Methyl sulphone metabolites of polychlorinated biphenyls and p,p´-DDE in Swedish human milk.

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Summary. The methyl sulphone metabolites of polychlorinated biphenyls and p,p´-DDE were extracted from pooled milk samples by liquid-gel partitioning using Lipidex 5000. Purifications and separations were made by column chromatography on aluminium oxide and gel permeation using Bio-Beads S-X3. Milk sampled in Stockholm during the periods from 1972 to 1991 was analysed. The levels of PCB and DDE methyl sulphones decreased during the time course studied and followed the decrease in the levels of total PCB and p,p´-DDE in the samples.

1. Introduction

In Sweden the use of polychlorinated biphenyls (PCBs) was restricted in 1972 and may presently be used only in closed systems (capacitors and transformers). Despite of the restricted use these compounds still circulate in the environment and are found in, e.g., adipose tissue and mother´s milk. DDE is a metabolite of DDT, which was a commonly used insecticide in the 1940´s-60´s. In Sweden the use of DDT was restricted in 1970 and totally banned in 1975. However, this pesticide is still used in certain countries with malaria problems[1)]. In the metabolism of lipophilic substances usually polar compounds are formed, which can be more easily excreted than the original compounds. However, the methylsulphonyl metabolites of DDE and certain chlorinated biphenyls are lipophilic and they accumulate in the body. The PCB methyl sulphones ($MeSO_2$-PCB) and DDE methyl sulphones ($MeSO_2$-DDE) were first identified in seal blubber from the Baltic[2)]. Since then such metabolites have been found in several species of animals and in man[3-6)]. It has also been shown that mice injected with $MeSO_2$-DDE will transfer the substance to their milk[7)]. These facts makes it of interest to analyse the levels of PCB and DDE methyl sulphones in human milk.

2. Experimental

Gas chromatography-mass spectrometry (GC/MS)
GC/MS analyses were performed with a VG 70-250 mass spectrometer equipped with a HP 5890A gas chromatograph and a VG-250 data system, VG Analytical. Gas chromatography was performed using a fused silica SE-54 capillary column (25m x 0.32mm I.D., 0.25 μm film thickness) with helium as a carrier gas. An all-glass falling

needle injector was used with a injector temperature of 270°C. The oven temperature was 190°C for 0.2 min, programmed to 230°C at 5°C/min, hold for 1 min, programmed to 235°C at 1°C/min, hold for 2.5 min, programmed to 260°C at 20°C/min and hold for 10 min. Electron ionization (EI) was performed in an EI only ion source at an electron energy of 30 eV. The temperature of the source was 260°C. The acceleration voltage was 6 kV and the resolution at 293 amu was 7000-9000. The MS was operated in a selected ion recording mode. For each compound two ions of the molecular ion cluster were monitored. Ions from perfluorokerosene was used as reference mass for correction of mass spectrometer drift (lock mass).

Samples
Pooled samples from the Mother's Milk Centre in Stockholm were analysed. Milk from immigrants was not included in the samples.

Method
Extraction. The extraction was performed according to previously described multicomponent method for organochlorine contaminants[8]. A sample of milk (10 ml) was weighed into a flask with a Teflon-lined screw cap. Internal standard (3-methylsulphonyl-4-methyl-5,2',3',4',5'-pentachlorobiphenyl) was added and thoroughly mixed with the milk. Then, formic acid (10 ml) was added and finally Lipidex 5000 (5.0 g). The mixture was shaken at 35°C for 2.5 h and then transferred to a column (2 cm I.D.). The solvent was drained and the gel was eluted with 30% metanol (40 ml) followed by 50% methaol (40 ml). Organochlorine compounds and some fat were eluted with acetonitrile (75 ml). Remaining lipids were eluted with chloroform/methanol/hexane (1:1:1, by vol.; 60 ml).

