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Levels and Enantiomeric Signatures of Methyl Sulfonyl PCB and DDE Metabolites in Livers of Harbor Porpoises (*Phocoena phocoena*) from the Southern North Sea

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The concentration of 26 methyl sulfonyl metabolites of polychlorinated biphenyls ($MeSO_2$-PCBs) and of *p,p′*-DDE ($MeSO_2$-DDE) were determined in 19 liver samples from harbor porpoises (*Phocoena phocoena*) stranded between 1997 and 2000 on the Belgian and French North Sea Coasts. The total concentration of $MeSO_2$-PCBs ranged from 39 to 4221 ng/g lipid weight (lw) and were generally higher in adults (age >2 yr, range 969−4 221 ng/g lw) than in juveniles (age <2 yr, range 39−1815 ng/g lw). The concentrations of $MeSO_2$-DDE were generally also higher in adults (21−96 ng/g lw) than in juveniles (0.5−60 ng/g lw). Congeners 3- and 4-$MeSO_2$-CB101 were the dominating metabolites in all samples. Due to their preferential retention in the liver, the $MeSO_2$-PCB congeners could be divided into two groups. The first group was dominated by the 3-$MeSO_2$-PCB congeners and consisted of $MeSO_2$-CB31, -CB49, -CB52, -CB87, and -CB101, which all have a 2,5-chlorine substitution in the phenyl ring containing the methyl sulfonyl group. The second group was dominated by the 4-$MeSO_2$-PCB congeners and consisted of $MeSO_2$-CB64, -CB91, -CB110, and -CB132, which all have a 2,3,6-chlorine substitution. The ratios of sum of PCBs/sum of $MeSO_2$-PCBs and *p,p′*-DDE/$MeSO_2$-DDE differed greatly between individual subjects and ranged from 15 to 419 and from 17 to 1088, respectively. The ratio between the precursor PCB congeners and their corresponding metabolites ranged from 0.6 (CB49) to 175 (CB174). Enantiomeric fractions (EFs) for $MeSO_2$-PCB atropisomers, which include 3-$MeSO_2$-CB132, 3-$MeSO_2$-CB149, 4-$MeSO_2$-CB149, 3-$MeSO_2$-CB174, and 4-$MeSO_2$-CB174, were also measured in 8 out of the 19 subjects. High enantiomeric excess (EF > 0.73 or EF < 0.23) for the measured chiral $MeSO_2$-PCB congeners was found in all samples. This result may suggest that one atropisomer may be preferentially formed in harbor porpoises or that the atropisomers are retained in a highly selective manner.

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Introduction

Methyl sulfones ($MeSO_2$-) of polychlorinated biphenyls (PCBs) and 1,1-*bis*-(4-chlorophenyl)-2,2-dichloroethene (*p,p′*-DDE) are stable and persistent metabolites of PCBs and *p,p′*-DDE (*1*). The enzyme-mediated biotransformation depends both on the congener structure and on the metabolic capacity of the species. The majority of the persistent and bioaccumulative $MeSO_2$-PCBs are congeners that originate from PCBs possessing 3−7 chlorine atoms and a 2,5-dichloro or a 2,3,6-trichloro substitution pattern on one of the biphenyl rings, while only 2- and 3-$MeSO_2$-DDE have been found in environmental samples until now (*1*). The initial phase I metabolism is mediated by cytochrome P450 2B-like (CYP2B) isoenzymes (*1*). The resulting PCB epoxide intermediate undergoes a phase II conjugation reaction with glutathione. The dehydration and hydrolysis of the glutathionyl-PCB occurs via the mercapturic acid pathway to form a cysteine conjugate, which is converted to a PCB thiol via a biliary excretion into the gastrointestinal tract and further to methylthio-PCB via C−S lyase and *S*-adenosylmethionine mediation. A following two-step CYP enzyme-mediated oxidation results in persistent 3- or 4-$MeSO_2$-PCBs (*1*).

Although the ecotoxicological effects of these metabolites are unclear, there is evidence to suggest that they are able to bind to the intracellular receptor proteins transthyretin and uteroglobin, preventing the binding of endogenous ligands, such as thyroxin and progesterone (*2*). Depending on the structure, certain $MeSO_2$-PCBs have been shown to inhibit aryl hydrocarbon (Ah) hydroxylase activity induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in human lymphoblastoid cells (*3*).

Since their first determination in Baltic grey seal blubber (*4*), the presence of $MeSO_2$-PCBs and -DDE in different species has been reported (*1*). It was found that these contaminants are ubiquitous and bioaccumulate in the environment (*5*−*10*). However, there is only little information available on the distribution of $MeSO_2$-PCBs and -DDE metabolites in marine mammals.

