PCB AND DDE METHYL SULPHONES - PERSISTENT ENVIRONMENTAL CONTAMINANTS

Åke Bergman
Department of Environmental Chemistry, Wallenberg Laboratory, Stockholm University, S-106 91 Stockholm, Sweden

Jensen first detected the presence of polychlorinated biphenyls (PCB) in the environment in 1966[1] and ten years later Jensen and Jansson reported on the determination of PCB methyl sulfones in blubber from grey seal[2]. Simultaneously, Mio *et al* identified both two isomeric methylthio- and two methylsulfonyl-2,5,2',5'-tetrachlorobiphenyls as metabolites of 2,5,2',5'-tetrachlorobiphenyl (CB-52[3]) in mice[4] Also a DDE methyl sulfone ($MeSO_2$-DDE) was detected in the seal blubber[2], identified as 3-methylsulfonyl-4,4'-DDE by comparison to the authentic synthesized standard[5]. Further, a strong retention of radioactivity in lung bronchial mucosa in mice dosed with some individual ^{14}C-labelled chlorinated biphenyls (CBs)[6] was found to be due to reversibly bound 4-methylsulfonyl-CB metabolites of the CBs studied[7]; an exception was 2,4,5,2',4',5'-hexaCB (CB-153) that was accumulated in the bronchial mucosa as such. After some early reports[e.g. 4,8,9], a large number of studies on PCB and DDE methyl sulfones have been published[e.g. 10-12 and references cited therein]. It must be emphasized that $MeSO_2$-CBs are among the most abundant xenobiotics in mammals, e.g. in grey seals from the Baltic they are the third most abundant class of anthropogenic substances[13], and are often found in concentrations of 1/10 to 1/20 of the total PCB.

The aim of the present work is to describe the development of PCB and DDE methyl sulfone research. Environmental levels and the retention of these PCB and DDE metabolites in mammals are discussed.

More than one hundred individual PCB methyl sulfones ($MeSO_2$-CBs) have been synthesized so far. The main methods for synthesis of $MeSO_2$-CBs are: i: methane thiolate nucleophlic displacement of a chlorine atom in 4-position of a CB with at least one 2,4,5-trichloro-substituted phenyl ring[14,15]; ii: Cadogan diaryl coupling between a chlorinated aniline and a polychlorothioanisole or between a methylsulfonyl-polychloroaniline and a polychlorobenzene[15]; iii: diazotation of amino-CBs and coupling with methanethiolate[16]. Buser *et al*[17] have shown that $MeSO_2$-CBs can be prepared by γ-irradiation of CBs in dimethyl sulfide and oxidation of the methylthio-CB produced. A number of radiolabelled $MeSO_2$-CBs have also been prepared, either with the label in one of the biphenyl rings or in the methyl group[18,19].

The physico-chemical characteristics of several synthetic $MeSO_2$-CBs have been described including their NMR-spectra[20]. A majority of the $MeSO_2$-CBs prepared have been analyzed by mass spectrometry, with both electron ionization (EI) and negative ion chemical ionization (NICI), for determining of structure specific fragmentation patterns for this class of compounds[11,15,16,17]. In contrast to PCB methyl sulfides it is not possible to unambigously determine the position of the methyl sulfone group unless this group occupies an *ortho*-position. MS(NICI) gives limited structural information for the $MeSO_2$-CBs. However, this method is valuable since it is at least 500 times more sensitive than MS(EI). It is notable that 2-$MeSO_2$-DDE and 3-$MeSO_2$-DDE show very different mass spectra both in EI and NICI mode[11]. In general, environmental samples were analyzed for $MeSO_2$-

CBs by gas chromatography with electron capture detection (GC(ECD)) and MS was mainly used to confirm the identity of the compounds. However, recently GC with atomic emission detection (AED) was utilized for analysis of PCB and DDE methyl sulfones, with specific detection of sulfur[21]. This method is advantageous for samples still containing PCB and other organochlorine substances (OCS) since these compounds will not interfere in the analysis.

