MULTIDIMENSIONAL HRGC-HRMS ENANTIOMER SEPARATION - A SUITABLE METHOD FOR THE DETERMINATION OF ENVIRONMENTAL BEHAVIOUR OF (+/-)-o, p´-DDT AND ITS METABOLITE (+/-)-o, p´-DDD

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Introduction

Dichloro**d**iphenyl-**t**richloro ethane (DDT) and its metabolites (DDD, DDE) are still ubiquitously found all over the world, even in snow of the polar regions [1-3], due to worldwide use for several decades and their undesirably long persistence. All members of the DDT family, for a large part, enter the environment through wastewater discharges, an important route to surface and ground water and marine environment. Another route is long-range atmospheric transport [4] which is now considered more important than was thought before. DDTs accumulate in sediments because of their low solubility in water and their tendency to adhere to particulate matter. They are bioaccumulated and after penetration of the cell membranes deposited long-term in body fat, in the liver and other organs of fishes and humans and these stored lipophilic compounds are to different extent excreted in mothers´ milk [5]. Elevated levels (0.1 mg kg^{-1}) of DDT including isomers such as o,p´- and o,o´-DDT, and metabolites, mainly however p,p´-DDE, have been found in fishes from the Baltic Sea [6] and in rivers or lakes due to frequent application of DDT in the environs in the past. [7, 8]. Swackhamer and Hites [4] have observed that the levels of organochlorine compounds like DDTs were not similar between fish species and inconsistence among compounds. They conclude that such differences indicate that physico-chemical properties alone do not determine bioaccumulation of these compounds. The application of DDT is banned since 1974 in Germany and many other countries, but still practised in Asia and Africa. The enrichment of DDTs in soil is estimated to be about 300, 000 tons world-wide [9].

Technical DDT is a mixture containing 77% p,p´-DDT, 1% DDE and 21.1% o,p´-DDT. The last of them and its metabolite o,p´-DDD are chiral compounds in the DDT family also accumulating especially in food chains of fishes and fish-eating birds [10]. Normally the chiral components exist as racemates in the technical products. Abiotic and biotic conversions take place in compartments, air, soil, and water, and degradation of the (-)-enantiomer to polar substances and enrichment of the unchanged (+)-enantiomer was observed. Furthermore, biological transformation of chiral compounds may be stereoselective, uptake and excretion of the (+)- and (-)-enantiomer may be very different [11-14].

Therefore, the enantiomer ratios (ER) of o,p´-DDT and o,p´-DDD in cod liver and fish oil samples were determined using a chiral HRGC capillary from BGB-Analytik (Switzerland) with 20% of tert.-butyldimethylsilylated ß-CD as chiral selector dissolved in OV 1701. Previous results and experience with several chiral CD phases have shown that the choice of a tailor-made column is still a „trial and error“ procedure [15-17]. Reproducible quantitative results in our group have been only achieved using the above-mentioned BSCD capillary column.

It has been attempted in this study to elucidate the environmental behaviour of (+/-)-o,p´-DDT and its chiral analogue DDD.

Materials and Methods

Chemicals: Standards (o,p´-DDT and o,p´-DDD) were provided by Dr. Ehrenstorfer GmbH (Augsburg, Germany). The organic solvents were of purity grade for residue analysis, Na_2SO_4 was from Merck (Darmstadt, Germany) and Biobeads SX3 were from BioRad, Germany.
Samples: Cod liver oils and fish oils originate from different countries and were purchased on local markets. All the samples were kept under -12 °C until use. A 5 g portion of each oil was dissolved in 25 ml of a cyclohexane/ethylacetate mixture (1:1) as stock solution.
Clean-up method: The organohalogen pesticides were separated from the fat by GPC (column 50 x 3.5 cm, filled with 60 g Biobeads SX3 (200/400 mesh)). 5 ml of each stock solution were applied on the column and eluted with 125-240 ml of cyclohexane/ethylacetate (1:1). A mini silica gel column chromatography of the eluate followed (column 20 x 1 cm, filled with glass wool/heated sea sand/1 g silica gel 60 (70-230 mesh), preconditioned by heating to 130 °C for 5 h and deactivated with 1.5% of water/anhydrous Na_2SO_4/glass wool in n-hexane). The pesticides were eluted with 8 ml of n-hexane/toluol (65:35) and 8 ml of toluol.
Multidimensional HRGC-ECD analysis: A *SiCHROMAT 2-8 double-oven GC* with live-T-technique and two ECDs (280 °C, make-up gas N_2) was used. Achiral column: DB5 (J&W), 60 m x 0.32 mm x 0.25 µm; temperature program: 100-150 °C/30 °C min^{-1}, 150-250 °C/2 °C min^{-1}; Chiral column: BGB-172 with 20% BSCD, 30 m x 0.32 mm x 0.20 µm; temperature program: 80 °C (individual time), to 150 °C/30 °C min^{-1}, 150-250 °C/1 °C min^{-1}; injector: 240 °C; injection volume 1 or 2 µl; carrier gas, 1st column: H_2, 1.5 bar and 2nd colum: H_2, 0.65 bar.
HRGC-MS analysis: *MS Finnigan Model 4500*, ion source temperature 140 °C, EI ionization energy 70 eV, accelerating voltage 950 V, emission current 250 µA; *GC Finnigan 9600*, column: DB 5 (J&W), 30 m x 0.25 mm x 0.25 µm, temperature program: 100 °C (5 min), 100-150 °C/5 °C min^{-1}, 150-250 °C/2 °C min^{-1} (10 min); *Evaluation:* MASPEC Data System for MS-Windows (MSS, Manchester), vers. 2.11, NIST-Library

