Methyl Sulfonyl PCB and DDE in grey seal tissues as determined by GC-AED and GC-ECD

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Introduction

Certain PCB and DDE methyl sulfone congeners ($MeSO_2$-CB, $MeSO_2$-DDE), metabolites of polychlorinated biphenyls (PCB) and 2,2-bis(4-chlorophenyl)-1,1,1-trichlorethene (DDE) are specifically retained and enriched in different organs of mammals [1,2]. Concentrations of $MeSO_2$-CB in blubber of seals are, in general, approximately 1/20 of the concentration of PCB [3]. Sufficient toxicological data are not yet available in order to evaluate the toxicity of $MeSO_2$-CB. However, $MeSO_2$-DDE is highly toxic in the adrenal cortex (*zona fasciculata*) of mice and some other animals. $MeSO_2$-CB with a sulfone group in meta-position have been shown to significantly induce phenobarbital inducible enzymes in the liver.

Applying gas chromatography (GC) with electron capture detection (ECD) or mass spectrometry detection (MS), laborious clean up steps prior to GC analysis are necessary to separate aryl methyl sulfones from extracted lipids and parent compounds. Determination of the methyl sulfones can be difficult due to restrictions that regard the selectivity of the ECD response or the dependence of the MS response on compound structure or instrumental parameters. Gas chromatography with atomic emission detection (GC-AED) enables to determine $MeSO_2$-CB in the presence of abundant and coeluting PCB when using the sulfur channel in conjunction with the chlorine channel. Quantification of congeners for which standard compounds are lacking is possible due to a largely compound independent response for PCB or dioxin congeners [4]. The high selectivities of the sulfur and chlorine channels may simplify both sample clean-up procedures and the determination of the analytes. Amounts of 20 pg aryl methyl sulfone are detectable by using the AED sulfur selective trace or 50 pg using the chlorine trace.

The aim of the present study is to investigate specific accumulation of individual $MeSO_2$-CB congeners and $MeSO_2$-DDE in the Baltic grey seal lung, liver and adipose tissues by element selective AED and by ECD. Distribution of the methyl sulfones between different tissues has been compared. Results from both detection techniques have been compared.

Materials and Methods

Instrumental parameters (GC-AED, GC-ECD, HPLC), chemicals and samples used are described elsewhere in detail [5]. Blubber, liver and lung samples were obtained from three male grey seals (*Halichoerus grypus*) from the Baltic. The liver and lung samples were homogenized, blubber was melted and filtrated. All samples were extracted [6] and were spiked with 3-methylsulfonyl-4-methyl-2',3',4',5,5'-pentachlorobiphenyl before homogenization. The environmental contaminants in the extracts were fractionated by gel permeation chromatography (GPC). The PCB/$MeSO_2$-CB fraction was partitioned with dimethylsulphoxide (DMSO)[1]. The isolated $MeSO_2$-CBs were analysed by GC-AED and GC-ECD. In order to improve clean-up efficiencies and to minimize possible interferences of PCBs in the determination of $MeSO_2$-CBs two additional sample clean-up procedures were applied to seal blubber. The DMSO purification of PCB/$MeSO_2$-CB fraction obtained from GPC was substituted either by HPLC using nitrophenylpropyl silica gel as the stationary phase and hexane:dichloromethane (1:1) as the mobile phase, or by a second fractionation on GPC.

Results

Clean-up : The HPLC method using nitrophenylpropyl silica gel yielded the best preconcentration of $MeSO_2$-CB (see Table 1).

Table 1 : Selectivities of different prefractionation methods applied for a seal blubber sample.

Clean-up	A	B
GPC (2x)	0.02±0.00	0.26±0.01
GPC+DMSO	0.05±0.01	0.74±0.13
GPC+NO_2-col.	0.13±0.01	5.26±0.13

A = ratio of summarized amounts of all $MeSO_2$-CB congeners identified by standards, divided by all PCB congeners present in the sample; B = ratio of summarized amounts of all $MeSO_2$-CB identified by standards, divided by PCB eluting between the first and the last eluting $MeSO_2$-CB (3-52 and 4-174).

Analysis of $MeSO_2$-CB in seal blubber, liver and lung

The total concentrations of $MeSO_2$-CB in the organs of three seals (in ppm[a]) and reproducibility of the determination are summarized in Table 2.

Table 2

	Total Concentration[b]		Specified Concentration[c]			RSD[d] (%)	
Method	AED-S	AED-Cl	AED-S	AED-Cl	ECD	AED-S	AED-Cl
Sample							
Blubber 1	1.1	1.5	1.0	1.1	0.9	4.3	4.7
Blubber 2	1.6	2.0	1.5	1.6	1.3	3.3	5.2
Blubber 3	0.9	1.1	0.8	0.8	0.9	3.4	4.6
Liver 1	26.1*	20.8*	25.2	19.8	16.4	5.2	4.6
Liver 2	17.3*	16.1*	16.5	15.3	17.0	5.9	4.8
Liver 3	11.7*	10.9*	11.4	10.5	8.7	5.7	4.8
Lung 1	1.6	---	1.5	---	1.4	5.5	---
Lung 2	1.9	---	1.8	---	1.3	6.3	---
Lung 3	1.2	---	1.1	---	0.7	5.7	---

Legend to Table 2 : a- related to 1 g of lipids in the tissue. b- total concentration : concentrations of all 'S and Cl containing compounds' which by their S/Cl ratio and by their retention time are considered to be $MeSO_2$-CB (retention time between that of the first and the last $MeSO_2$-CB standard). c- specified concentration : concentrations of $MeSO_2$-CB identified by standards. d- calculated from three replicates of the analysis of one particular sample for each congener and then averaged for all congeners. *- calculated concentration using different levels of I.S.

