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Methylsulfonyl PCB and DDE metabolites and their enantioselective gas chromatographic separation in human adipose tissues, seal blubber and pelican muscle

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Abstract

In the present investigation, eleven human adipose tissue samples, two seal blubber samples and two pelican muscles samples were analyzed with regard to their concentrations of PCB parent compounds as well as to the respective chiral methylsulfonyl metabolites 3-$MeSO_2$-CB 91, 4-$MeSO_2$-CB 91, 3-$MeSO_2$-CB 95, 4-$MeSO_2$-CB 95, 3-$MeSO_2$-CB 149, 4-$MeSO_2$-CB 149, 3-$MeSO_2$-CB 132, 4-$MeSO_2$-CB 132, 3-$MeSO_2$-CB 174, and 4-$MeSO_2$-CB 174 and the achiral metabolites 3-$MeSO_2$-CB 49, 4-$MeSO_2$-CB 49, 3-$MeSO_2$-CB 101, 4-$MeSO_2$-CB 101, 3-$MeSO_2$-CB 110, 4-$MeSO_2$-CB 110 and 3-$MeSO_2$-DDE. In order to verify enantioselective transformation processes and to compare the different enzymatic transformation pathways in birds and mammals, the enantioselective excesses of the chiral PCB-metabolites were determined by enantioselective gas chromatography with electron capture and mass spectrometric detection using modified cyclodextrin phases, including heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl-)-β-cyclodextrin/OV1701 (1:1) for the parent PCBs and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl-)-β-cyclodextrin/SE52 (1:4) for the metabolites, respectively.

Keywords: PCB; Methyl sulfones; Metabolites; Enantioselective gas chromatography; Adipose tissue

1. Introduction

Methyl sulfone derivatives are known to represent primary metabolic products of PCBs ($MeSO_2$-CB) and DDE ($MeSO_2$-DDE). These metabolites are formed via the mercapturic acid pathway (Bakke et al., 1982; Klasson-Wehler et al., 1987; Bakke et al., 1995) and belong to the group of persistent, lipophilic compounds which accumulate in the adipose, lung, liver and kidney tissues of mammals exposed to PCBs. In Jensen and Jansson (1976) reported the identification of PCB methyl sulfones as metabolites of PCBs in Baltic grey seal blubber. Methyl sulfones are moderately polar compounds that are only slightly less hydrophobic than the parent PCBs, and their partition coefficients fulfill the requirements for bioaccumulation through the food chain. The highest concentrations have been found in kidney and lung tissues of seals, otters, beluga whales, polar bears, fishes and in human tissues (Haraguchi et al., 1986; Bergman et al., 1994; Janák et al., 1998). From a toxicological point of view some of the $MeSO_2$-PCBs have been shown by Kato and coworkers to induce strongly P-450 cytochrome enzymes (Kato et al., 1995; Kato et al., 1997). The strong localization of $MeSO_2$-PCBs to the lungs has been discussed in relation to the respiratory distress observed among Yusho patients in Japan (Nakanishi et al., 1985).

Similar to their parent compounds, PCB metabolites may exhibit axial chirality if both phenyl rings possess an asymmetric chlorine substitution pattern. Seventy-eight out of 209 tri- and tetra-*ortho*-chlorine-substituted PCB congeners

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theoretically show hindered rotation around the phenyl–phenyl bond, which gives rise to axial chirality, and nineteen of the chiral PCBs are predicted to form stable atropisomers under most environmental conditions (Kaiser, 1974), at least 12 of which (PCBs 45, 84, 88, 91, 95, 132, 136, 144, 149, 171, 174, and 183) have been detected in commercial PCB mixtures above 1% (w/w) (Frame et al., 1996). Since the introduction of a methylsulfonyl group at the 2- or 3-position will add an additional element of asymmetry, these metabolites may be chiral even if the parent PCB is not. From a large number of theoretically possible PCB metabolites, only about half of them are chiral (Nezel et al., 1997). Of these congeners, only a fraction may be environmentally stable due to tri- or tetra-*ortho* substitution. PCB enantiomers can be separated on derivatised cyclodextrin based chromatographic columns (Hardt et al., 1994). The relevance of enantioselective analysis is supported by studies in which atropisomers of PCBs have been shown to exhibit a differential potency to induce several xenobiotic-metabolizing enzymes or accumulate uroporphyrin (Püttmann et al., 1990). It may be assumed that the atropisomers of metabolites possess similar characteristics.

