Enantiomeric determination of chiral persistent organic pollutants and their metabolites

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Enantiomeric determination of chiral persistent organic pollutants and their metabolites

E. Eljarrat, P. Guerra, D. Barceló

Many persistent organic pollutants (POPs) are chiral and are generally released into the environment as racemates, but frequently undergo alterations in enantiomeric composition as soon as they are subjected to biochemical processes. Enantiospecific analysis of chiral POPs is important, since enantiomers of chiral compounds often exhibit differences in biological activity, and most biochemical processes in nature are stereospecific. The effects and the environmental fate of the enantiomers of chiral pollutants therefore need to be investigated separately. Chiral separation of enantiomers is one of the most challenging tasks for any analytical technique. We discuss different aspects of enantiospecific analysis of chiral POPs, including classical POPs and their metabolites, as well as some emerging POPs.

Abbreviations: APCI, Atmospheric pressure chemical ionization; CD, Cyclodextrin; CE, Capillary electrophoresis; CHL, Chlordane; CRM, Certified reference material; CSP, Chiral stationary phase; DDD, Dichlorodiphenyl dichloroethane; DDE, Dichlorodiphenyl dichloroethylene; DDT, Dichlorodiphenyl trichloroethane; ECD, Electron capture detection; ECNI, Electron capture negative ionization; EF, Enantiomeric fraction; EI, Electron ionization; ESI, Electrospray ionization; GC, Gas chromatography; GCxGC, Comprehensive two-dimensional GC; HBCD, Hexabromocyclododecane; HCH, Hexachlorocyclohexane; HEPX, *Cis*-heptachlor-exo-epoxide; IT, Ion trap; LC, Liquid chromatography; LOD, Limit of detection; MDGC, Multidimensional GC; MEKC, Micellar electrokinetic chromatography; $MeSO_2$-CBs, Polychlorinated biphenyl methyl sulfones; MS, Mass spectrometry; MS^2, Tandem mass spectrometry; NICI, Negative ion chemical ionization; OXY, Oxychlordane; PBB, Polybrominated biphenyl; PCB, Polychlorinated biphenyl; PCCH, Pentachlorocyclohexene; PCDD, Polychlorinated dibenzo-*p*-dioxin; PCDF, Polychlorinated dibenzofuran; POP, Persistent organic pollutant; QqLIT, Quadrupole linear ion trap; QqQ, Triple quadrupole; SIM, Selected ion monitoring; SRM, Selected reaction monitoring; TBECH, Tetrabromoethylcyclohexane

Keywords: Brominated flame retardant; Capillary electrophoresis; Chiral POP; Enantiospecific analysis; Gas chromatography; Liquid chromatography; Mass spectrometry; Metabolite; Organochlorine pesticide; Persistent organic pollutant

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1. Introduction

A chiral object or molecule is not superimposable on its mirror image, whereas an achiral object can be superimposed on its mirror image. An alternative definition lies in the lack of symmetrical elements, so a chiral object lacks reflectional symmetry. Commonly known chiral objects are a person's right and left hands, snail shells, and screws threaded clockwise or counterclockwise.

For chiral molecules, a tetrahedral carbon atom bound to four different substituents is most common. The carbon atom is the stereogenic center and the two possible structures behave like the image and the mirror image of each other and are not superimposable. These structures are called enantiomers. While enantiomers have identical physical properties (e.g., melting points, mass spectra and retention times on achiral stationary phases) their biological behavior (e.g., stability in a chiral surrounding) can be different.

Biological processes usually proceed with stereoselectivity or enantioselectivity, so results from enantioselective investigations of chiral compounds may add valuable information on the bioavailability and the accumulation of anthropogenic contaminants in the food web. This is because chiral contaminants originally applied as racemates (enantiomeric fraction (EF) = 0.5) may prefer depletion or accumulation of one enantiomer in biological systems. A deviation of EF from 0.5 in environmental samples is a direct indicator of bioactivity of the given chiral compound.

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The importance of chiral analysis in environmental science was established during the 1990s because:

(i) a significant number of all organic chemicals are chiral;
(ii) these chemicals are released into the environment as racemates;
(iii) enantiomers frequently exhibit different toxicological and other biological activities; and,
(iv) most biochemical processes in nature are stereospecific.

Some pollutants have shown chirality, so their different enantiomers may have different toxicity. Furthermore, some non-chiral pollutants can give rise to different degradation products that can be chiral in nature showing, therefore, different toxicity. Today, information about enantiomeric distribution in environmental samples exists for a large number of chiral chemicals, including classical persistent organic pollutants (POPs) [e.g., polychlorinated biphenyls (PCBs), chlordanes (CHLs) and chlorinated bornanes]. However, few studies have investigated the fate of emerging chiral POPs in the environment [e.g., hexabromocyclododecane (HBCD) or tetrabromoethylcyclohexane (TBECH)].

We review the published analytical methods for chiral separation and determination of chiral POPs. We survey the current state-of-the-art and outline perspectives.

2. Chiral POPs

POPs have been shown to exhibit potentially harmful effects in man and the environment. In addition to being persistent, they are typically lipophilic (therefore bioaccumulative), semi-volatile and toxic. Some POPs have been deliberately produced by industry for a wide variety of applications (e.g., pesticides and PCBs). Others are accidentally formed or eventually released as byproducts from various activities, such as industrial or combustion processes [e.g., polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs)].

Since 1995, the international community has been working on a legally-binding instrument to eliminate POPs. Different organizations initiated an assessment process, which, in December 2000, resulted in the POPs Convention. Initial action was taken on 12 POPs: aldrin; CHL; dichlorodiphenyl trichloroethane (DDT); dieldrin; endrin; heptachlor; hexachlorobenzene; mirex; toxaphene; PCBs; PCDDs; and, PCDFs. Some of these POPs are chiral, and the degradation of these contaminants sometimes results in persistent chiral metabolites. Next, we describe these chiral POPs and their chiral metabolites, but we also describe some emerging chiral POPs that were not included in the POP Convention list, although they are potential candidates (Table 1).

2.1. CHL

CHL was one of the most heavily used pesticides until its restriction in the 1980s and subsequent world-wide ban in 1997. CHL is not a single chemical, but is a mixture of many related chemicals, comprising at least 147 different compounds. Technical CHL mainly comprises hepta-, octa- and nona-chlorinated bicyclopentadienes. The main components are *trans*-CHL, *cis*-CHL and *trans*-nonachlor. Other abundant components are heptachlor, *cis*-nonachlor and various chlordenes.

Heptachlor was itself a pesticide. It is rapidly degraded in the environment to heptachlor-*exo*-epoxide (HEPX), also called *cis*-heptachlorepoxide. Similarly, *trans*-CHL, *cis*-CHL and the nonachlors are mainly degraded to persistent metabolite oxychlordane (OXY). The metabolite products are often more toxic than their parent compounds. Many of the CHLs and CHL-related compounds are chiral, including the epoxidized metabolites HEPX and OXY. A review by Hegeman and Laane [1] summarized the different biological properties and the environmental fate of each enantiomer.

2.2. DDT

DDT was one of the first synthetic pesticides to gain wide acceptance. Initially, its use greatly enhanced crop yields, but pest species rapidly developed resistance so that its use in agriculture in the USA began to decline by 1959. It was effective longer in controlling mosquito-borne malaria and is still used for that purpose in some tropical countries.

Because of environmental concerns, the use of DDT was banned in the USA and in some other countries in the early 1970s. However, by that time, it was being distributed globally.

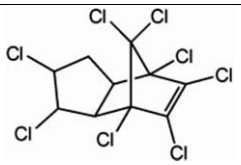
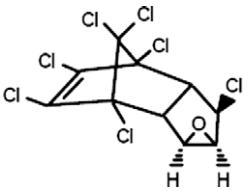
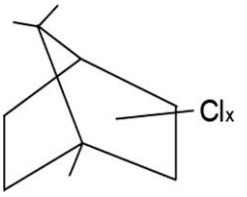
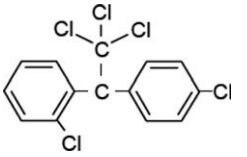
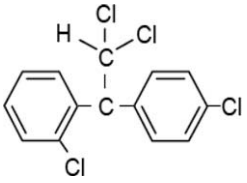
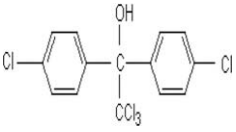
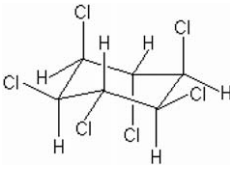
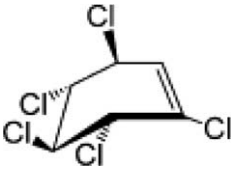
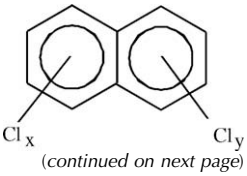
Both DDD (dichlorodiphenyl dichloroethane) and DDE (dichlorodiphenyl dichloroethylene) existed as by-products in commercial DDT formulations, and both may be formed by environmental degradation of DDT. DDD was also used to kill pests, but its use has also been banned. One form of DDD has been used medically to treat cancer of the adrenal gland.

