

Methylsulfonyl Metabolites of PCBs and DDE in Human Milk in Sweden, 1972–1992

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A multicomponent method used for analysis of organochlorine pesticides, polychlorinated biphenyls (PCBs), naphthalenes, dibenzo-*p*-dioxins, and dibenzofurans was adapted for the analysis of methylsulfonyl metabolites of chlorinated biphenyls (MeSO₂-CBs) and of *p,p'*-DDE (MeSO₂-DDE) in human milk. The extraction and initial purification was made by liquid-gel partitioning. Additional purification and separation steps were achieved by adsorption and gel permeation chromatography. The mean recoveries of 23 MeSO₂-CBs and MeSO₂-DDE standards, added to the milk before extraction, were 80–97%. Human milk sampled in Stockholm during 1972, 1976, 1980, 1984/85, 1990, 1991, and 1992 was analyzed by GC–MS. During the time course studied, the concentrations of MeSO₂-CBs decreased from approximately 9 to 2 ng/g lipids and of MeSO₂-DDE from 5 to 0.4 ng/g lipids. The concentrations of MeSO₂-CBs and MeSO₂-DDE correlated to the levels of total PCB and *p,p'*-DDE, respectively. 3-MeSO₂-DDE was the major isomer of the aryl methyl sulfones studied in the milk. PCB methyl sulfones with five and six chlorine atoms in the molecule were predominant among the PCB methyl sulfones. Generally, the concentrations of 4-MeSO₂-CBs were higher than the corresponding 3-MeSO₂-CB compound. The major MeSO₂-CBs in the milk were 4-MeSO₂-2,5,2',3',4'-pentaCB (4-87) and 4-MeSO₂-2,3,6,2',4',5'-hexaCB (4-149). **Key words:** DDE, environmental pollutants, human milk, methyl sulfone, polychlorinated biphenyls. *Environ Health Perspect* 104:766–773 (1996)

Polychlorinated biphenyls (PCBs) and 1,1-bis(4-chlorophenyl)-2,2-dichloroethene (*p,p'*-DDE) are among the most widely spread environmental contaminants known. PCBs have mainly been used as plasticizers, dielectric fluids, and hydraulic oils. They were first detected in a white-tailed sea eagle in 1966 (1) and soon after were detected in human milk (2). In Sweden the use of PCBs was restricted in 1972 and was fully phased-out in 1995. Despite worldwide restrictions of these compounds, PCBs are still distributed to the environment and circulate between different compartments of the system. The persistence and lipophilic character of PCBs lead to their ubiquitous distribution as environmental contaminants [e.g., found in blood (3) and mother's milk (4)].

DDE is a metabolite of 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane (DDT), which was a commonly used insecticide from the 1940s to the 1960s. In Sweden the use of DDT as an insecticide in homes, gardens, and agriculture was prohibited in 1970, with an exception made for use on conifer plants until 1974. However, this pesticide is still used in certain countries, particularly where malaria is a problem of great concern (5).

In animals, lipophilic compounds are usually metabolized to more hydrophilic compounds that are more easily excreted than the parent substances. Metabolites of persistent environmental contaminants, however, may also have hydrophobic prop-

erties and can thus be accumulated in the body, such as methyl sulfone metabolites of chlorinated biphenyls (MeSO₂-CBs) and of DDE (MeSO₂-DDE) (6,7), or they may have specific protein-binding properties, such as certain MeSO₂-CBs (8–11), hydroxy-CBs (12), and MeSO₂-DDE (13,14). These properties lead to the retention of certain MeSO₂-CBs in lung, kidney, and uterine fluid (8–10) and MeSO₂-DDE in adrenal tissue (13,14).

A major metabolic pathway of aromatic organochlorine compounds proceeds via P450-mediated formation of arene oxide intermediates, with subsequent formation of either hydroxylated metabolites or mercapturic acid pathway (MAP) metabolites after reaction between glutathione and the epoxide (15–17). The cysteine conjugates formed via MAP may form aryl thiols due to C–S lyase-induced cleavage of the C–S bond in the conjugate (16). The aryl thiol formed is enzymatically methylated and then subsequently oxidized to the corresponding aryl methyl sulfone (15,16). The structures of the formed compounds are exemplified by 3-MeSO₂-DDE, 4-MeSO₂-CB149, and 3-MeSO₂-CB149 (Fig. 1).

As environmental contaminants, MeSO₂-PCBs and MeSO₂-DDE were first identified in seal blubber from the Baltic (6). Since then such metabolites have been found in several species of animals from this and other parts of the world (7,18–20). In human samples, MeSO₂-

PCBs were first reported in adipose tissue and milk from a woman exposed to PCBs in a capacitor factory in Japan (19). MeSO₂-PCBs and MeSO₂-DDE were also identified in adipose, liver, and lung tissue from Yusho patients as well as in a control person (20–23).

Considering these results and the fact that PCBs and DDE are major environmental contaminants present in human milk in Sweden, it is of interest to investigate the occurrence of methyl sulfone metabolites of PCBs and DDE in human milk. The aim of the present study was to develop a method for simultaneous analysis of PCB (and other chlorinated compounds) and its methylsulfonyl metabolites and to determine the concentrations of these compounds in human milk sampled during different time periods to visualize any trends over time for the PCB and DDE methyl sulfones.

