Enantiospecific Analysis and Environmental Behavior of Chiral Persistent Organic Pollutants (POPs)

by

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Akademisk avhandling

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ABSTRACT

Many persistent organic pollutants (POPs) are chiral. These pollutants are generally released into the environment as racemates, but frequently undergo alterations in enantiomeric composition as soon as they are subjected to life chemistry processes. Enantiospecific analysis of chiral POPs is important since enantiomers of chiral compounds often exhibit differences in biological activity, and most biochemical processes in nature are stereospecific. For abiotic processes, such as air-water gas exchange, deposition and long-range air transport, enantiomeric patterns of POPs may be used as chemical markers.

The aim of the work described in this thesis was to improve our knowledge about the presence and fate of enantiomers of chiral POPs in the environment to provide a sound basis for accurate risk assessment. The compounds included were organochlorine (OC) pesticides (α-HCH, chlordanes and o,p'-DDT), atropisomeric PCBs and some of their respective metabolites (heptachlor-exo-epoxide, oxychlordane and MeSO₂-PCBs).

Analytical methods for chiral PCBs were developed, and the elution sequences of (+) and (−)-enantiomers were determined. Enantiomeric fraction (EF) was proposed as a better reflector of chiral composition than the conventional enantiomeric ratio (ER).

Enantioselective bioprocessing in various compartments was studied, with the main emphasis on factors controlling chiral composition in biota. Correlations were detected between changes in EFs and differences in trophic levels. The changes were, however, not consistent for all compounds. Instead, the enantiomeric composition was found to be species-specific in the polar bear food chain and in aquatic species from the Baltic Sea. The EFs of some POPs in Baltic seals were related to nutritional status and biotransformation capacity.

Enantiomeric and isomeric patterns were used to investigate abiotic processes in the southern Baltic Sea environment and EFs were used to study soil as a source of atmospheric heptachlor-exo-epoxide.

Keywords: chiral, persistent organic pollutants, POPs, organochlorine compounds, OCs, PCBs, analysis, mass spectrometry, MS, gas
chromatography, GC, enantiomers, atropisomers, food chain, polar bear, seal, fish, Baltic Sea, Arctic, air-water gas exchange, soil
LIST OF ORIGINAL PAPERS

This thesis is based on the following papers, which are referred to in the text by the corresponding Roman numerals:


VII. Wiberg K, Bergman A, Olsson M, Roos A, Blomkvist G, Haglund P. Concentrations and enantiomer fractions (EFs) of organochlorine compounds (OCs) in Baltic species that were hit by reproductive impairment. Manuscript for submission to *Environmental Toxicology and Chemistry*


X. Wiberg K, Brorström-Lundén E, Wängberg I, Bidleman TF, Haglund P. 2001. Concentrations and fluxes of hexachlorocyclohexanes (HCHs) and chiral composition of α-HCH in environmental samples from the southern Baltic Sea. *Environmental Science and Technology*, in press.

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ABBREVIATIONS
CD     Cyclodextrin
CHLs   Chlordane-related compounds
CNS    Central nervous system
Cs     Water solubility for solid phase
CSP    Chiral stationary phase
CTT    Compounds of technical toxaphene
CYP    Cytochrome P450
DDD    1,1-Dichloro-2,2-\textit{bis}(4-chlorophenyl)ethane
DDE    1,1-Dichloro-2,2-\textit{bis}(4-chlorophenyl)ethene
DDT    1,1,1-Trichloro-2,2-\textit{bis}(4-chlorophenyl)ethane
ECD    Electron capture detection
ECNI   Electron capture negative ionization
EF     Enantiomer fraction
EI     Electron impact ionization
ER     Enantiomer ratio
GC     Gas chromatography
HCH    Hexachlorocyclohexane
HEPX   Heptachlor-\textit{exo}-epoxide
$H_{LC}$ Henry’s law constant
HR     High resolution
$K_{ow}$ Octanol-water partition constant
LR     Low resolution
MD     Multi dimensional
MS     Mass spectrometer, mass spectrometry
NIST   National Institute of Standards and Technology
OCs    Organochlorine compounds
OXY    Oxychlordane
PCB    Polychlorinated biphenyl
PCCH   Pentachlorocyclohexane
PM     Permethylated
POP    Persistent organic pollutant
$P_s$  Vapor pressure for solid phase
QA     Quality assurance
QC     Quality control
RP     Reversed phase
SIM    Selected ion monitoring
SRM    Standard reference material
TBDMS  \textit{tert}-butyldimethylsilylated
$t_R$  Retention time
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1. INTRODUCTION

In 1848, when examining tiny crystals of tartaric acid, Louis Pasteur noticed that the crystals came in two asymmetric forms that were mirror images of each other. He sorted the two forms with a pair of tweezers into two piles and discovered that a solution of one form would rotate polarized light clockwise, while a solution of the other form would rotate the light anticlockwise, and an equal mix of the two would not rotate the light at all. This was the first chemical experiment in which chirality was demonstrated.

In 1874, the two scientists J. A. Le Bel and Dr. J.H van’t Hoff independently argued that the spatial arrangement of four groups around a central carbon atom is tetrahedral. As often, when unconventional scientists depart from traditional ways of thinking, they were harshly criticized. Dr. van’t Hoff’s work was dismissed as “...childish fantasy...” and he was criticized for having “...no taste for accurate chemical research...” (Solomons, 1980). Nevertheless, abundant evidence supporting their hypothesis accumulated, and in 1901, van’t Hoff was the first recipient of the Nobel Prize for Chemistry. The works of Van’t Hoff and Le Bel marked the beginning of the field stereo-chemistry.

**Figure 1.** Pasteur, *Munsey's Magazine*, May 1897
The constituent atoms of stereoisomers are attached in the same order, and differ only in the arrangement of their atoms in space. There are two general categories of stereoisomers: enantiomers and diastereomers. Enantiomers are stereoisomers whose molecules are mirror reflections of each other, while diastereomers are stereoisomers whose molecules are not mirror reflections of each other. Compounds that exist as enantiomers are chiral. The word chiral comes from the Greek word *cheir*, meaning “hand”, and is used since pairs of enantiomers differ from each other in the same way as left and right hands (i.e. they are non-super-imposable mirror images of each other, Figure 2). Most of the chemical and physical properties of an enantiomeric pair are identical. One difference, however, is in their effects on polarized light, as observed by Pasteur. A substance capable of causing polarized light to rotate is said to be optically active.

Chiral compounds are designated (+) or (−) to indicate molecules that induce clockwise (dextrorotatory) and counter-clockwise (levorotatory) optical rotation, respectively. A mixture containing equal amounts of two
enantiomers, known as a racemic mixture or racemate, is optically inactive. R and S designations are used to describe the absolute configuration, and do not tell anything about the optical rotation. In this nomenclature, groups or atoms that are attached to the chiral atom are assigned a priority on the basis of the atomic number of the atom that is directly attached to the chiral carbon. The group with the lowest preference is directed away from the viewer, and a path is traced depending on the priority of the remaining groups. If the direction described by this path is clockwise, the enantiomer is designated R (from the Latin word rectus, meaning right), and if the direction is counter-clockwise, the enantiomer is designated S (sinister, meaning left).

Most macromolecules (proteins, nucleic acids and polysaccharides) possess characteristic chiral structures. In many cases, one of the enantiomers is dominant, e.g. almost all naturally occurring amino acids have the same (L) configuration at the chiral carbon. Because of their chiral stereospecificity, enantiomers react and interact differently with other chiral compounds. Therefore, the biological activity of one enantiomer may vary tremendously compared to its mirror image. One enantiomer may have a desired effect as a drug, for instance, while the other may be poisonous. A dramatic example is the drug Neurosedyn, sold during the early 1960s as a racemic mixture, causing thousands of birth defects. The tragedy arose because one of the enantiomers of thalidomide (the active component of neurosedyn) is teratogenic. As a result of this and many other similar cases, nowadays the preparation and control of enantiomerically pure compounds is of immense interest to the pharmaceutical and agrochemical industries. In fact, the 2001 Nobel Prize in Chemistry was awarded to three scientists for their development of molecules that can catalyze important reactions so that only one of the mirror image forms is produced.

Interest in environmentally stable chiral compounds is relatively recent. König et al. separated racemic α-hexachlorocyclohexane (α-HCH) by chiral gas chromatography (GC) using cyclodextrin (CD) as a chiral stationary phase (CSP). The cited authors suggested that this technique could be used for enantioselective analysis of chiral pollutants (König et al., 1989). Shortly thereafter, chiral environmental analyses of several POPs were reported, such as α-HCH (Kallenborn et al., 1991; Faller et al., 1996).
1991a; Faller et al., 1991b), chlordanes (Buser and Müller, 1992; Buser et al., 1992; Buser and Müller, 1993), \textit{o,p'}-DDT (Oehme et al., 1994; Buser and Müller, 1995a), toxaphenes (Buser and Müller, 1994) and atropisomeric PCBs (Glausch et al., 1994; Hühnerfuss et al., 1995; Glausch et al., 1995). By then it was well known that organochlorine (OC) pesticides and other persistent organic compounds (POPs) were ubiquitously distributed throughout the world. In spite of restrictions and bans, these lipophilic compounds are found in all environmental compartments, even in such remote areas as the Arctic and Antarctic. Other characteristics of POPs (and many of their metabolites) are that they bioaccumulate and that they are suspected to cause immunological, reproductive, and teratogenic dysfunction in wildlife and man.

Since 1) a significant number of all organic chemicals are chiral, 2) these chemicals are released into the environment as racemates, 3) enantiomers frequently exhibit different toxicological and other biological activities, and 4) most biochemical processes in nature are stereospecific, the importance of chiral analysis in environmental science was established during the 1990s. Most studies focused on the highly persistent OC pesticides, atropisomeric PCBs and their respective metabolites, but there was also growing concern about the ecotoxicological effects of chiral pesticides (Armstrong et al., 1993) and pharmaceutical drugs (Buser et al., 1998) in current use. Besides anxieties about their biological properties, chiral pollutants also started to receive attention as chemical markers. The first example was demonstrated by Faller et al. (Faller et al., 1991b), who found specific enantiomeric ratios of \textit{\alpha}-HCH in seawater from different parts of the North Sea. This report was followed by several studies showing that monitoring the relative abundance of enantiomers in abiotic compartments could be useful for tracing sources and studying air-surface gas exchange parameters (Jantunen and Bidleman, 1995b; Ridal et al., 1997; Papers \textbf{VIII} and \textbf{IX}).

