PCB METHYL SULPHONES IN GREY SEAL AND OTTER FROM SWEDISH ENVIRONMENT

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ABSTRACT

Methyl sulphone metabolites of PCB and DDE were isolated from grey seal (*Halichoerus grypus)* in the Baltic Sea and from otter *(Lutra lutra)* in northern Sweden. Main components of the PCB methyl sulphones were identified as penta- and hexachlorinated biphenyl congeners in both cases. GC analyses of ten different seal tissues from one individual revealed a specific and different PCB methyl sulphone pattern in liver and lung compared to all other tissues.

INTRODUCTION

Methyl sulphone metabolites of PCB were first identified in high concentration in seal blubber from the Baltic Sea in 1976 (1). Since then, these products have been detected in a large number of species from the Japanese environment (2) and in human tissues, especially in the lung of Yusho patients (3). The PCB methyl sulphones are stable and lipophilic metabolites formed via the mercapturic acid pathway (4). The major concern for this type of metabolites is concentrated to their characteristic patterns of tissue localization, e.g. as shown by accumulation in lung, kidney and uterine fluid of mice (5). Populations of seals in the Baltic Sea and otter in the archipelagoes of the Baltic and in freshwaters of Sweden have decreased severely during this century. The decreasing populations of seals and otter are considered to be caused by organochlorine compounds, namely PCB (6, 7). It is thus necessary to improve our knowledge of the fate of individual PCB congeners and their metabolites in these endangered species. In the present paper, structures of PCB methyl sulphone congeners present in seal and otter in the Swedish environment are identified. Furthermore the levels and composition in ten tissue extracts from a seal are compared.

EXPERIMENTAL

Materials: One grey seal (*Halichoerus grypus*), a two year old male (92 kg) was collected from the Bothanian Sea, north of Gävle for analysis of tissues, e.g. adrenal, prostate, large intestine, testis, liver, lung, muscle, brain and kidney. Another juvenile grey seal, male (60 kg) from the Bothanian Bay, south east of Holmsund for analysis of blubber. Blubber from an otter (*Lutra lutra*), male (6.8 kg), run over by a car in 1984, from Jokkmokk in northern Sweden was analyzed.

Isolation and identification: Tissue samples were homogenized and extracted with dichloromethane (DCM). After the solvent was evaporated, the extract from blubber of the seal and otter was injected onto a GPC column (Bio Beads S-X3, 50 g) eluted with DCM : n-hexane (1:1) at a flow-rate of 4 ml/min. The extract from other tissues was treated with DMSO according to Jensen (personal communication). The PCB and PCB methyl sulphone compounds were isolated in one fraction and subjected to an aluminium oxide column (3g, neutral), and eluted with 8% of DCM in n-hexane (60 ml) for PCBs, and a fraction containing methyl aryl sulphones was successively eluted with DCM (50 ml). The latter fraction was analyzed by GC/MS (NCI) and GC/ECD. GC/MS was performed on a Finigan 4500 with a fused silica capillary DB-5 column (30 m x 0.25 mm i.d., J&W Sci.). The MS was operated in negative ion CI mode using methane as reagent gas. The temperature was programmed from 70°C (2 min) to 220°C at a rate of 20°C/min and then to 280°C at a rate of 3°C/min. The electron multiplier was 1650 V and electron energy 70 eV. GC/ECD (^{63}Ni) was performed on a Shimadzu GC 9A using a fused silica SPB-5 column (30 m x 0.25 mm i.d., Supelco) and with the same temperature program as for the GC/MS analysis. Structural identification of the sulphones was performed by comparison of their mass spectra and GC retention times with those of 90 authentic reference compounds synthesized earlier (8). The level of the PCB methyl sulphones was based on the integrated peak area on GC/ECD from the sample relative to that of the internal standard (3-methylsulphonyl-4-methyl-2',3',4',5,5'-pentachlorobiphenyl).

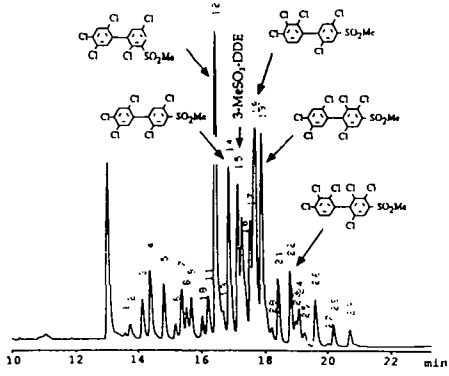

Figure 1. ECD gas chromatogram of $MeSO_2$-PCB fraction isolated from seal blubber.