The methods used to isolate aryl methyl sulfones include liquid–liquid partitioning (6,18,24) and various chromatographic methods (7,18,20,25). The final analyses are primarily performed by GC electron-capture detection (e.g., 7,18,20,24), mass spectrometry in electron ionization (EI) (25), or negative ion chemical ionization mode (27), but recently detection by GC atomic emission detector was described (28). Our work is discussed here in relation to these analytical procedures.

Materials and Methods

Pooled samples of human milk from the Mothers' Milk Centre in Stockholm were analyzed. The milk was from native Swedish women living in the Stockholm area. Equal amounts of milk from 10–20 women were mixed and stored at -20°C.

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We gratefully acknowledge the personnel at the Mothers' Milk Centre in Stockholm for collecting the samples. We thank Koichi Haraguchi for synthesis of several of the standard compounds and for making valuable comments on the manuscript. This study was supported by grants from the Swedish Environmental Protection Agency, the Swedish Medical Research Council, Stiftelsen Ragnhild och Einar Lundströms Minne, and Karolinska Institutet.

Received 28 September 1995; accepted 1 April 1996.

Several of the archived samples were pooled by mixing equal amounts of samples from the same time period. The average age of the women in the pools was 27–28 years, except for 1992 when the average age was 29 years. In each period, 55–60% of the women who supplied milk were nursing their first infant. The majority of the rest were nursing their second child.

Methanol, *n*-hexane, acetonitrile, dichloromethane, and trichloromethane were of HPLC-grade (Rathburn, Walkburn, Scotland) and were redistilled before use. Formic acid was of *pro analysi* quality (Merck, Darmstadt, Germany). Water was deionized and purified with a Milli Q cartridge system (Millipore, Bedford, Massachusetts). The MeSO₂-CBs used as standard compounds are listed in Table 1. The synthesis of these compounds has been described elsewhere (29–30). 3-MeSO₂-4,4'-

DDE was synthesized as described by Bergman and Wachtmeister (31). 3-Methylsulfonyl-4-methyl-5,2',3',4',5'-pentachlorobiphenyl was used as an internal standard. The Lipidex 5000 gel was from Packard Instruments (Downers Grove, Illinois). The Lipidex was washed and stored in methanol at 4°C (32). Immediately before use, the gel was rinsed with methanol in a separating funnel equipped with a sintered-glass disc and a polytetrafluoroethylene stopcock. For a 20-g portion of Lipidex, 2 × 25 ml of methanol was used. Most of the remaining methanol in the gel was removed with a gentle stream of nitrogen applied from the top of the funnel. The nitrogen, quality 5.5 from AGA (Stockholm, Sweden) was purified with moisture and oxygen filters (Chrompack, Middelburg, The Netherlands). Aluminum oxide 90 (activity grade II–III) from Merck was acti-

vated at 800°C for 4 hr and partly deactivated by adding water, corresponding to a concentration of 5% water (w/w). Bio-Beads S-X3, 200–400 mesh, was purchased from Bio-Rad Laboratories (Richmond, California).

All glassware was washed with detergents in an ultrasonic bath and rinsed thoroughly with tap water, deionized water and Milli Q water and then heated overnight at 280°C. The glassware was rinsed with hexane before use. The glass chromatographic columns used had ID of 2 and 1 cm.

GC/MS analyses were performed with a VG 70-250 mass spectrometer equipped with an HP 5890A gas chromatograph and a VG-250 data system (VG Analytical, Manchester, UK). Gas chromatography was performed using a fused silica SE-54 capillary column (25 m × 0.32 mm ID, 0.25-μm film thickness; Quadrex, New Haven, Connecticut) with helium as a carrier gas. An all-glass falling needle injector was used with an injector temperature at 270°C. The oven temperature was 190°C for 0.1 min, programmed to 230°C at 5°C/min, hold for 0.2 min, programmed to 235°C at 1°C/min, hold for 3 min, programmed to 270°C at 9°C/min, and hold for 8 min. EI was performed in an "EI-only" ion source at the electron energy of 31 eV and the trap current of 500 μA. The source temperature was 260°C. The acceleration voltage was 6 kV and the resolution at m/z 293 was 7000–9000. The MS was operated in a selected ion recording mode.

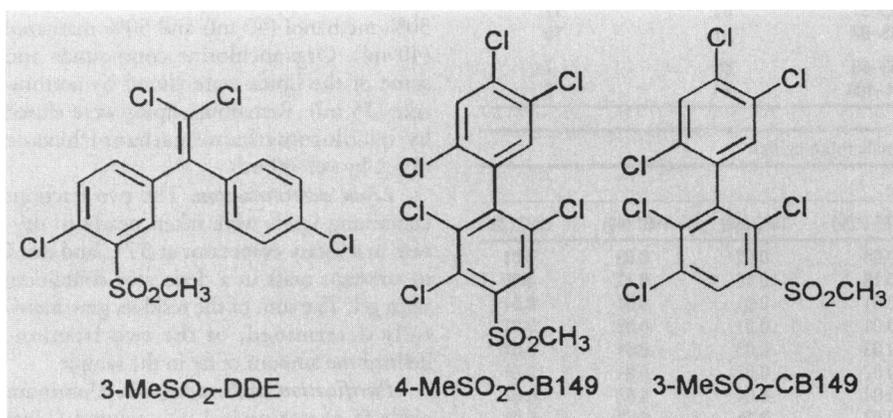


Figure 1. Structures of 3-MeSO₂-DDE, 4-MeSO₂-CB149, and 3-MeSO₂-CB149.

