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Enantioselective Determination of Toxaphene Components in Fish, Monkey Adipose Tissue from a Feeding Study and Human Milk

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1. Introduction

Toxaphene (ISO common name: camphechlor) is probably the most complex pesticide and more than one million tons of it have been used since 1946 [1]. It is a mixture consisting of polychlorinated monoterpenes, predominately chlorobornanes (CHBs). According to Vetter[2], there are 16128 possible enantiomeric pairs of chlorobornane congeners. Only 512 congeners of toxaphene are achiral compounds.
Fortunately, enantiomeric ratios can be determined without the availability of the individual enantiomers, since chiral compounds have identical chemical and physical properties in gas chromatographic detectors including the mass spectrometer (MS). Buser and Müller [3] studied the enantiomeric composition of some components of different technical toxaphene products. All products showed similar isomeric and enantiomeric compositions and no or only small deviations from an enantiomer ratio of 1. In an investigation of two toxaphene congeners which accumulate in high amounts in seal blubber, Ochme[4] has found small differences from a 1:1 enantiomer ratio only.

We have detected high concentrations of three chiral chlorobornanes in fish[5] and human milk from women with a high share of seafood in their diet[6]. In addition, the same toxaphene components have been found in the adipose tissue of monkeys treated one year with the whole complex mixture of toxaphene[7]. The enantiomers of these three chlorobornanes may show different uptake, metabolism,

and excretion in the food chain. In contrast, abiotic degradation processes should be the same for both optical isomers, and enantiomeric composition is not changed in this case. Therefore, an enantiomer ratio in biological samples close to 1 indicates a low degree of metabolism. Otherwise, if deviations from the value of 1 are found, biological transformation has occurred and different toxic properties of both enantiomers have to be expected.

2. Experimental

Samples

Marine fish samples were caught during cruises of the research vessel "Walther Herwig III" or were supplied by Staatliches Veterinäramt, Bremerhaven, Germany. Farmed salmons were obtained from wholesalers in Hamburg, Germany. The samples were deep-frozen at -18 °C until analysis. In most cases, pooled samples of at least five fishes were used.
Fat extracted from the adipose tissue of cynomologus monkeys was obtained from animals of a Canadian primate feeding study [7]. From a former study[6], extracts of four individual human milk samples and one pooled sample from 9 mothers living on the Faroe Islands were used

Extraction

Fat extraction from fish tissue was performed either using the AOAC method 970.52 L(e), which has been specifically developed for extraction of organochlorine pesticide multiresidues from fish[8], or by using the more quantitative modified Bligh and Dyer method[9].

Cleanup of fish and adipose tissue

The lipids from 0.5g fish oil were removed by gel permeation chromatography, fitted with a 2.5 × 40 cm column containing 50 g Bio-Beads SX-3 (Bio Rad Laboratories, 200 - 400 mesh). The mobile phase, consisting of cyclohexane/ethylacetate (1:1), was introduced into the column at a flow rate of 5 ml/min. The toxaphene congeners eluted together with PCBs, other chlorinated pesticides such as chlordane components or DDT and metabolites in the range of 95 - 150 ml.
After solvent removal, the sample was dissolved in isooctane and then applied to a 0.7 × 23 cm chromatography column packed with 1 g silica gel (Merck No. 7734, deactivated with 1.5 % water) and with 1 cm dried sodium sulfate on the top. Organochlorine pesticides and PCBs were fractionated by consecutive elution with 8 ml hexane (fraction 1) and 8 ml hexane/toluene (65:35 v/v, fraction 2). The hexane fraction contained all of the PCB, the p,p'-DDE congeners and the toxaphene congener **1**. Some chlordane/nonachlor and pp'-DDT were found in the first fraction, as well. The second eluent

(hexane/toluene) eluted the stronger polar chlorinated compounds from the column. This fraction of extracts contained toxaphene congener **2**, beside p,p'-DDD, p,p'-DDT and o,p'-DDT, the bulk of chlorobornanes and chlordanes.
From monkey adipose tissue, 23 mg of fat were directly applied to a silica gel column as described above without fat removal by GPC.