The aim of the work outlined in this thesis was to generate knowledge about the presence and fate of chiral POPs in the environment, to provide a sound basis for accurate risk assessment. Towards this end, work has been done within a broad range of current research fields related to chiral POPs,
so the scope of the thesis is extensive. After a short presentation of the chiral compounds covered, three disciplines are discussed in detail. First, attention is drawn to the analytical methods used for determining chiral pollutants (Papers I and II), and how chiral composition is best expressed (Paper III). Then enantioselective bioprocessing in environmental compartments is discussed, with the main emphasis on factors controlling chiral composition in biota (Papers IV-VII). The third part deals with chiral pollutants as chemical markers for identifying sources and quantifying various transport processes (Papers VIII-X).
2. CHIRAL PERSISTENT ORGANIC POLLUTANTS (POPs)

2.1 Background

To determine whether a molecule is chiral or not one has to look for the presence or absence of certain symmetric elements. A molecule will be chiral if it 1) possesses a center of asymmetry (a stereo or chiral center), 2) lacks a plane of symmetry, or 3) lacks any n-fold (where n=even number) alternating axes of symmetry. The most common kind of chiral center is an asymmetric carbon atom (also called a chiral or stereogenic atom), a carbon atom to which four different atoms or groups are bound (Figure 2). A molecule can, however, possess a chiral center even if it lacks a chiral atom. Most molecules are chiral also if more than one chiral center is present, but they might be achiral, depending on which groups or atoms are attached and how they are arranged in space. Molecules that are chiral due to hindered rotation about a single bond exhibit axial chirality and the isomers are called atropisomers.

The most intensively studied chiral POPs are α-HCH, chlordanes, o,p’-DDT, toxaphene and atropisomeric PCBs. Many of these compounds have environmentally stable metabolites, which may also be chiral. In the following sections, background information is given on selected chiral POPs and related compounds. DDTs and toxaphenes are only briefly reviewed since these they are given little attention in this thesis. Among the compounds discussed, chlordane, heptachlor, DDT and toxaphene are posted on the UNEP* 12 list of chemicals that are banned under the Global POPs Protocol.

2.2 Organochlorine (OC) pesticides

2.2.1 Hexachlorocyclohexanes (HCHs)
Technical HCH was introduced in the 1940s as a broad-spectrum pesticide. Its manufacture involves photochlorination of benzene, which yields a mixture of hexachlorinated cyclohexanes. The main isomer is $\alpha$-HCH (55-80%), but other stable HCH-isomers ($\beta$-, $\gamma$-, $\delta$-, and $\varepsilon$-HCH) are also present (Breivik et al., 1999). The isomers differ in their axial-equatorial substitution pattern around the ring (Figure 3), but only $\alpha$-HCH is chiral. The insecticidal activity is principally attributed to $\gamma$-HCH, which is also a pesticide in its own right (lindane, >99% $\gamma$-HCH). Considerable amounts of technical HCH have been used globally. Approximately 10 million tonnes was applied from 1948 to 1996, with quantities peaking in the 1970s and early 1980s (Li, 1999). From the 1970s, bans or severe restrictions were introduced, and today its use has been phased out in most countries.

The log $K_{ow}$ of HCHs is about 4 (Table 1), i.e. they are moderately hydrophobic compounds. However, compared to other OC pesticides, the HCHs are generally more water-soluble and volatile (Table 1), which promotes atmospheric transport and thus worldwide distribution. As pesticides, HCHs primarily affect the central nervous system (CNS). In insects, $\gamma$-HCH stimulates the CNS and at a high dose it will cause death. However, HCH also induces hepatic nodules and hepatocellular carcinoma in rats, the $\alpha$-isomer being the most potent isomer in this respect. The $\beta$-isomer has the greatest physical and metabolic stability, which is reflected in its environmental persistence. Since several studies indicate that $\beta$-HCH has estrogenic effects, this isomer may currently be of most toxicological concern. Extensive reviews on the toxicity and environmental fates of HCH-isomers were recently published (Willett et al., 1998; Walker et al., 1999).
As for most chiral POPs, there have been few enantiospecific toxicity studies of α-HCH. However, Möller et al. (1996) showed that the (+)-enantiomer was more effective than the (–)-form in cytotoxic and growth stimulation bioassays of primary rat hepatocytes. In another study, Portig et al. (1998) found that intravenous injection of single (+) or (–) enantiomers of HCH into male rats did not cause twitches, while an equal dose of racemate did.

2.2.2 Chlordanes

The cyclodiene pesticide chlordane was introduced into the United States in 1947 (Eisler, 1990). It is produced by cycloaddition of cyclopentadiene and hexachlorocyclopentadiene (Diels-Alder fusion), whereby the intermediate chlordene is formed. Chlordene is subsequently further chlorinated to produce the final product chlordane, which contains 64-67% (w/w) chlorine. Chlordane has been heavily used in the United States, Mexico, Southeast Asia, Australia and New Zealand for agriculture, on lawns and gardens, and as a termicide, and today chlordane and its metabolites occur globally, including remote areas such as the Arctic and Antarctic. After toxaphene, it was the second most important OC insecticide in the U.S. in 1976-77 (Nomeir and Hajjar, 1987).
Technical chlordane mainly consists of hepta-, octa- and nona- chlorinated bicyclopentadienes. The main components are: cis-chlordane (α-) 8-13%, trans-chlordane (γ-) 8-15% and trans-nonachlor 6-7% (Mattina et al., 1999). Other abundant components are heptachlor, cis-nonachlor and various chlordenes (Miyazaki et al., 1985). A detailed analysis of the technical mixture showed that it consists of > 140 related compounds, 120 of which have been tentatively identified (Dearth and Hites, 1996). There were other chlordane-related products. Heptachlor was itself a pesticide, and a high purity chlordane product containing 74% cis- and 24% trans-chlordane also used to be commercially available (Nomeir and Hajjar, 1987). However, all production throughout the world stopped in 1997.

Heptachlor is rapidly degraded in the environment to heptachlor-exo-epoxide (HEPX), also called isomer B or cis-heptachlorepoxide. Heptachlor-endo-epoxide (trans-heptachlorepoxide or isomer A) is also known, but this isomer is not environmentally stable. HEPX is a persistent compound that is abundantly present in biota. Similarly, cis-chlordane, trans-chlordane and the nonachlors are mainly degraded to the persistent metabolite oxychlordane (OXY) (Nomeir and Hajjar, 1987). Photodegradation of heptachlor and cis-chlordane mainly yields caged and half-caged photoproduts (those derived from heptachlor being dubbed ‘photoheptachlor’), which have also been detected in biota (Buser and Müller, 1993). HEPX may also be formed photochemically from heptachlor, but the extent of its formation is an order of magnitude lower than that of photoheptachlor (Buser and Müller, 1993).

Many of the chlordanes and chlordane-related compounds (CHLs) are chiral, including some of the minor components of the technical mixture, the epoxidized metabolites (HEPX and OXY), and the photoconversion products (Buser and Müller, 1992; Buser et al., 1992; Buser and Müller, 1993). The structural formulas for chiral and achiral chlordanes and chlordane metabolites discussed in this thesis are given in Figure 4. Depending on the number of chlorines on each ring, the octa- and nonachlorodanes are classified as ‘5+3’, ‘6+3’ and ‘6+2’ types. The nomenclature for the MC compounds and U82 follow Miyazaki et al.
Chapter 2. Chiral persistent organic pollutants (POPs) (1985) and Dearth and Hites (1996), respectively. The structure of U82 was unknown until recently (Karlsson et al., 1999).

Physico-chemical properties of several CHLs are summarized in Table 1. Due to their highly hydrophobic character, in combination with low water solubility and relatively low vapor pressure, these compounds tend to partition into soils and sediments. The degree of bioaccumulation and biomagnification is highly variable for different CHLs, depending on the structure and thus stability of the molecules. Generally, high relative accumulation and biomagnification factors (BMFs) have been found for compounds with three chlorines on ring 1 (Figure 4) and for the epoxides (the metabolites) (Muir et al., 1988; Dearth and Hites, 1991; Strandberg et al., 1997; Strandberg et al., 1998; Paper VI).

Chlordane hazards to fish, wildlife and invertebrates were reviewed by Eisler (1990) and comprehensive toxicological profiles for chlordane and heptachlor
**Figure 4.** Chemical structures of some chlordane related compounds.
and heptachlor-epoxide have been compiled, respectively (Duerkensen-Hughes et al., 1993; Abadin et al., 1994). Many aquatic species are adversely affected by technical chlordane at aqueous concentrations between 0.2 and 3.0 µg/L. In mammals, chlordane has been shown to cause liver cancer, and in another study, growth was inhibited by diets containing between 0.76 and 5.0 mg chlordane per kg of feed. The metabolites and photoco nversion products are often more toxic than their parent compounds. Again, enantiospecific studies are rare. However, two such studies were conducted by the group of Prof. A. Miyazaki (Miyazaki et al., 1979; Miyazaki et al., 1980). They found that the insecticidal toxicity of several cyclodienes differed significantly between enantiomers, as well as between enantiomers and racemates.

2.2.3 DDTs

DDT, or 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane, is the best known and also the most controversial OC pesticide. It was introduced in the 1940s for use in agriculture, forestry and public health programs, and has since been used worldwide. The peak quantities were applied around 1960. Thereafter, bans and restrictions have greatly limited its use, although some developing counties still use large quantities of it, particularly for malaria control. Although DDT is one of the UNEP 12 compounds, it has clearance for use against malaria. Its cumulative production has so far exceeded 1 million tonnes. The commercial product contains about 70% \textit{p,p\textsuperscript{'}}-DDT (the only isomer with insecticidal properties), 15% \textit{o,p\textsuperscript{'}}-DDT and various impurities.

DDT has several environmentally stable metabolites, of which DDE is the most abundant, often accounting for 70-80% of the DDT-related compounds (DDTs) in mammalian lipid. Like the chlordanes, they partition mostly into soils and sediments (Table 1). The various members of the DDT family are among the most prevalent environmental pollutants, and have been suspected to cause reproductive damage in wildlife (Carson, 1962; Helle et al., 1976a; Helander et al., 1982; Olsson et al., 1994). Some of the \textit{o,p\textsuperscript{'}}-analsogs are chiral. Structures of chiral and achiral DDT
compounds are given in Figure 5. The \( o,p' \)-DDT isomer has received a lot of attention due to its

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\text{Cl} & \text{Cl} & \text{Cl} \\
\text{Cl} & \text{Cl} & \text{Cl} \\
\text{Cl} & \text{Cl} & \text{Cl} \\
\end{array}
\]

\( \text{p,p}'\text{-DDT} \) (achiral)

\[
\begin{array}{ccc}
\text{Cl} & \text{Cl} & \text{Cl} \\
\text{Cl} & \text{Cl} & \text{Cl} \\
\text{Cl} & \text{Cl} & \text{Cl} \\
\text{Cl} & \text{Cl} & \text{Cl} \\
\end{array}
\]

\( \text{o,p}'\text{-DDT} \) (chiral)

\[
\begin{array}{ccc}
\text{H} & \text{Cl} & \text{Cl} \\
\text{Cl} & \text{Cl} & \text{Cl} \\
\text{Cl} & \text{Cl} & \text{Cl} \\
\text{Cl} & \text{Cl} & \text{Cl} \\
\end{array}
\]

\( \text{o,p}'\text{-DDD} \) (chiral)

\[
\begin{array}{ccc}
\text{Cl} & \text{Cl} & \text{Cl} \\
\text{Cl} & \text{Cl} & \text{Cl} \\
\text{Cl} & \text{Cl} & \text{Cl} \\
\text{Cl} & \text{Cl} & \text{Cl} \\
\end{array}
\]

\( \text{p,p}'\text{-DDE} \) (achiral)

\[
\begin{array}{ccc}
\text{H} & \text{Cl} & \text{Cl} \\
\text{Cl} & \text{Cl} & \text{Cl} \\
\text{Cl} & \text{Cl} & \text{Cl} \\
\text{Cl} & \text{Cl} & \text{Cl} \\
\end{array}
\]

\( \text{p,p}'\text{-DDMU} \) (achiral)

\[\text{Figure 5. Chemical structures of p,p}'\text{-DDT and related compounds.}\]

estrogenic activity (Bulger and Kupfer, 1983). Among the \( o,p' \)-DDT enantiomers, the (−)-form was shown to be significantly more estrogenic than its mirror image (McBlain et al., 1976; McBlain, 1987).

