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Publisher Taylor & Francis

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International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713640455>

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Jos Mes^a; Davida Turton^a; David Davies^a; Wing-Fung Sun^a; Pui-Yan Lau^a; Dorcas Weber^a

^a Health and Welfare Canada, Health Protection Branch, Food Directorate, Tunney's Pasture, Ottawa, Canada

To cite this Article Mes, Jos , Turton, Davida , Davies, David , Sun, Wing-Fung , Lau, Pui-Yan and Weber, Dorcas(1987) 'The Routine Analysis of Some Specific Isomers of Polychlorinated Biphenyl Congeners in Human Milk', International Journal of Environmental Analytical Chemistry, 28: 3, 197 — 205

To link to this Article: DOI: 10.1080/03067318708081861

URL: <http://dx.doi.org/10.1080/03067318708081861>

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The Routine Analysis of Some Specific Isomers of Polychlorinated Biphenyl Congeners in Human Milk

JOS MES, DAVIDA TURTON, DAVID DAVIES, WING-FUNG SUN, PUI-YAN LAU and DORCAS WEBER

Health and Welfare Canada, Health Protection Branch, Food Directorate, Tunney's Pasture, Ottawa, Canada K1A 0L2

(Received August 5, 1986)

A mixture of 14 polychlorinated biphenyl (PCB) isomers of various congeners, representing approximately 70% of all human milk PCBs reported in the literature, was made up to identify and approximate their residues in human milk. Individual isomer levels varied from 5 to 103 nanograms per gram of milkfat with the 2,2',4,4',5,5'-hexachlorobiphenyl isomer as the major PCB contaminant of human milk. PCB isomer numbers 74, 118, 153, 138 and 180 made up approximately 75% of all PCBs as measured by this mixture.

There was close agreement of total PCB isomer content in breast milk between electron capture gas chromatography and gas chromatographic mass spectrometry determinations. A major interference was encountered however for PCB isomer no. 52, whose residue level in the breast milk was approximately $3 \times$ higher by gas liquid chromatography than by mass spectrometry.

KEY WORDS: PCB, isomer, human milk.

INTRODUCTION

Until recently most polychlorinated biphenyl (PCB) residues in human milk were estimated by using all or a selected number of peaks above a given threshold, which did not interfere with other known chlorinated hydrocarbon and/or unknown residues in the resulting complex gas chromatographic (GC) pattern of the sample.¹

These selected PCB peaks in the sample were then directly related to the corresponding peaks in a commercial PCB mixture of known chlorine content, such as the Aroclor[®] series. This non-specific quantitation approach was the result of a limited separation efficiency of the packed GC column and the lack of pure isomers for each of the PCB congeners. The use of capillary GC and the synthesis of all PCB isomers, many of which are now commercially available, have opened the way for a PCB quantitation approach based on individual isomers.² Many isomers have been identified in human breast milk and their toxicological evaluation initiated.³⁻⁷

This paper describes experimental conditions for the routine analysis of some 14 PCB isomers in human milk. These 14 isomers represent approximately 70% of all breast milk PCBs reported in the literature.^{3,6} Most of these isomers are major components of breast milk and range in concentration from 0.6 to 12%.

EXPERIMENTAL

Samples and chemicals

Breast milk samples were collected as previously described by Mes *et al.*⁸

All solvents were glass distilled and free of interfering residues as tested by gas chromatography (concentration 250:1). Adsorbents were washed and prepared as earlier reported.^{9,10}

Individual isomers (99% pure) were obtained from Ultra Scientific Inc. (Hope, R.I., U.S.A.), except for the 2,3',4,4'-pentachlorobiphenyl (95% pure), which was a gift from Dr. H. Newsome (Health Protection Branch, Ottawa, Canada). The 2,4,4',5-tetra-, 2,3,3',4,4'-penta-, 2,2',3,4,4',5,5'-hepta- and 2,2',3,4',5,5',6-heptachlorobiphenyl congeners were gifts from Dr. S. Safe (Texas A&M University, College Station, Texas, U.S.A.).