Table 1. Structures of the MeSO₂-CBs analyzed and their parent compounds

Methyl sulfones	Parent compound
3-MeSO ₂ -2,5,2',4'-tetraCB (3-49) ^a	2,4,2',5'-tetraCB (CB-49)
4-MeSO ₂ -2,5,2',4'-tetraCB (4-49)	2,4,2',5'-tetraCB (CB-49)
3-MeSO ₂ -2,5,2',5'-tetraCB (3-52)	2,5,2',5'-tetraCB (CB-52)
4-MeSO ₂ -2,5,2',5'-tetraCB (4-52)	2,5,2',5'-tetraCB (CB-52)
3-MeSO ₂ -2,5,6,4'-tetraCB (3-64)	2,3,6,4'-tetraCB (CB-64)
4-MeSO ₂ -2,3,6,4'-tetraCB (4-64)	2,3,6,4'-tetraCB (CB-64)
3-MeSO ₂ -2,5,3',4'-tetraCB (3-70)	2,5,3',4'-tetraCB (CB-70)
4-MeSO ₂ -2,5,3',4'-tetraCB (4-70)	2,5,3',4'-tetraCB (CB-70)
3-MeSO ₂ -2,5,2',3',4'-pentaCB (3-87)	2,3,4,2',5'-pentaCB (CB-87)
4-MeSO ₂ -2,5,2',3',4'-pentaCB (4-87)	2,3,4,2',5'-pentaCB (CB-87)
3-MeSO ₂ -2,5,6,2',4'-pentaCB (3-91)	2,3,6,2',4'-pentaCB (CB-91)
4-MeSO ₂ -2,3,6,2',4'-pentaCB (4-91)	2,3,6,2',4'-pentaCB (CB-91)
3-MeSO ₂ -2,5,2',4',5'-pentaCB (3-101)	2,4,5,2',5'-pentaCB (CB-101)
4-MeSO ₂ -2,5,2',4',5'-pentaCB (4-101)	2,4,5,2',5'-pentaCB (CB-101)
3-MeSO ₂ -2,5,6,3',4'-pentaCB (3-110)	2,3,6,3',4'-pentaCB (CB-110)
3-MeSO ₂ -2,5,6,2',3',4'-hexaCB (3-132)	2,3,4,2',3',6'-hexaCB (CB-132)
4-MeSO ₂ -2,3,6,2',3',4'-hexaCB (4-132)	2,3,4,2',3',6'-hexaCB (CB-132)
3-MeSO ₂ -2,5,2',3',4',5'-hexaCB (3-141)	2,3,4,5,2',5'-hexaCB (CB-141)
4-MeSO ₂ -2,5,2',3',4',5'-hexaCB (4-141)	2,3,4,5,2',5'-hexaCB (CB-141)
3-MeSO ₂ -2,5,6,2',4',5'-hexaCB (3-149)	2,3,6,2',4',5'-hexaCB (CB-149)
4-MeSO ₂ -2,3,6,2',4',5'-hexaCB (4-149)	2,3,6,2',4',5'-hexaCB (CB-149)
3-MeSO ₂ -2,5,6,2',3',4',5'-heptaCB (3-174)	2,3,4,5,2',3',6'-heptaCB (CB-174)
4-MeSO ₂ -2,3,6,2',3',4',5'-heptaCB (4-174)	2,3,4,5,2',3',6'-heptaCB (CB-174)

^aPCB congener numbers, according to Ballschmiter (34), are given in parentheses.

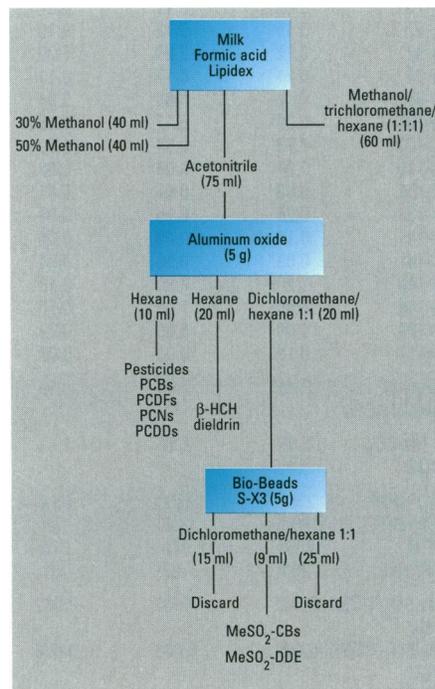


Figure 2. Scheme of method for analysis of aryl methyl sulfones.