The chlorine atom at the *ortho* position gives chirality to molecules *o,p′* -DDT and *o,p′*-DDD. The *o,p′*-DDT isomer has received a lot of attention due to its estrogenic activity. However, (–)-*o,p*'-DDT was reported to be a weak estrogen mimic and endocrine disruptor, whereas its mirror image [(+)-*o,p*'-DDT] is inactive [2].

2.3. Hexachlorocyclohexane (HCH)

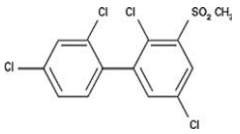
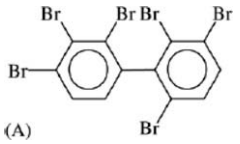
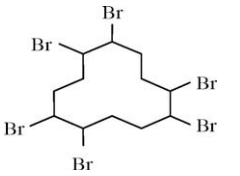
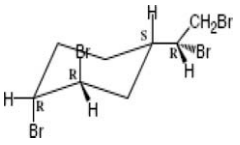
Technical HCH was introduced in the 1940s as a broad-spectrum pesticide. Its use was phased out in Europe during the 1970s and 1980s. Usage of technical HCH continued in China until 1983 and the former Soviet Union until 1990, but, even by 1996, its use had not

Table 1. List of classical and emerging chiral POPs, and their chiral metabolites

Group of POP	Acronym	Chiral compounds	Structure
Chlordane	CHL	*trans*-CHL, *cis*-CHL, octaCHLs (U81, U82, MC5, MC7), nonaCHL (MC6), chlordene, heptachlor	
CHL metabolites		*Cis*-heptachlor-exo-epoxide (HEPX), oxychlordane (OXY), *cis*-chlordene-epoxide	
Chlorinated bornane		B7-515*, B7-1059, B7-1146, B7-1453, B8-531, B8-786, B8-789, B8-806, B8-809, B8-1413, B8-1414, B8-1945, B8-2229, B9-415, B9-418, B9-1025, B9-1679, B9-2206	
Dichlorodiphenyl trichloroethane	DDT	*o,p′*	
DDT metabolites: Dichlorodiphenyl dichloroethane	DDD	*o,p′*	
Dicofol		*o,p′*	
Hexachlorocyclohexane	HCH	α	
HCH metabolites: Pentachlorocyclohexene	PCCH		
Polychlorinated biphenyl	PCB	45, 84, 88, 91, 95, 132, 136, 144, 149, 171, 174, 183	

(*continued on next page*)

Table 1. (*continued*)

Group of POP	Acronym	Chiral compounds	Structure
PCB metabolites: PCB methyl sulfones	$MeSO_2$-CBs	3-91, 4-91, 3-95, 4-95, 3-132, 4-132, 3-149, 4-149, 3-174, 4-174	
Polybrominated biphenyls	PBB	132, 149	
Hexabromocyclododecane	HBCD	α, β and γ	
Tetrabromoethylcyclohexane	TBECH	α, β, γ and δ	

*Systematic code names given by Andrews and Vetter [6].

been banned in India and some countries in Africa and South America.

Its manufacture involves photochlorination of benzene, which yields a mixture of hexachlorinated cyclohexanes. The main isomer is α-HCH (55–80%), but other stable HCH isomers (β, γ, δ and ε) are also present. Only α-HCH is chiral. However, some achiral pollutants degrade into the chiral metabolites. And, all HCH isomers may form chiral metabolites [e.g., penta-chlorocyclohexenes (PCCHs)].

Extensive reviews on the toxicity and environmental fates of HCH isomers were published [3,4].

2.4. Toxaphene

Toxaphene was introduced in 1945 as a non-systematic pesticide. It is chlorinated camphene (isomerized to bornanes), but there are also chlorinated terpenes. Technical toxaphene products comprise several hundred bicyclic components, most of which are chlorobornanes. Theoretically, 32,767 chlorinated bornanes are possible, and only 511 are achiral, while 16,128 exist as enantiomeric pairs [5], so it is obvious that most chlorobornanes in samples are chiral. Table 1 lists all chlorobornane-congener standards with enantiomers of known structure separated so far, using the systematic code names given by Andrews and Vetter [6]. Only a few chlorobornanes accumulate in organisms; however, all the compounds identified or isolated are chiral.

2.5. PCBs

PCB was introduced as an industrial chemical in the 1920s because of its high thermal and chemical stability, together with its electrical-insulating properties. PCB is the collective name for 209 mono- through deca- chlorinated biphenyls. Depending on the number of chlorines in *ortho* positions, PCBs are classified as: non-, mono-, di-, tri- and tetra-*ortho* congeners. The tri- and tetra-chlorinated congeners have restricted rotation about the phenyl-phenyl bond. The congeners with restricted rotation and an asymmetric chlorine substitution pattern on both ring systems exhibit axial chirality and exist as atropisomeric (enantiomeric) pairs in the environment. Of 209 theoretically possible PCBs, 78 occur as atropisomers. Of the chiral PCBs, 19 are predicted to form stable atropisomers under most environmental conditions, of which at least 12 (PCBs 45, 84, 88, 91, 95, 132, 136, 144, 149, 171, 174 and 183) have been detected in commercial PCB mixtures [7].

Püttmenn et al. [8] studied the biological activity of PCB atropisomers. Interestingly, they found that the atropisomers exhibit differential potency in inducing several xeniobotic-metabolizing enzymes (Cytochrome P450 enzymes), indicating that chirality plays a role in recognition events associated with these biological processes.

Methyl-sulfone derivatives are known to represent primary metabolic products of PCBs ($MeSO_2$-CB). These metabolites belong to the group of persistent, lipophilic

compounds that accumulate in the adipose, lung, liver and kidney tissues of mammals exposed to PCBs. Similar to their parent compounds, PCB metabolites may exhibit axial chirality if both phenyl rings possess an asymmetric chlorine-substitution pattern. Since the introduction of a methylsulfonyl group at the 2- or 3- position will add an additional element of asymmetry, these metabolites may be chiral even if the parent PCB is not. Of the 837 theoretically possible $MeSO_2$-CBs, 456 are chiral, of which 170 may be environmentally stable due to tri- or tetra-chloro-*ortho* substitution. However, few chiral $MeSO_2$-CBs are environmentally relevant. Of the 28 $MeSO_2$-CBs most frequently detected in wildlife and humans, eight congeners (3- and 4-$MeSO_2$-CB91, -CB95, -CB132 and -CB149) are chiral.

2.6. Dicofol

Dicofol is a non-systematic acaricide or miticide currently registered in the USA and Canada for use on a wide variety of crops. This agrochemical has been identified as a potential POP candidate, and it has been implicated as a potential endocrine-disrupting compound. The technical product is usually synthesized from technical DDT and comprises approximately 80% and 20% of *p,p′*- and *o,p′*-dicofol isomers.

The hydroxylated analogue of *o,p′*-DDT, *o,p′*-dicofol, is chiral due its unsymmetrically-substituted carbon atom. While previous investigations have demonstrated the enantiomer-specific estrogen activity of *o,p′*-DDT, suggesting that the (−)-enantiomer is the more active estrogen mimic in mammalian systems, the stereospecific activity of *o,p′*-dicofol has not been reported.

2.7. Polybrominated biphenyls (PBBs)

PBBs have been extensively applied as flame retardants in textiles, electronic equipment and plastics. In 1973, an accidental contamination of human food with PBBs in Michigan, USA, revealed the toxicological threat of this group of chemicals.

Despite continuous reduction in the worldwide annual production since the late 1970s and slightly declining levels in the environment in the past decade, the ubiquitous presence of PBBs has been documented in a wide range of samples. Although PBBs have exactly the same number of congeners as PCBs (i.e. 209 from mono- to deca-BBs), the number of congeners found in environmental samples is smaller than PCBs.

