Isolation and determination of methylsulfonyl-PCBs in biological samples by sulfuric acid partitioning, adsorption chromatography and GC-ECD

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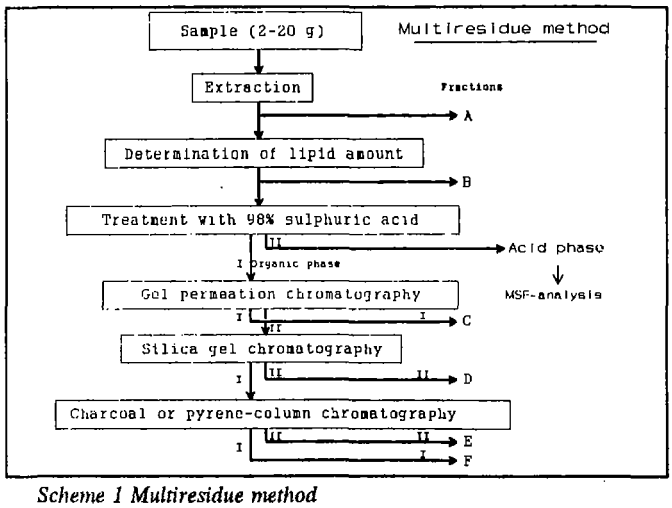
Methylsulfonyl ($MeSO_2$) metabolites of polychlorobiphenyls (PCBs) have been found in humans[1], in tissues of animals fed with PCBs[2] and in free living mammals such as seals and otter[3,4]. They are presumably formed from PCBs that possess two adjacent non-chlorine substituted carbon atoms in one of the phenylrings. The mechanism is supposed to proceed via an areneoxide intermediate and glutathione conjugation[5]. Theoretically over 840 $MeSO_2$-PCB congeners are possible, but until now not more than some 80-90 congeners have been synthesized[6], and the number of congeners positively identified in biological samples amounts to about 60. Effects of methylsulfonyl metabolites have been shown, e.g., in mice[7] and respiratory stress in Yusho victims is probably related etiologically to the $MeSO_2$-PCBs[7].

Most of the analytical methods used at present are not developed for monitoring purposes. The aim of the present work is to develop an analytical method for aromatic methylsulfonyl compounds in the same piece of sample that is used for the determination of other organic micropollutants, e.g. DDT, PCB, pesticides, planar compounds .

In our laboratory a multiresidue method has been developed[8] for the analysis of a variety of persistent organic microcontaminants (see Scheme 1). This method results in 6 fractions, each containing a particular set of contaminants from the original sample. Until now, the $MeSO_2$-PCBs and other methylsulfonyl metabolites were not recovered in the existing method.

Aromatic methylsulfonyl compounds dissolve in sulphuric acid without decomposing. In the multiresidue method, sample extracts are treated with sulphuric acid. The acid layer, which is usually discarded, contains the methylsulfonyl



Scheme 1 Multiresidue method

compounds. These may be recovered when the acidic phase is treated by additional liquid/liquid extraction and clean-up steps.

Experimental

Biological samples were homogenized and - after addition of the internal standard (3-methyl-4-$MeSO_2$-2,2'4'5,5'pentachlorobiphenyl) - extracted according to a method described earlier[8]. Extracts were concentrated to 1 ml and 1 ml of concentrated sulfuric acid was added. After shaking and centrifuging, the organic phase was decanted and stored for analysis of PCBs. The acidic phase was placed in an icebath and diluted 1:5(v:v) with cold distilled water. The resulting aqueous solution was extracted twice with 2ml n-hexane. Combined organic layers were concentrated and transferred to 0.5 ml of a 9:1 (v:v) n-hexane:diethyl ether mixture and cleaned up on an aluminiumoxide (1.0g) adsorption chromatographic column (slightly deactivated with water), eluting with 12 ml 9:1(v:v) n-hexane:diethyl ether and subsequently with 9 ml diethyl ether. The $MeSO_2$-PCB fraction eluted in ml 11-20. Additional clean up was performed by concentrating the fraction from the alumina column to 1 ml (n-hexane), transferring this to a second column containing 1 g of silica and eluting with 5ml n-hexane and 11 ml of n-hexane:diethyl ether (75:25, v:v). The $MeSO_2$-PCB fraction eluted in ml 7-15. This fraction was concentrated to 0.5 ml, 1 ml iso-octane containing the injection standard (PCB 189) was added and the solution was further concentrated to 1 ml. 2 μl of the final solution was injected (splitless) into a Hewlett-Packard HP5890 GC equipped with a 63Ni ECD, and analyzed using a 25m x0.2mm HP Ultra-2 fused silica column (film thickness, 0.33 μm) and the following temperature programme: 90°C(1.5 min)-30°/min-200°-5°/min-280°. He was used as the carrier gas.