The first to separate methylsulfonyl enantiomers were Ellerichmann et al. (1998), while the absolute structures of some particularly relevant derivatives were determined by Döbler et al. (2002) and Pham-Tuan et al. (2005). A comparative study of the separation of stable methylsulfonyl enantiomers on four different CD capillary GC columns was reported by Wiberg et al. (1998). Enantiomeric excesses of several atropisomeric methylsulfonyl PCBs have been found in different human, rat, whale tissue (Letcher et al., 1995; Larsson et al., 1999, 2002; Hühnerfuss et al., 2003) and in Baltic Guillemot egg samples (Jörundsdóttir et al., 2006). Some of the congeners are exclusively present in one enantiomeric form only. This is in contrast with the chiral PCB parents determined so far in biota. This proved that the atropisomers of parent PCBs are subjected to an enantioselective transformation and possibly, in addition, to an enantioselective transformation of their methylsulfonyl metabolites (Hühnerfuss et al., 2003).

Atropisomeric PCBs and $MeSO_2$-PCBs are assumed to represent good chiral indicators, because many enzymatic systems can recognize these activating substrates enantioselectively. According to the numerous possible substrates, it is necessary that they possess highly selective recognizing mechanisms, in order to allow the proper substrate only to bind to the receptor of the enzyme. As atropisomeric $MeSO_2$-PCBs can exert biomimetic effects on the glucocorticoid receptor, they are predestined for studies about their metabolisation- and effect-mechanisms in the living organism.

2. Materials and Methods

2.1. Samples

Human adipose tissue samples were obtained from the Department of Pathology, Faculty Hospital of Charles University Prague. Seal blubber samples and pelican muscles were obtained from Zoo Prague and Zoo Ústí nad Labem, both seals died after escape during flooding in 2002.

2.2. Chemicals

PCB parent compounds were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Their chiral methylsulfonyl metabolites 3-$MeSO_2$-CB 91, 4-$MeSO_2$-CB 91, 3-$MeSO_2$-CB 95, 4-$MeSO_2$-CB 95, 3-$MeSO_2$-CB 149, 4-$MeSO_2$-CB 149, 3-$MeSO_2$-CB 132, 4-$MeSO_2$-CB 132, 3-$MeSO_2$-CB 174, and 4-$MeSO_2$-CB 174 and the achiral metabolites 3-$MeSO_2$-CB 49, 4-$MeSO_2$-CB 49, 3-$MeSO_2$-CB 101, 4-$MeSO_2$-CB 101, 3-$MeSO_2$-CB 110, 4-$MeSO_2$-CB 110 and 3-$MeSO_2$-DDE were purchased from Cambridge Isotope Laboratories (USA).

2.3. Sample preparation

Letcher et al. (1995) developed an integrated analytical method for methylsulfonyl metabolites of PCBs in biological tissues, based on liquid–liquid extraction, GPC fractionation and adsorption chromatographic clean-up. Homogenized samples were dehydrated by grinding with sodium sulphate. Isolation and sample preparation were performed by extraction with *n*-hexane:acetone (2:1, v/v). Enrichment and cleanup procedure involved gel permeation chromatography using Bio Beads S-X3 gel and adsorption chromatography on 33% KOH/silica gel, Florisil, and 2.3% H_2O deactivated basic alumina columns. The GPC elution curves of PCB methyl sulfones are similar to the curves of parent PCB congeners when using Bio Beads S-X3 gel.

2.4. Determination by gas chromatography–mass spectrometry

$MeSO_2$-CB were determined in biological samples by gas chromatography with electron capture detection using simultaneous injection in splitless mode onto two capillary columns with different polarity of stationary phases, DB–5 (5% diphenyl–95% dimethyl polysiloxane) and DB–17 (50% diphenyl–50% dimethyl polysiloxane) both with the same parameters (60 m, 0.25 mm, 0.25 μm film thickness) installed in a HP 5890 series II gas chromatograph with electronic pressure control, dual automatic liquid sampler and two electron capture detectors. The oven temperature was programmed from 80 °C (splitless period, 2.5 min) to 220 °C at a rate of 30 °C/min and then 2–280 °C, held for 30 min.

