Bis(4-chlorophenyl) sulfone and PCB methyl sulfone metabolites

Trends and chirality in the Baltic Sea environment

Karin Norström

Department of Environmental Chemistry
Stockholm University
2006
Abstract

The Baltic Sea was earlier identified as a highly polluted brackish water body and still is. The high concentrations of polychlorinated biphenyls (PCBs), \(p,p^\prime\)-DDT and related compounds led to severe effects on several species in the Baltic region. However, the situation has improved significantly since the 1970’s resulting in lower exposures to a range of pollutants and healthier wildlife populations. Independent of this positive trend there are still new chemicals leaking into the Baltic Sea environment. The objective of this thesis is to improve the knowledge of sulfone containing compounds and pollutant metabolites in wildlife, with special interest in bis(4-chlorophenyl) sulfone (BCPS) distribution, temporal trend and exposure levels, and the methylsulfonyl-PCBs (MeSO\(_2\)-PCBs). The latter are of particular interest for chiral MeSO\(_2\)-PCBs. BCPS is used for the production of high temperature polymers and was detected as an environmental contaminant ten years ago. PCBs, \(p,p^\prime\)-DDT and related compounds are still of scientific interest.

BCPS is biomagnified and especially in the bird guillemot which has levels of up to 2000 ng BCPS/g fat compared to the grey seal with concentrations of about 60 ng/g fat. The seal levels are similar to the herring, the prey of the bird and seal, with concentrations of 30 ng BCPS/g fat. The guillemot concentration of BCPS has been similar over the last 30 years with a minimal, but significant, annual decline of 1.6%. The reason for the slow decline is not yet understood. Also MeSO\(_2\)-PCBs and 3-MeSO\(_2\)-DDE show a small decrease over time in guillemot egg (3 and 9%, respectively), which is less then for the parent compounds. This shows that the sulfone metabolites are more persistent than their precursors in the guillemot. Furthermore, all these sulfone containing compounds showed a specific retention to liver comparing different tissues in grey seal. The atropisomers of the chiral MeSO\(_2\)-PCB were analysed in both the guillemot and the grey seal and showed to occur in a skewed relationship. This is particularly pronounced in seals where one atropisomer of each chiral congener is very dominating. The dominating atropisomers have been identified with an absolute \(R\) configuration, in both grey seal and guillemot. An enantioselective metabolism was indicated to occur when experimentally tested by CB-132 in rat.

This thesis is stressing the high specificity in wildlife for one atropisomer in the pair of chiral PCB methyl sulfones being PCB metabolites, and the high BCPS concentrations in guillemot hatching in the Baltic proper.
Till
Daniel, Hannes och Melker
Front cover:
Ylva Ceder
List of original papers

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


Papers I, II and III are reprinted with the kind permission of the publishers of respective journal.
# Table of contents

Abstract ...................................................................................................................................... ii  
List of original papers ................................................................................................................ v  
Table of contents ....................................................................................................................... vi  
Abbreviations .......................................................................................................................... viii  

1 Introduction ................................................................................................................................ 1  
1.1 Aims and structure of the thesis ........................................................................................... 2  

2 Background ................................................................................................................................ 4  
2.1 BCPS - A “new” contaminant ................................................................................................ 4  
2.2 Chiral persistent organic pollutants .................................................................................... 4  
2.2.1 MeSO₂-PCBs .................................................................................................................... 6  
2.2.2 Enantiomeric signatures .................................................................................................... 6  
2.2.3 Chiral composition of POPs in biota ................................................................................ 7  

3 Persistent organohalogen compounds ......................................................................................... 9  
3.1 BCPS ........................................................................................................................................ 9  
3.1.1 Biological activity .............................................................................................................. 10  
3.1.2 Concentrations in biota ...................................................................................................... 10  
3.2 PCB ........................................................................................................................................ 11  
3.2.1 Chiral PCBs ....................................................................................................................... 12  
3.2.2 Metabolism of PCBs ......................................................................................................... 13  
3.3 Methylsulfonyl-PCBs ........................................................................................................... 14  
3.3.1 Nomenclature ................................................................................................................... 16  
3.3.2 MeSO₂-PCB concentrations in biota ................................................................................ 17  
3.3.3 Chiral MeSO₂-PCBs in biota ............................................................................................ 20  
3.3.4 Selective retention and some biological effects of MeSO₂-PCBs .................................... 20  
3.4 3-MeSO₂-DDE ...................................................................................................................... 22  
3.5 TCPMe .................................................................................................................................... 22  

4 Selection of samples ................................................................................................................... 24  
4.1 Species .................................................................................................................................... 25  
4.2 Number of samples ............................................................................................................... 26  
4.3 Time trend studies .................................................................................................................. 26  

5 Analytical methodology ............................................................................................................. 28  
5.1 Extraction ............................................................................................................................... 28  
5.2 Lipid removal .......................................................................................................................... 29
**Abbreviations**

BCPS  | bis(4-chlorodiphenyl) sulfone
---|---
CD   | cyclodextrin
$p,p'$-DDD | 1,1-dichloro-2,2-bis(4-chlorophenyl) ethane
$p,p'$-DDE | 1,1-dichloro-2,2-bis(4-chlorophenyl) ethene
$p,p'$-DDT | 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane
DDTs | DDT, DDE and DDD
ECD  | electron capture detector
ECNI | electron capture negative ionisation
EF   | enantiomeric fraction
EI   | electron ionisation
ER   | enantiomeric ratio
GC   | gas chromatography
GPC  | gel permeation chromatography
HBCDD | hexabromocyclododecane
HCH  | hexachlorocyclohexane
IUPAC | International Union of Pure Applied Chemistry
LRM  | laboratory reference material
LOD  | limit of detection
LOQ  | limit of quantification
MAP  | mercapturic acid pathway
3-MeSO$_2$-DDE | 1,1-dichloro-2-(3-methylsulfonyl-4-chlorophenyl)-2-(4-chlorophenyl) ethene
MeSO$_2$-PCBs | methylsulfonyl-polychlorinated biphenyls
MS   | mass spectrometry
PCB  | polychlorinated biphenyl
PFOS | perfluorooctane sulfonic acid
POPs | persistent organic pollutants
SIM  | selected ion monitoring
TCPMe | tris(4-chlorophenyl) methane
1 Introduction

The Baltic Sea is one of the most contaminated marine environments of the world. The exchange of water from the North Sea is limited and the inflow of contributing fresh water from surrounding countries is contaminated with both nutrients and environmental contaminants from urbanised and industrialised areas. To that adds the airborne pollution from surrounding and remote sources.

A “Non-toxic environment” is one of 15 national environmental goals which the Swedish Parliament has agreed to and this goal is aimed to be reached by the year of 2020 [1]. It states that the environment should be free from substances and metals that have been produced by the society which pose a threat to humans and the ecosystem. This means that the levels of exogenous substances in the environment should be close to zero. Several steps are included to reach the goal and one of them is an increased knowledge about anthropogenic chemicals.

International co-operation is a prerequisite for reaching the goal of a non-toxic environment, particularly important for all states surrounding the Baltic Sea. The international organisation, the Helsinki Commission (HELCOM), is striving for an unpolluted Baltic and a sea in ecological balance. A document has been signed by all states bordering the Baltic Sea and the European Community reiterating this goal [2]. Russia and the members of the EU have also accepted the Stockholm convention of persistent organic pollutants (POPs) from 2001 [3]. The aim is to stop the production and use of twelve chemicals or classes of chemicals which are persistent, bioaccumulative, toxic and able to undergo long range transport. The polychlorinated biphenyls (PCBs) are for example one of the defined groups of chemicals included in this convention. REACH (Registration, Evaluation, Authorisation of Chemicals), the new EU legislation for registration and limiting the use of hazardous chemicals within the union [4] is an other effort for improving the environment. Despite these international objectives, some Baltic fatty fish species of major commercial value are not allowed to be sold on the EU market because of too high concentrations of certain POPs, i.e. dioxins (polychlorinated dibenzo-p-dioxines (PCDDs) and dibenzofuranes (PCDFs)).

Today the eutrophication and the decreasing stocks of fish due to over-fishing comprise beside the POPs threats to the Baltic Sea. Still, a continuous monitoring of POPs is highly relevant, especially since organohalogenated substances continuously are produced and used in different applications by the industry and found to increase in the environment [5,6]. The Swedish
Monitoring Program follows the levels of several POPs in the Baltic Sea annually [6-10]. The program was established in the late 1960s due to high concentrations of PCB and DDTs found in the Swedish environment and especially in the Baltic [11]. PCBs and \( p,p' \)-DDE were causing a decrease of e.g. white-tailed sea eagles, seals and otters in the Baltic region due to impaired reproduction [12-15]. A couple of years after the severe effects were documented in the 1960s, \( p,p' \)-DDT was banned and the use of PCB was restricted. A total ban of PCB came first in 1995. The levels of DDTs and PCBs in the environment started to decrease and gradually, populations of endangered species increased [16-18]. Later on, when the brominated flame retardants came in focus as environmental pollutants of concern in the 1980s and the 1990s, concentrations of these chemicals were shown to increase [6,9,19,20]. It still took many years before actions were taken to ban some of them within the EU [21]. Other persistent and bioaccumulative compounds known to increase in the environment but for which no actions have been taken so far are e.g. the flame retardant hexabromocyclododecane (HBCDD) [6] and the more recently reported perfluorinated compounds (PFCs) [5], used as surfactants.

Many organohalogenated compounds found in biota are lipophilic, persistent and can be both bioaccumulated and biomagnified. For some contaminants, e.g. the surfactant perfluorooctane sulfonic acid (PFOS) and the fungicide pentachlorophenol (PCP), other mechanisms are involved causing them to retain in biota [22,23]. It is important to generate knowledge about compounds by assessing their chemical structures to predict their fate in the environment. Also metabolites that are formed need to be considered since they can possess totally different properties than their parent compounds and cause unexpected effects.

Another aspect is that many POPs released into nature are chiral. They are often detected as one compound even though they, from a biological point of view, are considered as two different compounds (see below). Approximately 25% of all modern pesticides are chiral [24].

### 1.1 Aims and structure of the thesis

This thesis is based on four separate scientific Papers (I-IV) with the common main goal; to improve the understanding of sulfone containing compounds and pollutant metabolites in wildlife. Special interests are bis(4-chlorophenyl) sulfone (BCPS) distribution, temporal trend and exposure levels, and formation of PCB and \( p,p' \)-DDE methyl sulfone metabolites (MeSO\(_2\)-PCBs and 3-MeSO\(_2\)-DDE). The chirality of the MeSO\(_2\)-PCBs is of particular interest. The chemical analyses are performed mainly on environmental...
samples (Papers I-III) but also on tissue samples from laboratory animals (Papers IV).

Focus in this thesis has been directed to the Baltic environment, harbouring a vast number of pollutants from agricultural, industrial and urbanised areas creating a complex mixture representative of a modern society. PCBs and \( p,p' \)-DDE have been analysed for comparative reasons and to study the relative retention, geographical distribution as well as their relative historical occurrence in the environment.

Marine and fresh water species from various areas and trophic levels were selected for BCPS analyses (Paper I). Guillemot eggs were chosen to study temporal trends of BCPS and methylsulfonyl metabolites of PCB and \( p,p' \)-DDE (Paper II).

Both the guillemot and the grey seal are wildlife species with high body burdens in which the chiral MeSO\(_2\)-PCBs were studied (Papers II and III). In the seals, different organs were analysed and the importance of the sulfone group regarding tissue distribution of BCPS, PCB and \( p,p' \)-DDE metabolites was also assessed (Paper III).

The particularly skewed retention of the MeSO\(_2\)-PCB atropisomers in wildlife called for further studies. To improve the understanding of enantioselective processes, the chiral composition of an atropisomeric PCB congener and its metabolites were studied experimentally in rat (Paper IV).
2 Background

2.1 BCPS - A "new" contaminant

After the disintegration of the Soviet Union it became possible to start looking into the POP pollution situation in the East European countries. Perch from the Gulf of Riga was collected in 1995 for analyses of traditional contaminants such as PCBs and DDTs but also for “new” POPs. Bis(4-chlorophenyl) sulfone, used today in the production of thermostable polymers was first identified here [25]. Notably, concentrations of BCPS were detected in the same range as CB-138, one of the major and most persistent PCB congeners known. Samples from the Swedish Environmental Specimen Bank at the Swedish Museum of Natural History were used and BCPS was found also at higher trophic levels as in grey seals and white tailed sea eagle eggs sampled at the Swedish east coast. This indicated that BCPS was biomagnified. BCPS was also detected in human livers [26]. The studies of perch from Latvian rivers and coastal areas indicated local sources of contamination [27,28]. The environmental concentrations of BCPS also seemed to increase since concentrations in white-tailed sea eagle eggs sampled over the period 1971-1991 went from non-detectable levels up to 600 ppb on lipid weight basis [16]. BCPS has also been detected in sediment and fresh waters in Europe [29-32] as well as in the marine water of the German Bight with concentrations up to 1.5 ng/L [33]. BCPS is further described in chapter 3.1.

2.2 Chiral persistent organic pollutants

Chiral substances are mirror images of each other generally called enantiomers. Chiral POPs are released into the environment as racemates, i.e. the two enantiomers occur in a 1:1 relationship, unless the compound is applied as one enantiomer. The latter may be relevant for pharmaceuticals and possibly some pesticides but not for other chemicals.

A new aspect of environmental contaminants was introduced when König and co-workers (1989) first separated the chiral hexachlorohexane (HCH) isomer, α-HCH by gas chromatography applying cyclodextrin as a chiral stationary phase [34]. Two enantiomers of a chiral compound are not likely affected differently by abiotic processes. In contrast, it is likely that enantiomers behave differently in biological systems [35,36]. Faller et al. (1991) showed that the enrichment of (+)α-HCH in the North Sea was enantioselective due to microbial degradation and shortly thereafter Kallenborn et al. (1991) showed
that the degradation of $\alpha$-HCH in eider duck is an enantioselective process [36,37].

Optical isomerism differs depending on the structure of the chiral compound (Figure 2.1). The most common way to define chirality of a compound is if the compound has an asymmetric centre, i.e. there is an atom with four different substituents, e.g. $o,p'$-DDT (Figure 2.1a). Compounds are also chiral if they are non-superimposable with their mirror images, even though they do not contain any asymmetric atoms. Cyclic environmental pollutants exhibit axial chirality if the structures do not contain any plane of symmetry, as $\alpha$-HCH (Figure 2.1b). PCBs and MeSO$_2$-PCBs with asymmetric chlorine substitution of both rings and with at least three chlorines in ortho position form a pair of enantiomers because of the hindered rotation around the C-C single bond (Figure 2.1c and 2.1d). These enantiomers are then referred to as atropisomers.

The two forms of a chiral compound rotate plane-polarized light clockwise (+) or counter-clockwise (-). Apart from this, the enantiomers have identical physical and chemical properties. If the absolute configuration is known of the two enantiomers, the $R$ (rectus) and $S$ (sinister) designations are used, depending on the arrangement of the substituents of the molecule in space. It should be stressed that there is no relation between the absolute structure of the compound and its ability to rotate light in one direction or the other.

