A Column Chromatography Approach to PCB and DDT Methyl Sulphone Metabolite Isolation From Biological Matrices

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1. Introduction

Monitoring of methyl sulphone ($MeSO_2$-) PCB and -DDE metabolites in environmental samples has been largely ignored because they escaped detection in standard organochlorine analysis procedures. $MeSO_2$-PCB and -DDE compounds accumulate in fatty tissue, resist further metabolic degradation and have similar lipophilicity to other organochlorines. In liver, muscle and adipose tissues of wild mammalian species, at least 22 $MeSO_2$-PCB and 2 $MeSO_2$-DDE compounds have been structurally identified at total levels ranging from 2% to 20% of total PCB and DDE levels[1,2,3]. Some $MeSO_2$-PCB bind to specific proteins and induce P450 monooxygenase enzymes in laboratory rodents[4] and may have been involved in adrenocortical hyperplasia and other physiological abnormalities in Baltic seals[5].

Physicochemical characteristics amenable to tissue extraction and separation from other organochlorines (OCs), such as Lewis basicity, acid/base stability and polarity have been exploited in liquid phase partitioning-based methodologies used so far. Analytical approaches have varied considerably, are laborious, potentially destructive to other OCs, give variable recoveries, and have had limited validation for quantiative analysis[3,6]. Column chromatography in combination with gel-permeation chromatography is a more dependable alternative to liquid phase partitioning. This combination of techniques takes advantage of the polarity characteristics of aryl methyl sulphone compounds, which are higher than those of most other biologically persistent OCs, and the smaller molecular size of aryl methyl sulphones than that of biogenic coextracting compounds, mainly triglycerides. We have developed such an approach facilitating the simultaneous determination of OCs and $MeSO_2$-PCB and -DDE metabolites from a variety of animal tissues. The method is potentially suitable for determination of hydroxy-PCB (HO-PCB) metabolites. The method was validated by replicate determination of percent recoveries of: 1) a standard mixture of 15 $MeSO_2$-PCBs and 3-$MeSO_2$-DDE which are biologically significant and an internal standard (IS) of 3-$MeSO_2$-2-CH_3-5-2',3',4',5'-Cl_5CB (IS) spiked to herring gull egg, whole smelt, polar bear liver and polar bear adipose tissue homogenates, and 2) 15 $MeSO_2$-PCBs 3-$MeSO_2$-DDE, IS, 11 PCBs and tris(4-chlorophenyl)methanol (TCPM) spiked to contaminant-free lipid extracts of the same samples

prepared by gel-permeation chromatography. $MeSO_2$-PCB and -DDE method detection limits (MDL) determined by GC/ECD (gas chromatography/electron capture detection) and GC/NIMS (gas chromatography/methane chemical ionization, negative ion mass spectrometry) are compared to instrumental detection limits (IDL) to demonstrate sample clean-up efficiency.

2. Methods

Tissue Samples. Samples archived at -40°C in the specimen bank at the National Wildlife Research Centre in Hull, Quebec, Canada were used. Lipid extract weights and $MeSO_2$-PCB and -DDE spiking levels (Table 1) were chosen to simulate co-extracted lipid loads and $MeSO_2$-metabolite levels encountered in the analysis of polar bear liver and adipose tissue[1,2,3].

Chemical Standards. $MeSO_2$-PCB and -DDE and 3-$MeSO_2$-2-CH_3-5-2',3',4',5'-Cl_5CB (IS) standards were synthesized as described previously[7]. We thank Dr. Hiroaki Kuroki for the gift of some of the $MeSO_2$-PCB standards. The $MeSO_2$ metabolite chemical name was abbreviated and simplified (Table 1) from the IUPAC derived numbering system of PCBs. The 3M- and 4M- prefix denotes the *meta*- and *para*- positions of the $MeSO_2$-functional group relative to the biphenyl carbon-carbon bond. The PCB mixture was the NRC (National Research Council of Canada) standard CLB-1, solution D. Tris(4-chlorophenyl)methanol (TCPM) was purchased from MTM Research Chemicals.