Several PBB congeners, containing 2–4 bromine orthosubstituents, cannot rotate about the interannular phenyl-phenyl bond due to steric hindrance. Such PBBs, which additionally possess a non-symmetric substitution pattern on both aromatic rings, form pairs of axially-chiral compounds (atropisomers). Analogously, 19 axially-chiral PCBs proved to form stable atropisomers under environmental conditions. However, due to the more bulky bromine substituent, it was assumed that more than 19 PBB congeners would form stable atropisomers. Some of the PBBs found in the environment are axially chiral (PBB 132 and PBB 149). However, no studies investigating the fate of atropisomeric PBBs in the environment have been published so far.

2.8. HBCDs

HBCDs are high-production-volume chemicals used as flame retardants for plastics and textiles. They are currently produced in quantities exceeding 20,000 tonnes per year. Despite this, the correct stereochemistry of most HBCDs is still not known. Considering all elements of symmetry, 16 different stereoisomers, including six pairs of enantiomers as well as four meso forms, are possible theoretically.

Technical HBCD is produced industrially by adding bromine to *cis,trans,trans*-1,5,9-cyclododecatriene, with the resulting mixture that contains three predominant stereoisomers, α-, β- and γ-HBCD. Normally, the γ isomer is the most dominant in commercial mixtures (in the range 75–89%), followed by the α and β isomers (10–13% and 1–12%, respectively) [9]. α-, β- and γ-HBCD diastereoisomers are chiral, so technical HBCD has three pairs of enantiomers.

HBCDs are highly lipophilic and accumulate in biota. Increasing environmental concentrations of HBCDs have been observed. As such, HBCDs have to be considered as potential emerging POPs. As regards their toxicology, only a few effects studies have been carried out and none of them addressed effects at the level of individual HBCD stereoisomers or enantiomers. It was shown that HBCDs stimulate the thyroid-responsive element, indicating that HBCDs might interfere with the thyroid-hormone system [10].

2.9. TBECH

TBECH is a commercial brominated flame retardant used as an additive in polystyrene and polyurethane. Production of TBECH was reported to be 4–225 metric tonnes in 2002 [11]. Recent work by Muir et al. [12] identified TBECH as a possible persistent and bioaccumulative organohalogen chemical, and a new study reported the finding of TBECH in beluga whales in the Canadian Arctic [13]. Also of great importance are studies that indicated that TBECH may have adverse health impacts on humans [14]. These recent studies highlight the importance of learning more about the stereochemistry and the fate of this emerging POP in the environment.

TBECH can exist as four pairs of enantiomers (α-, β-, γ- and δ-TBECH). The possibility of four pairs of enantiomers is due to the presence of the four chiral carbons in the structure. α- and β-TBECH are the major components of the technical mixture.

Very little is known about the presence of this brominated flame retardant in the environment, and no

Table 2. Recent analytical techniques used for chiral POP determinations in environmental and biotic matrices

Analyte	Matrix	Instrumental technique*	Ref.
PCBs (19 chiral congeners)	Food	GC-ECD Heart-cut MDGC-ECD GCxGC-μECD	[26]
PCBs (95,132,149 and 174)	Soil, air, herring, human milk and compost	GCxGC-QqQ-MS	[28]
PCBs (95,132,1149 and 174), α-HCH, *o,p′*-DDD, *o,p′*-DDT	Soil	GCxGC-QqQ-MS	[30]
$MeSO_2$-CBs (3-91, 4-91, 3-95, 4-95, 3-132, 4-132, 3-149, 4-149, 3-174, 4-174)	Human adipose tissue, seal blubber and pelican muscle	GC-ECD GC-NICI-MS	[20]
$MeSO_2$-CBs (3-91, 4-91, 3-132, 4-132, 3-149, 4-149, 3-174, 4-174)	Liver, lung and adipose tissue of grey seal	GC-ECNI-MS	[21]
Toxaphenes (B8-1413, B7-515, B8-1414, B8-2229 and B9-1679)	Real-life samples	Heart-cut MDGC-ECD GCxGC-μECD	[29]
PBBs (132 and 149)	White-tailed sea-eagle egg	GC-EI-MS^2	[32]
PBBs (101, 132, 149, 153, 174 and 180)	Technical mixture	GC-ECD GC-ECNI-MS	[33]
HBCDs (α, β and γ)	Fish liver and muscle	LC-MS^2	[35]
HBCDs (α, β and γ)	Biological tissues	LC-MS^2	[40]
HBCDs (α, β and γ)	Air samples	LC-MS^2	[41]
HBCDs (α, β and γ)	Marine food web	LC-MS^2	[42]
HBCDs (α, β and γ)	Food (pork, lean fish, butter)	LC-IT-MS^2	[43]
HBCDs (α, β and γ)	Human breast milk	LC-QqLIT-MS^2	[44]
HBCDs (α, β and γ)	Sediment	LC-QqLIT-MS^2	[34]

*NICI, Negative ion chemical ionization; ECNI, Electron capture negative ionization; EI, Electron ionization.

studies investigating its chirality have been published so far.

3. Chiral separation

Separation of chiral compounds is an interesting, challenging topic of research in many areas of analytical chemistry, especially in the biomedical, pharmaceutical, and environmental fields where pure enantiomeric forms are widely required. Due to the similar physical and chemical properties of the enantiomers, their resolution is very difficult. The techniques most frequently used for chiral separations have been gas chromatography (GC), liquid chromatography (LC) and capillary electrophoresis (CE). GC has been used for the determination of some chiral POPs (e.g., DDT, PCBs and CHLs). However, in view of the limitations of GC for the analysis of some chiral POPs (e.g., HBCD and TBECH), LC is the technique of choice.

Separation techniques in environmental analysis are typically coupled to mass spectrometry (MS) and tandem MS (MS^2), as these provide the sensitivity and the selectivity needed. Besides chromatography, some publications appeared in the field of chiral resolution using CE. Table 2 shows a review of recent analytical techniques used for chiral-POP determinations in different environmental and biotic matrices.

3.1. GC methods

Chiral separations using GC can be performed indirectly or directly.

The indirect approach involves derivatization of the chiral compound with a chiral auxiliary. The resulting diastereoisomers are subsequently separated on an achiral stationary phase. Important prerequisites are:

- availability of reactive functionalities of the analyte and the auxiliary being closely juxtaposed to the stereogenic elements;
- absence of racemization during derivatization;
- absence of kinetic resolution;
- absence of diastereoisomeric fractionation upon sample handling and injection;
- absence of diastereoisomeric bias upon detection; and,
- quantitative enantiomeric purity of the auxiliary.

The direct approach utilizes a non-racemic chiral stationary phase (CSP) as a selector, which need not be enantiomerically pure. This approach is straightforward and is the most commonly used.

GC using modified cyclodextrin (CD) as a CSP is the method of choice to separate the enantiomers of chiral organochlorines. The first capillary GC separation of enantiomers was published in 1966, and the first demonstration of CD as a successful CSP was reported in 1983 [15]. Cyclodextrins are cyclic α-(1-4) glucose oligomers with six, seven or eight glucose units,

corresponding to α-, β- and γ-CD, respectively (Fig. 1). In naturally-occurring cyclodextrins, the R groups are hydroxyl groups. These hydroxyl groups can be chemically converted to other groups (e.g., methyl and hydroxypropyl).

As regards PCBs, chiral congeners have been separated using columns with different types of CSP mainly based on β-CD.

Chirasil-Dex (permethyl-β-CD) is the most commonly used chiral column, due to its stability and its ability to resolve some of the main chiral PCBs found in the environment [16]. The elution order has been found to be (−/+) for PCBs 84, 132, 136, and 176 and (+/−) for PCBs 135 and 174.

The retention characteristics of all 19 tri- and tetra-*ortho* atropisomeric PCBs have also been investigated using Chirasil-Dex. Nine of the atropisomers could be separated using this chiral selector. PCBs 95, 132, and 149 were completely resolved and PCBs 84, 91, 135, 136, 174, and 176 were partially separated. All of the separated congeners are 2,3,6-substituted in at least one ring, and, conversely, none of the congeners that lacks 2,3,6-substitution could be separated. Thus, chiral recognition and enantiomer separation seem to be strongly governed by 2,3,6-substitution.