Results and Discussion

Chiral column: The selected chiral phase must fulfill the following main preconditions for a special enantiomer separation problem:

- a relatively high percentage of chiral selector without reducing the selectivity
- polarity of achiral part as high as possible without reducing the selectivity
- covalent binding of the chiral selector to the polysiloxan bone guaranteeing thermal stability and low column bleeding
- splitless/on column injection and column conditioning at higher temperatures

The separation efficiency of the used BSCD capillary column was characterized by the chiral separation factors $\alpha = t_R/t_S$ (ratio of the relative retention times of the later and earlier eluting enantiomers). Schurig and Schleimer [18] postulated that the lower limit for a quantitative separation of a pair of enantiomers on a HRGC capillary is 1.01. The α values of o,p´-DDT and o, p´-DDD have been isothermally determined as 1.02 and 1.008. Therefore, (+)- and (-) enantiomer of o,p´-DDD are not baseline-separated, but sufficiently enough for a quantitative determination of both enantiomers. Enriched standards (Ehrenstorfer) for the confirmation of the elution order of the (+)- and (-)-enantiomers of o,p´-DDT and o,p´-DDD were not available. But the fact that all

(+)-enantiomers of chiral organohalogens elute first, with the exception of α-HCH, led to conclude the same elution order, and additional aspects such as peak coalescence at the isoenantioselective temperature or inversion of peak order (> 240 °C) play no role, in this case.
The multidimensional HRGC is the method of choice for the determination of enantiomer ratios in complex matrices [19, 20]. The cleaned-up oil samples examined contained always more than 20 peaks and the pre-separation on the achiral column with a heart-cut to the chiral column eliminates separation problems, like coelution with other pesticides, contaminants or congeners during a single-column run.
Residue analysis of o,p´-DDT and o,p´-DDD in cod liver and fish oil samples: (+/-)-o,p´-DDT and (+/-)-o,p´-DDD could be detected quantitatively in 12 oil samples by HRGC/MS-EI/SIM analysis (Table 1). The enantiomer ratio of o,p´-DDD by multidimensional HRGC-ECD analysis could not be determined in some of the samples under the chromatographic conditions (Table 2).

Table 1. Determination of o, p´-DDT and o,p´-DDD in fish and cod liver oils

Oil samples	o, p´-DDT (ng g^{-1})	o,p´-DDD (ng g^{-1})
Cod liver oil 9 (UK)	3.5	2.3
Cod liver oil 10 (UK)	2.5	1.6
Cod liver oil 11 (UK)	1.6	0.8
Cod liver oil 12 (UK)	1.2	0.5
Cod liver oil 17 (USA)	8.1	2.3
Cod liver oil 18 (USA)	3.1	1.6
Cod liver oil 24 (France)	3.9	2.0
Cod liver oil 25 (France)	11.8	8.5
Cod liver oil 27 (Iceland)	1.4	0.4
Cod liver oil 28 (Iceland)	8.6	3.8
Cod liver oil 31 (Iceland)	3.0	1.2
Fish oil 32 (Spain)	4.1	3.0

Table 2. Enantiomer ratios of o,p´-DDT and o,p´-DDD in fish/cod liver oils

Oil samples	ER [(+)/(-)] of o,p´-DDT	ER [(+)/(-)] of o,p´-DDD
Cod liver oils	0.00 - 0.16	0.70
Cod liver oil 10 (UK)	0.06 - 0.32	1.00

In seawater the enantiomer ratio (ER) is always 1 i.e., a racemic ratio of the enantiomers and no enzymatically induced degradation [21]. The ER of (+/-)-o,p´-DDD, a conversion product of o,p´-DDT, in cod liver and fish oil samples is about 1 (Table 2) indicating a racemic ratio too. The ER of (+/-)-o,p´-DDT in the same cod liver/fish oil samples differs from 0.00-0.16/0.06-0.32. The apparent contradiction in the results may be explained as due to an enzyme-induced stereospecific reductive dechlorination/degradation of (+)-o,p´-DDT to an intermediate carbonium ion. In a second step a partial/total racemization of this carbonium ion to (+/-)-o,p´-DDD follows (Fig. 1).
Further experiments are necessary to confirm this possible biodegradation pathway of (+)-o,p´-DDT to racemic (+/-)-o,p´-DDD, including the influencing parameters such as food chains of certain fish species in defined draught regions.

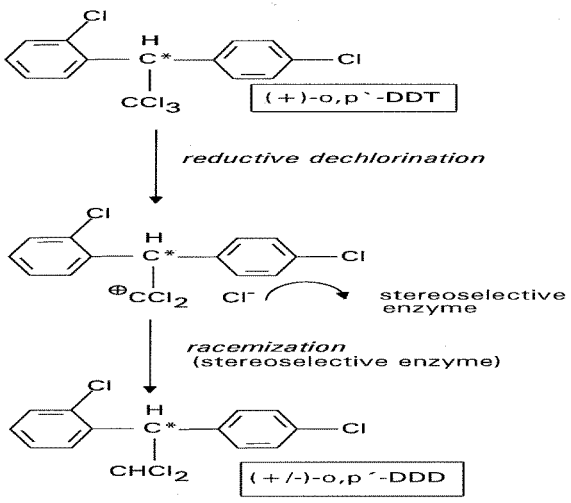

Figure 1: Possible stereospecific biodegradation of (+)-o,p´-DDT to racemic (+/-)-o,p´-DDD

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