The identity of the $MeSO_2$-PCBs was assessed by relative retention times and incidentally confirmed using a HP GC-MSD, operating in the EI mode (70 eV) and equipped with the same type of column as the GC-ECD instrument.

All materials used were analytical grade. All glassware was thoroughly rinsed with organic solvents. Solvents were distilled before use. All $MeSO_2$-PCBs and 3-$MeSO_2$-DDE standards were a gift of the Wallenberg Laboratory, Stockholm University.

Internal standards were PCB-189 (injection standard) and 3-$MeSO_2$-4-methyl-2',3',4',5,5'-pentachlorobiphenyl (procedural standard).

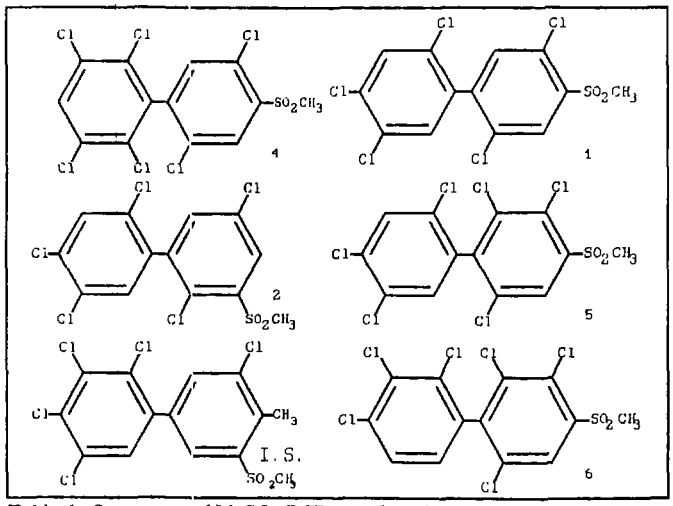


Table 1. Structures of $MeSO_2$-PCBs used in this study

1: 4-$MeSO_2$-253'4'5'-pentaCB, 2: 3-$MeSO_2$-253'4'5'-pentaCB, 4: 4-$MeSO_2$-252'3'5'6'-hexaCB, 5: 4-$MeSO_2$-2352'4'5'-hexaCB, 6: 4-$MeSO_2$-2352'3'4'-pentaCB, i.s. internal standard

Results and discussion

Quality control

Several experiments were carried out to establish the quality of the data. First, the recovery of the extraction and subsequent acid partitioning was assessed by applying a standard solution of $MeSO_2$ compounds to these procedures. Next, the recovery of the clean up on the aluminiumoxide column was established using the same standard solution. Finally, recoveries of $MeSO_2$ compounds for the entire procedure were assessed. The results are shown in Table 2. Recoveries for the compounds from Table 2, when analyzed according to Schemes 1 and 2, are all within the 75-100 % range.