Fat determination. The two fractions containing lipids were taken nearly to dryness in a rotary evaporator at 35°C and dried to constant mass in a desiccator with silica gel. The sum of the residue of the two fractions, gravimetrically determined, defined the amount of fat in the sample.

Purification and separation. Partly deactivated aluminium oxide (5 g) was packed in a column (1 cm I.D.) and washed with hexane (10 ml). The residue from the acetonitrile fraction was transferred with hexane to the column. The sample on the column was concentrated by evaporation of hexane with a gentle stream of nitrogen. The column was eluted with hexane (10 ml) that elutes pesticides, PCBs, PCDDs and PCDFs, an additional portion of hexane (20 ml) elutes β-HCH and dieldrin. Finally, 50% dichloromethane in hexane (17 ml) was used for the elution of PCB and DDE methyl sulphones. Bio-Beads S-X3 (5 g) were equlibrated for 2 h in dichloromethane/hexane (1:1, v/v) and then transferred to a column (1 cm I.D.). The solvent was drained and the gel washed with dichloromethane/hexane (1:1, v/v, 20 ml). The fraction from aluminium oxide, containing the PCB and DDE methyl sulphones, was evaporated to *ca* 0.5 ml, transferred to a small centrifuge tube and concentrated to *ca* 50 μl with nitrogen and then quantitatively transferred to the column. The gel was eluted with dichloromethane/hexane (1:1) at a rate of 20 drops /min. The first 15 ml were discarded. The following 8 ml were evaporated to *ca* 50 μl and analysed by GC/MS.

3. Results and discussion

In the present study extraction of organochlorine compounds was made with the Lipidex

gel. By this procedure repeated extractions and centrifugations were avoided. The following chromatography on aluminium oxide retaines the PCB and DDE methyl sulphones and a separation from pesticides, PCB, PCN, PCDD and PCDF is easily achieved. Remaining fat was eliminated by gel permeation on an open column. Further purification was not needed. The study include the compounds listed in Table 1. The synthesis of the reference compounds have been described elsewere[9,10]. The determinations were made by GC/MS using EI and selected ion recording. The detection limit for the different compounds was 0.01-0.05 ng/g milk fat. The mean recoveries of added $MeSO_2$-PCBs and $MeSO_2$-DDE were 71-90 %.

Table 1. Structures of the studied $MeSO_2$-PCBs and their parent compounds. PCB numbers according to Ballschmiter *et al.*[11] are given in parenthesis.

Methyl sulphones	Parent compound
3-$MeSO_2$-2,5,2',5'-tetraCB (3-52)	2,5,2',5'-tetraCB (PCB 52)
4-$MeSO_2$-2,5,2',5'-tetraCB (4-52)	"
3-$MeSO_2$-2,5,2',4'-tetraCB (3-49)	2,4,2',5'-tetraCB (PCB 49)
4-$MeSO_2$-2,5,2',4'-tetraCB (4-49)	"
3-$MeSO_2$-2,5,6,4'-tetraCB (3-64)	2,3,6,4'-tetraCB (PCB 64)
4-$MeSO_2$-2,3,6,4'-tetraCB (4-64)	"
3-$MeSO_2$-2,5,6,2',4'-pentaCB (3-91)	2,3,6,2',4',-pentaCB (PCB 91)
4-$MeSO_2$-2,3,6,2',4'-pentaCB (4-91)	"
3-$MeSO_2$-2,5,3',4'-tetraCB (3-70)	2,5,3',4'-tetraCB (PCB 70)
4-$MeSO_2$-2,5,3',4'-tetraCB (4-70)	"
3-$MeSO_2$-2,5,2',4',5'-pentaCB (3-101)	2,4,5,2',5'-pentaCB (PCB 101)
4-$MeSO_2$-2,5,2',4',5'-pentaCB (4-101)	"
3-$MeSO_2$-2,5,2',3',4'-pentaCB (3-87)	2,3,4,2',5'-pentaCB (PCB 87)
4-$MeSO_2$-2,5,2',3',4'-pentaCB (4-87)	"
3-$MeSO_2$-2,5,6,3',4'-pentaCB (3-110)	2,3,6,3',4'-pentaCB (PCB 110)
4-$MeSO_2$-2,3,6,3',4'-pentaCB (4-110)	"
3-$MeSO_2$-2,5,6,2',4',5'-hexaCB (3-149)	2,3,6,2',4',5'-hexaCB (PCB 149)
4-$MeSO_2$-2,3,6,2',4',5'-hexaCB (4-149)	"
3-$MeSO_2$-2,5,6,2',3',4'-hexaCB (3-132)	2,3,4,2',3',6'-hexaCB (PCB 132)
4-$MeSO_2$-2,3,6,2',3',4'-hexaCB (4-132)	"
3-$MeSO_2$-2,5,2',3',4',5'-hexaCB (3-141)	2,3,4,5,2',5'-hexaCB (PCB 141)
4-$MeSO_2$-2,5,2',3',4',5'-hexaCB (4-141)	"
3-$MeSO_2$-2,5,6,2',3',4',5'-heptaCB (3-174)	2,3,4,5,2',3',6'-heptaCB (PCB 174)
4-$MeSO_2$-2,3,6,2',3',4',5'-heptaCB (4-174)	"

