Chromatographic Enantiomer Separation of Chiral Xenobiotics and their Metabolites - a Versatile Tool for Process Studies in Marine and Terrestrial Ecosystems

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1. Introduction

The application of classical achiral stationary phases in capillary gas chromatography [cGC] and high-performance liquid chromatography [HPLC] allows insight into the distribution of xenobiotics in marine and terrestrial ecosystems. Furthermore, limited information about the fate of these compounds is accessible, in particular, metabolites can be determined. A deepened study of the various processes that may give rise to metabolization in ecosystems, be it microbial, enzymatic or photochemical processes, has to account for the very property that has been assumed to be closely related to *life* since the basic studies of Pasteur: *chirality*. Homochirality appears to be a requirement for the functioning of enzymes and nucleic acids, while the specific (partial) incorporation of the respective opposite enantiomers of amino acids and carbohydrates, respectively, may block the active sites and thus give rise to potent toxines [1].

In general, it is assumed that the chiral environment of such active sites is able to select monomers of matching chirality out of a racemic mixture thus giving rise to the formation of diastereomeric complexes between the active sites of enzymes and chiral xenobiotics and their metabolites, respectively. Diastereomeric complexes and salts are known to exhibit physical properties, molecular orders (e.g., packaging) and structures that may be quite different from those of homochiral domains of the respective components [2]. As a consequence, the velocities of the metabolization process of the enantiomers of chiral environmental pollutants may be significantly different. In some cases, only one enantiomer is being decomposed, while the second enantiomer is being accumulated in the environment. In the present paper, examples for both possibilites will be shown.

2. Material and Methods

The environmental chiral or prochiral xenobiotics thus far investigated are summarized in Table 1. The respective references do not give a comprehensive survey, but they represent examples allowing easy access to the analysis of the respective compound.

The introduction of modified cyclodextrins in cGC and the application of this technique to residual analysis can be considered to represent the historic breakthrough of enantioselective analysis of chiral organochlorine compounds. In 1989, *König et al.* [3] succeeded in the enantiomer separation of α-HCH on undiluted octakis(3-*O*-butyryl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin. In this pa-

per the authors stated that the successful enantiomer separation of this compound may be used to study the enantioselective degradation of α-HCH in the environment. Two years later, after some modification of the preparation procedure of this chiral stationary phase (which was not commercially available at that time) *Faller et al.* were the first to prove the enantioselective microbial degradation of α-HCH in the marine ecosystem [4] and *Kallenborn et al.* demonstrated the enantioselective metabolisation of this compound in Eider ducks (*Somateria mollissima* L.) [5]. In 1994, the two α-HCH enantiomers became accessible by HPLC using a preparative column with the chiral stationary phase ChiraDex (Merck, Germany) thus allowing the determination of the absolute structures of the enantiomers by X-ray analysis [25]. Meanwhile, reference materials of the enantiomers of many environmental xenobiotics can be obtained by preparative enantioselective gas chromatography [26].

For a comprehensive survey on the subsequent further development of the chiral stationary modified cyclodextrin phases the reader should refer to the recent review article by *Vetter and Schurig* [49].

