ENANTIOSELECTIVE DETERMINATION OF ATROPISOMERIC PCBs AFTER LIQUID CHROMATOGRAPHIC ENRICHMENT

Manuela Schwinge, Walter Vetter, and Bernd Luckas

Friedrich-Schiller-Universität Jena, Institut für Ernährungswissenschaften,
Dornburger Str. 25, D-07743 Jena, Germany

Introduction

Due to the different behavior of enantiomers in a chiral environment, the relationship between chirality and biological properties plays an important role in pesticide chemistry [1]. However, chirality was ignored for chloropesticides and atropisomeric PCB congeners applied in million tons in different industrial uses [2]. Present in technical mixtures as racemates, these components may undergo enantioselective biodegradation and/or bioacumulation. Chromatographic enantioseparation of chiral xenobiotics and their metabolites is a versatile tool for process studies in marine and terrestrial ecosystems [3]. Furthermore, enantioselective studies of chiral organochlorines may give deeper insights into the environmental fate of these substances in the environment.

19 of the 209 PCB congener exist as stable atropisomers [4], and the 19 standards have been enantioseparated during the last years [5-9]. However, determination of enantioratios of PCB atropisomers in environmental samples is more difficult, and comparably few results have been published so far [2]. The main problem is to avoid coelutions with other organochlorines. For this reason, multidimensional gas chromatography (MDGC) was suggested [10]. Goal of this presentation was the development of a simple method suitable for the determination of ERs of PCB atropisomers with GC/ECD and GC/MS without MDGC system.

Material and Methods

Organochlorine standards. Atropisomeric PCB 95, 132, 149, and further PCB and organochlorine standards were from Promochem, Wesel and Dr. Ehrenstorfer, Augsburg (both Germany).

Biological samples. Blubber was available from harbor seals (*Phoca vitulina*) from the German North Sea coast and grey seals (*Halichoerus grypus*) from the Baltic Sea and Iceland.

Sample clean-up. The sample matrix was either separated by acid digestion and liquid-liquid partitioning [11] or combined microwave-assisted extraction and gel-permeation chromatography [12]. After that, a PCB/toxaphene group separation was performed according to the modified method of Krock et al. [13][14]. Finally, the PCB atropisomers were enriched with the liquid chromatgraphic method below.

Liquid chromatographic enrichment of PCB Atropisomers. A mixture of 3 g Envicarb (Supelco, catalog no. 57210-U) and 3 g Celite 545-AW (Supelco, catalog no. 2-0199) was slurry packed into a 20 x 1.0 cm class column. The column was rinsed with 25 mL of n-hexane. The sample concentrate (diluted in 0.5 mL n-hexane) was placed onto the column and the sample was eluted with n-hexane/toluene (95:5, v:v). Fractions of 5 mL were separately collected and analyzed after concentration in a rotavapor and blowing down with nitrogen.

Enantioselective gas chromatography. Enantioseparations were performed on a HP 5890 series II (Hewlett-Packard) gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD). A

Gerstel KAS injection system was used for sample introduction. The injector temperature was set at 60°C, then raised at 12°C/sec to 270°C (8 min). He was used as carrier gas at a constant flow of 1.36 mL/min. The detector temperature was set at 270°C.
The GC chiral stationary phase consisted of 10% permethylated ß-cyclodextrin chemically bonded to CP-Sil 5 (ß-PMCD, CHIRASIL-DEX, Chrompack, The Netherlands). The column parameters were: 25 m length, 0.25 mm internal diameter, and 0.25 µm film thickness. The GC oven was programmed as follows: 120°C, 2 min, then 10°C to 160°C (15 min), then 10°C to 190°C (20 min), then 10°C/min to 230°C which was held for 20 min.
Peak confirmation was performed with GC/MS in the electron impact ionization mode on a HP 5971 MSD (Hewlett-Packard). Penta-, hexa-, and heptachloro-PCBs were detected with the SIM masses 324/326, 358/360, and 374/376, respectively. The 25 m x 0.25 mm i. d. fused-silica capillary column was coated with 0.25 µm ß-PMCD (see above).

