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Review

Enantioselective determination of chiral organochlorine compounds in biota by gas chromatography on modified cyclodextrins

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Abstract

Approaches to the gas chromatographic enantiomer separation of chiral organochlorines (α-hexachlorocyclohexane, *cis*- and *trans*-chlordane, heptachlor, heptachlorepoxide, oxychlordane, *o,p'*-DDT, compounds of technical toxaphene and stable atropisomeric polychlorinated biphenyls) are reviewed. Chiral stationary phases based on cyclodextrin derivatives and used for the gas chromatographic enantiomer separation of the chiral organochlorines are described. Enantiomeric ratios of chiral organochlorines in technical mixtures and biological samples are reported and discussed. © 1997 Elsevier Science B.V.

Keywords: Reviews; Water analysis; Soil; Environmental analysis; Enantiomer separation; Chiral stationary phases, GC; Enantiomeric ratio; Cyclodextrin-based stationary phases; Organochlorine compounds; Pesticides; Polychlorinated biphenyls

Contents

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1. Introduction

Due to the different behaviour of enantiomers in a chiral environment, the relationship between chirality and biological properties plays an important role in pesticide chemistry [1]. According to data published at the beginning of the 1980s, 537 of 550 pesticides were synthetic products and only seven of 90 chiral pesticides were applied as enantiopure compounds [1]. Ten years later, the Pesticide Manual lists one order of magnitude more pesticides [2] but the percentage of enantiopure pesticides has not significantly increased.

Organochlorine compounds belong to the category of synthetic products. Although many organochlorine pesticides are chiral and exist as pairs of enantiomers—in the case of α-hexachlorocyclohexane (α-HCH) this was already realized in 1949 [3]—this fact was not considered during the production climax between 1950 and 1980. Chirality was also ignored for atropisomeric congeners of polychlorinated biphenyls applied in million tons in different industrial uses.

Anthropogenic organochlorines accumulate in the environment as ubiquitous and persistent pollutants: these lipophilic xenobiotics are found in biota yielding ppt–ppm concentrations in fatty tissue. The determination of enantiomeric ratios (ERs) of traces of organochlorines by high-resolution gas chromatography (HRGC) was possible with the introduction of suitable chiral stationary phases (CSPs) a decade ago. Since then, a number of CSPs with modified (O-derivatized) cyclodextrins have been applied for the enantiomer separation of chiral organochlorines [4–7]. These cyclic oligosaccharides contain 6–8 α-D-glucose units linked together by α-1,4 glycoside bonds [8]. Suitable CSP for the enantiomer separation of organochlorine compounds by GC have cyclodextrins alkylated, acylated and/or silylated in the terminal 2,3 and 6-positions. The application of HRGC with different CSPs allowed to collect data in a number of different matrices. ERs of several organochlorines have been determined in air, water, as well as in low and high trophic animals.

Technical HCH, DDT, chlordane and toxaphene represent the most applied organochlorine pesticides [9]. However, it is not always the insecticidal active compound of the technical product which is chiral. E.g., the insecticidal γ-HCH isomer lindane is achiral but the major compound of technical HCH, α-HCH,

is chiral. The chiral *o,p*-DDT is a minor compound in technical DDT which is dominated by the achiral insecticide *p,p'*-DDT. The chiral oxychlordane is a metabolite of chiral chlordane compounds. Polychlorinated biphenyls may include nineteen stable atropisomers [10] and the agrochemical toxaphene more than 100 chiral compounds.

The present comprehensive review summarizes the gas chromatographic techniques employed, the applied CSPs and the ERs of chiral organochlorines observed in water, soil and biological samples.

2. Quantitation of mixtures of enantiomers — definitions

For the quantitation of a mixture of enantiomers several terms have been used in the literature. Enantiomers (from Greek *enantios meros*: the other, opposite part) are defined as one of a pair of molecular entities which are mirror images of each other and non-superimposable [11]. Any chiral molecule exists as a pair of enantiomers. Note that this definition does not explicitly reserve specific names for the two distinct entities. To describe enantioenrichment of chiral organochlorines in samples the terms enantiomeric excess and enantiomeric ratios are most frequently used. However, several other terms have also been applied [12] which are of minor interest for the present survey.

2.1. Definition of the enantiomeric ratio (er and ER)

The er is directly obtained by peak integration of NMR spectra and of chromatograms. The term er is defined in Eq. (1).

$$\mathrm{er} = \frac{E_1}{E_2} \tag{1}$$

Unfortunately, the term enantiomeric ratio er was defined using E_1 as the major enantiomer [13–16]. However, this definition would not allow to distinguish which enantiomer is enantioenriched. Therefore, in the field of organochlorine research, the following differentiation is customary:

If E_1 is the major enantiomer, the enantiomeric ratio is given with the conventional small letters as "er".

If E_1 is not necessarily the major enantiomer the enantiomeric ratio is given with capital letters as "ER".

ER is the term of choice to define enantiomeric ratios of organochlorine compounds in residual analysis. In environmental chemistry, ERs are mostly based on the ratio dextrorotary/levorotary enantiomer or—if the sign of the specific rotation of the individual enantiomers is not known—on the ratio of the first/second eluted enantiomer under defined chromatographic conditions. ER = 1 for a racemic mixture, ER < 1 if $E_1 < E_2$ (optimum value 0) and ER > 1 if $E_1 > E_2$ (optimum value ∞).

ER is a direct measure of the kinetic ratio k_{E1}/k_{E2} in enantioselective reactions $-\Delta\Delta G^{\#} = -\Delta\Delta H^{\#} + T\Delta\Delta S^{\#} = RT \ln \mathrm{ER}$ [14,15].

Note that the term of enantiomeric composition has also been used as a term for the proportion of $E_1{:}E_2$ or as the mole fraction of enantiomers of chiral compounds present in a mixture [17].

2.2. Definition of the enantiomeric excess (ee)

The ee which expresses the excess of one enantiomer over the other is defined in Eq. (2).

$$\mathrm{ee} = \frac{E_1 - E_2}{E_1 + E_2} \tag{2}$$

where E_1 and E_2 are the amounts of the enantiomers and E_1 is the major enantiomer. The magnitude of the ee extends from ee = 0 for the racemic mixture to ee = 1 for pure E_1.

In practice, ee is often quoted as a percentage (see Eq. (3)):

$$\%\mathrm{ee} = \frac{E_1 - E_2}{E_1 + E_2} \cdot 100 = \%E_1 - \%E_2 \tag{3}$$

Note that for a mixture of the proportions $E_1{:}E_2 = 99{:}1$, % $E_1 = 99$ but % ee = 98.

The expression enantiomeric purity has also been used as a synonym for ee but also for other definitions [13,18–20].

As in the case of ERs, there is a need to define ee without the requirement that E_1 is the major enantiomer. Unless negative values are accepted for ee, the

major enantiomer is not identified by the ee definition.

The enantiomeric excess ee is compatible with the law of mixing (mixing of samples of different ee) and it is useful for calculating the corrected ee when auxiliary compounds with ee<1 are used in enantioselective synthesis [14]. % ee is equivalent to the optical purity % op measured by polarimetry in the absence of nonlinear effects [13].

2.3. Relationship between ER and ee

Eqs. (4) and (5) show the relationship between ER and ee.

$$ER = \frac{(1 + ee)}{(1 - ee)} \quad (4)$$

$$ee = \frac{(ER - 1)}{(ER + 1)} \quad (5)$$

In principle, both % ee and ER are useful for the quantitation of a mixture of enantiomers but ER will be the term of choice in this survey.

3. Nomenclature of modified cyclodextrins

Modified or O-terminated cyclodextrins may have three different substituents, and the chemical name of a CSP is composed according the following rules of the International Union of Pure and Applied Chemistry (IUPAC): (i) substituents are ordered with respect to the first letter in the name following "O" with the exception of prefixes like "*n*" in *n*-pentyl and "*tert.*-" in "*tert.*-butyl". Numbering of the positions is not considered. Example: "6-O-*tert.*-butyl-2-O-ethyl-3-O-methyl". (ii) Prefixes "di" or "tri" are not considered in front of "O" but after "O": example: "2,6-di-O-methyl-3-O-di-*tert.*-butylmethylsilyl".

4. Enantioselective gas chromatography with modified cyclodextrins

4.1. The state-of-the-art

The first (albeit unsuccessful) attempt to separate the enantiomers of chiral saturated aliphatic hydrocarbons, devoid of any functionality, was performed in 1981 using permethylated β-cyclodextrin diluted with OV-101 or squalane and coated onto a glass capillary column by Kim in the Tübingen laboratory [21]. The first successful gas chromatographic separation of enantiomers was demonstrated by Koscielski et al. [22]. They separated the apolar racemic hydrocarbons α- and β-pinene on packed columns coated with native α-cyclodextrin dissolved in formamide. Despite a large separation factor α, the columns had a limited life time and efficiency was poor. In another pioneering publication, Juvancz and coworkers demonstrated that alkylated cyclodextrins can be employed in capillary columns for high-resolution enantiomer separation. Thus, molten permethylated β-cyclodextrin (heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin) was used at high temperatures [23–25]. In order to overcome the problems associated with the high melting point of permethylated cyclodextrin, two different strategies were followed afterwards:

(i) Schurig and coworkers dissolved permethylated β-cyclodextrin in moderately polar polysiloxanes (e.g., OV-1701, see Table 1 below) [26–29]. By diluting the chiral selector, the inherent enantioselectivity of cyclodextrins is combined with the unique gas chromatographic properties of polysiloxanes. A logical extension of this approach consisted in the fixation of the enantioselective cyclodextrin selector to the polysiloxane backbone by a permanent chemical linkage [30,31]. The fixation of the resulting Chirasil-Dex stationary phases on the fused-silica surface by thermal immobilization represented another refinement of the methodology [32]. The immobilized Chirasil-Dex stationary phase (commercially available from Chrompack International, Middelburg, Netherlands) is resistant to column bleeding, it is compatible with solvent intake (and thus amenable to on-column injection techniques) and is insensitive to temperature shock. The temperature range lies between −25°C to 250°C. The immobilization of Chirasil-Dex was a prerequisite for extending the scope of enantiomer separation to involatile enantiomers using supercritical fluid chromatography (SFC), open tubular electrochromatography (OT-EC) and open tubular liquid chromatography (OT-LC) [33,34].

Table 1
McReynolds indices of CSPs and achiral stationary phases

Stationary phase	McReynolds index[a]
Heptakis(2,3,6-tri-O-*n*-pentyl)-β-cyclodextrin	647 [5]
Heptakis(3-O-methyl-2,6-di-O-*n*-pentyl)-β-cyclodextrin	756 [5]
Heptakis(3-O-acetyl-2,6-di-O-*n*-pentyl)-β-cyclodextrin	1540 [5]
Octakis(3-O-acetyl-2,6-di-O-*n*-pentyl)-γ-cyclodextrin	1603 [5]
100% dimethylpolysiloxane (OV-1; OV-101; CP-Sil 5, DB-1)	229 [5,79]
94% methyl, 5% phenyl, 1% vinyl polysiloxane (SE 54)	336 [79]
14% phenylcyanopropyl, 86% dimethylpolysiloxane (OV-1701, CP-Sil 19)	705 [79]
50% dimethyl, 50% diphenylpolysiloxane (OV-17; Rtx 50; CP-Sil 24)	884 [5]

[a] McReynolds index $I = I_{benzene} + I_{n\text{-butanol}} + I_{2\text{-pentanone}} + I_{nitropropane} + I_{pyridine}$.

(ii) König and coworkers discovered that per-*n*-pentylated cyclodextrins are fluid at room temperature. Consequently, the following cyclodextrin derivatives (trade name Lipodex) were used in the undiluted form for the separation of enantiomers of many classes of compounds using deactivated glass capillary columns and fused-silica columns (commercially available from Macherey–Nagel, Düren, Germany) [5,35–38]:

Hexakis(2,3,6-tri-O-*n*-pentyl)-α-cyclodextrin	(Lipodex A)
Hexakis(3-O-acetyl-2,6-di-O-*n*-pentyl)-α-cyclodextrin	(Lipodex B)
Heptakis(2,3,6-tri-O-*n*-pentyl)-β-cyclodextrin	(Lipodex C)
Heptakis(3-O-acetyl-2,6-di-O-*n*-pentyl)-β-cyclodextrin	(Lipodex D)
Octakis(3-O-butanoyl-2,6-di-O-*n*-pentyl)-γ-cyclodextrin	(Lipodex E)

The following more polar cyclodextrin derivatives, coated on fused-silica capillary columns, have been applied by Armstrong and coworkers (these and further CSPs are commercially available from Astec, USA) [39–42]:

Hexakis(O-(*S*)-2-hydroxypropyl)-per-O-methyl)-α-cyclodextrin	(PMHP-α-CD)
Heptakis(O-(*S*)-2-hydroxypropyl)-per-O-methyl)-β-cyclodextrin	(PMHP-β-CD)
Hexakis(2,6-di-O-*n*-pentyl)-α-cyclodextrin	(dipentyl-α-CD)
Heptakis(2,6-di-O-*n*-pentyl)-β-cyclodextrin	(dipentyl-β-CD)
Heptakis(2,6-di-O-*n*-pentyl-3-O-trifluoroacetyl)-β-cyclodextrin	(DPTFA-β-CD)

The presence of three hydroxyl groups of different reactivity offers an enormous number of possible cyclodextrin (α, β, γ) derivatives, cf. Table 6 in Ref. [43].

Blum and Aichholz introduced per-*tert.*-butyldimethylsilylated β-cyclodextrin in enantioselective gas chromatography [44] (commercially available from BGB, Adliswil, Switzerland). The regioselective introduction of the bulky *tert.*-butyldimethylsilyl group (a typical protecting group for synthesis purposes) at the primary C_6-hydroxy groups influences the conformation of the cyclodextrin and blocks the entrance at the smaller rim. This may have an important impact on enantioselectivity. Thus, heptakis(2,3-di-O-acetyl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin and heptakis(6-O-*tert.*-butyldimethylsilyl-2,3-di - O - methyl)-β-cyclodextrin have been employed as useful complementary CSPs [45,46].

A combination of the advances (i) and (ii), i.e., the dissolution of various cyclodextrin derivatives in polysiloxanes, e.g., OV-1701, as well as the use of Chirasil-Dex type CSPs, proved the most useful approach to enantiomer separation.