Table 2. Recoveries of aryl methyl sulfones added to milk

MeSO ₂ -CB	Amount added (ng/ml)	n	Recovery		
			Range (%)	Average (%)	Relative SD (%)
3-49	0.41-0.75	7	71-118	86	14
4-49	0.42-0.81	7	77-110	90	10
3-52	0.41	6	80-113	90	11
4-52	0.42	6	80-93	87	4
3-64	0.41	6	79-100	88	7
4-64	0.12-0.41	7	80-98	87	7
3-70	0.19-0.42	7	80-95	87	7
4-70	0.41	6	75-104	90	9
3-87	0.41-1.52	7	72-96	87	8
4-87	0.41-2.12	5	78-102	97	10
3-91	0.42-0.49	7	69-107	89	12
4-91	0.42	6	81-102	93	8
3-101	0.40-4.19	5	81-97	92	6
4-101	0.41-1.47	5	79-101	94	9
3-110	0.08-0.41	7	73-96	89	9
3-132	0.41	7	75-101	89	9
4-132	0.41-1.02	7	72-88	84	7
3-141	0.40	6	71-100	86	13
4-141	0.41	6	73-108	85	11
3-149	0.41-0.75	7	78-110	93	11
4-149	0.41-1.13	5	81-109	84	11
3-174	0.40	6	63-97	87	12
4-174	0.41	6	66-98	87	10
MeSO ₂ -DDE	0.44-1.34	7	69-89	80	7
Internal standard	0.42	7	74-100	84	8

Table 3. Levels of MeSO₂-CBs and MeSO₂-DDE in human milk (ng/g lipids)

Compound	Year						
	1972 (75) ^a	1976 (153)	1980 (199)	1984/85 (102)	1990 (40)	1991 (40)	1992 (20)
3-49	0.14	0.10	0.06	0.04	0.02	0.03	0.01
4-49	0.46	0.34	0.26	0.16	0.10	0.12	0.05
3-52	0.07	0.05	0.01	0.01	<0.01	0.01	0.01
4-52	0.06	0.06	0.02	0.01	0.01	0.03	0.01
3-64	0.03	0.08	0.06	0.03	0.03	0.01	0.01
4-64	0.10	0.10	0.05	0.02	0.02	0.01	0.01
3-70	0.12	0.09	0.07	0.04	0.04	0.03	0.01
4-70	0.09	0.11	0.07	0.03	0.15	0.05	0.01
3-87	0.15	0.12	0.10	0.06	0.05	0.02	0.04
4-87	2.13	2.09	1.20	0.70	0.94	0.67	0.33
3-91	0.21	0.17	0.11	0.08	0.06	0.04	0.04
4-91	0.12	0.08	0.04	0.02	0.03	0.02	0.02
3-101	0.20	0.17	0.16	0.15	0.12	0.09	0.06
4-101	0.78	0.64	0.52	0.32	0.33	0.28	0.13
3-110	0.04	0.07	0.03	0.02	0.02	0.01	0.01
3-132	0.08	0.06	0.04	0.03	0.04	0.01	0.03
4-132	0.25	0.15	0.07	0.05	0.06	0.04	0.04
3-141	0.11	0.09	0.04	0.12	0.06	0.01	0.02
4-141	0.63	0.56	0.34	0.19	0.25	0.14	0.11
3-149	1.23	0.80	0.48	0.34	0.41	0.20	0.24
4-149	2.00	1.87	0.70	0.64	1.00	0.59	0.35
3-174	0.09	0.09	0.08	0.03	<0.03	0.04	0.02
4-174	0.15	0.09	0.05	0.02	0.04	0.01	0.01
Sum of MeSO ₂ -CBs	9.24	7.98	4.56	3.11	3.66	2.46	1.57
3-MeSO ₂ -DDE	5.05	2.80	1.81	0.79	0.64	0.40	0.46
<i>p,p'</i> -DDE	2300	1500	1055	500	375	260	251
<i>p,p'</i> -DDT	630	340	185	61	46	36	32
PCB	1050	910	810	600	650	511	410
CB-153	208	197	152	103	116	106	96
MeSO ₂ -DDE/DDE	0.002	0.002	0.002	0.002	0.002	0.002	0.002
MeSO ₂ -PCB/PCB	0.009	0.009	0.006	0.005	0.006	0.005	0.004
MeSO ₂ -PCB/ CB-153	0.04	0.04	0.03	0.03	0.03	0.02	0.02

^aNumber of women in parentheses.

For each compound, two ions of the molecular ion cluster were monitored. Ions from perfluorokerosene were used as reference masses for correction of mass spectrometer drift (lock mass).

A scheme of the analytical method is shown in Figure 2.

Extraction. The extraction was performed as previously described for multi-component analysis of organochlorine contaminants in human milk (33). A sample of milk (10 ml) was weighed into a flask with a Teflon-lined screw cap. Internal standard (100 μ l of 41.4 pg 3-methylsulfonyl-4-methyl-5,2',3',4',5'-pentachlorobiphenyl/ μ l) 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 hr and then transferred to a glass column (2 cm ID). The solvent was drained and, in consecutive steps, was washed with 30% methanol (40 ml) and 50% methanol (40 ml). Organochlorine compounds and some of the lipids were eluted by acetonitrile (75 ml). Remaining lipids were eluted by trichloromethane/methanol/hexane (1:1:1 by vol, 60 ml).