![Chemical structures of chiral compounds.](image)

Figure 2.1. Chemical structures of chiral compounds. a) $o,p'$-DDT; b) $\alpha$-HCH; c) CB-149; d) 5-MeSO$_2$-CB149. * Marks the chirality in a) due to an asymmetric centre and in c) and d) due to steric hindrance (atropisomerism).
The most well studied chiral POPs including their metabolites are the PCBs (chapter 3.2), \( \alpha \)-HCH (the only chiral and main isomer in the production of HCH), chlordane compounds (used as a pesticide (termiteicide)), toxaphene (a \( p,p' \)-DDT substitute) and \( o,p' \)-DDT (impurity in technically produced \( p,p' \)-DDT) and are reviewed by Kallenborn and Hünherfuss [38]. All occur globally, including remote areas as in the Arctic and Antarctic [39]. Other chiral compounds under study are polybrominated biphenyls and HBCDD [40,41].

### 2.2.1 MeSO\(_2\)-PCBs

PCBs are metabolised to MeSO\(_2\)-PCBs via the mercapturic acid pathway (MAP) [42]. The MeSO\(_2\)-PCBs were first identified in grey seal blubber [43] and mice faeces [44]. Shortly thereafter in human milk [45] and in lungs from mice [46]. Twenty eight MeSO\(_2\)-PCB congeners have been identified in biota by structure, ten of them being chiral [47,48]. The chiral composition of MeSO\(_2\)-PCB congeners have been studied in several species from the marine environment [48,49] but also in laboratory rats [50] and human livers [26]. Especially in wildlife and humans is the dominance of one atropisomer of each chiral congener very pronounced [26,48,49] and enantioselective formation or excretion has been suggested to be the cause.

The lipophilicity of MeSO\(_2\)-PCBs is only slightly lower compared to their parent PCB congeners and therefore accumulated in fatty tissues. However, these metabolites can also be selectively retained in tissues due to their capacity to bind to proteins in the lung and liver [51-55]. MeSO\(_2\)-PCBs are discussed in more detail in chapter 3.3.

### 2.2.2 Enantiomeric signatures

To describe the relationship of two enantiomers, the enantiomeric signature, enantiomeric ratios (ER) or enantiomeric fractions (EF) are used. ER is defined as the ratio of the peak area of the (+) enantiomer (\( A_+ \)) over the peak area of the (-) enantiomer (\( A_- \)), i.e.

\[
ER = \frac{A_+}{A_-}
\]

A racemic mixture has an ER of 1.0. ER can range from zero to infinity which limits its usefulness e.g. in graphical data presentations. Harner et al. (2000) proposed a chiral signature defined as enantiomeric fraction (EF) where:

\[
EF = \frac{A_+}{(A_+ + A_-)} \text{ or } EF = \frac{A_1}{(A_1 + A_2)}
\]

If the optical rotation is unknown, \( A_1 \) and \( A_2 \) are used instead representing the first and second eluting enantiomer when analysed on a chiral column [56].
When the absolute configurations of the enantiomers are known, the EF = AR/(AR + AS)

The EF can only range from 0.0 to 1.0 and an EF of 0.5 represents a racemic mixture. Each unit of deviation from a racemic value in both directions, up or down, is equivalent in contrast to ERs. ER values can be estimated to EFs by the equation EF = ER/(1+ER) [56,57].

The enantiomeric signatures of chiral pollutants are suggested to be used as tools for exposure processes, biotransformation capacities and trophic transfer but also to act as chemical tracers for air-surface exchange and atmospheric long-range transport [58,59]. An example of the enantiomeric signature as a tool is the racemic or very close to racemic ERs of o,p'-DDT compared to other pesticides found in plasma from Russian women, indicating fresh releases and local sources of p,p'-DDT [60].

2.2.3 Chiral composition of POPs in biota

The alterations of the chiral composition of POPs that occur by biological processes are far from understood and several mechanisms can be involved. Species from different parts of the food chain have been studied, from marine to terrestrial species [61-64], including humans [26,65-67]. In general, species from low trophic levels show EFs or ERs close to a racemate for different POPs studied [61-64]. Further up in the food chain the deviations from a racemic EF increase. The nearly racemic relationships in low trophic organisms have been suggested to be due to poor or negligible capacity of biotransformation [64] but it is believed that enantiomeric excess in higher trophic organisms arises because metabolism occurs enantioselectively [68].

Enantioselective metabolism is supported for atropisomeric PCBs with skewed EFs in the grey seal liver and blubber [69]. Bioaccumulation may be enantioselective, as based on a greater deviation from a racemic EF of atropisomeric PCBs in blubber than in liver [70]. Enantiomeric excess can also be due to enantioselective transport or hindered transfer as seen in brains of experimental rats dosed with α-HCH [71]. In brains of seals and dolphins, only (+)α-HCH was found (ER>50) but in the liver and blubber of the seal, ER was 1.5-2 [72]. The strong dominance of one atropisomer of the chiral MeSO₂-PCBs seen in wildlife, humans and in laboratory rats could arise from either enantioselective formation or enantioselective retention [26,48-50]. Factors that may influence EFs are e.g. age [63,73,74], tissue/organ [69,75], concentration [67] and species specificity [75,76].

Enantiomers can possess different biological activities, e.g. the atropisomers of 4-MeSO₂-CB149 have shown different biological activities in laboratory tests and the atropisomers of 5-MeSO₂-CB149 showed differences in body
clearance in mice dosed with racemic 5-MeSO2-CB149 [77]. Differences have also been shown for PCBs where the atropisomers have a different potency to induce drug metabolising enzymes [78,79]. Furthermore, the estrogenic activity of o,p'-DDT is due to the (-)-enantiomer [80].

In the pharmaceutical industry, chirality is very important because the enantiomers have different effects. A tragic and well-known example of enantiomers having different biological activity is thalidomide, the active component of the drug Neurosedyn, sold in the early 1960s as a racemate. One of the enantiomers showed to be teratogenic which caused severe birth defects. The preparation and control of pharmaceuticals as pure enantiomers is significant today, and the Nobel Prize 2001 was awarded scientists for their development of molecules which enantioselective catalysts reactions leading to the formation of only one enantiomer. A recent example is Losec which consisted of two enantiomers, but today only the active one is included in the medicine.
3 Persistent organohalogen compounds

The substances and substance classes analysed in this thesis and presented below are shown in Figure 3.1. They are all neutral and bioaccumulating compounds. The main focus in this chapter is given to BCPS, MeSO$_2$-PCBs and 3-MeSO$_2$-DDE. These sulfone containing compounds have the property of acting as Lewis bases which is utilized in analytical methodology (chapter 5).

![Structures of chemicals and chemical classes for compounds analysed in papers I-IV.](image)

**Figure 3.1.** Structures of chemicals and chemical classes for compounds analysed in papers I-IV.

3.1 BCPS

Pure BCPS (CAS 80-07-9) is a crystalline substance with a melting point of 148-150°C. The water solubility is less than 0.1 g/100 ml at 20°C and the calculated partition coefficient log $K_{ow}$ is 4.1, modelled by ACD (Advanced Chemistry Development). BCPS is classified as a high production volume chemical which means that it is produced at a level greater than 1,000 tonnes per year in at least one of the member countries of OECD (Organisation for economic co-operation and development). The main field of application of BCPS is in the production of thermostable polymers for the plastic industry, such as Polysulfones and Polyether sulfones [81]. These polymers have high resistance to burning and in most applications no flame-retardant additives are needed [81]. Good electrical insulation properties and a high resistance to
hydrolysis are examples of other properties of these polymers which make their field of application wide. Ultrason® from the German company BASF is an example of an industrial product containing Polysulfones or Polyethers with a wide range of applications e.g. microwave ovens and medical equipments. In the past, BCPS was reported both as a pesticide in Russia [82] and as the major contaminant in pesticide production [83].

### 3.1.1 Biological activity

Increased liver to body size has shown to be the most prominent effect in BCPS dosed rats [84,85]. Adipose tissue contained by far the highest levels of BCPS as shown in two different experimental studies in rats [85,86]. BCPS has shown to be slowly excreted primarily in faeces in the form as metabolites [86]. Only small amounts of unmetabolised BCPS were detected in faeces. The conjugated metabolites detected were suggested to arise from hydroxylation products through hepatic phase I and II metabolism involving enzymes inducible by BCPS [85,86]. BCPS has structural resemblance with p,p'-DDT due to the two phenyl rings with chlorines in the two para-positions. Similarities regarding the potency to induce microsomal enzyme activity in rats [87] have been shown for BCPS and p,p'-DDT as well as similarities in insecticidal activity [88].

Due to its high production and use, BCPS was selected by the U.S. National Toxicology Program for toxicity characterization and carcinogenicity studies [84]. BCPS was administrated to mice and rats for 14 weeks and for two years (rats only) with no evidence of carcinogenicity and a no observed adverse effect level (NOAEL) of 1.5 mg/kg body weight was suggested [84].

### 3.1.2 Concentrations in biota

The levels of BCPS reported in biota are reviewed in Table 3.1. BCPS is detected in grey seals, birds and fish from both fresh and marine waters. The highest concentrations are found in birds. The reports are mainly from the Baltic region but BCPS has also been found in plasma of glaucous gulls from the Norwegian arctic [89] and in fish (bream) sampled at four different locations in two German rivers, Elbe and Rhein (Paper I). BCPS has been detected in guillemot eggs in remote areas such as Iceland and the Faroe Islands, but at very low concentrations (Jörundsdottir, pers. commun.). In human liver, the concentration of BCPS was estimated to be five times the level of the MeSO_2-PCB congener detected in highest concentration, 5'-MeSO_2-CB132 [26].

So far, no sources have been identified that can explain the findings of BCPS in the environment. An effort was made to find a source of BCPS by
analysing granulate and finished plastic samples from BASF for BCPS residues but found to be no major contributors of free BCPS [90].

Table 3.1. Concentrations of BCPS (ranges ng/g fat) reported in wildlife. References to the scientific reports are given in the table.

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>Sampling year</th>
<th>Tissue</th>
<th>n(^a)</th>
<th>BCPS range ng/g fat</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perch</td>
<td>Latvia</td>
<td>1994</td>
<td>muscle</td>
<td>30</td>
<td>40-100</td>
<td>[25]</td>
</tr>
<tr>
<td>Perch</td>
<td>Latvia</td>
<td>1994-95</td>
<td>muscle</td>
<td>62</td>
<td>38-100</td>
<td>[27]</td>
</tr>
<tr>
<td>Perch</td>
<td>Latvia</td>
<td>1997</td>
<td>muscle</td>
<td>23</td>
<td>28-190</td>
<td>[28]</td>
</tr>
<tr>
<td>Salmon</td>
<td>Sweden</td>
<td>1971</td>
<td>muscle</td>
<td></td>
<td>1 pool (10)</td>
<td>8.7</td>
</tr>
<tr>
<td>Salmon</td>
<td>Sweden</td>
<td>1996</td>
<td>muscle</td>
<td></td>
<td>2 pool (4)</td>
<td>31,33</td>
</tr>
<tr>
<td>Perch</td>
<td>Sweden</td>
<td>1998</td>
<td>muscle</td>
<td></td>
<td>4 pool (5)</td>
<td>15-37</td>
</tr>
<tr>
<td>Baltic herring</td>
<td>Sweden</td>
<td>1996-98</td>
<td>muscle</td>
<td></td>
<td>2 pool (10)</td>
<td>29,31</td>
</tr>
<tr>
<td>Arctic char</td>
<td>Sweden</td>
<td>1996</td>
<td>muscle</td>
<td></td>
<td>1 pool (5)</td>
<td>1.8</td>
</tr>
<tr>
<td>Bream</td>
<td>Germany</td>
<td>1997</td>
<td>muscle</td>
<td>4</td>
<td></td>
<td>3.5-35</td>
</tr>
<tr>
<td>Bird</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White-tailed sea eagle</td>
<td>Sweden</td>
<td>1987</td>
<td>egg</td>
<td>1</td>
<td></td>
<td>500</td>
</tr>
<tr>
<td>White-tailed sea eagle</td>
<td>Sweden</td>
<td>1971-91</td>
<td>egg</td>
<td>21</td>
<td></td>
<td>n.d.-610</td>
</tr>
<tr>
<td>Guillemot</td>
<td>Sweden</td>
<td>1989</td>
<td>muscle</td>
<td>2 pool (5)</td>
<td>1600, 1900</td>
<td>P I</td>
</tr>
<tr>
<td>Guillemot</td>
<td>Sweden</td>
<td>1971-01</td>
<td>egg</td>
<td>35</td>
<td></td>
<td>760-2600</td>
</tr>
<tr>
<td>Herring gull</td>
<td>Canada</td>
<td>1989</td>
<td>egg</td>
<td>−</td>
<td></td>
<td>i.d.</td>
</tr>
<tr>
<td>Glaucous gull</td>
<td>Norway</td>
<td>2002-04</td>
<td>plasma</td>
<td>87</td>
<td></td>
<td>5.2-143</td>
</tr>
<tr>
<td>Mammal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grey seal</td>
<td>Sweden</td>
<td>1993</td>
<td>blubber</td>
<td>3</td>
<td></td>
<td>53-88</td>
</tr>
<tr>
<td>Grey seal</td>
<td>Sweden</td>
<td>1995-97</td>
<td>blubber</td>
<td>1 pool (5)</td>
<td>68</td>
<td>P I</td>
</tr>
<tr>
<td>Grey seal</td>
<td>Sweden</td>
<td>1996-97</td>
<td>blubber</td>
<td>4</td>
<td></td>
<td>49-470</td>
</tr>
<tr>
<td>Grey seal</td>
<td>Sweden</td>
<td>2000-01</td>
<td>liver</td>
<td>10</td>
<td></td>
<td>55-700</td>
</tr>
<tr>
<td>Grey seal</td>
<td>Sweden</td>
<td>2000-01</td>
<td>lung</td>
<td>10</td>
<td></td>
<td>21-98</td>
</tr>
<tr>
<td>Grey seal</td>
<td>Sweden</td>
<td>2000-01</td>
<td>blubber</td>
<td>10</td>
<td></td>
<td>41-240</td>
</tr>
<tr>
<td>Human</td>
<td>Germany</td>
<td></td>
<td>liver</td>
<td>6</td>
<td></td>
<td>~2-40</td>
</tr>
</tbody>
</table>

P I = Paper I et cetera, i.d. = identified; n.d. = not detected
a) pooled or individual samples, y pool (x), y = number of pools and x = number of samples in the pool

3.2 PCB

Polychlorinated biphenyls comprise 209 structurally different compounds, PCB congeners, with the empirical formula \(C_{12}H_{10-x}Cl_x\). Each congener has been assigned a systematic number by Ballschmiter and co-workers [92,93] following International Union of Applied Chemistry (IUPAC) rules, numbers that are routinely used as abbreviations of PCBs nowadays. The commercial production of PCB started in 1929 for applications in transformers, as dielectric fluids in capacitors, in paints, heat transfer fluids, lubricants and more [94]. The PCBs were produced and sold as mixtures with trade names
such as Aroclor (U.S.), Kanechlor (Japan), Pheneclor (France), Sovol (former USSR) and Clophen (Germany) followed by a number designating their degree of chlorination.

Since the first discovery of PCBs in biota in 1966, PCBs have shown to be globally distributed contaminants and have been identified in air, water, sediments, wildlife and humans [94-96]. Due to high persistence and lipophilicity, several of the PCB congeners are strongly biomagnified in the food webs. The biological half-life of PCB congeners is determined by the position and number of chlorine atoms. In general, PCBs with more than five chlorines and with para-chlorines are more resistant to metabolism than PCBs with vicinal hydrogens in the meta-para (lateral) positions [42].