2.2.4 Toxaphenes

Toxaphene, or Camphechlor, was introduced in 1945 as a non-systemic pesticide. It largely replaced DDT in North America, and in the mid 1970s, toxaphene was the most widely applied pesticide in the United States. Other major consumers were Brazil, the former USSR, the former German Democratic Republic and Central America. Most registrations of toxaphene were cancelled, but there are indications that its use has continued in some parts of the world (Voldner and Li, 1995).
Toxaphene is chlorinated camphene (isomerized to bornanes), but there are also chlorinated terpenes. Technical toxaphene products consist of several hundred bicyclic components, most of which are chlorobornanes (Figure 6).

![General structure of chlorinated bornane.](image)

Some components of technical toxaphene (CTTs) might be non-racemic in the technical mixture because their precursors are non-racemic (Buser and Müller, 1994). CTTs cover a relatively wide range of physico-chemical properties. Long range atmospheric transport of these compounds clearly occurs and they have worldwide distributions. The environmental fate of toxaphene congeners was recently reviewed by Vetter and Oehme (2000). Most CTTs are chiral. Due to their diversity, it is a formidable task to analyze the CTTs with achiral methods, and enantiospecific analysis is even more challenging. However, several chiral CTTs have been separated successfully and enantioselective environmental studies of both sediments and biota have been done (Vetter et al., 1998; Vetter and Maruya, 2000). The persistence of the CTTs varies, which facilitates analysis of environmental samples, particularly biota.

### 2.3 Polychlorinated biphenyls (PCBs)

#### 2.3.1 Parent PCBs

PCB was introduced as an industrial chemical in the 1920s because of its high thermal and chemical stability, together with its electrical insulating properties. It has been extensively used, leading to a total production of
more than 1 million tonnes (Erickson, 1997). It has been applied in numerous fields, but it was especially favored as a component of dielectric oils in capacitors and transformers. The first evidence of PCB contamination of biota was found in the 1960s by Jensen (1966), and today it is well known

![Mirror Plane](image)

**Figure 7.** Optical isomers of 2,2',3,6-tetrachlorobiphenyl - a PCB atropisomer. Reprinted from Chemosphere, 32, Haglund, P., Isolation and characterisation of polychlorinated biphenyl (PCB) atropisomers, Page 2134, 1996, with permission from Elsevier Science.

that PCB is one of the most prevalent pollutants world-wide. Most producers reduced or stopped production in the 1970s, and thereafter the environmental levels started to decline. However, PCB is still of great environmental concern due to the substantial amounts stockpiled at numerous sites, leakage from dumps and landfills, atmospheric deposition, the persistence and toxicity of PCB metabolites and lack of knowledge concerning its toxicological effects, especially following chronic exposure.

PCB is the collective name for 209 mono- through deca-chlorinated biphenyls. Depending on the number of chlorines in *ortho*-positions, PCBs are classified as: non-, mono-, di-, tri- and tetra- *ortho* congeners. The tri- and tetra-chlorinated congeners have restricted rotation about the phenyl-phenyl σ-bond at physiological and ambient temperatures. The congeners with hindered rotation and asymmetric chlorine substitution pattern on of both ring systems exhibits axial chirality and exists as atropisomeric (enantiomeric)
Chapter 2. Chiral persistent organic pollutants (POPs)

pairs in the environment. The structure of an atropisomeric PCB is given in Figure 7. Seventy-eight out of 209 theoretically possible PCBs occur as atropisomers. Nineteen of the chiral PCBs are predicted to form stable atropisomers under most environmental conditions (Kaiser, 1974), of which at least 12 (PCBs 45, 84, 88, 91, 95, 132, 136, 144, 149, 171, 174, and 183) have been detected in commercial PCB mixtures at levels greater than 1% (w/w) (Frame et al., 1996).

The properties of the PCBs depend highly on their degree of chlorination and chlorine substitution patterns. In general, atropisomeric PCBs have high degrees of chlorination, resulting in high hydrophobicity, low water solubility and low vapor pressure (Table 1), although the atropisomeric PCBs have higher vapor pressure than di-, mono-, and non-ortho congeners within the same homologue group. Non and mono-ortho PCBs can acquire a planar configuration, and may thereby bind to the Aryl hydrocarbon receptor (AhR). The activation of this receptor induces certain xenobiotic-metabolizing enzymes (especially CYP1 types), and cause oxidative damage. Atropisomeric PCBs are non-planar and do not induce dioxin-like toxic responses. However, multi-ortho PCBs have been shown to induce CYP2 enzymes and cause a broad range of biochemical and toxicological effects, which are different from those stimulated by dioxins (Safe, 1994). There is a great variability in persistence among the PCBs, which also depends on the chlorine substitution pattern. PCB congeners with more than five chlorines and para-chlorine substituents are slowly transformed metabolically.

The biological activity of PCB atropisomers was studied by Püttmann et al. (1990) and Rodman et al. (1991). Interestingly, they found that the atropisomers exhibit differential potency to induce several xenobiotic-metabolizing enzymes (Cytochrome P450 (CYP) enzymes) and the accumulation of uroporphyrin (Rodman et al., 1991), indicating that chirality plays a role in recognition events associated with these biological processes.
2.3.2 Methyl sulfonyl- (MeSO$_2$-) containing PCB metabolites

Methyl sulfonyl- (MeSO$_2$-) containing metabolites of PCBs have been found to persist in organisms. The MeSO$_2$-CBs can exist as 2-, 3- and 4-MeSO$_2$-substituted congeners, but only the 3- and 4- substituted compounds are frequently detected in biota (Letcher et al., 1998). Like their CB precursors, MeSO$_2$-CB congeners exhibit axial chirality if both of the phenyl rings have an asymmetric chlorine substitution pattern. Since the introduction of a MeSO$_2$-group at the 2- or 3- position will add an additional element of asymmetry, MeSO$_2$-CBs may be chiral even if the parent PCB is not. Of the 837 theoretically possible MeSO$_2$-CBs, 456 are chiral, of which 170 may be environmentally stable due to tri- or tetra-chloro-ortho substitution (Nezel et al., 1997). However, few chiral MeSO$_2$-CBs are environmentally relevant. Of the 28 MeSO$_2$-PCBs most frequently detected in wildlife and humans, eight congeners (3- and 4- MeSO$_2$-CB91, -CB95, -CB132 and -CB149) are chiral, and together they have been shown to account for 17% of the total MeSO$_2$-PCB content in ringed seal (Phoca hispida) and polar bear (Ursus maritimus) lipids (Paper II) and human liver (Ellerichmann et al., 1998). Structures of some environmentally relevant chiral MeSO$_2$-CBs are given in Figure 8.

The MeSO$_2$-PCBs are only slightly less hydrophobic than the parent compounds. Several studies have shown that MeSO$_2$-PCBs have toxicological potential, e.g. they show tissue-selective retention, induction of CYP enzymes, and endocrine-related effects (Letcher et al., 2000). Based on tissue concentrations and pathological findings, a tentative suggestion has been made that persistent MeSO$_2$-CBs and MeSO$_2$-DDEs may contribute to the disease complex observed in Baltic seals (Olsson et al., 1994). Enantiospecific toxicity studies of MeSO$_2$-PCBs are currently under way (Kallenborn et al., 2001).
Figure 8. Chemical structures of the major atropisomeric MeSO₂ (MS) - CBs found in biota.
Table 1. 
Selected physico-chemical properties of some POPs at 20-25°C

<table>
<thead>
<tr>
<th>Compound</th>
<th>Log $K_{OW}$</th>
<th>HLC (Pa m$^3$ mol$^{-1}$)</th>
<th>$C_S$ (g m$^{-3}$)</th>
<th>$P_S$ (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-HCH</td>
<td>3.9$^a$</td>
<td>0.65$^b$</td>
<td>1.0</td>
<td>3 * 10$^{-3}$</td>
</tr>
<tr>
<td>β-HCH</td>
<td>3.9$^a$</td>
<td>0.12</td>
<td>0.10</td>
<td>4 * 10$^{-5}$</td>
</tr>
<tr>
<td>γ-HCH</td>
<td>3.7$^a$</td>
<td>0.27$^b$</td>
<td>7.0</td>
<td>3 * 10$^{-3}$</td>
</tr>
<tr>
<td>cis-chlordane</td>
<td>6.10$^c$</td>
<td>16$^d$</td>
<td>NR</td>
<td>4.0 * 10$^{-4}$</td>
</tr>
<tr>
<td>trans-chlordane</td>
<td>6.22$^e$</td>
<td>11$^d$</td>
<td>NR</td>
<td>5.2 * 10$^{-4}$</td>
</tr>
<tr>
<td>heptachlor</td>
<td>5.44</td>
<td>112</td>
<td>0.056</td>
<td>5.3 * 10$^{-2}$</td>
</tr>
<tr>
<td>cis-nonachlor</td>
<td>6.08$^c$</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>trans-nonachlor</td>
<td>6.35$^c$</td>
<td>49$^d$</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>MC6 (nonachlor III)</td>
<td>6.06$^c$</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>heptachlor epoxide</td>
<td>5.40</td>
<td>3.4</td>
<td>0.35</td>
<td>3.5 * 10$^{-4}$</td>
</tr>
<tr>
<td>oxychlordane</td>
<td>4.43$^e$</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>$p,p'$-DDT</td>
<td>6.00</td>
<td>1.31</td>
<td>0.003</td>
<td>2.0 * 10$^{-5}$</td>
</tr>
<tr>
<td>$o,p'$-DDT</td>
<td>5.65</td>
<td>NR</td>
<td>0.085</td>
<td>NR</td>
</tr>
<tr>
<td>Toxaphene</td>
<td>5.50</td>
<td>1.1</td>
<td>3.0</td>
<td>5.3 * 10$^{-4}$</td>
</tr>
<tr>
<td>PCB 88 (-Cl$_5$, tri-ortho)</td>
<td>6.50</td>
<td>137</td>
<td>0.02</td>
<td>2.8 * 10$^{-3}$</td>
</tr>
<tr>
<td>PCB 136 (-Cl$_6$, tri-ortho)</td>
<td>6.70</td>
<td>93</td>
<td>0.01</td>
<td>8.0 * 10$^{-4}$</td>
</tr>
<tr>
<td>PCB 171 (-Cl$_7$, tri-ortho)</td>
<td>6.70</td>
<td>30</td>
<td>0.0004</td>
<td>2.7 * 10$^{-5}$</td>
</tr>
</tbody>
</table>

HLC: Henry’s law constant; $C_S$ and $P_S$: saturated water solubility and vapor pressure for solid phase, respectively; NR: not reported

$^a$Willett et al., 1998 $^b$Jantunen and Bidleman, 2000 $^c$Simpson et al., 1995 $^d$Iwata et al., 1993b $^e$Mortimer and Connell, 1995
Values without references were selected from Mackay et al. (1997).
3. ENANTIOSPECIFIC TRACE ANALYSIS OF CHIRAL POPs

3.1 Background

Enantioselective trace analysis of OC compounds is preferably performed with high-resolution gas chromatography (HRGC) using modified cyclodextrin (CD) as a chiral stationary phase (CSP). This technique is relatively new. The first capillary GC separation of enantiomers was published in 1966, and the first demonstration of CD as a successful CSP was reported in 1983 (Koscielski et al., 1983).

