DETERMINATION AND ENVIRONMENTAL BEHAVIOR OF PERSISTENT HALOGENATED CHIRAL CONTAMINANTS

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Abstract
The occurrence of persistent chiral environmental contaminants is reported and the particular aspects of their analysis in relation to the stereochemistry of these compounds are discussed. The necessity of enantioselective determinations is emphasized which provides additional information on possible environmental degradation pathways of these contaminants, aids in the distinction of biotic from abiotc processes and is necessary to assess biological effects such as endocrine disruption in biota.

Chiral Compounds, Their Properties and Environmental Behavior
Many of the different groups of halogenated contaminants include chiral congeners. This is the case with the hexachlorocyclohexanes (HCHs), the chlordanes, toxaphenes, PCBs, PCTs, DDT, and others. Despite this wider occurrence of chiral contaminants, only relatively recently have they attracted a wider interest in environmental chemistry. This is largely due to the former unavailabilty of analytical techniques for the enantioselective determination of such compounds, particularily so at the trace level concentrations required for environmental analyses.
The chemical and physical properties of enantiomers are identical except that they rotate the plane of polarized light in opposite directions and that they react differently in a chiral environment (1). Enantiomers may thus show a different behavior in biotic processes (chiral environment) whereas their behavior is identical in abiotic processes. For some organochlorine contaminants (PCBs, chlordanes, toxaphenes, others) drastic changes in congener and isomer composition were observed in the course of environmental transformation and degradation. These changes can be biotically and/or abiotically mediated. An enantioselective determination of chiral compounds and their conversion products (metabolites) in environmental and biological samples may thus give additional information on possible degradation pathways and allow a distinction of enantioselective biotic (microbial, enzymatic) from non-enantioselective abiotic processes (chemical, photochemical, distribution, transport). Generally, only biotic processes will affect enantiomeric composition and change enantiomeric ratios (ERs).
Molecular asymmetry (chirality) is a fundamental aspect of some molecules leading to closely related, non-superimposable stereoisomers (enantiomers) that are like image and mirror-image. The structural elements that lead to chirality (among others) are the presence of a chiral center, such as an asymmetrically substituted C-atom (C-chirality), or the presence of a chiral axis (axial-chirality) (1). The compounds considered here are:
HCHs. *α-HCH* is the most abundant component (≈65%) of technical HCH and a global environmental contaminant. α-HCH is the only chiral HCH isomer (C-chiral); the absolute configurations of its enantiomers can be inferred from those of the related

inositols (1). All HCH isomers can form chiral metabolites such as the tetra- and pentachlorocyclohexenes (TCCHs, PCCHs). Enantiomerization of (+)-α-HCH into (-)-α-HCH, or vice- versa, is an unlikely process because it requires changing the position (equatorial/ axial) of two chlorines. Isomerization of α-HCH into γ-HCH is much more likely because it would require changing of only one chlorine in its position. The structural relationship of an α-HCH isomer is thus closer to γ-HCH than to its own enantiomer. α-HCH and some of the PCCHs have been enantiomerically resolved and enantiomer ratios in biota reported (2-4).

PCBs. PCBs are among the most abundant global contaminants. Theoretically, there are 209 PCB isomers (mono- to decachloro); around 150 have been detected in the various technical formulations and several dozens in environmental samples. Suitable asymmetric substitution of both phenyl rings leads to axial chirality of all non-planar conformations. There are thus 78 PCBs that are inherently axial-chiral (5). However, many of them can interconvert via coplanar *cis* or *trans* transition states (rotation about the central C-C bond). There are 19 chiral tri- or tetraortho-substituted PCBs which have rotational barriers sufficiently high (>80 kJ M^{-1}) to be conformationally stable to racemization. Several of these chiral PCBs have been enantiomerically resolved (6-7). Presumably, many PCB metabolites are chiral. This includes metabolites not only from chiral but also from achiral PCB parent congeners. E.g. many of the sulfur-containing metabolites (methylthio and their oxygenated analogs) are axial-chiral. Model calculations have shown that these metabolites are of about equal thermal stability as the chiral PCB parent compounds (8). Dihydrodiols, reactive intermediate metabolites of PCBs, are C-chiral. However, so far little is known about the chiral aspects of these metabolites (9).

Axial-chiral compounds can interconvert under suitable thermal conditions (enantiomerize, racemize), and enantiomers of such compounds (rotamers) are much more prone to racemization than enantiomers of C-chiral compounds. Hence, racemization could proceed not only under environmental conditions (if barriers are low) but even more so at higher temperatures such as those experienced in HRGC. The enantioselective analytical techniques actually used for such analyses will, therefore, have to depend on the thermal stability of the stereoisomers. In order to adress these problems properly, it is important to know the magnitude of the rotational barriers.

Chlordane. Technical chlordane is a complex mixture of various chemically similar compounds (10) and some of the major components such as *cis*- and *trans*-chlordane, heptachlor, and component MC5, are chiral. Other components are achiral (prochiral), such as *cis*- and *trans*-nonachlor, but they can still form chiral metabolites. The two major persistant metabolites, *cis*-heptachlorepoxide (HEP) and oxychlordane (OXY), are chiral. All major chiral chlordane components and the two major metabolites have been enantiomerically resolved. Enantiomeric ratios were 1:1 in the technical or synthetic mixtures but they differed in biological samples (11-13).