Due to the possibility of false positive results when using ECD detection, all the results were confirmed by GC/MS operated in negative chemical ionization mode using an Agilent 6890 N gas chromatograph with 5973 N mass selective detector. Separation was performed on a HP-5MS fused silica capillary column (5% diphenyl–95%

dimethyl polysiloxane, 30 m, 0,25 mm, 0.25 μm) and the oven temperature was programmed from 90 °C (splitless, period 2 min) to 230 °C at a rate of 25 °C/min and then 1.5 °C to 280 °C, held for 10 min. GC/MS-NICI (reagent gas methane, ion source 150 °C, quadrupole 150 °C) was used to confirm $MeSO_2$-CB and $MeSO_2$-DDE identities by total ion current scanning (TIC) from 50 amu to 550 amu. For quantitation of trace amounts of the analytes, MS-NICI was operated in the selected ion monitoring mode (SIM, dwell time 100 ms per ion) scanning for *m/z*: 370 for $MeSO_2$-tetraCB, 404 for $MeSO_2$-pentaCB, 438 for $MeSO_2$-hexaCB, 472 for $MeSO_2$-heptaCB and 396 for 3-$MeSO_2$-DDE. The high sensitivity of $MeSO_2$-CB and $MeSO_2$-DDE in GC/MS-NICI has proven to be of great value in the analysis of this type of xenobiotics (Haraguchi et al., 1993).

2.5. Enantioselective gas chromatography

In order to verify enantioselective transformation processes and to compare the different enzymatic transformation pathways in birds and different kinds of mammals, the enantioselective excesses of the chiral PCB-metabolites were determined by enantioselective gas chromatography with electron capture and mass spectrometric detection using modified cyclodextrin phases, including heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl-)-β-cyclodextrin/OV1701 (1:1) for the parent PCBs and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl-)-β-cyclodextrin/SE52 (1:4) for the metabolites, respectively. These columns were used by Ellerichmann et al. (1998). for the enantiomeric separations of chiral $MeSO_2$-PCBs in human liver (Ellerichmann et al., 1998). Enantioselective gas chromatography was performed on HP 5890II and Agilent 6890 N instruments equipped with the chiral columns mentioned above. Oven temperature was programmed from 70 °C (splitless period, 2.5 min) to 200 °C at a rate of 20 °C/min and then held for 200 min.

3. Results and Discussion

3.1. Method validation

Method validation and quantitation of results were performed by using commercially available reference materials containing PCBs and chlorinated pesticides (Dr. Ehrenstorfer, Augsburg, Germany) with no methyl sulfones present. This material was spiked with $MeSO_2$-PCBs reference solutions prepared from commercially available methyl sulfone standards (Cambridge Isotope Laboratories, USA). Selected validation parameters are shown in Table 1.

3.2. Analysis of human adipose, pelican muscle and seal blubber

The individual congeners of PCB methyl sulfones were identified, separated and quantified successfully in human adipose tissue, pelican muscle and seal blubber by gas chromatographic methods with ECD and MS-NICI detection (Figs. 1 and 2).

In human adipose tissue samples, 3-$MeSO_2$-DDE, 4-$MeSO_2$-CB 49, 4-$MeSO_2$-CB 101 and 3-$MeSO_2$-CB 110 were detected as predominant methyl sulfonyl metabolites (Table 3). 3-$MeSO_2$-DDE was found to be present in the human adipose tissue at the highest concentration among all methyl sulfones analyzed. With the exception of 3-$MeSO_2$-DDE all analyte levels were below 5 ng/g of lipids, The concentration of which is too low for further enantioselective gas chromatographic analysis using a chiral stationary phase (see Fig. 3).