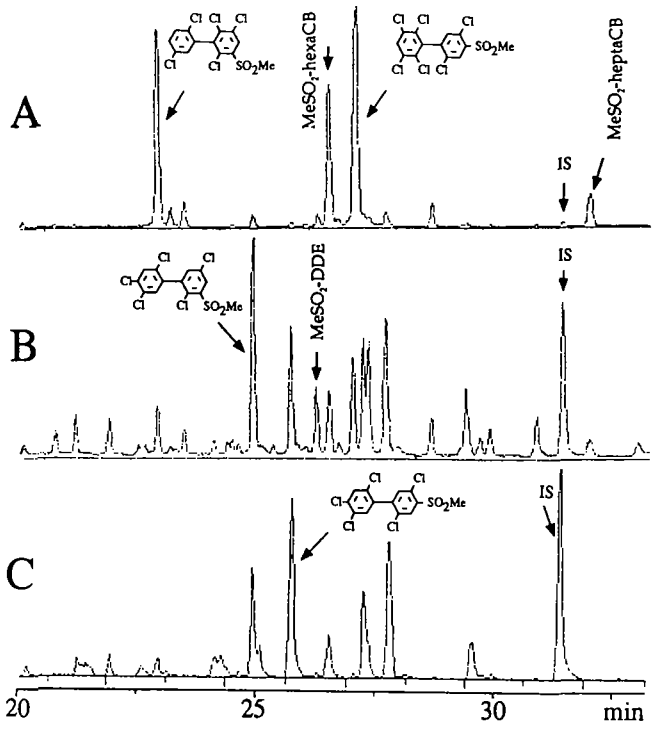

Figure 2. Total ion chromatograms of $MeSO_2$-PCBs in seal liver (A), seal blubber (B) and otter blubber (C).

Table 1. Tissue levels of PCBs and $MeSO_2$-PCBs in gray seal and otter.

Tissue	Sample (g)	Neutral fat (mg)	Total PCB (ug/g fat)	Total $MeSO_2$-PCB (ug/g fat	ug/g f.w.)
Seal 1					
Blubber	2,3	2000	NA	3,0	2,8
Seal 2					
Blubber	9,2	7480	27,4	NA	NA
Adrenal	5,8	860	9,8	2,7	0,4
Prostate	10,7	530	21,0	0,6	0,03
Intestine	10,9	70	17,3	0,7	<0,01
Testis	10,0	120	15,3	0,7	<0,01
Liver	10,4	140	27,1	28,5	0,4
Lung	12,3	80	20,1	15,0	0,1
Muscle	10,3	70	12,3	0,5	<0,01
Brain	10,6	610	2,1	0,4	0,02
Kidney	10,6	200	13,8	1,5	0,03
Otter					
Blubber	2,2	2000	NA	0,4	0,3

NA = Not analyzed

RESULTS AND DISCUSSION

More than 25 different PCB methyl sulphones, containing three to seven chlorine atoms, and/or two DDE methyl sulphones were detected in seal and otter fat. A gas chromatogram (ECD) of PCB and DDE methyl sulphones in seal blubber is shown in Figure 1. Main components were identified as 3-$MeSO_2$-2,2',4',5,5'-pentaCB, 4-$MeSO_2$-2,2',4',5,5'-pentaCB, 3-$MeSO_2$-2,2',3',4',5-pentaCB, 4-$MeSO_2$-2,2',3',4',5-pentaCB, 4-$MeSO_2$-2,2',3',5,5',6'-hexaCB, 4-$MeSO_2$-2,2',3,4',5',6-hexaCB, 4-$MeSO_2$-2,2',3,3',4',6-hexaCB, 4-$MeSO_2$-2,2',3',4',5,5'-hexaCB and 3-$MeSO_2$-DDE, on the basis of comparison of mass spectra and GC retention times with reference compounds using two different capillary columns. Different tissues in one individual seal were analyzed in order to compare the composition and levels of the PCB methyl sulphones with the blubber. As shown in Figure 2, the total ion chromatogram (TIC) for the sulphones in liver was significantly different from the peak pattern in the blubber. Three of the major peaks that appeared in the TIC in the liver sample were identified as 3-$MeSO_2$-2,2',5,5',6-pentaCB, 4-$MeSO_2$-2,2',3',5,5',6'-hexaCB and a $MeSO_2$-hexaCB with an unknown structure. The same characteristic GC-peak pattern was also observed in the lung. However, all the other seven tissues analyzed contained the same components as that observed in the blubber. The components in otter blubber were also compared with those in the seal (Figure 2). Relative peak ratio within the components in the otter was different from that in the seal. Especially it contained 4-$MeSO_2$-2,2',4',5 ,5'-pentaCB as the major component. It should be noted that 3-$MeSO_2$-DDE was not detected in the otter sample, although the levels of DDE are found at a similar order of magnitude as PCB in Swedish freshwater fish. The concentrations of $MeSO_2$-PCB in the seal and otter are listed in Table 1. The levels of the PCB methyl sulphones in the blubber of the seal and otter were estimated to be 2.8 and 0.3 ug/g, respectively. The tissue levels of the $MeSO_2$-PCB in the seal were 0.4 ug/g in the adrenal, 0.4 ug/g in the liver and 0.1 ug/g in the lung, whereas those in the other tissue were below 0.04 ug/g. The level of $MeSO_2$-PCB in the seal blubber is estimated to approximately 1/10 of that of PCB, and were found to be accumulated in the liver and lung at almost similar levels as PCB. Also in the adrenal tissue *(homogenate of cortex and medulla)*, $MeSO_2$-PCB was high and accounted roughly for 1/4. This condition needs to be investigated further since the disease complex *(hyperadrenocorticism)* of Baltic seals seems to be caused by a primary lesion of the adrenal cortex leading to secondary reactions in other organs (6).

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