3.2.1 Chiral PCBs

Only 19 PCB congeners fulfil the requirements for being chiral, having rotational barriers high enough to be stable under general environmental conditions [97]. The atropisomers are stable enough to be isolated in pure form after being applied to specific chromatographic separation procedures. These 19 congeners are shown in Table 3.2. Twelve of these congeners are present in commercial PCB mixtures at levels greater than 1% w/w [98]. These PCB congeners are marked in Table 3.2 as well as those five PCBs that are precursors to the atropisomeric MeSO₂-PCB metabolites formed and retained in biota.

Atropisomeric PCBs have mainly been studied in marine mammals [62,69,70,73,76,99] but also in humans [65,66]. Harju suggests that the degree of deviation from a racemic ratio correlate with the presence of vicinal hydrogens in both meta-para and ortho-meta positions and, e.g. EFs for CB-91, CB-95 and CB-132 deviates more from that of a racemic mixture than CB-149 and CB-174 in grey seals [69,100]. The dominance of one atropisomer in biota is not due to de-racimerisation, potentially caused by high temperatures during e.g. GC analysis [101,102]. Furthermore, atropisomeric PCBs with a chlorine also in the meta position, adjacent to the ortho chlorine, have increased rotation barriers which is called “buttressing” effect [100].

The absolute structures of individual PCB atropisomers have not yet been determined. Instead, optical rotation and elution order on specific chiral analytical columns are known for several of the congeners [101,103].
Table 3.2. Chlorine substitution pattern of the 19 stable atropisomeric PCB congeners. The PCBs marked in bold text exist in >1% in technical PCB mixtures [98] and those marked in italic are PCB congeners forming MeSO₂-PCB metabolites that have been reported present in biota.

<table>
<thead>
<tr>
<th></th>
<th>2'</th>
<th>2',3'</th>
<th>2',4'</th>
<th>2',5'</th>
<th>2',3',4'</th>
<th>2',3',5'</th>
<th>2',3',6'</th>
<th>2',4',5'</th>
<th>2',3',4',6'</th>
</tr>
</thead>
<tbody>
<tr>
<td>234</td>
<td>132</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>235</td>
<td>135</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>236</td>
<td>45</td>
<td>84</td>
<td>91</td>
<td>95</td>
<td>136</td>
<td>149</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2345</td>
<td></td>
<td>174</td>
<td>196</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2346</td>
<td>88</td>
<td>131</td>
<td>139</td>
<td>144</td>
<td>171</td>
<td>175</td>
<td>176</td>
<td>183</td>
<td>197</td>
</tr>
</tbody>
</table>

3.2.2 Metabolism of PCBs

Lipophilic exogenous substances, present in biota, are subjected to metabolism often leading to the formation of polar water soluble compounds, facilitating their excretion. The metabolism is simply divided in two phases; phase 1 alters the molecule by oxidation, reduction or hydrolysis adding or transforming a functional group, which in phase 2 can be conjugated to glucuronic acid, sulfate or to glutathione. Metabolism may cause negative effects, e.g. PCBs forming reactive intermediates, arene oxides, quinones and semiquinones, with a potential to react with DNA [104]. Also, the resulting metabolites have different biological activity then the parent compound which make them a second class of environmental contaminants. This is the case with hydroxylated PCBs (OH-PCBs) and MeSO₂-PCBs [42,105]. A short review of the formation of methylsulfonyl containing metabolites is presented [42,105] and illustrated in Figure 3.2, with CB-132 as an example. The route for MeSO₂-PCBs formation was first clarified by Bakke in the early 1980’s [106,107].

The PCB molecule is initially oxidised to an arene epoxide by the hepatic cytochrome P450 system, preferentially in the meta-para position. The epoxide reacts with the endogenous tripeptide glutathione (GSH). GSH conjugation occurs spontaneously and/or via glutathione-S-transferase (GST). The formed adduct is dehydrated and the glutathionyl-PCB (PCB-SG) conjugate undergoes peptidase hydrolysis through the mercapturic acid pathway (MAP) to form a cysteine conjugate. This conjugate is excreted in the bile and then into the gastrointestinal tract where C-S β-lyase cleaves the C-S bond of the cysteine conjugate and a thiol-substituted PCB (PCB-SH) is formed. The PCB-SH undergoes methylation in an S-methyltransferase (SAM) catalyzed reaction with S-adenosylmethionine. The free PCB thiol can
also undergo conjugation with glucoronic acid or with sulfate. The SAM methylated product, a PCB methyl sulfide (PCB-MeS) is oxidised in two steps to a sulfoxide (MeSO-PCB) and finally a sulfone (MeSO$_2$-PCB) is formed. The route of MeSO$_2$-PCB formation is built on enterohepatic circulation since the intestinal microflora is requested for the C-S cleavage of the cystein conjugate [106].

The arene oxide initially formed may also be deactivated to a dihydrodiol which is dehydrated to an OH-PCB [42,105]. When the arene oxide is formed in a meta-para position with a meta-hydrogen and a para-chlorine, both meta- and para-OH-PCBs can be formed as a result of a 1,2-shift. OH-PCBs may be formed via direct insertion of the hydroxyl group. All OH-PCBs can be further conjugated with glucuronic acid or with sulfate, increasing the water solubility of the compound.

Depending on the chlorine substitution pattern of the PCB congener and animal species, different cytochrome P450 enzymes are involved [42], potentially resulting in different metabolic products. The MeSO$_2$-PCBs are formed from tri- to heptachlorinated PCB congeners with either 2,5-dichloro- or 2,3,6-trichloro-substitution pattern in the methylsulfonyl containing phenyl ring [47]. Also, MeSO$_2$-PCBs with a low number of chlorines and/or unsubstituted meta-para carbons may be further metabolised [108]. The PCB metabolites are mainly excreted via the bile [109].

### 3.3 Methylsulfonyl-PCBs

Methylsulfonyl-PCBs are slightly less hydrophobic than the corresponding parent PCB congener, which is due to the polar MeSO$_2$-functional group. The parent PCBs have log $K_{ow}$ values of 4.5-8.2 [110] while MeSO$_2$-PCBs have log $K_{ow}$ of 4-6.5 [42]. The identified MeSO$_2$-PCBs in wildlife are meta and para substituted MeSO$_2$ congeners occurring in isomer pairs derived from the same PCB precursor. Fourteen MeSO$_2$-PCB pairs have been identified by structure in wildlife but additional congeners have been detected [47,111]. Recently a large number of MeSO$_2$-PCBs was indicated in human plasma [112].
**Figure. 3.2.** Metabolic pathway of CB-132 visualizing the formation of 4’-MeSO₂-CB132. The isomer 5’-MeSO₂-CB132, also formed in this process, is not shown in the figure.
3.3.1 Nomenclature

The abbreviation of MeSO$_2$-PCBs often found in older literature is based on the meta or para position of the MeSO$_2$ group and referred to as 3- or 4-MeSO$_2$-CB. In modern literature and in this thesis the abbreviation system used is based on that suggested by Letcher et al. (2000) [42]. In this abbreviation system the number of the metabolite congener in question is decided by first omitting the MeSO$_2$-group to identify the PCB precursor with the PCB number given originally [93]. Then note which of the phenyl rings that contains the unprimed and primed chlorine atoms respectively. From this, identify the position of the MeSO$_2$-group and give it an unprimed or primed number depending on which carbon it is attached to. When the full chemical name is written, IUPAC rules are applied where the MeSO$_2$-group get higher priority than the chlorines. This abbreviation number system is demonstrated and described in Figure 3.3 where the structure of 5’-MeSO$_2$-CB132 with full chemical (Figure 3.3a) and abbreviated name (Figure 3.3b) is shown. An additional system was recently proposed by Maervoet et al. (2004) with the difference that also the full chemical name should be written according to the principles applied for abbreviation [113]. The suggested change is not following IUPAC rules.

![Diagram](image_url)

**Figure 3.3.** a) Full chemical name of MeSO$_2$-PCB following IUPAC rules where the MeSO$_2$-group are numbered with a higher priority than the chlorines and b) abbreviated name where the congener number is given as described by Ballschmiter et al. (1993) and the MeSO$_2$-group is numbered and primed from this [93].
3.3.2 MeSO$_2$-PCB concentrations in biota

Methylsulfonyl-PCB metabolites, discovered in 1976 until 1999, have been reported in marine and terrestrial mammals, birds and fish from Europe, North America and Japan as reviewed elsewhere [42]. The number of individual MeSO$_2$-PCBs quantified and the congener pattern varies from study to study depending on species, tissue and exposure. In general, the concentrations in biota, humans excluded, are in the order of mammals > birds > fish. By calculating the ratio MeSO$_2$-PCB/PCB, the metabolic capacity of a species is indicated [114]. ΣMeSO$_2$-PCB concentrations reported in wildlife from 2000 to 2005 are presented in Table 3.3.

**Mammals** The grey seal from the Baltic Sea is a frequently studied species with previously reported concentrations of ΣMeSO$_2$-PCB up to 110 µg/g fat in the blubber which is 15% of ΣPCB [115]. In grey seal liver, the ratio ΣMeSO$_2$-PCB/ΣPCB has been shown to be up to 1.1 [42]. MeSO$_2$-PCBs are also among the most abundant organohalogen substances in Swedish otter and Canadian polar bear [116,117]. The ratio ΣMeSO$_2$-PCB/ΣPCB in adipose tissues of marine mammals is similar between species (~0.01-0.1) but seals have in general higher concentrations of ΣMeSO$_2$-PCBs than whales and dolphins (Table 3.3) [42].

Metabolism of PCBs rather than accumulation of MeSO$_2$-PCBs from the diet is most likely the source in marine mammals with fish and lower trophic species as prey [114]. In contrast, polar bears have shown to accumulate MeSO$_2$-PCBs via the diet which was suggested to be the major source of their MeSO$_2$-PCB concentration [47]. Exposure of MeSO$_2$-PCBs may also be from transplacental transfer due to the higher concentrations in pilot whale foetus than the cow [118].

**Birds** A lower number of MeSO$_2$-PCBs are detected in birds compared to mammals [89,119-121] and the ratio of ΣMeSO$_2$-PCB/ΣPCB is about 1/10$^\text{th}$ compared to mammals. In glaucous gull eggs from the Arctic Norway, this ratio was similar in plasma and egg but the congener pattern was different in the two compartments [89]. The plasma was dominated by an unidentified hexachlorinated MeSO$_2$-PCB congener followed by 5’-MeSO$_2$-CB132 whereas the egg was dominated by pentachlorinated congeners [89]. MeSO$_2$-PCBs have also been detected in pelican muscle but no concentrations were reported [122].

**Fish** MeSO$_2$-PCBs in fish are rarely reported. Fish appear to have a low capacity to metabolise PCBs [123]. Still, the low number of PCB congeners with 2,5- or 2,3,6- chlorine substitution patterns and a ΣMeSO$_2$-PCB/ΣPCB ratio of 0.1 suggested that the MeSO$_2$-PCBs detected in the deepwater sculpin is a result of metabolism [123]. 3’-MeSO$_2$-CB101 was detected in the highest
**Table. 3.3.** Arithmetic mean concentrations and ranges (ng/g fat) of ΣMeSO₂-PCB and MeSO₂-DDE reported from wildlife. References to the scientific reports are given in the table.

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>Sampling year</th>
<th>Tissue</th>
<th>n</th>
<th>ΣMeSO₂-PCB</th>
<th>No. c</th>
<th>ΣMeSO₂-PCB / ΣPCB</th>
<th>3-MeSO₂-DDE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mean</td>
<td>range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deepwater sculpin</td>
<td>Great Lakes</td>
<td>1997</td>
<td>-</td>
<td>10</td>
<td>7.0</td>
<td>-</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Burbot</td>
<td></td>
<td>1998</td>
<td>-</td>
<td>12</td>
<td>40</td>
<td>-</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Bird</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guillemot²</td>
<td>Baltic Sea</td>
<td>1971-2001</td>
<td>egg</td>
<td>5</td>
<td>16-3447</td>
<td>-</td>
<td>5.6-54</td>
<td>&lt;8-12</td>
</tr>
<tr>
<td>Glaucous gull</td>
<td>Bear Island</td>
<td>2002, 2004</td>
<td>plasma/female</td>
<td>42</td>
<td>410</td>
<td>66-1200</td>
<td>0.01</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2002, 2004</td>
<td>plasma/male</td>
<td>45</td>
<td>360</td>
<td>68-1100</td>
<td>0.007</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>egg</td>
<td>30</td>
<td>92</td>
<td>38-160</td>
<td>0.008</td>
<td>29</td>
</tr>
<tr>
<td>Mammal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beluga whale</td>
<td>Hudson bay</td>
<td>1993-94</td>
<td>blubber/male</td>
<td>7</td>
<td>160</td>
<td>120-190</td>
<td>0.04</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>St. Lawrence</td>
<td>1994-96</td>
<td>blubber/male</td>
<td>30</td>
<td>230</td>
<td>22-1000</td>
<td>0.03</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>St. Lawrence</td>
<td>1994-96</td>
<td>blubber/female</td>
<td>3</td>
<td>180</td>
<td>35-410</td>
<td>0.02</td>
<td>1.8</td>
</tr>
<tr>
<td>Bowhead whale</td>
<td>Alaska</td>
<td>1997-2000</td>
<td>blubber</td>
<td>20</td>
<td>6.2</td>
<td>5.4-7.0</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>Harbour porpoise</td>
<td>Baltic Sea</td>
<td>1996</td>
<td>blubber</td>
<td>3</td>
<td>74</td>
<td>54-110</td>
<td>0.006</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1996</td>
<td>nuchal fat</td>
<td>3</td>
<td>62</td>
<td>37-97</td>
<td>0.005</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1996</td>
<td>liver</td>
<td>3</td>
<td>310</td>
<td>150-490</td>
<td>0.025</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1996</td>
<td>muscle</td>
<td>3</td>
<td>160</td>
<td>100-250</td>
<td>0.022</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1996</td>
<td>brain</td>
<td>3</td>
<td>74</td>
<td>15-130</td>
<td>0.023</td>
<td>8.9</td>
</tr>
<tr>
<td>Harbour porpoise</td>
<td>North Sea</td>
<td>1997-00</td>
<td>liver/juvenile</td>
<td>11</td>
<td>490</td>
<td>39-1800</td>
<td>0.015</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1997-00</td>
<td>liver/adult</td>
<td>8</td>
<td>1900</td>
<td>970-4200</td>
<td>0.013</td>
<td>49</td>
</tr>
<tr>
<td>Grey seal²</td>
<td>Baltic Sea</td>
<td>2000-01</td>
<td>blubber</td>
<td>10</td>
<td>1700</td>
<td>640-5200</td>
<td>0.001</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2000-01</td>
<td>liver</td>
<td>10</td>
<td>42000</td>
<td>20000-770</td>
<td>0.013</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>lung</td>
<td>10</td>
<td>850</td>
<td>460-2500</td>
<td>0.015</td>
<td>57</td>
</tr>
<tr>
<td>Harbour seal</td>
<td>Germany</td>
<td>1988</td>
<td>blubber</td>
<td>10</td>
<td>1200</td>
<td>210-4300</td>
<td>0.066</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1988</td>
<td>lung</td>
<td>10</td>
<td>960</td>
<td>190-3300</td>
<td>0.044</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1988</td>
<td>uterus</td>
<td>10</td>
<td>610</td>
<td>150-1400</td>
<td>0.036</td>
<td>0.04</td>
</tr>
<tr>
<td>Striped dolphin</td>
<td>Mediterranean</td>
<td>1990-92</td>
<td>blubber</td>
<td>12</td>
<td>520</td>
<td>200-1600</td>
<td>0.0069</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1990-92</td>
<td>lung</td>
<td>12</td>
<td>160</td>
<td>10-420</td>
<td>0.0065</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1990-92</td>
<td>uterus</td>
<td>12</td>
<td>50</td>
<td>10-100</td>
<td>0.0024</td>
<td>0.01</td>
</tr>
<tr>
<td>Pilot whale</td>
<td>Coast of Maine</td>
<td>1990</td>
<td>blubber/mother</td>
<td>3</td>
<td>62</td>
<td>32-69</td>
<td>0.024</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1990</td>
<td>blubber/foetus</td>
<td>3</td>
<td>32</td>
<td>12-51</td>
<td>0.015</td>
<td>29</td>
</tr>
<tr>
<td>Polar bear³</td>
<td>East Greenland</td>
<td>1999-01</td>
<td>adipose</td>
<td>19</td>
<td>700</td>
<td>130-3900</td>
<td>0.07</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1999-01</td>
<td>whole blood</td>
<td>19</td>
<td>1200</td>
<td>310-7300</td>
<td>0.19</td>
<td>26</td>
</tr>
<tr>
<td>Polar bear</td>
<td>Alaska</td>
<td>1996-02</td>
<td>adipose/ male</td>
<td>7</td>
<td>150</td>
<td>110-290</td>
<td>0.051</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>Canada</td>
<td>2001-02</td>
<td>adipose</td>
<td>64</td>
<td>120</td>
<td>0.91-270</td>
<td>0.055</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Svalbard</td>
<td>2002</td>
<td>adipose</td>
<td>15</td>
<td>200</td>
<td>76-460</td>
<td>0.033</td>
<td>-</td>
</tr>
</tbody>
</table>

P II = Paper II et cetera; n.d. = not detected. a) geometric mean, b) median concentrations, c) maximum number of congeners detected
Table 3.4. Enantiomeric fractions (EF=A₁/(A₁+A₂)) and enantiomeric ratios (ER=A₁/A₂) of atropisomeric MeSO₂-PCBs reported from wildlife and humans. The chiral GC column used for respective study is shown. References to the scientific reports are given in the table.