Cyclodextrins are cyclic α-(1→4) glucose oligomers with 6, 7, or 8 glucose units corresponding to α-, β- and γ-CD, respectively (Figure 9). They are torus-shaped molecules with specific cavity dimensions, in which one entrance is slightly wider than the other (Figure 9). The inner part of the cavity is hydrophobic, while the outer is hydrophilic. The hydroxy groups at positions 2-, 3- (at the wide end) and 6- (at the narrow end) can be derivatized to form modified CDs with different selectivity (α) and better thermal stability than pure CD. The thermal stability is further improved by dissolving the CD in a conventional achiral stationary phase (e.g. polysiloxane). Even more thermally stable and weakly-bleeding columns are obtained if the chiral selector is chemically bonded (immobilized) to the polysiloxane backbone.

The enantiospecific retention occurs through selective interactions between the enantiomer and the CD-molecules. The enantioselective resolution on derivatized CD may involve several retention mechanisms. One of the mechanisms through which chiral recognition may occur is the formation of an inclusion complex. In this process the analyte forms a host-guest complex with the CD cavity, and one of the enantiomers is retained more strongly than the other if it has a stronger interaction with the functional groups at the rim of the torus. A second possible mechanism involves the formation of an external multiple association with the top, side and/or bottom of the CD (Berthod et al., 1992). Most enantioselective separations
are governed by enthalpy control, and a reduction in temperature normally improves the separation factor ($\alpha$) (Schurig, 1994). Optimum resolution, however, is obtained at a temperature where $\alpha$ is high and peak broadening is moderate.

In contrast to isomer separation, isothermal separations have proven to give maximum enantioselective $\alpha$ values.

Although a broad selection of modified CD capillary GC columns are available, it is often difficult to enantiospecifically resolve all chiral analytes of interest using a single CSP column. Even if the chiral pairs are resolved, the enantiomers of different isomers may overlap. Other types of

Figure 9. Chemical structure and geometry of cyclodextrin.
co-elution problems are also often encountered. Therefore, tandem-columns consisting of an achiral column connected to a chiral column or vice versa have been introduced and used in conventional GCs to separate enantiomers of POPs in environmental samples (Oehme et al., 1994; Papers V and VII).

An even more sophisticated technique to overcome the co-elution problem is to employ multidimensional (MD) GC, which involves separation on two serial columns with different selectivity. In addition, the columns are separately temperature controlled, and the eluate from the first column is directed into the second column during a pre-determined time for subsequent separation. MDGC has been successfully used for separation of PCB atropisomers and CTTs in environmental samples (Glausch et al., 1995; Glausch and Schurig, 1996; Blanch et al., 1996; Benicka et al., 1996; Blanch et al., 1999). In comprehensive two-dimensional GC (GCxGC), the compounds are trapped between the first and the second column. The trapped compounds are focused and then transferred to the second column. The process is repeated continuously, and in this way, a complete chromatogram from the first column can be analyzed on the second column.

In all trace compound analysis it is important to choose a sensitive and highly selective detection method. For multi-halogenated compounds electron capture detection (ECD) fulfills both criteria. However, this method has a serious drawback since it does not allow co-eluting electron capturing compounds to be distinguished. In a mass spectrometer (MS), the analytes are ionized and fragmented, and then the target ions are selected on the basis of mass to charge (m/z) ratios and linked to the detector. This largely eliminates problems associated with co-eluting compounds, and enables individual isotopes to be monitored. Another advantage of MS is that it can screen out interference from column bleed, which is a big problem when using ECD with non-bonded chiral columns. Use of a quadrupole MS results in low- (unit mass) resolution (LR) ion selection, while a magnetic sector instrument provides high-resolution (HR) data. Common ionization methods in trace analysis of halogenated POPs are electron impact ionization (EI) and electron capture negative ion chemical
ionization (ECNI), the second of which is more selective. Very high selectivity is obtained with MS/MS\(^n\) configurations, in which the mass selection is done in two (n=2) or more (n>2) consecutive fragmentation and m/z isolation steps. In an ion trap MS/MS\(^n\) instrument, the mass selection is achieved by trapping ions from the first fragmentation step, ejecting unwanted m/z ions, re-fragmenting, ejecting again, and so on until satisfactory selectivity is attained. The target m/z ions are then ejected and transferred to the detector.

Comprehensive reviews on the analytical chemistry of chiral POPs have recently been published (Vetter and Schurig, 1997; Kallenborn et al., 2001).

### 3.2 Method development

#### 3.2.1 Chromatographic separation

The ultimate goal for chiral chromatography of trace compounds is the complete enantiospecific resolution of all compounds of interest, while avoiding co-elution of compounds (unless the detector can resolve co-eluants) in the shortest possible time and maintaining acceptable signal-to-noise ratios. The choice of a suitable CSP is therefore crucial for satisfactory results. Chromatographic resolution modeling of chiral POPs is in its infancy, and thus trial-and-error is often the only option. However, occasionally some guidance from the literature is available. In Paper I, nine of nineteen stable atropisomeric PCBs were separated on a 2,3,6-\(O\)-methyl (permethylated, PM)-\(\beta\)-CD column. All of the PCBs that were 2,3,6-substituted on at least one ring were separated (except PCB 45). Conversely, none of the congeners in which at least one ring was 2,3,4,6-substituted, and neither ring 2,3,6-substituted, could be separated. Similar results were obtained in reversed phase- (RP-) HPLC separations on PMCD (Haglund, 1996a). In a later study, good enantiomer separation of PCBs with a 2,3,4,6-pattern and poor separation of those with 2,3,6-substitution was found using the 2,3,6-\(O\)-tert-butyldimethylsilylated...
Chapter 3. Enantiospecific trace analysis of chiral POPs

(TBDMS)-β-CD column, i.e. this phase exhibit an inverse chlorine substitution preference (Vetter et al., 1997).

In Paper II, a systematic search was described for a suitable CSP capillary column to resolve the enantiomers of atropisomeric MeSO₂-PCBs present in ringed seal and polar bear (Figure 8). Since the 2,3,6-O-TBDMS-β-CD and 2,3,6-O-PM-β-CD columns had proven to be efficient in separating atropisomeric parent PCBs, two columns with such chiral selectors (#1 and #2, respectively) were chosen. In order to evaluate the importance of the CD-substituents, a β-CD column with TBDMS at the 6-O-position and methyl groups at the 2- and 3-O-positions was also tested (#3). Finally, an identically modified 2,3-di-O-methyl-6-O-TBDMS γ-CD was selected (#4), to assess the importance of the cavity diameter.

The GC oven temperature program was optimized, and an isothermal (final) separation temperature of 230 °C was selected. A lower final temperature (210-220 °C) resulted in marginally improved resolution, but also led to excessive retention times and lower signal to noise ratios. Since the method was designed for trace analysis of environmental samples, the higher final temperature was preferred.

The 2,3,6-O-TBDMS-β-CD column was by far the most selective column for the MeSO₂-CBs tested (Table 1 in Paper II). From our resolution data, it was clear that the separation of enantiomers was enhanced by 3-MeSO₂-substitution as compared to 4-MeSO₂-substitution of the PCB molecule. A favorable partial inclusion complex for one of the enantiomers of the 3-MeSO₂-PCBs pairs may explain this. MeSO₂-CB enantiomeric resolution was poor or non-existent with columns #2-4, suggesting that the enantioselective interactions took place mainly at the 2,3-O-side of the 2,3,6-O-TBDMS CDs. However, explaining this type of phenomenon is far from straightforward, and other possible chiral recognition mechanisms cannot be excluded. Ellerichmann et al. (1998) used a homemade (non-commercial) 2,3-di-O-methyl-6-O-tert-hexylDMS β-CD column. It differed from column #3 only in the substituents at position 6, and yet they achieved good enantiomeric resolution for eight of the atropisomeric
MeSO$_2$-CBs that were tested in our study (Figure 8), notably also for the 4-MeSO$_2$-CBs.

The size of the CD cavity is apparently an important factor for the separation of enantiomers. The only difference between columns 3 and 4 was the number of glucose units in the CD molecule: seven in $\beta$-CD, and eight in $\gamma$-CD. Although none of these columns was suitable for chiral recognition of MeSO$_2$-CBs, the $\beta$-CD gave somewhat better selectivity.

3.2.2 Determination of elution sequences of enantiomers

For accurate risk assessments and comparison of results from different studies, it is essential to identify the (+) and (−) enantiomers. The study outlined in Paper I described the determination of elution sequences of (+) and (−) atropisomeric PCBs on a PM-$\beta$-CD GC column (Chirasil-Dex). Isolation of enantiomers was obtained earlier by RP-HPLC using two serially connected columns filled with silica that had been surface modified with covalently bonded PMCD (Haglund, 1996b). By repeated injection/fractionation cycles for each atropisomeric PCB, milligram quantities of pure (>98%) enantiomers were isolated, and subsequently characterized by polarimetry. Mixtures of these enantiomers were prepared and analyzed using chiral GC-ECD. Six of the nine pairs were completely or partially resolved on Chirasil-Dex and so were readily identified by comparing retention times ($t_R$) of the enantiomers with those of racemic standards.

3.2.3 Detection

In Paper II, two different MS detection methods, EI ion trap MS/MS and ECNI quadropole MS, were compared for the analysis of atropisomeric MeSO$_2$-CBs in tissues of ringed seal and polar bear. For the ion trap analysis, a MS/MS method had to be developed and optimized for MeSO$_2$-CBs sensitivity. In a first step, full scan and daughter ion scans were conducted for selected congeners from each homolog group. Once the
major fragments had been identified, the settings for isolating the parent ion and forming daughter ions (collision induced dissociation, CID, conditions) were optimized. Finally, the limits of detection were determined.

The use of GC- ion trap MS/MS and GC-ECNI-MS-selected ion monitoring (SIM) techniques resulted in similar MeSO₂-CB chromatograms, although different ionization and selection mechanisms were involved (Figure 10). Thus, with appropriate clean-up steps, both techniques appear to be suitable for detecting MeSO₂-CBs in extracts derived from biological samples. Under the conditions of our analysis using the two different instruments, ECNI was able to detect 2 to 5 fold lower amounts of MeSO₂-CBs than the ion trap MS/MS. However, it is important to remember that different instruments

![Figure 10](image-url)  
**Figure 10.** EI-MS/MS and ECNI-LRMS-SIM chromatograms showing chiral analysis of atropisomeric MeSO₂-CBs in polar bear and ringed seal (Paper II).

were used for these experiments. Furthermore, when choosing between these techniques, other aspects also have to be considered. For example, in
contrast to ECNI-SIM, the MS/MS technique provides daughter ion spectra, which contain valuable qualitative information.