4.2. Thermodynamics of enantioselectivity in gas chromatography

In gas chromatography, enantioselectivity is governed by thermodynamics and it is defined by the free enthalpy (Gibbs energy) difference $-\Delta_{R,S}$ (ΔG) of the diastereomeric associates between the cyclodextrin selector R′ and the enantiomers *R* and *S* of the racemic selectand. For temperature dependent investigations the Gibbs–Helmholtz Eq. (6) applies

[47] (K_R and K_S refer to the formation constants of the diastereomeric association between the selector R′ and the enantiomers R and S whereby arbitrarily $K_R > K_S$)

$$-\Delta_{R,S}(\Delta G) = -\Delta_{R,S}(\Delta H) + T\Delta_{R,S}(\Delta S) = RT \ln K_R/K_S \quad (6)$$

which may be rewritten as the Van't Hoff plot

$$\frac{-\Delta_{R,S}(\Delta G)}{T} = \frac{-\Delta_{R,S}(\Delta H)}{T} + \Delta_{R,S}(\Delta S) = R \ln K_R/K_S \quad (7)$$

As expected for an association process, $-\Delta_{R,S}(\Delta H)$ and $\Delta_{R,S}(\Delta S)$ compensate each other in determining $-\Delta_{R,S}(\Delta G)$. Therefore, an isoenantioselective temperature T_{iso} exists at which enantiomers cannot be separated due to peak coalescence [47–50].

$$T_{iso} = \Delta_{R,S}(\Delta H)/\Delta_{R,S}(\Delta S) \text{ for } -\Delta_{R,S}(\Delta G) = 0 \quad (8)$$

Below T_{iso}, enantiomer separation is enthalpy-controlled and the R enantiomer is eluted after the S enantiomer while above T_{iso} enantiomer separation is entropy-controlled and the S enantiomer is eluted after the R enantiomer (reversal of the elution order, peak inversion). Thus, peak reversals may occur at different temperatures as the result of entropy/enthalpy compensation. There are only rare cases of peak inversions reported thus far [48–50]. Only when the Van't Hoff plot is linear over a wide temperature range including T_{iso} [48,50], the thermodynamic principle of entropy/enthalpy compensation is clearly established. In principle, temperature dependent multimodal recognition mechanisms opposing each other, may also account for the observation of an isoenantioselective temperature [51–53].

It is important to realize that most enantiomer separations are governed by enthalpy control. Therefore, the temperature of the measurement should be decreased at an acceptable level in order to increase the separation factor α.

4.3. Precision of gas chromatographic enantiomer analysis

The precision of ERs determined by gas chromatography is high [54–56]. It is always advantageous if the minor enantiomer is eluted as the first peak from the gas chromatographic column. The change of the elution order can easily be obtained by change of the chirality of the CSP. Unfortunately, this strategy is not feasible for stationary phases based on carbohydrates such as cyclodextrins (all D configuration). In certain cases, the elution order can be reversed by the use of different cyclodextrin derivatives.

4.4. Sources of error of gas chromatographic enantiomer analysis

Despite the great success of gas chromatography for determining enantiomeric ratios ER, potential sources of error should also be considered. They have recently been summarized [57]. The most important are mentioned herein: (i) decomposition of the analyte at high temperatures during chromatography (the enantiomer which spends a longer time in the column will be lost preferentially causing an error in ERs), (ii) co-elution of impurities spuriously increasing peak-areas, (iii) "enantiomerization" of configurationally labile enantiomers causing peak distortions due to inversion of configuration during enantiomer separation, (iv) peak distortions caused by an inadequate instrumentation and (v) nonlinear detector response.

In general, the error in ERs due to decomposition of the sample can be reduced if the difference of the residence time in the column is minimized for both enantiomers. This goal may be realized by using short columns, high pressure drops, ambient temperatures and CSPs exhibiting only small separation factors α. A rather trivial but frequent cause for the deviation from the expected 1:1 ratio of a racemic mixture consists of the co-elution of impurities [58].

Errors due to (i) and (ii) can usually be recognized by conducting the ER determination with stationary phases of opposite chirality. This procedure is precluded for cyclodextrins. Errors due to (ii) can also be recognized, and eliminated, by changing the chromatographic parameters such as temperature, carrier gas flow or by the use of pre-columns with different polarities in multidimensional GC. The verification of the expected 1:1 ratio of a racemic

mixture is mandatory in enantiomer analysis by chromatography. Deviations from the original ER of a mixture of enantiomers may, in principle, arise during sample manipulation via self-association of the enantiomers under non-ideal conditions ("EE-effect" [59]). This effect, whereby the relative amounts of two enantiomers induce an observable difference between them [59], may lead to accidental fractionation of enantiomers during work-up, isolation or injection. On-column injection would eliminate this possible source of error. Selected chiral compounds, e.g., atropisomers, may be prone to enantiomerization during enantiomer separation. This effect can give rise to characteristic chromatographic interconversion profiles which have been observed with cyclodextrins [60,61]. The determination of ER will of course be biased by such an effect.

Peak distortions having their origin in instrumental shortcomings may also obscure enantiomer analysis by gas chromatography. An inadequate oven heating may cause thermal peak splitting when open-tubular columns made from vitreous materials of low thermal capacities are used. These periodical fluctuations in the chromatographic elution profiles are predominant at large retention factors and are recognized by the co-called "christmas-tree" shape of the chromatographic peaks [62].

The response to a racemic mixture of enantiomers is strictly 1:1 for nonchiroptic detection devices, since enantiomers cannot be distinguished in an achiral environment. Indeed, a racemic composition (obtained by synthesis in a achiral environment) represents an ideal equimolar mixture (save for minute statistical differences) which is useful in its own right for testing the precision of integration facilities which should produce a correct ER of 1. In enantiomer analysis, however, a linear detector response is indispensable. Thus, for the correct determination of, e.g., 0.1% of an enantiomerically impurity, linearity within a concentration range of at least three orders of magnitude is required. It is generally accepted that the flame ionization detector (FID) fulfils this requirement. The linear response range of the electron capture detector (ECD) is low in this respect with only two to three orders of magnitude. It is therefore recommended to verify the linear detector response via dilution experiments of an enriched mixture.

4.5. Assignment of absolute configurations by GC with CSPs

The determination of absolute configurations of chiral analytes is an important task in enantiomer analysis. Absolute configurations of minute amounts of chiral samples may be determined directly, and free of chiroptical evidence, by GC via coinjection of reference compounds with the known stereochemistry. Absolute configurations may also be predicted indirectly by empirical rules which correlate the absolute configuration and the order of elution for enantiomers belonging to the same class of compounds or to homologous series of compounds. Although consistent relationships between the order of elution and absolute configuration of homologous compounds have been observed in many instances [56], remarkable inconsistencies, e.g., in complexation GC, have also been detected [57]. As a rule, such comparisons, if any, should be restricted to measurements at the same temperature since peak inversions may arise at different temperatures as the result of enthalpy vs. entropy control of chiral recognition. Thus, changes of the elution order of enantiomers below and above the isoenantioselective temperature T_{iso} have indeed been observed [48–50]. Therefore, the assignment of absolute configurations by GC may be quite ambiguous and absolute configurations should preferentially by obtained by other evidence.

4.6. Method of enantiomer labelling

The GC determination of enantiomeric ratios ERs can be used for the quantification of enantiomers in complex matrices with a known quantity of the pure enantiomer added as an internal standard [63]. To our knowledge, this method has not been used yet in the realm of chiral organochlorines. In the absence of diastereoisomeric effects between enantiomers in nonideal solutions ("EE-effect" [59]), the enantiomeric ratio is not influenced by sample manipulations (achiral derivatization, dilution, injection, detection, chemical and physical losses). With the method of "enantiomer labelling" [63] a known quantity of an enantiopure standard is added to the mixture (or an aliquot of it) and the amount of the enantiomer originally present is calculated from the

change of ER after the addition of the standard [55]. The method of enantiomer labelling presupposes the precise knowledge of the ERs of the sample and the standards.

A comparative study of enantiomer and isotopic labelling in enantiomer analysis has also been carried out with GC–MS selected ion monitoring [64].

4.7. Practical recommendations

The merit of cyclodextrin derivatives for GC enantiomer separation is the great spectrum of resolvable classes of compounds. With a few exceptions enantiomer separation with modified cyclodextrins is characterized by low separation factors α, and, as a (beneficial) consequence, reduced analysis times. The use of high efficient capillary columns is mandatory. Many types of cyclodextrin derivatives coated onto fused-silica capillary columns are commercially available (cf. pp. 148–150 in Ref. [65]). Factors such as availability, prize, performance and reproducibility should guide the analyst when selecting cyclodextrin stationary phases in commercial columns. Information on long term stability of columns are, unfortunately, not readily available from commercial vendors. De novo or published chiral test mixtures should be used to compare columns from different sources [66–68]. Immobilized chiral stationary phases of the type Chirasil-Dex have the advantage of solvent compatibility, resistance to temperature shock and longevity. For non-volatile chiral organochlorines enantiomer separation by SFC [32,69] may become important in the future. Enantiomer separation on Chirasil-Dex-type stationary phases can be performed in the usual temperature range of 25–250°C. For special applications it is also possible to use temperatures down to −25°C [70].

4.7.1. Selection of column parameters

The dimensions of commercial columns are typically 10–30 m×0.25 mm I.D. while the film thickness of the chiral stationary phase is usually $d_f = 0.25$ μm. Column miniaturization may have important merits in terms of shorter analysis times [71], improvement of detectability and for unified enantioselective capillary chromatography [33,34]. The latter term implies that one individual column can be used for enantiomer analysis by capillary GC, SFC fluid chromatography, open-tubular electrochromatography and OT-LC. Since enantiomer separation represents a binary separation system the whole elution window required for multicomponent mixtures need not be exploited unless enantiomers are detected in complex matrices. With shorter columns, the elution temperature can be decreased whereby the chiral separation factor α is increased in the common enthalpy-controlled region of enantioselectivity [48,49]. The loss of efficiency is compensated by the gain of selectivity leading to comparable resolution factors R_s. The shorter analysis times increase the narrowness of peaks and hence the detectability of the enantiomers. Further miniaturization via reduction of the internal diameter of the columns to 0.1 and 0.05 mm I.D. requires smaller films of the stationary phase in order to keep the phase ratio β constant. The reduced amount of stationary phase decreases the sample capacity. The reduced signal-to-noise ratio may become critical in regard to the precision of the ER determination.

4.7.2. Chiral separation factor α and peak resolution

The chiral separation factor α is related to enantioselectivity and is thus a temperature-dependent quantity. α can therefore not be defined in temperature-programmed runs. In diluted systems the chiral separation factor α is also concentration-dependent. It has been implied by theoretical considerations, and verified by experiments, that α does not increase linearly with the concentration of the cyclodextrin derivative in the polysiloxane [72]. The experimental data imply that the optimum is often reached at low concentrations and, consequently, no further improvement of selectivity is gained above a cyclodextrin weight percentage of approximately 30% for permethylated β-cyclodextrin or 50% for derivatives with high-molecular-masses, i.e., γ-cyclodextrins containing *n*-pentyl groups. Nowadays, the use of undiluted cyclodextrins [5] is recommended only in special cases.

The ultimate goal of a successful enantiomer separation is resolution in a short time. Unfortunately, the peak resolution R_s is not always quoted in the literature, although this data is important to assess efficiency and selectivity at the same time. In a recent collection of separation factors α, obtained

by capillary GC on cyclodextrin CSPs, the important information on efficiency and retention at a given selectivity α is omitted [73]. Unfortunately, there is no universal cyclodextrin phase available at present and column selection is currently a matter of trial and error. In a thorough study, permethylated β-cyclodextrin dissolved in polysiloxanes [26] turned out to be the most versatile chiral stationary phase [74]. On a given enantioselective column, the parameters column length, temperature, film thickness, cyclodextrin concentration, mobile phase velocity and their influence on the chiral separation factor α, retention factor k and efficiency H have to be carefully balanced. Some variables can not be freely selected when commercial columns are acquired.

4.7.3. Helpful literature sources and data bases

A full account on the use of cyclodextrins in GC enantiomer separations including pertinent applications in general enantiomer analysis is given in Refs. [5,21,43,65]. Current information, inter alia, on the use of cyclodextrins in GC are available from Cyclodextrin News abstract service [75]. Information on enantiomer separations by GC can be retrieved from the CHIRBASE data bank [76].

4.7.4. Configuration of modified cyclodextrins

Of disadvantage is the fact that cyclodextrins are available only in the D-configurated form. Thus, the rigorous proof of a given (novel) enantiomer separation by using the racemic selector (D,L), producing peak coalescence, or by using the mirror-image selector (L), resulting in peak inversion, cannot be provided with cyclodextrins. The unavailability of L-configurated cyclodextrins also precludes the useful peak-switching technique in enantiomer analysis causing the minor enantiomer to elute as the first peak [21].

4.8. Problems in the determination of enantiomeric ratios in environmental samples

Organochlorine compounds are present in samples at particularly low levels and in a complex mixture. Consequently, the separation of the components in samples requires the technique with the highest efficiency, i.e., HRGC. Since organochlorine compounds must be determined in ultra-trace levels in complex matrices, the highly selective detection techniques electron-capture detection (GC–ECD) or mass spectrometry with selected ion monitoring detection (GC–MS-SIM) are mandatory.

Dönnecke et al. reported efficiency values in GC for several organochlorine compounds ranging from 2500 to 13 000 plates per meter on heptakis(2,6-di-O-methyl-3-O-*n*-pentyl)-β-cyclodextrin [77]. Coelutions can not be excluded even by group separation prior to injection and application of 50 m columns. In residual analysis, confirmation of the result on a second stationary phase with different polarity is recommended.

Every enantiomer separation adds one more peak to the number to be resolved in samples and the proper determination of enantiomer ratios (ERs) is a difficult task which requires improved techniques. Usually, columns consisting of more than 30 m length are not recommended. This is partly due to the unavoidable polarity of CSPs which may lead to extended retention times. Polarities of stationary phases are usually expressed as the sum of the five McReynolds indices (see Table 1) [78,79]. The higher the sum of the McReynolds indices the more polar a phase is. As can be seen from the data in Table 1, the polarity of typical CSPs is similar or higher than OV-1701.