3. Process Studies

3.1 Microbial degradation

In 1991, *Faller et al.* [4] raised the question "Do marine bacteria degrade α-HCH stereoselectively?", and they were the first to answer this question in the affirmative by applying cGC with heptakis(3-*O*-butyryl-2,6-di-*O*-*n*-pentyl)-β-cyclodextrin as a chiral stationary phase to residual analysis of environmental samples. In a subsequent systematic investigation by *Faller et al.* [6] 16 water samples representing all parts of the North Sea that are interesting both from an oceanographic and chemical view were analysed with regard to enantiomeric ratios (ER) of the α-HCH enantiomers. The ER is directly obtained by peak integration of the gas chromatogram and dividing the peak area of the first eluting (+)-α-HCH through the peak area of the second eluting (-)-α-HCH enantiomer, i.e., ER = E_1/E_2. The results thus obtained revealed different microbial degradation pathways in the North Sea: while in the eastern part of the North Sea including the German Bight and the Skagerrak a preferential degradation of the (+)-α-HCH was observed (ER ~ 0.85), in the area east off the coast of Great Britain preferably (-)-α-HCH is degraded (ER ~ 1.15) and (+)-α-HCH appears to be more resistant to microbiological attack. In 1992, the preferential degradation of (+)-α-HCH in the eastern part of the North Sea was confirmed by analysis of additional water samples from the German Bight and from the Baltic Sea [8]. The average ER value calculated from 21 water samples turned out to be ER = 0.87 ± 0.05. Furthermore, a verification of the conclusions drawn from the North Sea results was accomplished by systematic laboratory degradation measurements using a culture of marine microorganisms which is known to be representative for the German Bight and presumably also for the Baltic Sea. Both these laboratory studies and the latter North Sea investigation by *Hühnerfuss et al.* [8] for the first time included the determinantion of the ER of the metabolites β-pentachlorocyclohexene (β-PCCH) and γ-pentachlorocyclohexene (γ-PCCH).

Similar observations of different microbial degradation pathways of α-HCH were reported by *Bidleman and coworkers* [17, and lit. cited therein]: the enantiomeric ratios of dissolved α-HCH in arctic waters were generally ER > 1.00 in the Bering-Chukchi Seas, indicating preferential microbial degradation of (-)-α-HCH, while depletion of the (+)-α-HCH was found in the Arctic Ocean and Greenland Sea, with ERs < 1.00.

Table 1: Environmental chiral xenobiotics thus far reported in the literature.

Substance	Standards/Water/Tissue/Air [References]
α-HCH	Standards [25,29,36,40]; water [4,6-10,13,14-17]; Eider duck: liver, muscle, kidney, brain [5,9,10]; blue mussel [9,10]; seal: blubber, brain, liver, lung [10-12,25]; fish liver [9,11]; whale blubber [12]; seagull eggs [10]; roe deer liver [28]; sheep: fat, liver, brain [24]; air/rain [12-16]
β-PCCH	water [7,8,10,36]; air [8]
γ-PCCH	water [7,8,36]; air [8]
PCCH II, III, V-VIII	standards [36]
heptachlor	standards [22,25,40]
cis-chlordane	standards [22,25,29,40]; water [17]; herring, salmon, seal, penguin [37,39]; cod liver oil [44]
trans-chlordane	standards [22,25,29,40]; water [17]; herring, salmon, seal, penguin [37,39]; cod liver oil [44]
chlordene	standards [22]
cis-heptachlorepoxide	standards [22,25,29,40]; water [17]; seagull eggs [10,25]; herring, seal [19,25,39]; cod liver oil [44]; roe deer liver [25,28]; hare liver [25]; rat liver [18]; human adipose [39]
trans-heptachlorepoxide	standards [25,29,39,40]
oxychlordane	standards [22,25,29,40]; seagull eggs [10,25]; herring, salmon [39]; cod liver oil [44]; hare liver [25]; roe deer liver [25,28]; seal [25,37,39]; human adipose [39];
octachlordanes MC4, MC5, MC7	herring,salmon, seal, penguin "[37,39,44]
and nonachlor III MC6	dto.
photo-*cis*-chlordanes [2,5-, and 1,5-]	standards [40]; herring, seal, salmon, penguin [39];
photoheptachlor	standard [17,40] herring,salmon, penguin [39]; seal [20,39]; polar bear, human plasma [20];
bromocyclen	standards [26,29]; water [34]; fish [27,34];
o,p'-DDT	standards [21,40]
o,p'-DDD	standards [40]
Hepta-, octa-, nonachlorobornanes	herring, salmon, seal, penguin [41]
toxaphene Parlar26/32/40/41/44/62	standards [42,43]
toxaphene Parlar38/39/42/51/58/63	standards [43]
toxaphene Parlar50	standards [42]
PCB 45/139	standard [29,45]
PCB 84	standard [29,48]
PCB 88	standard [29]; blue mussel [30]
PCB 91/136	standard [29,31]
PCB 95	standard [29,31,45]; human milk, doe liver, eel [32]
PCB 131/135/175	standard [29]
PCB 132	standard [29,31,46,48]; doe liver, eel [32]; human milk [32,33]
PCB 144	standard [44]

