Chiral PCB methyl sulfones and their metabolic formation

Norström K, Bergman Å
Department of Environmental Chemistry, Stockholm University, SE-10691 Stockholm, Sweden

Introduction

The PCBs and their methylsulfonyl metabolites ($MeSO_2$-PCBs) with asymmetric substitution of both phenyl rings are chiral. Those congeners with three or four chlorines in *ortho* position are stable due to hindered rotation around the C-C bond and exist, even at elevated temperatures, as individual atropisomers (enantiomers). The two enantiomers of a chiral compound are optically active and are designated (+) and (-) enantiomers to indicate the clockwise and counter-clockwise rotation of plane-polarized light, respectively. Apart from this, the enantiomers have identical physical and chemical properties. If the absolute configuration is known, *R* and *S* designations are used, depending of the arrangement of the atoms or substituents of a molecule in space. A new aspect of environmental pollutants became of interest when enantiomers could be separated from each other by modified cyclodextrin as a stationary GC or HPLC phase[1].

Twelve of the chiral PCBs (CB-45, 84, 91, 95, 132, 135, 136, 144, 149, 171, 174 and 183) have been detected in commercial PCB mixtures at levels greater than 1% (w/w)[2] and about half of them are found in biota[3-6]. Technical PCBs are released into the environment as racemates but this relationship between the atropisomers alters when they are subjected to biological systems. The alteration of the chiral composition is often found to be greater further up in the food chain. PCBs undergo Cytochrome P450 mediated metabolism forming both polychlorobiphenylols and methylsulfonyl-PCBs after multistep transformations subsequent to arene oxide formation[7]. The methylsulfonyl-PCBs were first reported thirty years ago, being retained in seal blubber[8], specifically retained in lung tissue[9,10] and as metabolites of CB-52[11]. After intensive synthesis of authentic reference $MeSO_2$-PCB standards by Japanese and Swedish researchers in the 1980s it was possible to identify a large number of $MeSO_2$-PCB congeners in different species of seals, polar bears and otters[7,12].

Ten chiral $MeSO_2$-PCBs have been reported from biota and they constitute a great part of $\Sigma MeSO_2$-PCBs in several species[13,14]. Primarily one atropisomer of the $MeSO_2$-PCB atropisomer pair is dominating in mammals (Table 1). In birds, both atropisomers of a chiral $MeSO_2$-PCB are present at a relative higher degree even if one of them is dominating (Table 1). It has been shown that if the $MeSO_2$-group is in *para*-position, it is the first eluting atropisomer on a chiral GC column that is enriched. For *meta*-substituted congeners, it is the second eluting atropisomer that is enriched. In grey seal tissues and in guillemot eggs, all the enriched atropisomers were shown to have an absolute configuration of *R*[12].

The aim of the present study was to experimentally study if the strong dominance of one atropisomer of a chiral $MeSO_2$-PCB congener is due to enantioselective formation or retention in rats. 2,2',3,3',4,6'-Hexachlorobiphenyl (CB-132) was selected for the study and was administered to rats both as individual atropisomers and as racemate.

Materials and methods

Isolation of pure CB-132 atropisomers: Crystalline CB-132 was dissolved in acetonitrile containing 8% water. Aliquots were separated by HPLC and fractions of the two atropisomers were collected. The first atropisomer (CB-132:A1) eluted after 9.54 minutes and the second (CB-132:A2) after 10.34 minutes. The purity of CB-132:A1 and CB-132:A2 was 99.4% and 99.2%, respectively. Other chemicals used for the study are presented elsewhere[12]. Instrumentation and methodologies applied are presented in full detail in the thesis of Norström[12].

Animals and treatment: The present study was performed according to the approved IRB 363/03 and 20/04 protocols. Racemic CB-132, CB-132:A1 and CB-132:A2 were dissolved in diethyl ether and mixed with peanut oil. The mixtures were kept at 40°C and stirred over night under a gentle flow of nitrogen for evaporation of the diethyl ether. Twelve male Wistar rats (200 g) (Scanbur BK, Sollentuna, Sweden) were used in the present study. They were kept in cages with free access to pellet diet and water and were allowed to acclimatize during 5 days prior to dosing. Each of the substances (0.2 mg/atropisomer/rat) were given to a group of four rats at a single occasion. After four days the rats were sacrificed using carbon dioxide and tissues for analysis were removed.

