$MeSO_2$-PCBs IN HUMAN BLOOD FROM SWEDEN, THE FAROE ISLANDS AND SLOVAKIA

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Introduction

The $MeSO_2$-PCBs were first reported in 1976 as metabolites of polychlorinated biphenyls (PCBs) detected in grey seal blubber[1], as experimentally determined metabolites of 2,2',5,'-tetrachlorobiphenyl (CB-52)[2] and as metabolites being retained in mouse lung tissue after dosed with certain PCB congeners[3]. The metabolic route of $MeSO_2$-PCBs formation was not clarified until the early 1980's through a series of studies performed under the guidance of J. Bakke[4]. The impact of the gastrointestinal micro flora was evident after studies applying bile duct cannulated rats and germfree animals [4,5]. The identification of $MeSO_2$-PCBs in wildlife initiated extensive efforts to synthesise $MeSO_2$-PCB congener standards which lead to identification and quantification of about 30 $MeSO_2$-PCB congeners in marine mammals[6]. $MeSO_2$-PCBs are much more abundant metabolites in mammals than in birds and fish, recently reviewed by Norström[7].

Efforts were not made until rather recently to detect and to quantify $MeSO_2$-PCBs in human blood. In humans, they were first detected in a woman working at a capacitor factory in Japan in 1970's[8]. Our present knowledge on $MeSO_2$-PCB concentrations in humans is summarized in Table 1. Around 40 $MeSO_2$-PCB congeners have been indicated in human tissues[9]. From a temporal trend study on banked (1972-1992) mother's milk samples in Sweden, the levels (sum of 23 congeners) were between 2 and 9 ppb on fat weight basis[10].

The aim of this study is to compare and discuss the methylsulfonyl-PCB pattern in humans living close to a heavily PCB contaminating area in Eastern Slovakia, a Swedish cohort of men and selected subject pairs (mothers and their 7 years old children) with the highest content of PCB within a larger cohort due to their life style (diet) in The Faroe Islands.

Material and methods

Study groups: Ten Swedish men participated in this study by giving plasma at four different occasions; 1988, 1991, 2001 and 2002[11]. Serum from nine Faroese mothers and from their seven years old children with the highest PCB concentrations were selected for analysis from the cohort presented elsewhere[12]. Also, 122 and 175 serum samples from Slovakia were analysed representing people living in the Michalovce district (known PCB contaminated area) and the Stropkov/Svidnik (reference) area, respectively[13].

Chemical analysis: The chemicals used, extraction of serum, lipid determination, partitioning with an aqueous alkaline solution, procedure for analysis have been described in detail elsewhere[13,14]. Lipids were removed from the extracts by use of concentrated sulfuric acid. The details for the analysis from the samples from Slovakia can be found elsewhere[13]. The sulfuric acid phase from the Swedish and Faroese samples was kept after other studies and worked up as will follow below.
The sulfuric acid phase (2 ml) was dissolved with equal volumes of deionised water and extracted twice with n-hexane (4 ml). After reduction of the solvent under a gentle stream of nitrogen the samples were further purified on a multilayer column prepared as follow: A Pasteur pipette packed from bottom to top with 0.1 g of activated silica gel (300°C over night), 0.4 g of impregnated silica with 0.85 M potassium hydroxide (2:1, weight basis) and 0.8 g of silica gel with sulfuric acid (90%) (2:1, weight basis). This column was first eluted with a mixture of n-hexane : dichloromethane (1:1, 8 ml, discarded) and afterwards with pure dichloromethane (28 ml) containing the $MeSO_2$-PCBs and $MeSO_2$-DDE. The solvent was evaporated and replaced by n-hexane to a final analysis volume of 50 µl. One microliter was used for the instrumental analysis. The reference compounds used were synthesised in house.

Table 1. Median and range (min-max) concentrations (ng/g lipid weight) of methylsulfonyl-PCBs and -DDE in humans from four European countries and from Canada. The data provided from plasma, in bold, are discussed in this abstract.