### 3.3 Data presentation

There are many different ways to express chiral composition. Until recently, the enantiomeric ratio (ER) was the most frequently used descriptor for the relative abundance of environmental pollutants. ER is defined as:

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

where \(A_+\) and \(A_-\) correspond to the peak areas (or heights) of the (+) and (−) enantiomers (assuming equal molar response factors), respectively. Thus, a racemic mixture has an ER of 1.00. However, there are several limitations in using ER. When used graphically the ER gives a misleading representation of data. Because of the way it is defined, the ER can range from 0 to infinity. Therefore, a unit change in ER away from unity in the downward direction (i.e. < 1) is not equivalent to the same unit change in the opposite direction. Complications may also arise when ER is employed in mathematical expressions.

In Paper III, we proposed that a better representation of the chiral signature is the enantiomer fraction (EF):

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

where \(A_1\) and \(A_2\) are the first and last eluting enantiomers on chiral column ‘X’ when the identity of the (+) and (−) forms is not known. The relation between EF and ER can be expressed either as \(EF = ER / (ER +1)\) or \(EF = 1/(1+1/ER)\).

The EF can only range from 0 to 1.0 with EF=0.5 representing a racemic mixture. Each unit of deviation from the racemic value (0.5), both in the upward and downward direction, is equivalent. Because it is a proper
fraction, the EF can also be applied more naturally in mathematical fate expressions.

The authors of Paper III represented three different research groups. In a contemporaneous paper yet another research group (De Geus et al., 2000) proposed the EF concept as a preferable alternative to ER. They underlined the advantage with a linear scale, and quoted an example from literature where incorrect mean and standard deviation (SD) of ER values were reported since individual ER data were used in the statistical calculations.

3.4 Quality assurance and quality control

In all trace analysis, quality assurance (QA) and quality control (QC) are essential for obtaining data with high precision and accuracy. For EF determinations, peak identification and peak area determinations are crucial steps.

To help ensure correct peak identification and acceptable peak area determination, the following conditions were applied in the chiral analyses in Papers I, II and IV-X:

- Detection was by MS and two or more ions of the molecular ion isotope cluster of the analyte were always monitored. The abundance ratio of the isotope ions had to be within \( \pm 5\text{-}10\% \) relative to the theoretical value for results to be accepted.

- The elution order of enantiomers was determined on each individual CSP-column (for compounds where enantio-enriched or enantio-pure reference standards were available).

- GC retention times had to be within \( \pm 2\text{s} \) compared to reference standards.

- The signal to noise (S/N) ratio had to be greater than 5.

- Racemic standards had to yield EFs of \( 0.50 \pm 0.01 \) (or ERs of \( 1.00 \pm 0.02 \)).
Field and lab blanks were checked for possible interferences in the retention windows of the target compound.

Enantiomeric profiles were compared between samples within the same matrix type.

It is important to note that each individual column had to be checked, since the elution order may be reversed in different columns, even if the same CSP is used (Oehme et al., 1997). Moreover, even if correct isotope ratios are derived, each peak should be carefully examined, in order to exclude co-elution of fragments of more highly chlorinated compounds or other interferences. Note also that co-elution of highly abundant compounds with non-interfering m/z ions may affect enantiomeric resolution (Buser and Müller, 1992). Instead of using enantio-enriched chlordane standards, it is possible to assign the enantiomers by injecting the cod liver oil Standard Reference Material (SRM) 1588 (National Institute of Standards and Technology, NIST), for which isomeric and enantiomeric ratios of chlordane residues have been determined (Mössner et al., 1992; Oehme et al., 1994; Müller et al., 1997).

EF determinations can be checked by using more than one CSP, preferably CSPs with reversed enantiospecific elution sequences (Paper VI). Use of different MS ionization and mass selection techniques (Paper II) is also desirable, but can hardly be regarded as a prerequisite. Analysis of replicates (Paper IV) and repeated injection of samples (Papers I, II and IV-X) yields important information on the measurement uncertainty of the analysis.
4. ALTERATION OF CHIRAL COMPOSITION OF POPs

4.1 Background

The technical pesticide mixtures and the PCB products are generally produced and emitted as racemates, and they will remain racemic as long as they are subjected exclusively to achiral interactions and reactions. Most abiotic reactions (e.g. hydrolysis and photolysis) and processes (e.g. partitioning, leaching, volatilization and atmospheric deposition) are not enantioselective. Analogously, chiral abiotic transformation products will not be formed enantioselectively. For example, solid phase photolysis by natural sunlight of racemic heptachlor in a study by Buser and Müller (1993) yielded racemic photoheptachlor and racemic HEPX. Illumination of non-racemic α-HCH yielded β-pentachlorocyclohexane (β-PCCH) of similar non-racemic composition (Hühnerfuss et al., 1992). However, the abiotic interaction (adsorption) between a crystal surface and a chiral compound may result in enantio-enriched or even homochiral composition (Hazen et al., 2001).

Once the racemic mixtures come into contact with life chemistry processes, the chiral composition frequently alters. The changes are generally small in sea surface water, soil and sediments (Bidleman et al., 2001b) and more pronounced in deep water (Jantunen and Bidleman, 1996; Harner et al., 1999) and biota, especially in mammals and birds (Vetter and Schurig, 1997; Vetter, 2001; Kallenborn et al., 2001; Fisk et al., 2001b). Volatilization of bioprocessed chiral POPs from water and soil frequently result in non-racemic composition of boundary air layers, i.e. air up to some tens of meters above the surface (Bidleman et al., 2001b).

In abiotic compartments, non-racemic composition is suggested to be due to enantioselective degradation by microbes present in the medium (Faller et al., 1991a; Falconer et al., 1997; Aigner et al., 1998; Lewis et al., 1999; Helm et al., 2000; Harner et al., 2000a), while in higher organisms the enantioselective alteration mechanisms are much more complex. Pharmacokinetic processes such as metabolism, excretion, distribution and
absorption may all be stereospecific (Landoni et al., 1997). For drugs, it has been shown that stereospecificity commonly arises in biotransformation, binding to plasma and tissue proteins, and renal clearance processes (Tucker and Lennard, 1990). In contrast, passive processes like diffusion across gastrointestinal and other membranes do not involve macromolecular interactions and are therefore not likely to be stereospecific (Landoni et al., 1997).

Laboratory experiments have confirmed that enantioselective metabolism of POPs occurs. Parent compounds and their metabolites were studied and preferential enantioselective degradation was found in sewage sludge (Buser and Müller, 1993), in water (by marine microbes) (Ludwig et al., 1992), and in rat liver homogenates (Buser and Müller, 1995b). Moreover, bioassays have confirmed that POP enantiomers may differentially induce the formation of CYP enzymes (Rodman et al., 1991).

In contrast, reductive dechlorination of \( o,p' \)-DDT to \( o,p' \)-DDD in the presence of plant (phytodegradation), cultures from anaerobic freshwater sediments, porphyrin hemat in or human hemoglobin occurred non-enantioselectively in a study by Garrison et al. (2000). The pronounced enantioselective excesses of (+)-\( \alpha \)-HCH found in the brain of several species (Mössner et al., 1992; Möller and Hühnerfuss, 1993; Hühnerfuss et al., 1993; Iwata et al., 1998) has prompted the hypothesis that stereospecific mechanisms other than degradation may be important for the chiral composition of POPs in organisms. Results from in vivo experiments indicated that metabolism is not responsible for the enantiomeric excess in brain, but rather enantioselective transport, or constrained enantioselective passage, across the blood-brain barrier (Ulrich et al., 2001).

For metabolites, it is not only enantioselective depletion that matters, but also their formation. Müller and Buser (1994) have shown that abiotic transformation of (+) and (–) trans-chlordane yields (+) and (–) OXY, respectively. The EFs of the parent compounds may thus be essential determinants of the chiral composition of metabolites.
Chapter 4. Alteration of chiral composition of POPs

In summary, the extent of the alteration of the chiral composition of POPs in environmental compartments is fairly well known, but the mechanisms influencing these changes are far from fully understood. There is a current search for factors that control EFs in abiotic and biotic compartments, involving both in situ investigations and experimental in vitro and in vivo investigations. In many cases, screening studies have been done, which need to be followed by more comprehensive work. In the following sections, factors that may influence chiral composition are discussed along with chiral data from various environmental compartments.

4.2 Soil

Chiral studies of o,p'-DDT, chlordanes, HEPX, α-HCH and toxaphene in soil have been conducted mostly on samples from U.S.A (Ohio, Indiana, Illinois, Pennsylvania, Connecticut, Alabama, Hawaii) (Aigner et al., 1998; Eitzer et al., 2001; Bidleman et al., 2001b; Paper IV), together with some from Canada (British Columbia) (Falconer et al., 1997), U.K (Meijer et al., 2001) and Germany (Müller et al., 1992).

In Paper IV, the results from chiral analysis of Alabama soils are presented and discussed. The enantiomeric composition of OC pesticides was investigated in 32 agricultural and three cemetery soil samples from sites scattered throughout the state. The EFs of o,p'-DDT showed great variability, ranging from 0.41 to 0.57, while the EFs of chlordanes and chlordane metabolites were less variable and generally differed significantly from racemic (Figure 11). Enantiomeric excesses of (−)-trans-chlordane, (+)-cis-chlordane, the second eluting enantiomer of MC5, (+)-HEPX and (+)-OXY were found in a large majority of the samples. The enantiomeric composition of α-HCH was racemic or close to racemic. This consistency makes it possible to use enantiomeric signatures to follow soil-air exchange of CHLs and elucidate chlordane sources.

The EFs derived for the Alabama soils were compared with chiral data of other soils (Table 2 in Paper IV and Table 2 in Bidleman et al., 2001b).
The enantiomeric signatures found in Alabama soils (Paper IV) were shown to be similar to those from other sites. Although small regional differences occurred, some common features were clear. In general, the examined soils exhibited

enantiomeric excesses of $(-)\text{-trans}$-chlordane, $(+)$-cis-chlordane and $(+)$-HEPX, ambivalent $o,p'$-DDT and OXY data, and little or no enantioselective degradation of $\alpha$-HCH.

The causes underlying the variability in the enantiomeric composition of chiral pesticides in agricultural soils are poorly understood. Attempts to find soil variables that correlate with the EFs have largely been in vain (Paper IV; Aigner et al., 1998). Recent research has, however, provided insight into how environmental changes, such as deforestation, nutrient enrichment and warming, may shift the enantioselective microbial degradation of chiral pollutants in soils, even to the point of reversing degradation preferences (Lewis et al., 1999). A possible explanation could be that under different environmental conditions different groups of related microbial genotypes tend to dominate.
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4.3 Water

Most aquatic studies of chiral POPs have focused on α-HCH, which is the predominant OC compound detected in the ocean environment (Iwata et al., 1993a). Paper X reports on chiral α-HCH data gathered from sea surface water and other environmental samples from the southern Baltic Sea. In summer 1997 and winter 1998, paired boundary air and surface water samples were collected during cruises in the southern Baltic Sea. Simultaneously, ‘wet and dry’ deposition samples were taken at Gotland Island, located close to the air and water sampling area. Water samples taken shortly after the flooding of the Oder and Wisla Rivers in summer 1997 were also included in the study.

