen compd. 58 (2002) 105-108.

Åkerblom N., Olsson K., Berg A. H., Andersson P. L., Tysklind M., Förlin L., and Norrgren L. Impact of polychlorinated naphthalenes (PCNs) in juvenile Baltic salmon, *Salmon Salar*: Evaluation of estrogenic effects, development, and CYP1A induction.
Arch. Environ. Contam. Toxicol. 38 (2000) 225-233.

Appendix A

Systematic numbering of PCB compounds. The number is used as a synonym for the corresponding PCB compound in tables and figures. The PCBs are grouped depending on the number of substituted *ortho*-chlorine atoms in the molecule.

PCB[a]	Structure	PCB	Structure
Non-ortho	(20)		
2	3-MonoCB	31	2,4',5-TriCB
3	4-MonoCB	33	2',3,4-TriCB
11	3,3'-DiCB	34	2',3,5-TriCB
12	3,4-DiCB	55	2,3,3',4-TCB
13	3,4'-DiCB	56	2,3,3',4'-TCB
14	3,5-DiCB	57	2,3,3',5-TCB
15	4,4'-DiCB	58	2,3,3',5'-TCB
35	3,3',4-TriCB	60	2,3,4,4'-TCB
36	3,3',5-TriCB	61	2,3,4,5-TCB
37	3,4,4'-TriCB	63	2,3,4',5-TCB
38	3,4,5-TriCB	66	2,3',4,4'-TCB
39	3,4',5-TriCB	67	2,3',4,5-TCB
77	3,3',4,4'-TCB	68	2,3',4,5'-TCB
78	3,3',4,5-TCB	70	2,3',4',5-TCB
79	3,3',4,5'-TCB	72	2,3',5,5'-TCB
80	3,3',5,5'-TCB	74	2,4,4',5-TCB
81	3,4,4',5-TCB	76	2',3,4,5-TCB
126	3,3',4,4',5-PeCB	**105**[b]	**2,3,3',4,4'-PeCB**
127	3,3',4,5,5'-PeCB	106	2,3,3',4,5-PeCB
169	3,3',4,4',5,5'-HxCB	107	2,3,3',4',5-PeCB
Mono-ortho	(48)	108	2,3,3',4,5'-PeCB
1	2-MonoCB	111	2,3,3',5,5'-PeCB
5	2,3-DiCB	114	2,3,4,4',5-PeCB
6	2,3'-DiCB	**118**[b]	**2,3',4,4',5-PeCB**
7	2,4-DiCB	120	2,3',4,5,5'-PeCB
8	2,4'-DiCB	122	2',3,3',4,5-PeCB
9	2,5-DiCB	**123**[b]	**2',3,4,4',5-PeCB**
20	2,3,3'-TriCB	124	2',3,4,5,5'-PeCB
21	2,3,4-TriCB	**156**[b]	**2,3,3',4,4',5-HxCB**
22	2,3,4'-TriCB	157	2,3,3',4,4',5'-HxCB
23	2,3,5-TriCB	159	2,3,3',4,5,5'-HxCB
25	2,3',4-TriCB	162	2,3,3',4',5,5'-HxCB
26	2,3',5-TriCB	167	2,3',4,4',5,5'-HxCB
28	2,4,4'-TriCB	189	2,3,3',4,4',5,5'-HpCB
29	2,4,5-TriCB		

[a] IUPAC numbers are used for the different PCBs. [b] Bolded PCBs are dioxin-like PCBs analysed in paper V.

Appendix A (continued)