The determination of ERs by application of GC and CSPs is a highly selective method. ERs were sometimes presented as precise as with three digits after the comma [80]. To verify such detailed results, one must take into account the nonsymmetric peak shapes often observed even in enantiomer separations of racemates and the poor linear range of ECD which usually does not allow a direct comparison of peak areas or heights in standard and samples for the determination of ERs in the latter.

The access to enantiopure or enantioenriched standards is a prerequisite to establish the elution order of enantiomers. This task has been solved for α-HCH [81,82], chlordane compounds [83,84] and atropisomeric PCBs [85]. The elution order of enantiomers on a CSP can not be predicted a priori and several instances of peak reversals have been reported [82,86,87]. Therefore, caution has to be exercised by comparing elution orders from one column to another.

In regard to sample clean-up, standard methods for

the determination of chiral organochlorines have been applied. In some studies coelutions with other compounds were noted [82,84,88]. Buser and Müller reported complexation and saturation of the chiral selector caused by achiral interferents in samples investigated by GC–MS [84]. Injection of different concentrations of samples led to variations in the ERs caused by interferents. Note that the interferents had other m/z values and were not recorded by the mass spectrometer but caused lower resolution of the compound on the CSP [84]. In such cases higher separation temperatures are recommended [89]. Although carrying out an enantiomeric separation at higher temperature decreases the separation factor, it significantly increases the column capacity due to the increase in the average amount of analyte in the gas phase and its corresponding decrease in the stationary phase [89].

Pfaffenberger et al. mentioned interferences from the sample matrix which were not observed on an achiral column although an additional clean-up step had been introduced [90]. In some cases the determination of ERs in multicomponent mixtures was possible by application of multidimensional GC in the case of atropisomeric PCBs [91] or single reaction monitoring experiments in MS–MS for chiral compounds of technical toxaphene [92].

Loss of a chiral compound during the sample clean-up will not influence the ER. However, the recovery rates of chiral compounds play an important role for the interpretation of results if the ER in a species (or class of samples) is proportional to the organochlorine level. Fortunately, there are only few reports with inversion of ERs in the same matrix in dependence of the region [93], the level [82] and on the species [88,94]. However, these examples show that a number of samples is necessary for a correct judgment of ERs observed in biological samples.

Another problem arises from the relatively low separation factors α obtained by GC on modified cyclodextrins [89]. This is mainly attributed to weak Van der Waals interactions between analyte and the CSP [7]. Very often no baseline separation can be obtained and the chromatographic quantitation of low amounts of an enantiomer in the presence of the mirror image can be difficult [95]. In this context deconvolution has been suggested to improve the precision of ERs even when no baseline separation was obtained or in the presence of minor interferents [96].

A further important point is the quality of standard solutions. In few cases wrongly labelled ampoules have been distributed and the check if the compound in the mixture is really that on the label is an important task for quality control.

All-in-all, every compound (or class of compounds) requires a special optimization procedure for the determination of ERs which are discussed in the sections which follow.

5. Enantioselective determination of α-HCH

1,2,3,4,5,6-Hexachlorocyclohexanes (HCHs) were the first organochlorine compounds successfully applied as insecticides [97]. HCHs are synthesized by photochlorination of benzene [98]. Five of the eight possible isomers of 1,2,3,4,5,6-hexachlorocyclohexanes are present in technical mixtures. Later it was shown that only the gamma-isomer, *aaaeee*-1,2,3,4,5,6-hexachlorocyclohexane (γ-HCH) which only accounts for approx. 15% of the technical HCH was responsible for the expected insecticidal properties of technical HCH. In honor of the contributions of Van der Linden to the chemistry of HCHs, the γ-HCH was coined Lindane [98]. After this observation technical HCH was replaced by pure γ-HCH. However, even today high amounts of *aaeeee*-1,2,3,4,5,6-hexachlorocyclohexane (α-HCH) can still be detected in environmental samples [98].

α-HCH, representing the major component in technical HCH (approx. 60%), is the only chiral hexachlorocyclohexane isomer [3]. This fact has already been used in 1949 to assign the *aaeeee*-configuration of the chloro substituents to α-HCH [3]. As a synthetic product, technical HCH contains the enantiomers of α-HCH in the racemic ratio 1:1 [99]. Reactive chiral bases like 2,3-dimethoxystrychnidin-10-one (brucine) have been used to degrade one of the enantiomers and allowed to study the properties of pure α-HCH enantiomers. In the seventies, toxicologic investigations carried out with pure enantiomers of α-HCH revealed no statistically

significant differences in acute toxicity of pure enantiomers and racemic α-HCH [100].

The introduction of modified cyclodextrins in GC and the application of this technique to residual analysis consisted an important breakthrough of enantioselective analysis of chiral organochlorine compounds. In 1989, König et al. succeeded in the enantiomer separation of α-HCH on undiluted octakis(3-O-butyryl-2,6-di-O-*n*-pentyl)-γ-cyclodextrin (see Fig. 1). In this paper the authors stated that the enantiomer separation of this compound may be used to study the enantioselective degradation of α-HCH in the environment [101]. Two years later, Kallenborn et al. demonstrated the enantioselective degradation of α-HCH in biota [102]. In the following, great attention has been paid to the enantioselective determination of α-HCH in biological samples.

Newest data, obtained after the preparation and isolation of pure α-HCH enantiomers, followed by the determination of their absolute configuration [103], imply a slight difference in the cytotoxicity of the enantiomers [104]. Anyhow, ERs in biota deviating from 1.0 alone are an indication for different toxic potentials of the enantiomers due to the different period over which the two enantiomers are effective in the respective body [82].

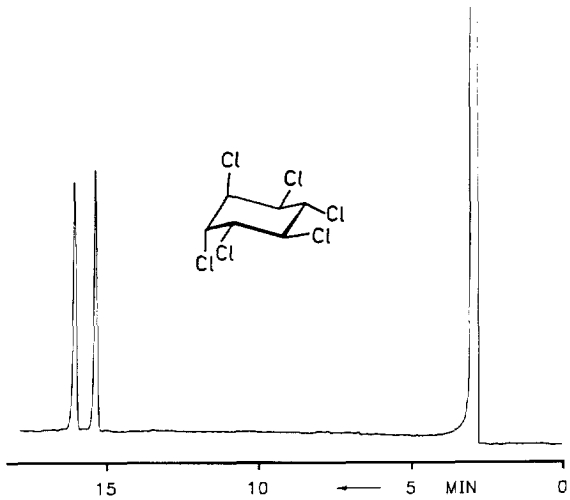


Fig. 1. First successful gas chromatographic enantiomer separation of a chiral organochlorine pesticide. The enantiomers of α-HCH standard solution were separated on a 50 m Pyrex glass capillary column (0.25 mm I.D.) coated with octakis(3-O-butyryl-2,6-di-O-*n*-pentyl)-γ-cyclodextrin at 190°C by GC–FID [101].

5.1. CSPs applied for the enantiomer separation of α-HCH

As reported above, the first GC enantiomer separation of α-HCH was carried out on undiluted octakis(3-O-butyryl-2,6-di-O-*n*-pentyl)-γ-cyclodextrin [101]. Baseline separation was obtained at a temperature of 190°C [101]. This CSP was also used in the pioneering work of Hühnerfuss and coworkers to screen the enantiomers of α-HCH in environmental samples [102,103,105,106]. Yet, the enantiomer separation of α-HCH was relatively poor on this phase. Even on a 60 m column baseline separation was not obtained [102] except at retention times exceeding 1 h [105]. Another undiluted modified cyclodextrin phase frequently used was heptakis(2-O-methyl-3,6-di-O-*n*-pentyl)-β-cyclodextrin [81,107] and heptakis(3-O-acetyl-2,6-di-O-*n*-pentyl)-β-cyclodextrin [108].

Faller et al. stated that the standard procedure for the production of CSPs [5] had to be improved to meet the requirements of residual analysis and quantitation with GC–ECD [105]. At that time, temperatures above 150°C led to high bleeding rates in the ECD which was not observed in the flame-ionization detection (FID) [105]. A similar problem was mentioned by Buser et al. who stated that undiluted modified cyclodextrins present limitations for general use including low thermal stability and poor inertness [109]. Though the columns consisting of modified cyclodextrins diluted in polysiloxanes, introduced by one of the present authors, showed a somewhat reduced chiral selectivity compared to undiluted cyclodextrins, they appeared to be more suitable for chiral analyses of real biological samples [109].

Furthermore, CSPs of high polarity lead to extraordinary long GC run times with some organochlorine compounds present in environmental samples. This is a particular problem in the case of CSPs with undiluted cyclodextrins. From 1992 on, most of the enantiomer separations of α-HCH were carried out on columns consisting of a mixture of the CSP dissolved in (or bonded to) a polysiloxane as intro-

duced by Schurig and Nowotny [26]. Due to the polarity of peralkylated cyclodextrins, medium polar polysiloxanes were necessary to dilute CSPs in achiral polysiloxanes such as OV-1701 and PS086 (85% dimethyl, 15% diphenylpolysiloxane) [4,52].-Consequently, octakis(3-O-butyryl-2,6-di-O-*n*-pentyl)-γ-cyclodextrin [81,90,93,105,107,110,111], octakis(2,3,6-tri-O-*n*-pentyl)-γ-cyclodextrin, octakis(2,6-di-O-methyl-3-O-*n*-pentyl)-γ-cyclodextrin [112] and heptakis(2,3,6-tri-O-*n*-pentyl)-β-cyclodextrin [81,106,107,113] were also used in mixtures with OV-1701 [106,113] to separate the enantiomers of α-HCH.

The introduction of chemical bonded (immobilized) phases allowed also to use 100% of apolar dimethylpolysiloxane as an achiral matrix of the column [30,31]. Enantiomer separation of α-HCH was also obtained on polysiloxane bonded heptakis(2,3,6-tri-O-trifluoroacetyl)-β-cyclodextrin (Chirasil-Dex-TFA) [4].

2,3,6-Tri-O-methylated cyclodextrins diluted in OV-1701, PS086 or CP-Sil 8 based on the standard procedure of Schurig and Nowotny [26] are the most often used CSPs for the enantiomer separation of α-HCH in biological samples. A preference of β-cyclodextrin [80] [82,87,108,114–120] as compared to γ-cyclodextrin [82,87,116,121] can be noticed but some groups used the effect of the inverse elution order of α-HCH enantiomers on these phases (see Fig. 2) [82,87]. Frequently, phases consisting of *tert.*-butyldimethylsilylated β-cyclodextrin [82,122], introduced by Blum and Aichholz [44], were used [82,86]. However, this CSP which showed excellent enantiomer separations for other organochlorine compounds exhibited only a poor [82] or no separation of α-HCH enantiomers [86]. On the other hand, Vetter et al. used *tert.*-butyldimethylsilylated β-cyclodextrin in PS086 obtained from two different manufacturers and, unexpectedly, observed an inverse elution order of α-HCH enantiomers [123]. The peak inversion shown by injection of enantioenriched enantiomers was obtained at comparable elution temperatures and a crossing of the isoenantioselective temperature, T_{iso}, can not be the reason for the peak inversion. To our knowledge, this is the only report on an inversion of the elution order probably caused by the synthesis procedure of a CSP. Although unusual, these observations should be kept in mind when elution orders of CSPs are compared. A definitive order of elution can only be established by injection of enantioenriched standards. In addition to the CSPs mentioned above a good separation of α-HCH enantiomers was obtained on 35% heptakis(6-O-*tert.*-butyldimethylsilyl-2,3-di-O-methyl)-β-cyclodextrin in OV-1701 [82].

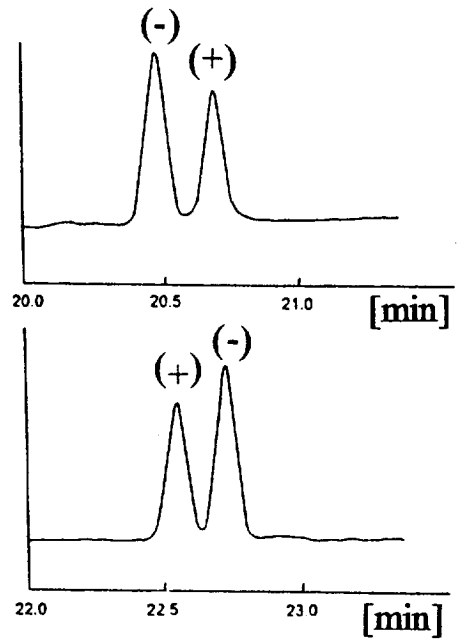


Fig. 2. Enantiomer separation of an enantioenriched α-HCH standard solution demonstrating the inversed elution order on permethylated γ-cyclodextrin (above) and permethylated β-cyclodextrin (below) [87].

At present, permethylated β-cyclodextrin seems to be the phase of choice for the enantiomer separation of α-HCH in biological samples. This column type is commercially available from a number of manufacturers. In this context immobilized permethylated cyclodextrins of the Chirasil-Dex type are favorable due to the involatility of the CSP (no bleeding in ECD) which has also been suggested by Dönnecke et al. [77].

5.2. Determination of ERs of α-HCH in biological samples

The enantiomer screening of α-HCH in biological samples is more established than for other organo-

chlorine compounds. Indeed, α-HCH was the first organochlorine compound that was enantiomer separated and a multitude of CSPs can be employed. Additionally, α-HCH is an early eluting organochlorine compound and therefore, the number of potential coelutions is limited after a good sample clean-up. This allows the application of relatively short GC columns as well as low separation temperatures. Consequently, mostly GC–ECD has been applied to study the enantioselective accumulation of α-HCH which was distributed as racemate [101].

Besides the general precautions during sample clean-up, two special methods are worth mentioning. In an additional sample clean-up step, Kallenborn et al. fractionated α-HCH by application of HPLC [102] which allow a proper separation of the enantiomers by GC–ECD. This technique was applied in further publications by Hühnerfuss and coworkers [90,113]. In another paper the use of an internal standard for the exact determination of ERs was suggested [82]. For this purpose perdeuterated α-HCH (α-PDHCH) was used. Mixtures of α-HCH and α-PDHCH were baseline separated in four peaks whereby α-PDHCH enantiomers eluted before the respective α-HCH enantiomers [82,120]. Apart from this, the samples were injected into the gas chromatographs as it was carried out for achiral determination of organochlorines. In the case of α-HCH, the elution order of the enantiomers on different CSPs is well known and ERs given in the following sections refer always to the ratio (+)-α-HCH/(−)-α-HCH.