Preparation of standard solution

A mixture of specific isomers of several PCB congeners was made up in hexane with the concentration of each component as given in

[®] Monsanto Chemical Co.

TABLE I
Concentration of PCB isomers in standard solution

BZ No. ^a	Z No. ^b	C1 substitution pattern (in order of GC elution)	pg/ μ l
28	54	2,4,4'	2.4
52	99	2,2',5,5'	2.1
74	D4	2,4,4',5	6.0
66	56	2,3',4,4'	1.0
101	D9	2,2',4,5,5'	0.5
151	B91	2,2',3,5,5',6	0.5
118	D6	2,3',4,4',5	9.1
153	DD	2,2',4,4',5,5'	15.3
105	76	2,3,3',4,4'	1.6
138	7D	2,2',3,4,4',5'	6.7
187	BD1	2,2',3,4',5,5',6	2.9
183	7D1	2,2',3,4,4',5',6	3.3
156	F6	2,3,3',4,4',5	1.5
180	FD	2,2',3,4,4',5,5'	6.9

^aBallschmider and Zell numbering system (Ref. 12).

^bZitko numbering system (Ref. 13).

Table I. The composition of isomers in this mixture (Figure 1) simulates that found in human milk.^{3,4} For GC mass spectrometry (GC-MS) analysis the same solution was diluted 1.5 times with hexane.

Extraction

The extraction of breast milk samples has previously been described by Mes *et al.*¹¹ and can briefly be summarized as follows: 10 g samples were centrifuged at 600 \times g for 20 min and the milky waterphase removed from the fat by aspiration. The fat was then extracted with acetone:benzene (19:1, v/v), filtered and the solvent evaporated. The oily residue was redissolved in hexane and dried over anhydrous Na₂SO₄. The dried hexane was filtered, concentrated, transferred to a 50 ml graduated centrifuge tube and diluted to 20 ml. The lipid content was determined gravimetrically using a 1 ml aliquot.

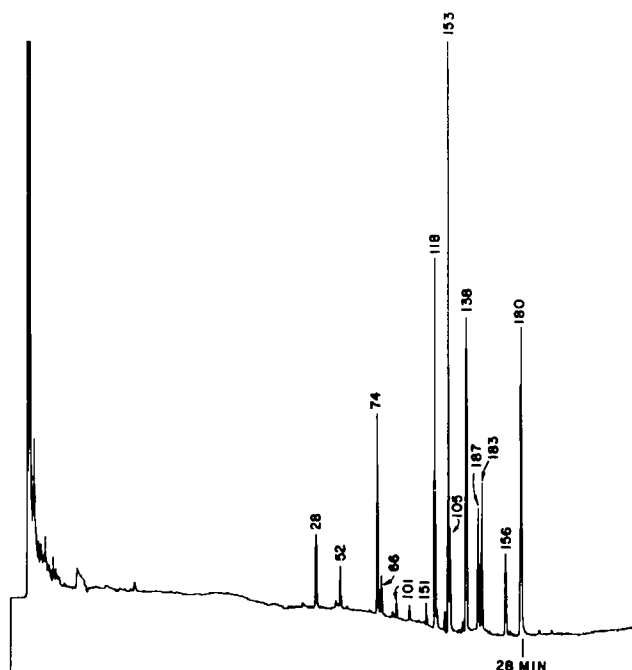


FIGURE 1 The GC elution pattern of a standard PCB isomer mixture simulating that found in human milk. The numbers relate to the PCB isomers listed in Table I.