PCB	Structure	PCB	Structure
Di-ortho	(72)		
4	2,2'-DiCB	109	2,3,3',4,6-PeCB
10	2,6-DiCB	110	2,3,3',4',6-PeCB
16	2,2',3-TriCB	112	2,3,3',5,6-PeCB
17	2,2',4-TriCB	113	2,3,3',5',6-PeCB
18	2,2',5-TriCB	115	2,3,4,4',6-PeCB
24	2,3,6-TriCB	116	2,3,4,5,6-PeCB
27	2,3',6-TriCB	117	2,3,4',5,6-PeCB
30	2,4,6-TriCB	119	2,3',4,4',6-PeCB
32	2,4',6-TriCB	121	2,3',4,5',6-PeCB
40	2,2',3,3'-TCB	125	2',3,4,5,6'-PeCB
41	2,2',3,4-TCB	128	2,2',3,3',4,4'-HxCB
42	2,2',3,4'-TCB	129	2,2',3,3',4,5-HxCB
43	2,2',3,5-TCB	130	2,2',3,3',4,5'-HxCB
44	2,2',3,5'-TCB	133	2,2',3,3',5,5'-HxCB
47	2,2',4,4'-TCB	137	2,2',3,4,4',5-HxCB
48	2,2',4,5-TCB	138	2,2',3,4,4',5'-HxCB
49	2,2',4,5'-TCB	141	2,2',3,4,5,5'-HxCB
52	2,2',5,5'-TCB	146	2,2',3,4',5,5'-HxCB
59	2,3,3',6-TCB	153	2,2',4,4',5,5'-HxCB
62	2,3,4,6-TCB	158	2,3,3',4,4',6-HxCB
64	2,3,4',6-TCB	160	2,3,3',4,5,6-HxCB
65	2,3,5,6-TCB	161	2,3,3',4,5',6-HxCB
69	2,3',4,6-TCB	163	2,3,3',4',5,6-HxCB
71	2,3',4',6-TCB	164	2,3,3',4',5',6-HxCB
73	2,3',5',6-TCB	165	2,3,3',5,5',6-HxCB
75	2,4,4',6-TCB	166	2,3,4,4',5,6-HxCB
82	2,2',3,3',4-PeCB	168	2,3',4,4',5',6-HxCB
83	2,2',3,3',5-PeCB	170	2,2',3,3',4,4',5-HpCB
85	2,2',3,4,4'-PeCB	172	2,2',3,3',4,5,5'-HpCB
86	2,2',3,4,5-PeCB	180	2,2',3,4,4',5,5'-HpCB
87	2,2',3,4,5'-PeCB	190	2,3,3',4,4',5,6-HpCB
90	2,2',3,4',5-PeCB	191	2,3,3',4,4',5',6-HpCB
92	2,2',3,5,5'-PeCB	192	2,3,3',4,5,5',6-HpCB
97	2,2',3',4,5-PeCB	193	2,3,3',4',5,5',6-HpCB
99	2,2',4,4',5-PeCB	194	2,2',3,3',4,4',5,5'-OCB
101	2,2',4,5,5'-PeCB	205	2,3,3',4,4',5,5',6-OCB

Appendix A (continued)