Aryl methyl sulfones are semi-polar compounds with slightly lower octanol/water partitioning coefficient than the corresponding parent compounds. The sulfone group can act as a Lewis base, a property often used for clean-up of samples. Hence a hexane solution containing the sulfones can be partitioned into concentrated sulfuric acid, and reextracted from the acid after dilution with water and addition of another portion of hexane[2,22]. Aryl methyl sulfones may also be partitioned from hexane to dimethyl sulfoxide, a method frequently used for clean-up of environmental samples[2,22]. Several chromatographic methods have been used for clean-up of aryl methyl sulfones prior to GC analysis, e.g. aluminum oxide[22], Florisil[23] and phosphoric acid/silica gel (unpublished). The difference in polarity between most OCS and aryl methyl sulfones makes these separations successful.

In comparison with synthetic $MeSO_2$-CBs, only 30 compounds have been identified in mammals[11] and a lower number of sulfones in fish, birds and other organisms[13,24]. 2-$MeSO_2$-CBs have been detected in environmental samples but were also identified in animals experimentally dosed with 2,4,2',4'-tetraCB[25]. In contrast, 2-$MeSO_2$-DDE has occasionally been detected in samples from certain mammalian species even though 3-$MeSO_2$-DDE is the major sulfone metabolite of DDE[10]. The structures of all major $MeSO_2$-CBs are dependent on the structure of the parent CB and on the metabolism of the individual CBs. It is well known today that the concentrations of CBs with vicinal hydrogen atoms, in at least one of the phenyl rings of the molecule, in organisms at high trophic levels are low or below the detection limit, cf. e.g. CBs in minks dosed with technical PCB[22]. Among these CBs, compounds with at least one 2,5-dichloro- or 2,3,6-trichloro-substituted phenyl ring form two isomeric $MeSO_2$-CBs and the sulfone group may be localized to the *meta*- or *para*-positions in these phenyl rings. Both the 3- and 4-$MeSO_2$-CBs accumulate in adipose tissue of mammals. According to Schultz *et al*[26], 17 and 14 CBs with chlorine substituents in 2,5- or 2,3,6-positions are present in the technical PCB products, Clophen A50 and A60, respectively. The other phenyl ring may be substituted in the 2,5- or 2,3,6-positions or substituted with at least a chlorine atom in the 4-position. The CBs which are metabolized to isomeric $MeSO_2$-CBs and accumulate in biota are listed in Table 1 together with abbreviated names for the $MeSO_2$-CBs.

CBs with vicinal hydrogen atoms as described above are readily oxidized to arene oxides via oxidation by cytochrome P450-dependent enzymes[27]. Arene oxides with chlorine atoms on both sides of the 3,4-positions readily react with glutathione (GSH). The nucleophilic attack by GSH of the 3,4-arene oxide may occur in either one of the 3- or 4-positions. The glutathione conjugate formed is transformed via the mercapturic acid pathway to a cysteine conjugate that is cleaved to thiol-substituted CBs by a C-S-lyase present in the intestinal microflora[28]. These aryl thiols are methylated by adenosyl-methionine to the corresponding methyl sulfides and the sulfides are oxidized to aryl methyl sulfones, via an intermediate formation of methylsulfinyl-CBs[29].

After most of the relevant $MeSO_2$-CBs present in environmental samples had been synthesized several reports of $MeSO_2$-CBs and $MeSO_2$-DDE concentrations in such samples have been reported. For example, all three species of seals living in the Baltic have been extensively studied and analyzed for PCB and $MeSO_2$-CBs and -DDE[30]. Among juvenile seals, the grey seals show the highest concentrations (12 µg/g lipid) and harbour seals the lowest (2 µg/g). No significant differences in the sulfone composition were observed among the seal species, even though certain dissimilarities were observed. For other mammals, e.g. otter, mink, polar bear and humans, the $MeSO_2$-CB concentrations are lower

but the ratio to PCB is still similar[10]. A typical gas chromatogram of the $MeSO_2$-CB fraction from a sample of mink pups from mothers dosed with Clophen A50 for half a year is shown in Figure 1.

The metabolic capacity of xenobiotics is lower in fish than in mammals, which may explain the low levels of sulfones detected in fish. No experimental data describing formation of sulfones in fish and their capacity for this type of metabolism has been reported. On the other hand, birds feeding on fish such as guillemot and white tailed sea eagle are at the top of the food-web and high levels of xenobiotics may be expected. This is also true for most of the traditional OCS but it is not valid for $MeSO_2$-CBs or -DDE. Significantly different peak patterns and lower concentrations of $MeSO_2$-CBs are found in these species. The ratio between PCB and $MeSO_2$-CBs is approximately 1000/1[31]. This is an indication that birds do not form aryl methyl sulfones to the same extent as mammals. It is not known if birds metabolize CBs to methyl sulfones and it can not be excluded that the trace levels in the birds are due to uptake via food.