3-$MeSO_2$-DDE was found to be present in the milk samples at the highest concentration among all methyl sulphones analysed. The level of this compound was about 5 ng/g fat in milk from 1972 and has declined to 0.4 ng/g fat in 1991. The decrease correlated to the decline in the levels of p,p'-DDE during the same time period[12].
The most abudant $MeSO_2$-PCB originated from 2,3,4,2',5'-pentaCB (PCB 87), Fig.1. The precursors to the PCB methyl sulphones are not found at higher levels in the milk. Of the given precursors only 2,5,2',5'-tetraCB (PCB 52) and 2,4,5,2',5'-CB (PCB 101) have been determined at levels about 1 ng/g fat. The present investigation indicates that certain PCBs are very effectively metabolised to $MeSO_2$-derivatives which are accumulated in the adipose tissue and excreted with milk.

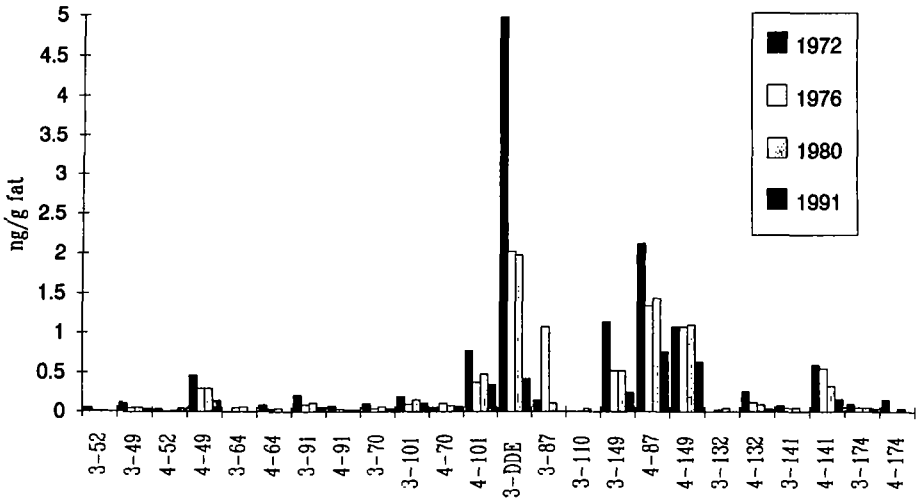

Fig. 1. Levels of $MeSO_2$-DDE and $MeSO_2$-PCBs in Swedish human milk sampled during different years from 75, 78, 83 and 20 mothers, respectively.

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5. References

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