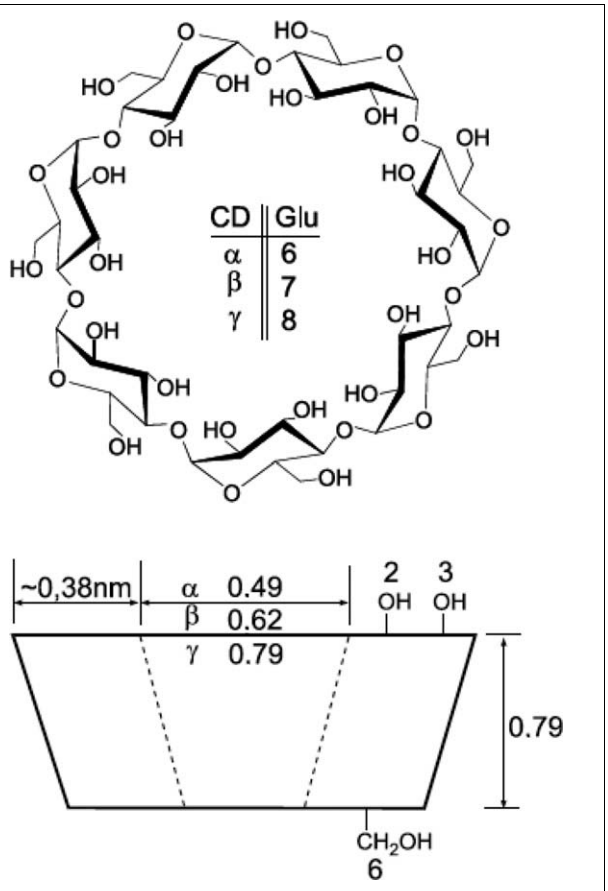


Figure 1. Chemical structure and geometry of cyclodextrin.

α- and γ-CD have been also employed [17], the latter for congeners that usually resist separation into enantiomers with β-CD (i.e. PCBs 88, 139, 196 and 197).

Fluoroacetyl-γ-CD has been successfully used, but, due to its low thermal stability [18], it is not really suitable for routine analysis.

However, there have been several advances in separation using columns with β-CD stationary phases specifically developed for enantiomeric separation (e.g., PCB 144 has been resolved into enantiomers by using *tert*-butyldimethylsilylated β-CD columns [19]).

Metabolites of environmental pollutants have generally been studied less than parent compounds. They are generally present at much lower concentrations, and often require more extensive and rigorous clean-up and processing techniques in order to analyze them reliably from environmental matrices. Some studies have been carried out in order to determine chiral $MeSO_2$-CB [20,21]. The enantiomeric separation was obtained using an heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-CD column (SE52 (1:4)). However, enantiomers of congeners of methyl sulfones with 3-$MeSO_2$ substitution were better resolved than the corresponding 4-$MeSO_2$ pairs.

Despite the environmental importance of toxaphene, only a few studies have focused on the enantiomer separation of chlorobornanes. This is partly due to difficulties inherent in the separation of chlorobornane enantiomers on several CSPs.

A *tert*-butyldimethylsilylated β-CD column (β-BSCD) was used for the enantioselective separation of toxaphene congeners. While the enantiomers of eight chlorinated bornane standards were resolved on β-BSCD, so far the determination in environmental samples has been reported for only B8-1413, B9-1679 and B8-2229 [22].

A silylated heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-CD column (β-TBDM) was also applied to toxaphene studies, showing the enantiomeric separation of an abundant octachlorobornane, B8-1412, in different biological samples [23]. This chlorobornane was not resolved into enantiomers on β-BSCD.

A heptakis(2,3,6-tri-*O*-methyl)-β-CD (β-PMCD) was tested and separated enantiomers of four chlorinated bornanes, as well as that of B8-1412 [24]. The advantage of this last column, with the β-PMCD (Chirasil-Dex) phase, is the good reproducibility of the CSP. Chiral-resolution characteristics obtained on this CSP can therefore be easily reproduced on any other commercially-available CSP of the same type. This is not the case for β-BSCD and β-TBDM.

CD stationary phases have also been tested for enantioselective separation of α-HCH and chlordanes and their metabolites [25]. Four CSPs containing modified

CDs diluted in, or chemically bonded to, a non-chiral phase were used to resolve chiral organochlorine compounds (e.g., α-HCH, oxychlordane, *cis*- and *trans*-chlordane, *cis*- and *trans*-heptachloroepoxide, PCB-95, PCB-132, PCB-149 and chiral toxaphenes). The chiral columns tested were:

(a) β-PMCD, 10% heptakis-(2,3,6-tri-*O*-methyl)-β-CD = permethylated CD in dimethylpolysiloxane, CP-Sil 5;
(b) γ-PMCD, 10% octakis-(2,3,6-tri-*O*-methyl)-γ-CD = permethylated CD in dimethylpolysiloxane, CP-Sil 5;
(c) β-TBDM, 35% heptakis(6-*O*-t-butyldimethylsilyl-2,3-di-*O*-methyl)-β-CD in 85% methyl-, 7% phenyl-, 7% cyanopropil-, 1% vinyl-polysiloxane, OV1701; and,
(d) β-BSCD, 10% heptakis-2,3,6-tri-*O*-butyldimethylsilylated β-CD in 85% dimethyl-, 15% diphenyl-polysiloxane, PS086.

None of the CSPs resolved all chiral organochlorine compounds. However, the β-TBDM column separated all compounds under investigation, except PCB-95 and chiral toxaphenes.

Although a broad selection of modified CD capillary GC columns are available, it is often difficult to resolve all chiral analytes of interest enantiospecifically. Even if the chiral pairs are resolved, the enantiomers of different isomers may overlap. Moreover, enantiomer separation of standard compounds is only one prerequisite for the suitability of a CSP. Real samples contain many more components that may interfere with the target components. Different approaches can be used to obtain an improvement:

(a) multidimensional GC (MDGC);
(b) MS^2 detection; and,
(c) LC enrichment.

3.1.1. MDGC. MDGC techniques [e.g., heart-cut MDGC and lately comprehensive two-dimensional GC (GCxGC)] are regarded as powerful options to improve chiral POP separations.

Heart-cut MDGC permits co-eluting congeners on a pre-column to be transferred to a second GC column with a different selectivity, allowing their separation. This technique has been used for chiral separations. Bordajandi et al. [26] applied heart-cut MDGC for the enantiomeric separation of chiral PCBs. This methodology improved the separation, compared to conventional, one-dimensional GC, and enantiomeric fractions of chiral PCBs could be determined free from interferences. Optimized column combination used a DB-5 column as achiral pre-column followed by Chirasil-Dex as chiral column for the determination of PCB-91, 95, 132, 135, 136, 149 and 174. A different combination, a DB-5 followed by a BGB-172, was used for the analysis of PCB-171 and 183 (Fig. 2).

These tandem columns, comprising an achiral column connected to a chiral column or vice versa, have been used in conventional GC to separate enantiomers of other families of POPs in environmental samples. Oehme et al. [27] obtained a simultaneous enantioselective separation of chlordanes, a nonachlor compound, and *o*,*p*'-DDT in environmental samples using tandem capillary columns, one coated with 90% biscyanopropyl, 10% phenylcyanopropylpolysiloxane (RT_x 2330), and the other with dimethyl-t-butyl-silylated-β-CD dissolved in 85% methyl, 15% phenyl polysiloxane (PS-086). This combination enabled the enantioselective separation of all the chiral chlordanes present in fish-oil reference material SRM1588, without any overlap between enantiomers of different isomers. This capillary-column combination can also separate the enantiomers of other chiral polychlorinated pesticides (e.g., *o*,*p*′-DDT, oxy-chlordane, a chiral nonachlor, and heptachlor *exo*-epoxide).

The main limitations of heart-cut MDGC, relating to the number of target compounds that can be transferred to the second column in a single run and, therefore, the time required, have led to the evaluation of other GC options.

An even more sophisticated technique for overcoming the co-elution problem is to employ GCxGC. Compared to MDGC, GCxGC allows the entire extract, not just fractions, to be analyzed by both columns. Moreover, the focusing process during the GCxGC run increases the signal-to-noise ratio and that improves the limits of detection (LODs). Recently, GCxGC successfully separated PCB atropisomers, *o*,*p*′-DDT, α-HCH and toxaphene enantiomers in environmental and food samples [26,28–30].