Analytical Methodology. Extraction and separation is outlined in Figure 1. The procedure forextraction, gel-permeation chromatography (GPC) and OC elution from Florisil® substantially followed the method of Norstrom et al.[10] (F1, PCBs: 35 mL hexane; F2, chlordanes: 36 mL 15% dichloromethane/hexane; F3, TCPM: 60 mL 50% dichloromethane/hexane; F4, aryl methyl sulphones: 80 mL of 7% methanol/dichloromethane), except that a 33% KOH/silica gel column, eluted with 50 mL 50% dichloromethane/hexane, was interposed between GPC and Florisil® chromatography. The alumina column was eluted first with 10 mL 50% dichloromethane/hexane, and $MeSO_2$-PCBs and -DDE were eluted with a further 40 mL of the same solvent mixture. All GC/ECD and GC/NIMS conditions have been described elsewhere[1]. GC/NIMS analysis for $MeSO_2$-PCBs and 3M-DDE was performed by 1) total ion monitoring (GC/TNIMS) from 83 to 550 amu, 2) single ion monitoring (GC/SNIMS) of the dominant $(M)^-$ and $(M+2)^-$ ions in the molecular ion cluster of each of the $MeSO_2$-PCB tetra- to hepta-chloro isomer groups, IS and 3M-DDE (i.e. m/z of 368/370, 404/406, 438/440, 472/474, 418/420 and 394/396, respectively). Percent recovery (Table 1) was determined by external GC/ECD peak area comparison to individual standards. Spiked $MeSO_2$-PCB and -DDE levels approximately matched endogenous levels in polar bear liver and adipose substrates to avoid disproportionate congener GC/ECD peak area subtraction. Approximately 1 ng/µl per congener of the $MeSO_2$-PCB and -DDE mixture was serially diluted for IDL determination according to the procedure outlined elsewhere[11]. Replicate (n=7) 0.5 g wet weight equivalents of polar bear liver, contaminant-free lipid extract were spiked with the standard mixture at concentrations near the IDL for MDL determination.

3. Results and Discussion

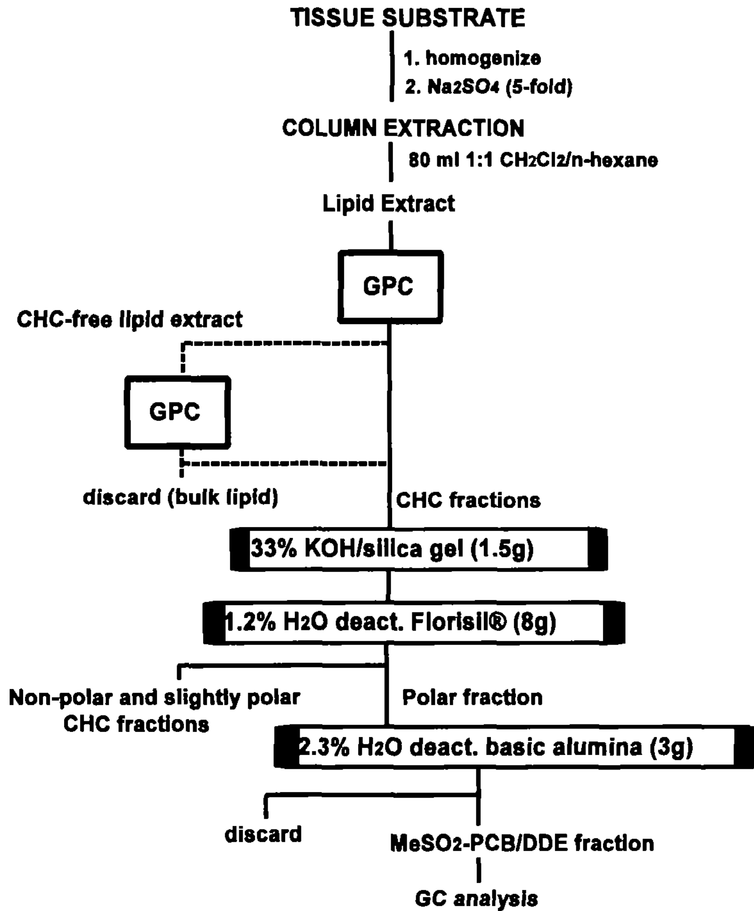

Figure 1. Analytical methodology schematic for CHC and $MeSO_2$-PCB and -DDE sample analysis. Chromatographic steps in boxes.