It is well-known that some PCBs exhibit axial chirality, and 19 of the chiral PCBs are predicted to form stable atropisomers under most environmental conditions (*11*). Chiral $MeSO_2$-PCBs may be formed when chiral PCBs or even achiral PCBs are metabolized in biota (*12*). Therefore, a detailed study of bioprocesses may give additional information on the PCB fate or metabolism. While a relatively large amount of information is available on the distribution of chiral PCBs in ecosystems (*13*), only very few enantioselective determinations of $MeSO_2$-PCBs metabolites have been performed on environmental samples (*13*−*17*).

It was reported that the number of cetaceans in North European waters has drastically decreased during the past decades (*18*) and that high levels of contaminants were presumably one of the main anthropogenic causes. To understand their potential toxicological effects, levels of organohalogenated contaminants, including PCBs, organochlorine pesticides, and polybrominated diphenyl ethers (PBDEs), have been previously determined in liver samples

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TABLE 1. Individual Characteristics and $MeSO_2$-PCB and -DDE Levels[a]

sample no.	age	sex	length (cm)	weight (kg)	blubber (mm)	lipid (%)	sum $MeSO_2$-PCBs (ng/g lw)	sum PCBs (ng/g lw)	3-$MeSO_2$-DDE (ng/g lw)	*p,p′*-DDE (ng/g lw)
97/944	j	f	118	28	25	3.9	1 027	15 986	60	997
99/923	j	f	116	17	8	6.2	1 005	33 823	46	2 354
00/974	j	f	114	22	20	15.8	39	1 866	0.5	311
97/759	j	m	108	21	11	1.6	191	7 952	3	843
97/967	j	m	118	22	10	3.6	380	24 994	5	2 066
99/924	j	m	106	17	4	3.8	1 815	38 345	15	1 692
99/1041	j	m	103	16	8	7.1	212	41 881	3	3 405
99/1094	j	m	108	15	8	3.1	113	16 745	3	1 795
99/1374	j	m	80	7	6	21.7	104	13 268	1	1 085
00/212	j	m	99	16	8	10.8	277	116 445	16	12 614
00/600	j	m	103	27	8	12.0	268	63 286	3	2 999
mean			107	19	11	8.1	494	34 054	14	2 742
mean jf			116	22	18	8.6	690	17 225	36	1 221
mean jm			103	18	8	8.0	420	40 365	6	3 312
97/1237	a	f	151	39	11	4.1	969	50 144	24	3 546
98/254	a	f	152	36	10	4.1	1 238	79 803	21	3 447
99/707	a	f	134	30	15	6.2	2 492	36 119	24	1 543
98/356	a	m	144	33	13	9.2	1 992	404 455	33	24 346
00/307	a	m	142	41	10	7.5	1 453	59 848	96	3 695
00/342	a	m	144	43	14	6.7	1 225	86 078	44	5 899
00/559	a	m	149	37	8	3.4	1 747	76 393	94	4 575
00/1019	a	m	150	39	9	3.2	4 221	359 745	58	2 574
mean			146	37	11	5.5	1 917	144 073	49	6 203
mean af			146	35	12	4.8	1 566	55 355	23	2 845
mean am			146	39	11	6.0	2 128	197 303	65	8 218

[a] Expressed in ng/g lipid weight (lw) in harbor porpoise liver samples from the Belgian and French North Sea Coast. j, juvenile; a, adult; m, male; f, female.

from harbor porpoises (*Phocoena phocoena*) (*19*). The present study aims to further identify and quantify $MeSO_2$-PCBs and -DDE metabolites in the same specimens. In addition, the enantioselective formation of $MeSO_2$-PCBs and relationships between the metabolites and their precursor congeners were also investigated.

Experimental Section

Samples. Liver samples were collected from 19 harbor porpoises (*P. phocoena*) stranded on the Belgian and French North Sea Coasts (Southern North Sea) between 1997 and 2000. Sample details are shown in Table 1. All the samples were kept at −20 °C until analysis. Complete data for 74 mono- to tetra-*o*-PCB congeners, three hexachlorocyclohexane isomers (α-, *β*-, and *γ*-), hexachlorobenzene, DDTs, and 8 polybrominated diphenyl ether (PBDE) congeners in the same samples have been previously reported (*19*).