5.3. ERs of α-HCH in technical α-HCH, air and water samples

In a recent study, several CSPs were used to determine the ER in technical α-HCH and ratios ranging from 1.00–1.10 on different columns by using the same standard solution were observed [82]. It was concluded that the ER of technical α-HCH is 1.00 and apparent deviations in the standard were due to artifacts during the GC enantiomer separation. In view of this, it is obvious that the determination of ERs at low trophic levels has to be carried out carefully. Data on ERs of α-HCH in air are rare. Müller et al. presented ERs of 1.02(0) in ambient air compared to 1.00(2) in racemic technical α-HCH [80] and Falconer et al. confirmed these results by ERs of 1.00±0.04 versus 1.00±0.01 in the reference standard [121]. These data are in contradiction to a multi group publication presenting ERs in air ranging from 0.86 to 1.03 [107]. Unfortunately, no experimental data was given. Recently, Jantunen and Bidleman also found in air samples higher deviations from 1.00, ranging from approx. 1.10–0.95 [118].

Enantioenriched α-HCH in sea water was already reported by Faller et al. in 1991 [93]. The authors found ERs ranging from 0.80–1.19. However, this data should not be interpreted as ranges measured in one region but the authors decisively claimed regional differences in the ERs, e.g., in several stations from the Baltic Sea, Skagerrak and the Eastern part of the North Sea (−)-α-HCH was predominant while in the central North Sea and east of Great Britain (+)-α-HCH was more abundant [93,107]. Falconer et al. found ERs of 0.93±0.06 in sea water. In fresh water, seasonal variations were found from 0.88–0.99 in late June to 0.65 to 0.86 in mid-July in Arctic Lake Amituk [121]. Even at approx. 20 m depth the α-HCH ER was 0.77, this value being within the values in surface water [121]. In a later study, however, Jantunen and Bidleman reported ERs depending on the water depth in Beaufort Sea and Canadian Basin with ERs ≤1.0 in surface water and ERs ≤0.4 at 200–350 m depth, and in some deep water samples (+)-α-HCH was nearly absent [118].

In Arctic snow samples, Falconer et al. found ERs close to 1.0 (0.99–1.01) except for one sample which contained a preference of (−)-α-HCH with an ER of 0.91 [87] while Müller et al. found an ER of 1.07 (GC–ECD) or 1.03 (GC–MS) in a rain sample from Norway. All these data suggest microbial degradation of α-HCH in favour of the (+)- or (−)-α-HCH enantiomers as suggested by Faller et al. [105]. Enantioselective microbial degradation of α-HCH was also reported in sewage sludge [119]. An interesting pathway, i.e., the enantioselective transformation of γ-HCH in α-HCH is disputed [105,119].

5.4. ERs of α-HCH in terrestrial animals

ERs of α-HCH were also determined in some terrestrial species. In fat and liver of seven samples

from sheep ERs<1 were observed while sheep brain showed a higher level of (+)-α-HCH with ERs of 1.4–3.8 [81,113].

In roe deer (*Capreolus capreolus*) livers from two remote regions in Germany particular low ERs ranging from 0.4 to 0.03 were measured [90]. The ER determined in blubber of a polar bear sample by GC–ECD was 1.18 and by GC–ECNI-MS 1.16 [82].

5.5. ER of α-HCH in marine biota at low trophic levels

Pfaffenberger et al. analyzed blue mussels (*Mytilus edulis* L.) from two locations of the German Wadden Sea coast (North Sea) and found ERs (mean values) ranging from 0.89±0.14 (with one value exceeding ER=1.0) [110]. They found in blue mussel samples similar values with a wider range compared to adjacent water samples. From this fact they concluded that blue mussels are able to accumulate organochlorines like α-HCH but no enantioselective degradation occurs at this low trophic level [110]. In another study, ERs of 0.67–0.89 were determined which also coincided with the status in the surrounding water [81]. From this result, it can not be concluded that predominant levels of the (−)-α-HCH enantiomer are typical values for blue mussels since in some sea water samples ERs>1 have been found (see above). The proof of the expectation that mussels from these areas also show ERs>1 is still missing. As a consequence, data of ERs of α-HCH in blue mussels and at other biota at low trophic levels without detailing the exact origin of the samples are only of limited significance. Yet, blue mussels might be used as bioindicators for the assessment of the ERs ratio of sea water since bioaccumulation is not so dependent from the season than water samples.

A similar picture was also observed in different fish species. In liver of flounder (*Platychthys flesus* L.) from the German Bight (North Sea)—a region with ERs>1 in sea water (information extracted from [93,110]), ERs ranging from 0.80–0.94 [81,107] showed no pronounced enantioenrichment of α-HCH. In a cod liver sample from the North Atlantic (SRM 1588) an ER of approx. 1.00 was determined [108].

5.6. ER of α-HCH in biota at high trophic levels

Enantioenrichment in biota at high trophic levels was expected to be more pronounced compared to lower trophic levels due to more specialized enzyme systems of the former. Consequently, a number of studies have been carried out with top predators such as marine mammals and marine birds. Different tissues of common Eider ducks (*Somateria mollissima* L.) were investigated by Hühnerfuss and coworkers [81,102,107,110]. In kidney and muscle α-HCH ERs were approx. 1.6 and 7.0, respectively. This represents a clear trend towards higher levels of the (+)-α-HCH enantiomer. Common Eider ducks are almost exclusively feeding on (blue) mussels which, however, showed higher levels of the (−)-α-HCH enantiomer. In liver samples of common Eider ducks the α-HCH ERs ranged from 1.4 to nearly infinity [81,102] and in brain from 1.0 to nearly infinity [81]. Furthermore, no correlation between α-HCH concentration and ER was found [110], but there was some evidence that the ability to enantioselective biodegradation of α-HCH is dependent on the physical condition of the animals [110].

Additionally to common Eider ducks, blubber and tissue of different seal and cetacean species have been investigated. In marine mammals it can be assumed that species specific degradation is more pronounced than the influence of the surrounding environment. For the sake of simplicity the following data are given without details of the catching area of samples which was often not exactly defined in the following references.

The adipose tissue (blubber) of harbour seals (*Phoca vitulina*) [80,88,103,107,111,116,124], northern fur seals (*Callorhinus ursinus*) [108], grey seals (*Halichoerus grypus*) [124] and harp seals (*Phoca groenlandica*) [116] exhibited α-HCH ERs from 1.0–4.5. These ERs in marine biota at high trophic levels are in agreement with the higher (+)-α-HCH value determined in common Eider ducks (see above). However, investigations of blubber samples of three hooded seals (*Cystophory cristata*) revealed α-HCH ERs<1 [115,116]. Moreover, higher levels of (−)-α-HCH in Antarctic Weddell seal (*Leptonychotes Weddelli*) blubber determined by GC–ECNI-MS were mentioned [82]. These exceptions

from the rule that (+)-α-HCH is enantioenriched in seal species confirms that a clear specification of a species is necessary.

Among the northern fur seal (*Callorhinus ursinus*) blubber samples analyzed by Mössner et al. [108], there was also a sample of a still born pup and seal milk. Interestingly, "normal" northern fur seal blubber had α-HCH ERs comparable to the ERs in fur seal milk and a pup. On the other hand, a sick northern fur seal showed a lower ER compared to healthy samples [108], and this confirms the observation of Pfaffenberger et al. in common Eider ducks (*Somateria mollissima* L.) [110].

In brain samples of harbour seals (*Phoca vitulina*) the enantioenrichment of α-HCH was more pronounced compared to blubber (see Fig. 3). In brain of harbour seals, Hühnerfuss et al. found ERs of 7.9–infinity [81]. It was assumed that only (+)-α-HCH is able to penetrate the blood–brain barrier [103]. The blood–brain barrier greatly inhibits the transport of non-lipid-soluble substances such as proteins, and obviously also certain fat-soluble organic pollutants [103]. In brain, liver and lung of northern fur seal (*Callorhinus ursinus*) α-HCH ERs

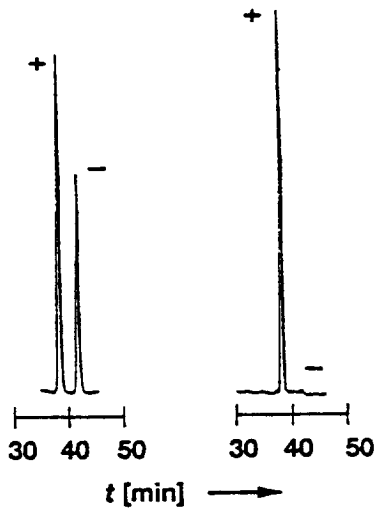


Fig. 3. Enantiomer separation of α-HCH in blubber (left) and brain (right) of a purified harbour seal (*Phoca vitulina*) extract on 50% octakis(3-O-butyryl-2,6-di-O-*n*-pentyl)-γ-cyclodextrin in OV-1701 by GC–ECD [103].

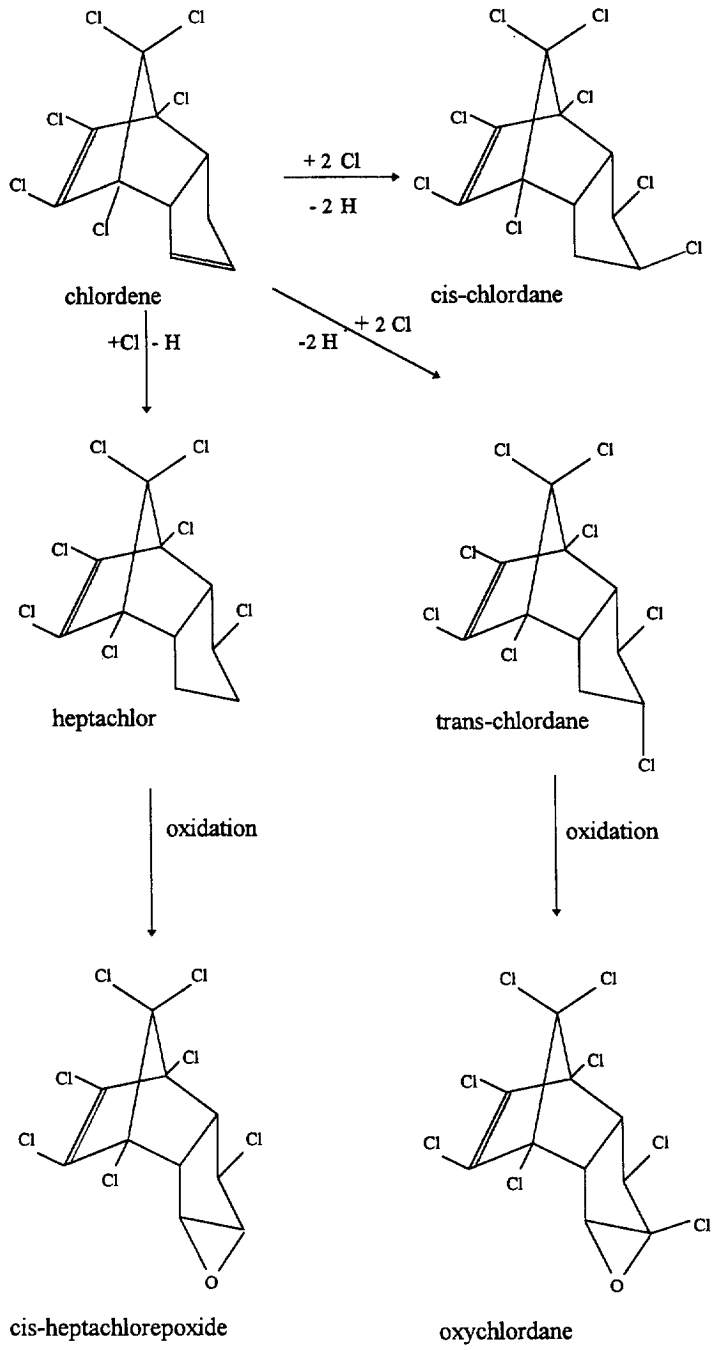
of 1.2–1.9 which was in the range of blubber samples but brain showed ERs of approx. 30 [108].

ERs in cetaceans were determined in blubber of harbour porpoises (*Phocoena phocoena*) [80,116] from different regions, in white-beaked dolphins (*Lagenorhynchus albirostris*) [116], Dall's porpoise (*Phocoenoides dalli*) [117], Baird's beaked whale (*Berardius bairdii*) [117], northern right whale dolphin (*Lissodelphis borealis*) [117], Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) [117], common dolphin (*Delphinus delphis*) [117], striped dolphin (*Stenella coerulealba*) [117], melon-headed whale (*Peoponocephala electra*) [117], Fraser's dolphin (*Lagenodelphis hosei*) [117], spinner dolphin (*Stenella longirostris*) [117] and Indo-Pacific humpbacked dolphin (*Sousa chinensis*) [117]. The α-HCH ER in the samples ranged between 1.1 and 3.9.

6. Enantioselective determination of chlordane-related compounds

Scheme 1 shows structure and chemical names of the most important chlordane-related compounds. The term chlordane-related compounds is used here as a synonym for compounds of technical chlordane, (*cis*-) heptachlor and their major metabolites. From an environmental point of view, chlordane-related compounds are the most important bicyclic cyclodiene pesticides. Technical chlordane is synthesized by a Diels-Alder reaction between hexachlorocyclopentadiene and cyclopentadiene. The resulting hexachloro compound, chlordene, is further chlorinated yielding technical chlordane. Technical chlordane consists of more than 100 compounds [125] and the chiral *cis*- and *trans*-chlordane are both the major compounds (approx. 15% each) and the insecticidal relevant parts of the formulation [126]. Several other compounds of technical chlordane are also chiral (see below). One important compound of technical chlordane is heptachlor which itself was also used as an insecticide.

