CONGENER PATTERN AND ENANTIOMER RATIOS OF TOXAPHENES IN HUMAN MILK

Sonja Skopp[1], Michael Oehme[1] and Peter Fürst[2]

[1]Organic Analytical Chemistry, University of Basel, Neuhausstr. 31, 4057 Basel, Switzerland
[2] Chemical and Veterinary Control Laboratory (CVUA), Sperlichstr. 19, 48151 Münster, Germany

Introduction

Toxaphene is a pesticide mainly consisting of hundreds of chlorobornanes. It is found world wide in the environment. Due to differences in the persistency of congeners a much smaller number of toxaphene compounds is found in biota compared to the technical mixture. About 20 major congeners are found in fish, eight in marine mammals and only two in humans, Parlar #26 and #50[1].

Most toxaphene compounds are chiral. Bioaccumulation and metabolism in biota is usually enantioselective[1]. Recent progress in the development of derivatized cyclodextrins as chiral stationary phases enables the determination of enantiomer ratios (ER) of chiral environmental pollutants, even at trace levels by capillary gas chromatography (HRGC)[2,3,4].

Human milk is the most favorable nutrition for infants. Unfortunately, human breast milk also contains traces of persistent organochlorines (OC). This leads to exposure throughout the breast-feeding period. The levels of OC in milk depend on many factors such as mothers age, number of deliveries and lactation periods, place of residence, changes in the mother's weight during lactation or nutrition.

The aim of this study was to investigate the toxaphene congener and enantiomer pattern in 8 human milk samples from Germany and to determine the enantiomer ratios (ER) of some dominant congeners.

Materials and Methods

Reference compounds and solvents. A standard mixture containing 22 toxaphene congeners (#11, #12, #15, #21, #25, #26, #31, #32, #38, #39, #40, #41, #42a/b, #44, #50, #51, #56, #58, #59, #62, #63 and #69) at concentrations of 400 pg/µL each in iso-octane was purchased from Promochem (Wesel, Germany). It was diluted to 100 pg/µL with iso-octane (Scharlau, Barcelona, Spain). A reference standard containing the additional toxaphenes B7-1453 (~ 2 ng/µL), B8-1412 (~ 0.8 ng/µl) and #44 in iso-octane was made available by Walter Vetter (University of Jena, Germany). Solid ε- hexachlorocyclohexane (ε-HCH, >99 %, Promochem) was diluted to 250 pg/µL with ethylacetate (nanograde quality, Promochem) and used as syringe spike.

Milk samples and cleanup. Milk samples were collected from volunteering nursing mothers living in North-Rhine-Westphalia (Germany). Here, the regional government offers all nursing mothers to analyze their breast milk for OC, polychlorinated biphenyls (since early 1970's) and dioxins (from 1984).

All women were asked to fill in a questionnaire concerning personal data, living conditions, food consumption or smoking habits (see Table 1). Seven human milk samples and one pooled sample from Germany (1992 /93) were selected for this study.

100-200 mL milk were extracted with 4 mL of a aqueous solution of 35 % (w/v) potassium oxalate (p.a., Merck, Darmstadt, Germany), 200 mL of ethanol, 100 mL of diethyl ether and 140 mL of n-pentane (all solvents of nanograde quality, Promochem, Wesel, Germany). The combined organic layers were washed with a 2 % (w/v) solution of sodium sulfate (p.a., Merck, Darmstadt, Germany) and dried over anhydrous sodium sulfate (p.a., Merck) for 30 min. The solvent was removed on a rotary evaporator (Büchi, Flawil, Switzerland) at 40 °C and the extract dried under a gentle stream of nitrogen (Messer Griessheim, Krefeld, Germany) before weighing the extractable lipids. Afterwards, lipids were removed by gel permeation chromatography (GPC, Autoprep 1001, INCI, Columbia, USA) with a column (38 cm long, 25 mm i.d.) filled with 30 g of Biobeads S-X3 (Biorad, München, Germany) using cyclohexane/ethylacetate (1+1, v/v) as eluent. The fraction between 20-42 min was collected at a flow rate of 5 mL/min. Further clean-up was carried out by column chromatography (glass column, 10 cm long, 8 mm i.d.) on 1 g of silica (deactivated with 1.5% water, silica 60, 70-230 mesh, Merck, Darmstadt, Germany) using 8 mL of n-hexane (eluate 0), 8 mL n-hexane/toluene (60:35, v/v, eluate 1) and 8 mL of toluene (eluate 2). 1 mL (samples 1998) or 0.1 mL (samples 1999) of ε-HCH solution was added to each eluate. Toxaphenes were determined in eluate 1. A whale blubber sample (QUASIMEME laboratory performance study, round 14) was used for comparison[5].