PCB 149	standard [29,31,48]; blue mussel [30]; human milk, doe liver, eel [32];
PCB 171	standard [48]; blue mussel [30]
PCB 174	standard [29,47,48]; blue mussel [30]
PCB 176	standard [29,47]
PCB 183	standard [29,48]; blue mussel [30]
PCB 196/197	not yet separated
PCB methyl sulfones: 3-91, 4-91, 3-95, 4-95, 3-149, 4-149, 3-132, 4-132, 3-174, 4-174	standards [35, these proceedings]
PCB methyl sulfones: 3-132, 3-149	human liver [35; these proceedings]
2-(2,4-dichlorophenoxy)propionic acid	standard [22]; water [10,23]
2-(4-chloro-2-methylphenoxy)propionic acid	standard [22]; water [38]
2-(2,4,5-trichlorophenoxy)propionic acid	standard [22]
(S)-bioallethrin/acephate/malaoxon/bromoacil	standards [29]
fonofos	standard [22]
methamidophos, trichlofon	standards [22,29]

3.2 Enzymatic degradation

After the pioneering investigation by *Kallenborn et al.* who demonstrated the enantioselective metabolisation of α-HCH in Eider ducks (*Somateria mollissima* L.) [5] increasing attention has been paid to the chromatographic enantiomer separation of chiral xenobiotics and their metabolites in environmental samples. A comprehensive survey on the various aspects reported in the literature during the last years would be beyond the scope of this key note lecture. Some representative studies as well as the respective references are summarized in **Table 1**. For a more detailed information the reader should refer to the review by *Vetter and Schurig* [49] and to the monography by *Kallenborn and Hühnerfuss* [50] which will allow deepened insight into the application of enantioselective analysis with chiral stationary phases to enzymatic degradation studies.

Basically, very different enzymatic degradation pathways become visible: in tissue extracts of muscle, liver, and kidney of Eider ducks [5] and in liver samples of flounders [9], (+)-α-HCH was clearly enriched; almost pure (+)-α-HCH was present in liver extracts of Eider ducks [5], roe-deer [28] as well as in brain tissue extracts of Eider ducks and of harbour seals [26]. The latter results are insofar notable as they imply an enantioselective permeation of (+)-α-HCH through the Blood-Brain Barrier (BBB). The same enantioselectivity of the BBB was also observed for brain tissues of sheep [24] and humans [51], although in fat and liver tissues of sheep a preferential enrichment of the (-)-enantiomer was determined reflecting quite different enzymatic systems.

Another notable phenomenon revealed by enantioselective gas chromatography was reported by *Pfaffenberger et al.* [28] who observed a correlation between the concentrations of α-HCH, *cis*-heptachlorepoxide and oxychlordane, respectively, in roe-deer liver samples and the enantiomeric ratios of these compounds, which indicated that higher concentrations of these xenobiotics resulted in stronger decomposition of the (+)-enantiomer of α-HCH, while higher levels of *cis*-heptachloepoxide and oxychlordane appeared to lead to a faster decomposition of the respective (-)-enantiomer or a preferential formation of the respective (+)-enantiomer. Similar observations were made by *Pfaffenberger et al.* [27] for bromocyclen in trout liver tissue.

During the last three years special emphasis was placed upon the enantiomer separation of atropisomeric PCBs [29-33,42,43,45-48] and their chiral PCB methyl sulfons [35] in environ-

mental samples. This aspect will be pursued in the subsequent paper by *Ellerichmann et al.* [see this issue].