Sodium sulphate sample dehydration prior to spiking maximized penetration of $MeSO_2$-PCB and -DDE standard spike into tissue matrix (Figure 1). The size exclusion and non-destructive characteristics of gel permeation chromatography (GPC) and the use of 1:1 DCM/n-hexane at flow rates of ~5 ml/min permitted >90% separation of the long-chain lipids[8]. Incomplete sample takeup by the GPC was the crucial step in reducing overall contaminant recovery. The recoveries (Table 1) of ~80% can be improved if GPC operation is optimized. A 33% KOH/silica gel column step was incorporated to assist in removing residual lipids, since approximately half the total lipid extract weight could be removed using this column (not shown). Bergman et al.[9] have successfully partitioned HO-PCB compounds from plasma into alcoholic base. The KOH/silica gel column step will most likely trap HO-PCBs efficiently. We are currently testing the possibility of recovering HO-PCBs from the KOH/silica gel column for quantitative analysis. A 1.2% H_2O deactivated Florisil® column has been used for clean-up and separation of neutral to slightly polar OCs for polar bear fat[10]. An additional 7% MeOH/DCM fraction effectively eluted the $MeSO_2$-PCB and -DDE compounds with improved isolation from residual biogenic material in contrast to neat MeOH, which was applied recently to Florisil® clean-up of Baltic seal samples[12]. Final separation from residual polar interferences was achieved with 2.3% H_2O deactivated basic alumina.

Absolute mean recoveries of PCB, $MeSO_2$-PCB and -DDE congeners in the four tissue types were ~75-83% (Table 1), similar to chemical partitioning methodologies[3,6,7]. Polar bear liver is high in complex organic compounds such as retinyl esters, whereas egg extract contains cholesterol. The constancy of the recovery among tissue matrix types and amounts (wet weight 0.5-15g and lipid weight 0.01-0.66 g) attested to the ruggedness of the methodology. The low absolute recovery standard deviation of 3-5% indicated there was little discrimination by number and position of chlorines. Variation in replicate analysis (3-13%) was also low. The mean congener percent recovery relative to the IS ranged from 88%±6% to 108%±7% for all substrates, indicating that recovery of the IS is a good measure of overall $MeSO_2$-PCB and -DDE recovery in environmental samples. PCB-121 elutes in an area of the chromatogram relatively free of interference in biological samples, and it is not present in Aroclors®. If PCB-121 was chosen as an example for internal standard corrections, the corrected mean PCB recoveries for the contaminant-free lipid extracts would range from 94%±6% to 113%±10% (Table 1). However, recoveries of lower chlorinated PCB congeners can be lower than in those of higher chlorinated PCBs, e.g. in herring gull eggs (Table 1), due to volatility losses. Therefore, a range of low to high chlorinated PCB internal standards is preferable to one in the mid-range.

The IDL ranges for the various $MeSO_2$-PCB and -DDE congeners were 0.02-0.13 pg for GC/SNIMS, 0.18-0.31 pg for GC/ECD, and 0.33-6.0 pg for GC/TNIMS. The MDL ranges were 0.11-0.71 pg for GC/SNIMS and 1.5-2.7 pg for GC/ECD. The MDL for GC/TNIMS has yet to be determined. These MDLs were lower than previously attained[3,6,7,11], and also indicated the clean-up efficiency of the method. GC/NIMS may be useful for ultratrace determination of $MeSO_2$-PCB compounds since its sensitivity is 500-1000 times greater than electron impact (EI)[7]. However, non-linearity of GC/NIMS at low and sub-pg levels limits the practical lower limit of quantitative analysis to 11-75 pg. The practical lower limit of quantitative analysis was lowest for GC/ECD, 11-30 pg, although analyte selectivity of ECD was not as good as NIMS. The samples analyzed to date did not appear to have significant co-eluting, electron-capturing compounds in the tetrachloro- to heptachloro-$MeSO_2$-PCB range of the chromatogram.

Table 1. Percent recovery of individual MeSO2-PCBs and -DDE and PCBs determined by GC/ECD from various tissue matrices