Table 1: Summary of information about mothers delivering milk samples

Sample (year)	Age [years]	Weight [kg]	Height [m]	Period of nursing	Lactation period [months]
461 (1998)	36	65	1.72	First	1.1
473 (1998)	34	68	1.66	First	4.3
481 (1998)	36	65	1.73	First	5.0
19 (1999)	32	70	1.63	Third	23.0
302 (1999)	34	65	1.78	Second	8.0
402 (1999)	33	69	1.76	First	2.7
412 (1999)	36	56	1.76	First	6.0

Instrumentation. A HP 5890II gas chromatograph/HP 5989B mass spectrometer system was employed in the negative ion chemical ionisation (NICI) mode using methane as reagent gas (1.2 mbar source pressure). The injector temperature was 200 °C and the interface temperature 250 °C. The ion source was kept at 200 °C and the quadrupole at 100 °C. Compounds were detected in the single ion monitoring mode (SIM) using the $[M\text{-}Cl]^-$ fragments. For the internal standard ε-HCH, the ions at m/z 255/257 were recorded.

HRGC capillaries. The three following capillaries were employed for enantioselective separations: Column A, 19 m length, 0.25 mm i.d., coated with 0.2 µm of 10 % heptakis(2,3,6-*O*-*tert*-butyldimethlylsilyl)-β-CD (TBDMS-CD) in OV 1701 (BGB 172, BGB-Analytik, Anwil, Switzerland); column B, 12 m length, 0.25 mm i.d., coated with 0.15 µm of 25 % octakis(2,3,6-tri-

O-ethyl)-γ-cyclodextrin (TEG-CD1) in OV-1701 (home-made[2]) and column C, 12 m length, 0.25 mm i.d., coated with 0.15 µm of 25 % octakis(2,3,6-tri-*O*-ethyl)-γ-cyclodextrin (TEG-CD2) in PS086 (home-made[2]).Separations were performed with the following temperature program: Column A, 100 °C, isothermal for 2 min, then 2.5 °C to 220 °C, isothermal for 10 min; column B and C, 100 °C, isothermal for 2 min, 30 °C/min up to 150 °C, 1 °C up to 230 °C, isothermal for 10 min.

Results and Discussion

Congener pattern. A typical enantiomer selective pattern of a human milk samples is shown in Fig. 1. The most abundant toxaphenes were #50 and #26 as typical for human samples[1,6]. In agreement with earlier studies, also #44 and #41 were present whereas #40 was missing[6]. #40 cannot be separated from #41 on commonly used achiral phases such as phenyl-methyl-polysiloxane but on TBDMS-CD. Further congeners detected were B7-1453 in some and B8-1412 in all analyzed samples, They have not been identified in human material before but are abundant in fish and marine mammals[1]. Additionally, #63 was also clearly present. #62 was not found due to its high detection limit (10-20 pg) in NICI-MS.