PCB	Structure	PCB	Structure
Tri-ortho	(48)		
19	2,2',6-TriCB	177	2,2',3,3',4',5,6-HpCB
45	2,2',3,6-TCB	178	2,2',3,3',5,5',6-HpCB
46	2,2',3,6'-TCB	181	2,2',3,4,4',5,6-HpCB
50	2,2',4,6-TCB	182	2,2',3,4,4',5,6'-HpCB
51	2,2',4,6'-TCB	183	2,2',3,4,4',5',6-HpCB
53	2,2',5,6'-TCB	185	2,2',3,4,5,5',6-HpCB
84	2,2',3,3',6-PeCB	187	2,2',3,4',5,5',6-HpCB
88	2,2',3,4,6-PeCB	195	2,2',3,3',4,4',5,6-OCB
89	2,2',3,4,6'-PeCB	196	2,2',3,3',4,4',5',6-OCB
91	2,2',3,4',6-PeCB	198	2,2',3,3',4,5,5',6-OCB
93	2,2',3,5,6-PeCB	199	2,2',3,3',4,5,5',6'-OCB
94	2,2',3,5,6'-PeCB	203	2,2',3,4,4',5,5',6-OCB
95	2,2',3,5',6-PeCB	206	2,2',3,3',4,4',5,5',6-NCB
98	2,2',3',4,6-PeCB	*Tetra-ortho*	(21)
100	2,2',4,4',6-PeCB	54	2,2',6,6'-TCB
102	2,2',4,5,6'-PeCB	96	2,2',3,6,6'-PeCB
103	2,2',4,5',6-PeCB	104	2,2',4,6,6'-PeCB
131	2,2',3,3',4,6-HxCB	136	2,2',3,3',6,6'-HxCB
132	2,2',3,3',4,6'-HxCB	145	2,2',3,4,6,6'-HxCB
134	2,2',3,3',5,6-HxCB	150	2,2',3,4',6,6'-HxCB
135	2,2',3,3',5,6'-HxCB	152	2,2',3,5,6,6'-HxCB
139	2,2',3,4,4',6-HxCB	155	2,2',4,4',6,6'-HxCB
140	2,2',3,4,4',6'-HxCB	176	2,2',3,3',4,6,6'-HpCB
142	2,2',3,4,5,6-HxCB	179	2,2',3,3',5,6,6'-HpCB
143	2,2',3,4,5,6'-HxCB	184	2,2',3,4,4',6,6'-HpCB
144	2,2',3,4,5',6-HxCB	186	2,2',3,4,5,6,6'-HpCB
147	2,2',3,4',5,6-HxCB	188	2,2',3,4',5,6,6'-HpCB
148	2,2',3,4',5,6'-HxCB	197	2,2',3,3',4,4',6,6'-OCB
149	2,2',3,4',5',6-HxCB	200	2,2',3,3',4,5,6,6'-OCB
151	2,2',3,5,5',6-HxCB	201	2,2',3,3',4,5',6,6'-OCB
154	2,2',4,4',5,6'-HxCB	202	2,2',3,3',5,5',6,6'-OCB
171	2,2',3,3',4,4',6-HpCB	204	2,2',3,4,4',5,6,6'-OCB
173	2,2',3,3',4,5,6-HpCB	207	2,2',3,3',4,4',5,6,6'-NCB
174	2,2',3,3',4,5,6'-HpCB	208	2,2',3,3',4,5,5',6,6'-NCB
175	2,2',3,3',4,5',6-HpCB	209	2,2',3,3',4,4',5,5',6,6'-DCB

Appendix B

Exact masses for the major molecular cluster ions of methyl-PCDFs.

No. of chlorines	Methyl-PCDF							
	Methyl-PCDF	**Di-methyl-PCDF**	**Tri-methyl-PCDF**	**Tetra-methyl-PCDF**	**Penta-methyl-PCDF**	**Hexa-methyl-PCDF**	**Hepta-methyl-PCDF**	**Octa-methyl-PCDF**
0	182.0732	196.0888	210.1045	224.1201	238.1358	252.1514	266.1671	280.1827
1	216.0342	230.0498	244.0655	258.0811	272.0968	286.1124	300.1281	
	218.0312	232.0469	246.0625	260.0782	274.0938	288.1095	302.1251	
2	249.9952	264.0109	278.0265	292.0422	306.0578	320.0735		
	251.9923	266.0079	280.0236	294.0392	308.0549	322.0705		
	253.9893	268.0050	282.0206	296.0363	310.0519	324.0676		
3	283.9562	297.9719	311.9875	326.0032	340.0188			
	285.9533	299.9689	313.9846	328.0002	342.0159			
	287.9503	301.9660	315.9816	329.9973	344.0129			
	289.9474	303.9630	**317.9787**	331.9943	346.0100			
4	**317.9173**	331.9329	345.9486	359.9642				
	319.9143	**333.9300**	347.9456	361.9613				
	321.9114	335.9270	349.9427	363.9583				
	323.9084	337.9241	**351.9397**	365.9554				
	325.9055	339.9211	353.9368	**367.9524**				
5	**351.8783**	365.8940	379.9096					
	353.8754	**367.8910**	381.9067					
	355.8724	369.8881	383.9037					
	357.8695	371.8851	**385.9008**					
	359.8665	373.8822	387.8978					
6	**385.8393**	399.8550						
	387.8364	**401.8520**						
	389.8334	403.8491						
	391.8305	405.8461						
	393.8275	407.8432						
7	**419.8004**							
	421.7974							
	423.7945							
	425.7915							
	427.7886							

* Bolded methyl-PCDF masses are similar to the most abundant ^{13}C-PCDD and ^{13}C-PCDF molecular ion masses.

Appendix B (continued)

Exact masses for the major molecular cluster ions of ethyl-PCDFs.