PCB methyl sulfones were among the first metabolites of xenobiotics observed to be localized with high selectivity to other tissues than adipose tissues. Thus, 4-$MeSO_2$-CBs of CB-31, CB-49, CB-52, CB-70 and CB-101 were identified in lung tissue[7]. Some of these CBs were also retained in intraluminal uterine fluid (CB-31) and in the kidney (CB-31, CB-49, CB-101)[32]. More recently, structure dependent retention of $MeSO_2$-CBs have been observed in the liver of mammals. For example, grey seal livers contain basically three $MeSO_2$-CBs but their total concentration exceeds the concentration of total $MeSO_2$-CBs detected in blubber from the same individuals[11]. It is notable that major differences in the liver composition of $MeSO_2$-CBs are observed depending on species. This is discussed in more detail by Haraguchi *et al*[33]. The mechanisms involved in the selective retention in the mammalian livers are still unknown but Larsen has shown that 3- and 4-$MeSO_2$-CB-101 bind to a fatty acid binding protein (FABP) with a molecular weight of 14.000 dalton as further discussed by Larsen[34].

Even though both 2- and 3-$MeSO_2$-DDE are formed in the metabolism of DDE the latter metabolite dominates in the environmental samples analyzed. The ratios observed between total DDT and the sulfones varies with animal and tissue[30,35,36]. Also 3-$MeSO_2$-DDE show a strong, but different, selective localization in the adrenal cortex of e.g. mice[37]. However, the identity of the reactive intermediate that leads to the covalent binding of this DDE methyl sulfone is still unclear.

The retention of both DDE and PCB methyl sulfones is dependent on the possibility for further metabolism. $MeSO_2$-DDE may undergo metabolic transformations in the ethene-group and/or the other phenyl ring. These transformations decrease the halflife of $MeSO_2$-DDE. $MeSO_2$-CBs containing two phenyl rings with hydrogen atoms in vicinal positions (CB-52, CB-95 and CB-136) may undergo further metabolism after formation of an initial methyl sulfone metabolite since another arene oxide may be formed that may lead to a variety of metabolites including hydroxylated PCB methyl sulfones and bis(methylsulfonyl)-substituted CBs. In fact, the concentrations of 3- or 4-$MeSO_2$-CB-52 and 3- or 4-$MeSO_2$-CB-95 are low in most environmental samples which is probably due to further metabolism of an initial methylsulfonyl-CB.

Methylsulfonyl-metabolites of DDE and PCB are ubiquitous environmental contaminants, at least in mammals. Even though the effects of the different sulfones still needs further investigation, it is obvious that these metabolites of PCB and DDT influence the toxicokinetics of these well known environmental contaminants through their selective tissue retention.

Table 1. Chlorinated biphenyls (CBs) present in four different commercial PCB products and their corresponding $MeSO_2$-CB metabolites. Methyl sulfone metabolites of the CBs given in this table have been determined in environmental samples.