Chemicals. The 26 $MeSO_2$-PCB standard reference congeners (details in Figure 1 and Supporting Information), 3-$MeSO_2$-4,4′-DDE, and 3-$MeSO_2$-4-methyl-2′,3′,4′,5,5′-pentachlorobiphenyl, which was used as internal standard (IS), were synthesized as previously reported (*20*) at the Daiichi College of Pharmaceutical Sciences (Japan). The chemical names of $MeSO_2$-PCB metabolites were simplified on the basis of the IUPAC-derived numbering system of the parent PCBs (*1*). All solvents were of pesticide grade (Merck, Darmstadt, Germany). Concentrated sulfuric acid (95−97%) was obtained from Merck. Anhydrous Na_2SO_4 (Merck) was heated prior to use at 600 °C for 6 h in a muffle furnace to destroy all organic contaminants. Florisil (60−100 mesh) (Supelco, Bornem, Belgium) was activated at 130 °C overnight and then deactivated with 2% (w/w) water. Potassium hydroxide (KOH)/silica gel (33% KOH, w/w) was prepared by dissolving KOH into methanol and adding it to dry silica gel 60 (70−230 mesh) (Merck). The mixture was then solvent evaporated at 80 °C and heated at 150−170 °C for 4 h.

Analytical Procedures. The method used for the determination of $MeSO_2$-PCBs and -DDE was described elsewhere (*21*). Briefly, about 1−2 g of liver sample was mixed with anhydrous Na_2SO_4, and after the addition of 1 ng of IS, the sample was extracted with 100 mL of *n*-hexane/acetone (3/1, v/v) in a Soxhlet system (B-811 Büchi, Brussels, Belgium) operated in hot extraction mode. The extract was twice treated with concentrated sulfuric acid, and the acid layers were combined. After dilution of the acidic phase with cold water (50%, v/v), the methyl sulfones were back-extracted to *n*-hexane. The organic phase containing $MeSO_2$-PCBs and -DDE was cleaned up by a column packed with 1 g o basic silica (33% KOH) and 5 g of anhydrous Na_2SO_4 and eluted with 10 mL of dichloromethane (DCM). After concentration and solvent exchange into *n*-hexane, the extract was further purified on a Florisil column (8 g). The column was first eluted with 40 mL of DCM/*n*-hexane (50/50, v/v) and 10 mL of DCM that were discarded, and then the methyl sulfonyl metabolite fraction was eluted with 40 mL of DCM. After solvent evaporation to dryness, the residue was dissolved in 100 *μ*L of isooctane.

Quality control measures included procedural blanks and recovery checking of spiked samples (*21*). The recoveries of individual $MeSO_2$-PCB congeners were estimated through the analysis of 0.3 g of sunflower oil spiked with 1 ng of each compound and ranged from 73 to 112% with a mean value of 89%. Seven replicates from 0.3 g of sunflower oil spiked with the standard mixture containing 26 $MeSO_2$-PCBs and 3-$MeSO_2$-DDE at concentrations of 0.33 ng/g lw were analyzed for the determination of the corresponding limit of detection (LOD). The LOD was here defined as three times the standard deviation of the measured values for the spiked sample.

Instrumentation. The determination of $MeSO_2$-PCBs and -DDE was performed on a Hewlett-Packard (Palo Alto, CA) gas chromatograph (GC) 6890 equipped with a 5973 quadrupole mass spectrometer (MS) detector operated in electron capture negative ionization (ECNI) mode. A total of 2 *μ*L of