Cleanup and separation

An aliquot of hexane extract containing not more than 250mg of milkfat was concentrated under a gentle stream of N_2 to ~ 1 ml and subsequently chromatographed on a semi-micro combined Florisil-silicic acid column as earlier described.¹⁰ All PCBs were collected in 40 ml of 2% dichloromethane in hexane, but not completely separated from other chlorinated hydrocarbon residues such as chlorinated benzenes and *p,p'*-DDE. The dichloromethane was removed by concentrating the PCB fraction to ~ 1 ml on an all glass rotatory evaporator ($< 30^\circ C$). The residual solvent was then quantitatively transferred to a graduated 15 ml centrifuge tube. This hexane fraction was either evaporated under a gentle stream of N_2 or diluted with hexane to an exact volume before GC analysis.

Identification and quantitation

The PCB fraction was chromatographed on a DB-5 (J&W Scientific Inc.) fused silica capillary column (0.24 mm I.D. \times 30 m), using a Varian model 3700 GC with a splitless injector (Scientific Glass & Engineering, Australia) and a ^{63}Ni electron capture detector (ECD). Injector and detector temperatures were 240 and 300°C respectively. After an initial 7 min at 130°C the column temperature was increased to 230°C at a rate of 6°C/min and kept at the final temperature for 30 min. The linear velocity of the He carrier gas at 130°C was 47 cm/sec. The make-up gasflow was adjusted to 30 ml N_2 /min. Aliquots (0.5 μl) of the standard solution were injected before and after every three sample runs (0.5 μl aliquots).

PCBs were quantitated by relating peakheights in the sample GC pattern with those of identical retention time and known concentrations in the standard.

Confirmation

The GC-MS analysis was carried out using a similar GC instrument and column as above. For tri- and tetra-chlorobiphenyls the column temperature was kept at 80°C for 1 min, followed by an increase to 140°C in 1 min and a final programming to 230°C at a rate of 3°C/min. For the higher chlorinated congeners the final rate of the temperature program was 4°C/min. A VG Multispec Data System was used to carry out multiple ion detection (MID) analysis on a VG Analytical ZAB-2F mass-spectrometer with an ion source temperature of 250°C, an electron energy of 70 eV and a resolution of 1500 (10% valley definition). Dwell time ranged from 75–100 msec depending on the number of ions monitored.

Pooled PCB fractions were monitored for the following ions (m/z): 256 and 258 for tri-, 290 and 292 for tetra-, 324 and 326 for penta-, 358 and 360 for hexa- and 394 and 396 for heptachlorobiphenyl. PCBs were also quantitated in pooled PCB fractions by GC-MS, using the same PCB isomers as for GC-ECD.

Controls

Three breast milk samples were fortified with a mixture of isomers (No. 28, 52, 101, 105 and 187) at levels of ~ 1 ng/g whole milk.

Acetone was used as fortification solvent and the fortified samples were left standing at room temperature for 1 hr before extraction. Solvent blanks were run through the entire analytical procedure, concurrently with the fortified samples.

RESULTS AND DISCUSSION

Recoveries of specific isomers from fortified samples ranged from 77–108%, depending on the isomer. Solvent blanks indicated a possible background interference of ~10% in the determination of specific PCB isomers.

All isomers listed in Table I were identified in the breast milk and confirmed by GC-MS. The ratio of total PCB isomer content as determined by GC-ECD and GC-MS was 0.92, indicating good agreement between the two modes of analysis. Only isomer no. 52 was overestimated by the GC-ECD technique by a factor of 3, indicating considerable interference.

Table II shows the contribution of each individual isomer to the total PCB content of whole breast milk and milkfat as measured by the specific isomer mixture of Table I. The results indicate that the 2,4,4',5-tetrachlorobiphenyl (No. 74), the 2,3',4,4',5-pentachlorobiphenyl (No. 118), the 2,2',4,4',5,5'- and 2,2',3,4,4',5'-hexachlorobiphenyl (No. 153 and 138) and 2,2',3,4,4',5,5'-heptachlorobiphenyls (No. 180) are the major contributors: making up approximately 75% of all measured PCB isomers (Figure 2). This percentage closely agrees with percentages (~67%) calculated from literature data on specific isomer distribution in human milk, using the same isomers as in this study.^{3,6}

One of the advantages of using specific PCB isomers for the quantitation of PCBs in human milk lies in its ability to compare isomer specific PCB data as shown in Table III. The results indicate no isomer differed by more than a factor of two for breast milk samples from parts of New York State (U.S.A.) and the Province of Ontario (Canada) around Lake Ontario.