In biological samples the major residual contaminants of chlordane-related compounds are the achiral *trans*-nonachlor and the chiral oxychlordane. The chiral oxychlordane is the common metabolite of *cis*- and *trans*-chlordane in biota [127]. A further im-



Scheme 1. Structures and IUPAC names of major chlordane-related compounds. Chlordene: 4,5,6,7,8,8-hexachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanoindene. Heptachlor: 1-*exo*,4,5,6,7,8,8-heptachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanoindene (*cis*-heptachlor). *cis*-Chlordane: 1-*exo*,2-*exo*,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanoindane. *trans*-Chlordane: 1-*exo*,2-*endo*,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanoindane. Heptachlorepoxide: 1-*exo*,4,5,6,7,8,8-heptachloro-2,3-*exo*-epoxy-3*a*,4,7,7*a*-tetrahydro-4,7-methanoindene (*cis*-heptachlorepoxide). Oxychlordane: 1-*exo*,2-*exo*,4,5,6,7,8,8-octachloro-2,3-*exo*-epoxy-3*a*,4,7,7*a*-tetrahydro-4,7-methanoindene.

portant compound is (*cis*-) heptachlorepoxide, the major metabolite of heptachlor.

6.1. CSPs applied for the enantiomer separation of chlordane-related compounds

The enantiomer separation of chlordane-related compounds is more difficult than the enantiomer separation of α-HCH. The group of chlordane consists of several compounds and some of them can not be distinguished by GC–MS. Unfortunately only few reports exist which deal with the simultaneous separation of several compounds attributed to chlordane. At present no CSP is available which separates all enantiomers and isomers of this class of compounds. Therefore, the following description of the columns applied to separate chlordane-related compounds is rudimentary. However, to provide a maximum of information, the components were discussed individually in the order *cis*- and *trans*-chlordane; oxychlordane; heptachlor/heptachlorepoxide and minor components of technical chlordane.

The first enantiomer separation of the most important chlordane compounds was presented by König et al. in 1991 [128]. For the enantiomer separation of *cis*- and *trans*-chlordane the authors used heptakis(3-O-methyl-2,6-di-O-*n*-pentyl)-β-cyclodextrin, heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin and heptakis(2-O-methyl-3,6-di-O-*n*-pentyl)-β-cyclodextrin (see Fig. 4). Though all CSPs mentioned above separated the enantiomers of *cis*- and *trans*-chlordane, the separation in four peaks was only obtained on the latter CSP [128]. On the former two CSPs, the second peak of *trans*-chlordane coeluted with the first eluted peak of *cis*-chlordane. However, also on heptakis(2-O-methyl-3,6-di-O-*n*-pentyl)-β-cyclodextrin no baseline separation was obtained and it was doubtful if the separation and the stability of the column would be sufficient to apply the column for the separation of *cis*- and *trans*-chlordane in residual analysis [109]. Therefore, further CSPs have been tested to optimize the enantiomer separation of *cis*- and *trans*-chlordane in order to obtain four peaks.

Buser et al. used 10–20% heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin in PS086 but baseline separation of *cis*- and *trans*-chlordane enantiomers was not possible with this CSP [109]. The authors

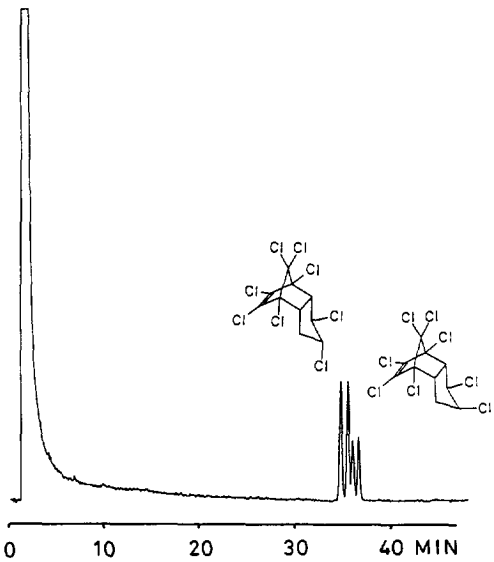


Fig. 4. Enantiomer separation of *trans*- and *cis*-chlordane on a 25 m Pyrex glass capillary column (0.25 mm I.D.) coated with heptakis(2-O-methyl-3,6-di-O-*n*-pentyl)-β-cyclodextrin at 160°C by GC–FID [128].

obtained an improved separation of the chlordane enantiomers with higher amounts of chiral selector (20% instead of 10%) but with a decreased separation of the *cis*- and *trans*-chlordane isomers. With 10% of the CSP in PS086 coelution of the second enantiomer of *cis*-chlordane and the first enantiomer *trans*-chlordane was the consequence [82,109]. In a later publication Buser and Müller used also 25% *tert.*-butyldimethylsilylated β-cyclodextrin in PS086, prepared according to Blum and Aichholz [44], and 30% hexakis(2,3,6-tri-O-ethyl)-α-cyclodextrin in OV-1701 [129]. To our knowledge the latter is the only α-cyclodextrin selector which was used to separate enantiomers of chiral organochlorines. The column separated the enantiomers of *cis*-chlordane but the enantiomers of *trans*-chlordane coeluted [129]. The authors compared three CSPs regarding the enantiomer separation of *cis*- and *trans*-chlordane and nine further chlordane-related compounds. The best enantiomer separation was obtained on 25% *tert.*-butyldimethylsilylated β-cyclodextrin in PS086. However, neither a chromatogram nor retention times were presented and therefore, it is elusive if the column separated *cis*- and *trans*-chlordane in four peaks. In a follow-up account, Buser and Müller

used 30% *tert.*-butyldimethylsilylated β-cyclodextrin in PS086 [84] but were not able to separate the first eluted peaks of *cis*- and *trans*-chlordane. This was confirmed in a paper published by Oehme et al. who used 10% *tert.*-butyldimethylsilylated β-cyclodextrin in PS086 [130]. However, this group offered a solution by combining the chiral phase with a achiral Rt_x-2330 column [130]. While the achiral column was responsible for the separation of the chlordane isomers, the enantiomer separation was obtained afterwards on the 10% *tert.*-butyldimethylsilylated β-cyclodextrin phase. Though *cis*-chlordane was not baseline separated the tandem technique represented a good example to demonstrate a simple solution for this problem. Buser and Müller experienced problems in the separation of technical chlordane on 30% *tert.*-butyldimethylsilylated β-cyclodextrin in PS086 [84] since coelution was observed. On the other hand, in another report baseline separation was obtained on 30% *tert.*-butyldimethylsilylated β-cyclodextrin in PS086 (BGB Analytik, Adliswil, Switzerland) [123]. Some of the authors found that *trans*-chlordane and four other organochlorines (including oxychlordane and three compounds of technical toxaphene) were separated at temperatures as high as 275°C on this phase. This is one of the highest temperatures applied for a GC enantiomer separation. Recently, the separation of *cis*- and *trans*-chlordane in four peaks was also obtained on 35% heptakis(6-O-*tert.*-butyldimethylsilyl-2,3-di-O-methyl)-β-cyclodextrin in OV-1701 [82,88]. Note that modification of the 6-position of this phase was erroneously described as 6-O-*tert.*-butyl instead of 6-O-*tert.*-butyldimethylsilyl in the paper by Müller et al. [88].

The enantiomers of oxychlordane were not separated on heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin in PS086 [129] or CP-Sil 5 [82] which was recommended for the separation of α-HCH enantiomers (see above). Also on heptakis(2,3,6-tri-O-ethyl)-β-cyclodextrin no enantioselective separation of oxychlordane was obtained [129], whereas CSPs containing the chiral selector 30% *tert.*-butyldimethylsilylated β-cyclodextrin enabled the separation of oxychlordane enantiomers [82,129,130]. This suggests that more bulky substituents at the smaller rim of cyclodextrins are necessary to separate oxychlordane enantiomers. In the first article dealing with enantiomer separation of chlordane-related compounds, König et al. reported that the enantiomers of oxychlordane were separated on several CSPs without giving any details [5,128]. One of the columns is, however, concerned with heptakis(2-O-methyl-3,6-di-O-*n*-pentyl)-β-cyclodextrin which was used by Pfaffenberger et al. [90] and Hühnerfuss et al. [107,131]. Later some of the authors used a CSP consisting of 50% heptakis(2,3-di-O-methyl-6-O-thexyldimethylsilyl)-β-cyclodextrin in OV-1701 to separate oxychlordane enantiomers [thexyl=2-(2,3-dimethyl)-butyl] [112]. The undiluted cyclodextrin derivative was also used in preparative GC [83]. Finally, a column consisting of 35% heptakis(6-O-*tert.*- butyldimethylsilyl-2,3-di-O-methyl)-β-cyclodextrin in OV-1701 also baseline separated oxychlordane enantiomers [82].

The enantiomer separation of heptachlor and heptachlorepoxide was also subject of several studies [77,82,83,86,90,128,129]. In 1991, the first separation was published using heptakis(2-O-methyl-3,6-di-O-*n*-pentyl)-β-cyclodextrin [128]. Later, enantiomers of heptachlorepoxide were separated on heptakis(2,6-di-O-methyl-3-O-*n*-pentyl)-β-cyclodextrin by GC and SFC [77]. König et al. also reported α-values for heptachlor on 50% octakis(2,6-di-O-methyl-3-O-*n*-pentyl)-γ-cyclodextrin in OV-1701 and 50% heptakis(2,6-di-O-methyl-3-O-*n*-pentyl)-β-cyclodextrin in OV-1701. The γ-cyclodextrin phase showed comparable α-values at 10°C lower separation temperature [132].

Both heptachlorepoxide and heptachlor were separated on hexakis(2,3,6-tri-O-ethyl)-α-cyclodextrin in OV-1701 [129] while *tert.*-butyldimethylsilylated β-cyclodextrin diluted in PS086 only separated heptachlorepoxide [129,130]. Buser and Müller have also tested mixtures of two modified cyclodextrins and found no enantiomer separation. They stated that enantioselectivity is not additive [129]. Heptachlorepoxide was also separated on 35% heptakis(6-O-*tert.*-butyldimethylsilyl-2,3-di-O-methyl)-β-cyclodextrin and the authors reported at least similar separation power for the enantiomers of heptachlorepoxide as on a heptakis(2-O-methyl-3,6-di-O-*n*-pentyl)-β-cyclodextrin column [82].

In addition to the major compounds of chlordane,

several minor constituents in technical chlordane, photoproducts and their levels in biota were also subject of some studies [84,109,129,130].

6.2. Determination of ERs of chlordane-related compounds in biological samples

In biota, residues of chlordane-related compounds are usually dominated by *trans*-nonachlor and oxychlordane [133]. A further important component is heptachlorepoxide. While *trans*-nonachlor is achiral, oxychlordane and heptachlorepoxide are recalcitrant metabolites of chlordane and heptachlor, respectively. Otherwise the major components, not present in technical chlordane, are biodegradation products of chiral parent compounds. Müller and Buser showed that oxidation of (+)-*trans*-chlordane leads to one oxychlordane enantiomer and, as expected, (−)-*trans*-chlordane to the other one while racemic *trans*-chlordane yielded racemic oxychlordane [86]. Buser and Müller were also the first to separate oxychlordane and heptachlorepoxide in biological samples [84] followed by Hühnerfuss et al. who claimed this result for themselves [107]. In both articles the elution order of the enantiomers was not known. Already in 1992 Armstrong and Pitha found that elution orders on different CSPs can not be predicted [39]. Since enantioenriched standards of chlordane-related compounds were not available at the outset, ERs are difficult to interpret in terms of the identity of the enantiomers. In 1994, however, both König et al. [83] and Müller and Buser [86] obtained enantioenriched standards by preparative enantiomer separation including oxychlordane, *cis*- and *trans*-chlordane and determined the sign of rotation of the respective enantiomers.

Buser and Müller published enantioselective determination of oxychlordane and other chlordane-related compounds in biological samples [84]. Since the elution order of enantiomers was not known, they referred the data to the elution order on *tert.*-butyldimethylsilylated β-cyclodextrin [84]. In later work the authors determined the rotation of the oxychlordane enantiomers and confirmed that the (+)-oxychlordane eluted in front of (−)-oxychlordane from this CSP. Therefore, the ER data published by Buser and Müller for oxychlordane [84] can now be interpreted as $ER_{(+)}$. Hühnerfuss et al. commenting on results of Buser and Müller did neither mention the exact kind of species nor the fact that ERs are given with respect to the elution order and the sign of rotation of the enantiomer [107]. In the case of seal blubber we were able to reconstruct the facts (see below). In some cases it might be problematic that the identification of the species was omitted. As outlined in Section 4.1, there is strong evidence that biodegradation occurs species specific and even in related species inverse ERs can be revealed. On 35% heptakis(6-O-*tert.*-butyldimethylsilyl-2,3-di-O-methyl)-β-cyclodextrin in OV-1701 the peak with the shorter retention time originated from (−)-oxychlordane which was shown by injection of commercially available enantioenriched oxychlordane [82].

6.3. ERs of compounds present in technical chlordane

Buser and Müller studied the composition of technical chlordane by determining ERs of the chiral components [84]. Due to the complex mixture of technical chlordane a number of coelutions were observed but the use of three different CSPs allowed to separate the major compounds *cis*-chlordane, *trans*-chlordane, heptachlor as well as the minor compounds MC5, MC6 and U82. The structure of U82 is still unknown and therefore, the evidence of the chirality is an important information toward the structure elucidation of this and other compound. As expected, for all compounds, ERs close to 1 were observed confirming that the synthesis of chlordane leads to a racemic mixture [84].

6.4. ERs of oxychlordane and heptachlorepoxide in terrestrial animals

In terrestrial animals only ERs of the metabolites oxychlordane and heptachlorepoxide have been investigated so far. In roe-deer (*Capreolus capreolus*) livers from Schleswig-Holstein (north of Germany) and Baden-Württemberg (south of Germany) high levels of the epoxides were measured by GC–ECD. In Schleswig-Holstein oxychlordane ERs ranged from 9–17 (levels from 10–60 μg/kg fat) and heptachlorepoxide ERs ranged from 1–9 (levels

from 10–100 μg/kg fat). In Baden-Württemberg the levels were in the same order but in some of the samples inferferences were observed which precluded a determination of the ERs. The ERs determined in four samples from Baden-Württemberg confirm an excess of the (+)-enantiomers with higher ERs for oxychlordane [90]. Hühnerfuss et al. found an excess of the (+)-epoxides in hare liver but the ERs (oxychlordane: 1–1.5; heptachlorepoxide: 2.5–3.7) were lower compared to roe deer though the levels were in the same order in both species [131]. Levels and ERs of oxychlordane were recently published in liver of polar foxes (*Alopex lagopus*) [82,88]. Interestingly, all samples exhibited ERs<1 with the exception of a single sample with the highest concentration of oxychlordane which had an ER>1. The ERs were confirmed on two different CSPs on GC–ECD and also on GC–EI-MS. This is the only remarkable example of an inversion of the ER within one species which depends on the concentration on an organochlorine compound.

