Multidimensional gas chromatographic enantiomer quantification of some polycyclic xenobiotics in cod liver and fish oils

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Introduction

Many of the polychlorinated insecticides, such as chlordane, heptachlor, aldrin, dieldrin, and toxaphene, were extensively used in some countries (U.S.) but reportedly not in others, e.g., not in Scandinavia [1]. The fact that they are very persistent and detected in biota from remote areas, for example the Arctic and Antarctic [2-4], points to global distribution mechanisms, such as long-range transport [5-9]. They can now be found in specimens of all trophic levels, generally together with many other halogenated compounds, such as DDT and its metabolites or γ-, β- and α-HCH. Furthermore, residues of the conversion products especially of the cyclodiene insecticides are found because of their increased stability in the environment in comparison to that of the original pesticides. Whereas most of these substances are achiral, several cyclodiene compounds, many of their metabolites and photoconversion products as well as many toxaphene components are chiral, and their two enantiomeric forms differ in biological properties.

Biological transformation of chiral compounds can be stereoselective, and uptake and excretion of the (+)- and (-)-isomer may thus be very different [10-13]. The enantiomers can have different activities and toxicities, which has been shown for instance for members of the group of cyclodiene insecticides [14,15]. Therefore, achiral analyses of chiral compounds will give only part of information, and chiral analysis is required for a full understanding of the biological behavior of such compounds.

Previously, the enantiomer resolution of cis- and trans-chlordane, oxychlordane, cis- and trans-heptachlorepoxide, photo-heptachlor, photo-cis-chlordane, o,p´-DDT, and α-HCH was achieved by chiral HRGC with α- and β-cyclodextrin derivatives as chiral selectors [16-18]. The experience in all cases is that small changes in the steric structure of molecules drastically change the enantioselective interaction with the stationary phase. Therefore, enantiomer separation by HRGC is still based on empirical selection of the stationary phase.

Materials and Methods

Chemicals: Standards (heptachlor, cis- and trans-chlordane, o,p´-DDT, o,p´-DDD, α-HCH, and toxaphene components) were provided by Dr. Ehrensdorfer GmbH

(Augsburg, Germany). The photoconversion products of dieldrin were synthesized by irradiation with UV-light and purified by recrystallisation as described previously [19-21]. Organic solvents were of purity grade for residue analysis. Na_2SO_4 was from Merck, Germany. Biobeads SX3 was from BioRad, Germany.

Samples: Cod liver oils and fish oils were obtained from different countries. The samples were kept under -12°C until use. 2g of the oil was dissolved in 25ml cyclohexane/ethylacetate (1:1) and separated from the fat by GPC (SX3; l = 40cm; ∅ = 2.5cm).

High resolution gas chromatography: All samples were comparatively analyzed using three chiral HRGC column systems (see Table 1). The commercial chiral columns were obtained from Macherey & Nagel (Düren), Chrompack GmbH (Frankfurt), W.A. König (University Hamburg), and from BGB-Analytik (Switzerland). The actual column dimensions and operating conditions are listed in Table 1. All three columns have a film thickness of 0.25μm. Chrompack gas chromatographs (2001 model) equipped with electron capture detectors (280°C, make-up gas nitrogen) and split injectors (220°C) were used with hydrogen as carrier gas (1ml/min). All samples were diluted in acetone and 1μl was injected at a split ratio of approximately 1:100. Chrompack software (Maestro) was employed for registration and quantification.

Multidimensional gas chromatography: A Sichromat 2-8 with live-T-technique was used (1. column: DB5, 60m, i.d. 0.32mm, film thickness 0.25μm; temp. program: 100°C to 150°C with 30°/min, 150°C to 250°C with 2°/min; 2. column: BGB-A2 (20% BSCD); 30m, i.d. 0.32mm, film thickness 0.20μm; temp. program: 80°C (39min) to 180°C with 30°/min, 180°C to 250°C with 1°/min).

Table 1. Chiral HRGC columns used

No.	chiral selector	column material, dimensions	column temp[d] (rate)
1[a]	Heptakis(6-O-TBDMS-2,3-di-O-methyl) - β-CD 50% dissolved in OV1701	fused silica, 11m x 0.25mm	70-115-180°C (1.5°C/min)
2[b]	FS-Hydrodex-β-3P	fused silica, 25m x 0.25mm	70-115-190°C (1°C/min)
3[c]	CP-Chirasil-Dex CB β-CD bound to dimethylpolysiloxane	fused silica, 25m x 0.25mm	70-115-190°C (1°C/min)
4[d]	tert-butyldimethyl-silinated β-CD	fused silica, 30m x 0.32mm	80(39min)-180-250°C (1°C/min)

[a]W.A. König, University Hamburg (Germany); [b]Macherey & Nagel, Düren (Germany); [c]Chrompack, Frankfurt (Germany); [d]BGB-Analytik (Switzerland) injection, intermediate, and final hold temperatures; program rate between intermediate and final hold temperature listed; program rate between injection and intermediate temperature 30°C/min.

Results and Discussion

Selection of the chiral column: All reference compounds and the photoconversion products were analyzed on the four chiral HRGC column systems. The stationary phases were different in enantiomer seperation. Column 4 separated more enantiomer pairs than the other three. Column 1 and 2 had short lifetimes and low temperature stabilities. Column 3 was acceptable for the analysis of some of the chiral substances, but with restrictions. Therefore, of all columns tested only the fourth is suitable for quantification of the selected chiral pesticides.

Residue analysis of chiral compounds in cod liver and fish oils: 13 cod liver oil samples and 11 fish oil samples were investigated (Table 2). The enantiomer ratios [ER] of cis-chlordane, trans-chlordane, photodieldrin, α-HCH, o,p'-DDD, and toxaphene #26 in fish oils are nearly 1.0, while the ERs of the same substances are significantly different from 1.0 in cod liver oils. It can be assumed that these pesticides occur only as racemates (ER = 1) in sea-water. Direct accumulation from sea-water through skin and gills as well as accumulation by ingestion of contaminated food without biodegradation should not lead to any change in the enantiomer ratio in the fish. This situation is mirrored by the ERs in the fish oil samples. The significant change in ER in the cod liver oil samples points to the involvement of a metabolic pathway either in fish liver or, more specifically, in codfish or somewhere else in the marine environment, i. e. the food chain. Contrary to that, the ER values of o,p'-DDT and toxaphene #44 are remarkable different from 1 in the cod liver oils as well as in the fish oils. In these cases metabolic pathways can be assumed which are independent from the fish species either because all fish can metabolize these substances, or because the metabolizing species belong to a common part of the food chain.

Table 2. Enantiomer ratios (+/–) of selected organochlorine pesticides in cod liver oils and fish oils separated on column 4

Substance	Cod Liver Oil	Fish Oil
cis-chlordane	0.50-1.02	1.00-1.18
trans-chlordane	0.80-3.49	1.00-1.06
photodieldrin	0.97-1.02	0.90-1.02
α-HCH	1.00-1.23	1.00
o,p'-DDT	0.00-0.16	0.06-0.32
o,p'-DDD	0.57-0.70	1.00
toxaphene #26	1,00-1,41	1,00-1,02
toxaphene #44	1,10-1,28	1,20 1,28
toxaphene #50	1,00-1,11	1,01-1,02
toxaphene #62	1,01-1,09	1,75-1,89

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