ENANTIOMERIC SEPARATION OF CHIRAL METHYLSULPHONYL PCB CONGENERS IN LIVER AND ADIPOSE TISSUE FROM RATS DOSED WITH A50:

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Introduction

Methylsulphonyl substituted polychlorinated biphenyls ($MeSO_2$-PCBs) are one type of PCB metabolites (1) which are formed via the mercapturic acid pathway from reactions of glutathion with an aren oxide intermediate of PCB (2).
Methylsulphonyl-PCBs were first identified in the blubber from Baltic grey seal in 1976 (3). Since then, $MeSO_2$-PCBs have been identified in fish, birds, mammals and humans (4). The retention of $MeSO_2$-PCBs in tissue is related to their physico-chemical properties as well as to their capacity to bind to proteins in the body, e.g. in liver and lung (5,6).

It has been shown that PCBs as well as $MeSO_2$-PCBs with a non- symmetrical substitution pattern of the chlorine atoms on both phenyl rings and with at least three chlorine atoms in the *ortho*-positions can form stable PCB rotational enantiomers, or atropisomers. The atropisomers were shown to consist of as much as 20% of the total $MeSO_2$-PCBs concentration in the fat of ringed seal and polar bear and in human liver (7,8).

Enantioselective analysis of PCBs in biota has shown enantiomeric ratios similar to those of technical products (racemic) as well as enantiomeric excess (9-12). While high enantiomeric excess has been shown for 3'-$MeSO_2$-CB132 and 3-$MeSO_2$-CB149 in human liver (8), and 3- and 4- $MeSO_2$-CB91 and 3-$MeSO_2$-CB149 in polar bear fat.

The aim of the present study was *i)* to look for changes in the congener pattern of chiral $MeSO_2$-PCBs and to determine enantiomeric ratios over time, *ii*) to look for similarities or differences in the enantiomeric ratios in different tissues in rats dosed with a technical mixture of PCB.

Material and methods

Chemicals: 3-$MeSO_2$-2,2',4',5,6-pentaCB (3-$MeSO_2$-CB91), 4-$MeSO_2$-2,2',3,4',6-pentaCB (4-$MeSO_2$-CB91), 3'-$MeSO_2$-2,2',3,4,5',6'-hexaCB (3'-$MeSO_2$-CB132), 4'-$MeSO_2$-2,2',3,4,5',6'-hexaCB (4'-$MeSO_2$-CB132), 3-$MeSO_2$-2,2',4',5,5',6-hexaCB (3-$MeSO_2$-CB149), 4-$MeSO_2$-2,2',3,4',5',6-hexaCB (4-$MeSO_2$-CB149) were used as analytical standards (13).

Analysis: Quantitative analysis of the tissue samples were performed on a Varian GC 3400 under the same conditions as described elsewhere (14). The enantioselective gas chromatography/mass spectrometry analysis were carried out under same conditions as described in detail by Ellerichmann and co-workers (8), with the exception of an alternative cyclodextrin column (15 m, i.d. 0.25 mm, film thickness not measured, 6-Tx-2,3-Me-Beta-Cd/80% SE 52).
Samples: Rats were given one single, oral dose of a commercial PCB product (Chlophen A50) dissolved in corn oil (25 mg/kg b.w.). The rats were sacrificed after 1, 2, 4 and 8 weeks and liver, lung, kidney, adrenal and fat were removed and kept frozen untill the analysis.
In the present study livers and fat were worked up as described in detail elsewhere (15).

Results and discussion

In all samples analysed the concentrations of the 4-$MeSO_2$-CB91 and 4'-$MeSO_2$-CB132 are slightly higher than those of the 3-$MeSO_2$-PCB isomers in fat and liver. Still the ratio 4-$MeSO_2$-PCBs/3-$MeSO_2$-PCBs is almost 1:1 for these $MeSO_2$-PCB congeners in both liver and fat. However 3-/4-$MeSO_2$-CB149 seems to differ from the others with a concentration ratio of at least 1:2 in both fat and liver.

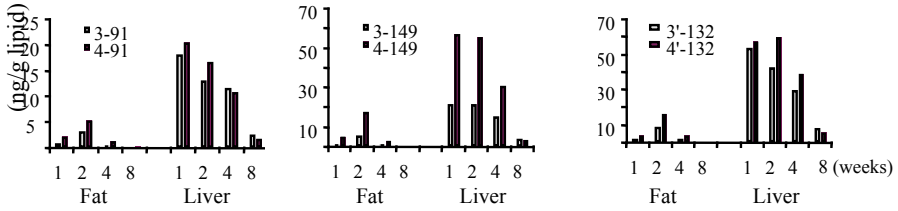

Fig 1: The concentration ratios of each chiral 3-/4-$MeSO_2$-PCB congener from the different times in fat and liver from rat.

On the cyclodextrin column used we have been able to separate the enantiomeric pairs of 4-$MeSO_2$-CB91, 3'-$MeSO_2$-CB132, 4'-$MeSO_2$-CB132, 3-$MeSO_2$-CB149, 4-$MeSO_2$-CB149. With the exception of 3'-$MeSO_2$-CB132, baseline separation was received for all enantiomeric pairs. It was not possible to separate 3-$MeSO_2$-CB91into its two enantiomers.