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissue</th>
<th>GC-column</th>
<th>4-MeSO₂-CB91 EF</th>
<th>5-MeSO₂-CB149 EF</th>
<th>4-MeSO₂-CB149 ER</th>
<th>5'-MeSO₂-CB132 EF</th>
<th>4'-MeSO₂-CB132 ER</th>
<th>5'-MeSO₂-CB174 EF</th>
<th>4'-MeSO₂-CB174 ER</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bird</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guillemot</td>
<td>egg</td>
<td>1:1 heptakis (2,3-di-O-Me-6-O-t-hexyl)-β-CD OV 1701</td>
<td>0.67 - 0.97</td>
<td>0.41 - 0.97</td>
<td>0.52 - 1.0</td>
<td>0.032-0.090</td>
<td>0.98-0.99</td>
<td>&lt;0.010-0.024</td>
<td>0.99-1.0</td>
<td>P II</td>
</tr>
<tr>
<td>Pelican*</td>
<td>muscle</td>
<td>1:4 heptakis (2,3-di-O-Me-6-O-TBDMS)-β-CD SE52</td>
<td>-</td>
<td>0.43 - 0.75</td>
<td>0.75 - 1.6</td>
<td>-</td>
<td>0.68 - 2.1</td>
<td>-</td>
<td>-</td>
<td>[122]</td>
</tr>
<tr>
<td>Mammal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grey seal</td>
<td>blubber</td>
<td>1:1 heptakis (2,3-di-O-Me-6-O-t-hexyl)-β-CD OV 1701</td>
<td>0.94-0.98</td>
<td>0.24 - 0.32</td>
<td>0.32 - 1.0</td>
<td>0.023-0.14</td>
<td>0.98-0.99</td>
<td>&lt;0.010-0.040</td>
<td>0.99-1.0</td>
<td>P III</td>
</tr>
<tr>
<td></td>
<td>liver</td>
<td>1:1 heptakis (2,3-di-O-Me-6-O-TBDMS)-β-CD SE52</td>
<td>0.76</td>
<td>0.31 - 0.36</td>
<td>0.53 - 1.2</td>
<td>-</td>
<td>0.79 - 3.8</td>
<td>-</td>
<td>-</td>
<td>[122]</td>
</tr>
<tr>
<td></td>
<td>lung</td>
<td>20% 2,3,6-O-TBDMS-β-CD in OV-1701</td>
<td>~0.8 - 0.8</td>
<td>0.24 - 0.24</td>
<td>0.32 - 1.0</td>
<td>~0.8 - 3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[48]</td>
</tr>
<tr>
<td>Arctic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ringed seal*</td>
<td>blubber</td>
<td>1:4 heptakis (2,3-di-O-Me-6-O-TBDMS)-β-CD SE52</td>
<td>0.91</td>
<td>0.09 - 0.1</td>
<td>0.24 - 1.0</td>
<td>~0.9 - 0.9</td>
<td>0.91 - 0.9</td>
<td>~0.9 - 0.9</td>
<td>-</td>
<td>[48]</td>
</tr>
<tr>
<td>Seal*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harbour</td>
<td>liver</td>
<td>Chirasil-Dex</td>
<td>-</td>
<td>&lt;0.02-0.20</td>
<td>0.95-0.99</td>
<td>0.06-0.20</td>
<td>-</td>
<td>0.10-0.23</td>
<td>&gt;0.73-0.89</td>
<td>[49]</td>
</tr>
<tr>
<td>porpoise</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arctic</td>
<td>adipose</td>
<td>20% 2,3,6-O-TBDMS-β-CD in OV-1701</td>
<td>&gt;0.9 - 10</td>
<td>0.09 - 0.1</td>
<td>0.24 - 1.0</td>
<td>&gt;0.9 - 10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[48]</td>
</tr>
<tr>
<td>polar bear*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>liver</td>
<td>1:1 heptakis (2,3-di-O-Me-6-O-THBMS)-β-CD OV 1701</td>
<td>-</td>
<td>0 - 0.01</td>
<td>0.82 - 1.0</td>
<td>-</td>
<td>0.91 - 1.0</td>
<td>-</td>
<td>-</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>lung</td>
<td>1:4 heptakis (2,3-di-O-Me-6-O-t-hexyl-β-CD) SE52</td>
<td>0.77</td>
<td>0.04 - 0.43</td>
<td>0.66 - 0.24</td>
<td>-</td>
<td>0.91 - 1.0</td>
<td>-</td>
<td>-</td>
<td>[50]</td>
</tr>
<tr>
<td>Laboratory animals</td>
<td>Wistar rat</td>
<td>adipose</td>
<td>1:1 heptakis (2,3-di-O-Me-6-O-t-hexyl)-β-CD OV 1701</td>
<td>-</td>
<td>- - -</td>
<td>- - -</td>
<td>0</td>
<td>0.95 - 1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>liver</td>
<td>1:1 heptakis (2,3-di-O-Me-6-O-t-hexyl)-β-CD OV 1701</td>
<td>-</td>
<td>- - -</td>
<td>- - -</td>
<td>0</td>
<td>0.95 - 1.0</td>
<td>-</td>
<td>-</td>
<td>P IV</td>
</tr>
<tr>
<td></td>
<td>lung</td>
<td>1:4 heptakis (2,3-di-O-Me-6-O-t-hexyl-β-CD) SE52</td>
<td>0.77</td>
<td>0.04 - 0.43</td>
<td>0.66 - 0.24</td>
<td>-</td>
<td>0.91 - 1.0</td>
<td>-</td>
<td>-</td>
<td>[50]</td>
</tr>
<tr>
<td></td>
<td>adipose</td>
<td>1:1 heptakis (2,3-di-O-Me-6-O-t-hexyl)-β-CD OV 1701</td>
<td>-</td>
<td>- - -</td>
<td>- - -</td>
<td>0</td>
<td>0.95 - 1.0</td>
<td>-</td>
<td>-</td>
<td>P IV</td>
</tr>
<tr>
<td></td>
<td>liver</td>
<td>1:4 heptakis (2,3-di-O-Me-6-O-t-hexyl-β-CD) SE52</td>
<td>0.77</td>
<td>0.04 - 0.43</td>
<td>0.66 - 0.24</td>
<td>-</td>
<td>0.91 - 1.0</td>
<td>-</td>
<td>-</td>
<td>[50]</td>
</tr>
<tr>
<td>Lab. rat</td>
<td>adipose</td>
<td>1:1 heptakis (2,3-di-O-Me-6-O-t-hexyl)-β-CD OV 1701</td>
<td>-</td>
<td>- - -</td>
<td>- - -</td>
<td>0</td>
<td>0.95 - 1.0</td>
<td>-</td>
<td>-</td>
<td>P IV</td>
</tr>
<tr>
<td>Lab. rat</td>
<td>liver</td>
<td>1:4 heptakis (2,3-di-O-Me-6-O-t-hexyl-β-CD) SE52</td>
<td>0.77</td>
<td>0.04 - 0.43</td>
<td>0.66 - 0.24</td>
<td>-</td>
<td>0.91 - 1.0</td>
<td>-</td>
<td>-</td>
<td>[50]</td>
</tr>
<tr>
<td>Sprague</td>
<td>adipose</td>
<td>1:1 heptakis (2,3-di-O-Me-6-O-t-hexyl)-β-CD OV 1701</td>
<td>-</td>
<td>- - -</td>
<td>- - -</td>
<td>0</td>
<td>0.95 - 1.0</td>
<td>-</td>
<td>-</td>
<td>P IV</td>
</tr>
<tr>
<td>Dawley rat</td>
<td>liver</td>
<td>1:4 heptakis (2,3-di-O-Me-6-O-t-hexyl-β-CD) SE52</td>
<td>0.77</td>
<td>0.04 - 0.43</td>
<td>0.66 - 0.24</td>
<td>-</td>
<td>0.91 - 1.0</td>
<td>-</td>
<td>-</td>
<td>[50]</td>
</tr>
</tbody>
</table>

P II = Paper II et cetera. a) ERs are originally reported but calculated to EFs by EF=ER/(1+ER)
concentration followed by 4’-MeSO₂-CB174 and 4-MeSO₂-CB149. Low concentrations of MeSO₂-PCBs have also been detected in cod liver [128].

**Humans** MeSO₂-PCBs in human plasma, milk, adipose, liver and lung have recently been reviewed by Hofvander 2006 [112]. Three dominating congeners are reported, 4-MeSO₂-CB149, 4’-MeSO₂-CB101 and 4’-MeSO₂-CB87 as well as ΣMeSO₂-PCB. Plasma from mothers and their seven year old children from the Faroe Islands contained ΣMeSO₂-PCB (11 congeners) concentrations of approximately 70 ppb on lipid weight basis. This was more than ten times higher than other concentrations reported in human plasma and was suggested to be accumulated from the diet (birds and sea mammals) as an additional source to internal metabolism. Also, people living in an area contaminated by a previous PCB manufacturing plant in Slovakia showed levels of 4.2 ppb (lipid weight) ΣMeSO₂-PCB (3 congeners included) compared to 1.5 ppb in the control area [129]. As many as 51 individual MeSO₂-PCB congeners were detected in the Slovakian plasma and partly identified by their mass spectrum.

### 3.3.3 Chiral MeSO₂-PCBs in biota

The five chiral MeSO₂-PCB isomer pairs detected in biota, *meta* and *para* substituted MeSO₂-CB91, MeSO₂-CB95, MeSO₂-CB132, MeSO₂-CB149 and MeSO₂-CB174 are shown in Figure 3.4 (Papers II and III) [26,48,49,122]. These congeners have also been reported in human tissues [112,130,131]. In human liver, 5’-MeSO₂-CB132 constituted 76% of ΣMeSO₂-PCB [131]. Table 3.4 reviews the EFs and ERs calculated for seven atropisomeric MeSO₂-PCB congeners reported in wildlife, humans and laboratory rats. All EFs are based on \( A_1/(A_1+A_2) \) and ERs on \( A_1/A_2 \). Irrespective of the chiral column used, \( A_1 \) dominates for the *para*-substituted congeners and \( A_2 \) for the *meta*-substituted congeners. The ERs have been calculated to EFs as described in chapter 2.2.2 to facilitate comparisons. The absolute structures for the individual atropisomers of some MeSO₂-PCB congeners have been determined [127] and are shown in Table 3.4. The respective absolute configuration, \( R \) or \( S \), corresponds to the first or second eluting atropisomer determined on the column 1:1 heptakis (2,3-di-\( O\)-Me-6-\( O\)-tert-hexyl)-\( β\)-CD OV 1701, used in Papers II-IV.

### 3.3.4 Selective retention and some biological effects of MeSO₂-PCBs

Like the PCBs, the MeSO₂-PCBs are distributed to lipid rich tissues but also specific mechanisms for accumulation of MeSO₂-PCBs in the liver and lung have been observed (Papers III and IV, Table 3.3) [42]. The concentrations of MeSO₂-PCBs on a lipid weight basis are highest in liver followed by blubber.
and lung both in terrestrial and marine mammals (Table 3.3) [42]. In lung and uterus of mice and rats, MeSO₂-PCBs bind reversibly to an uteroglobin-like protein also called Clara-cell secretory protein (CCSP) and to uteroglobin (UG), leading to a selective accumulation in these tissues [51,54,55]. The hormone progesterone is the only endogenous ligand for these proteins known so far. The importance of CCSP in MeSO₂-PCB binding was shown in mice lacking the CCSP gene which could not accumulate MeSO₂-PCBs in lung and kidney[55]. CCSP- and UG-like proteins have also been found in two species of seals [125]. The slower decrease of MeSO₂-PCBs in rat lung compared to adipose tissue was suggested to be due to CCSP binding [50].

The reason for the strong retention in liver is unknown but binding of MeSO₂-PCB to a fatty acid binding protein (FABP) has been shown in rat intestinal mucosa and liver [52,53]. This protein is thought to be involved in absorption, intracellular transport and metabolism of free fatty acids. In general, para-
substituted congeners dominate in lung and meta-substituted in liver which can be seen both in laboratory studies, wildlife and humans (Papers III IV) [42,51,116,131,132].

The toxicological consequences of MeSO$_2$-PCB binding to CCSP and UG is not known but could possibly lead to reproduction failure caused by the higher binding affinity to UG than progesterone which may effect the protection of an embryo as discussed by Troisi et al. (2001) [125]. Reproductive effects have been seen in minks dosed with MeSO$_2$-PCBs with increased litter size and decreased kit survival as a consequence [133]. MeSO$_2$-PCBs have also been shown to inhibit the glucocorticoid synthesis which affects for example the anti-inflammatory functions in the body [134]. The biological activity of MeSO$_2$-PCBs has been shown to be dependent of the substitution pattern and position of the MeSO$_2$-group regarding the potential to induce drug metabolising enzymes [135-137].