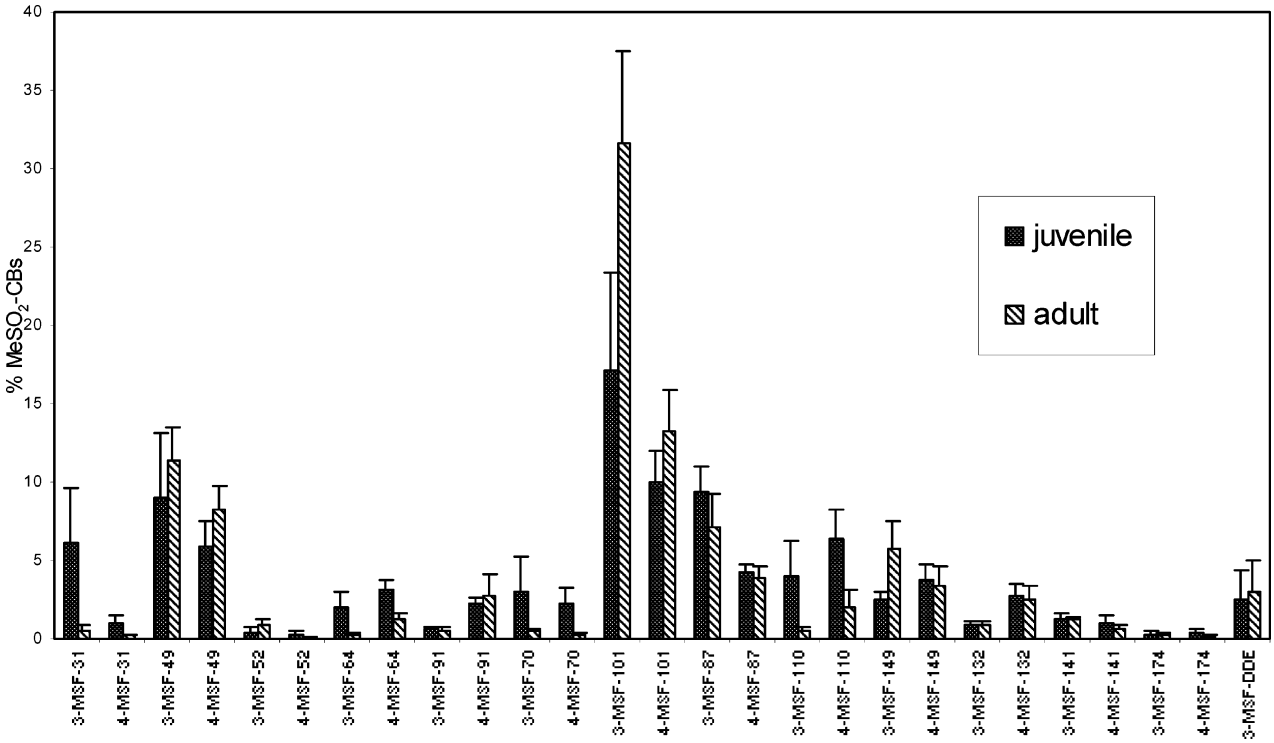

FIGURE 1. Distribution of individual $MeSO_2$-PCB and -DDE congeners (%) in juvenile and adult harbor porpoises. The error bars represent the standard deviation of the percentage mean for each congener.

extract was injected in solvent vent mode. An AT-5MS capillary column (50 m × 0.18 mm i.d. × 0.25 μm film thickness) (Alltech, Lokeren, Belgium) was used. The oven temperature was programmed as follows: 80 °C, held for 2.5 min, then at 20 °C/min to 250 °C, and finally at 5 °C/min to 290 °C, held for 40 min. The carrier gas was helium, and methane was used as the moderating gas. The ion source and quadrupole temperatures were 250 and 150 °C, respectively. The identification of analytes was based on the comparison of retention times and mass spectrum with appropriate individual standards. Quantitative determination was done using a multi-level calibration curve covering the range of expected analyte concentrations in real samples.

Enantioselective analysis of $MeSO_2$-PCB metabolites in porpoise liver samples was performed with the same instrumentation as described above. The enantiomeric separation was carried out on a Chirasil-Dex column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) (Chrompack, Raritan, NJ), which was connected with a Press-Tight connector (Agilent, Palo Alto, CA) to a DB-XLB precolumn (3 m × 0.25 mm i.d) (J&W Scientific, Folsom, CA). The carrier gas was helium with constant pressure of 2.8 bar. The injector system was operated in large volume injection mode. The parameters for solvent vent were as follows: initial temperature 60 °C, held for 1 min, then at 600 °C/min to 300 °C, held for 300 min. The vent and purge times were 0.9 and 2.5 min, respectively. A 25-μL (5 × 5 μL) sample aliquot was injected. The ion source and quadrupole temperatures were 250 and 150 °C, respectively. Initial oven temperature was 50 °C, held for 3 min, then at 15 °C/min to 120 °C, and finally at 5 °C/min to 190 °C, held for 330 min. Three characteristic ions were monitored for each analyte (*21*). Identification was based on retention times and the ion intensity ratios compared with those obtained from the corresponding individual standards.

Enantiomeric ratios (ERs) for the chiral $MeSO_2$-PCB atropisomers were quantified as the ratio between the areas of the first-eluting to the second-eluting enantiomer, while enantiomeric fractions (EFs) were computed as suggested by Harner et al. (*22*):

$$EF = \frac{ER}{1 + ER}$$

Statistical Analyses. For comparison between groups, two-tailed Student *t*-tests were carried out using Statistica for Windows, version 5.0 (StatSoft, 1995, Tulsa, OK) to determine whether any differences were statistically significant ($p < 0.05$).