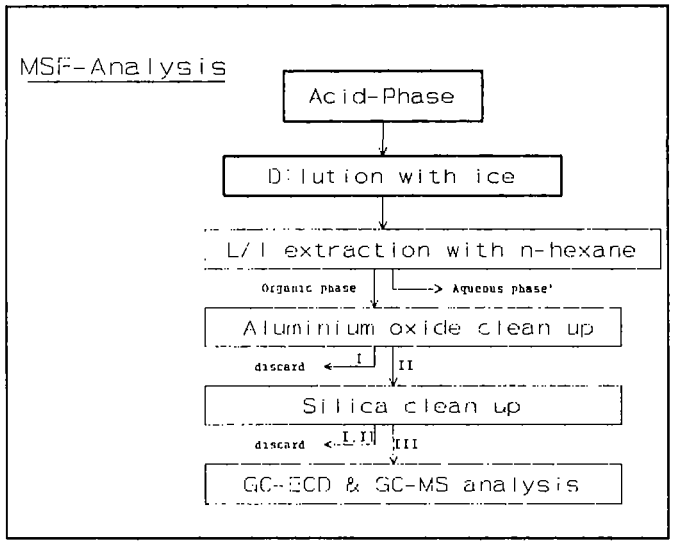
Table 2. Results of recovery experiments of a standard solution for control of the analytical procedure (all results based on 2 or 3 replicates)

Compound (Code: see Table 1)	*Recovery (%) Acid treatment*	*Al_2O_3 Clean up*	*Whole procedure*
1	*93*	*95*	*93*
2	*94*	*94*	*94*
4	*93*	*94*	*84*
5	*86*	*nd*	*84*
6	*95*	*93*	*87*
3-$MeSO_2$-DDE	*104*	*95*	*77*
Int. standard	*100*	*84*	*100*

results of each treatment established in separate experiments

Analysis of biological samples

Here we show some results of a series of treatments which are summarized in Scheme 2. The $MeSO_2$-metabolites (see Table 1) can be partitioned into an apolar phase by first diluting the acid phase with water and subsequently shaking with, e.g., n-hexane. Subsequent clean up is necessary, as can be seen from Figures 1 and 2. This was achieved through common absorption chromatography methods (see e.g.[9]). The method was applied to tissue samples of otter and Grey seal and to White-tailed eagle eggs.



Scheme 2. Further treatment of $MeSO_2$-fraction

Seal blubber samples were properly cleaned by aluminium oxide only (Fig. 1), whereas for otter liver aluminium oxide clean up is unsatisfactory and an additional silica

clean up was necessary to remove substances interfering with the ECD (cf. Fig. 2). The same preliminary result was observed for White-tailed eagle eggs.

As can be seen from Fig. 3, the peak patterns in otter liver and Grey seal blubber are entirely different. Among the major peaks in otter liver are 3-$MeSO_2$-2,2',4',5,5'-pentaCB and 4-$MeSO_2$-2,2',3',5,5',6'-hexaCB, whereas e.g. 3-$MeSO_2$-DDE was not present (see Fig. 3a and b). These findings and the peak pattern found are in agreement with the findings of Haraguchi et al.[4] who compared grey seal blubber and liver and otter blubber. Some earlier eluting (retention times: 15-27 min) ECD-sensitive peaks are also present in the otter liver extract. Some of these compounds are perhaps $MeSO_2$-benzenes or methylthio-metabolites, both of which have been reported to be present in animals and plants[10]. In seal blubber (cf. Fig. 3b), more $MeSO_2$-PCB peaks were found than in the otter liver extract. Among the major peaks were 3-$MeSO_2$-2,2',4',5,5'-pentaCB, 4-$MeSO_2$-2,2',4',5,5'-pentaCB, 4-$MeSO_2$-2,2',3',5,5',6'-hexaCB, 3-$MeSO_2$-DDE, 4-$MeSO_2$-2,2',3,4',5',6-hexaCB and 4-$MeSO_2$-2,2',3,3',4',6-hexaCB. The peak pattern was almost identical to the pattern found in the study of Haraguchi et al.[4]. The concentration levels (Table 3) found in five species of Grey seal from the Baltic range from 0.5-3 $\mu g.g^{-1}$ (lipid weight) for the sum of five $MeSO_2$-PCBs, 0.1-0.3 $\mu g.g^{-1}$ $MeSO_2$-DDE and 35-80 $\mu g.g^{-1}$ total PCBs. The mean ratio (sum of five $MeSO_2$-PCBs/total PCB) in Grey seal blubber is 2.5%. The ratio (total $MeSO_2$-metabolites/total PCBs) is estimated to be between 5 and 10%, which corresponds to literature findings[4].

