ENANTIOSELECTIVE TRANSFORMATION OF ATROPISOMERIC PCBs OR OF THEIR METHYLSULFONYL METABOLITES BY RAT HEPATOCYTES ?

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Introduction

Approximately ten years after the discovery of PCBs in the environment [1], Jensen and Jansson reported on the identification of methylsulfonyl-PCBs ($MeSO_2$-PCBs) in Baltic Grey seal blubber [2]. Over the years thereafter, $MeSO_2$-PCBs have been detected in fish, birds and mammals including humans [3]. Some of the $MeSO_2$-PCBs in biota have also been observed to be selectively and strongly retained in lung and liver tissue of mammals including man [3]. The $MeSO_2$-PCBs formed are persistent and only slightly less hydrophobic than their parent compounds which make them long lasting contaminants in the biosphere. From a toxicological point of view several of the 3-$MeSO_2$-PCBs have been shown by Kato and co-workers to induce P-450 cytochrome enzymes such as P450 2B1, 2B2, 3A2 and 2C6 [4,5]. As a consequence, it is tentatively assumed that a part of the toxic effects induced by PCBs in the environment may be subject to the presence of these PCB metabolites. Furthermore, the main metabolites mentioned above are chiral and, accordingly, enantioselective transformation as well as differential toxic effects of the enantiomers have been postulated [6]. A verification of this conjecture, however, requires a systematic and comprehensive study including enantioselective separation of $MeSO_2$-PCB enantiomers, determination of the absolute structures of the separated enantiomers, transformation experiments with the parent compounds and the metabolites as well as systematic investigations of differential toxic effects of the $MeSO_2$-PCB enantiomers. First results of this ongoing research work have been recently reported [7-9]. Additional information and references can be found in Ph.D. theses by Ellerichmann [10], Wiberg [11], Larsson [12], and Peters [13].

The present study aimed at gaining deepened insight into the transformation of PCB parent compounds into methylsulfonyl PCBs by rat hepatocytes. As an example, first results will be presented that were obtained after incubation of rat hepatocytes with parent PCB 149 racemates and with the racemate of one of its potential metabolites, 3-$MeSO_2$-2,2´,4´,5,5´,6-hexachlorobiphenyl (abbreviated 3-149; see [14]), respectively. Thus the question can be answered as to whether the dramatic enantiomeric excess of the second eluting *R*-enantiomer found in rat liver extracts [7,12] is caused by enantioselective transformation of the atropisomeric parent PCB 149 or, alternatively, by subsequent enantioselective transformation of the metabolite 3-149, which is chiral as well (Fig. 1).

Experimental

The preparation of the methylsulfonyl-PCB standard 3-149 (Fig. 1) was published elsewhere [15,16]. The PCB 149 enantiomers were separated by GC-ECD using a chiral stationary phase

consisting of heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-*β*-cyclodextrin (2,3-Me-6-TBDMS-*β*-CD; 50 % in OV 1701; film thickness 0.1 µm; column diameter 0.25 mm; length 25 m), while the enantioselective separation of the metabolite 3-149 was achieved by application of a 2,3-Me-6-TBDMS-*β*-CD column in 20 % SE 52 (film thickness 0.1 µm; column diameter 0.25 mm; length 15 m). In both cases, a gas chromatograph GC 6000 Vega (Carlo Erba) was used equipped with a ^{63}Ni- ECD detector (Carlo Erba); the other experimental parameters comprised: basis temperature 558 K; make-up gas N_2 (150 kPa); carrier gas helium (150 kPa); on column injection; temperature programme for the separation of the PCB 149 enantiomers: 343 K/2 min - 40 K/min - 443 K/70 min; temperature programme for the separation of the 3-149 enantiomers: 343 K/2 min - 20 K/min - 433 K/25 min -1,2 K/min - 507 K -1,0 K/min - 518 K/30 min.

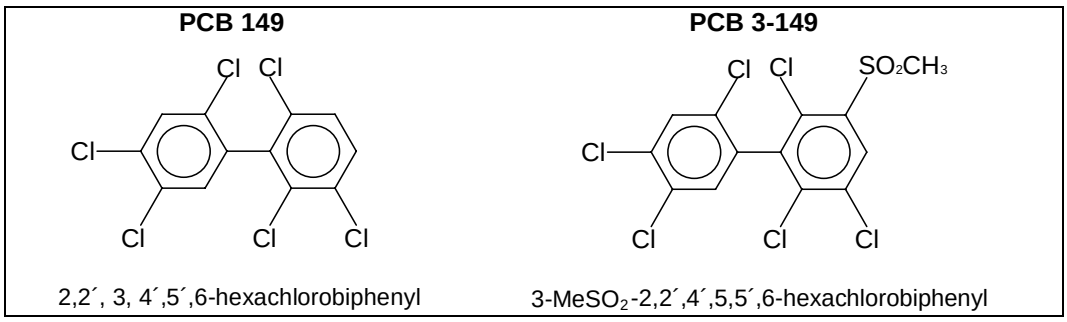

Figure 1: Structures of PCB 149 and its metabolite 3-MeSO_2-2,2´,4´,5,5´,6-hexachlorobiphenyl investigated in this work

The hepatocytes were prepared from male Wistar rats according to known procedures [13]. Thereafter, 10^7 living cells (5 x 10^5/mL) were transferred into multi-chamber Petri dishes. For each concentration of the test solution two dishes were applied, one of which containing PCB 149 and the metabolite 3-149, respectively, plus hepatocytes, while the second dish contained one of the two test compounds, however, no hepatocytes. In parallel, a blank control (negative control) including the solvents only and a positive control (containing 2-acetyl-aminofluoren) were included in the tests. 1 mL of a DMSO solution (2 x 10^{-3} mol/L) of PCB 149 and its transformation product 3-149, respectively, were added followed by an incubation period of 12 h. The reaction mixture was extracted by 150 mL of a *n*-hexane/acetone mixture (2:1) in a Soxhlet apparatus, the extract was condensed to 20 mL, followed by a change of the solvent (to *n*-hexane) and condensation to about 1 mL, which was analyzed by GC/ECD as described above.