Buser and Müller published chromatograms showing the enantiomer separation of oxychlordane and heptachlorepoxide in human adipose tissue of a male American (see Fig. 5) [84]. At that time the elution orders of the enantiomers were unknown but from data of the authors presented later [86] it can be concluded that in both cases the (+)-enantiomers were more abundant. In blubber of a polar bear (*Ursus maritimus*) the oxychlordane ER was also >1 [82].

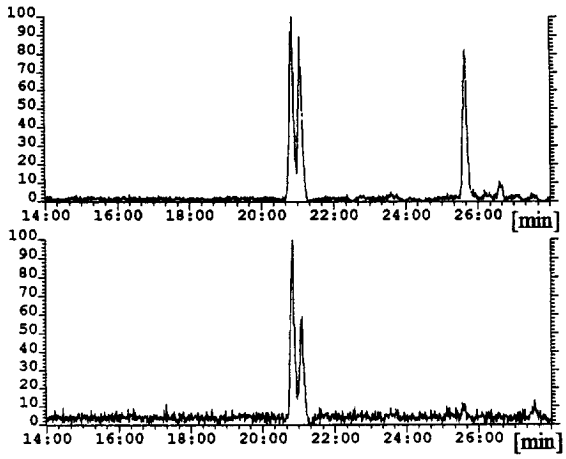


Fig. 5. Enantiomer separation of oxychlordane in blubber of a Baltic grey seal (*Halichoerus grypus*) (above) and human adipose tissue (below) on *tert.*-butyldimethylsilylated β-cyclodextrin by GC–ECNI-MS and recording of *m/z* 424 [84]. (+)-Oxychlordane eluted in front of (−)-oxychlordane [86].

6.5. ERs of oxychlordane and heptachlorepoxide in marine samples

In a Baltic cod liver sample and a Baltic herring sample the ER of oxychlordane was >1 [84]. Eggs of seagulls exhibited oxychlordane ERs of 1.5–2.3 ($n=5$) and heptachlorepoxide ERs of 1.6–2.7 ($n=2$) [83,107,131]. Only few sea mammal species have been analyzed for the ER of oxychlordane. Buser and Müller found coelutions with further organochlorines in a Baltic grey seal (*Halichoerus grypus*) sample which had an influence on the ERs though the interferent had a different mass in ECNI-MS [84]. The authors expected an oxychlordane ER>1 (see Fig. 5) which is in agreement with data in blubber of grey seal samples from Iceland [82,88]. However, harbour seal (*Phoca vitulina*) blubber from the same region showed oxychlordane ERs<1 [82,88]. Higher levels of the (−)-oxychlordane were also reported before in blubber of harbour seals from the German North Sea coast [83]. This result was surprising since both harbour and grey seals showed α-HCH ERs>1 (see above). No explanations were given by the authors, but they noted that both seal species were dissected, stored, transported and analyzed in the same way [82]. However, these data confirm that an explicit description of a species is very important. In blubber of a caspian seal (*Phoca caspica*) the oxychlordane ER was 2.0 [82]. König et al. studied the oxychlordane and (*cis*-) heptachlorepoxide ERs in blubber, liver and brain of two harbour seal samples [83]. No significant alteration in the ERs determined in blubber, liver and brain was observed which is in contrast to α-HCH (see Section 5.6).

7. Enantioselective determination of *o,p'*-DDT and *o,p'*-DDD

DDT is one of the most important pesticides with a broad field of application. The use of DDT as insecticide was the first effective protection against

Malaria transmitting insects. The application of DDT may have saved the life of millions of people [97]. The importance of DDT can be seen in the Nobel Price grant to Paul Müller for his discovery of the insecticidal properties of DDT. Already in 1974, the usage of DDT amounted to four billion English pounds [134]. On the other hand, the accumulation of DDT and its metabolites in fatty tissue was the starting point for the recognition of organochlorine compounds as persistent and hazardous environmental contaminants.

Typical technical DDT mixtures consist of 77.1% achiral *p,p'*-dichlorodiphenyltrichloroethane, *p,p'*-DDT, and 14.9% chiral *o,p'*-DDT. Further compounds detected in technical DDT are the achiral *p,p'*-dichlorodiphenyldichloroethene, *p,p'*-DDE, (4%), *o,p'*-DDE (0.1%), *p,p'*-dichlorodiphenyldichloroethane, *p,p'*-DDD, (0.3%), and the chiral *o,p'*-DDD (0.1%) together with approx. 3.5% unidentified compounds [135].

In 1993, the first enantiomer separation of an *o,p'*-DDT standard solution was obtained on 50% octakis (2,6-di-O-methyl-3-O-*n*-pentyl) -γ-cyclodextrin in OV-1701 [136]. Schurig used a heptakis(2,3,6-tri-O-trifluoroacetyl)-β-cyclodextrin (Chiralsil-Dex-TFA) (see Fig. 6) [4] and Oehme et al. separated the enantiomers of *o,p'*-DDT on a tandem combination of RT_x-2330 and *tert.*-butyldimethylsilylated β-cyclodextrin in PS086 [130]. Buser and Müller showed that *o,p'*-DDT and *o,p'*-DDD are separated on *tert.*-butyldimethylsilylated β-cyclodextrin in PS086 as single column [137] which was confirmed in one of the present laboratories.

Oehme et al. also determined *o,p'*-DDT in standard reference material cod liver oil (SRM 1588) and found the second eluted enantiomer more abundant than the first eluted enantiomer (see Fig. 7) [130]. This was the first report on enantioenriched *o,p'*-DDT in biological samples. The authors were not able to assign the elution order to the peaks but recently, Buser and Müller isolated enantiopure *o,p*-DDT and *o,p'*-DDD by chiral HPLC and determined also the elution order on CSPs [137]. On 30% *tert.*-butyldimethylsilylated β-cyclodextrin in OV-1701, (−)-*o,p'*-DDT eluted in front of (+)-*o,p'*-DDT. Since only the absolute configuration of *o,p'*-DDT was known the authors deduced the absolute configuration of *o,p'*-DDD via chemical analogy

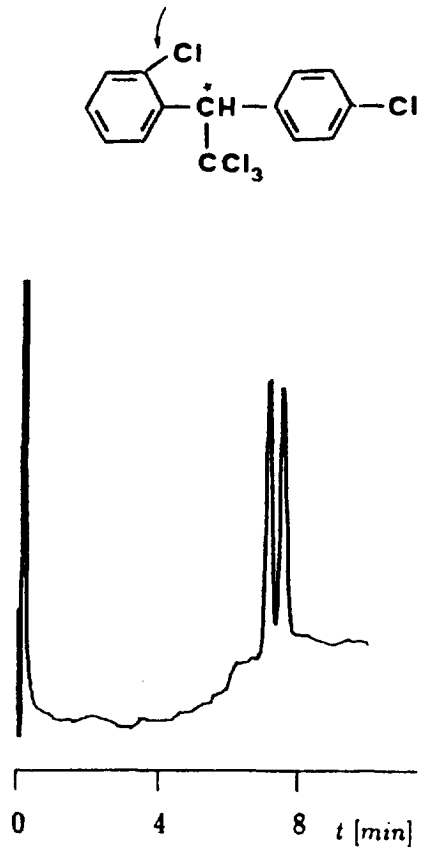


Fig. 6. Enantiomer separation of *o,p'*-DDT standard solution on a 10 m capillary column (0.25 mm ID) coated with heptakis(2,6-di-O-methyl-3-O-trifluoro-acetyl)-β-cyclodextrin bonded to dimethylpolysiloxane (Chirasil-Dex-TFA) [4].

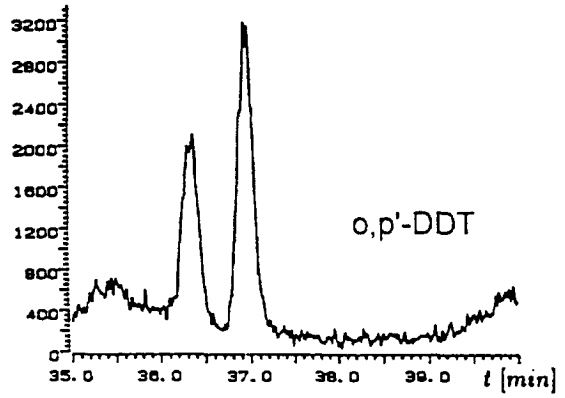


Fig. 7. Enantiomer separation of *o,p'*-DDT in cod liver oil (SRM 1588) on a tandem column consisting of RT_x-2330 and *tert.*-butyldimethylsilylated β-cyclodextrin by GC–ECNI-MS [130].

[137], a method which is often used in pharmacology [138]. Thus, reaction with $NaBH_4$ showed that (+)-*o,p'*-DDT is correlated with (−)-*o,p'*-DDD. On 30% *tert.*-butyldimethylsilylated β-cyclodextrin in OV-1701, the (+)-*o,p'*-DDD eluted in front of the (−)-*o,p'*-DDD [137]. As expected, both components were present in a racemic composition in technical DDT. Additionally, the authors described chirality of several unidentified components in technical DDT [137].

8. Enantioselective determination of compounds of technical toxaphene (CTTs)

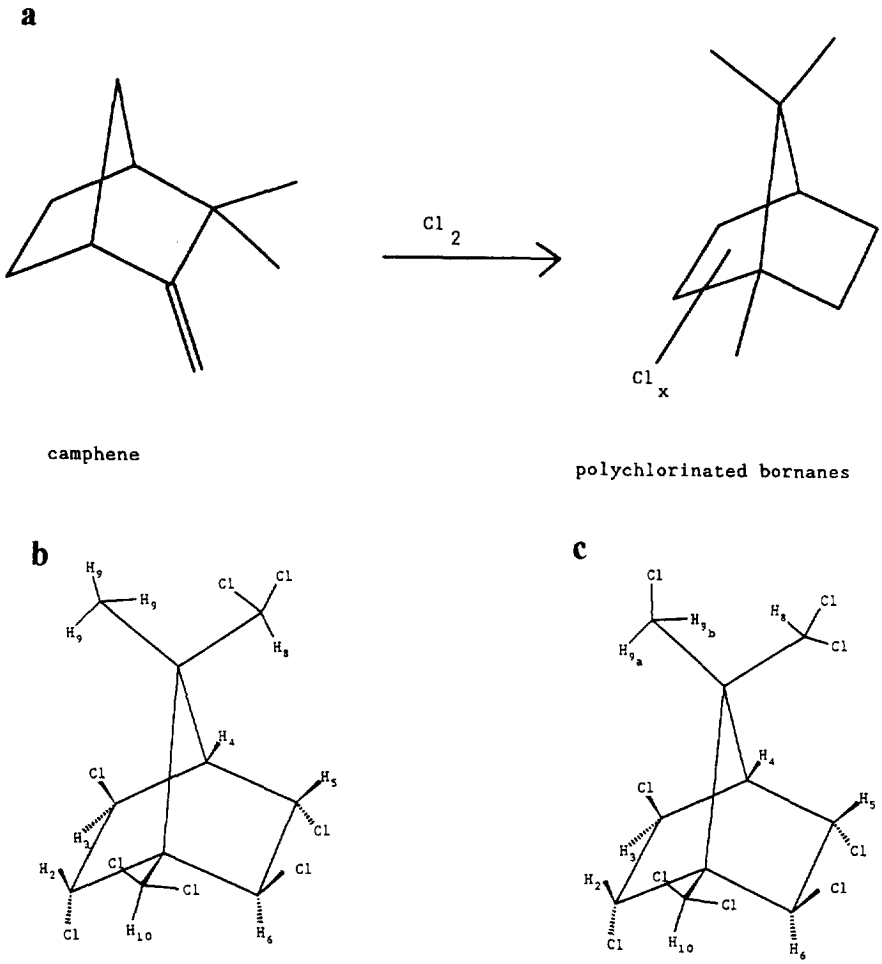
Toxaphene is one of the world's most applied organochlorine insecticides. Recent surveys showed that toxaphene is a major organochlorine contaminant of aquatic life in polar regions and lakes all over the world [139]. Technical toxaphene is synthesized by the exhaustive chlorination of camphene (chlorine content: 67–69%). A Wagner-Meerwein rearrangement during the synthesis leads mainly to polychlorinated 1.7.7-trimethyl-bicyclo[2.2.1]heptanes (bornanes) [140]. Scheme 2 shows the synthesis procedure and the structure of two major recalcitrant CTTs in biota. Several hundred compounds have been detected in the technical mixtures [141]. Theoretically 32 767 chlorinated bornanes are possible and only 511 are achiral while 16 128 exist as enantiomeric pairs [142]. Therefore, it is obvious that most of the CTTs in samples are chiral. Unfortunately, single toxaphene compounds isolated from the technical mixtures or detected in biological samples have been characterized by using several abbreviations. In order to unify the nomenclature, a system suggested by Andrews and Vetter has been applied in this study [143]. It starts with a letter and a number which reflect the carbon backbone (b for bornane) and the number of chlorine atoms present. This system is similar to the PCB numbering by Ballschmiter and Zell [164]. Alternative abbreviations including Parlar numbers are listed in Table 2. Table 2 lists all CTT congener standards of known structure enantiomer separated so far.

Hitherto, despite the environmental importance of toxaphene only few studies focused on the enantiomer separation of CTTs. This is partly due to difficulties inherent to the separation of CTT enantiomers on several CSPs. Recently, a list of twelve CSPs which resisted any enantiomer separation of the major octachlorobornane in biota, B8-1413 (Parlar No. 26), was presented [123]. Up to now, only CSPs consisting of different percentages of *tert.*-butyldimethylsilylated β-cyclodextrin in PS086 or OV-1701 separated the enantiomers of this octachlorobornane and other CTTs [82,92,123,148–154]. On the other hand, enantiomer separation of CTTs required high temperatures. On 20–30% *tert.*-butyldimethylsilylated β-cyclodextrin in PS086 separation temperatures between 220°C and 245°C were reported [123]. Three CTTs were even separated at 275°C on β-BSCD [153].