Concentrations of individual $MeSO_2$-CB congeners in blubber, liver and lung tissue of one seal (in ppb, related to one gram of lipids in the tissue) estimated by the AED sulfur (S) and chlorine (Cl) channel and by GC-ECD are summarized in Table 3. Both detection techniques showed mostly comparable results. Higher ECD values for the congeners 4-87 and 4-149 were caused by impurities in the respective standards, detectable on the AED carbon selective channel. Values were corrected in AED analysis using an average response factor for closely eluting compounds. Evaluating lung samples, several congeners detected and estimated in the sulfur mode of the AED could not be determined by ECD due to non-selective response. Four sulfone congeners were not detected by the AED due to an overloading of the plasma by matrix compounds. Variation of congener concentrations for samples of different seals was pronounced for some congeners (see Figure 1). The extent of variation was comparable in all tissues in AED analysis; in ECD analysis, larger dispersion of values was found for lung samples due to low sulfone concentrations.

Table 3

	blubber			liver			lung	
Method	S	Cl	EC	S	Cl	EC	S	ECD
Solute								
3-52	13	18	10	41	nd	46	nd	nd
3-49	32	50	27	43	nd	49	30	81
4-52	nd	nd	nd	nd	nd	nd	nd	nd
4-49	23	27	21	24	44	29	28	nd
3-64	8	nd	7	51	57	44	21	16
4-64	20	nd	19	3500	3800	4550	136	140
3-91	15	15	11	329	352	350	36	nd
4-91	10	13	7	15	nd	nd	nd	nd
3-70	28	29	29	38	nd	48	30	nd
3-101	327	322	260	355	390	390	213	145
4-70	11	nd	14	nd	nd	21	nd	nd
4-101	171	211	140	140	170	160	86	82
3-DDE	97	104	85	760	765	750	158	116
3-87	80	89	65	2080	2100	1920	157	72
3-110	13	15	nd	165	150	190	20	nd
3-149	56	62	46	6700	5200	6520	429	230
4-110	182	172	144	216	nd	200	126	nd
4-87	119	121	160	128	nd	250	97	170
4-149	108	118	120	176	175	290	68	nd
3-132	38	43	36	524	568	525	58	59
4-132	53	59	49	50	54	52	32	35
3-141	27	32	26	29	nd	28	nd	20
4-141	31	35	31	33	nd	32	nd	19
3-174	14	14	8	700	706	550	nd	25
4-174	15	17	11	15	nd	11	nd	16

nd : not detected.

It has been of interest to compare $MeSO_2$-CB pattern in seal organs since different accumulation potency has been reported for aryl methyl sulfones in mammalian organs. GC-AED and GC-ECD analyses showed partly different patterns of $MeSO_2$-CBs and $MeSO_2$-DDE present in blubber and lung (Figure 1). Differences between these tissues could be observed for the relative abundances of four congeners. However, the concentrations of these compounds in blubber and lung were similar. In agreement with previous reports, strong retention of some $MeSO_2$-CB congeners is observed in the liver. The toxicological impact of a high concentration of 3-149 in liver is at present not known but work is in progress to determine whether protein binding of this PCB methyl sulfone occurs.

3-149 was shown by Kato and co-workers to strongly induce P-450 dependent enzymes [7]. It is notable that no specific accumulation of 4-$MeSO_2$-CB congeners is observed in the grey seal lung tissue (Fig.1c). This can thus be taken as an indication for the lack of a uteroglobin-like protein in lung cells in contrast to what has been observed in lung tissue from mice, rats and humans [8]. The atomic emission technique significantly improved the determination of measured solutes compared with ECD. AED was also valuable for the monitoring of the prefractionation and to decrease the requirements of the sample clean-up.

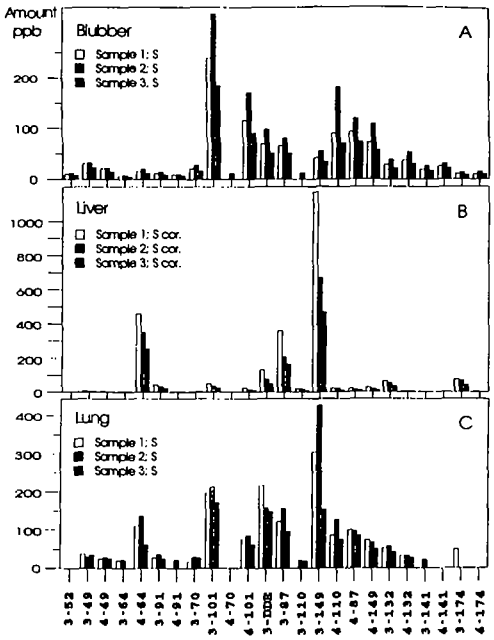

Figure 1 : Comparison of the patterns of the $MeSO_2$-CB concentrations in blubber (A), liver (B) and lung (C) for three seals estimated by GC-AED with sulfur selective detection.

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