Lipid 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 containing silica gel. The sum of the residue, gravimetrically determined, of the two fractions defined the amount of fat in the sample.

Purification and separation. Aluminum oxide (5 g) was packed in a column (1 cm ID) and washed with hexane (10 ml). Then the stopcock was closed and the residue from acetonitrile fraction was quantitatively transferred to the column with small portions of hexane. The sample on the column was concentrated by evaporation of the solvent with a gentle stream of nitrogen. Organochlorine compounds, such as pesticides, PCBs, polychlorinated naphthalenes, polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans, were eluted by hexane (10 ml) and β -hexachlorocyclohexane (β -HCH) and dieldrin in an additional fraction of hexane (20 ml). All MeSO₂-CBs and MeSO₂-DDE were obtained in the third fraction, collected when 50% dichloromethane in hexane (20 ml) was used as a mobile phase.

Bio-Beads S-X3 (5 g) were transferred to a beaker and dichloromethane/hexane (1:1, v/v) was added to cover the gel. The beaker was placed in an ultrasonic bath for a few seconds and then let equilibrate for 2 hr. The mixture was subsequently transferred to a column (1 cm ID). The solvent was drained and the gel was washed with dichloromethane/hexane (1:1, v/v, 20 ml). The vol-

ume of MeSO₂-CBs and MeSO₂-DDE-containing fraction was adjusted by evaporation to about 0.5 ml, transferred to a small, tapered centrifuge tube, concentrated to about 50 µl with nitrogen, and then quantitatively transferred with small volumes of dichloromethane/hexane (1:1) to the GPC column (Bio-Beads S-X3). The mobile phase, dichloromethane/hexane (1:1), was collected at a rate of 20 drops/min. The first 15 ml were discarded. The following 9 ml contained MeSO₂-CBs and MeSO₂-DDE. To purify the column from contaminants, it was washed with an additional 25 ml of the solvent mixture and discarded. The fraction containing MeSO₂-CBs and MeSO₂-DDE was concentrated to 50 µl and transferred quantitatively to the same column, and the procedure was repeated. The fraction containing MeSO₂-CBs and MeSO₂-DDE (9 ml) from this second fractionation was concentrated and about half of the sample was used for analysis by GC-MS.

Recovery studies. Recovery studies were performed by adding 50–100 µl of standard mixtures in hexane of MeSO₂-CBs (see Table 1), 3-MeSO₂-DDE and 3-methylsulfonyl-4-methyl-5,2',3',4',5'-pentachlorobiphenyl (the internal standard used) to the sample before extraction. Before determination by GC-MS, 2,3,4,5,2',3',4'-heptaCB (CB-170) (34) was added as an internal standard for volume correction.

Results

The congener-specific analyses of MeSO₂-CBs and MeSO₂-DDE were made by GC-MS using an "EI-only" ion source. At a resolution of 7000 and the electron energy of 31 eV, the detection limits of the compounds listed in Table 1 were 0.5–2 pg at S/N 2.5. Considerably lower sensitivity was obtained at 70 eV (detection limit 2–10 pg). In the investigation of milk, the detection limits of MeSO₂-CBs and MeSO₂-DDE were 0.01–0.05 ng/g lipid.

The mean recoveries of MeSO₂-CBs and MeSO₂-DDE added to the samples before extraction were 80–97% and of internal standard were 84% (Table 2). Congener-specific analyses of MeSO₂-CBs and MeSO₂-DDE in human milk collected in different years, starting from 1972, were performed. The concentrations of MeSO₂-CBs and MeSO₂-DDE are shown in Table 3. Of the metabolites studied, 3-MeSO₂-DDE had the highest concentration. The level of 3-MeSO₂-DDE was about 5 ng/g fat in milk from 1972 and declined successively with time to about 0.4 ng/g fat in 1991–1992. A decline in the concentrations of *p,p'*-DDT and *p,p'*-DDE in Swedish human milk has been demonstrat-

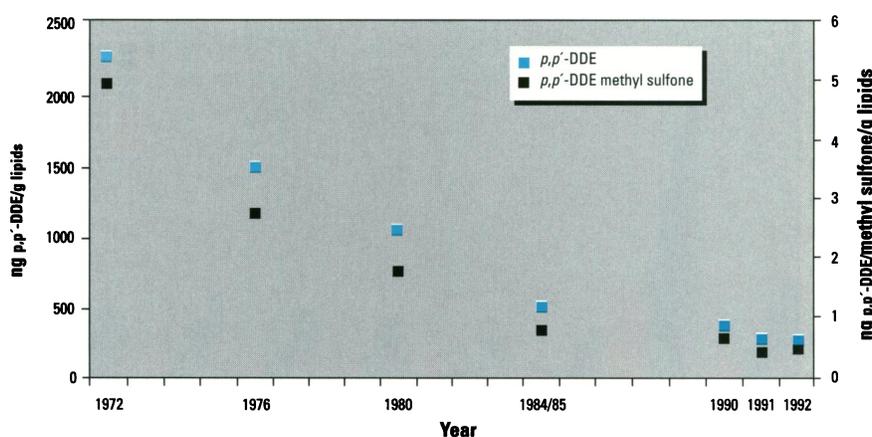


Figure 3. Concentrations of *p,p'*-DDE and *p,p'*-DDE methyl sulfone in human milk, 1972–1992.

ed previously (35,36). The concentrations of *p,p'*-DDT and *p,p'*-DDE in the present samples are shown in Table 3. There is a good correlation between the concentrations of 3-MeSO₂-DDE and corresponding decline in the concentration of *p,p'*-DDE (correlation coefficient of 0.99; Fig. 3). The ratio of the concentrations of 3-MeSO₂-DDE to *p,p'*-DDE was 0.002.