			Contaminant-free lipid extracts *				Tissue substrates **			
			Herring Gull Egg	Smelt	Polar Bear Liver	Polar Bear Adipose	Herring Gull Egg	Smelt	Polar Bear Liver	Polar Bear Adipose
		% Lipid					10.0	4.4	2.7	65.0
		Lipid extract wt. (g)	0.18	0.15	0.019	0.65				
		Wet wt. (g)					5.0	15.0	0.5	0.5
Congener Structural Formula	Compound Abbreviation	Spiking level *** (pg/ul)								
MeSO2-PCB and -DDE										
3-MeSO2-2,2',4',5-Cl4CB	3M-CB49	312	78±4	75±6	71±1	79±2	74±6	74±2	85±5	82±6
4-MeSO2-2,2',4',5-Cl4CB	4M-CB49	228	75±6	76±7	74±2	77±2	83±5	77±2	82±5	84±6
4-MeSO2-2,4',5,6-Cl4CB	4M-CB64	272 [136]	81±6	76±8	79±3	77±5	81±6	80±1	82±4	82±10
3-MeSO2-2,2',4',5,5'-Cl5CB	3M-CB101	264	70±7	74±2	72±1	79±1	75±6	83±3	80±5	84±7
4-MeSO2-2,2',4',5,5'-Cl5CB	4M-CB101	220 [330]	74±6	78±6	76±4	89±5	65±4	77±5	84±5	80±6
3-MeSO2-DDE	3M-DDE	595 [36]	85±5	78±8	71±1	78±2	74±4	75±1	88±.6	78±5
3-MeSO2-2,2',3',4',5-Cl5CB	3M-CB87	368 [552]	74±3	72±3	71±2	79±4	71±3	73±1	87±.5	77±6
3-MeSO2-2,2',4',5,5',6-Cl6CB	3M-CB149	472	82±4	77±8	79±4	82±3	68±4	80±4	n.s.	n.s.
4-MeSO2-2,2',3',4',5-Cl5CB	4M-CB87	368	83±4	75±8	76±4	79±2	80±6	76±2	n.s.	n.s.
4-MeSO2-2,2',4',5,5',6-Cl6CB	4M-CB149	211 [318]	83±7	78±7	83±6	87±2	72±10	75±2	87±5	85±10
3-MeSO2-2,2',3',4',5,6-Cl6CB	3M-CB132	414	80±5	76±9	77±2	78±1	77±4	81±2	n.s.	n.s.
4-MeSO2-2,2',3',4',5,6-Cl6CB	4M-CB132	604 [302]	81±6	78±9	78±3	79±3	72±3	80±2	75±8	88±4
3-MeSO2-2,2',3',4',5,5'-Cl6CB	3M-CB141	284 [109]	77±5	75±9	72±2	78±3	77±3	88±3	71±7	86±3
4-MeSO2-2,2',3',4',5,5'-Cl6CB	4M-CB141	436 [71]	80±7	75±8	76±3	79±3	-	75±1	81±7	85±2
3-MeSO2-2-CH3-2',3',4',5,5'-Cl5CB	IS	436	74±2	72±1	74±2	79±3	86±2	75±2	77±4	80±3
3-MeSO2-2,2',3',4',5,5',6-Cl7CB	3M-CB174	440 [44]	67±4	69±4	77±2	81±4	-	78±3	90±9	83±6
4-MeSO2-2,2',3',4',5,5',6-Cl7CB	4M-CB174	316 [32]	75±2	75±4	79±4	82±2	-	80±1	88±4	88±6
Absolute mean percent recovery			78±5	75±3	76±4	80±3	77±5	78±4	83±5	83±3
Relative mean percent recovery ****			105±7	105±3	102±5	102±4	88±6	104±5	108±7	104±4
PCB and TCPM										
2,2',5-Cl3CB	CB-18	295	71±4	79±.3	75±1	74±1				
2,2',6,6'-Cl4CB	CB-54	415	71±6	76±1	79±1	77±1				
2,4',5-Cl3CB	CB-31	165	71±6	77±3	84±3	76±4				
2,2',4',5-Cl4CB	CB-49	190	74±7	78±4	78±3	78±4				
2,2',3,5'-Cl4CB	CB-44	148	69±5	80±4	77±2	77±2				
2,2',3,3'-Cl4CB	CB-40	122	67±3	79±4	90±7	77±3				
2,3',4,5',6-Cl5CB	CB-121	78	67±5	81±2	90±3	78±3				
2,2',3,4,5-Cl5CB	CB-86	72	77±2	84±.3	81±5	82±1				
2,2',3',4',5-Cl5CB	CB-87	95	69±4	77±4	89±5	80±1				
3,3',4,4'-Cl4CB	CB-77	138	74±4	78±.3	83±2	81±2				
2,2',4,4',5,5'-Cl6CB	CB-153	52	86±7	77±2	90±3	86±2				
2,3,3',4,5,5'-Cl6CB	CB-159	30	84±5	84±2	88±9	80±3				
2,3,3',4,4',5-Cl6CB	CB-156	38	81±6	80±.4	82±3	78±2				
tris(4-chlorophenyl)methanol	TCPM	500	86±5	79±.2	82±3	78±3				
2,2',3,3',4,4',5,5',6,6'-Cl10CB	CB-209	42	84±7	81±.3	85±4	82±2				
Absolute mean percent recovery			75±7	79±1	84±5	79±3				
Relative mean percent recovery *****			113±10	98±3	94±6	101±4				

* n=3 replicate spiked matrices (standard deviation (S.D.) in brackets)
** calculated by difference from spiked (n=3) and unspiked (n=3) samples (S.D. in brackets)
*** spike amounts in square brackets applicable to polar bear liver and adipose tissue substrates only
**** relative to IS
***** relative to PCB-121
n.s. = no spike

4. References

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