Chemical analysis: Liver (5 g), lung (1-2.5 g) samples and faeces were homogenized in 50 mL Falcon® test tubes and extracted[15]. Adipose tissue (1 g) was homogenized in test tubes. The solvent volumes described originally[15] were reduced and adjusted for each sample size. After each extraction the tubes were centrifuged and the organic solvent transferred to a glass funnel containing phosphoric acid (25 mL, 0.1 M) in an aqueous 0.9% sodium chloride solution. The sample lipid weights were performed gravimetrically. The extracted lipids volumes were reduced by partitioning[16]. The samples were subsequently dissolved in hexane (3 mL) and CB-132 and its methylsulfonyl metabolites were separated from each other by sulfuric acid partitioning and further cleaned up by different sulfuric acid impregnated silica gel columns (1 g)[17]. The fraction containing methylsulfonyl-CB132 metabolites were cleaned up on an extra silica gel column impregnated with diluted sulfuric acid (0.5 g) with dichloromethane (15 mL) as the mobile phase. CB-189 and 5'-$MeSO_2$-4'-Me-CB106 were used as surrogate standards. Enantioselective analyses of CB-132 and the metabolites 5'-$MeSO_2$-CB132 and 4'-$MeSO_2$-CB132 were performed by GC-ECD equipped with a CP Chirasil-Dex column and a column coated with a mixture of OV1701 and heptakis (2,3-di-*O*-Me-6-*O*-*t*-hexyl)-*β*-CD (1:1), respectively.

Results and discussion

It was shown that one of the atropisomers of CB-132 had a higher rate of clearance compared to the other. The overall results of the study are presented in Figure 1. The enantiomeric fractions of CB-132, 5'-$MeSO_2$-CB132 and 4'-$MeSO_2$-CB132 in tissues and faeces of rats dosed with racemic CB-132 are presented in Table 2. The metabolites formed were only of the absolute configuration *R*. The results suggest that an enantioselective metabolism occurs of the parent compound. The detection of one atropisomer of CB-132 in lower concentration than the other indicates that the enantioselectivity in the metabolism occurs in the initial metabolising step when an arene epoxide is formed by the cytochrome P450 system. The absolute structures of the PCB atropisomers are not known but it seem reasonable to believe that the absolute structures of the atropisomers of CB-132 are the same as their metabolites formed.