Enantiomer selective determination

Gas chromatography (GC) was performed by using a Hewlett-Packard 5890 gas chromatograph equipped with a 30m × 0.35 mm × 0.25 µm fused silica column coated with a mixture of tert-butyldimethylsilylated-ß-cyclodextrin and 25%-phenyl-75%-methylpolysiloxane (BGB-172, BGB Analytik AG, Switzerland). For ^{63}Ni ECD, nitrogen was used as make-up gas. A typical oven temperature programme was as follows: initial temperature 90 °C held for 1 min, first ramp to 180 °C with 40 °C/min, second ramp to 220 °C with 1 °C/min. The temperatures of splitless injector and electron capture detector were 230 and 300 °C, respectively. Helium was used as carrier gas at a flow rate of 35-45 cm/s for all separations. GC/MS experiments were carried out with a Finnigan MAT95 mass spectrometer operating in the negative ionisation mode (ECNI-MS) using ammonia under a pressure of 0.5 torr as reactant gas. Ion source temperature and electron energy were set to 140 °C and 100 eV, respectively. ECNI-MS measurements were performed in selected ion monitoring (SIM), recording signals at 341, 343, 345, 375, 377, 379, 411, 413, 443, 445 and 447 amu. All measurements were made in duplicate or triplicate.

3. Results

Since many other chlorinated compounds are present in the extracts analysed, coelution of analytes with interferents is a particular problem. In chiral phase high-resolution gas chromatography (HRGC), small differences in the intensity of enatiomeric peaks are of basic interest most of the time. Even small interference peaks will significantly shift the enantiomeric ratio of such peak pairs. Therefore, chiral analysis was performed using the selective ECNI-MS detection in selected ion mode. In all extracts, two out of the three proposed toxaphene indicator compounds [10] could be detected and separated into the enantiomeric pairs. These chlorobornanes were (corrected nomenclature [11] used):

indicator compound **1**: 2-endo,3-exo,5-endo,6-exo,8, 8,10,10-octachlorobornane
indicator compound **2**: 2-endo,3-exo,5-endo,6-exo,8, 8,9,10,10-nonachlorobornane

Figure 1 shows the good separation of both enantiomers of the chlorobornanes **1** and **2** in the GC/MS chromatograms. Due to the low sensitivity of ECNI/MS for 2,2,5,5,8,9,9,10,10-nonachlorobornane (our indicator compound **3**), no data for this chlorobornane are available.

Figure 1: ECNI-MS/SIM chromatograms of the Baltic herring extract showing elution of toxaphene indicator components **1** (upper trace) and **2** (lower trace) on chiral BGB-172 column

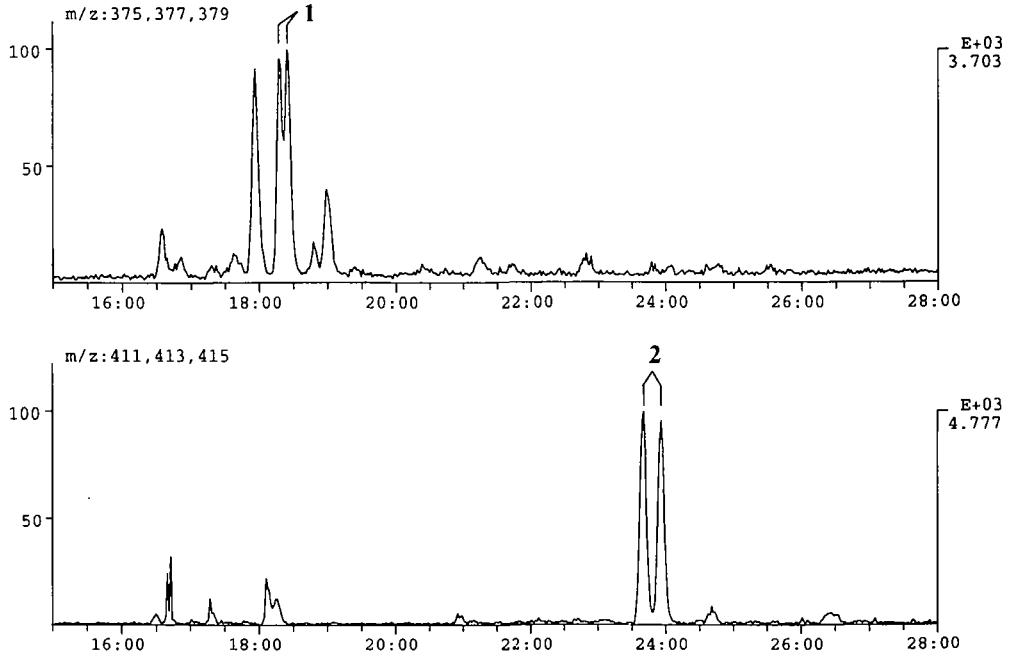
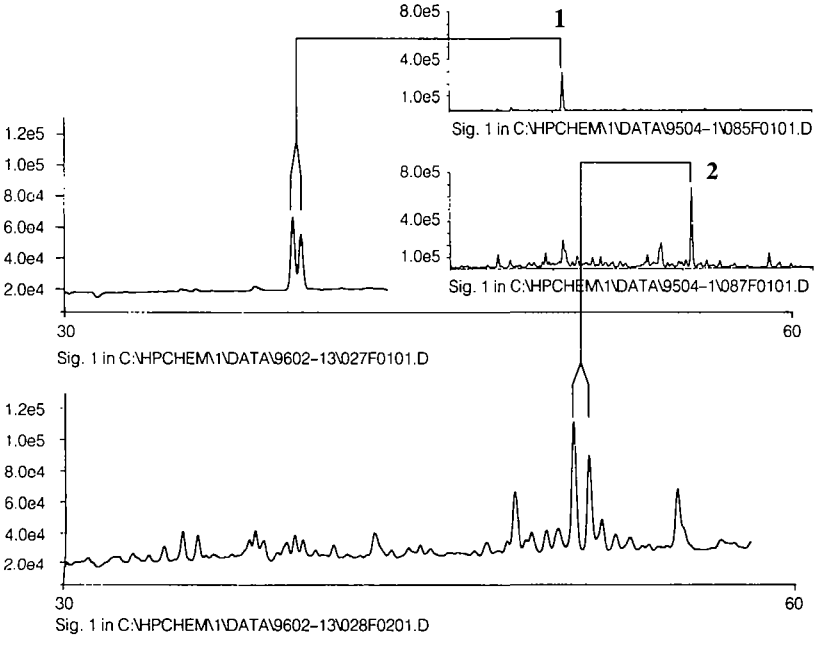