No. of chlorines	Ethyl-PCDF							
	Ethyl-PCDF	**Di-ethyl-PCDF**	**Tri-ethyl-PCDF**	**Tetra-ethyl-PCDF**	**Penta-ethyl-PCDF**	**Hexa-ethyl-PCDF**	**Hepta-ethyl-PCDF**	**Octa-ethyl-PCDF**
0	196.0888	224.1201	252.1514	280.1827	308.2140	336.2453	364.2766	392.3079
1	230.0498	258.0811	274.1124	314.1437	342.1750	370.2063	398.2376	
	232.0469	260.0782	276.1095	316.1408	344.1721	372.2034	400.2347	
2	264.0109	292.0422	308.0735	348.1048	376.1361	404.1674		
	266.0079	294.0392	310.0705	350.1018	378.1331	406.1644		
	268.0050	296.0363	312.0676	352.0989	380.1302	408.1615		
3	297.9719	326.0032	342.0345	382.0658	410.0971			
	299.9689	328.0002	344.0315	384.0628	412.0941			
	301.9660	329.9973	346.0286	386.0599	414.0912			
	303.9630	331.9943	348.0256	388.0569	416.0882			
4	331.9329	359.9642	375.9955	416.0268				
	333.9300*	361.9613	377.9926	418.0239				
	335.9270	363.9583	379.9896	420.0209				
	337.9241	365.9554	381.9867	422.0180				
	339.9211	**367.9524**	383.9837	424.0150				
5	365.8940	393.9253	409.9566					
	367.8910	395.9223	411.9536					
	369.8881	397.9194	413.9507					
	371.8851	399.9164	415.9477					
	373.8822	**401.9135**	417.9448					
6	399.8550	427.8863						
	401.8520	429.8833						
	403.8491	431.8804						
	405.8461	433.8774						
	407.8432	**435.8745**						
7	433.8160							
	435.8131							
	437.8101							
	439.8072							
	441.8042							

* Bolded ethyl-PCDF masses are similar to the most abundant ^{13}C-PCDD molecular ion masses.

Appendix C

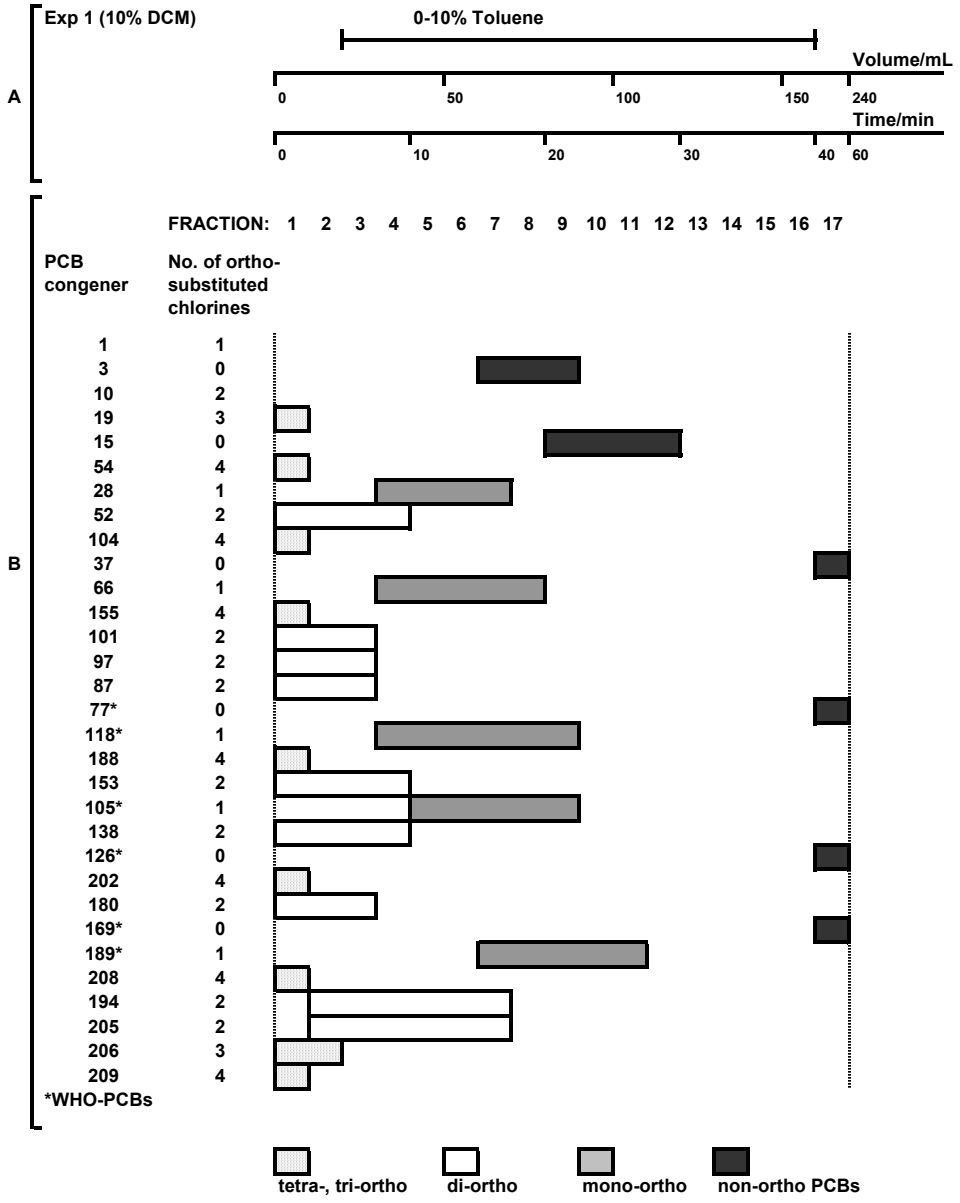
Major molecular cluster ions for PCDDs and PCDFs and their relative abundances.