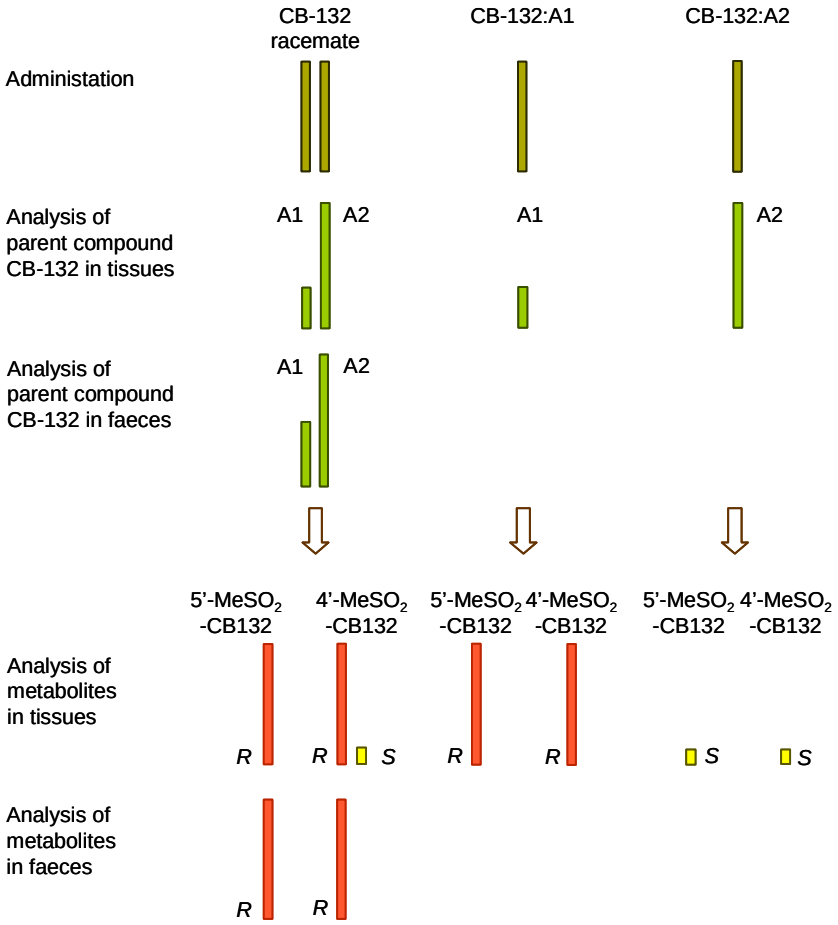

Figure 1. Experimental study of enantioselective metabolism in rats dosed with racemic CB-132 and the pure atropisomers, CB-132:1 and CB-132:2, respectively. The bars show the relative presence of the atropisomers of CB-132, 5'- and 4'-$MeSO_2$-CB132 in the tissues and faeces. *R* and *S* are the absolute configurations of the metabolites formed in each group of rats.

Table 1. Enantiomeric fractions (EF=$A_1/(A_1+A_2)$) of atropisomeric $MeSO_2$-PCBs reported from wildlife and humans. References to the scientific reports are given in the table.

Species	Tissue	4-$MeSO_2$-CB91	5-$MeSO_2$-CB149	4-$MeSO_2$-CB149	5'-$MeSO_2$-CB132	4'-$MeSO_2$-CB132	5'-$MeSO_2$-CB174	4'-$MeSO_2$-CB174
Bird								
Guillemot[17]	egg	0.67	0.41	0.52	-	0.65	0.27	0.56
[a]Pelican[18]	muscle	-	0.43	0.62	-	0.68	-	-
Mammal								
Grey seal[13]	blubber	0.94-0.97	0	0.99-1.0	0.032-0.090	0.98-0.99	<0.010-0.024	0.99-1.0
	liver	1	0	0.99-1.0	0	0.97-0.99	<0.010	n.d.
	lung	0.96-0.98	0	0.99-1.0	0.023-0.14	0.98-0.99	<0.010-0.040	0.99-1.0
[a]Arctic ringed seal[14]	blubber	~0.8	0.24	-	-	~0.8	-	-
[a]Seal[18]	blubber	0.76	0.26	0.53	-	0.79	-	-
Harbour porpoise[19]	liver	-	<0.02-0.20	0.95->0.99	0.06-0.20	-	0.10-0.23	>0.73-0.89
[a]Arctic polar bear[14]	adipose	>0.9	0.09	-	-	>0.9	-	-
Human[20]	liver	-	0	-	0	-	-	-
Laboratory animals								
Wistar rat[12]	adipose	-	-	-	0	0.95	-	-
	liver	-	-	-	0	0.95	-	-
	lung	-	-	-	0	0.93	-	-
Sprauge-dawley rat[21]	adipose	0.77	0.04	0.66	-	0.95	-	-
	liver	0.91	0.01	0.82	-	0.91	-	-
	lung	0.61	0.43	0.24	-	0.91	-	-

a) The originally reported ERs have been converted to EFs using the relationship EF=ER/ (1+ER)

Table 2. Enantiomeric fractions (EF) of 2,2',3,3',4,6'-hexaCB, 3-$MeSO_2$-2,2',3',4',5,6-hexaCB and 4-$MeSO_2$-2,2',3,3',4',6-hexaCB in tissues and faeces of rats dosed with racemic CB-132.

	EF *(range)*		
	$A_1/(A_1+A_2)$	$A_R/(A_R+A_S)$	$A_R/(A_R+A_S)$
Tissue	CB-132	5'-$MeSO_2$-CB132	4'-$MeSO_2$-CB132
Liver	0.18[a]	1	0.95
	(0.17-0.19)		*(0.94-0.96)*
Adipose	0.19	1	0.95
	(0.17-0.21)		*(0.95-0.96)*
Lung	0.17	1	0.93
	(0.16-0.18)		*(0.93-0.93)*
Faeces[b]	0.43; 0.41	1	1

n=3 for all EFs except [a] where n=4 and [b] which consists of pooled faeces from four rats collected at day four.

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