Country	year[a]	n	4'-$MeSO_2$-CB87	4'-$MeSO_2$-CB101	4-$MeSO_2$-CB149	Σ$MeSO_2$-PCB	3-$MeSO_2$-DDE	No of congeners[b]
Plasma								
Slovakia	**2001**							
Stropkov/Svidnik		**175**	**0.24 (LOQ-1.4)**	**0.22 (LOQ-1.4)**	**1.0[c] (0.19-9.2)**	**1.5 (0.34-11)**	**3.5 (0.31-73)**	**3**
Michalovce[13]		**122**	**0.85 (LOQ-22)**	**0.75 (LOQ-9.9)**	**2.7[c] (0.25-72)**	**4.2 (0.41-100)**	**6.3 (0.40-81)**	**3**
Faroe Islands								
maternal	**1994/95**	**10**	**5.1 (1.9-15)**	**4.5 (1.6-11)**	**7.5[c] (2.4-25)**	**73 (24-189)**	**3.6 (1.2-12)**	**11**
children	**2000/01**	**10**	**4.0 (1.7-6.5)**	**3.6 (1.3-6.6)**	**8.6[c] (3.4-17)**	**70 (23-103)**	**1.8 (0.34-7.6)**	**11**
Sweden	**2002**	**9**	**0.88 (0.14-2.4)**	**0.50 (0.14-1.5)**	**1.2[c] (0.28-4.6)**	**5.6 (0.90-17)**	**0.86 (LOQ-5.2)**	**11**
Sweden[15]	1997[d]	11				2.0 (0.81-5.6)		17
Milk								
Sweden[10]	1972	p[e]/75	2.1	0.78	2.0	9.2	5.1	23
Sweden[10]	1984/85	p[e]/102	0.70	0.32	0.64	3.1	0.79	23
Sweden[10]	1992	p[e]/20	0.33	0.13	0.35	1.6	0.46	23
Canada[16]	1992	50	0.33[f]	0.087[f]		0.81[f]	0.26[f]	12
Adipose tissue								
Sweden[17]	1997[d]	7	0.82 (0.48-3.6)	0.40 (0.21-2.6)	0.71 (0.41-5.1)	2.9 (2.0-9.0)	1.4 (0.12-8.9)	24
Sweden[18]	1994	5	1 (0.2-2)	0.3 (0.2-1)	1 (0.4-2)	6 (2)	9	24
Belgium[19]	2002	11	0.33[g] (0.13-0.98)	0.27[g] (0.12-0.93)	0.08[g] (nd-0.17)	1.57[g] (0.33-4.3)	1.2[g](nd-4.68)	26
Liver								
Sweden[17]	1997[d]	7	1.2 (0.61-2.61)	0.54 (0.18-1.5)	1.7 (0.69-11)	28 (12-358)	13 (0.41-31)	24
Sweden[18]	1994	5	1 (0.4-2)	0.3 (0.2-2)	1 (0.1-11)	34 (12-358)		24
Belgium[19]	2002	11	0.35[g] (nd-0.87)	0.23[g] (nd-1.0)	0.10[g](nd-0.42)	9.3[g] (1.7-27)	4.7[g] (1.0-21.9)	26
Lung								
Belgium[19]	2002	11	0.55[g] (nd-1.1)	0.46[g] (nd-1.2)	0.08[g] (nd-0.89)	2.7[g] (nd-12)	2.8[g] (nd-2.7)	26

nd=not detected; LOQ=limit of quantification; [a] Sampling year; [b] Number of $MeSO_2$-PCB congeners in the Σ$MeSO_2$-PCB; [c] include concentration of the $MeSO_2$-hexaCB (unknown); [d] the publication year; [e]pooled sample/number of subjects in the pool; [f] Data has been recalculated for a lipid content of 4%; [g] Mean values

Identification and quantification on GC-MS were performed using a Finnigan TSQ 700 mass spectrometer coupled with a Varian GC 3400 with a split/splitless injector operating in electron capture chemical ionization (ECNI) mode, tracing selected ion monitoring (SIM) for the masses corresponding to the $[M]^-$ and $[M+2]^-$ of tetra- to heptachlorinated $MeSO_2$-PCBs. A J&W DB5 MS capillary column (30 m × 0.25 mm i.d. and 0.25 µm film thickness) was used with temperature program of 80ºC (2 min) – 20ºC/min – 230ºC – 3ºC/min – 300ºC (5 min). Helium was used as carrier gas. The electron energy was 70 eV using methane as buffer gas. The transfer line temperature was 280ºC and the temperature in the ion-source was 150ºC.

Results and Discussion

The concentrations of $\Sigma MeSO_2$-PCB (11, 7 and 11 congeners) in serum from Swedish men analysed at four different occasions, the Michalovce and Stropkov/Svidnik (Slovakian cohorts) and mothers at delivery and their infants 7 years later (the Faroese groups) are presented in Figure 1. The percentage of the $MeSO_2$-PCB metabolites compared to the PCBs in serum is approximately 0.05 %. The Swedish fishermen and the Slovakian cohort living at the PCB contaminated site are rather similar and clearly different (higher) than the human subjects from the control area. A five fold higher concentration is observed in the Faroe Island subjects (Figure 1). An interesting aspect by comparing the Faroe Islands mother and child is that the levels are similarly high, which may be an indication of transfer from mother to child. The high Faroese level of $MeSO_2$-PCBs may be explained by another dietary intake of either PCBs or $MeSO_2$-PCBs.

A pooled sample was prepared from all sulfone samples of the Slovakian subjects and was analysed in full scan mode by GC/MS (ECNI) and found to contain as many as 50 $MeSO_2$-PCBs and two $MeSO_2$-DDEs.