Finally, it is interesting to note that in White-tailed eagle eggs analysed (chromatograms not shown here) no methylsulfone metabolites could be detected above the detection limit, despite the PCB concentrations levels in the eggs, which were in a range similar to the PCB concentrations found in seal blubber. Somewhat speculatively, this would imply that the metabolic pathway present in mammals does not occur in the egg and that if such a pathway would be present in the bird body, the resulting methylsulfones do not seem to be transferred to the egg.

Table 3 *Concentrations of $MeSO_2$-metabolites and PCBs found in biological samples (in $\mu g.g^{-1}$ on lipid base)*

Sample	*Total PCB*	*Sum of 5 $MeSO_2$-PCBs*	*3-$MeSO_2$-DDE*
Grey seal Blubber			
Seal 1	*63*	*1.0*	*0.18*
Seal 2	*69*	*0.7*	*0.14*
Seal 3	*77*	*2.9*	*0.52*
Seal 4	*37*	*0.6*	*0.17*
Seal 5	*42*	*1.4*	*0.19*
Otter liver	*nd*	*2.1*	*< 0.04*
White-tailed eagle egg			
egg 1	*50*	*< 0.04*	*< 0.04*
egg 2	*170*	*< 0.04*	*< 0.04*

Acknowledgements

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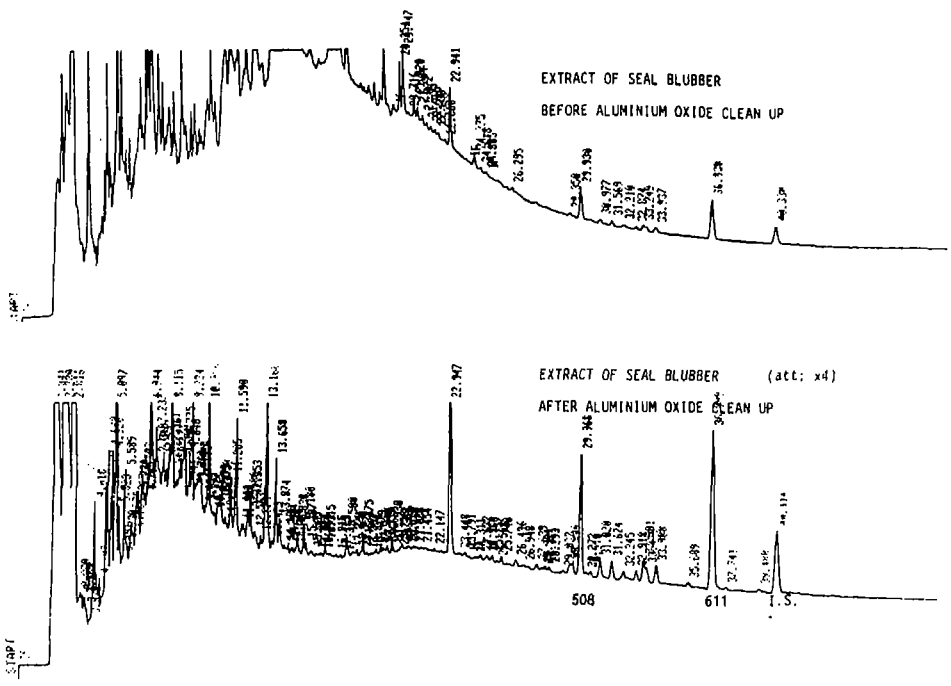