3.4 3-MeSO$_2$-DDE

1,1,1-Trichloro-2,2-bis(4-chlorophenyl) ethane (p,p'-DDT) has been and is used in tropical/sub-tropical regions even though it has been banned in most countries. p,p'-DDT is transformed to 1,1-dichloro-2,2-bis(4-chlorophenyl) ethene (p,p'-DDE) and 1,1-dichloro-2,2-bis(4-chlorophenyl) ethane (p,p'-DDD) in the environment. p,p'-DDE is known as a major contaminant in a large variety of species including humans [16,18,47,130,138] and is metabolised to 3-MeSO$_2$-DDE most probably in the same way as the MeSO$_2$-PCBs. 3-MeSO$_2$-DDE was detected in 1976 together with MeSO$_2$-PCBs in seal blubber and was the first methylsulfonyl metabolite to be structurally identified [43,139]. The concentrations of 3-MeSO$_2$-DDE in different species from wildlife published from the year 2000 and onwards are reviewed in Table 3.3. In wildlife, 3-MeSO$_2$-DDE does not occur as the major methylsulfonyl metabolite as is the case in humans [112]. 3-MeSO$_2$-DDE is present at the highest concentrations in liver tissue but the ratio 3-MeSO$_2$-DDE/p,p'-DDE is about 1/10$^\text{th}$ in all tissues compared to $\Sigma$MeSO$_2$-PCB/ΣPCB. Isomers of 3-MeSO$_2$-DDE, designated 2-MeSO$_2$-4,4'-DDE and MeSO$_2$-2,4'-DDE, have also been detected in beluga whale adipose tissue [114]. The most well known toxic effect of 3-MeSO$_2$-DDE is the cause of cell necrosis in the adrenal cortex [140].

3.5 TCPMe

Tris(4-chlorophenyl) methane (TCPMe) and the isomers 2,2',4'''-TCPMe and 2,4',4'''-TCPMe are formed as by-products in the synthesis of technical p,p'-DDT [141]. However, it is only 4,4',4'''-TCPMe that is found in
environmental biological samples. It was suggested that this was due to the higher resistance to metabolism of 4-chlorophenyl rings compared to the 2-chlorophenyl rings [141]. TCPMe was first detected in 1992 in peregrine falcon eggs from California, U.S. (2000 ng/g fat.) [142] and in ringed seal (*Phoca hispida*) from the Baltic Sea [143]. TCPMe has also been detected in human milk, adipose tissue, fish and sediments at levels in the low ppb range [144-146].

Tris(4-chlorophenyl) methanol (TCPM-OH) has been suggested to be a hydroxylation product of TCPMe, as a result of metabolic action either by microbial or direct chemical transformation in the environment [142]. However, TCPM-OH also has an industrial origin and is used in small amounts for the manufacture of e.g. agrochemicals and drugs [142]. TCPM-OH was detected already in 1989 in harbour seals [147] but was later found in samples collected in 1952 [142]. Far more work has been done on TCPM-OH than on TCPMe. Both compounds are reviewed by de Boer (2000) [144].
4 Selection of samples

The Swedish Monitoring program is today a part of the environmental goal “A non-toxic environment”, mentioned in chapter 1. The program was initiated to monitor mercury, \( p,p'\)-DDT and PCB contamination in marine and fresh waters with no known local discharges [148]. Specimen from terrestrial, fresh and marine waters have are collected and stored in the Environmental Specimen Bank at the Museum of Natural History in Stockholm. From the end of the 1960s, samples have been collected annually for long-term trend studies [149].

The choice of species as well as tissue has to be considered depending on the environmental pollutants studied and the aim of the study. Age, sex, size, relative lipid content and season for collection are examples of parameters that can influence the results [149,150] and must be considered to minimize sampled related variation. Species at different positions in food webs have various abilities to metabolise POPs and depending on the substance of interest, metabolite and/or parent compound, trophic level is also an important factor to take into consideration. Species at higher trophic levels are generally exposed to higher contaminant loads through biomagnification and through a longer life span, compared to species at lower levels. Analysis of higher trophic animals is a choice for the detection of “new” persistent POPs. In contrast, metabolism may lead to disappearance of contaminants in wildlife at higher trophic levels making analysis of lower organisms more valuable. Negative effects of POPs are often not shown until they reach top predators, e.g. the impairment of reproduction caused by \( p,p'\)-DDE and PCBs in white-tailed sea eagles [12], and in seals [13,14] and otters [15], respectively.

The analytical matrix is also important to consider. Muscle tissue is commonly used, and especially for fish since it is an important food for humans and the major feed for fish consuming animals. For birds, egg is a commonly used matrix to indicate the embryo exposure as well as the maternal female bird exposure. Levels of POPs have shown to correlate with the content of triglycerides in the tissues [151]. POPs are assumed to be evenly distributed in tissues with high triglyceride content such as blubber. Still, differences in contaminant levels are dependent on which part of the blubber that has been sampled [111]. The ability of a substance to retain in the body is also dependent on the matrix, e.g. OH-PCBs are retained in blood due to their protein binding properties [152,153] and only present at low levels in e.g. lipid rich tissues as blubber and adipose.
4.1 Species

To reach the aims of Papers I-III, the Swedish Environmental Specimen Bank was utilized and in Paper IV, laboratory animals were used.

**Fish** The species selected for analysis, as described in Paper I, were selected on basis of previous experiences concerning concentrations of PCB, \( p,p' \)-DDT and related compounds. The fish were collected in early autumn according to a scheduled plan within the Swedish Environmental Monitoring Programme. The fat content is high and the variation in contaminant concentrations is less when compared to the spring [150]. To describe spatial, trophic and temporal differences of BCPS, four species of fish were used, perch (\emph{Perca fluviatalis}), salmon (\emph{Salmo salar}), arctic char (\emph{Salvelinus alpinus}) and herring (\emph{Clupea harengus}). Perch is a lean fish with a stationary behaviour that makes it a good indicator of the local contamination situation. The arctic char is sampled in fresh water, Lake Vättern, and in a remote lake in the north of Sweden where no local industries or farms are located and there are no human settlements in the drainage area of the lake [7]. The Baltic herring is an important food source for both grey seal [154] and guillemot [155] which is used for comparisons of trophic levels. Herring younger than four years were selected for analysis since they have less variation in contaminant levels than older herring [150]. Young herring are also bound to their region even if they are not stationary [148].

**Seal** The Baltic grey seal (\emph{Halichoerus grypus}) are heavily contaminated with organohalogen substances due to their fish diet and through their long life span. The grey seals are distributed mainly along the central and north of the Swedish east coast. Grey seal blubber was used in Paper I to study the biomagnifying properties of BCPS in this species. In Paper III, liver, lung and blubber samples from ten grey seals were analysed regarding chiral MeSO\(_2\)-PCBs and the tissue distribution of sulfone containing compounds. The seals used in Papers I and III were all accidentally drowned in fishing nets and their health status was examined before being stored in the Specimen bank.

**Bird** The guillemots (\emph{Uria aagle}) used in Papers I and II are from the island Stora Karlsö west of Gotland. They are stationary in the Baltic and spend the winter in ice-free areas of the southern Baltic proper and return to the island in the beginning of the year. Besides herring, the guillemot also feeds on sprat (\emph{Sprattus sprattus}) [155], a clupeid with similar \( p,p' \)-DDT and PCB concentrations as herring [156]. The guillemots nest in colonies which make the population very homogeneous at the nestling location and reduce the variation in contaminant levels [148]. They breed in early May and normally one egg is laid. The collection of an egg does not affect the reproduction since a lost egg can be replaced about 15 days later [157]. Replacement eggs have
shown significantly higher contaminant levels than first laid eggs but of lower egg weight, shell weight and shell thickness [158]. In Paper II, replacement eggs were used and in Paper I muscle tissues were used.

**Laboratory animals** In order to study the enantioselective metabolism of chiral PCBs in Paper IV, a complete metabolic system was needed which only could be achieved through the use of laboratory animals. Male Wistar rats were chosen since they are commonly used in studies of PCB metabolism and toxicity[137,159,160].

### 4.2 Number of samples

In Paper I pooled samples were used due to the aim of the study – to survey the BCPS situation in the Swedish environment. The concentrations of POPs in a pooled sample will represent a mean value from several individuals and may be more representative of the specimen if many individuals are included compared to analysis of a few individuals. The draw back of using pooled samples is the risk of losing information due to the fact that biological parameters may influence the concentrations [149,150]. If pooled samples are used, then several pools are needed to estimate the variation. Also, to reduce the analytical variance it is advantageous to use individual samples. Contaminant concentrations are often skewed to the right and a pooled sample represents an arithmetic mean value while geometric means or median are used to reduce the influence of extreme values [149,161].

The fish species and the guillemot muscle in Paper I consisted of pooled samples and for these species the individual variation is small compared to the seals which were analysed both individually and as a pool. When concentrations are under the limit of detection, pooling of samples is necessary, which was done in order to study the chiral composition of the atropisomeric MeSO$_2$-PCBs in Paper II.

### 4.3 Time trend studies

Long time trend studies are necessary in evaluation of the development of pollutants in the environment. They make a base for the need of actions from the society or if actions that already have been taken are adequate. Guillemot eggs have been used for time trend studies for several compounds such as PCBs, DDTs, HCH isomers, PCDDs/PCDFs (dioxins) [7,8,158,162], PFOS, perfluorooctanoic acid (PFOA) [5], HBCDD, polybrominated diphenyl ethers (PBDEs) [6] and also for heavy metals [8]. To this list adds BCPS, MeSO$_2$-PCBs, 3-MeSO$_2$-DDE, *trans*-nonachlor and TCPMe (Paper II). To determine the occurrence of a time trend, the time period as well as the frequency of the sampling years are important as discussed elsewhere [161]. For time periods
of 10 years, the statistical power increases the more frequent the sampling occurs. In Paper II, five eggs were sampled every fifth year during a time period of 30 years.
5 Analytical methodology

The main purpose of this chapter is to present the analytical methods used in Papers I-IV, from extraction to instrumental analysis. A general scheme for the different analytical steps is shown in Figure 5.1. For more details, please see the experimental section in each of the Papers I-IV. The compounds analysed in this thesis are all associated with lipids in the various tissues and matrixes.

5.1 Extraction

The extraction method used in Papers I-III is referred to as the original Jensen method [156,163] where the tissues first are homogenised and dehydrated by using acetone/hexane (5:2). The lipids are then extracted with a mixture of hexane and diethyl ether and washed with an acidic water solution. In Papers I-III, the diethyl ether was replaced by methyl-tert-butyl ether. This method was accepted as a standard method by the Swedish Museum of Natural History in 1969 when the Environmental Monitoring Programme started [164]. The original Jensen method was developed to replace the chloroform used in the extraction method developed by Bligh and Dyer [165] with a non-halogenated solvent.

Triglycerides and phospholipids are the two major groups of lipids found in organisms but their relative proportions vary between species and tissue [166]. In lean fish such as pike and cod, the phospholipids constitute a relatively high proportion of the fat content, compared to fat fish such as herring and salmon which is dominated by triglyceride lipids. Recently it was shown that the original Jensen method did not extract the lipids of fish with a fat content <1% as efficiently as the Bligh and Dyer method and accordingly Jensen and co-workers modified the method [164]. In the modified Jensen method the acetone/hexane is replaced with isopropanol/diethyl ether which gave recoveries of the fat comparable with the Bligh and Dyer method [164]. The modified Jensen method was applied in Paper IV for extraction of all tissues and also for faeces from experimental rats.

In previous studies, Soxhlet extraction is the most frequently applied technique for extraction of environmental contaminants and their metabolites in faeces [167,168] but also for different tissues [123,128]. Grinding a tissue with sodium sulfate for dehydration and subsequent extraction with hexane/dichloromethane is another method that has been applied for analysis of MeSO₂-PCB [91,118,123].
5.2 Lipid removal

It is important to remove all the extracted lipids to achieve samples clean enough for instrumental analyses. An effective way to destruct lipids is by sulfuric acid but it is also destructive for substances like dieldrin and heptachloroepoxide. All compounds analysed in this thesis are resistant to strong acids and bases. However, sulfone containing substances such as BCPS, MeSO_2-PCBs and 3-MeSO_2-DDE are Lewis bases and form charged complexes with the acid. By diluting the acid with water, these compounds can be re-extracted to an organic solvent phase, usually hexane [169]. Unfortunately, some of the destructed lipid residues are difficult to remove. Two lipid removal steps have therefore been added for removal of the major part of the co-extracted lipids prior to further clean up.

**Acetonitrile partitioning** In Paper I, a time efficient method was applied to allow analysis of lipid amounts of 0.5 g. The extraction efficiency of BCPS with acetonitrile was controlled by the use of ¹⁴C-labelled BCPS. A first lipid reduction step was performed by partitioning the lipids with 5 ml acetonitrile, i.e. an approximate relationship of 1:9 (lipids:acetonitrile). The sulfone containing compounds do partition to a higher degree to acetonitrile compared to compounds such as PCB. The extracted lipids are therefore treated three times with acetonitrile to promote the partitioning of the sulfone containing compounds to acetonitrile [170]. Under these conditions about 10% of the lipids are still co-extracted and present in the acetonitrile phase. This method was also applied in Papers II and IV.

**Gel permeation chromatography (GPC)** In Paper III, GPC was used to reduce the major portion of the lipids. In this case a cross-linked polystyrene gel (BioBeads S-X3) was used. The size of the molecules of lipids and analyte is the basic separation mechanism but other factors that also effect the separation are choice of mobile phase, planarity and polarity of the compounds [171]. Therefore, separation between the compounds and the lipids are more or less efficient. In Paper III, hexane/dichloromethane was used as a mobile phase that gives a separation mainly based on the molecular volume even though the polarities of the compounds also have an effect.

In MeSO_2-PCB analyses, GPC is commonly used for removing the bulk of lipids [91,118] but also dialysis with semi-permeable membranes [111] and Lipidex 5000 [131] have been used. These methods and acetonitrile partitioning are all non-destructive for both lipids and analytes. Saponification with sodium hydroxide dissolved in alcohol is a destructive method for removal of lipids performed by e.g. Haraguchi et al. (1984) [169]. A drawback is that this method is also destructive for substances such as p,p'-
DDT, HCHs and other compounds easily eliminating hydrogen chloride or bromide.

**Figure 5.1.** General scheme for the analytical methods used in Papers I-IV.

### 5.3 Separation of substance classes and clean up

The chemical properties of sulfone containing compounds are utilized to separate them from other substances. Sulfuric acid is used for this purpose and the samples are simultaneously cleaned up from remaining lipid residues.
In Papers I and III, after the first lipid removal step, acetonitrile partitioning or GPC, the sample is transferred to a silica gel column which is impregnated with diluted sulfuric acid (90%). Compounds such as PCB and $p,p'$-DDE are first eluted by hexane. Sulfone containing compounds are subsequently eluted by increasing the polarity of the organic solvent, in this case dichloromethane was used. In Papers II and IV, the two substance classes are instead separated by hexane/conc. sulfuric acid partitioning. The hexane is removed and the acid phase is diluted with water (1:2) and sulfone containing compounds can be partitioned to an organic phase. After this separation, both classes of compounds are finally purified by a column packed with sulfuric acid (diluted for sulfone compounds) impregnated silica gel before analysis. Both ways of separating the substance classes give a complete separation of the sulfone containing substances from PCBs and $p,p'$-DDE. The compound TCPMe has in hexane/conc. sulfuric acid partitioning shown to be partly (25 %) partitioned to the acidic phase even though it is not a Lewis base (Paper II) [172].

MeSO$_2$-PCBs have also been separated from other compounds (PCBs etc.) by liquid liquid partitioning with dimethyl sulfoxide (DMSO) [111,173,174] and acetonitrile [169], or by adsorption chromatography using columns with pure silica gel [169], Florisil or basic alumina columns deactivated with water [91,124]. Effective multilayer columns with base- and acid-impregnated silica gel with a third layer of pure silica [112] or sodium sulfate [118] has been used for clean up and substance class separation.

5.4 Instrumental analysis

Gas chromatography (GC) applying a set of different analytical columns with varying properties has been used for analyte separations presented in this thesis. All compounds have first been analysed on a DB5 or CP-Sil 8CB (both with 5% phenyl polysiloxane) capillary columns for identification and quantification. Enantioselective analysis for the atropisomeric PCBs and MeSO$_2$-PCBs have then been performed using different chiral columns (Papers II-IV). The detection has either been performed with electron capture detector (ECD) or by mass spectrometry (MS) operated in electron capture negative ionisation (ECNI) - or electron ionisation (EI) mode.