GC/ECD with non-chiral stationary phases. Samples were analyzed on a HP 5890 series II GC equipped with two capillary columns and two ECDs. The capillary columns were coated with 0.25 µm CP-Sil 2 and 0.25 µm CP-Sil 8/C18. Both columns were 50 m long and had an internal diameter of 0.25 mm. Helium was used as the carrier gas and nitrogen was the make-up gas for the ECDs (detector temperatures 300°C). The samples were splitless injected (the split was closed for two min) at 250°C. After 2 min at 60°C, the GC oven was programmed at 25°C/min to 180°C (2 min), then at 2°C/min to 230°C (4 min), and at 15°C/min to 270°C (5 min).

Results and discussion

PCBs in environmental samples are complex mixtures, and atropisomers are usually much less abundant than the major congeners PCB 153 and PCB 138. Each enantioseparation adds one more peak to the chromatograms, and the determination of minor components is difficult. Furthermore, minor interferences of one enantiomer can already lead to a significant change of the ER. To overcome these problems, MDGC has been suggested [10]. However, MDGC systems were not available for us. Therefore, we developed a liquid chromatographic method for enrichment of PCBs atropisomers and separation of PCBs with less ortho-substituents which enabled enantioselective analyses of atropisomeric PCBs.
In the 1970s it was discovered that the elution of PCBs from active charcoal columns is depending on the number of ortho-substituents. This technique was widely used to isolate low abundant but very toxic non-ortho and mono-ortho PCBs. The principle of this PCB separation is that the more planar the π-system of the two phenyl rings the more the PCBs are bonded to charcoal. Mono-ortho and non-ortho PCBs are fixed on charcoal, and have to be eluted with an aromatic solvent (e. g. toluene). This process is replacing chromatography which can seen from the fact that even polar solvents do not elute the co-planar PCBs. The respective method in our institute is based on liquid chromatography with Envicarb/Celite (1:1) [15]. The Celite which was mixed with the charcoal has the function to decrease the packing of the charcoal in the column. This method was modified to obtain a fraction enriched with atropisomeric PCBs.
A pre-study with selected PCB standards showed that 6 g Envicarb/Celite (1:1) was the best compromise between consumption of chemicals and separation efficiency. Collection and analysis of 5 mL fractions revealed that atropisomers eluted after 15 mL from the column. The fraction 15-20 mL contained the bulk of the atropisomeric PCB 149, PCB 132, PCB 95, PCB 183, PCB 171,

and PCB 174 (main fraction). Lower but detectable amounts of the atropisomers eluted into the fraction from 20-25 mL (side fraction).
The main fraction (15-20 mL) contained between 50-90% of the atropisomers while (the di-ortho) PCB 153 accounted only for approximately 20% of the total PCB 153 level of the samples. Only 0-5% of PCB 138 and PCB 180 eluted into the main fraction and up to 90% of p,p´-DDE, p,p´-DDD, and p,p´-DDT were removed by the fractionating.
Note that the reported percentages were determined with real samples of a wide concentration range (e. g. PCB 149 ranged from 0.01-1.80 mg/kg and PCB 153 from 0.08-17 mg/kg). The elution profile was also a little bit depending on the level of highly concentrated organochlorines in the sample. The results obtained with standard solutions were even better. However, some variation of the elution was due to slight variances in the density of the column packing (and thereby the height of the column).
In seal blubber, the di-ortho PCB 153 which was up to ten 10 times more abundant than PCB 149 [16]. The concentration factor of PCB 149 to PCB 153 in the main fraction (15-20 mL) was 3-10. After the fractionation, PCB 149 was the 2nd to 5th most abundant compound in the ECD chromatogram of the main fraction. While the bulk of the atropisomers eluted into the main fraction (15-20 mL), clearly measurable levels (approx. 20% of PCB 149, see Table 1) were also obtained in the side fraction (20-25 mL). Enantioselective analysis of the side fraction was used to confirm the enantioratio of PCB 149 determined in the main fraction (see Table 1).

Table 1: Enantioratios of PCB 149 in the blubber of seals as determined in the main fraction (15-20 mL) and the side fraction (20-25 mL) of 6 g Envicarb/Celite (1:1)

#	Label	Sample	MAIN FRACTION		ER	SIDE FRACTION		ER
20	080888/4	p. v. NS	14743**	14370	**1.03**	3694	3957	**0.93**
18	240888/5	p. v. NS	45352	53274	**0.85**	10476	12297	**0.85**
21*	MW	h. g. BS	62734	62339	**1.01**	23510	22576	**1.04**
22	MW	h. g. BS	111566	111274	**1.00**	19510	18374	**1.06**
23	MW	h. g. BS	33162	32754	**1.01**	n. d.	n. d.	**n. d.**
24	MW	h. g. BS	85994	87047	**0.99**	n. d.	n. d.	**n. d.**
25	H67	h. g. IC	3522	3963	**0.89**	n. d.	n. d.	**n. d.**

p. v. = phoca vitulina; h. g. = halichoerus grypus; n. d. = not determined
NS = North Sea; BS = Baltic Sea; IC = Iceland;
* ER determined with EI-MS (m/z 360) = 1.00
** arbitrary counts integrated by the software