However it may still be desirable at this stage of analytical development, to compare PCB content in breastmilk obtained by addition of individual isomer contributions to that found by the use of commercial PCB mixtures.

TABLE II
Mean residue levels of specific PCB isomers in
Canadian breast milk

BZ No.	ng PCB isomer/g	
	Breast milk ^a	Milkfat ^a
28	0.4	11
52	0.4	11
74	1.4	37
66	0.2	5
101	0.3	8
151	0.2	5
118	2.1	56
153	3.9	103
105	0.7	19
138	3.1	82
187	0.5	13
183	0.3	8
156	0.6	16
180	1.8	48

^aMean of 75 samples with mean fat content of 3.78%.

TABLE III
A comparison of specific PCB isomers in human milk from different
locations

BZ No.	ng PCB isomer/g breast milk	
	Bush <i>et al.</i> ¹⁴ (N ^a = 40)	This study (N = 39)
153	4.3	3.2
105	0.5	1.0
138	3.4	2.1
156	0.7	1.0
180	2.0	1.2

^aN = number of samples.

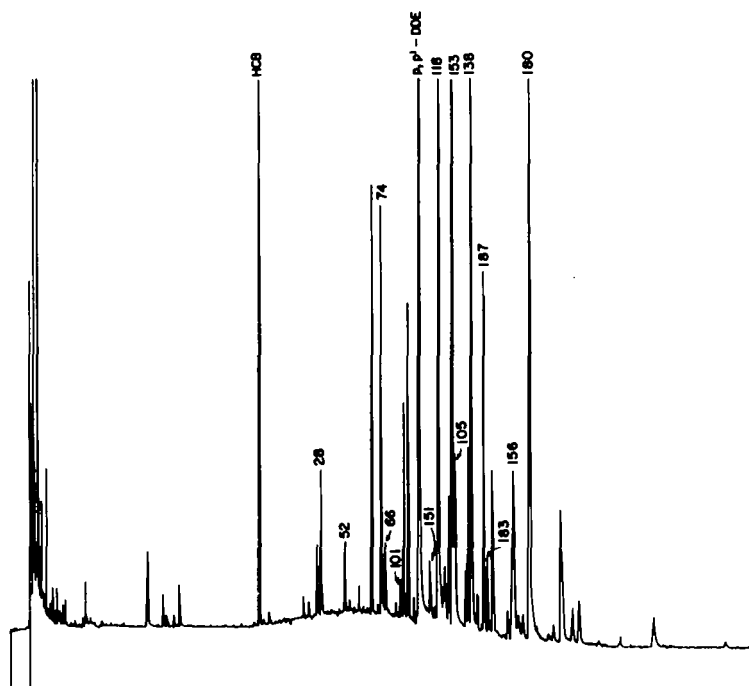


FIGURE 2 A typical GC elution pattern of PCB isomers found in breast milk of Canadian women. The numbers correspond to those in Figure 1.

Under our experimental conditions the specific isomer mixture measured $62 \pm 2\%$ of a known weight of Aroclor 1260. Therefore PCB breast milk levels based on Aroclor 1260, obtained under similar conditions, may be converted to "total isomer" PCBs by applying a conversion factor of 0.62. The mean "total isomer" PCB value calculated from Table II was 16 ng/g. The equivalent value of 27 ng/g based on Aroclor 1260, was earlier reported by Mes *et al.*¹¹

Peaks in the GC pattern of breast milk PCBs not presently accounted for by specific isomers may be included in future determinations, when isomers representing these peaks become readily available and thus increase analytical confidence to an even higher degree.

Acknowledgements

The authors wish to thank Drs J. Ryan and J. Iyengar for reviewing this manuscript.

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