GCxGC with micro electron-capture detection (μ-ECD) has been evaluated for the enantioseparation of five chiral toxaphenes typically found in real-life samples (B8-1413, B7-515, B8-1414, B8-2229 and B9-1679) [29]. Two enantioselective β-CD columns were evaluated as the first-dimension column [i.e. BGB-176SE (20% 2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl β-CD diluted in SE-52, 5% phenyl-95% methylpolysiloxane) and BGB-172 (25% 2,3,6- *tert*-butyldimethylsilyl β-CD diluted in PS086, 15% phenyl-85% methylpolysiloxane)], whereas three non-enantioselective columns were tested as second-dimension columns (i.e. HT-8, BPX-50 and Supelcowax-10). Preliminary results using one-dimensional GC-ECD demonstrated that BGB-172 separated all five toxaphenes into enantiomers (Fig. 3a) although there was overlap between the first enantiomer of B7-515 and the first enantiomer of B9-1679. The combination of BGB-172 x BPX-50 was finally selected because it provided a complete separation of all enantiomers (Fig. 3b).

3.1.2. MS^2. The use of MS^2 can also enhance enantiomeric separations. Bucheli and Brändli [28] showed

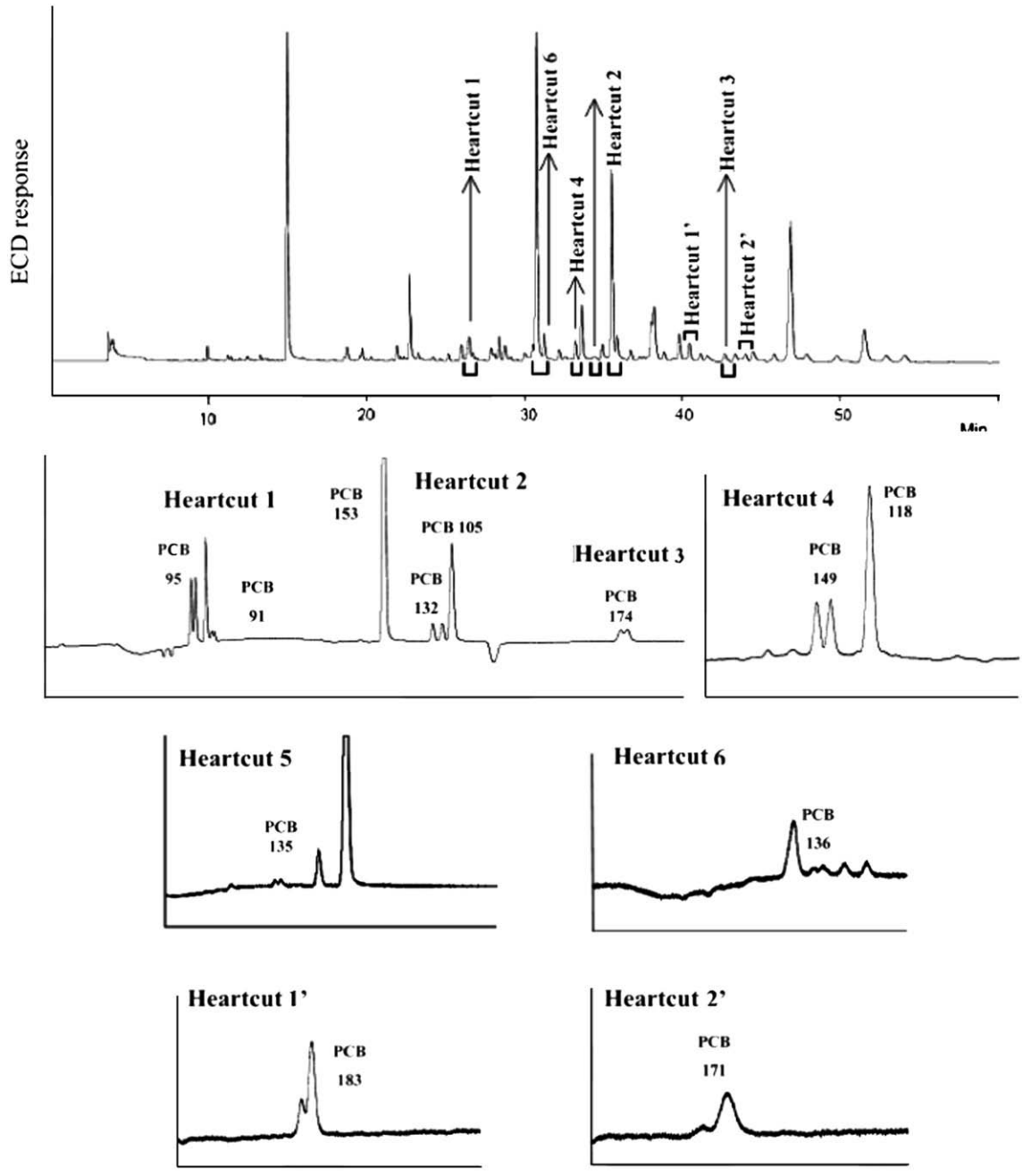


Figure 2. Chromatogram of a goat's milk sample and subsequent heart-cuts to Chirasil-Dex for the determination of congeners 91, 95, 132, 135, 149 and 174 (heart-cut 1 to heart-cut 6), and to BGB-172 for the determination of congeners 171 and 183 (heart-cut 1′ and 2′) (Reproduced from [26].

several advantages of heart-cut MDGC in combination with triple-quadrupole (QqQ) detection for the determination of atropisomeric PCBs in environmental samples. For instance, there was no discernable interference by co-eluting PCBs on Chirasil Dex (171 and 197 with 174; 176 with 132). These congeners are effectively separated on HT-8 and, thus, not transferred to the second column. Moreover, congeners 197 and 176 hold one more chlorine atom than the congeners of interest, so they did not appear in the (tandem) mass traces. Moreover, at 145 min., the total run time remained relatively short, compared to one-dimensional GC runs of 100–135 min.

These authors also compared the powers of separation and detection of different techniques (e.g., GC-MS, heart-cut MDGC-MS, GC-MS^2 and heart-cut MDGC-MS^2), for the determination of atropisomeric PCBs [28]. Fig. 4 illustrates the limits of one-dimensional separation and/or detection of atropisomeric PCBs in real samples, showing soil-sample chromatograms obtained with the