No. of chlorines	PCDD Exact mass	^{13}C-PCDD Exact mass	Relative abundance	PCDF Exact mass	^{13}C-PCDF Exact mass	Relative abundance
0	184.0524	196.0927		168.0575	180.0978	
1	218.0135	**230.0537***	100.00	202.0185	**214.0588**	100.00
	220.0105	232.0508	33.16	204.0156	216.0559	33.62
2	251.9745	**264.0147**	100.00	235.9796	**248.0198**	100.00
	253.9715	266.0118	65.79	237.9766	250.0169	66.24
	255.9686	268.0088	10.99	239.9737	252.0139	11.29
3	285.9355	**297.9758**	100.00	269.9406	**281.9809**	100.00
	287.9326	299.9728	98.41	271.9376	283.9779	98.87
	289.9296	301.9699	32.46	273.9347	285.9750	32.90
	291.9267	303.9669	3.64	275.9317	287.9720	3.79
4	319.8965	331.9368	76.31	303.9016	315.9419	76.05
	321.8936	**333.9338**	100.00	305.8987	**317.9389**	100.00
	323.8906	335.9309	49.27	307.8957	319.9360	49.55
	325.8877	337.9279	10.86	309.8928	321.9330	11.05
	327.8847	339.9250	0.92	311.8898	323.9301	0.97
5	353.8576	365.8978	61.10	337.8627	349.9029	60.93
	355.8546	**367.8949**	100.00	339.8597	**351.9000**	100.00
	357.8517	369.8919	65.57	341.8568	353.8970	65.84
	359.8487	371.8890	21.57	343.8538	355.8941	21.80
	361.8458	373.8860	3.57	345.8509	357.8911	3.66
6	387.8186	399.8589	50.95	371.8237	383.8639	50.83
	389.8156	**401.8559**	100.00	373.8207	**385.8610**	100.00
	391.8127	403.8530	81.87	375.8178	387.8580	82.14
	393.8097	405.8500	35.82	377.8148	389.8551	36.10
	395.8068	407.8471	8.85	379.8119	391.8521	8.99
7	421.7796	433.8199	43.69	405.7847	417.8250	43.60
	423.7767	**435.8169**	100.00	407.7818	**419.8220**	100.00
	425.7737	437.8140	98.18	409.7788	421.8191	98.44
	427.7708	439.8110	53.61	411.7759	423.8161	53.95
	429.7678	441.8081	17.60	413.7729	425.8132	17.81
8	455.7407	467.7809	33.40	439.7457	451.7860	33.27
	457.7377	469.7780	87.35	441.7428	453.7830	87.15
	459.7348	**471.7750**	100.00	443.7398	**455.7801**	100.00
	461.7318	473.7721	65.48	445.7369	457.7771	65.67
	463.7289	475.7691	26.83	447.7339	459.7742	27.02

* ^{13}C-labeled molecular ions having the highest abundances are bolded.