Results and Discussion

Levels of $MeSO_2$-PCBs and -DDE in Harbor Porpoise Livers. The total concentrations of $MeSO_2$-PCB and -DDE congeners together with levels of total PCBs and *p,p′*-DDE in liver samples from harbor porpoises ($n = 19$) are presented in Table 1. All concentrations are given in nanogram per gram lipid weight (ng/g lw). The 26 $MeSO_2$-PCBs and 3-$MeSO_2$-DDE could be quantitatively determined in most samples. The total concentration of $MeSO_2$-PCBs in the 19 animals ranged from 39 to 4221 ng/g lw (mean 1092 ng/g lw). The total $MeSO_2$-PCB levels were statistically higher ($p < 0.05$) in adult (age >2 yr, mean 1917 ng/g lw, range 969−4221 ng/g lw) than in juvenile (age <2 yr, mean 494 ng/g lw, range 39−1815 ng/g lw) individuals. $MeSO_2$-DDE was found in all samples and ranged from 0.4 to 96 ng/g lw. The mean concentrations of $MeSO_2$-DDE were also statistically higher ($p < 0.05$) in adults (49 ng/g lw) than in juveniles (14 ng/g lw). As compared to adults, a higher inter-individual variation in the juveniles was observed for $MeSO_2$-PCBs and -DDE as well as for the lipid content (Table 1). While concentrations of the sum of PCBs and *p,p′*-DDE were statistically higher in the adult males as compared with adult females (*19*), there was no significant difference in the total concentration of $MeSO_2$-PCBs and -DDE between male and female harbor porpoises for both adults and juveniles. This result was different from previous investigations in which the levels of $MeSO_2$-PCBs and $MeSO_2$-DDE in adult seals seemed to be

higher in females than in males (*6*). No significant correlations ($p < 0.05$) could be found between levels of $MeSO_2$-PCBs or $MeSO_2$-DDE and the length/weight ratio or blubber thickness.

The levels of $MeSO_2$-PCBs in harbor porpoises from the Southern North Sea (this study) were higher than in liver samples from three adult harbor porpoises caught in Swedish waters (sum of $MeSO_2$-PCBs 150, 280, and 480 ng/g lw, respectively), while the contamination level of $MeSO_2$-DDE in liver was found to be similar in both groups (*10*). Correspondingly, the mean concentrations of sum PCBs in the same samples from the North Sea (detailed data presented in ref *19*) were also higher than in porpoises from Swedish waters (36.4 vs 6.0 μg/g lw) (*10*).

The number and relative proportion of the $MeSO_2$-PCBs in tissues of marine mammals might present high intra- and inter-species variations. The concentration of total $MeSO_2$-PCBs in tissues from different animals collected from Canadian and Swedish marine environments (*7*) varied between 600 ng/g lw in the liver of Canadian beluga whale (*Delphinapteru leucas*) and 21 000 ng/g lw in the liver of Baltic grey seal (*Halichoerus grypus*). The total concentration of the $MeSO_2$-PCBs (6 congeners) in blubber samples of cetaceans collected from the Irish and Aegean Seas ranged from 30 to 580 ng/g lw, with the highest concentration being found in a harbor porpoise (*23*).

For harbor porpoises, PCBs and other neutral organohalogenated pollutants (such as *p*,*p*′-DDE or PBDEs) were reported to be similarly distributed between tissues with the ratio between their lipid-normalized concentrations in liver and blubber being 1.10 ± 0.10 ($n = 3$) (*24*). However, for $MeSO_2$-PCBs and -DDE, higher concentrations (up to five times) were reported in liver as compared to blubber (*10*). Therefore, due to the uneven distribution of methyl sulfonyl metabolites between cetacean liver and blubber (*10*), it may be assumed that the data reported by Troisi et al. (*23*) were in the same range as the present investigation.

Congener Distribution of $MeSO_2$-PCBs and -DDE. A larger number of $MeSO_2$-PCB congeners are present in porpoise liver, and the patterns are relatively similar for all individuals (see Supporting Information). The most abundant congeners are 3- and 4-$MeSO_2$-CB101 (about 40% of sum of $MeSO_2$-PCBs), followed by 3- and 4-$MeSO_2$-CB49, -CB87, and -CB149. This congener distribution was similar to that previously reported in seal, dolphin, whale, and harbor porpoises (*6*, *10*, *25*). The congener profile was somewhat different in polar bear adipose tissue, where 4-$MeSO_2$-CB87 was reported to be the main congener (*26*). Surprisingly, a different congener distribution pattern between juvenile and adult specimens could be observed (Figure 1). Both 3- and 4-$MeSO_2$-CB31, -CB64, -CB70, and -CB110 were more abundant in juvenile specimens, while $MeSO_2$-CB101 was present in a higher proportion in the adult individuals.

Due to the preferential retention in the liver, the $MeSO_2$-PCB congeners investigated in the present study could be divided into two groups. The first group is dominated by the 3-$MeSO_2$-PCB congeners and consists of $MeSO_2$-CB31, -CB49, -CB52, -CB87, and -CB101, which all have a 2,5-chlorine substitution in the phenyl ring containing the methyl sulfonyl group. The second group is dominated by the 4-$MeSO_2$-PCB congeners and consists of $MeSO_2$-CB64, -CB91, -CB110, and -CB132, which all have a 2,3,6-chlorine substitution. Apparently, the preferential formation in porpoises of 3- or 4-$MeSO_2$-PCBs depends on the different precursor PCB congeners.