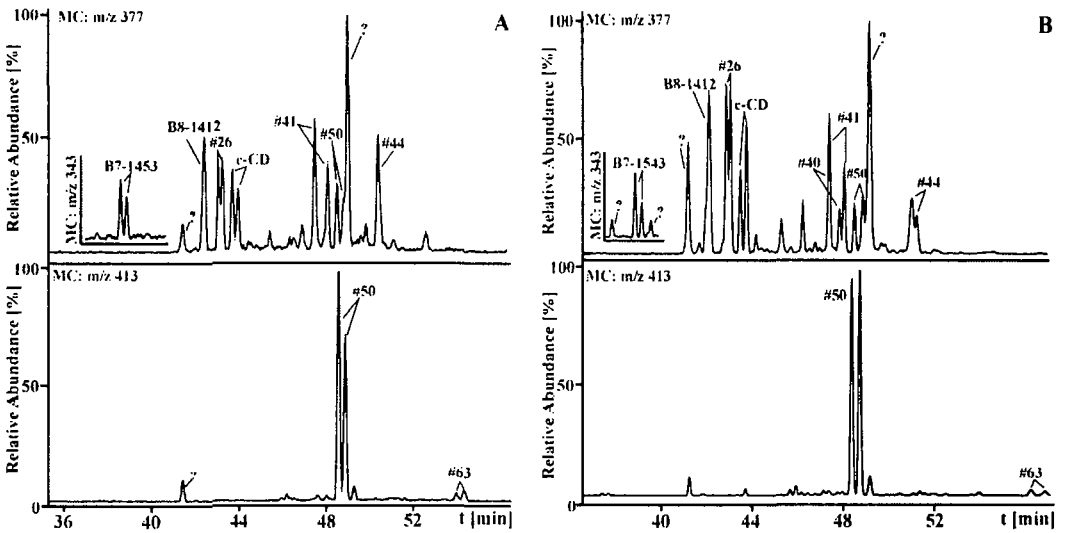

Figure 1: Enantiomer selective mass chromatograms of a human milk sample extract (no. 402, A) and a whale blubber sample (B) separated on TBDMS-CD. Identified toxaphene congeners, cis-chlordane (c-CD) and unknowns (?) are assigned.

Observed enantiomer ratios. So far, most data are available for ER of #26 and #50, which are the most stable congeners in biota including humans[1,3]. As can be seen from Table 2, deviations from racemic values are also observed in human milk. The ER for #26 was close to racemic for most of the samples, but decreased to 0.85 for pool 81 and sample no. 461. Alder et al.[3] found ER between 1.07 and 1.28 while values <1 were determined in this study. This indicates a possible reversion of enantiomer elution orders on the applied phase batches as also reported in the literature for other OC.

For #50 values varied between 1.37 (no. 412) and 1.72 (no. 302) and were in good agreement on all three phases. Since the enantiomer elution order for #50 is reversed between TBDMS-CD and TEG-CD, the ER were calculated similarly to the elution order of the TBDMS-CD. The ER found for #50 are comparable to those found in human milk and monkey adipose tissue as reported by Alder et al[3]. Moreover, the ER for B7-1453 (1.17 to 1.39), #41 (1.54 to 2.37) and #63 (0.53 to 0.87) also deviated significantly from the racemic value. Reference standards were racemic within the measuring precision (± 0.01- 0.05) except for #63 (0.87 ± 0.04). The presented results provide a further example for enantioselective transformation of chiral xenobiotics and are in line with other enantiomer selective studies of OC[3,4].

Table 2: Enantiomer ratios (ER) of B7-1453, #26, #41, #50 and #63 determined in eight human milk samples. Separations were carried out on TBDMS-CD only for B7-1453, #26, #41 and #63, and on all three phases for #50. The ER from TEG-CD were calculated similarly to the elution order of TBDMS-CD. Values for reference standards are given with their standard deviation.

Sample no.	B7-1453	#26 (B8-1413)	#41 (B8-1945)	#50 (B9-1679)	#63 (B9-2206)
Pool 81	1.35	0.85	1.98	1.46	0.77
461	1.30	0.85	2.03	1.47	0.67
473	1.28	0.97	2.37	1.45	0.71
481	1.38	0.92	2.11	1.56	0.87
19	1.39	0.97	1.64	1.47	0.62
302	1.17	n.e.r.	1.79	1.72	0.53
402	1.35	0.98	1.54	1.45	0.65
412	1.26	0.89	1.76	1.37	0.65
Reference	1.05 ± 0.08	0.97 ± 0.03	n.a. (co-el.)	1.04 ± 0.02	0.87 ± 0.04

n.a.: not analyzed, co-el.: coelutions, n.e.r.: not resolved in enantiomers

Acknowledgements

We gratefully acknowledge funding from the Swiss National Science Foundation (project no. 2000-056489.99). In addition, many thanks to Alexandra Jaus for providing the TEG-CD capillaries and Britta Kubicki and Ludger Wessel for the cleanup of the milk samples.

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