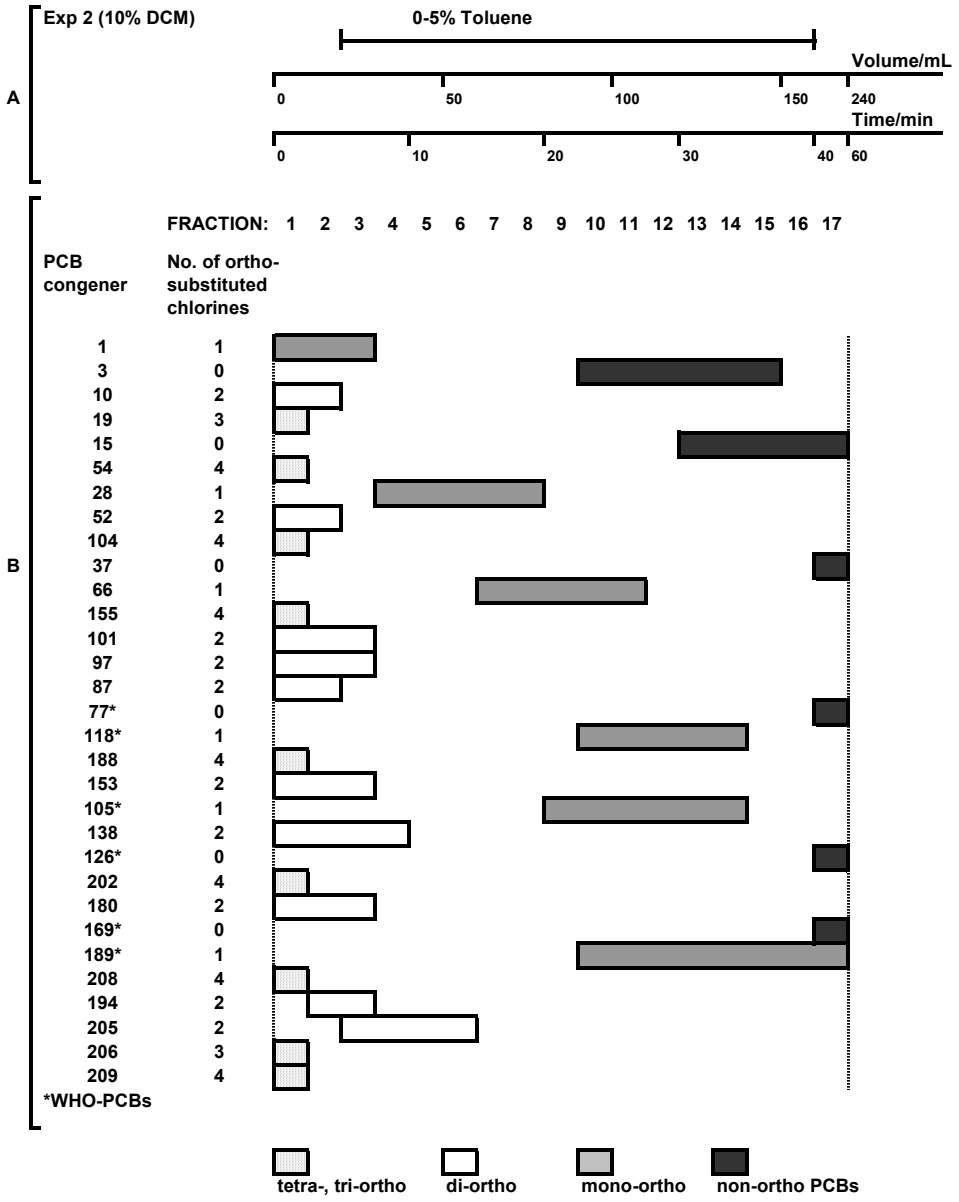
Appendix D

The elution order of a PCB mixture on the 100-mg PX-21 carbon column using the experimental conditions in Exp. 1 (Table 2).