3.3 Photochemical Degradation

In general, photochemical degradation of chiral xenobiotics is assumed to be largely non-enantioselective. This hypothesis forms the basis for a discrimination between biotic (enantioselective) and abiotic processes like photodecomposition. Systematic laboratory investigations on photochemical degradation of α-HCH were performed by *Hühnerfuss and coworkers* [8], while the groups of *Buser and Müller* [18], and *Parlar and coworkers* [40] studied the photoconversion products of cyclodiene insecticides, because of their higher toxicities and stabilities in the environment in comparison with their original pesticides.

Basically, photochemical degradation processes turned out to be nonenantioselective, as to be expected in an achiral environment. However, *Hühnerfuss et al.* showed that enantiomeric excesses, e.g., of β-PCCH, that may have been formed by enzymatic processes may be modified by photochemical processes provided that enzymatic processes become less important, for example due to seasonal variations of microbial activity [8].

3.4 Air/Sea Exchange Processes and Atmospheric Longe Range Transport

The water/air exchange of α-HCH and other halogenated xenobiotics is gas phase controlled and often described by water/air fugacity ratios, which reflect the saturation state of the water relative to the partial pressure of the respective compounds in air [15]. A fugacity ratio of 1.0 implies that the compounds in water and air are at equilibrium.

In addition to this classical approach, *Jantunen and Bidleman* [15] determined the enantiomers of α-HCH as tracers of air-water gas exchange. The α-HCH in air samples taken in 1993 and 1994 within 40 m off the sea surface was non-racemic and followed the same order of degradation as in surface water. Air over the Bering Sea and southern Chukchi Sea was depleted in (-)-α-HCH (ER > 1.00) but, as with the water, the selectivity reversed at higher latitudes. ERs in air portions of the Arctic Ocean and the northern Atlantic Ocean showed ER values < 1.00, from depletion of (+)-α-HCH. These results suggest that sea-to-air gas exchange is an important source of α-HCH to the marine boundary layer. Furthermore, this process should be taken into account when discussing the atmospheric long range transport of such xenobiotics to remote areas.

As the two enantiomers exhibit the same Henry's law constants, differences in the enantiomeric ratios in the air and the adjacent water column allows an estimate of the relative contributions of volatilization and deposition in the respective sea areas. For example, *Ridal et al.* [16] compared ER values of Lake Ontario water (average ER = 0.85 ± 0.02) with those in air samples measured at 10 m above the lake. The latter ER values showed a seasonal variability with values near 1.00 in spring and fall and minimum values in individual samples near 0.90 in summer. A simple air-water gas transfer model demonstrated that enantiomeric ratios < 1.00 in air are derived from equilibration of the air with the water during transport of the air mass over the lake and that as much as 60 % of the α-HCH in air above Lake Ontario was derived from the lake itself.

3.5. Enantioselective Toxic Effects

Differential effects of the α-HCH enantiomers on cytotoxicity and growth stimulation in primary rat hepatocytes were recently observed by *Möller et al.* [52]. The cytotoxic effect was deter-

mined as a parameter for the acute toxicity of α-HCH, while the growth stimulation may be associated with the chronic toxicity, e.g., tumor promotion. In both assays the (+)-α-HCH was more effective than the (-)-enantiomer. It may be concluded from these results that an enantioselective enrichment of (-)-α-HCH is associated with a lower risk factor than the accumulation of (+)-α-HCH. Thus, the quantitative determination of α-HCH as a mixture of both enantiomers may lead to an overestimation of the toxicity in those animal tissues, e.g., the liver of sheep [24], in which the more toxic (+)-enantiomer is preferentially degraded. For more details the reader should refer to ref. [52]. Furthermore, enantioselective toxic effects were discussed for the atropisomers of PCBs quantified in marine blue mussels [30, and literature cited therein].

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