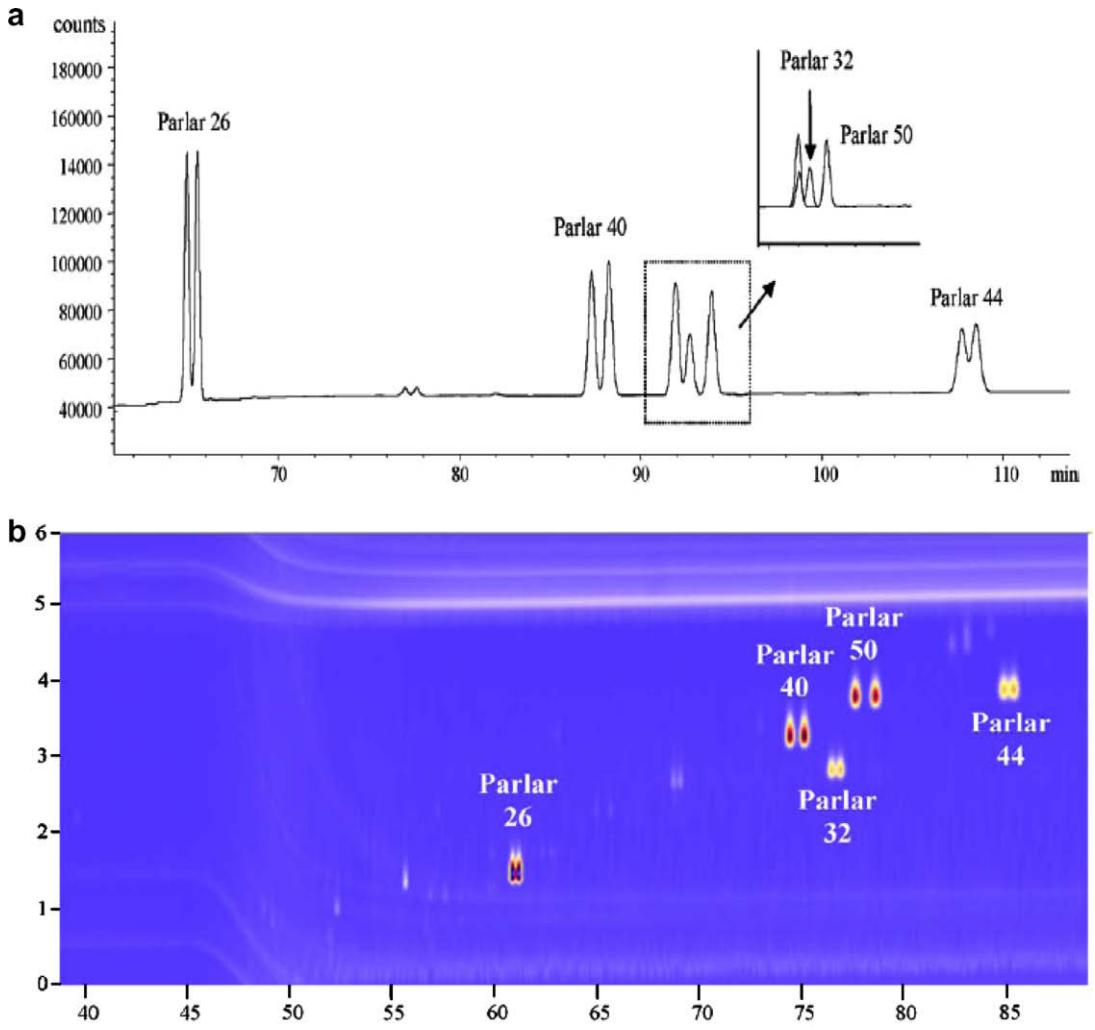


Figure 3. Enantiomer resolution of five toxaphenes by: (a) GC-ECD using a BGB-172 column; and, (b) GCxGC-μECD with BGB-172 × BPX-50 column combination. (Nomenclature: Parlar 26 = B8-1413; Parlar 32 = B7-515; Parlar 40 = B8-1414; Parlar 44 = B8-2229; and, Parlar 50 = B9-1679) (Reproduced from [29]).

four coupling techniques investigated. First of all, the increase in selectivity and sensitivity due to MS^2 was observed: for example, co-eluting compounds of PCB-132 (+) (Fig. 4a and Fig. 4b) and PCB-149 (−) (Fig. 4c and Fig. 4d) are efficiently eliminated when using MS^2. Moreover, although the peak shape and the intensity of the individual atropisomers of PCBs 132 and 174 appear to be free of any disturbances in Fig. 4b (heart-cut MDGC-MS), the relative abundance of the respective enantiomers changes when using MS^2 (Fig. 4a). Hence, apparently ''clean'' single-quadrupole mass traces do not guarantee artifact-free quantitation of EFs. However, the improved, lasting power of separation for atropisomeric PCBs when using heart-cut MDGC was observed (Fig. 4a and Fig. 4b). By comparison, the peak width and tailing of individual PCB atropisomers obtained with one-dimensional chiral chromatography were not satisfactory (Fig. 4d), even in MS^2 mode (Fig. 4c). In general, the Chirasil Dex column exhibited considerable susceptibility to damage by on-column injection of real sample extracts, especially if these were not subjected to rigorous clean up. No such problems were observed for any of the sample extracts when heart-cut fractions were applied.

3.1.3. LC enrichment. Finally, another approach to obtain an improvement in enantiomeric resolution is LC enrichment, which was applied for the enantiomeric separation of axially chiral PCBs [31] and PBBs [32,33]. Götsch et al. [32] used LC enrichment for the separation and the determination of enantiomers of PBB 132 and 149 in extracts from bird-egg samples. After a general clean-up procedure for organohalogens, two fractionation steps were employed in order to separate PBBs from most other organohalogen compounds that could have impaired the enantioselective analyses. PCBs were separated from PBBs on deactivated silica. The fraction collected quantitatively contained all PBBs being inves-

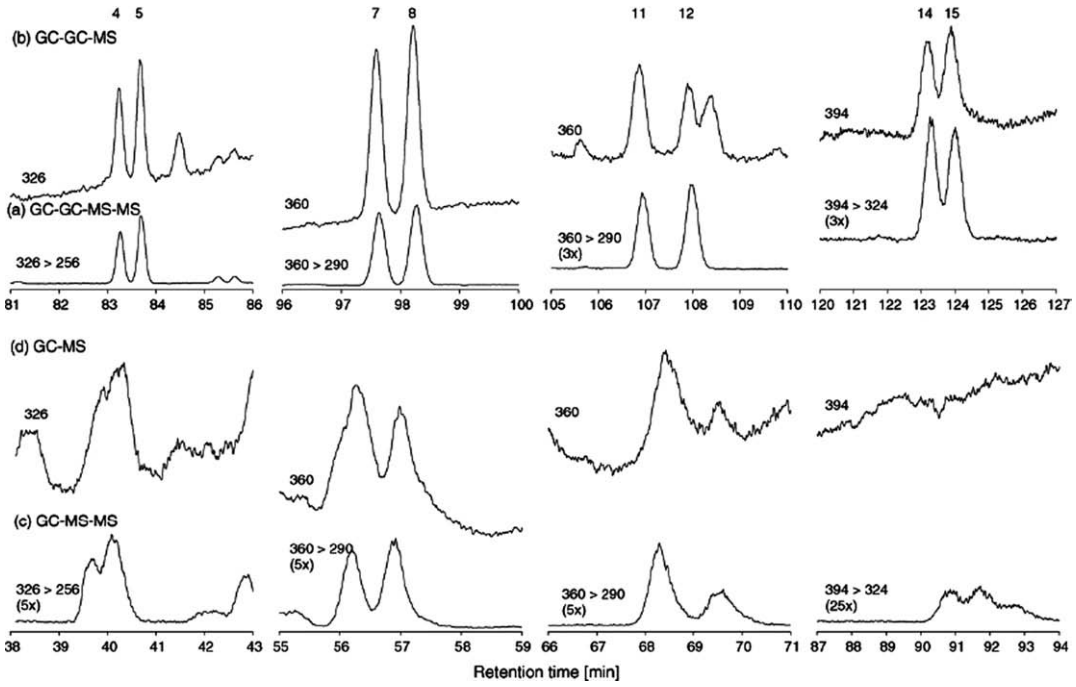


Figure 4. Chromatograms of a Swiss soil extract analyzed with: (a) heart-cut MDGC-MS^2; (b) heart-cut MDGC-MS; (c) GC-MS^2; and, (d) GC-MS. Monitored ions are indicated and magnification factors of MS^2 mass traces (a and c) are given in brackets. {Peak assignment: 4 = 95(E1); 5 = 95(E2); 7 = 149(−); 8 = 149(+); 11 = 132(−); 12 = 132(+); 14 = 174(+); and, 15 = 174(−)) (Reproduced from [28]}.

tigated. Only trace amounts of PCBs were left. However, PBDEs and most organochlorine pesticides have been shown to co-elute with the PBBs, so the concentrated sample extract was further fractionated using silica LC. This technique separated quantitatively PBBs 132 and 149 into two fractions. This was important, since these two congeners had to be treated separately in enantioselective separation. Furthermore, HCB, β-HCH, the nonachlor isomers, and PBDE 77 were separated from the PBBs. Of the standards tested, besides other PBB congeners, only heptachlor and *p,p′*-DDE partly eluted into the fractions where PBBs 132 and 149 were found. These fractions were then used for enantioselective studies.

3.2. LC methods

Chiral LC is also used in environmental problems, although the major applications of this method at present are pharmaceutical, probably because the separation power of chiral LC columns with complex environmental matrices is somewhat limited. The danger arises that the true chiral separation overlaps with compounds in the matrix thus giving unreliable results. High selectivity of respective detectors (e.g., MS^2) is therefore an essential prerequisite for the application of chiral LC columns in environmental sciences. It should also be noted that a multitude of different separation mechanisms could be used in LC. Anyway, the combination of the variety of columns with eluents is fascinating.