All the MeSO₂-CBs shown in Table 1 were identified in human milk. The profile of the MeSO₂-CBs was similar in the milk sampled in different years, but the concentrations changed with time. The most abundant MeSO₂-CB was compound 4-87, originating from 2,3,4,2',5'-pentaCB (CB-87), followed by 4-149, originating from 2,3,6,2',4',5'-hexaCB (CB-149). The compounds with methylsulfonyl group in position 4 (*para*) occur at higher levels than the corresponding compounds with methylsulfonyl group in position 3 (*meta*) (Fig. 4).

The MeSO₂-CBs in human milk originate from CBs that do not occur at high levels in milk. Of the precursors, only 2,5,2',5'-tetraCB (CB-52) and 2,4,5,2',5'-pentaCB (CB-101) were found in milk (to be published). The concentrations of MeSO₂-CBs decreased from 9.2 ng/g lipids in 1972 to 1.6 ng/g lipids in 1992 (Table 3) and correlated to the decline in the levels of total CBs by a correlation coefficient of 0.95 (Fig. 5). The total CB levels were determined by EC-GC using a packed column (35,36; in preparation). The ratios of the sum of MeSO₂-CBs (MeSO₂-PCB) to total CB (PCB) concentration decreased from 0.009 to 0.004 during the time course studied (Table 3). Comparisons were also made to 2,4,5,2',4',5'-hexaCB (CB-153), determined by congener specific analysis. This PCB congener does not contain adjacent, unsubstituted carbon atoms susceptible to metabolic reactions. Accordingly, it has a long half-life (37) and is the most abundant PCB congener in the human

milk. The decline of MeSO₂-CBs relative to CB-153 indicates a somewhat more rapid decline of MeSO₂-CBs in milk than of PCB over the course of this time period.

Discussion

The analytical methods used in previously reported investigations of MeSO₂-CBs and MeSO₂-DDE involve several steps of liquid-liquid partitioning and column chromatography (6,7,18,20,24,25). The aryl methyl sulfones were extracted from tissue samples by nonpolar solvents or medium-polarity mixtures of solvents (6,24,25,38). Different combinations of methods have been used for purification and separation from other organochlorine compounds, e.g., chromatography (Bio-Beads, silica gel, aluminum oxide) and partitioning between solvents of different polarity, e.g., between hexane and aqueous acetonitrile (24) and between hexane and dimethyl sulfoxide with subsequent reextraction of the analytes with methyl *tert*-butyl ether:hexane, after dilution of the dimethyl sulfoxide phase with water (7). Because MeSO₂-CBs and MeSO₂-DDE possess the character of a Lewis base, partitioning between hexane and concentrated sulfuric acid has frequently been used as a method for purification of these compounds. However, a method using exclusively chromatographic fractionations (gel permeation, silica gel modified with KOH, Florisil, and basic aluminum oxide) for separation of the methyl sulfone derivatives was recently reported (39).

In the present study the extraction procedure for MeSO₂-CBs and MeSO₂-DDE from milk differs from the previously described methods. Instead of partitioning between solvents, the extraction was accomplished by partitioning between the milk-formic acid mixture and a lipophilic gel, Lipidex 5000. The extraction between an aqueous solution and Lipidex resembles the extraction with a solvent of medium