A similar phenomenon could be observed in other reported data. In livers from adult Swedish harbor porpoises (*10*), it was found that, for $MeSO_2$-CB49, -CB87, and -CB149, the dominant congener was the 3-$MeSO_2$-substituted compound, while for $MeSO_2$-CB64 and -CB91, the dominant congener had a 4-$MeSO_2$ substitution. However, whether this phenomenon is specific for only some species of marine mammals or for specific marine areas is still unclear; therefore, further investigation is necessary.

Karlson et al. (*10*) have previously found that the distribution of $MeSO_2$-PCB congeners is similar in liver and blubber of harbor porpoises (*10*). This is in contrast with the distribution of $MeSO_2$-PCBs in human tissues reported by Weistrand and Norén (*9*) and Chu et al. (*27*), where 4-$MeSO_2$-CB87 and -CB101 were the main congeners in adipose tissue, while 3-$MeSO_2$-CB132 was the dominant congener in liver. Differences between mammalian species are probably due to variations in metabolic capabilities combined with differences in the levels and profiles of PCBs in their diet. While most of marine mammal species are predators having a high and consistent dietary intake of fish and benthic organisms, humans have a more diversified diet, including vegetables and different types of meat.

Relationships between $MeSO_2$-PCBs and Their Precursor PCBs. On the basis of the results obtained for PCBs by Covaci et al. (*19*), the ratios of sum of PCBs/sum of $MeSO_2$-PCBs and *p*,*p*′-DDE/$MeSO_2$-DDE for individual samples could be computed. It was found that they had a large variation ranging from 14 to 420 and from 17 to 1088, respectively. Positive and significant correlations between individual $MeSO_2$-PCBs and their precursor compounds were found only for CB91, CB132, CB149, and CB174 ($r = 0.563-0.752$, $p < 0.05$). No correlation was found between *p*,*p*′-DDE and $MeSO_2$-DDE, while a weak correlation ($r = 0.669$, $p < 0.01$) was found between the sum of $MeSO_2$-PCBs and the sum of PCBs. Furthermore, the correlation between the sum of $MeSO_2$-PCBs and $MeSO_2$-DDE was weaker ($r = 0.559$, $p < 0.02$) than the correlation between the sum of PCBs and *p*,*p*′-DDE ($r = 0.721$, $p < 0.01$). This can be explained by the fact that, although the rate of intake for PCBs and *p*,*p*′-DDE is relatively similar, the transformation rate into the corresponding methyl sulfonyl metabolites is very different.

It is well-known that CB153 is resistant to metabolization in many organisms, and it is assumed that CB153 holds the highest bioaccumulation potential for slowly metabolized lipophilic compounds (*28*). To minimize the variation among individuals, the concentration of $MeSO_2$-PCBs and their precursor PCBs was normalized to the concentration of CB153 in each specimen. Figure 2 shows the ratio between concentrations of $MeSO_2$-PCBs and CB153 and between concentrations of the precursor PCBs and CB153. Because the amount of CB64 was undetectable in all samples, $MeSO_2$-CB64 and its precursor were not shown in Figure 2. Obviously, for PCB congeners with 2,5- and 2,3,6-chlorine substitution, one of the main metabolization pathways is the formation of $MeSO_2$-PCBs. However, the range of ratios between CB153 and individual $MeSO_2$-PCBs is still large (from 3.7 to 92.7). A different level of enzyme induction as function of growth and health condition might provide an explanation for this variation. The high ratio sum of PCBs/sum of $MeSO_2$-PCBs also implies that harbor porpoises have a relatively low metabolic capacity as compared to terrestrial mammals (*6*, *26*, *29*, *30*) but higher than other marine mammals (*23*).

Enantiomeric Signatures of $MeSO_2$-PCBs in Porpoise Liver Samples. Although the separation of atropisomeric PCBs by GC with chiral stationary phases, their bioaccumulation, and fate has been extensively investigated, only little emphasis has been placed on atropisomeric $MeSO_2$-PCBs. Although, theoretically there are 837 $MeSO_2$-PCB congeners possible of which 456 congeners are chiral (*12*), there are no more than 30 $MeSO_2$-PCB congeners found in environmental samples of which only 10 are chiral. Some enantiomeric $MeSO_2$-PCBs pairs can be determined by enantioselective capillary GC without preseparation. Ellerichmann et al. (*15*) successfully used a capillary column coated with a 1:1 (w/w) mixture of OV 1701 and 2,3-di-*O*-