5.4.1 Detection

**GC-ECD** The ECD is highly sensitive for electrophilic substances such as halogenated and oxygen-containing compounds and it has been widely used for analyses of PCBs and chlorinated pesticides. The response of the ECD generally increases with the number of chlorines in the analyte but the
substitution pattern or molecule structure also influences the response. The
detection and identification by GC-ECD relies on comparison of the retention
times in the sample relative to retention times of authentic reference standards
and is therefore dependent of no co-eluting interferences.

**GC-MS** This detection technique gives additional information of compound
characteristics such as molecular weight and fragmentation pattern. Chlorine
containing compounds give rise to typical isotope clusters by the two isotopes
$^{35}\text{Cl}$ and $^{37}\text{Cl}$, depending on the number of chlorines. The selectivity and
sensitivity can be enhanced by using the selected ion monitoring (SIM)
technique. Interferences from co-eluting compounds with different m/z are
minimized. For electronegative compounds, ECNI is a selective and sensitive
soft ionisation method, giving little fragmentation of the analyte. Also in this
technique, the response is increased by increasing the number of chlorines of
the analyte. EI is less sensitive due to the extensive fragmentation of the
analyte compared to ECNI. On the other hand, EI is more informative
regarding the structure of the compound analysed.

ECNI with SIM was used for analysis of BCPS and the chiral MeSO$_2$-PCBs
by utilizing [M]$^-$ and [M+2]$^-$ ions. EI was utilized both to verify the identity of
BCPS in Paper I and to identify TCPMe in Paper II.

GC-MS is by far the most applied technique for the analysis of MeSO$_2$-PCBs
in different species and tissues [49,89,111,114,117,118,123,125,126].

### 5.5 Enantiomer specific analysis using cyclodextrin columns

Cyclodextrins (CD) are cyclic glucose oligomers with 6, 7 or 8 D-glucose
units corresponding to $\alpha$-, $\beta$-, and $\gamma$-CD, respectively, as shown in Figure 4.2.
CD has a shape of a hollow cone where the inner part of the cavity is
hydrophobic and the outer part is hydrophilic. Hydroxyl groups are attached
to carbons at position 2 and 3 at the wide end and at position 6 at the narrow
end of each glucose unit (Figure 5.2). These OH-groups can be derivatized to
form a great number of modified CD with different selectivity and an
enhanced thermal stability. The thermal stability is also increased by
dissolving the CD in a conventional achiral stationary phase, e.g. polysiloxan
(e.g. OV-1710) [175]. If the CD is chemically bound to the polysiloxane
backbone, the thermal stability is further improved [176]. The enantiospecific
retention occurs by the formation of a host-guest complex where one
enantiomer interacts more strongly with the functional groups at the edges of
the CD-molecule. Most enantiomeric separations are governed by entalphy
control, the separation increase with decreasing temperatures [176]. This is
reflected in the long retention times often seen in analyses of chiral
compounds, which is due to temperature programmes involving slow
temperature increases together with long isothermal temperatures at low temperatures.

In 1983, the first successful separation of enantiomers using CD was demonstrated [177] and in 1993, the first separation of PCB atropisomers was reported [178]. Several different β- and γ-cyclodextrin columns have since then been used as chiral selective phases for the separation of atropisomeric PCBs as reviewed by Harju (2003) [100]. In Paper IV, a Chirasil-Dex column (β-cyclodextrin) is used for the atropisomeric separation of CB-132. The chiral stationary phase Chirasil-Dex is chemically bound to the polysiloxane backbone which increases its usefulness in GC-ECD analysis due to lower bleeding rate at high temperatures [179]. Chirasil-Dex is frequently used for atropisomeric PCB analysis [62,70,73,103]. The great number of PCB congeners in biological samples is leading to co-elution problems in gas chromatography in general, and also in chiral column assisted gas chromatography. These problems may be overcome by using multidimensional gas chromatography (MDGC) and comprehensive two-dimensional gas chromatography (GC×GC) [100].

![Chemical structure of cyclodextrin. Derivatization of the hydroxyl groups can be performed in the 2-, 3- and 6-postions.](image)

**Figure 5.2.** Chemical structure of cyclodextrin. Derivatization of the hydroxyl groups can be performed in the 2-, 3- and 6-postions.

The enantiomer specific analysis of the chiral MeSO₂-PCBs in Papers II-IV was performed with a GC column coated with a mixture of heptakis (2,3-di-O-methyl-6-O-t-hexyl)-β-cyclodextrin and OV-1701 (1:1), prepared by König and co-workers [180]. Wiberg et al. (1998) has evaluated four different 30 m long commercial CD columns with different cavity diameters (β- or γ-CD)
differently derivatized [48]. Neither of them were as effective in resolving the same number of congeners as the shorter (10 m) non-commercial columns made available by König (Paper III) [26]. The different chiral columns used for atropisomeric separation of MeSO₂-PCBs detected in biota are shown in Table 3.4. Atropisomeric separation of MeSO₂-PCBs is often performed with modified non-bonded cyclodextrin mixtures but also the Chirasil-Dex column has been applied for these compounds. In the latter case extremely long temperature programs had to be used for the separation of the MeSO₂-PCB atropisomers [73].

5.6 Identification and quantification
Identification of PCBs, MeSO₂-PCBs and 3-MeSO₂-DDE were made by comparisons of GC-ECD retention times between the analyte and an authentic reference substance. PCBs and \( p,p' \)-DDE are well documented and known compounds regarding their elution order on a DB-5 column and also their relative abundance in different species are continuously documented. For BCPS, analysis by GC-MS was necessary for identity confirmation. In Paper I, the chlorine isotope ratio of BCPS were calculated in the samples and compared with a reference standard. Samples with the highest BCPS concentrations were also run in full scan mode (EI) for determination of the fragmentation pattern. GC-MS (EI) was also used in Paper III when suspiciously high concentrations of 4-MeSO₂-CB52 were detected in the guillemot samples by GC-ECD. The accurate mass and the identification were done by high resolution mass spectrometry (HRMS) and by comparisons with previously published chromatograms [142]. It was shown that TCPMe was the compound co-eluting with 4-MeSO₂-CB52. The chiral composition of the atropisomeric MeSO₂-PCBs in Papers II and III were calculated from GC-MS (ECNI) data to get correct enantiomeric fractions without interferences.

The quantification of the reported compounds was performed by the use of surrogate standards and reference standards with single point quantification. When using single point quantification it is important to work within the linear range of the detector, which was controlled by the establishment of calibration curves made from reference substance in a dilution series.

5.6.1 Limits of detection and quantification
When measuring low amounts of environmental pollutants in biological samples it is necessary to define the presence of a compound by defining the limit of detection (LOD) and limit of quantification (LOQ). They are dependent on the concentrations in blank solvent samples but also from the noise of the matrix and the instruments. LOD and LOQ have to be defined
each time an analysis is performed. If an analyte is found in the blank solvent samples, the LOD is based on this level and the LOQ is set from this. In Paper I, LOQ for BCPS was set at three times the LOD which was based on the mean background amount in the blank solvent samples. If the blank solvent samples do not contain any background amounts, the background from the matrix and from instrumental noise will determine the LOD and LOQ which are set from a signal to noise (S/N) ratio. In Papers II, III and IV, the LOQ was based on the lowest concentration quantified with a S/N ratio of five. The chiral \( \text{MeSO}_2 \)-PCBs could be quantified with a LOQ greater than three.

### 5.6.2 Data presentation

The concentrations of the POPs presented in the thesis are all lipid normalised. The advantages with expressing the concentrations on a lipid weight basis are that tissues and species with different lipid amount can be compared. The variance also becomes less compared to fresh weight basis. In order to get an appropriate lipid normalisation concentration of lipophilic contaminants in biological tissue an accurate lipid determination is important. However, comparing lipid normalised data from studies where different extraction methods with various extraction efficiency has been used may be a complicated factor in assessment of data on a fat weight basis [164,181].

In Papers I-III, \( 2,2',4,4',5,5' \)-hexaCB (CB-153) was used for comparisons to other substances that were analysed but also for comparison to other previously presented studies. CB-153 is one of the most abundant congener (by weight percent) in technical PCB mixtures [98] and it is also a congener with a long biological half-life [42]. Consequently it is often found in highest concentrations in biota.

The results in Papers II-IV, based on individual data, have been presented using different types of mean values. In Paper II, geometrical mean values are used since the individual variation is small and to reduce the influence of potential outliers. Median values together with ranges were considered to best describe the results in Paper III when the ranges in concentrations between the seals were wide. In Paper IV, only four individuals were included and therefore arithmetic means were used.

### 5.7 Quality of the analytical procedure

**Relevant surrogate standards** BCPS has in previous studies been quantified with \( 2,2\)-bis(4-flourophenyl)-1,1,1-trichloroethane as a surrogate standard [25,27,28]. A surrogate standard should be structurally similar to the compound of interest. Therefore, 2,4,4’-tri-chlorodiphenyl sulfone (Trifon) was synthesised as described in Paper I and this compound was used as a
A quantitative surrogate standard for the BCPS analyses performed in Papers I-III.

**Recovery** In every study, the recovery of each surrogate standard after the entire clean up is calculated. In Paper II, prior to the analysis of guillemot eggs, the method was evaluated by a recovery study using hens’ egg. Known amounts of several compounds of interest were added to this matrix which was similar to guillemot eggs and with no detectable background levels.

**Double samples** The analytical variance of the clean up and analysis was controlled by analysing double samples of the seal tissues in Papers I and III.

**Blank solvent samples** Systematic contamination from the solvents used throughout the analytical methods, instrumentation, laboratory equipment etc is controlled by running blank solvent samples in parallel with the biological samples.

**Laboratory reference material (LRM)** Muscle from a Baltic Sea salmon was minced and mixed thoroughly to become as homogenous as possible. It was then divided in portions of 10 g and kept at -80 °C. Five of these samples were initially analysed with the actual method to be used in Paper I. Portions of the LRM have then been analysed in parallel with the samples in Papers I, III and IV for comparison and quality control of the different methods used and is shown in Figure 5.3.

![Figure 5.3](attachment:image.png)

**Figure 5.3.** BCPS analyses of the laboratory reference material (LRM) at four different occasions. The line show the mean value (37 ng/g fat, n=17) ± one standard deviation (dashed line).

**Analyses with pure enantiomers** The elution order of enantiomers was determined on the two chiral columns using enantio-pure reference standards. EFs for both racemic and enantio-pure standards were calculated to ensure
that no racemisation or deracemisation processes occurred during the GC analysis, when the analytes were subjected to elevated temperatures.

**Control of the GC instrument** The linearity of the detector was controlled by running reference standards as dilution series. The stability of the detector over time was checked by running the same LRM samples at different occasions.
6 Results and discussion

Three main topics have been dealt with in the Papers I-IV, i.e. BCPS, PCB and DDE methyl sulfone metabolites, and chirality. The results from these studies are discussed below. For BCPS the geographical distribution, temporal trend and biomagnifying potential are highlighted and discussed in relation to PCBs and \( p,p' \)-DDE. The issues of MeSO\(_2\)-PCBs and 3-MeSO\(_2\)-DDE, i.e. retention, chirality in PCB metabolism and PCB methyl sulfone formation are discussed in different perspectives than previously in Papers II-IV.

6.1 BCPS in the environment

6.1.1 Geographical distribution

Today BCPS is mainly applied in the plastic industry but has a history as a pesticide [82]. Still, the introduction and distribution of BCPS to the environment is unknown. Possible leakages to the environment could have an origin from production and handling of BCPS as a monomer in the industry. There is no production of BCPS in Sweden but polymers produced from BCPS are imported. BCPS is detected in fish, seals and birds from the Swedish environment (Paper I) [16,25]. BCPS is a significant contaminant also in the Latvian environment where it has been studied extensively [25,27,28], while its distribution elsewhere is poorly known.

When the BCPS environmental distribution pattern is compared to that of CB-153 and \( p,p' \)-DDE it appears that BCPS has a different route to the environment than the traditional POPs (Paper I). This is shown in Figure 6.1 where the relative concentrations of BCPS, CB-153 and \( p,p' \)-DDE are compared in fish from Lake Vättern and the Baltic Sea, both sampling sites located at the same latitude. The two species of salmonids, arctic char and salmon representing fresh and marine species, respectively, were selected due to previous knowledge of PCB and \( p,p' \)-DDE in these two fatty fish species. BCPS was detected in low concentrations in arctic char from Lake Vättern (1.8 ng/g fat) compared to salmon from the Baltic Sea (32 ng/g fat). In contrast, CB-153 and \( p,p' \)-DDE were detected in higher concentrations in the Lake Vättern compared to the Baltic Sea fish. PCBs and \( p,p' \)-DDE are mainly considered as airborne pollutants [7] and Lake Vättern is an oligotrophic lake which is reflected in the high concentrations of PCBs and \( p,p' \)-DDE [182].

Indication of the discharges of BCPS to the Baltic Sea being waterborne can be gathered from the literature [27,28]. Concentrations of BCPS in perch from
the Gulf of Riga is about three times higher than in perch from the Swedish east coast (Table 3.1) [27]. The concentration of CB-153 is similar in Latvian coastal areas and similar to or even lower than in Sweden [27]. Valters and co-workers found increasing downstream levels of BCPS in perch from a Latvian river (53-160 ng/g fat) which confirms discharges to water [28]. A different pathway of BCPS to the environment compared to PCBs and \( p,p'-\text{DDE} \) is strengthened by the very low or non-detectable levels of BCPS in guillemots from the remote areas Iceland and the Faroe Islands (Jörundsdottir. pers. commun.). In the Baltic Sea, guillemots have levels of up to 1900 ng/g fat in muscle and egg (Papers I and II). PCBs are detected in 10 times lower concentrations in the remote areas compared to the Baltic Sea (Jörundsdottir. pers. commun.). However, BCPS have been reported in plasma of glaucous gulls from the Norwegian arctic at levels of 5-140 ng/g fat [89]. Furthermore, bream sampled at four different locations in two German rivers showed BCPS concentrations with up to ten times the difference between the sampling sites (3.4-34 ng/g fat) (Paper I). On the other hand, the PCB concentrations were similar at all sampling sites. Hence, the so far complex results indicate that BCPS has a more local distribution than the more well-known long range distributed \( p,p'-\text{DDT} \) and PCB.

![BCPS, CB-153, \( p,p'-\text{DDE} \) in Lake Vättern and Baltic Sea](image)

**Figure 6.1.** Relative concentrations of BCPS, CB-153 and \( p,p'-\text{DDE} \) in Lake Vättern arctic char and Baltic Sea salmon (Paper I).

### 6.1.2 Temporal trend

The temporal trend of BCPS in guillemot eggs sampled between 1971 and 2001 shows an annual decrease of 1.6% (Paper II). Even if this change is significant, it is remarkably small compared to the trends of contaminants such as DDTs and PCB in the Baltic during the same time period. The
negative temporal trend of CB-153 in Paper II was in good agreement of previous studies [7] which strengthens the results of BCPS in Paper II.

The concentrations of BCPS are quite similar throughout the time period (1000-1500 ng/g fat) (Paper II). Figure 6.2 illustrates the trend of BCPS in relation to temporal trends of other chemicals for which guillemot eggs have been used as a monitoring matrix. Both the flame retardant HBCDD and the surfactant PFOS are used today and show increasing trends [5,6]. The concentrations of HBCDD are presently twice the concentration in the 1970s [6]. The concentration of PFOS was close to zero in the beginning of the 1970s and reached levels of 1 ppm (fresh weight basis) in the late 1990s [5].