The EFs in the water ranged from 0.439 to 0.455, and thus the variation was very low (RSD 1.1%). The overall mean water EF value, of 0.445, implied that the ratio of the (+) and (−) enantiomers of α-HCH in the surface water of the southern Baltic Sea was about 45:55 all year round. Analysis of samples from both Arctic waters and different sections of the North Sea has shown that the enantio-enrichment in seawater does not always favor the (−) enantiomer (Faller et al., 1991b; Jantunen and Bidleman, 1998). The chiral composition of α-HCH in sea surface water from different regions was compared in Table 3 in Paper X and surface and deep water from oceans and lakes were compared in Bidleman et al. (Bidleman et al., 2001b). The enantiomeric compositions found in our study agree well with earlier Baltic Sea values and also with values from the connecting eastern part of the North Sea.

Different microbial populations have been suggested to be the cause of the reversed enantiomeric dominance in different regions of sea surface water (Faller et al., 1991b; Jantunen and Bidleman, 1998). For lakes, wetlands and streams, enantioselective degradation has been found to be greater in oligotrophic Arctic and sub-Arctic waters compared with warmer and more nutrient-rich lakes in the temperate climate zone (Law et al., 2001). The cited authors hypothesized that enantioselective degradation is favored by close contact between the chemicals and sediments in nutrient-poor waters.
Enantiospecific measurements of CHLs in water have only been reported from the Arctic Ocean (Jantunen and Bidleman, 1998). As in soils, HEPX was enriched in the (+)-enantiomer, while *cis* and *trans* chlordane were both racemic. In contrast, chlordanes in Lake Ontario water were found to be non-racemic (Terry Bidleman, personal communication).

### 4.4 Air and deposition

The chiral composition of POPs in the air is much more variable than in water and soil. There is a constant input from racemic and non-racemic sources by volatilization of new and aged residues. The mixing is rapid and the air masses can be transported long distances within a few days.

In the Baltic boundary air (Paper X), *α*-HCH was clearly non-racemic. The HCH water concentration is typically higher, by a factor of $10^4$, than in the overlying air, and the water column will therefore affect the composition of the boundary air layer (Ridal et al., 1997). Although the overall variation of *α*-HCH EF was low, the average of the summer values (EF=0.464, RSD 2.3%) was significantly lower than winter values (EF=0.481, RSD 0.7%) (modified t-test since the SDs were not equal, p=0.05), as a result of greater volatilization from the water during summer.

There have been several chiral studies of pesticides in the air above water and soil, including measurements of *α*-HCH (Table 1 in Paper IX; Table 3 in Paper X) and CHLs (Table 4 in Bidleman et al., 2001a). A trend generally observed is that air collected above open sea or soil during summer time shows the greatest deviation from racemic composition due to volatilization from the water or soil. Air-surface gas exchange is discussed in Chapter 5.

For the Baltic deposition samples (Paper X), a contrasting observation was made. Summer samples were close to racemic, while winter samples were similar to boundary air, i.e. somewhat enriched in (−)*α*-HCH. These results suggest that *α*-HCH in rain is scavenged mostly from background air rather than from boundary air. Not much is known about the enantiomeric composition of precipitation. In a study of *α*-HCH in samples
collected near Lake Ontario, rainwater was found to be racemic although boundary air was not (Ridal et al., 1997). The cited authors suggested that the rain scavenged nearly racemic $\alpha$-HCH from above the boundary layer, and it did not have time to re-equilibrate with the non-racemic air just above the lake. On the other hand, precipitation collected at the west coast of the Wadden Sea, Germany, in a location where the prevailing winds were from the sea, showed seasonal EF variation of $\alpha$-HCH with the largest deviations from racemic values occurring during the warmer months (Bethan et al., 2001), thus indicating that the rain mainly reflected boundary air.

4.5 Biota

Hydrophobic compounds, such as the POPs, tend to accumulate in the lipids of organisms. The net result of uptake, distribution and elimination of a substance through all routes of exposure, i.e. via air, water, soil, sediment and food, is called bioaccumulation. The term bioconcentration is used for concentration due to exposure via ambient water, whereas biomagnification is a special case of bioaccumulation in which there is an increase in levels of the compounds due to uptake from food.

As already outlined (section 4.1), enantioselective processes in biota may be complex, and they have been far from comprehensively explored. However, it is widely believed that the enantiomeric excess of (non-metabolite) POP enantiomers in organisms principally arises because metabolism occurs enantioselectively. Therefore, the biotransformation ability and capacity of specific organisms is of central importance to the degree of EF alteration that may occur.

There has been quite a number of chiral POP investigations of biota, ranging from marine to terrestrial species, and from low trophic level organisms to top predators including human beings (Papers II, V, VI and VII; Vetter and Schurig, 1997; Vetter, 2001; Kallenborn et al., 2001). Most of these investigations have been in situ studies, although there have also been various in vitro and in vivo experiments (see section 4.1). The
compounds of interest have included chiral OC pesticides, atropisomeric PCBs and their metabolites. The composition of chiral POPs may differ between species (due to differences in genetics, bioaccumulation and trophic level etc.), within populations (due to variations in age, sex, health- and nutrition-status etc.), between populations (due to differences in feeding habits, habitat and genetics etc.) and between tissues. The following sections highlight the search for relationships between chiral POP composition and some of the above-mentioned factors.

4.6 The search for factors that control the EF of chiral POPs in biota

4.6.1 Biotransformation ability and capacity

Biotransformation rates depend on the POP-responsive induction of CYP enzymes. An increase in the level of contamination will therefore often result in elevated biotransformation rates (Letcher et al., 1996). A high proportion of metabolites relative to parent compounds in an organism indicates a high biotransformation capacity. In Paper VII, the proportion of chlordane metabolites (HEPX and OXY) in relation to the combined level of CHLs in gray seal blubber was shown to be linearly correlated with several EFs of OCs. Another measure of biotransformation capacity is the proportion of a stable isomer or congener present relative to the total level of the compound group. For instance, the chiral composition of α-HCH in blubber of male Dall’s porpoise (Phocoenoides dalli) has been found to be linearly related to the β-HCH/ΣHCH ratio (Tanabe et al., 1996). Similar results have been found for species of the North Water Polynya (Canadian Arctic) marine food web (Moisey et al., 2001).

These findings further support the hypothesis that alterations in EFs in biota are largely due to enantioselective biotransformation. Another measure of biotransformation capacity is the CYP enzyme content, since induction of CYP enzymes in hepatic microsomes may be used as biomarkers for xenobiotic exposure. In Paper VI, the dependence of the CYP2B isozyme protein levels on chlordane and HCH concentrations and the ERs was investigated with multivariate statistics. The variance in the CYP2B protein levels could be explained primarily by the variance in
4.6.2 Bioaccumulation

The main routes of POP uptake and elimination for water-breathing organisms are uptake and loss via gills, uptake from food (mainly by feeding on lower trophic level species, biomagnification), metabolism and excretion. Bioconcentration is an important route of exposure for compounds with log $K_{\text{ow}}<4-5$. Uptake from food (biomagnification) becomes increasingly important for compounds with increasing $K_{\text{ow}}$ values up to about log $K_{\text{ow}}$ 7.

Metabolism may be enantioselective, while the other uptake and elimination processes are believed to principally proceed without enantioselectivity. Since fish and invertebrates generally have low metabolic capacity, it is likely that these species and water exhibit similar enantiomeric composition of POPs. In Paper VI, $\alpha$-HCH, trans-chlordane, cis-chlordane and HEPX of Arctic cod ($Boreogadus saida$) were found to exhibit similar chiral composition as the seawater from the Canada Basin of the Arctic Ocean (Jantunen and Bidleman, 1998). Chiral resemblance of $\alpha$-HCH was also found between water, invertebrates and fish from Northwater Polynya (Moisey et al., 2001) and between water, mussels and fish from the North Sea (Pfaffenberger et al., 1992). Furthermore, the EF profile of salmon ($Salmo salar$) showed very close resemblance to that of herring ($Clupea harengus$) from different locations of the Baltic Sea (Paper V and VII). For $\alpha$-HCH, the EF of the seawater was known (Paper X), and it was similar to that of the fish. Where differences in chiral composition between water and water-breathing organisms exist they may be due to enantiospecific metabolism, feeding from higher trophic level animals, or perhaps the food web is based in sediment.
The ability to biotransform POPs generally increases with trophic level, and therefore EFs can reflect trophic transfers. In order to explore the relation between changes in EF and trophic levels, the polar bear food chain in the Resolute Bay (Canadian Arctic) was studied (Paper VI). This food chain is simple because of the limited biodiversity in the Arctic marine environment. The polar bear is the top predator, eating mostly blubber of ringed seal, which in turn consumes largely Arctic cod and amphipods. The chiral composition of \( \alpha \)-HCH and CHLs was examined in cod, ringed seal and polar bear. The cod showed near-racemic mixtures for most of the compounds. In contrast, ringed seal and polar bear samples were frequently non-racemic, probably due to enantiomer-specific biotransformation (Figure 12).

As (\(+\)-\( \alpha \))-HCH was transferred up the food chain, it became more abundant relative to (\(-\)-\( \alpha \))-HCH (Figure 13), while for the CHLs, there were no uniform trends linking the chiral changes and trophic levels.

Apparent chiral biomagnification factors (BFs) were calculated, and up to a 20-fold difference in the BFs between enantiomers was found, clearly demonstrating that enantiomers of chiral POPs may significantly differ in environmental persistence. BFs normalized to CB-153 indicated that OXY was formed by ringed seals, and metabolized by polar bears. However, the chiral composition did not change significantly as a result of these biotransformations. Moisey et al. (2001) determined BFs for enantiomers of \( \alpha \)-HCH in cod to ringed seal (North Water Polynya), and found similar values to ours (BFs = 2-3, and slightly higher values for the (\(+\))-enantiomer).
Chapter 4. Alteration of chiral composition of POPs

Figure 12. GC-MS chromatogram of enantiomers of trans-chlordane (TC), cis-chlordane (CC) and MC5 in Arctic cod and ringed seal.
Figure 13. The enantiomeric ratios (ERs) ± SD of α-HCH in the polar bear food chain. Abbreviations: RS, ringed seal; PB, polar bear (Paper VI).

Dramatic EF changes in α-HCH from one trophic level to the next have also been reported for mussel and eider duck (Pfaffenberger et al., 1992), and in the chiral study of α-HCH in zooplankton, Arctic cod, seabirds and ringed seal, a linear relationship between EF and trophic level has been found (Moisey et al., 2001). It is, however, apparent from the results in Paper VI that EFs do not always change uniformly throughout the food chain. Further indications of this were found in a study of seven species of Arctic seabirds, where EFs of CHLs failed to predict the trophic levels of the birds (Fisk et al., 2001b).