Figure 1. Effect of clean up over Al_2O_3 on extracts of Grey seal

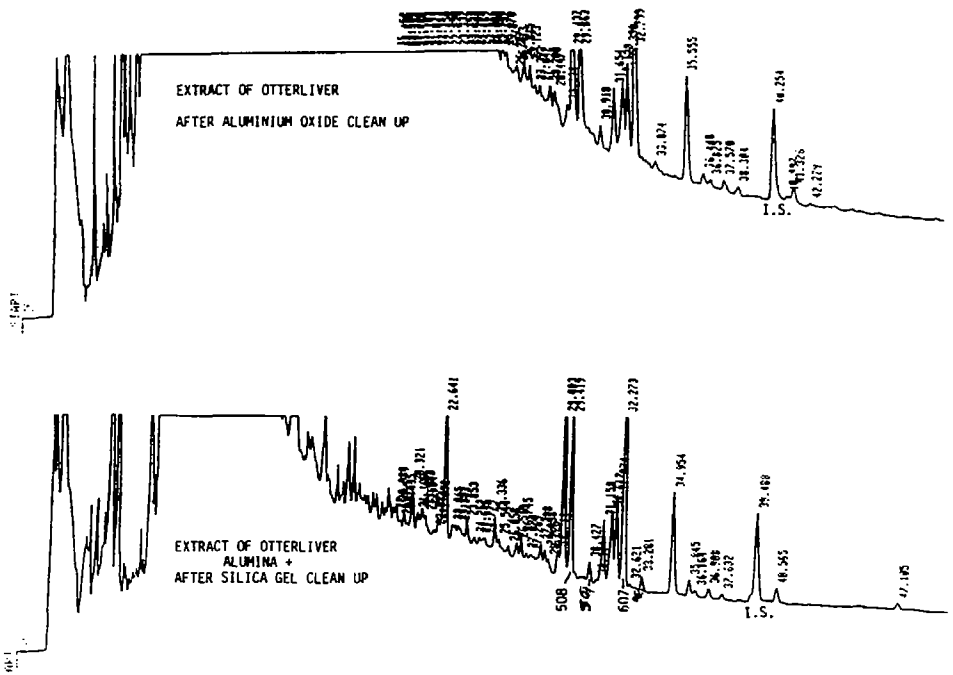

Figure 2. Effect of silica clean up on extract of otter liver after Al_2O_3 clean up

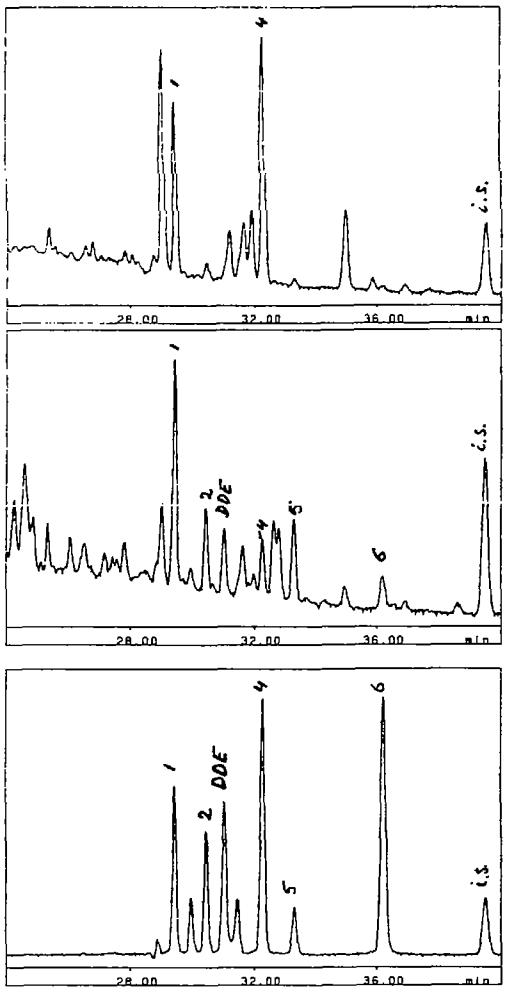

Figure 3. GC-ECD chromatograms of extracts of a (top) otter liver, b (middle) Grey seal blubber, c (bottom) standard mixture. Numbers refer to Table 1