It was indicated that the levels of BCPS in the environment was increasing in white tailed sea eagle eggs sampled between 1971-1991, [16]. This was thought to be a result of an increase in the production of thermostable polymers. Accordingly, an increase was also expected to be seen in guillemot eggs. High temperature polymers have been mass-produced since 1965 [183] and if an increase in the plastic production occurs but in parallel with a better care of chemicals by the industry, this could lead to only minor changes over time. This would then explain both the small decline of BCPS and also the even levels of BCPS in the guillemot eggs. This has to be taken as a speculation since the origin of BCPS in the environment is unknown.

![Figure 6.2. Temporal trend of BCPS in relation to some other chemicals for which guillemot eggs have been used for monitoring (Paper II) [5-7]. Each of the pollutants has its own scale.](image)

### 6.1.3 Biomagnifying potential

The biomagnifying potential of BCPS was shown by comparisons of the concentrations between the Baltic guillemot and grey seal, both feeding mostly on herring (Papers I-III). It was shown that the biomagnifying
potential of BCPS is species dependent. Baltic seals have in general BCPS blubber concentrations only slightly higher (41-98 ng/g fat) or similar to that of herring (30 ng/g fat) (Papers I and II). However, individual seal data in both Papers I and II showed concentrations of up to 470 and 240 ng/g fat, respectively. In the seal with the highest concentration, the lipid content in the blubber was only 32%, indicating poor health. Also the concentrations of CB-153 and \( p,p' \)-DDE in these individuals were very different from other seal individuals. Besides, the individual variation in BCPS levels was small. When BCPS concentrations where compared in different seal tissues, it was shown that BCPS had highest retention to the liver with concentrations in the range of 55-700 ng/g fat (Paper III). Based on the data in this thesis it seems reasonable to conclude that BCPS is poorly biomagnified in grey seal.

The BCPS concentration in the guillemot muscle was 50 times higher compared to the herring (Paper I). The guillemot seems in general to accumulate BCPS to a higher degree than the seals. Rather high concentrations of BCPS have also been detected in white tailed sea eagles (610 ng/g fat) [16]. It is yet too early to tell if birds in general do bioaccumulate BCPS to a higher degree than fish and mammals.

BCPS has previously been detected in human livers (15 ng/g fat) [26]. MeSO\(_2\)-PCBs were recently studied in human plasma from men living in a PCB contaminated area in Slovakia [129]. BCPS was detected as a significant contaminant in their blood (Hovander, pers. commun.). This is an intriguing result that requires further studies.

### 6.2 MeSO\(_2\)-PCBs and 3-MeSO\(_2\)-DDE

#### 6.2.1 Unknown MeSO\(_2\)-PCB congeners in Baltic Sea grey seals

The MeSO\(_2\)-PCBs analysed in grey seal liver, lung and blubber (Paper III) were identified and quantified applying GC-ECD equipped with a DB-5 column. The identification was made by comparisons of the retention times in the samples with reference standards containing a total of 24 MeSO\(_2\)-PCB congeners and also 3-MeSO\(_2\)-DDE. Recently, GC-MS analyses were performed on these seal samples and it was found that the peak reported as 3-MeSO\(_2\)-DDE co-eluted with a hexachlorinated (Cl\(_6\)) MeSO\(_2\)-PCB congener as shown in Figure 6.3. This unknown Cl\(_6\) congener corresponds with the retention time and MS fragmentation pattern to the authentic reference compound, 5’-MeSO\(_2\)-CB135 (Figure 6.3). Due to the lack of other reference standards it can of course not be excluded that this identification is incorrect. However, the precursor to 5’-MeSO\(_2\)-CB135 is CB-135 which is present in several technical PCB mixtures at levels of approximately 1% [98]. CB-135
Figure 6.3. GC-MS (ECNI) chromatogram of MeSO₂-PCBs and 3-MeSO₂-DDE in grey seal blubber. Fragmentation of the co-eluting hexachlorinated MeSO₂-PCB congener and 3-MeSO₂-DDE is shown. Marked peaks are 1) 3'-MeSO₂-CB101, 2) 4'-MeSO₂-CB101, 3) 3'-MeSO₂-CB87, 4) 5-MeSO₂-CB149, 5) 4-MeSO₂-CB110, 6) 4'-MeSO₂-CB87 and 5'-MeSO₂-CB132.

has vicinal hydrogens in the meta-para position (Table 3.2) and can undergo metabolism to form methylsulfonyl metabolites. From this, it is likely that this metabolite is 5'-MeSO₂-CB-135. Furthermore, CB-135 and its metabolites are chiral with stable atropisomers [97].

The GC-MS analysis was performed using a DB-5 MS column which separated the peak identified as 4-MeSO₂-CB149 into two peaks in the seal samples (Figure 6.3). This was not shown in the GC-ECD analysis. This co-eluting peak was also a hexachlorinated MeSO₂-PCB congener but with a different fragmentation pattern than 4-MeSO₂-CB149. This unknown
congener was present in blubber, liver and lung tissues. In blubber 4-MeSO$_2$-CB149 was reported as the congener detected in second highest concentration. This unknown congener has also been detected in human plasma [112] and ringed seal blubber [47].

It is likely that the concentrations of 3-MeSO$_2$-DDE and 4-MeSO$_2$-CB149 reported in Paper III are overestimated. However, 3-MeSO$_2$-DDE and 4-MeSO$_2$-CB149 in the pooled sample of five guillemot eggs from 1976 did not co-elute with any of the unknown Cl$_6$ MeSO$_2$-PCB congeners.

6.2.2 Tissue specific retention

MeSO$_2$-PCBs were analysed in different tissues in grey seal and in experimental rat (Papers III and IV). In the seal, highest concentration of ΣMeSO$_2$-PCBs was detected in the liver. This is in accordance with previous studies [42,111]. However, in the rat which only contained 5′-MeSO$_2$-CB132 and 4′-MeSO$_2$-CB132 (Paper IV), highest concentration was detected in the lung. Para-substituted congeners are known to have a specific retention in the lung due to protein binding [51,54]. In biota, the para-substituted congeners seem in general to be formed to a higher degree than the meta-substituted congeners [42]. This specific retention in the lung for para-substituted MeSO$_2$-PCBs was less clear in the seals (Paper III).

Meta-substituted congeners are retained primarily in the liver compared to other tissues [42,116,132] and this was clearly shown both in the seals and in the rats (Papers III and IV). Protein binding is believed to be the major mechanism behind the specific retention also to liver [52,53]. The different affinity for meta- or para-substituted MeSO$_2$-PCB congeners is probably due to the fact that different proteins are involved in the binding mechanisms in liver and lung, respectively.

It has been discussed by Karlsson et al. (2000) that the lipid content in the liver could be an additional reason for the accumulation of MeSO$_2$-PCBs [111]. For harbour porpoises it has been shown that the fat content in the liver constitutes to a lesser proportion of triglycerides compared to the blubber [184]. Instead the liver contains more polar lipids as phospholipids and cholesterol. Compounds such as PCB congeners and p,p′-DDE have shown to correlate with the content of triglycerides in a tissue and that phospholipids are less able to dissolve these substances [151]. PCBs and p,p′-DDE are also found in highest concentrations in lipid rich tissues such as blubber and adipose tissue in mammals (Papers III and IV). All sulfone-containing compounds analysed in this thesis (BCPS, 3-MeSO$_2$-DDE and MeSO$_2$-PCBs) independent of origin, were detected in highest concentrations in the liver. They are slightly less hydrophobic compared to PCBs and p,p′-DDE and it
seems reasonable to believe that the lipid composition in the liver is an additional explanation to the higher concentrations for these compounds in the liver compared to other tissues.

6.2.3 Temporal trend of MeSO\textsubscript{2}-PCBs

The PCB concentrations in the guillemot show the exposure from the feed while the MeSO\textsubscript{2}-PCBs reflect the bird metabolism. In guillemot eggs, the level of ΣPCB today is about 5% of the levels in the 1970s [7]. Even though, the present concentrations of the three MeSO\textsubscript{2}-PCB congeners reported in Paper II are 50% of their concentrations in the 1970s. This is also reflected in the temporal increase in the ratio of metabolite to parent compound as shown in Paper II. It is possible that the bird has a poor transfer of MeSO\textsubscript{2} metabolites to the egg. In glaucous gulls, higher concentrations of ΣMeSO\textsubscript{2}-PCBs were found in the lipids of plasma compared to the egg (Table 3.3) [89]. Also the MeSO\textsubscript{2}-PCB congener pattern was different in the two compartments. The plasma was dominated by hexachlorinated congeners while the egg was dominated by tetra- and pentachlorinated congeners and a poor transfer of higher chlorinated MeSO\textsubscript{2}-PCB congeners was suggested [89].

In the glaucous gull, BCPS was reported in the plasma but not in the egg [89]. BCPS concentrations were similar in the guillemot muscle and egg (Papers I and II). This indicates that even if MeSO\textsubscript{2}-PCBs have a poor transfer to the egg, this is not the case for BCPS in the guillemot.

6.3 Chiral MeSO\textsubscript{2}-PCBs

6.3.1 EFs of chiral MeSO\textsubscript{2}-PCBs in grey seals and guillemots

For the enantioselective analyses of chiral MeSO\textsubscript{2}-PCBs in grey seal and guillemot eggs sampled in 1976, the same chiral GC-column was applied (Papers II and III). The EFs, defined as EF = A\textsubscript{R}/(A\textsubscript{R}+A\textsubscript{S}) (chapter 2.2.2) in the two species are shown in Figure 5.4. In both species, the atropisomer with the \textit{R} configuration is dominating. This dominance is more pronounced in the seal compared to the guillemot. The enantioselective analyses of atropisomeric MeSO\textsubscript{2}-PCBs reported in wildlife are reviewed in Table 3.4, including grey seal and guillemot. Even though different chiral GC columns have been applied, EFs are >0.5 for \textit{para}-substituted congeners and <0.5 for \textit{meta}-substituted congeners. Also in the pelican and seal analysed by Karasek \textit{et al.} (2004), were the dominating atropisomers of \textit{R}-configuration [122]. It seems likely that the dominating atropisomer of chiral MeSO\textsubscript{2}-PCB congeners in biota has an \textit{R}-configuration, regardless of species. Similarly as in
guillemot are the S-atropisomers in the pelican present in a relatively higher degree compared to seal [122]. Generally, the grey seal show a stronger dominance for one atropisomer for each chiral MeSO$_2$-congener compared to the guillemot (Table 3.4).

![Figure 6.4](image-url)  
**Figure 6.4.** Enantiomeric fractions (EF = $A_R/(A_R+A_S)$) in grey seal blubber and guillemot egg sampled 1976 (Papers II and III).

### 6.3.2 Enantioselective metabolism of PCB

To study possible enantioselective processes causing the strong retention of one atropisomer for a chiral MeSO$_2$-PCB congener in mammals (Figure 6.4 and Table 3.4), a laboratory experiment was performed (Paper IV). The experiment and results are illustrated in Figure 5.5. Group of rats were dosed with racemic CB-132 and the pure atropisomers; CB-132:A1 and CB-132:A2. The results showed a stronger retention of CB-132:A2 than CB-132:A1 and a strong retention for the 5’- and 4’-MeSO$_2$-CB132 atropisomers with $R$-configurations.

Enantioselective metabolism of the parent compound is suggested since CB-132:A1 was the precursor to the $R$-atropisomers of the metabolites. This enantioselectivity in the metabolism would at least occur in the initial step when an arene oxide is formed as shown in Figure 3.2 in chapter 3.2.2. However, it can not be excluded that other steps in the metabolism are enantioselective as well. In fact, CB-132:A2 was detected in 2-3 times higher concentrations than CB-132:A1. In the opposite, the MeSO$_2$-metabolites formed from CB-132:A2 was detected in about 20 times lower concentrations than those formed from CB-132:A1. The enantioselective analysis of CB-132
in faeces showed a similar EF as in the tissues with a dominance of the second eluting atropisomer. This shows that other possible enantioselective processes such as excretion and uptake can be excluded.

It has been discussed by Müller and Kohler (2004) [68] that the enantioselective enzymatic degradation of chiral pollutants could be due to the occurrence of one enzyme degrading the enantiomers at different rates, or that two enzymes occur, one for each enantiomer. From the laboratory
experiment, it seems most reasonable that only one enzyme exists, preferentially degrading the R-atropisomer. However, the possibility that there exists one enzyme for each atropisomer can not be excluded. In a previous study where rats were dosed with high amounts of technical PCB, both atropisomers of 5-MeSO₂-CB149 and 4-MeSO₂-CB149 [50] showed a clear presence. Notably, the S-atropisomer of 4-MeSO₂-CB149 was dominating in the lung [50]. It is possible that the differences in metabolising rates of the two enzymes are due to different induction potentials, or that one of the enzymes was saturated by the high dose of PCB given.

This study also shows the advantage by using absolute configurations when expressing EFs. For example, the fact that all dominating atropisomers are of R-configurations is emphasised (Figure 6.4). The animal experiment showed that the R-atropisomers of the metabolites were formed from CB-132:A1 which very likely also has the R-configuration. The absolute structure is important since it is believed that the active site(s) of the metabolising enzyme(s) has a greater affinity for one of the absolute configurations of a chiral compound [68]. EF based on R/S for both parent compound and metabolite would stress this. However, for the atropisomers of chiral PCBs only the optical rotation is known [101,103].

### 6.3.3 EFs of chiral PCBs and MeSO₂-PCBs in the marine environment

The chiral composition of atropisomeric PCBs has been studied in several species and examples are presented here for which the chiral MeSO₂-PCBs have been analysed. It should be stressed that the PCB and MeSO₂-PCB studies are performed independently of each other.

EFs for several atropisomeric PCBs have been determined in Baltic grey seals, arctic ringed seals and harbour porpoises [62,69,73]. In both the seal species, a relationship between the substitution pattern and the deviation from a racemic EF was found. The chiral composition was more altered for congeners with vicinal hydrogens in meta/para- or ortho/meta position in both rings (CB-91, CB-95 and CB-132) compared to CB-149 and CB-174 with only one pair of vicinal hydrogens [62,69]. CB-91, CB-95 and CB-132 are due to their structure (Table 3.2) more susceptible to metabolism which could be a reflection of the degree of altered EF from a racemic value. In the harbour porpoise, the chiral composition was more altered for adult individuals than juveniles who had nearly racemic EFs for CB-95, CB-132 and CB-149. [73].

Enantioselective metabolism was in all studies suggested to be a reason for the observed altered chiral compositions of the atropisomeric PCBs in the different studies. Also, the dominating atropisomer of the parent compounds
was similar in the seal species and the porpoise. For the chiral MeSO₂-PCBs, a strong dominance for one atropisomer has been shown in these three marine species (Table 3.4) [48,49].

The dominance for one atropisomer of the chiral MeSO₂-PCBs has been suggested to be due to enantioselective excretion which was shown in mice dosed with pure atropisomers of 5′-MeSO₂-CB149 [77]. Enantioselective excretion is not believed to be the reason for the enantioselective retention seen in the study on rat (Paper IV). However, enantioselective retention could be another explanation for the additional enantioselective dominance in wildlife if an animal is exposed to MeSO₂-PCBs via the diet. In polar bear, which mainly feeds on ringed seal blubber, it was shown that the concentration of 4'-MeSO₂-CB132 in the adipose tissue was solely accumulated from the diet [47]. Wiberg et al. (1998) showed an increase in enantiomeric ratio ER for 4′-MeSO₂-CB132 from the seal to the bear. This indicates that enantioselective retention or excretion also occurs in wildlife [48].