In contrast to technical toxaphene, only few CTTs accumulate in higher trophic organisms and all of the compounds identified or isolated so far are chiral. Recently, congener specific quantitation of CTT residues was possible after synthesis and isolation of more than 20 single CTTs [155–157].

8.1. CSPs applied for the enantiomer separation of CTTs

The first enantiomer separation of two CTTs was published in 1994 by Buser and Müller [92,149] and Kallenborn et al. [148]. The columns contained 30% *tert.*-butyldimethylsilylated β-cyclodextrin in OV-1701 [92,148,149], a mixture of *tert.*-butyldimethylsilylated and perethylated β-cyclodextrin in OV-1701 [149] and 10% *tert.*-butyldimethylsilylated β-cyclodextrin in PS086 [148,150]. Later, columns consisting of 20–30% *tert.*-butyldimethylsilylated β-cyclodextrin in PS086 were applied [82,123,151–154]. However, the separation efficiencies were not reproducible since the CSPs were obtained from different manufacturers. CSPs containing *tert.*-butyldimethylsilylated β-cyclodextrin were introduced by Blum and Aichholz in 1990 [44], and it was shown that the synthesis of BSCD does not lead to a single product but to a mixture of several compounds. Slight changes from batch to batch are likely to occur. Maas and coworkers introduced defined CSPs with *tert.*-butyldimethyl groups in 6- and 2,6-position while the other positions (2,3 or 3) of the cyclodextrin were modified with other groups



Scheme 2. Synthesis pathway of toxaphene and structures and IUPAC names of the two major recalcitrant CTTs in biota. (a) Toxaphene synthesis starting from camphene and leading to polychlorinated bornanes. (b) B8-1413 (Parlar No. 26): 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10-octachlorobornane. (c) B9-1679 (Parlar No. 50): 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10-nonachlorobornane.

[52,158,159], but these columns lost the capability of enantiomer separation of the major octachlorobornane in biota 2-*endo*,3-*exo*,5-*endo*,6-*exo*, 8,8,10,10-octachlorobornane, B8-1413 (see Scheme 2) [82,123]. Also columns consisting of various alkylated cyclodextrins failed to separate the enantiomers of this compound. Therefore, *tert.*-butyldimethylsilylated β-cyclodextrin columns introduced by Blum and Aichholz are so far the only available CSPs on which the enantiomer separation of CTTs was achieved. Fig. 8 shows the enantiomer separation of a mixture of six major recalcitrant CTT standards on β-BSCD.

8.2. Determination of ERs of CTTs

One of the most remarkable observation in the enantioselectivity of organochlorine compounds is the small levorotary rotation of different technical toxaphene mixtures detected with chiroptical detector [92]. Furthermore, enantioselective gas chromatography in combination with MS–MS analysis of technical toxaphene mixtures using selected reaction monitoring (SRM) showed some compounds in a racemic composition while others were enantioenriched. However, as outlined above, toxaphene consists of several hundreds of compounds. Though

Table 2
Systematic code names [143], chemical names, Parlar numbers and further abbreviations of CTTs and reference to studies on the enantiomer separation of *tert.*-butyldimethylsilylated cyclodextrins

Systematic code [143]	Chemical structure enantiomer a (IUPAC name)	Chemical structure enantiomer b	Parlar number	Further abbreviations	Ref.
B7-515	2,2,5-*endo*,6-*exo*,8,9,10	2-*exo*,3-*endo*,6,6,8,9,10	32	Toxicant B [144]	[150,123]
B7-1059	2-*exo*,3-*endo*,6-*endo*,8,9,10,10	2-*endo*,5-*endo*,6-*exo*,8,9,10,10	–	Tox3157 [145]	[150]
B7-1146	2-*exo*,5,5,9,9,10,10	3,3,6-*exo*,8,8,10,10	–	Tox2439 [145]	[150]
B7-1453	2-*exo*,3-*endo*,5-*exo*,9,9,10,10	3-*exo*,5-*endo*,6-*exo*,8,8,10,10	–	TOX7 [161]	[123,154]
B8-531	2,2,3-*exo*,5-*endo*,6-*exo*,8,9,10	2-*exo*,3-*endo*,6,6,8,9,10	39	-	[150]
B8-786	2,2,5,5,8,9,10,10	3,3,6,6,8,9,10,10	51	–	[150]
B8-789	2,2,5,5,9,9,10,10	3,3,6,6,8,8,10,10	38	–	[150]
B8-806	2,2,5-*endo*,6-*exo*,8,8,9,10	2-*exo*,3-*endo*,6,6,8,9,9,10	42	Toxicant A [144]	[150]
B8-809	2,2,5-*endo*,6-*exo*,8,9,9,10	2-*exo*,3-*endo*,6,6,8,8,9,10	42	Toxicant A [144]	[150]
B8-1413[a]	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10	2-*exo*,3-*endo*,5-*exo*,6-*endo*,9,9,10,10	26	T2 [146], TOX8 [147]	[82,92,123,148–154]
B8-1414	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,9,10,10	2-*exo*,3-*endo*,5-*exo*,6-*endo*,8,9,10,10	40	–	[150,123,148,149]
B8-1945	2-*exo*,3-*endo*,5-*exo*,8,9,9,10,10	3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10	41	–	[150,123,148]
B8-2229	2-*exo*,5,5,8,9,9,10,10	3,3,6-*exo*,8,8,9,10,10	44	–	[150,123,148]
B9-415	2,2,3-*endo*,5,5,8,9,10,10	3,3,5-*endo*,6,6,8,9,10,10	58	–	[150]
B9-418	2,2,3-*exo*,5,5,9,9,10,10	3,3,5-*exo*,6,6,8,8,10,10	–	Tox7047 [145]	[150]
B9-1025	2,2,5,5,8,9,9,10,10	3,3,6,6,8,8,9,10,10	62	–	[150,123,148,149]
B9-1679[a]	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10	2-*exo*,3-*endo*,5-*exo*,6-*endo*,8,9,9,10,10	50	T12 [146], TOX9 [147] Toxicant A_C [144]	[82,92] [123,148–154]
B9-2206	2-*exo*,3-*endo*,5-*exo*,6-*exo*,8,8,9,10,10	2-*exo*,3-*exo*,5-*endo*,6-*exo*,8,9,9,10,10	63	[150]	[150]

Note that the structures adapt IUPAC rules [147] and sometimes deviate from literature citations.
[a] Formulas are shown in Scheme 2.

SRM in MS–MS is a selective method to investigate CTTs [160], coelutions with further CTTs can not be excluded and there was a strong skepticism among other scientists referring to nonracemic composition of synthetic toxaphene. Single CTT standards synthesized by Burhenne et al. [155], Hainzl et al. [156] and Tribulovich et al. [157] lead to racemates [123,148,150]. However, recently a heptachlorobornane B7-1453 (TOX7) isolated from the technical product Melipax (former German Democratic Republic) [161] showed different peak abundance of the enantiomers [123], and constant ERs of 1.26±0.03 when determined by both GC–EI-MS and GC–ECNI-MS for several selected ions [154]. The

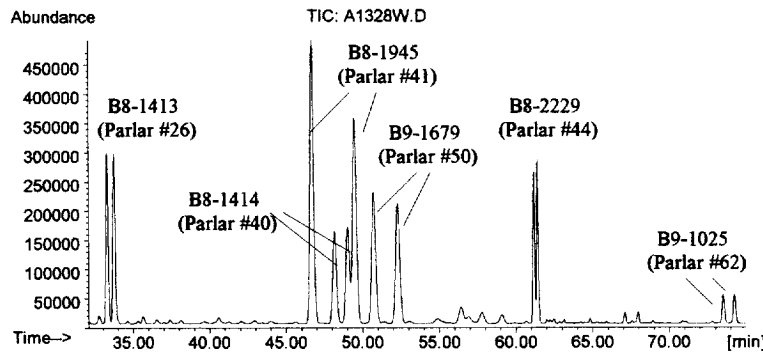


Fig. 8. Enantiomer separation of six persistent CTTs on 25% *tert.*-butyldimethylsilylated β-cyclodextrin in PS086 by GC–ECNI-MS [152]. Multiple ion chromatogram, 20–47 min: *m/z* 341, *m/z* 343, *m/z* 377, *m/z* 379, *m/z* 381; 47–80 min *m/z* 377, *m/z* 379, *m/z* 411, *m/z* 413, *m/z* 415. Note that the second eluted peak of B8-1945 is lower abundant than the first eluted peak since both signals appeared in different time windows. Both enantiomers were present in equal amounts.

TIC showed no interferences and coelution with another compound could be excluded. Therefore, there is strong evidence that B7-1453 and maybe other CTTs are present in a nonracemic composition in technical mixtures. An explanation is provided by the use of natural precursors (α-pinene, terpene and camphene) in the toxaphene synthesis. This observation, however, makes the judgment of ERs of CTTs in biota critical. It must be expected that deviations from a racemic composition in biological samples may not only arise from enantioselective biodegradation and therefore, ERs of CTTs in biota must be interpreted with great care.

8.3. ERs of CTTs in biological samples

The first two reports of ERs of two major abundant CTTs in biological samples focused on the determination in blubber of a harbour seal (*Phoca vitulina*) [148,149]. In both studies the same sample was used from which the major CTTs in biota have been isolated [162]. B9-1679 showed good agreement with ERs of 1.06 vs. 1.08 but B8-1413 deviated remarkably with an ER of 1.02 [148] versus 1.12 [149]. It is important to note that baseline separation of the B8-1413 enantiomers was not obtained in both studies mentioned above and this might be the explanation for the discrepancy of the results.

The most complete study so far has been published by Alder et al. [151]. The authors found B8-1413 in different fish species with ERs ranging from 0.91–1.13, in monkey adipose tissue 1.30 and in human milk 1.07–1.28. The ERs of B9-1679 in the same samples ranged from 1.08–1.13 in fish, 1.4 in monkey adipose and 1.06–1.33 in human milk [151]. In both cases Alder et al. found the first peak more abundant in warm blooded animals and confirmed an ER of B9-1679 determined in Antarctic penguin by Buser and Müller [149]. In Antarctic seal blubber the first peak of B9-1679 was also abundant [82,123]. The ER of B7-1453 in a cod liver (*Gadhus morhua*) sample from the Baltic Sea was 1.0. However, the ER of B7-1453 isolated from technical mixture was approx. 1.26 (see Fig. 9). This example presents the unique case of an organochlorine compound which has been applied in nonracemic composition and an ER of 1.0 in biota must therefore be linked to an enantioselective accumulation of this compound resulting in a racemic composition (see Fig. 9).

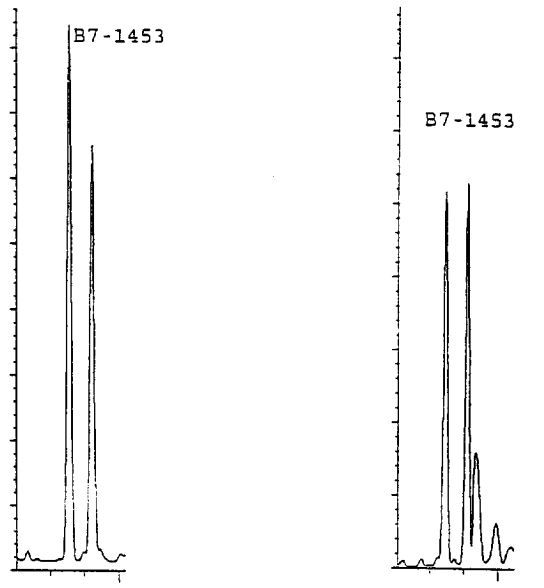


Fig. 9. Enantiomer separation of B7-1453 by GC–ECNI-MS (conditions as in Fig. 8). Left: nonracemic composition in the isolate from the technical product Melipax; right: isolate from cod liver extract.

9. Enantioselective determination of atropisomeric polychlorinated biphenyls (PCBs)

In contrast to all classes of organochlorine compounds discussed so far PCBs are not pesticides but industrial compounds with a wide range of application. From the 1940s on large amounts of PCBs have been used but following the discovery of PCBs in environmental samples in 1966 [163], the production was more and more diminished and finally discontinued.

The biological properties of PCBs are depending critically on the number of *ortho* substituents. Portrayed in simplified terms, non- and mono-*ortho*-PCBs represent most of the toxic congeners, di-*ortho*-PCBs are the most abundant congeners in biological samples, and among the tri- and tetra-*ortho*-PCBs stable atropisomers are found. Requirement for chirality of PCBs is a nonsymmetrical substitution pattern on both phenyl rings and hin-

dered rotation about the phenyl-phenyl axis. 78 of the 209 PCB congeners are nonsymmetrically substituted. Stable PCB atropisomers are compounds which are chiral at ambient temperatures (>80°C) requiring three or four *ortho*-chloro substituents. Among di-*ortho* substituted phenyl rings only 2,3,6- and 2,3,4,6- and among the mono-*ortho* substituted, eight nonsymmetric substitution patterns fulfill the requirement mentioned above. Combination of these substitution patterns lead to the nineteen stable atropisomeric PCBs as predicted by Kaiser [10].

In the mid-1980s, LC on triacetylcellulose has been used for the first time to separate the enantiomers of PCB 88, PCB 139 and PCB 197 [165,166]. Toxicological tests established different toxicity of the (+)- and (−)-enantiomers of PCB 139 [167]. In 1993, the first GC separations of atropisomeric PCBs were published [122,168] and the year after the first report on the enantioselective determination of an atropisomeric PCB in biological samples appeared [91]. Recently, isolation of enantiopure atropisomeric PCBs by application of chiral HPLC was reported [169–172] followed by a study of the elution order of PCB atropisomers in enantioselective gas chromatography [85]. The axial chirality of atropisomers is linked to steric hindrance and, consequently, the temperature is the determining factor for the conformational stability of atropisomers. At 0°C–30°C several di-*ortho*-PCBs like PCB 40 were also separated into enantiomers by application of HPLC and modified cyclodextrins [171].