4.6.3 Species

The non-uniform EF trends found for the polar bear food chain in Paper VI suggest that there are species-specific differences in the biotransformation of enantiomers. In Paper V, the chiral composition of α-HCH and CHLs were compared between herring and three different seal species; ringed seal (Phoca hispida), gray seal (Halichoerus grypus), and harbor seal (Phoca vitulina); all collected from points along the Swedish coastline. The results indicated strongly that species-specific differences are important factors influencing the chiral composition of pollutants in Baltic seals, even for the closely related Phoca species. It is possible that the types and characteristics of the biotransformation enzymes vary between species (Fisk et al., 2001a).

Results from earlier and recent chiral α-HCH and chlordane studies also support the idea of enantioselective, species-dependent metabolism (Buser et al., 1992; Tanabe et al., 1996; Iwata et al., 1998; Fisk et al., 2001a).

4.6.4 Sex and age
Several ecological and physiological factors could contribute to different chiral POP composition between individuals of different sexes and ages within a population. Since higher levels can induce CYP enzyme activity (i.e. increase the biotransformation capacity), gender-specific and age-related biotransformation rates could arise if the OC residues are higher in males relative to females or in older individuals compared to the young. The OC concentration in adipose tissue versus age has been studied for several species of mammals, and the trends observed differed depending on the species and type of OC (Norstrom and Muir, 1994). Positive relationships between OC concentrations and age are more frequently found among males than females, and moreover males are generally more heavily contaminated than females (Norstrom and Muir, 1994). The lower levels of OCs in females is due to the fact that they have the advantage of being able to transfer some of their burden to offspring during lactation.

Enantiospecific accumulation of $\alpha$-HCH and CHLs in male and female herring (Paper V), gray seal, ringed seal, harbor seal (Paper V) and ringed seal (Paper VI) were studied. In all cases, the EF profiles were similar between males and females. EF ranges of male and female herring overlapped and EFs of male and female harbor seals were close to identical. Gray and ringed seal exhibited some chiral differences between the sexes. However, all surveys included few individuals, and the results may not be representative for the species.

The EFs have been found to be independent of sex in ringed seals from the Canadian Arctic (CHLs) (Fisk et al., 2001a) and double-crested cormorants ($Phalacrocorax auritus$) from the Great Lakes ($\alpha$-HCH; Iwata et al., 1998). Hitherto, only one study has reported pronounced gender-specific differences in EFs of chiral POPs. Several chlordanes showed significantly different enantiomeric patterns in male versus female cod ($Gadus morhua$), even to the point where the preferential enantiomeric depletion was reversed (Karlsson et al., 2000). The cited authors speculated that this was due to gender-specific enantioselective accumulation/metabolism.

We also assessed the relation between age and EFs of OCs in male polar bear muscle (Paper VI) and female gray seal blubber (Paper VII). In bear,
U82 deviated more from racemic with age, while MC6 and OXY became closer to racemic with age. Significant linear relations were found, and the compounds that showed a linear correlation between ERs and polar bear age (OXY, MC6, U82) are known to be the most persistent chiral CHLs in a Baltic Sea marine food chain (Strandberg et al., 1997). In female gray seal, the EFs of α-HCH, MC5 and MC6 were linearly correlated with age, and all these compounds deviated more from racemic with increasing age.

A significant linear correlation between the EF of α-HCH and age has also been found for ringed seals (Fisk et al., 2001a), but an earlier search for correlations between the age of northern fur seal (*Callorhinia ursinus*) and double-crested cormorants and chiral composition of α-HCH, was in vain (Paper VI; Iwata et al., 1998). A lack of age- and sex-related trends have also been observed for the chiral composition of OXY in gray seal and harbor seal (*Phoculina vitulina* L.) from Iceland (Kallenborn et al., 2001). Naturally, age-related EF changes may be more or less concealed by major pollution sources such as the ambient water (bioconcentration) and contaminated food (biomagnification). Age-EF relationships are therefore more likely to be found for species with high metabolic capacity and long life spans, and for compounds that are very persistent and have large differences in half-life between their enantiomers.

4.6.5 Nutritional and health status

A starved, unhealthy gray seal (Paper V), and diseased eider ducks (Pfaffenberger et al., 1992) were reported to exhibit atypical enantiomeric composition of OCs. In order to investigate whether alteration in EFs may be indicative of poor physical and/or nutritional status, the enantiomeric composition of α-HCH, CHLs and *o,p'-DDT* in female gray seal and salmon from the Baltic Sea was determined (Paper VII).

In the late 1970s, gray and ringed seal were found to be affected by a disease complex characterized by primary lesions in the adrenals (Helle et al., 1976a; Helle et al., 1976b; Bergman and Olsson, 1986). Secondary reactions in other organs, including occlusions and stenoses of the uterus, highly reduced the capacity for reproduction. Many of the diseased
individuals were also found to be starved. There were strong indications that exposure to OC pollutants, especially PCB and DDT, were related to the disease (Olsson et al., 1994). So, for the analyses reported in Paper VII, samples from adult females with variations in nutritional status, health and age were selected. The samples were divided into five subgroups (A-E) and three main groups (AB, CD and E) depending on health and nutrition condition.

Another species in the Baltic Sea affected by severe reproductive failure, as part of a disease syndrome named M74, was *Salmo salar*, the Baltic salmon (Bengtsson et al., 1999). Muscle from healthy female sea-run Baltic salmon and from salmon that produced offspring with the M74 syndrome was also included in the study (Paper VII).

Significant differences in enantiomeric composition were found between the seal groups (Figure 14), but not between M74 and non-M74 salmon.

The relationships between variables describing age, health, nutritional-status and chemical variables (concentrations and EFs) of selected OCs were investigated using uni- and multi-variate analyses (linear regression and Partial Least Squares regression, PLS). Our results suggested that highly altered EFs might be good indicators of starvation and high biotransformation capacity for gray seal. More studies, including both healthy and sick animals of similar age, are needed to find out whether atypical EFs also can be used as indicators of poor health status.

Deviations from racemic composition of OC pollutants were positively correlated with age, which in turn strongly correlated with symptoms linked to the seal disease complex that may lead to reproductive failure. It was therefore not possible to state that strong deviation from racemic composition is directly related to poor health or reproductive ability. On the other hand, a link could not be excluded on the basis of our data. According to our results, it is however unlikely that enantiomeric composition of OC pesticides in salmon muscle is linked to the disease syndrome M74.
In a recent study of CHLs in adipose tissue from patients suffering from Non-Hodgkin’s lymphoma and a control cohort, it was concluded that the chiral composition of the CHLs was not associated with the disease (Karlsson, 2001).

4.6.6 Populations

Different populations of the same species occupy different habitats and may feed upon different prey. This may lead to significant differences in exposure and chiral composition of POPs.
The influence of collection site on the enantioselective accumulation of α-HCH and CHLs was studied in three-year-old herring from three locations in the Baltic Sea, ranging from the Bothnian Bay in the north to the Baltic proper in the south (Paper V). There were no clear spatial trends in the EF profiles, indicating that the environmental variations among habitats within the Baltic Sea do not give rise to significant alterations in EFs.

Similarly, ringed seals from two different populations in the Canadian Arctic (east and west Northwater Polynya) exhibited similar EFs of CHLs (Fisk et al., 2001a). However, it has been shown that under certain circumstances chiral differences between populations will arise. For example, the composition of α-HCH enantiomers in double-crested cormorants from Lake Michigan was significantly non-racemic, while the Lake Superior population showed close to racemic values, and in a study by (Iwata et al., 1998), EFs in Dall’s porpoise (Phocoenoides dalli) from the Bering Sea were found to differ significantly between populations from the North Pacific and Japan Sea (Tanabe et al., 1996).

4.6.7 Tissues

Apart from the brain and spinal marrow investigations discussed in section 4.1, few other chiral POP tissue distribution studies have been carried out.

In the liver, the metabolic capacity is higher than in other organs. Therefore, one would expect larger deviations from racemic EFs in the liver compared to the blubber. In Papers V and VI, the compositions of α-HCH and chlordane enantiomers were investigated in blubber and liver tissues of gray seal, ringed seal, harbor seal and polar bear. Many of the compounds, including α-HCH, MC5, MC6 and MC7, followed the expected pattern. However, the trend was not seen for all the compounds. For example, the octachlordane U82 showed an opposite trend in all of the species, with higher deviations from racemic in the blubber than in the liver. Other compounds, including metabolites, were more variable. For the
metabolites, increased transformation rates in the liver could be concealed by the simultaneous formation.

Larger deviations from racemic values in liver compared to blubber have also been found for $\alpha$-HCH in Icelandic polar bear (Klobes et al., 1998) and for HEPX in harbor seal (Kallenborn et al., 2001). No such differences were seen for $\alpha$-HCH in fur seal (*Callorhinus ursinus*), although the impression that differences were observed was mistakenly given in Paper V. Neither were any differences detected in chlordane EFs between liver and fat of Arctic seabirds (Fisk et al., 2001b) and in muscle, gonad and liver of cod, the chiral composition showed great consistency (Karlsson et al., 2000). The results discussed here indicate that animals with large fat reserves, such as seals, are more likely to exhibit differences in chiral POP composition between tissues.
Chapter 5. Enantiomers of chiral POPs as chemical markers for abiotic processes

5. ENANTIOMERS OF CHIRAL POPs AS CHEMICAL MARKERS FOR ABIOTIC PROCESSES

5.1 Background

The physicochemical properties that affect diffusive exchange (water solubility, vapor pressure, Henry’s law constant, $K_{ow}$) are equal for enantiomers, and thus chiral signatures of water and soil are retained upon volatilization. Therefore, the enantiomeric composition of a chiral pollutant in air can yield information on whether the compound has been subjected to deposition and revolatilization in the environment. A composition similar to the technical product (close to racemic) indicates fairly fresh release, while enantio-enrichment suggests that the compound was released some time ago and has since been subjected to recycling from water and soil (Paper IX).

Advective transport (passive transport of a compound via a moving medium) is also non-enantioselective. EFs can therefore also be used as chemical markers within compartments, e.g. as tracers of the origin of air and water masses and to differentiate between young (surface) water from older (deeper) water. Pollutants that are present as non-racemic mixtures are often referred to as ‘aged’, ‘weathered’, ‘recycled’ or even ‘ghost of the past’ residues.

The enantiomeric signatures of chiral pesticides in the boundary air layer (i.e. tens of meters above the surface) depend on the EFs of the volatilized and advected compounds. It is possible to use EFs for gauging the relative input of two sources (e.g. volatilized and advected compounds). In a water-air system, the fraction released from the water to the boundary layer ($F_w$) is:

$$F_w = \frac{EF_m - EF_a}{EF_w - EF_a}$$

(1)
where $EF_m$, $EF_w$ and $EF_a$ are the EFs of the boundary layer, the water and the background air, respectively (Figure 15) (Bidleman and Falconer, 1999a; Paper III). In a soil-water system the $EF_w$ is replaced by $EF_s$, which is the EF

$$f_{\text{water}} = \frac{(EF_{\text{boundary air}} - EF_{\text{background air}})}{(EF_{\text{water}} - EF_{\text{background air}})}$$

**Figure 15.** The fraction of the chiral pollutant that has volatilized from water to boundary air ($f_{\text{water}}$) can be estimated from the EFs of the surface water, boundary air and background air.

in the soil. Note that the erroneous formula (1 and 2) in Paper IX were corrected in Bidleman and Falconer (1999a).