DDT. DDT was one of the most important pesticides and is still valuable in combating vector-transmitted deseases such as malaria. Technical DDT is a mixture of isomers of which 4,4`-DDT is the only one with insecticidal properties. 2,4`-DDT, the second major component (≈25%), is chiral and the two enantiomers differ in estrogenic activity. 2,4`-DDT has been enantiomerically resolved and the enantiomeric ratios in technical DDT was 1:1 (14). Preliminary data indicated the estrogenically more potent R-(-)-enantiomer to be less abundant in human adipose tissue and biota (14,15). 4,4`-DDT is prochiral. The two chlorophenyl groups are enantiotopic and have a different behavior in a chiral environment. Metabolic action may thus affect the two chlorophenyl groups differently, and the two enantiomers of the chiral metabolites

need not be formed in equal amounts. This is expected to be the case in situations such as ring hydroxylation leading to chiral hydroxy-DDT metabolites.

Toxaphene. Toxaphene was a widely used pesticide produced by the chlorination of camphene. It constitutes probably the most diverse pesticide mixture with several hundred components (theoretically >30,000)(16). Most of the components are chiral although the exact structure of only about a dozen components is known. Camphene, the starting material is chiral and is occurring naturally in the (+)- as well as in the (-)-form. Technically, camphene is also produced from α-pinene (turpentine) which itself is chiral and occurrs in the different forms from different locations. In environmental samples a drastically reduced pattern of isomers and congeners is found. The finding of small but significant deviations from the exact 1:1 enantiomeric ratio for racemic mixtures for some key components in technical toxaphene was reported (17), but still is controversial. However, the nonracemic composition of the toxaphene components in environmental biological samples is undisputed and indicates the involvement of biological processes in the environmental tranformation of these compounds. Several toxaphene key components have been enantiomerically resolved (15,18).

Analytical Considerations

Extraction and Clean-Up. There are some analytical aspects particular to enantioselective determinations of chiral environmental contaminants. Since the chemical properties of enantiomers are almost identical there should be no discrimination of one enantiomer over the other during extraction or clean-up. Inefficiences (losses) encountered can change isomer composition but they cannot change enantiomeric composition. Enantiomeric composition is thus much less subject to analytical variation. Previously analyzed samples can be reanalyzed using chiral HRGC and valid results on enantiomer composition still be obtained although the clean-up procedures might not have been optimal for a particular chiral compound.

Detection. Enantiomers have also identical physico-chemical properties (except chiroptical properties). Generally, the analytical techniques cannot distinguish among enantiomers and therefore the MS properties (SIM response, mass spectra, SRM transitions, fragmention patterns) under all ionization conditions (EI, ECNI) are identical. Response ratios of enantiomers are thus identical and enantiomeric composition (ERs) can be directly determined from peak area ratios, assuming that no interference from other compounds is encountered. Pure enantiomers are not required since detector response can be calibrated using the racemates.

Enantiomer Resolution. Enantiomers cannot be resolved by the normal analytical techniques such as HRGC, HPLC or CE. However, recently HRGC columns prepared by dilution of chiral cyclodextrin derivatives in polysiloxane stationary phases were described (19, 20). These columns were applied to the enantioselective detection of various chiral compounds in environmental and biological samples.

The chiral columns now in use show excellent GC behavior and allow the analysis of a broad range of environmental contaminants. Each column with a specific selector has its merits, but usually none resolves the enantiomers of all chiral compounds. Of particular use as so-called chiral selectors were cyclodextrin (CD) derivatives which included alkylated, acylated or silylated derivatives of α-CD, β-CD or γ-CD (6, 7, 12, 19-21). They are usually added to polysiloxane stationary phases (10-50%, relative amounts). Enantiomer resolution and enantiomer elution sequence are depending on these chiral selectors and vary among different analytes (formation of guest-host complexes). They are hardly predictable but have to be checked for each particular case. Beside alkylated CDs, underivatized CDs are also used in chiral HPLC and CE. In the presence of reactive groups in a chiral compound (carboxylic, others) the preparation of diastereo-meric derivatives with a suitable chiral reagent is another way

(e.g. esters with L-menthol). The diastereomers may then be resolved by normal analytical techniques.

Reference Compounds. *Pure enantiomers or fractions enriched with one or the other enantiomer are initially required to establish enantiomer elution sequences.* Enantiomers can be isolated from the racemates using chiral HRGC or HPLC (13, 21). Chiroptical detection allowed the assignment of the (+)- and (-)-enantiomers of several chiral contaminants although some were almost unresolved and showed no apparent enantiomer resolution by other detection means (13). These methods were used for the isolation of the enantiomers of α-HCH, several chlordane and DDT compounds, and PCBs, which were then used for an unambiguous assignment of the enantiomers in subsequent analyses using chiral HRGC. The absolute configurations of these enantiomers may be known, such as with many chlordane compounds, or they can be sometimes inferred from chemical analogy, as was shown with some other chlordane and DDT compounds (13, 14).

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