9.1. Enantiomerization of atropisomeric PCBs

The conformational integrity of the enantiomers of atropisomeric PCBs is important in enantiomer analysis and in the assessment of biochemical transformations. Rotation about the central single-bond of atropisomeric biphenyls leads to enantiomerization via a planar transition state. When the rate of enantiomerization is comparable to the time scale of the GC enantiomer separation, characteristic peak profiles are obtained which are characterized by the appearance of a plateau between the terminal peaks of the enantiomers [122,173,174]. By peak form analysis, the activation parameters or enantiomerization ($\Delta G^{\#}$) can be determined by dynamic gas chromatography [60]. If enantiomerization is fast within the chromatographic time scale, coalescence will arise resulting in only one peak.

It is remarkable that the enantiomers of three- and tetra-*ortho* substituted PCBs show two distinct elution profiles upon GC on polysiloxane bonded permethylated β-cyclodextrin (Chirasil-Dex) at elevated temperatures. Thus, the elution profile of the enantiomers of atropisomeric PCB 132 (for structure see Fig. 11) shows no sign of a plateau-formation at 170°C [168,170] despite the apparent low barrier of enantiomerization of $\Delta G^{\#} = 116$ kJ/mol calculated by the AM1 formalism [175]. The discrepancy was interpreted by Krupcik et al. by the presence of the cyclodextrin derivative which was believed to enhance the activation barrier of PCB 132 [175].

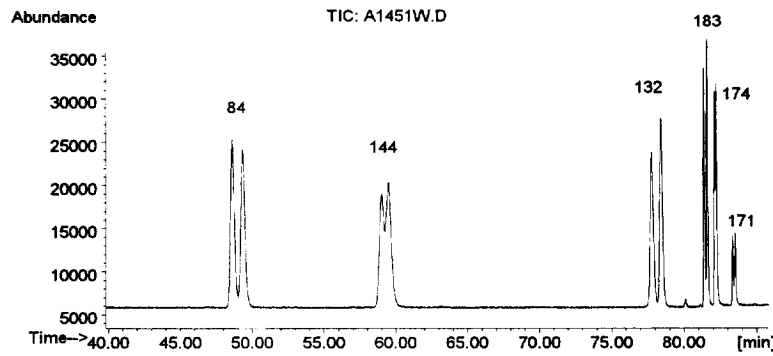


Fig. 10. Enantiomer separation of PCB 132 in Clophen A60 by multidimensional GC and ECD. Left: separation on the achiral first column (DB-5). Right: enantiomer separation of the "heart cut" on immobilized heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (Chirasil-Dex) [91].

However, Schurig et al. determined the experimental barrier of the enantiomerization of PCB 132 via racemization kinetics with isolated enantiomers of PCB 132 (obtained by preparative LC on Chirasil-Dex coated on Nucleosil) [170] and by a new stopped flow technique [170,176]. The high activation barrier of $\Delta G^{\#} = 182$ kJ/mol (280–300°C) is in agreement with the absence of interconversion profiles in the gas chromatogram (see Figs. 10 and 11) and lends support to the notion that the enantiomeric bias of PCB 132 observed in vivo is not due to a deracemization process in the presence of chiral biogenic material [170]. It was also shown that the semiempirical approach is strongly inferior to ab initio methods in calculating the rotational barrier of PCB 132 with data ranging from $\Delta E = 118$ kJ/mol (PM3) to $\Delta E = 240$ kJ/mol (*HF*/6−31 *G**) [177].

It should be noted that the phenomenon of enantiomerization is relevant for dichloro *ortho*, *ortho'*-PCBs with rotational barriers expected to be low. However, at common GC temperatures for gas chromatographic enantiomer separations of atropisomeric PCBs (>150°C), no enantiomerization is likely to occur for the nineteen PCB congeners shown in Table 3, and the study of enantioenrichment of these atropisomeric PCB in biological samples is feasible.

9.2. CSPs applied for the enantiomer separation of atropisomeric PCBs

Enantiomer separations of atropisomeric PCBs were carried out on columns consisting of polysiloxane bonded (immobilized) heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (Chirasil-Dex) [85,91,96, 168,170,175,178–181] and on 50% octakis(2,6-di-O-methyl-3-O-*n*-pentyl)-γ-cyclodextrin in OV-1701 [122]. Several PCB atropisomers were separated on 35% or 50% heptakis(6-O-*tert.*-butyl-2,3-di-O-methyl)-β-cyclodextrin in OV-1701 [82,182,183], 50% heptakis(2,3-di-O-methyl-6-O-thexyldimethylsilyl)-β-cyclodextrin in OV-1701 [112], and 25% *tert.*-butyldimethylsilylated β-cyclodextrin in PS086 [184]. The latter CSP separated the enantiomers of PCB 144 (see Fig. 10) which was not possible on the other mentioned CSPs. Table 3 lists the atropisomeric PCBs together with the chiral GC stationary phases which have been applied, including the first report on the enantiomer separation with the respective CSP.

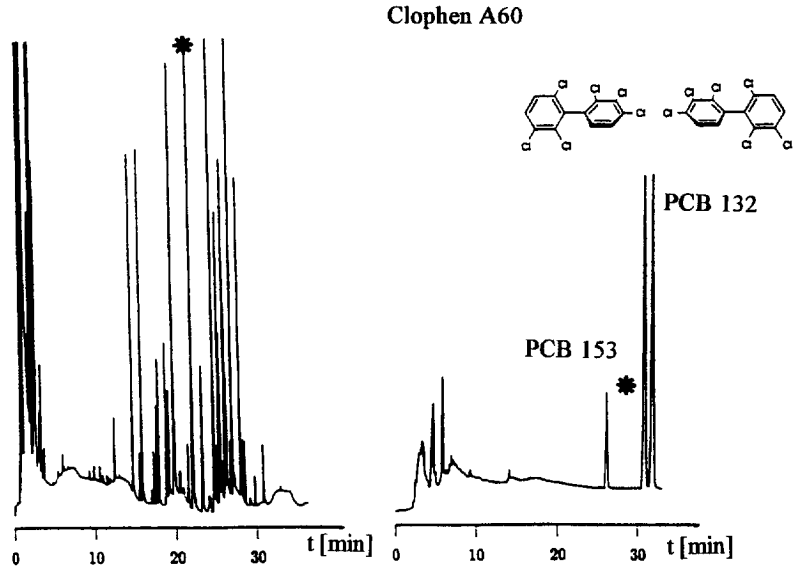


Fig. 11. Enantiomer separation of atropisomeric PCB standard solutions on 25% *tert.*-butyldimethylsilylated β-cyclodextrin in PS086 by GC–EI-MS [184].

Table 3
Successful gas chromatographic separation of the nineteen stable atropisomeric PCBs on some CSPs and first reports on the enantiomer separation

	Ring A	Ring B′	2,3,6-Tri-O-methyl-β-cyclodextrin	2,3-Di-O-methyl-6-O-thexyldimethylsilyl β-cyclodextrin	2,6-Di-O-methyl-3-O-*n*-pentyl-γ-cyclodextrin	6-O-*tert.*-Butyldimethylsilyl-2,3-di-O-methyl-β-cyclodextrin	*tert.*-Butyldimethylsilylated β-cyclodextrin
PCB 45 (4/3)[b]	2,3,6	2		[112]	[122]		
PCB 84 (5/3)	2,3,6	2,3	[168][a]	[112][a]			[184][a]
PCB 88 (5/3)	2,3,4,6	2			[112][a]		
PCB 91 (5/3)	2,3,6	2,4	[168]	[112]			
PCB 95 (5/3)	2,3,6	2,5	[168]	[112]	[122]		
PCB 131 (6/3)	2,3,4,6	2,3		[112]		[112][a]	
PCB 132 (6/3)	2,3,4	2,3,6	[168]		[112]	[82][a]	[184]
PCB 135 (6/3)	2,3,5	2,3,6	[168]		[112][a]		
PCB 136 (6/4)	2,3,6	2,3,6	[168]	[112]			
PCB 139 (6/3)	2,3,4,6	2,4			[122]		
PCB 144 (6/3)	2,3,4,6	2,5					[184]
PCB 149 (6/3)	2,3,6	2,4,5	[168][a]	[112][a]		[182]	[184]
PCB 171 (7/3)	2,3,4,6	2,3,4					[184]
PCB 174 (7/3)	2,3,4,5	2,3,6	[85]	[112]			[184]
PCB 175 (7/3)	2,3,4,6	2,3,5		[112]			
PCB 176 (7/4)	2,3,4,6	2,3,6	[85]	[112]			
PCB 183 (7/3)	2,3,4,6	2,4,5		[112]			[184]
PCB 196 (8/3)	2,3,4,5	2,3,4,6					
PCB 197 (8/4)	2,3,4,6	2,3,4,6					

[a] No chromatogram shown.
[b] Number in parentheses reflect degree of chlorination and number of *ortho* substituents.

On heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin bonded to polysiloxane (Chirasil-Dex) nine of the nineteen atropisomeric PCBs were separated but the enantiomer separation of 2,3,4,6-substituted atropisomeric PCBs was problematic [85]. None of the nine 2,3,4,6-substituted PCBs, except for PCB 176, with additional substitution at 2,3,6-position could be separated [85]. However, seven of them have been separated on other phases (see Table 3). The missing two, PCB 196 and PCB 197 have been separated by LC [165,166,171]. So far, most separations were obtained on 50% heptakis(2,3-di-O-methyl-6-O-thexyldimethylsilyl)-β-cyclodextrin in OV-1701 (see Table 3). Haglund isolated enantiopure PCBs by chiral HPLC and determined the elution order of six PCB atropisomers on immobilized heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (Chirasil-Dex). While the (−)-enantiomers of PCB 84, PCB 132, PCB 136 and PCB 176 eluted in front of the (+)-enantiomers, the elution order of PCB 135 and PCB 175 was inverse [85].

The separation of various PCB atropisomers vividly confirms the prediction of Kaiser on their conformational stability [10].

9.3. ERs of atropisomeric PCBs in technical mixtures and biota

Most of the nineteen stable PCB atropisomers have been detected in technical PCB mixtures [185]. In 1994, Glausch et al. succeeded in the enantiomer separation of PCB 95, PCB 132 and PCB 149 in Clophen A60 (Bayer, Germany) (see Fig. 11) [91]. Due to the complex mixture of Clophen A60, the authors used multidimensional gas chromatography (MDGC) for an unambiguous determination of the congeners. After separation on an achiral DB-5 column heart cuts of the PCBs were subsequently separated on the chiral CSP consisting of immobilized heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (Chirasil-Dex).

At lower trophic levels, sediment samples were analyzed by MDGC–ECD [96,180].

Glausch et al. found no enantioenrichment of PCB

95, PCB 132 and PCB 149 in sediment samples from a small river in southern Germany [180] while Benická et al. measured ERs of approx. 0.6 for PCB 95 and ER = 1 for PCB 91 [96]. Unfortunately, only little information about the origin of the samples was available in the latter report. As outlined above, the presentation of the exact origin of a sample is mandatory for the evaluation of ERs.

Glausch et al. found a first indication of a higher abundance of the second eluted peak of PCB 132 in human milk samples [91]. A more detailed description of the PCB 132 ERs in human milk samples was presented later. Although MDGC was used, the unambiguous determination of PCB 132 ERs was difficult [178]. To confirm the results, two achiral stationary phases of different polarity were applied, as well as GC–MS in the SIM mode. Finally, ERs of PCB 132 in human milk ranged from 0.4 to 0.9 [178]. This appears to confirm the higher enantioenrichment of the second eluted PCB 132 enantiomer. Since Haglund and Wiberg used the same CSP as Glausch et al. (i.e., Chirasil-Dex), the elution order of PCB 132 published in Ref. [85] allows the conclusion that in the human milk samples measured by Glausch et al. [178] (+)-PCB 132 was the more abundant enantiomer.

Recently, Blanch et al. also used MDGC for the determination of ERs of PCB 95, PCB 132 and PCB 149 in liver of shark (*C. coelolepis*) [179]. While PCB 95 and PCB 149 were present in racemic compositions, the second eluted enantiomer of PCB 132 (i.e., (+)-PCB 132 acc. to Ref. [85]) predominated at a small degree. The ER of PCB 132 ranged from 0.75 to 0.89 [179]. Hühnerfuss et al. identified five atropisomeric PCBs in blue mussels (*Mytilus edulis* L.) on an achiral stationary phase [182]. Higher levels of PCB 88, PCB 149, PCB 171, PCB 174 and PCB 183 were determined in spring as compared to autumn [182]. In this study only the atropisomers of PCB 149 were separated and ERs of PCB 149 between 1.0 and 1.2 were determined by GC–ECD, showing a weak enantioenrichment of the first eluted enantiomer [182]. Recently, Ramos et al. analyzed nine atropisomeric PCBs in two otter samples and a cow's milk sample [94,181]. The ERs were occasionally extremely high in the two samples. Although no details were presented, the study is the most comprehensive in environmental PCB samples thus far [94]. Enantioenrichment of PCB 149 in blubber of an adult female harbour seal (*Phoca vitulina*) sample from Iceland was determined on heptakis(6-O-*tert.*-butyldimethylsilyl-2,3-di-O-methyl)-β-cyclodextrin. The first eluted peak was significantly higher than the second eluted peak [82]. In a later study, enantioenriched PCB 149 was also determined in blubber of further harbour seals (*Phoca vitulina*), grey seals (*Halichoerus grypus*) and a caspian seal (*Phoca Caspica*) [186].

10. Conclusions

A versatile GC instrument is available to study the ERs of chiral organochlorines in different biogenic matrices. Although these advances have been achieved only recently, a large body of evidence on the role of enantioselectivity in environmental chemistry is already available. Unfortunately, no clear picture on mechanisms of chiral discrimination emerges from the data reported thus far. Therefore, much more research is needed to correlate the observed enantioselectivity with enzymatic degradation, chiral fractionation in the presence of primitive biogenic material (bacteria) and highly developed body functions in high trophic biota. Additionally, in vitro and in vivo laboratory studies of the enantioselectivity of biotransformation and toxicokinetics of organochlorine pesticides should be carried out. At present the results are not amenable to a simple rationalization of enantioselective phenomena in the environment. As more and more data accumulate in the literature, rather emphatic statements at the outset, e.g., that "the detection of enantiomeric bias in living systems may be linked to an inherent time scale applicable to dating purposes" [168] will have to be reconsidered. Nevertheless, the phenomenon of chirality will continue to play a significant role in the elucidation of subtle differences the enantiomers of chiral organochlorines exhibit in the environment.

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