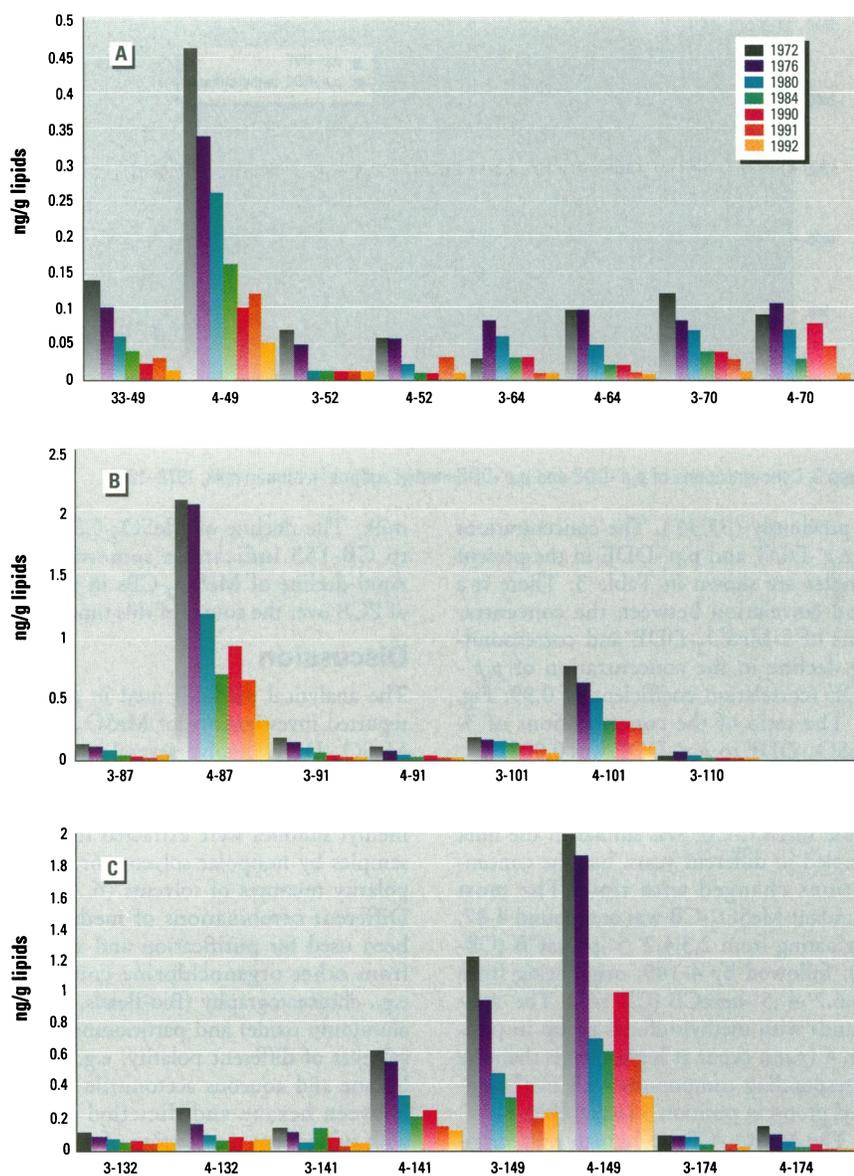


Figure 4. Concentrations of individual MeSO₂-CBs in human milk from 1972–1992. (A) Compounds with four chlorine atoms in the molecule, (B) compounds with five chlorine atoms, and (C) compounds with six to seven chlorine atoms.

polarity. The advantage is that the procedure can be done in one step. The column bed can subsequently be eluted with solvents of different polarity, facilitating partial separation of lipids and an initial purification of the analyte fraction. By this procedure about 60% of the lipids in the milk sample were separated from the analytes. In addition two chromatographic systems, aluminum oxide and Bio-Beads S-X3 were used. By repeating the gel permeation step once, sufficiently clean extracts were obtained for GC–MS analysis.

The recoveries of added MeSO₂-CBs and MeSO₂-DDE (mean 80–97%) with a relative standard deviation of 4–14% (Table 2) were considered satisfactory for

the analysis using a small sample size (10 ml milk).

In the milk samples, 3-MeSO₂-DDE was the major aryl methyl sulfone compound (about 5–0.4 ng/g lipids), whereas only traces could be discerned of 2-MeSO₂-DDE. The ratio of MeSO₂-DDE/DDE (0.002) was constant in the samples from different years. In Japanese human samples (adipose, lung and liver tissue) the ratios were somewhat higher (0.007–0.009). A high species-specific organ selectivity has been demonstrated for MeSO₂-DDE in wild animals (7), but no concentrations in milk from these animals or in human milk have previously been reported. The present results are in accor-

dance with the observations that MeSO₂-DDE is the major aryl methyl sulfone in Swedish human adipose tissue (40). However, high concentration of 2-MeSO₂-DDE and 3-MeSO₂-DDE (about 0.9 and 0.4 µg/g fat, respectively) were reported in the liver of polar bear (Canada). In grey seal the levels were higher in the liver than in adipose tissue, and the levels of 2-MeSO₂- and 3-MeSO₂-DDE were equal. In otter and mink, no 2-MeSO₂-DDE was detected and the levels of 3-MeSO₂-DDE were about equal in liver and muscle, calculated on lipid weight basis (7). The results indicate differences in exposure and/or metabolism.

MeSO₂-DDE is a potent toxicant for the adrenal cortex in mouse (13) and also in the human adrenal glands as observed as *in vitro* bioactivation of this compound in human adrenal gland (14). The irreversible binding of MeSO₂-DDE in the zona fasciculata of the adrenals led to the formation of necrotic cells and inhibition of glucocorticoid hormone synthesis (13,41). Also, *p,p'*-DDE was recently reported to be a strong androgen receptor antagonist (42); the potency of the corresponding MeSO₂-DDE is not yet known. Due to the potential toxicity of DDE and MeSO₂-DDE, it is necessary to reduce the exposure to DDT/DDE, especially in areas where DDT is still used.

Generally, the precursors to the MeSO₂-CBs were not found in the milk, indicating an effective metabolism of the precursor CBs. The MeSO₂-CBs originate from CBs with chlorine atoms in 2,5- or 2,3,6-positions of at least one of the phenyl rings of the PCB congener (8). In these compounds there are unsubstituted *meta/para* positions adjacent to two chlorine atoms. This strongly facilitates the reaction between the PCB arene oxide and glutathione and formation of the two isomeric 3- and 4-MeSO₂-PCBs, as observed in minks dosed with a technical preparation of the PCB Clophen A50 (43).