Limitations of GC in the analysis of some emerging chiral POPs (e.g., HBCD and TBECH) have led to the development of LC approaches to analysis. Because HBCD diastereoisomers can interconvert at temperatures above 160°C and do not elute from GC columns below this temperature, early measurements of HBCD using GC were not reliable, and more accurate analyses required LC-MS^2. Achiral environmental analysis of HBCD is nontrivial, but enantiomer analysis is even more challenging. The individual enantiomers of α-, β- and γ-HBCD were not resolved using chiral chromatographic columns (e.g., ChiraDex or ChiraSep); but, good resolution was achieved on a β-CD Nucleodex column (Fig. 5) [34]. The elution order of each enantiomer was assigned following Janák et al. [35]. Good separations were obtained for (−)α-, (−)β- and (−)γ-HBCD. Only in the case of (+)α- and (+)β-, and (+)β- and (+)γ-HBCD separations, there was some minor co-elution (at less than 5% of the baseline). However, unequal areas of the two peaks for racemic γ-HBCD were observed, in contrast with most chiral chromatography, where the detector has identical responses to enantiomers present at equal concentrations. This observation could be attributed to differential matrix effects during electrospray ionization (ESI). It is well known that ESI is subject to sample-matrix effects that can enhance or suppress the signal for target analytes and can adversely affect their quantitation [36,37]. Matrix effects are caused by co-eluting compounds that interfere with ionization of the analyte

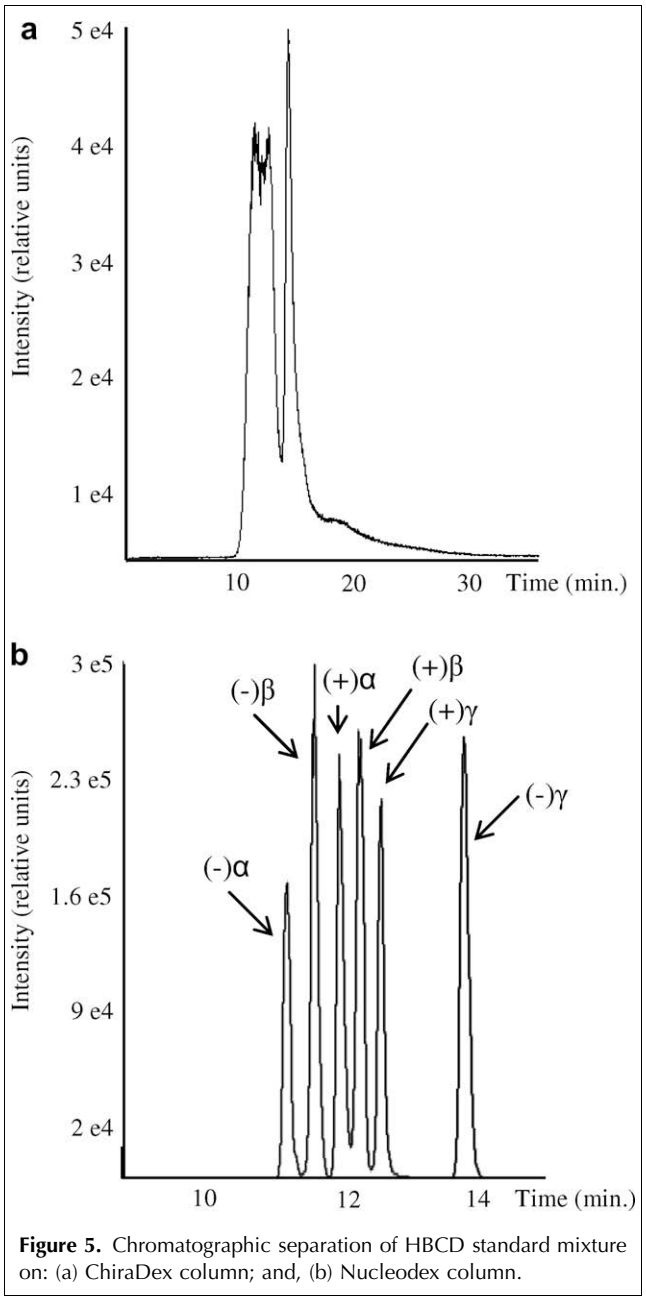


Figure 5. Chromatographic separation of HBCD standard mixture on: (a) ChiraDex column; and, (b) Nucleodex column.

and can affect EF calculations. The enantiomeric composition is usually expressed as EF, which is the preferred descriptor of chiral signatures in environmental samples [38] and which is normally calculated from the peak areas of the enantiomeric pairs using Equation (1):

$$EF = \frac{A_+}{(A_+ + A_-)} \quad (1)$$

where A_+ and A_- are the peak area of eluting enantiomers. In order to avoid matrix effects in EF calculations, Marvin et al. [39] introduced corrected EF values, calculated using Equation (2). This correction is based on the use of isotopic labeled standards (HBCDs) since d_{18}-labelled enantiomeric analogues behave in an identical manner to their native counterparts.

$$EF_{corrected} = \frac{(([A_+]/[A_{+d18}]) \times pg_{+d18})}{(([A_+]/[A_{+d18}]) \times pg_{+d18}) + (([A_-]/[A_{-d18}]) \times pg_{-d18})} \quad (2)$$

where A_{+d18} and A_{-d18} are the peak areas of eluting labeled enantiomers.

Other potential means of reducing the effect of the matrix on signal intensity include standard addition. However, standard addition is time consuming and is not well suited to environmental analysis where there is a demand for large sample throughput.

Different MS approaches have been developed for the enantiomeric determination of HBCDs, always working in ESI mode. An LC-MS^2 method involving a triple quadrupole (QqQ) instrument was used for analysis of HBCDs in biological and air samples [35,40–42].

LC-ion trap (IT)-MS^2 was applied by Gómara et al. [43] for the analysis of different food samples, including pork meat, lean fish and butter. This method was based on the formation of a chlorine adduct (m/z 676.6) of the (±)α-, (±)β- and (±)γ-HBCD enantiomers and their further fragmentation into their stable quasi-molecular ion (m/z 640.6). This method solved problems relating to the low mass cut-off of the IT and the variable amounts of other adduct peaks in the samples.

More recently, Guerra et al. developed an enantiomeric method for HBCDs using a LC-quadrupole linear ion trap (QqLIT)-MS^2 instrument. This method was applied to sediment and human breast-milk samples [34,44]. Similarly to LC-QqQ-MS methods, data acquisition was performed in selected reaction monitoring (SRM) mode. The $[M-H]^- \rightarrow Br^-$ transitions at m/z 640.6 → 78.9 and 640.6 → 80.9 were monitored for unlabeled HBCD, whereas labeled HBCD were monitored at the m/z 652.6 → 78.9 and 652.6 → 80.9 transitions. Instrumental LODs and LOQs were 0.3–1.5 injected pg and 1–6 injected pg, respectively. These values are at least one order of magnitude lower than those obtained by LC-IT-MS^2 [43], with instrumental LODs of 30–86 injected pg.

We should point out that MS^2-detection conditions must be optimized to the highest relative intensity. However, in enantiomeric analysis, special attention must also be given to EF values. For example, Fig. 6 shows optimization of the temperature of the Turbo-IonSpray source, modifying its value in the range 350–500°C. The relative response of a standard solution of γ-HBCD was better when the temperature was higher (Fig. 6b). However, the EF value (0.62) deviated significantly from the optimal racemic value (0.5). When the temperature was set at 350°C (Fig. 6a), the sensitivity

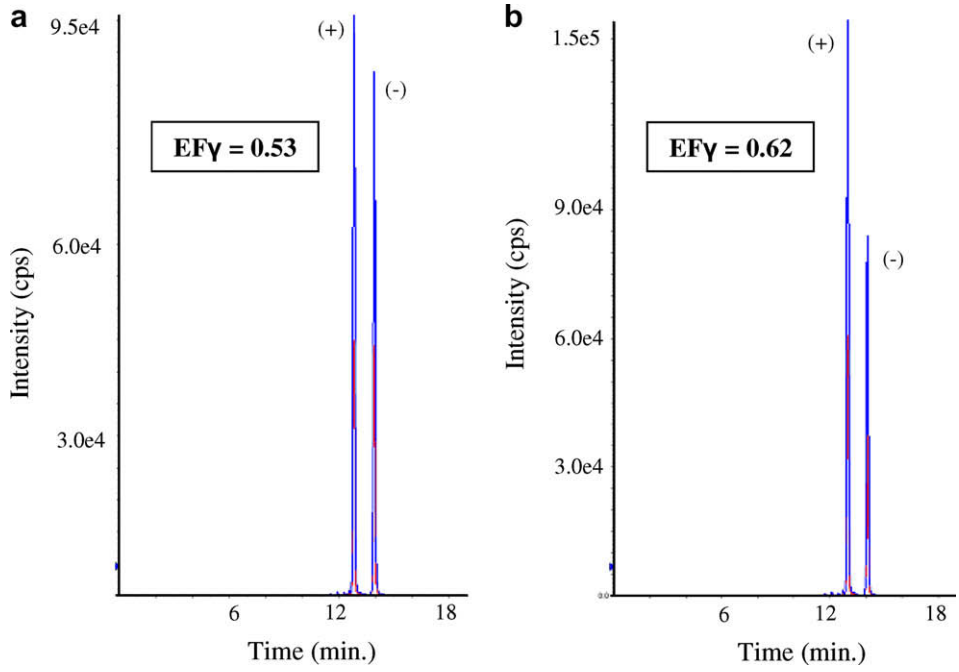


Figure 6. Chromatographic separation of γ-HBCD standard mixture at different temperature of TurboIonSpray source: a) 350°C; and, b) 500°C.

was slightly lower, but the racemic value was maintained (0.53).