Concentrations, enantiomeric ratios and enantiomeric fractions of $MeSO_2$-CB determined for pelican muscle and seal blubber samples are summarized in Tables 4 and 5, respectively. The concentrations for seal samples are comparable with those already reported earlier (Haraguchi

Table 1
Method validation parameters for GC/MS-NICI analysis of $MeSO_2$-CBs in biotic matrices (column DB-5MS)

$MeSO_2$-CB	Recovery (%) spike level: 10 ng/g of lipids	Repeatability, RSD_r (%)	LOQ (ng/g of lipids)
3–49	87	8	0.2
4–49	91	7	0.1
3–91	83	7	0.2
4–91	84	5	0.2
4–95	78	9	0.3
3–101	88	7	0.2
4–101	85	6	0.1
3–110	82	9	0.2
4–110	85	7	0.3
3–132	81	8	0.3
4–132	77	10	0.3
3–149	89	6	0.1
4–149	85	8	0.2
3–174	69	11	0.3
4–174	73	12	0.4
3-DDE	88	9	0.3

Table 2
The numbers refer to the $MeSO_2$-CBs in chromatograms

Number	1	2	3	4	5	6	7	8
$MeSO_2$-CBs	3–49	4–49	3–91	4–95	4–91	3–101	4–101	3-DDE
Number	9	10	11	12	13	14	15	16
$MeSO_2$-CBs	3–110	3–149	4–110	4–149	3–132	4–132	3–174	4–174

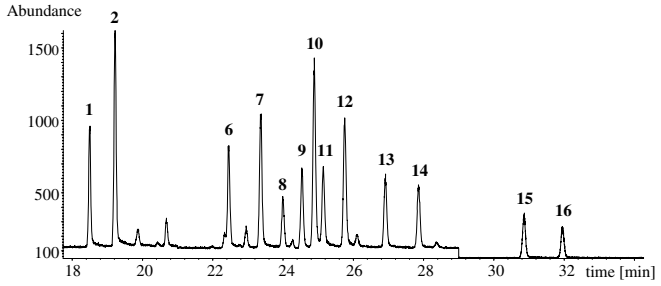

Fig. 1. GC/MS-NICI chromatogram of a selected standard mixture (4–4.4 ng/ml) of $MeSO_2$-CBs recorded in SIM mode. The numbers refer to the $MeSO_2$-CBs specified in Table 2.

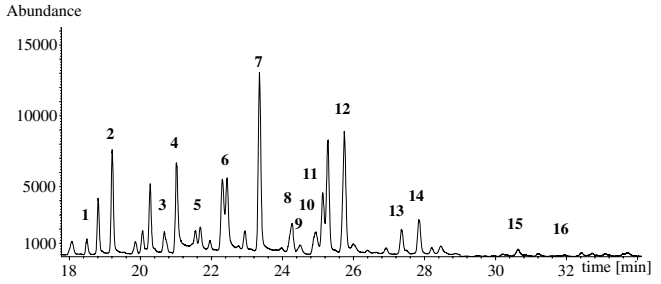

Fig. 2. GC/MS-NICI chromatogram of seal blubber recorded in SIM mode (numbers Table 2).

et al., 1993; Wiberg et al., 1998), while the concentrations of methylsulfonyl metabolites of PCB and DDE in pelican muscles have not been published yet. The results in Tables 4 and 5 show 4-$MeSO_2$-CB 149 as a major chiral PCB methyl sulfone in both animals. Characteristic differences were found for 4-$MeSO_2$-CB 91, which was found in relatively high concentrations with regard to the chiral methyl sulfones in seal blubber, while in pelican muscle its concentration was below the limit of quantitation.

With regard to the enantiomeric excesses, the present data can be compared with results published by Wiberg et al. (1998) for Arctic ringed seals (Wiberg et al., 1998): Particularly, high enantioselective transformation was

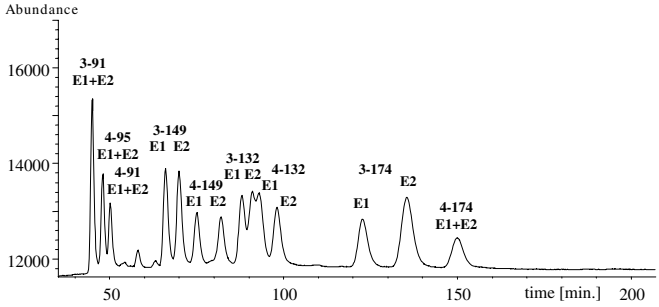

Fig. 3. GC/ECD chromatogram of a standard mixture (25 ng/ml) of selected chiral $MeSO_2$-CBs.