In milk, the PCB metabolites with the MeSO₂ group in the 4-position are present at higher concentrations than the corresponding compounds with the MeSO₂ group in 3-position, except for 3-91, which is present at a higher concentration than 4-91 (see Table 3). The major MeSO₂-CBs were 4-87 and 4-149. Both 4-87 and 4-149 were the predominant MeSO₂-CBs in human adipose tissue (40) and thus consistent with the results of the present study. In adipose and lung tissue from Yusho patients, MeSO₂-CB 4-87 was also the predominant PCB methyl sulfone, whereas a quite different profile was reported in the

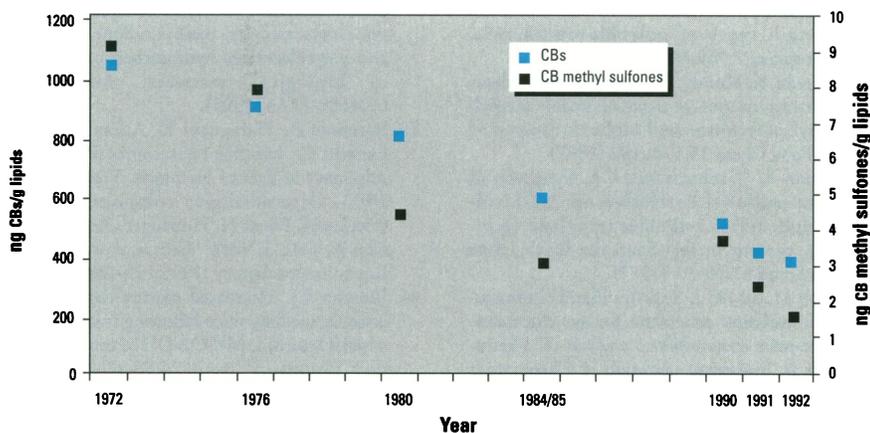


Figure 5. Concentrations of total chlorinated biphenyls (CBs) and sum of CB methyl sulfones in human milk sampled 1972–1992.

control sample with 4-MeSO₂-2,5,4'-triCB as the dominating and 4-MeSO₂-2,5,2',4'-CB (4-49) occurring in second highest level (20). A different profile of MeSO₂-CBs is found in tissues of grey seal, otter, and mink (7,18). In these species 3-MeSO₂-2,5,2',4',5'-pentaCB, originating from CB-101, is the predominant PCB methyl sulfone. The results indicated differences in exposure and/or metabolism of DDE and PCBs, which may be of importance in possible toxic effects of these compounds.

The concentrations of MeSO₂-DDE and MeSO₂-PCB in milk decreased during the time course studied, in accordance with the previously reported decline of DDT, DDE and PCB in Swedish human milk (35,36; in preparation). In a study of milk from an occupationally exposed mother, a decline in the concentration of PCB, from 14,000 to 3700 ng/g lipids, and of MeSO₂-PCB, from 590 to 150 ng/g lipids, during 16 months of milk excretion was reported (44). At the time of that study, MeSO₂-PCB congener-specific analysis was not possible. However, the concentration of MeSO₂-PCB was estimated to 0.05 of the PCB concentration. In the present investigation the concentration ratios of MeSO₂-PCB relative to PCB were 0.009–0.004 and declined during the 20 years studied. In a Japanese study, corresponding ratios in adipose tissues were 0.02 and 0.04 from two control persons and 0.004 and 0.006 from two Yushu patients (23). The profile of the MeSO₂-PCB in these samples differed from the profile in Swedish human milk. In the present study the congeners with 5 and 6 chlorine in the molecule dominated, while in Japanese adipose tissue the lower chlorinated congeners were the dominating MeSO₂-CBs. In Swedish blood plasma, the profile of MeSO₂-PCB is similar to that in the milk (Weistrand and Norén, in prepara-

tion). Approximately twice as much 4-MeSO₂-CBs are present in the milk as are 3-MeSO₂-CBs. The latter type of metabolites have recently been reported as potent inducers of several hepatic microsomal drug-metabolizing enzymes while the 4-MeSO₂-CBs congeners tested were inactive (45). On the other hand, certain 4-MeSO₂-CBs are strongly retained in lung bronchial mucosa due to their binding to uteroglobin-like macromolecules in the Clara cells (9). It is also supposed that the chronic lung dysfunction symptoms in Yushu patients may have been caused by MeSO₂-PCB (46). It may thus be emphasized that the MeSO₂-CBs are also of potential toxicological importance and the presence of these PCB metabolites in human milk cannot be neglected. Further studies of their toxicological role are needed.

The data show that restrictions on the use of DDT and PCBs have led to decreased levels of these compounds and their methyl sulfone metabolites in human milk. The present study is a first attempt at congener-specific analysis of MeSO₂-DDE and MeSO₂-PCB in milk, and it should be followed up by analysis of other matrices of human tissue to investigate specific retention.

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