**Conclusion** Since several biological processes can be enantioselective leading to enrichment of one enantiomer over the other is it difficult to determine which step is the most critical. The laboratory experiment (Paper IV) shows the advantage to study the chiral composition of both the parent compound and the metabolites to further understand the enantioselective biotransformation processes. Also, analyses of faeces showed to be informative for this purpose. Problems with chiral analyses of PCBs in biota which are precursors to MeSO₂-PCBs are sometimes in low concentrations due to being easily metabolised. Moreover, the great number of PCB congeners complicates the enantioselective analysis due to co-elution problems requiring advanced techniques for analysis, e.g. multidimensional GC [69]. Fractionation of the PCBs according to their chlorine substitution pattern by HPLC has also been used to facilitate the analysis [73].

### 6.4 Metabolism in birds

A different metabolic capacity in guillemot than grey seal is indicated in several ways. In mammals, it is known that the metabolism of PCBs to OH- and MeSO₂-PCBs occurs via an epoxide in *meta-para* position mediated by CYP2B enzymes from the cytochrome P450 system [42,185]. The higher ratio ΣMeSO₂-PCB/ΣPCB in mammals compared to birds is a reflection of the higher metabolic capacity [42]. The metabolism of *p,p′*-DDE to 3-MeSO₂-DDE is believed to occur as for PCBs but via an epoxide in the *ortho-meta* position [42]. Guillemots have similar levels of DDE as seals but 10 times lower 3-MeSO₂-DDE levels (Papers II and III). It is possible that the CYP2B
enzymes also metabolises BCPS via an epoxide in the \emph{ortho-meta} position. Metabolites of BCPS have been found in rats [86].

The fact that guillemots have lower concentrations of 3-MeSO$_2$-DDE and higher concentrations of BCPS than seals indicate a lower rate of metabolism and especially for compounds with \emph{para}-substituted phenyl rings. This is supported by the high concentrations of TCPMe found in birds compared to mammals. The concentrations of TCPMe in the guillemot eggs sampled in 2001 was approximately 1000 ng/g fat (Paper II). Previous studies have shown that birds accumulate TCPMe to a higher degree than mammals, e.g. ringed seal from the Baltic Sea contained 200 ng/g fat in the blubber [186] while white-tailed sea eagles from Poland contained 11000 ng/g fat in the egg, 5300 ng/g in the breast muscle and 20000 ng/g in the liver [187]. TCPMe has also been detected in high concentrations in peregrine falcon eggs (2000 ng/g fat) [142].

The different metabolism in guillemots compared to the seals is also indicated by the presence of both atropisomers of the chiral MeSO$_2$-PCBs in a relatively higher degree (Figure 6.4). An unknown factor is the microbial activity in the gastrointestinal tract of the bird, a factor that will influence the MeSO$_2$-PCB and –DDE formation.

It is evident that we need more studies on birds and metabolism of anthropogenic compounds. In particular the chirality in metabolism needs to be addressed.
Concluding remarks

The work presented in this thesis has been performed in collaboration with the Contaminant Research Group at the Swedish Museum of Natural History and with the Institute for Organic Chemistry at the University of Hamburg. Their contributions to the thesis have increased the knowledge of BCPS and the chiral MeSO₂-PCBs in biota, in particular.

The detection of BCPS in the environment and especially the high concentrations in the guillemot calls for further biomonitoring activities. BCPS may not be harmful to the ecosystem at the present concentrations, but different species are differently susceptible to contaminants which call for caution since our present toxicological knowledge about this compound is still limited. Furthermore, it is notably that whereas concentrations of many other environmental pollutants decrease over time the concentrations of BCPS seem almost stable since the early 1970s. Effects on ecosystems may also arise from a combination of chemicals, a potential “cocktail effect”. It is accordingly important to apply our knowledge of what we learn, e.g. how compounds behave once they reach the environment and the wildlife. The structure of a compound can give indications of their environmental fate. It is important to consider the metabolism of contaminants, i.e. which metabolites are formed since they have different physical and chemical properties making them accumulative or excreted in other ways than their parent compounds or distributed differently throughout the body.

The liver is the target tissue for the sulfone containing compounds as shown in this thesis. The liver is also the tissue often most affected in toxicity tests. Therefore the choice of matrix should be considered depending of the aim of the study. It is also important to emphasise the usefulness of analysis of different species from different levels in the food chain and to include both mammals and birds of prey since they bioaccumulate and metabolise pollutants differently. For BCPS, the specific retention to seal liver and the accumulation in guillemot muscle and egg makes the bird liver especially interesting for BCPS analysis and effect studies.

The enantiomeric signatures of chiral environmental pollutants can be useful markers for exposure and determination of biotransformation capacities. The different metabolic capacities between guillemot and grey seal are indicated in several ways. The enantiomeric signatures of the chiral MeSO₂-PCBs in the two species were proving this. The metabolism of environmental pollutants in birds is rather unknown and the MeSO₂-PCB congener pattern has shown to differ between plasma and egg [89]. Further analyses of the chiral
composition in different compartments and tissues in the bird may tell us more about bird metabolism, retention and transfer mechanisms.

When an active mechanism is involved in a transfer process, protein binding or enzyme mediated reaction, it is reasonable to believe that there will be a large degree of enantioselectivity due to higher affinity to one enantiomer. Lots of questions still exist of the enantioselectivity of the different enzymes involved in the metabolism of chiral pollutants. For chiral PCBs, the P450 system most likely prioritises $R$-structures, but several enzymes are involved in the further metabolism to the final methylsulfonyl product. The specific affinity for one enantiomer may also play an important role in the toxicity of a parent compound or its metabolite.

This thesis has addressed specific questions in the frame of “A non-toxic environment” in the future. Both the result of only very slowly decreasing concentrations of BCPS in the guillemot eggs and the complicating result on high enantiomeric specificity of PCB methyl sulfoines in biota show that much more efforts are required to increase the understanding of chemicals to reach a less polluted environment, not the least research to identify new potential pollutants and their metabolites.
8 Populärvetenskaplig sammanfattning

Östersjön är ett mycket förorenat hav på grund av utsläpp från industri och jordbruk från kringliggande länder. Hög halter av polyklorerade bifenyler (PCB) och DDT upptäcktes på 60-talet vilka visade sig vara orsaken till nedgången hos flera djurarter som livnär sig på östersjöfisk. Dessa ämnen har med tiden förbjudits och populationerna i Östersjöregionerna har börjat återhämta sig.

Idag finns direktiv inom EU för en bättre kemikaliehantering och även förbud mot produktion av flera kemikalieklasser. Sveriges regering har också antagit målet ”En giftfri miljö” där vår miljö ska vara fri från ämnen som skapats eller utvunnits i samhället. Detta mål ska vara uppnått senast år 2020.

Ständigt hittas ”nya” industriellt producerade kemikalier i miljön som visar sig persistenta, dvs de är långlivade och kan anrikas i näringskedjan. Bis(4-klorfenyl) sulfon (BCPS) är ett sådant ämne som produceras i stora mängder för plastindustrin. BCPS har tidigare också använts som bekämpningsmedel, om än i begränsad omfattning. PCB metaboliseras till bl.a. metylsulfonyl-PCB (MeSO\(_2\)-PCB) vilka också är persistenta. BCPS och MeSO\(_2\)-PCBer har strukturella och kemiska likheter vilket har varit en bas för denna avhandling. Målet med avhandlingen är att få mer kunskap om dessa sulfon-föreningar angående deras spridning, halter i miljön och tidstrender genom att sätta resultaten i relation till välstudierade kemikalier som t.ex. PCB och DDT. Särskilt intresse har riktats till kirala MeSO\(_2\)-PCB.

BCPS analyserades i en rad djurarter från svenska insjöar och från Östersjön för att studera dess spridningsmönster och potential att anrikas i rovdjur. Tidstrenden av BCPS har studerats i ägg från sillgrisslor från Stora Karlsö. Proverna för dessa ändamål hämtades från provbanken på Naturhistoriska Riksmuseet. Det visade sig att BCPS har ett annat spridningssätt till miljön än t.ex. PCB och DDT. BCPS tycks spridas mer via vatten än PCB och DDT, för vilka spridning via luften är av stor betydelse. Gråsäl och sillgrisslor från Östersjön innehåller alla BCPS liksom den fisk de livnär sig på. Särskilt höga halter hittades i sillgrisslornas ägg och muskel. Minskningen av BCPS i sillgrissla de senaste 30 åren har dessutom varit mycket liten (1.6% per år endast). Det visade sig att även MeSO\(_2\)-PCB minskar långsamt i sillgrisslan, långsammare än de PCBer de metaboliseras från. Både BCPS och MeSO\(_2\)-PCB studerades i olika organ från säl. Dessa substanser hittades i högst koncentration i levern medan PCB främst återfinns i späcket.

Ett antal föroreningar som hittas i miljön är kirala, dvs. de förekommer som spegelbilder av varandra. För kirala PCBer kallas de två spegelformerna för

Gräsälar och sillgrisslor från Östersjön är kontaminerade med PCB via födan och det var därför intressant att studera de kiralna PCB metaboliterna i dessa arter. Det visade sig att kiralna MeSO$_2$-PCB i säl nästan enbart bestod av en atropisomer medan sillgrissleäggen innehöll båda två där en atropisomer fanns i något högre halt än den andra. För att studera anledningen till att det förekommer mer av en atropisomer än den andra av de kiralna PCB metaboliterna gjordes ett djurförsök. Råttor doserades med en kiral PCB förening, CB-132. Resultatet visade att atropisomererna hos en kiral PCB förening metaboliseras olika och att detta är anledningen till att en MeSO$_2$-PCB atropisomer förekommer mer än den andra i råttor.

De höga halterna av BCPS i sillgrissla visar att det är viktigt att ha en fortsatt miljöövervakning av BCPS i naturen. Även om vi idag inte känner till några biologiska effekter av BCPS kan inte effekter av ämnet på någon ekologisk nivå uteslutas. Inte heller kan dess eventuella inverkan i kombination med andra miljöföreoreningar förutses. Inte minst av dessa skäl är det oroande att halterna sjunker ytterst långsamt i miljön.


Avhandlingen ger stöd för fortsatt och intensifierad forskning inom området kemikalier och miljö.
9 Acknowledgement

Det är lite oerkligt att ha nått hit, till det sista kapitlet och det är faktiskt med blandade
känslor som de sista raderna skrivs. Tänker på åren på miljökemi som har varit fantasistiskt
givande på så många sätt. Det är en väldigt stimulerande miljö att jobba i, där det är roligt
varje dag. Jag trivs helt enkelt. Och det beror framför allt på er alla som finns och har
funnits med på miljökemi. Därför är det också en märklig känsla att faktiskt vara klar och
att nu snart stå på ”andra” sidan. Ni är många som genom åren har bidragit till den värme
och trivsamhet som finns på institutionen, som jag har jobbat med och som gjort det hela
så rolig och intressant.

Jag vill först tacka min handledare Åke – för din enorma förmåga att förmedla energi med
ditt positiva och inspirerande sätt. Din dörr är alltid öppen och även i de mest hektiska tider
tar du dig tid. Jag går alltid ut ur ditt rum fylld med ny entusiasm och vilja, och med
känslan av att göra ett bra och intressant arbete.

Mats - med dig som biträdsande handledare har jag fått förmånen att få andra infallsvinklar
för att du ofta ser saker från ett annat perspektiv vilket har varit inspirerande och lärorikt.

Sören - jag har fått ta del av dina erfarenheter och ditt kunnande på lab. Du tar dig alltid tid
att lyssna på problem och komma på hundra sätt att lösa dem på.

Tack alla mina medförfattare, utan er hade jag inte stått här. Genom alla många och långa
diskussioner har jag lärt mig oehört mycket av er. Anders – du gav mig bästa starten, tack
också för soffan, Stina – jag är glad över att få ha jobbat med dig och som ledde mig in i
kiralitetens värld, Hrönn – du är så positiv och okomplicerad och en bra rumskamrat,
Ioannis – din vilja att hjälpa och ge kunskap likaväl som din vilja att förstå har gett mig
mycket, Johan – du är alltid hjälpfull och du tar dig tid, Johanna – du är ett bra
ifrågasättande bollplank som jag har haft mycket roligt med, Virginia – your firm hand
with the rats was needed when I only felt sorry for them, Anders ”Bingen” – du har
bidragit med mycket ”power”.

Tack alla ni som finns runt mig dagligen eller ibland, ni bidrar alla till den glada och
speciella stämning som genomsyrar miljökemi. Ulrika – för att jag alltid kan komma till
dig, du är glad och bjuder mycket på dig själv, Anita – jag blir glad av dig, Britta – du är
en vän även utan för jobbet, Per – du har bidragit med så mycket knäppa saker som bara
du kan och det uppskattar jag, Anna C – tack för ditt sällskap, promenader och kvällsfikor,
Maria – för ditt stöd och din uppmuntran, Tina – tycker om att prata med dig, Jana – för
sällskap längs vägen, Lotta, Jeanette, Birgit, Hans, Kaj - hoppas du har det bra,
Lillemor, Tati, Ronnie – bästa ass. partner, Rick, Lisa, Cristina, Ulla, Linda – fortsätt


Tack ni i miljögiftsgruppen på Riksmuseet för all hjälp med prover och ert alltid lika välkommande sätt.

Hrönn, Anna C, Maria A, Rick, Britta, Ioannis, Ulrika och Linda - extra tack till er alla för all hjälp med avhandlingen vilket gjorde att jag fick en bra tillvaro och att paniken uteblev.

Jag är förunnad att ha många och vädligt fina vänner. Ni är alla speciella och viktiga för mig och jag vill ta tillfället iakt och tacka även er, för utan er…;


Jag vill också tacka alla i min stora härliga Norströms familj. Speciellt Åke och Gunilla – ni står alltid där med öppna armar, tck för allt ni gör för oss och tck för Dalarna.

Min fantastiska Ceder familj. Nisse och Ylva, Mamma och Pappa – ni ger så mycket och ni finns alltid där för mig, Nisse och Ylva. Tck för ert stora stöd och engagemang, för att ni tror på mig och för all er förståelse.

Min alldeles egen lilla familj. Melker och Hannes – jag blir alldeles varm inombords när jag tänker på er – det finaste har – längtar så efter era små armar om min hals.


♥ Tack alla!
10 References


30. Lucas, S. V., (1984), GC/MS analysis of organics in drinking water concentrates and advanced waste treatment concentrates, EPA-600/1-84-020, Health Effects Research Lab.


60. Kallenborn, R., Sandanger, T., and Odland, J., (2003), Enantioselective analysis of chiral organochlorines in plasma of delivering women from Arkhangelsk (Russia), *SETAC, Austin, USA, 24th annual meeting.*


104. Robertson, L. W. and Gupta, R. C., (2000), Metabolism of polychlorinated biphenyls (PCBs) generates electrophiles and reactive oxygen species that damage DNA, In: Molecular Drug Metabolism and Toxicology, Eds. Williams, G.M. and Aruoma, O.I., OICA International, 16-32.

105. Letcher, R. J., (1996), The ecological and analytical chemistry of chlorinated hydrocarbon contaminants and methyl sulfonyl-containing metabolites in the polar bear (Ursus maritimus) food chain., PhD Thesis, Department of Chemistry, Carlton University, Ottawa, Canada.