Table 4
Quantitation of enantiomers, enantiomeric ratios and enantiomeric fractions for pelican muscles

$MeSO_2$-CB	E1 (ng/g of lipids)	E2 (ng/g of lipids)	ER (E1/E2)	EF $E_1/(E_1 + E_2)$
3–132	n.d.	n.d.	–	–
4–132	2.3	1.1	2.09	0.68
3–149	2.7	3.6	0.75	0.43
4–149	11.8	7.3	1.62	0.62

Table 5
Quantitation of enantiomers, enantiomeric ratios and enantiomeric fractions for seal blubber

$MeSO_2$-CB	E1 (ng/g of lipids)	E2 (ng/g of lipids)	ER (E1/E2)	EF $E_1/(E_1 + E_2)$
4–91	7.2	2.3	3.13	0.76
3–132	n.d.	n.d.	–	–
4–132	11.8	3.1	3.81	0.79
3–149	2.9	8.2	0.35	0.26
4–149	31.4	27.3	1.15	0.53

observed for 3-$MeSO_2$-CB 149 (ER = 0.32) in seal blubber extracts. The authors attributed these low ER values to highly enantioselective formation, enantioselective metabolism,

Table 3
Concentrations of PCB and DDE methyl sulfones in human adipose tissue (ng/kg lipids)

Age/sex $MeSO_2$-CB	34/f	71/f	56/f	82/f	76/m	62/f	68/m	73/f	57/m	74/m	69/f
3–49	n.d.	0.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4–49	0.7	0.5	0.3	0.8	0.7	0.9	1.2	1.4	1.9	0.8	0.6
3–91	n.d.	0.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4–91	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4–95	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3–101	1.1	5.0	0.7	0.5	0.3	0.5	n.d.	0.8	2.6	n.d.	n.d.
4–101	n.d.	0.9	n.d.	0.5	1.9	0.6	0.6	0.8	3.6	3.7	n.d.
3–110	n.d.	3.7	2.3	2.5	0.3	n.d.	n.d.	n.d.	0.6	1.6	0.4
4–110	n.d.	0.9	0.3	0.6	0.3	n.d.	0.5	n.d.	0.6	n.d.	n.d.
3–132	n.d.	0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4–132	n.d.	1.1	0.7	0.4	0.4	0.4	n.d.	n.d.	0.3	n.d.	n.d.
3–149	n.d.	0.8	0.7	0.4	0.3	n.d.	n.d.	0.4	0.3	n.d.	n.d.
4–149	n.d.	1.1	0.2	0.5	0.8	0.9	0.3	0.5	0.4	n.d.	0.8
3-DDE	0.8	8.4	18.1	4.3	11.3	2.8	12.8	16.3	21.0	14.4	11.9

n.d. = not detected (below the limit of quantitation).

enantioselective transport across cell membranes, or a combination of the three. In the present study, a nearly the same ER value (ER = 0.35) was found for a seal sample stemmed from animals that had been kept in the Prague Zoo, which may indicate that the enzymatic transformation processes being responsible for the metabolisation of 3-$MeSO_2$-CB in seals lead to comparable enantiomeric excesses, regardless of the habitat of the respective animals.

The present data set is insofar unique as both the pelican and seal samples stem from animals of the Prague Zoo that had been fed by fish from the same source, i.e., the diet of these animals contained the same pollution pattern. As a consequence, the differences in composition of methylsulfonyl metabolites of PCBs reflect different metabolic transformation of the parent PCBs and/or of the transformation products by these animals.