Figure 2: ECD chromatograms of the monkey adipose tissue showing elution of toxaphene indicator components **1** and **2** on the achiral DB-5 column (upper traces) and on chiral BGB-172 column (lower traces)



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Additionally to the HRGC/ECNI-MS measurements, the monkey adipose tissue from the toxaphene feeding study could be analysed by GC/ECD. In the extracts of this sample, the toxaphene components **1** and **2** were clearly dominating (Figure 2), apart from chlorobornanes, other interfering pesticide residues were not detected.

In total, enatiomer ratios of eight fish samples, one adipose tissue and five human milk samples were determined. The results are presented in Table 1.

Table 1: Enatiomer ratio (first enantiomer/second enantiomer) of toxaphene indicator compounds **1** and **2** in fish, monkey adipose and human milk samples determined by GC/ECNI-MS (SIM)

Sample	Indicator compound **1**		Indicator compound **2**	
	concentration (ng/g fat)	enantiomer ratio	concentration (ng/g fat)	enantiomer ratio
Herring (Baltic Sea)	69	0,95 +/- 0,03	65	1,08 +/- 0,03
Saithe (North Atlantic)	126	1,08 +/- 0,03	180	1,07 +/- 0,03
Farmed salmon (Norway)	36	1,02 +/- 0,06	77	1,13 +/- 0,04
Redfish (North Atlantic)	153	1,10 +/- 0,10	358	1,08 +/- 0,07
Herring (North Sea)	31	1,06	61	1,08 +/- 0,03
Mackerel (North Atlantic)	23	0,98 +/- 0,03	30	1,08 +/- 0,03
Mackerel (North Sea)	16	1,13 +/- 0,12	32	1,11 +/- 0,03
Halibut (North Atlantic)	390	0,91 +/- 0,08	620	1,08 +/- 0,03
Monkey adipose tissue	3100	1,29 +/- 0,10	8000	1,44 +/- 0,05
Monkey adipose tissue [a)]	3100	1,31 +/- 0,03	8000	1,35 +/- 0,03
Human milk A	640	1,28 +/- 0,09	750	1,06 +/- 0,03
Human milk B	25	1,28 +/- 0,04	41	1,17 +/- 0,03
Human milk C	45	1,17 +/- 0,10	61	1,23 +/- 0,06
Human milk D	96	1,23 +/- 0,10	124	1,21 +/- 0,04
Human milk, pooled sample (N=9)	77	1,07 +/- 0,05	118	1,33 +/- 0,03

a) Identical extract determined with GC/ECD.

When taking the measuring error into account, the results exhibited only small differences from nearly racemic concentrations in all fish samples. In contrast, the first eluting enantiomers of chlorobornanes **1** and **2** in the samples from warm-blooded species (human milk and cynomologus monkey adipose tissue) showed a much higher enantiomeric excess. This is in accordance with observations by Buser[3)] who noticed an enatiomer ratio of 1,34 for chlorobornane **2** in Antarctic penguins.

4. Conclusions

Chiral toxaphene congeners can be separated into enantiomers by a commercially available capillary columns. The fact that enantiomer ratios of important chlorobornanes in the samples of warm-blooded species deviated more from unity than in other samples (fish) indicates a more efficient enantioselective biological metabolization process in this such species.

5. References

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