Another emerging POP that presented some limitations for GC analysis was TBECH. Technical TBECH comprises near equimolar amounts of two diastereomers, α and β. At temperatures above 120°C, a small amount of thermal conversion occurs with the α and β isomers, resulting in the formation of γ and δ isomers.

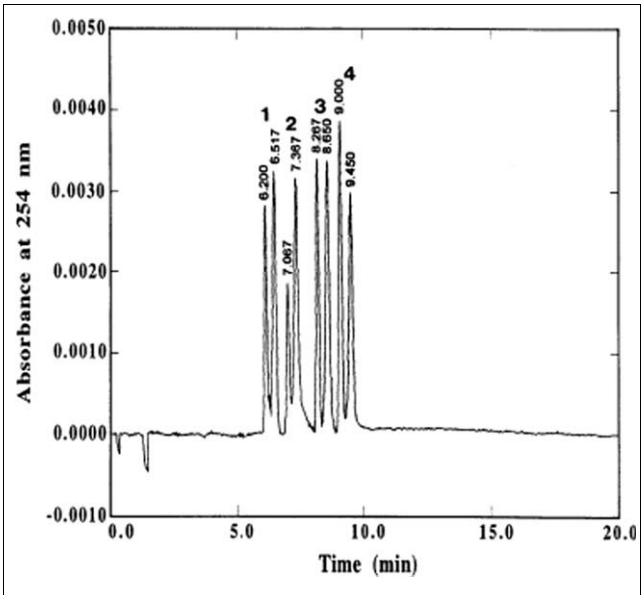


Figure 7. CE separation of the enantiomers of 1 (*o,p'*–DDT), 2 (*p,p'*–DDT), 3 (diclofop methyl) and 4 (silvex isooctyl ester) (Reproduced from [48].

There is only one published work dealing with isomeric determination of TBECH by LC-MS [45]. Partial separations of the four TBECH isomers could be achieved on a BEH C18 or BEH Shield RP_{18} column. There is good separation between isomeric pairs α/β and γ/δ. The γ/δ isomers show some separation with valleys of about 57% on the shield RP_{18} column and about 34% on the BEH C18 column. By contrast, separation of the α/β isomers was not seen under the LC conditions used.

With MS detection, the molecular ion for the four TBECH isomers could not be detected using ESI. However, the compounds were detected when bromide (Br^-) ion was monitored in SIM mode. Atmospheric pressure chemical ionization (APCI) conditions permitted observation of very weak molecular ion adducts, but detection of the bromide (Br^-) ion, monitored in SIM mode, was still more practical due to its stronger signal. Surprisingly, the response factor for the γ/δ isomers was approximately 100-fold greater than that for the α/β isomers. Finally, it should be pointed that no enantiomeric study has been yet carried out for this emerging contaminant.

3.3. CE methods

Since the first report in 1985 [46] showed the great possibilities of CE for the separation of chiral compounds, the number of publications on this topic has increased. Although chiral electromigration methods have mainly been used for enantioseparation of drugs and pharmaceuticals, they have also been applied to analyze chiral pollutants and their metabolites.

Chiral separations can be performed by LC, but CE can offer significant advantages (e.g., higher separation efficiency, speed of analysis, and flexibility, allowing the incorporation of various chiral selectors at different concentrations). In LC, chiral selectors are usually fixed onto the stationary phase, so the concentrations of the chiral selectors cannot be varied.

Enantiomers have identically the same charge-to-mass ratio, so they cannot be separated by CE without a suitable chiral selector being present. Enantiomers are separated with these chiral selectors bonded to the capillary wall, included in a gel or directly added into the separation electrolyte. The most convenient, easy way to perform chiral separations is to add the chiral selector to the supporting electrolyte. The chiral selectors used in CE mimic those used in LC and include natural and derivatized CDs, carbohydrates, proteins, antibiotics and crown ethers. CDs are by far the most widely used chiral additives in CE, as they are relatively cheap and water soluble, and have low UV activity, which permits use of low-wavelength UV for sensitive detection.

Only a few works have been published on enantiomeric separation of chiral POPs by CE techniques. Schmitt et al. [47] applied micellar electrokinetic chromatography (MEKC) to separate six DDT congeners, *o,p′* and *p,p′*-DDT, -DDD and -DDE, as well as the enantiomers of the chiral members of this series, *o,p′*-DDT and -DDD. MEKC is based on adding charged micelles to the running buffer, and separation then depends on the relative partitioning between water and the micellar phase. Finally, adding to the running buffer chiral selectors that selectively bind the different enantiomers allows electrophoretic separation of enantiomers.

Zhang and El Rassi [48] developed a method based on using capillary electrochromatography for chiral separation of some organochlorine pesticides (e.g., *o,p′*-DDT). A commercially-available idol-silica stationary phase was converted in situ to a CSP by dynamically coating it with hydroxypropyl-β-CD (HP-β-CD). Several parameters affecting enantioseparation were investigated, including the concentration of HP-β-CD, ionic strength, pH and organic modifier content of the mobile phase. Fig. 7 shows the results obtained for four chiral organochlorine pesticides under optimal conditions using a mobile phase of 1 mM sodium phosphate, 2.5 mM sodium borate and 2 mM HP-β-CD, and composed of 30% v/v aqueous buffer (pH 5.0).

Although GC has been the most frequently used technique to analyze PCB enantiomers, CE has also proved to be effective in this field. Chiral separation of atropisomeric PCBs by MEKC was first reported by Marina et al. [49,50], who separated the enantiomers of 12 PCBs (45, 84, 88, 91, 95, 132, 136, 139, 149, 171, 183 and 196) using γ-CD or mixtures of β-CD and γ-CD. More recently, García-Ruiz et al. [51] compared the use of different CDs for the chiral separation of the 19 atropisomeric PCBs. Anionic CDs (β-CD-phosphated, β-CD-sulfated, succinylated γ-CD and succinylated β-CD) and a cationic CD (6-monodeoxy-6-monoamino-β-CD) were tested. The best results were obtained with the cationic CD at a concentration of 30 mM in a 10 mM phosphate buffer at pH 2.0 containing 2 M urea, but individual chiral recognition of only 11 PCBs could be achieved. The presence of urea was found to be crucial in achieving the chiral resolution.

4. Conclusions and perspectives

Chiral analysis is a powerful tool for detecting and gaining insight into biochemical environmental fate processes affecting pollutants, which otherwise might remain unknown if analysis is purely achiral. A predictive capability for chiral pollutants would enable chemical manufacturers to produce chiral chemicals with enantiomer compositions that provide maximum benefit with minimum environmental impact.

The first step to be completed is development of analytical methods capable of separating enantiomers and providing reliable EF values.

For chiral POPs, GC is the most commonly used technique. In contrast with LC and CE methods, GC does not require optimization of mobile phases with respect to solvents, pH of buffers, modifiers and gradients, which is often cumbersome. Furthermore, the introduction of new chromatographic techniques (e.g., GCxGC) has improved the analysis of complex mixtures. In this sense, GCxGC could provide the required resolution for chiral separation.

However, for some new chiral POPs (e.g., HBCD), several analytical difficulties have been found with using GC techniques. Recently, new methodologies based on the use of LC have been developed, allowing enantiomer-specific determination. Furthermore, additional research is needed to evaluate the presence and the impact of some emerging chiral POPs on the environment, especially TBECH, for which there is a total lack of data. In order to achieve this goal, it is necessary to develop isomeric and enantiomeric analytical methods.

Although much work has been carried out in separating chiral pollutants by CE, it is important to realize that most of the methods have been developed with pure standards, possibly because of the relatively low sensitivity of CE. This limitation makes direct application of CE to real-life samples difficult, since POPs are usually present at very low concentrations, so still more effort is required in order to be able to analyze these compounds and their metabolites in real-life samples. In this sense, new chiral selectors are needed to extend the application of chiral CE analysis. The attainment of these new chiral selectors, compatible with MS detection, can open the way to application of CE-MS analysis, since this technique has hardly been used for chiral analysis of pollu-

tants due to inhibition of the MS signal by non-volatile chiral selectors (mainly CDs).

Finally, it is important to point out the need for a range of certified reference materials (CRMs) to provide chiral signatures for chiral POPs. There are none currently available, although the EFs of a number of chiral organochlorine pesticides and PCB atropisomers have been measured in different CRMs [52,53].

Acknowledgements

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