Results and discussion

The GC-ECD chromatograms obtained by analyses of the PCB 149 test series carried out in the presence and the absence of hepatocytes is shown in Figure 2, while the respective results for the transformation product 3-149 is displayed in Figure 3. Several clear conclusions can be drawn:

- Rat hepatocytes are able to transform the two PCB 149 enantiomers with comparable velocities thus giving rise to a nearly racemic metabolite 3-149;
- The subsequent further transformation of the two 3-149 enantiomers, as induced by rat hepatocytes, occurs with significantly different velocities; while the concentration of the first eluting *S*-enantiomer is significantly reduced, the second eluting *R*-enantiomer remains nearly unaffected after an incubation period of 12 hours.

- qualitatively, the laboratory *in vitro* results for the transformation of the metabolite 3-149 are in accordance with the enantiomeric excesses determined in rat tissues, after exposure of the animals to a technical PCB product, Clophen A50 (see Table 1; [7]).

In conclusion, the results obtained in this study suggest that the enantiomeric excesses of the metabolite 3-149 observed in *in vivo* experiments with rats [7] are caused by the following transformation process: the parent compound PCB149 is being transformed to 3-149 nearly racemically, followed by a highly enantioselective preferential transformation of the *S*-3-149; in contrast, *R*-3-149 is being transformed significantly slower and thus enriched, though in different rat tissues in different concentrations.

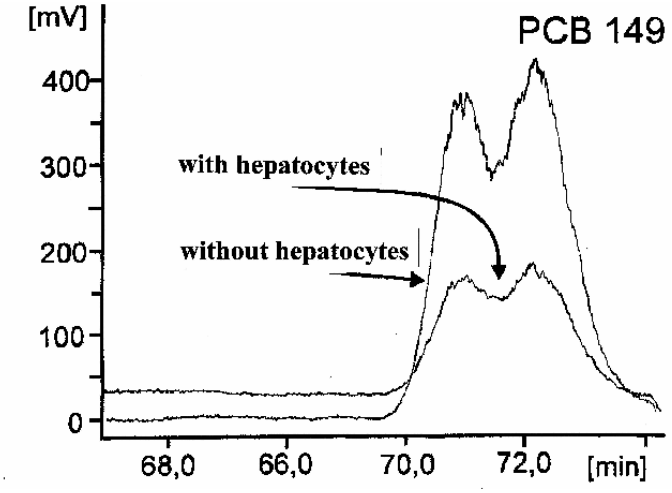

Figure 2: PCB 149 with and without hepatocytes after 12 h incubation: chiral 2,3-Me-6-TBDMS-β-CD column (50 % in OV 1701; see Experimental part)

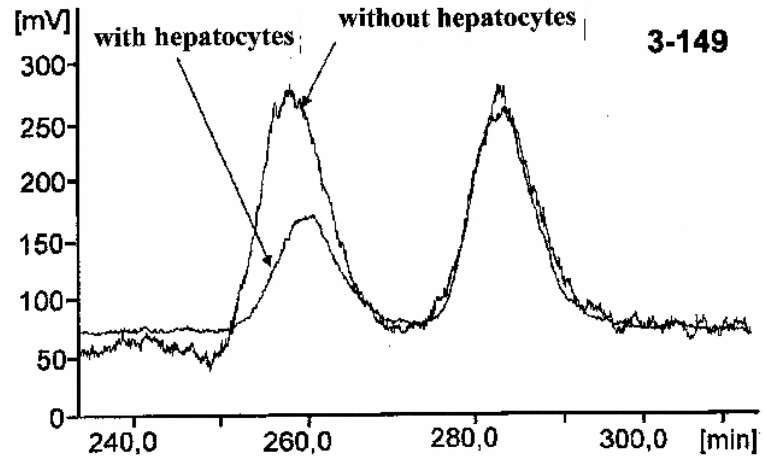

Figure 3: The metabolite 3-149 with and without hepatocytes after 12 h incubation: chiral 2,3-Me-6-TBDMS-β-CD column (20% in SE 52; see Experimental part)

Table 1: Mean values of enantiomeric fractions, i.e., first eluting versus the sum of both enantiomers of 3-149 in adipose, liver and lung of rat tissue extracts after exposure of the animals to a technical PCB product, Clophen A50; from [7]

PCB metabolite	Enantiomeric fractions		
	adipose	liver	lung
3-$MeSO_2$-CB149	0.04	< 0.01	0.43

Acknowledgements
This work could only be accomplished by a grant of the German Academic Exchange Service (DAAD) through the *International Quality Net (IQN) Programme.* This is gratefully acknowledged.

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