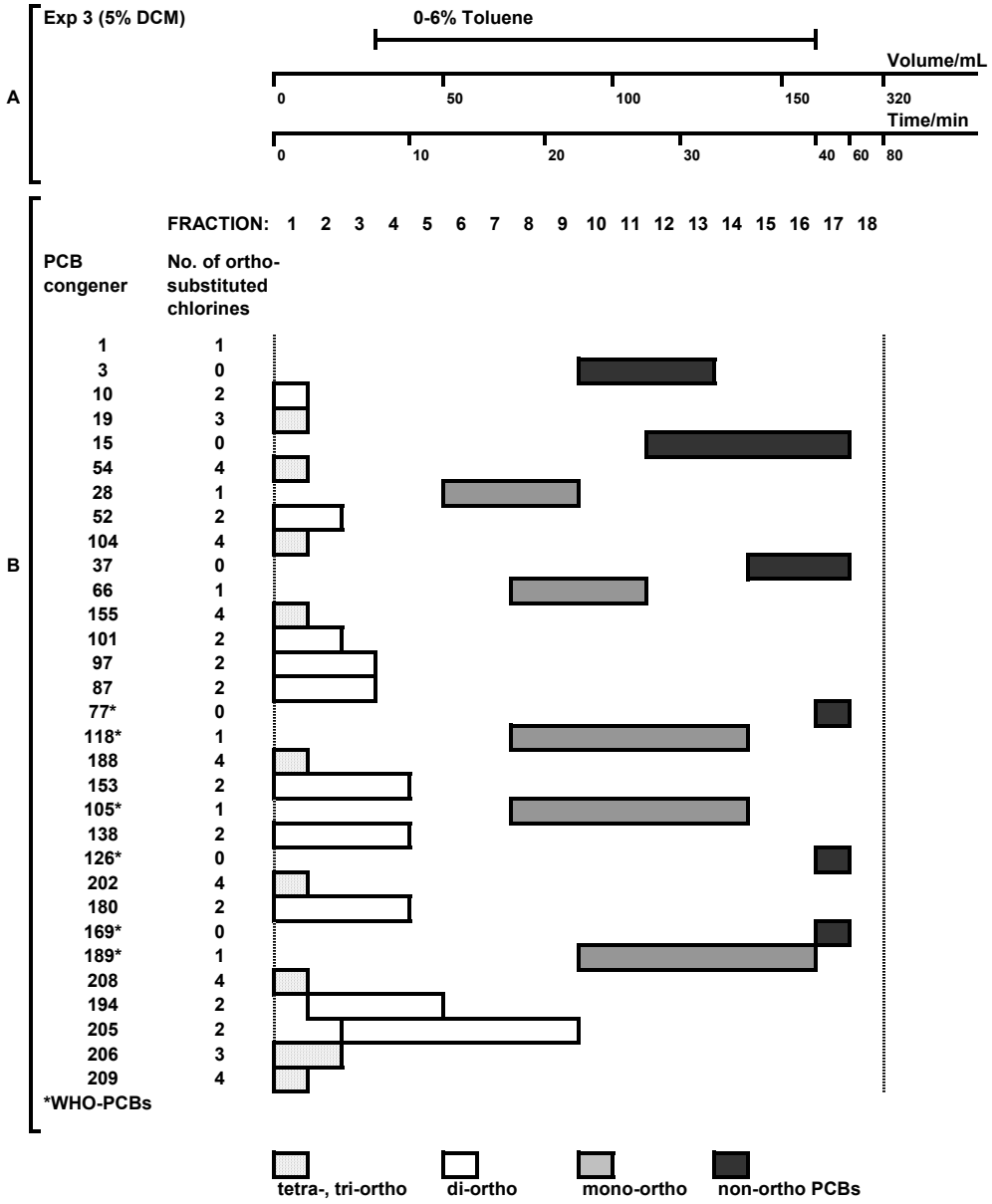
Appendix D (continued)

The elution order of a PCB mixture on the 100-mg PX-21 carbon column using the experimental conditions in Exp. 2 (Table 2).