For PCB enantioselective analysis of PCB in environmental samples, the enantiomeric ratios are usually racemic, or close to racemates, as in the technical products (11-14). However for enantioselective analysis of $MeSO_2$-PCBs high enantiomeric excess have been found (7,8). This may not be surprising since $MeSO_2$-PCBs are formed in a possibly enantioselective enzymatic process in the body.

In the enantiomeric separations of fat and liver no differences between the tissues were found. Neither did the enantiomeric ratios change over time.

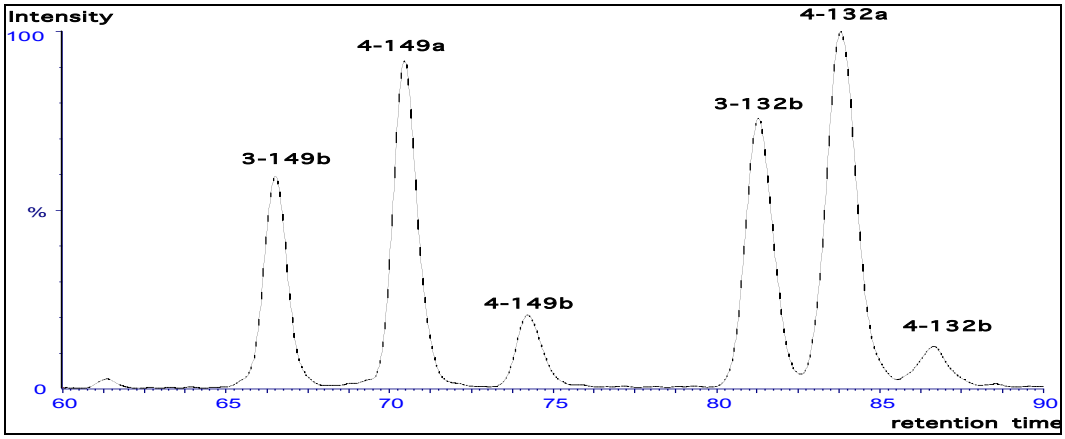

Fig 2: Enantioselective separation of $MeSO_2$-hexaPCBs in a rat liver sample, 2 weeks after a single dose of the commercial PCB-mixture, Chlophen A50.

In all the analysed samples only the second eluting enantiomer of 3'-$MeSO_2$-CB132 and 3-$MeSO_2$-CB149 were present. In contrast 4-$MeSO_2$-CB91, 4'-$MeSO_2$-CB132 and 4-$MeSO_2$-CB149 were dominated by the first enantiomer, although minor amounts of the second enantiomers were found. The enantiomeric ratios (the quotient a/b) for 4-$MeSO_2$-CB91 were in the range of 7-11, for 4-$MeSO_2$-CB149 2-8 and for 3'-$MeSO_2$-CB132 6-13. This indicates that either both enantiomers are formed or, if only one enantiomer is formed, that enantiomer is converted to both the optical forms. This is true at least for the 4- $MeSO_2$-PCBs. For the 3- $MeSO_2$-PCBs there were no indications of another enantiomer. Also enantiselective transport prosesses are possible.

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References

1. Preston PD and Miller EC, *Chem Biol Interact* ,**1984,** 50, 121-123
2. Bakke JE and Gustafsson JÅ , *Trends in Pharmacol. Sci.*, **1984**, 5, 517-521
3. Jensen S and Jansson B *Ambio,* **1976**, 5, 257
4. Letcher RJ, Klasson Wehler E and Bergman Å, **1999**, *The Handbook of Environmental Chemistry*, in press.
5. Lund J, Brandt I, Poellinger L, Bergman Å, Klasson Wehler E and Gustafsson JÅ, *Mol. Pharmacol.* **1985,** 27, 314-323
6. Larsen G.L., Bergman, Å., Klasson Wehler, E. and Bass, N.M., *Chem. Biol. Interactions*, **1991**, 77, 315-323.
7. Wiberg K, Letcher R, Sandau C, Duffe J, Norstrom R, Haglund P and Bidleman T, *Anal. Chem*, **1998**, 70, 3845-3852
8. Ellerichmann T, Bergman Å, Franke S, Hühnerfuss H, Jakobsson E, König WA and Larsson C, *Fresenius Envir Bull*, **1998,** 7, 244-257

9. Glauch A, Nicholson GJ, Fluck M, Schurig V, *J. High Resolut. Chromatogr.*, **1994**, 17, 347-349.
10. Glauch A, Hahn J, Schurig V., *Chemosphere*, **1995**, 30, 2079-2085.
11. Blanch GP, Glauch A, Schurig V, Serrano R, Gonzales MJ, *J. High Resolut. Chromatogr.*, **1996**, 19, 392-396.
12. Klobes U, Vetter W, Luckas B, Skirnisson K and Plötz J, *Chemosphere*, , 37, 2501-2512.
13. Haraguchi K, Kuroki H, Masuda Y, *J. Agricultural food chem.*, **1987**, 35, 178-182.
14. Haraguchi K, Athanasiadou M, Bergman Å, Hovander L, Jensen J, *Ambio*, **1992**, 21, 570-576.
15. Larsson C *et al*., manuscript in preparation.