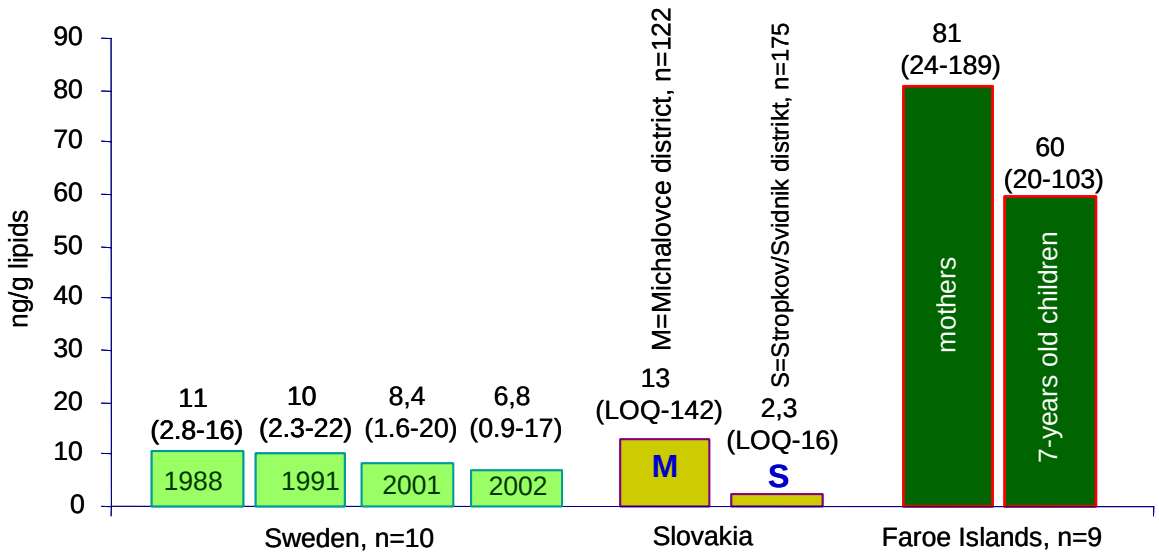

Figure 1. Mean concentrations of $MeSO_2$-PCB in men from Sweden, in humans from Slovakia and women and their 7 years old child's from the Faroe Islands. The mean values and ranges (min-max) in ng/g lipids are given.

In Figure 2, the internal distribution between the 4'-$MeSO_2$-CB101 and the 3'-$MeSO_2$-CB101 differ clearly between in the three countries. The pattern in the Slovakian cohorts, irrespective of district, shows a direct exposure of internally metabolized PCBs. On the other hand the Faroese population shows a different exposure pattern that possibly can be explained by their consumption of wildlife (pilot whale and fulmars). *Meta*-substituted $MeSO_2$-PCB have been indicated to dominate in wildlife[20]. The Swedish samples shows a mixture of directly metabolised PCBs and exposure of $MeSO_2$-PCBs dominated by *meta* substituted $MeSO_2$ PCB (Figure 2) from the high fish intake from the Baltic.

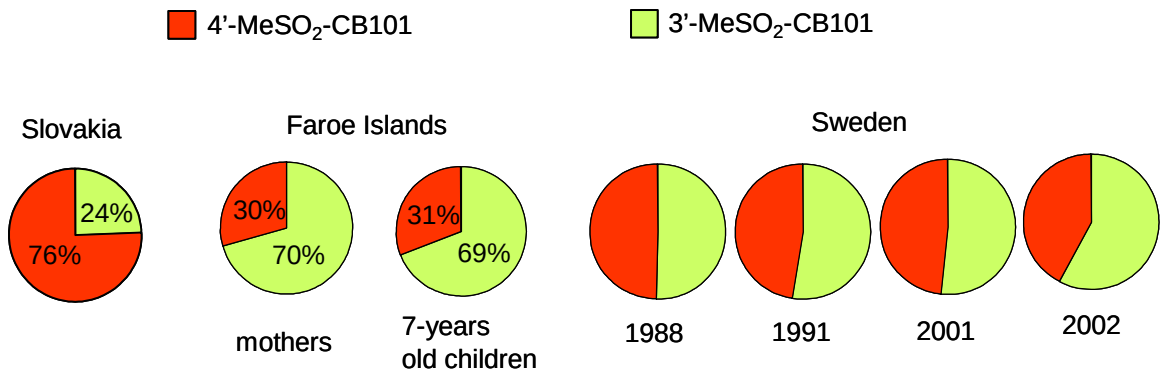

Figure 2. The 3'-MeSO$_2$-CB101 and 4'-MeSO$_2$-CB101 distribution in serum from humans from Slovakia, the Faroe Islands and Sweden.

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