Parent CB	Chlorine substitution pattern	Percent in A50/1254*	Percent in A60/1260*	$MeSO_2$-CB metabolite (Short form)
49	2,4-2',5'	1.96 1.64	- -	3-$MeSO_2$-CB49 (**3-49**) 4-$MeSO_2$-CB49 (**4-49**)
52	2,5-2',5'	5.53 5.18	0.75 0.56	3-$MeSO_2$-CB52 (**3-52**) 4-$MeSO_2$-CB52 (**4-52**)
64	2,3,6-4'	0.71 0.45	- -	3-$MeSO_2$-CB64 (**3-64**) 4-$MeSO_2$-CB64 (**4-64**)
70	2,5-3',4'	3.85 3.21	0.06 0.09	3-$MeSO_2$-CB70 (**3-70**) 4-$MeSO_2$-CB70 (**4-70**)
87	2,3,4-2',5'	4.22 3.78	1.13 0.77	3-$MeSO_2$-CB87 (**3-87**) 4-$MeSO_2$-CB87 (**4-87**)
91	2,3,6-2',4'	0.92 0.83	- -	3-$MeSO_2$-CB91 (**3-91**) 4-$MeSO_2$-CB91 (**4-91**)
95	2,3,6-2',5'	6.00 6.02	3.70 3.04	3-$MeSO_2$-CB95 (**3-95**) 4-$MeSO_2$-CB95 (**4-95**)
101	2,4,5-2',5'	7.72 7.94	5.21 5.02	3-$MeSO_2$-CB101 (**3-101**) 4-$MeSO_2$-CB101 (**4-101**)
110	2,3,6-3',4'	6.27 5.85	2.15 1.90	3-$MeSO_2$-CB110 (**3-110**) 4-$MeSO_2$-CB110 (**4-110**)
132	2,3,4-2',3',6'	2.57 1.98	4.52 3.69	3-$MeSO_2$-CB132 (**3-132**) 4-$MeSO_2$-CB132 (**4-132**)
141	2,3,4,5-2',5'	0.98 1.04	3.31 2.56	3-$MeSO_2$-CB141 (**3-141**) 4-$MeSO_2$-CB141 (**4-141**)
149	2,4,5-2',3',6'	4.50 2.21	8.57 7.83	3-$MeSO_2$-CB149 (**3-149**) 4-$MeSO_2$-CB149 (**4-149**)
174	2,3,4,5-2',3',6'	0.37 0.34	3.92 3.85	3-$MeSO_2$-CB174 (**3-174**) 4-$MeSO_2$-CB174 (**4-174**)

* Percent in Clophen A50/Aroclor 1254 and Clophen A60/Aroclor 1260 are given (Clophen upper figure).

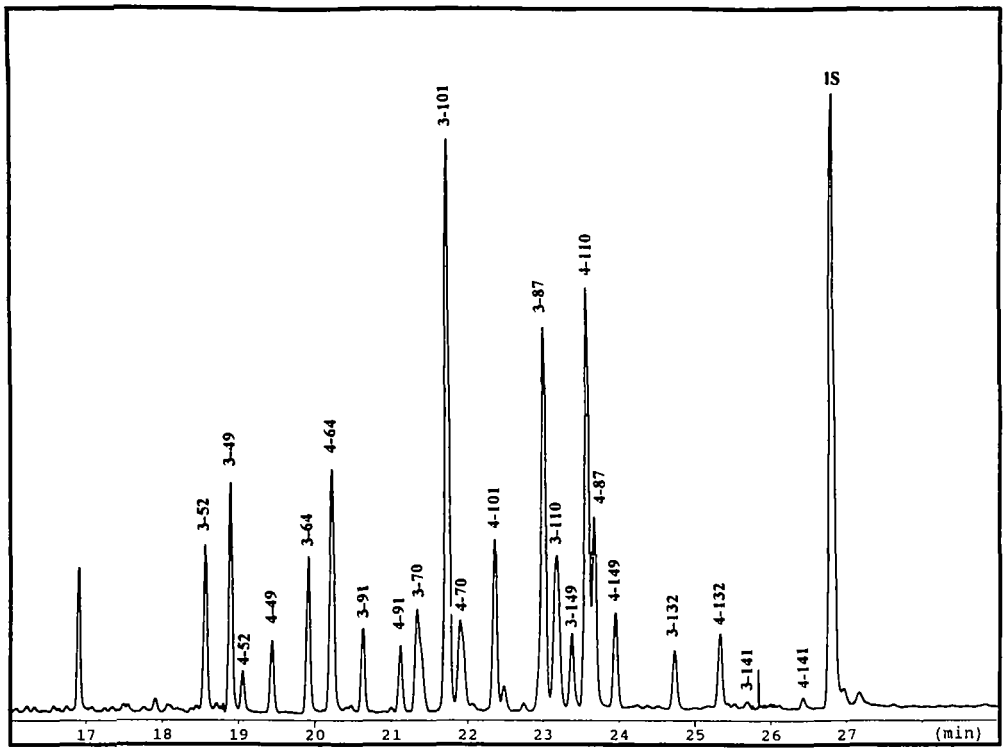

Figure 1. Gas chromatogram of PCB methyl sulfone metabolites present in mink pups after oral administration of Clophen A50 to their mothers for 6 months prior to parturition. The peaks correspond to the $MeSO_2$-CB structures listed in table 1(see short forms). The analysis was performed as described in reference 22.

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