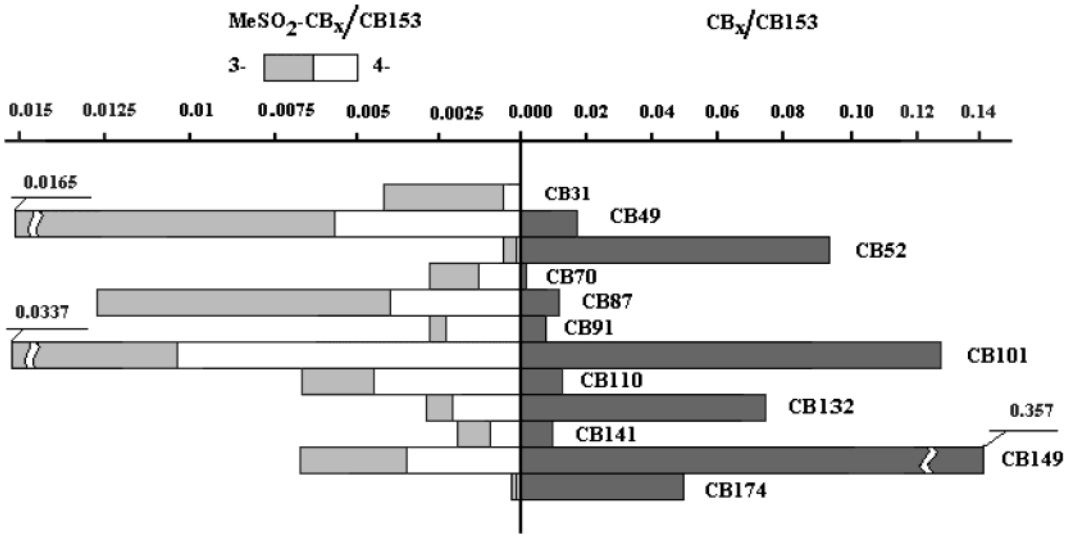

FIGURE 2. The ratio of $MeSO_2$-PCBs and their precursor PCB congeners vs concentration of CB153.

TABLE 2. Values of Enantiomeric Fractions in Porpoise Liver Samples ($n = 8$)[a]

sample no.	99/923	00/974	98/254	98/356	00/307	00/342	00/559	00/1019
age/sex	jf	jf	af	am	am	am	am	am
3-$MeSO_2$-CB132	<0.07	0.12	0.16	0.20	0.06	<0.12	<0.19	0.16
3-$MeSO_2$-CB149	0.20	0.03	0.04	0.10	<0.02	<0.03	<0.06	0.09
4-$MeSO_2$-CB149	>0.98	>0.99	>0.99	0.95	>0.98	>0.98	>0.94	>0.98
3-$MeSO_2$-CB174		0.19	0.20		0.10	0.19		0.23
4-$MeSO_2$-CB174		>0.89	>0.84		>0.73	>0.76		>0.76

[a] Key: j, juvenile; a, adult; m, male; f, female.

methyl-6-*O*-*tert*-hexyl-β-cyclodextrin to separate atropisomers of 3- and 4-substituted $MeSO_2$-CB91, $MeSO_2$-CB95, $MeSO_2$-CB132, $MeSO_2$-CB149, and $MeSO_2$-CB174, while Larsson et al. (*16*) used a capillary column coated with a 4:1 mixture of SE-52 and 2,3-di-*O*-methyl-6-O-*tert*-hexyl-β-cyclodextrin. Wiberg et al. (*14*) reported that these congeners (except 4-$MeSO_2$-CB95 and 4-$MeSO_2$-CB149) could also be separated with a commercially available BGB-172 column (BGB Analytik, Switzerland).

In our case, the separation of chiral $MeSO_2$-PCBs in porpoise livers on a BGB 172 column was unsatisfactory. Not only the retention time showed great shifts in different runs but also the enantioselectivity of the column was not stable and the elution order for some congeners changed over time. For example, in the beginning, 3-$MeSO_2$-CB132 (E_1) eluted after the pair of atropisomers of 4-$MeSO_2$-CB132 (E_1 and E_2). However, after a few runs, 3-$MeSO_2$-CB132 (E_1) coeluted with 4-$MeSO_2$-CB132 (E_2), and after some more runs, it coeluted with 4-$MeSO_2$-CB132 (E_1). A similar phenomenon was also observed in other enantioselective analyses (*31*), and it was postulated that the variation in enantioselectivity might arise from the loss at different rates of the chiral stationary phase. In the present study, the separation of some pairs of chiral $MeSO_2$-PCBs was achieved with a Chirasil-Dex capillary column (Chrompack, Middelburg, The Netherlands) coated with permethylated β-cyclodetrin bonded to the polysiloxane stationary phase, which provided more reproducible results than the BGB 172 column. However, a maximum oven temperature of 200 °C was used, and this resulted in long run times (>300 min), resulting in peak broadening and poorer detection limits. In these conditions, the quantification of $MeSO_2$-PCBs enantiomers present at low concentrations was very difficult even if a large injection volume (25 μL) was used.