Studies of other biota samples were reported by Ellerichmann et al. (1998) that analysed six human liver and two human lung samples for the content as well as for the enantiomeric excess of eight $MeSO_2$-PCBs, two of which (3-$MeSO_2$-CB 149 and 3-$MeSO_2$-CB 132) were detected in all liver samples. In all liver sample extracts, exclusively the second eluting enantiomers of the $MeSO_2$-PCBs 3-$MeSO_2$-CB 149 and 3-$MeSO_2$-CB 132 were encountered, i.e., not only a high congener selective (as already previously observed), but also a high enantioselective liver retention of these $MeSO_2$-PCBs was determined. None of the $MeSO_2$-PCBs included in the study by Haraguchi et al. (1993) were detected in the two human lung samples also analysed. During a joint Swedish/German investigation, enantiomeric excesses of chiral methylsulfonyl PCBs in liver and adipose tissues from rats dosed with Chlophen A50 were determined (Larsson et al., 1999, 2002). In all samples analysed, the concentrations of the 4-$MeSO_2$-CB 91 and 4′-$MeSO_2$-CB 132 were slightly higher than those of the 3-$MeSO_2$-PCB isomers in fat and liver. It is important to note that comparable enantiomeric ratios were found in fat and liver tissue extracts: in all samples analysed by Larsson et al. (1999, 2002), only the second eluting enantiomers of 3-$MeSO_2$-CB 132 and 3-$MeSO_2$-CB 149 were present. In contrast, 4-$MeSO_2$-CB 91, 4-$MeSO_2$-CB 132 and 4-$MeSO_2$-CB 149 were dominated by the first eluting enantiomers, although minor amounts of the second eluting enantiomers were also found. The ratios of the first and second eluting enantiomers (the quotient a/b) for 4-$MeSO_2$-CB91 were in the range 7–11, for 4-$MeSO_2$-CB 149 2–8 and for 3-$MeSO_2$-CB 132 6-13. This indicates that either both enantiomers are being formed or, if only one enantiomer is formed, that this enantiomer is converted to both 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. Furthermore, it cannot be excluded that enantioselective transport processes may also play an additional role. A surprising result was recently presented by Larsson et al. with regard to the enantiomeric excesses of $MeSO_2$-PCBs in rat lung sample extracts, determined for the same animals the adipose and liver tissues of whom had been analysed. It turned out that the second eluting enantiomers of 4-$MeSO_2$-CB 149 and 4-$MeSO_2$-CB 132 were clearly more abundant than the first eluting ones (Larsson et al., 2002).

Jörundsdóttir et al. (2006), who investigated time trends of methyl sulfone containing metabolites of PCBs from 1971 to 2001 in eggs of Guillemot (*Uria aalge*) hatching in the Baltic Proper, were the first to study chiral $MeSO_2$-PCBs in any bird species. Of the eight chiral $MeSO_2$-PCBs in the reference standard, it was not possible to separate 3-$MeSO_2$-CB 91 and 3-$MeSO_2$-CB 132 into its two atropisomers. Both of these two congeners were present in the sample. Complete baseline separation was only obtained for 4-$MeSO_2$-CB 149. Interestingly, for the six chiral $MeSO_2$-PCBs investigated both atropisomers were present. Almost racemic mixtures were observed for 4-$MeSO_2$-CB 149 and 4-MeSO2-CB 174 with enantiomeric fractions (EF) of 0.52 and 0.56, respectively. Although both atropisomers were present, a small enantiomeric excess was determined for the first eluting enantiomer for all *para*-substituted congeners, while the second eluting enantiomer showed a small dominance for all *meta*-substituted congeners.

4. Conclusions

The following conclusions can be drawn:

- Enantiomers of selected atropisomeric PCB methyl sulfones in pelican muscle tissue and seal blubber were separated and resolved from possible coelutants successfully by enantioselective capillary gas chromatography using a modified cyclodextrin as stationary phase.
- Enantiomers of congeners of methyl sulfones with 3-$MeSO_2$ substitution were better resolved using chiral stationary phases than the corresponding 4-$MeSO_2$ pairs.
- Concentrations of chiral metabolites in human samples were below the limit of detection using a chiral stationary phase. In both pelican and seal samples, the enantiomers with *R*-conformation were found in higher concentrations than those exhibiting the complementary *S*-structure. Possible explanations may include enantioselective enzymatic formation, transformation or transport mechanisms.
- The precursors of the PCB methyl sulfones have not been found at higher levels in human adipose tissue, which indicates that certain PCBs are very effectively metabolized to their methyl sulfonyl derivatives which are accumulated in the adipose tissue.

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