OC pesticide enantiomers have been utilized as tracers in fresh water (Falconer et al., 1995; Ridal et al., 1997; Helm et al., 2000; Law et al., 2001), sea water (Faller et al., 1991b; Jantunen and Bidleman, 1996; Harner et al., 1999; Harner et al., 2000), air (Paper X; Bidleman et al., 2001a) and deposition (Paper X; Bethan et al., 2001) and also in air-surface gas exchange studies (Bidleman et al., 2001; Papers IX and X). In the following sections, applications of chiral OC tracers in air, air-water and air-soil systems are presented.
Chapter 5. Enantiomers of chiral POPs as chemical markers for abiotic processes

5.2 Air transport and air-water gas exchange

The air-water gas exchange of a compound is a diffusive process that is best assessed through fugacity and flux calculations. To assess the relevant fugacities and fluxes, environmental temperatures, concentrations and wind-speed at the time of the sampling have to be determined. The relationships for fugacity and flux calculations are given in Paper X (derived from Bidleman and McConnell, 1995 and Harner et al., 2001).

The air-water gas exchange of POPs may exhibit long-term trends, seasonal cycles and short-term variations, leading to periods with higher or lower net volatilization of the pollutant. Long-term trends may be observed as a consequence of declining use and global fractionation, while seasonal cycles are due to changes in temperatures, air concentration, stratification and ice-cover of the water column. Short-term variations are likely because of constant shifts in the origins of air masses. There may also be latitudinal differences in net volatilization, due to latitudinal temperature and concentration differences.

For HCHs, these variations may be mirrored in isomeric and enantiomeric compositions of the boundary air, thus providing complementary information to fugacity and flux calculations. In analogy with the EF concept, the relation between $\alpha$- and $\gamma$-HCH isomers may be expressed as the fraction $\alpha$-HCH ($\text{Fr}_\alpha$), defined as $\alpha$-HCH / ($\alpha$-HCH + $\gamma$-HCH; Paper X). The $\alpha/\gamma$-ratio in technical HCH is 4-7 (Hinckley et al., 1991; Iwata et al., 1993a), which corresponds to a $\text{Fr}_\alpha$ value of 0.80-0.88, while a $\text{Fr}_\alpha$ (± SD) of 0.71 ± 0.10 reflects northern hemisphere background air (average of monthly measurements at Pallas, Finland, 1996-97; Brorström-Lundén et al., 2000). High $\text{Fr}_\alpha$ values indicate a technical HCH source, which is subsequently aged due to long range (LR) transport, whereas low values are typically associated with regional lindane usage (Oehme et al., 1996; Haugen et al., 1998).

Paper X describes a study of HCH air-water deposition in the southern Baltic Sea (see also 4.3 and 4.4), in which fluxes and fugacities were calculated, and enantiomers and isomers were used as tracers. According to the fugacity calculations, $\alpha$- and $\gamma$-HCH were close to air-sea partitioning...
equilibrium. However, both seasonal and short-term variations were seen in the fugacities, fluxes, and enantiomeric and isomeric patterns. For example, during summer, the boundary air showed larger deviations from racemic values (volatilization) and low Frα values in some cases (reflecting regional use of lindane). Short-term variations were correlated with air mass origins, as shown by back trajectories. The variations in air mass origin were clearly reflected in net air-

### Fraction α-HCH from Water in Boundary Air

- Background Air EF=0.500
- Background Air EF=0.485 (Rörvik)
- Background Air EF=0.492 and 0.481 (Hoburg)

![Graph showing fraction α-HCH from water to boundary air](image)

**Figure 16.** The fraction of the chiral pollutant that has volatilized from water to boundary air (f_water) can be estimated from the EFs of the surface water, boundary air and background air (Paper X).

... sea gas exchange parameters and isomeric and enantiomeric ‘signatures’ in boundary air. Enantiomer fractions were used to estimate the fraction of α-HCH in the boundary air layer that had volatilized from the water. During summer, the fraction was approximately 60%, while in the winter it was significantly lower (0-35%). The estimated fraction vary depending on the choice of EF in background air (Figure 16).
Seasonal variations were also found for EFs of α-HCH in Lake Ontario air (Ridal et al., 1997), and latitudinal variation was confirmed by measurements of Arctic and sub-Arctic waters (Jantunen and Bidleman, 1996). Fugacity and flux calculations, together with chiral analyses, suggest a that a long-term change in α-HCH Arctic Ocean-air exchange is occurring, in which the direction of net exchange has reversed in response to decreasing atmospheric levels of HCHs, from deposition in the 1980s to volatilization in the 1990s (Bidleman et al., 1995; Jantunen and Bidleman, 1995a; Jantunen and Bidleman, 1996). Recent measurements indicate that α-HCH is close to air-water equilibrium in the eastern Arctic Ocean (Harner et al., 1999). Ice-cover has been shown to hinder the gas-exchange since α-HCH was racemic in air above the ice, even though underlying water was oversaturated with non-racemic α-HCH (Jantunen and Bidleman, 1996).

5.3 Air-soil gas exchange

Chiral OC compounds that show consistency in preferential enantioselective degradation in soil can be used as global or regional tracers for air-soil gas exchange (Figure 17). For a large majority of the soils that have been analyzed, enantiomeric excesses of (−)-trans-chlordane, (+)-cis-chlordane and (+)-HEPX have been observed (see also 4.2). Chiral OCs that show little or ambivalent enantiomeric depletion (such as OXY, o,p′-DDT and α-HCH) may be used as local chemical markers (Jantunen and Bidleman, 1996; Finizio et al., 1998; Leone et al., 2001).

Evaporation of OC pesticide residues from soils is a significant source of atmospheric contamination. In Paper VIII, chiral composition of air and soil was used to investigate the source of atmospheric HEPX. Residues of heptachlor are racemic in air (Paper VIII; Buser and Müller, 1993), and the photolysis of heptachlor yields HEPX non-enantioselectively. Epoxidation of heptachlor also occurs in soils, mammals and houseflies (Donnelly et al., 1993), where transformation may be enantioselective. HEPX in air has been found to be distinctively non-racemic, with the (+)-enantiomer dominating, just as it does in soils (Paper VIII). These findings suggest
that the main source of HEPX is not photolysis of heptachlor (which may be a minor contributing factor), but rather emissions of HEPX from soils (Paper VIII). This hypothesis is also supported by the findings of Finizio et al. (1998) in a study investigating the emission of chiral pesticides from an agricultural soil in the Fraser Valley, British Columbia. The EFs of HEPX did not change with height above the soil, although the concentration declined upwards in the vertical profile.

The airborne chlordane in northern Alabama and the city of Columbia, South Carolina, was reported to be close to racemic by Jantunen et al. (2000), whilst chlordane in Alabama soils was non-racemic (Paper IV).

However, chlordane in indoor air was found to be racemic (Wiberg et al., 1997; Leone et al., 2000), and the concentrations in Alabama homes greatly exceeded those in the ambient air (geometric mean = 22 times higher than ambient). Therefore, emissions from houses treated with termiticide (chlordane) and long-range transport from countries where chlordane was recently used were suggested to be the most significant sources, rather than release from agricultural fields (Wiberg et al., 1997;
Chapter 5. Enantiomers of chiral POPs as chemical markers for abiotic processes

Paper IX). By contrast, ambient air in the Great Lakes region appeared to receive chlordane inputs from both non-racemic sources (revolatilized soil and water residues) and racemic sources (Paper IX; Aigner et al., 1998; Ulrich and Hites, 1998; Bidleman and Falconer, 1999b; Leone et al., 2001). A large achiral data-set, including data from the analysis of remote (Arctic) air sampled at stations in Canada, Russia and Finland over a 14-year period (1984-98), has been examined to discern temporal trends for chlordanes in background air. The most recent samples (1993-98) were analyzed enantioselectively. Isomeric and enantiomeric composition indicated that during recent years, greater proportions of chlordanes in the air have been recycled from soils (Bidleman et al., 2001a).
6. CONCLUDING REMARKS AND CALL FOR FUTURE RESEARCH

Our knowledge about the fate and distribution of enantiomers of chiral POPs has increased dramatically in the last decade. Nevertheless, many questions remain unanswered and several findings are puzzling.

There is a need for further research within the chiral pollutant field, and for this it is essential to have good analytical tools for trace (below ppm level) and ultra-trace (below ppt level) analysis. A versatile capillary CD column, which resolves the most important chiral OCs, is not presently available, but would be highly desirable. Use of such a column in a GCxGC system would increase the probability of obtaining unbiased enantiospecific results. The GCxGC technique is in a rapidly developing stage, and hopefully within a short time, difficulties with integration and other types of data processing will be overcome.

Another problem with current CD columns is that their performances often vary from batch-to-batch. This problem is attributed to poor reproducibility in the derivatization process. Some types of CSPs also change upon storage. Furthermore, the commercially available capillary CD columns generally have non-bonded phases, leading to excessive column bleeding and high chemical background levels in GC-MS and GC-ECD analysis. Chemically bonded columns would enable higher temperature separations, prolong column life, and improve S/N ratios.

There is also a need for additional enantio-enriched or pure enantiomer reference standards. Some of the minor chiral components in the technical products bioaccumulate and become increasingly important along the food chain. EFs for these are frequently reported, but often without assignment, making comparisons between studies difficult. A chiral intercalibration study for biota including the minor components is needed. This matrix is complex and knowledge about the accuracy of EF determination is largely lacking.
Enantioselective degradation appears to be central to changes in enantiomeric composition. However, the underlying causes of enantiomeric excess in various matrices are far from fully understood. How important are achiral mechanisms, such as adsorption to mineral surfaces, for chiral selectivity in environmental samples? How can waters in one region have reversed enantiomeric composition relative to a region nearby? Why are there gender specific differences in fish? What are the mechanisms behind species specific EFs? Apart from metabolism, are other pharmacokinetic processes, such as uptake, excretion and distribution, important for the chiral composition in biota? A few attempts have been made to isolate and identify microbial communities and enzymes responsible for these phenomena for use in *in vitro* experiments, but very few *in vivo* studies have been conducted. More such studies are needed.

There seems to be a link between the capacity of organisms to biotransform xenobiotics and their chiral POP composition, but very little is known about whether enantio-enrichment in organisms may be related to various factors affecting them, such as diseases and physiological changes. If that is the case, EFs may also be useful as markers for poor health and nutritional status of biota. Thus, the search for correlations between enantiomeric excesses and various parameters in biota should be continued. There is also a need for more enantiospecific toxicity studies.

Enantiomeric pattern analysis has proven to be very useful as chemical markers in abiotic processes. Studies of original sources of banned POPs may provide important knowledge for analyzing the fate of these unwanted compounds. Isomeric and enantiomeric pattern variables provide information that is complementary to contaminant levels, and should be included in environmental data-sets for optimal evaluation.
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