Five pairs of atropisomeric $MeSO_2$-PCBs (which include 3-$MeSO_2$-CB132, 3-$MeSO_2$-CB149, 4-$MeSO_2$-CB149, 3-$MeSO_2$-CB174, and 4-$MeSO_2$-CB174) could be separated and determined. The values of the EFs in porpoise liver samples are listed in Table 2. High enantiomeric excesses were found in all samples and for all the chiral $MeSO_2$-PCBs determined. In some cases, the dominance was so pronounced that only one atropisomer could be detected. In that case, for the calculation of EFs, the concentration of missing atropisomer was estimated as the limit of detection (S/N = 3/1). The enantioselective $MeSO_2$-PCB analysis showed excess and dominance of the second eluting enantiomer of 3-$MeSO_2$-CB149, 3-$MeSO_2$-CB149, 3-$MeSO_2$-CB174, while 4-$MeSO_2$-CB132 and 4-$MeSO_2$-CB174 were enriched in the first eluted enantiomer. Similar results were observed in grey seal tissues by Larsson et al. (*17*). However, no relationships could be found between the total concentration of $MeSO_2$-PCBs and EFs. This might be due to the limited data set and to the fact that EFs could not be measured with this method in low-concentrated samples (except sample no. 00/974).

High enantiomeric excess of $MeSO_2$-PCBs in mammals was also reported in other investigations (*14*, *16*). Wiberg et al. (*14*) reported the dominance in polar bears and ringed seals being so pronounced that in some cases only one enantiomer of chiral $MeSO_2$-PCBs studied could be detected with certainty. Ellerichmann et al. (*15*) have successfully separated eight atropisomeric $MeSO_2$-PCBs and found a strong enantiomeric excess of 3-$MeSO_2$-CB132 and 3-$MeSO_2$-CB149 in human liver. The changes in EFs of $MeSO_2$-PCB atropisomers were studied in rat tissues by Larsson et al. (*16*). Enantioselective $MeSO_2$-PCB analysis of lung samples showed an excess and dominance of the second eluting atropisomer of 4-$MeSO_2$-CB149.

Moreover, $MeSO_2$-PCBs presented higher enantiomeric excesses in marine mammals than their precursor PCBs. EFs of CB149 and CB132 were previously measured by Chu et al. (*32*) in some of the same porpoise liver samples used in the present study. EFs ranged from 0.56 to 0.63 and from 0.30

to 0.34 for CB149 and CB132, respectively. The enantiomeric composition of chiral $MeSO_2$-PCB residues reflect in a better way bioprocesses, because only bioprocesses can give rise to enantiomeric excesses of a chiral compound (*13*, *33*) and methyl sulfones are one of the main metabolites of PCBs. This implies that enantioselective metabolism may be one of the main reasons that influences the enantiomeric enrichment of CBs in marine mammals, although preferential degradation, metabolite formation, and transport across membranes have also been suggested (*34*). The metabolization rates of chiral PCBs to chiral $MeSO_2$-PCBs may be significantly different, which makes that one of the enantiomers accumulates in biota, while the other enantiomer tends to be metabolized faster. These findings may partly explain why the enantiomeric enrichment of $MeSO_2$-PCBs is more accentuated than of their precursor PCBs. Further studies are necessary, especially for the determination of elution order of optical active atropisomers of $MeSO_2$-PCBs and their precursor compounds in different chiral columns, to find the metabolization process of individual atropisomers.

The results indicate that $MeSO_2$-PCBs and -DDE are one of the major environmental pollutants in harbor porpoises. The $MeSO_2$-PCB congener pattern, the ratio between the 3- and 4-$MeSO_2$-substituted congeners, the ratio between metabolites and their precursor compounds, and enantiomeric compositions are all factors that must be investigated in future studies involving more mammalian species from different locations. Although the toxicological significance of $MeSO_2$-PCBs in wildlife is largely unknown, the levels of $MeSO_2$-PCBs in the marine mammals are high enough to merit further attention in ecotoxicological investigations.

Supporting Information Available

$MeSO_2$-PCBs and -DDE congener levels (ng/g lw) in porpoise liver samples from the North Sea. This material is available free of charge via the Internet at http://pubs.acs.org.

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