No. 21 to 24 represent the same sample (four independent sample clean-up procedures). Good agreement of the enantioratio of PCB 149 was obtained in the main fraction. Slightly higher ER were obtained in the side fraction (see Table 1). This points towards a co-elution in the side fraction since the ER of 1.00 was confirmed by GC/EI-MS (see footnote in Table 1).
The ER of PCB 149 in No. 20 varied a little bit between the two fractions. At the moment it is not clear if this is was caused by an interfering component or measuring insecurities in the side fraction due to the low PCB 149 levels. The ER values determined in the samples were in the

range of those reported earlier in seals from different marine regions [17][18]. These measurements suggest that PCB 149 is only weakly enantioenriched in the blubber of seals.
Further abundant atropisomeric PCBs in the samples were PCB 95, PCB 132, PCB 174, and PCB 183. The latter two, PCB 174 and PCB 183, were not enantioresolved on the ß-PMCD column. First results for PCB 95 in No. 22, 23, and 24 also revealed only weak if any enantioenrichment (ER = 0.99, 0.97, and 0.98). The triplicates agreed very well in the main fraction, but there was some deviation in the side fraction which points towards a coeluting component in the latter fraction. Further samples and tissues will be analyzed with the new liquid chromatographic technique.

References

1. van der Godt H, Timmerman H, Sterical Isomerism. *In*: Ariens EJ, van Rensen JJS (Eds.): Stereoselectivity of Pesticides - Biological and Chemical Problems. Elsevier Science, **1988**
2. Vetter W, Schurig V; *J. Chromatogr. A*, Review, **1997**, *774*, 143
3. Hühnerfuss H; *Organohalogen Compd*. **1998**, *35*, 319
4. Kaiser KLE; *Environ. Poll.* **1974**, *7*, 93
5. Mannschreck A, Pustet N, Robertson LW, Oesch F, Püttmann M; *Liebigs Ann. Chem.***1985**, *1985*, 2101
6. Schurig V, Glausch A; *Naturwissenschaften*, **1993**, *80*, 468
7. König WA, Gehrcke B, Runge T, Wolf C; *J. High Resol. Chromatogr.* **1993**, *16*, 376
8. Haglund P, Wiberg K; *J. High Resol. Chromatogr.* **1996**, *19*, 373
9. Vetter W , Klobes U, Luckas B, Hottinger G; *J. Chromatogr.* **1997**, *769*, 247
10. Glausch A, Nicholson GJ, Fluck M, Schurig V; *J. High Resol. Chromatogr.* **1994**, *17*, 347
11. Luckas B, Vetter W, Fischer P, Heidemann G, Plötz J; *Chemosphere* **1990**, *21*, 13
12. Vetter W, Weichbrodt M, Hummert K, Glotz D, Luckas B; *Chemosphere* **1998**, *37*, 2435
13. Krock B, Vetter W, Luckas B; *Chemosphere* **1997**, *35*, 1519
14. Klobes B, Vetter W, Luckas B, Hottinger G; *Organohalogen Compd.* **1998**, *35*, 359
15. Natzeck C, Vetter W, Luckas B, Moskopp G, Buijten J; *Chromatographia* **1995**, *41*, 585
16. Vetter W, Natzeck C, Luckas B, Heidemann G, Kiabi B, Karami M; *Chemosphere* **1995**, *30*, 1685
17. Klobes U, Vetter W, Luckas B, Skírnisson K, Plötz J; *Chemosphere* **1998**, *37*, 2497
18. Vetter W, Luckas B: Marine Organisms as Bioindicators for the Assessment of the Global Pollution with Organochlorines. *In*: Modern aspects in monitoring of environmental pollution in the sea (Ed. W. E. G. Müller). Schriften der Akademie gemeinnütziger Wissenschaften zu Erfurt, Mathematisch-Naturwissenschaftliche Klasse, Sitzungsberichte **1998**, *8*, 159