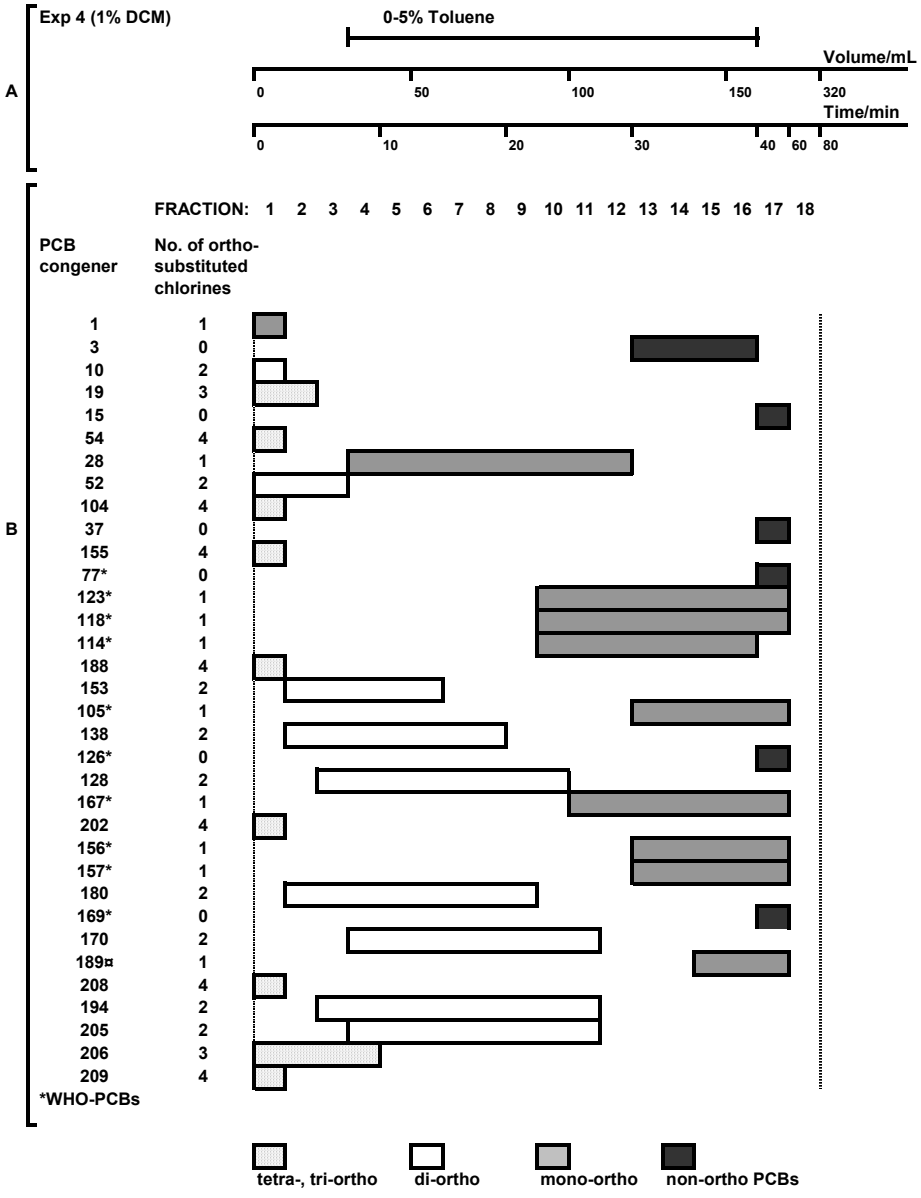
Appendix D (continued)

The elution order of a PCB mixture on the 100-mg PX-21 carbon column using the experimental conditions in Exp. 3 (Table 2).



Appendix D (continued)

The elution order of a PCB mixture on the 100-mg PX-21 carbon column using the experimental conditions in Exp. 4 (Table 2).



Appendix D (continued)

The elution order of a PCB mixture on the 100-mg PX-21 carbon column using the experimental conditions in Exp. 5 (Table 2).