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Comparison of Some Specific Polychlorinated Biphenyl Isomers in Human and Monkey Milk

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The presence and levels of polychlorinated biphenyls (PCBs) in monkey milk have been reported earlier as part of studies which investigated the overall toxicity of PCBs in commercial Aroclors[1] (Allen and Barsotti 1976; Truelove et al. 1982). Some of this information has served as a basis for an estimation of the potential health hazard of PCB contaminated breast milk to human infants (Morrison 1978). To further support such extrapolation from one primate situation to another, it would be desirable to know not only the levels of PCBs in the milk of these primates, but also the isomeric distribution in order to better evaluate the contribution of each isomer to the overall toxicity. A large concentration in breast milk of an isomer of relatively low toxicity may have the same effect on an infant as a smaller concentration of a highly toxic isomer. Furthermore, specific isomer analysis could stimulate toxicity studies on those isomers observed in human and monkey milk for which toxicity data are lacking.

This paper compares the relative amounts of 29 selected PCB isomers in human and monkey milk samples. The selection of isomers was based on the most prevalent PCB isomers in human milk as reported by Safe et al. (1985) and represented approximately 80% of all reported isomers. In addition, Aroclor 1254, whose toxicity in monkeys has been investigated recently by several investigators (Tryphonas et al. 1984, 1986), was analysed for the same 29 selected PCB isomers.

Human milk from a 1982 nation-wide survey was collected as described earlier by Mes et al. (1986) and pooled. Small amounts (0.2-1 mL) of monkey milk were manually expressed from several Rhesus monkeys, not previously exposed to PCBs other than through background levels in air, water and food. The monkey milk was collected in residue-free glass vials and pooled.

[1] A registered product of Monsanto Chemical Co.

All solvents were glass-distilled and free of interfering residues as tested by gas chromatography (GC) with electron-capture detection after concentration from 250 mL to 1 mL. Residue-free glassware was prepared as described previously (Mes and Davies 1978).

All 29 PCB isomers used in this study were 95-99% pure, except for the 2',3,4-trichloro-, 2,2',3,4-tetrachloro-, 2,2',4,4',5- and 2,3,3',4',6-pentachlorobiphenyls, which were 48, 34, 52 and 77% pure, respectively. The impurities (mainly other PCB isomers) did not interfere with analyses for the selected PCB isomers, except for one instance, which will be described later.

A detailed extraction procedure has been described earlier by Mes et al. (1986) and may briefly be summarized as follows: the pooled human and monkey milk samples (~10 g of each) were centrifuged, and the aqueous phase was removed. The milkfat was extracted with acetone:benzene (19:1, v/v), and the solvents were evaporated after filtering through glass wool. The residue was redissolved in hexane and dried over anhydrous Na_2SO_4. Out of a final 20-mL hexane extract, 1 mL was used for a gravimetric lipid determination.

An aliquot of hexane extract, containing not more than 250 mg lipid, was evaporated to <1 mL on a rotatory evaporator (<30°C), and the residue was chromatographed on a micro Florisil[2] column as described by Mes et al. (1980). PCBs were collected in 35 mL of hexane.

A 1-μl aliquot of the PCB fraction was chromatographed on a DB-5 (J&W Scientific Inc., Folsom, CA, U.S.A.) fused-silica capillary column (30 m x 0.24 mm i.d.), using a Varian 3500 Series GC with on-column injector and a Ni-63 electron-capture detector. The inlet temperature was programmed from 80° to 240°C at the rate of 160°/ min. The column temperature was kept at 130°C for 7 min, increased to 190°C at the rate of 4°/min, increased to 230°C at 3°/min and held at 230°C for 15 min. The detector temperature was 300°C.

The linear velocity of the helium carrier gas at 130°C was 20 cm/sec, and the nitrogen make-up gas was adjusted to 30 mL/min. A 1-μl standard solution, containing 5-60 pg of each PCB isomer (depending on individual isomer response), was gas-chromatographed before and after sample injections. Identification of the GC peaks was automated by using a Varian Vista 402 data system. Quantitation of individual PCB isomers was based on comparing peak heights of standard and sample.

The presence of individual isomers was confirmed by multiple reaction monitoring on a Taga 6000 E tandem MS/MS.

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[2] A registered product of Floridin Co.

RESULTS AND DISCUSSION

Table 1 shows the PCB content of pooled human and monkey milk samples based on the 29 selected PCB isomers. The fat content of the monkey milk was almost twice as high as that of human milk; this difference was also reflected in the total selected PCB isomer content on a milkfat basis.

Table 1. A comparison of total PCB isomer and fat levels in pooled human and monkey milk

Substrate	Sample weight (g)	% Fat	ng PCB isomers/g	
			Whole milk	Milkfat
Human milk	9.72	3.85	15.3	397
Monkey milk	10.42	6.09	9.3	153
Blank		N.A.[a]	<0.1	N.A.

[a] N.A. = not applicable

Table 2 shows the residue levels of all 29 individual PCB isomers calculated on a milkfat basis. As expected from the results given in Table 1, the levels of individual PCB isomers were in general higher in human milk than in monkey milk. PCB isomer numbers 74, 99, 118, 153, 138 and 180 were the main contributors to the overall higher PCB level in human milk. Furthermore, the monkey milk contained considerably lower levels of the 2,4,4',5-tetrachloro- and 2,2',4,4',5-pentachlorobiphenyl congeners (No. 74 and 99).

TABLE 2. Comparison of specific PCB isomer levels in human and monkey milk

PCB isomer No.[a]	Chlorine substitution pattern	ng/g Milkfat	
		Monkey milk	Human milk
28	2,4,4'	5	3
33	2',3,4	<1	2
52	2,2',5,5'	4	4
49	2,2',4,5'	1	2
44	2,2',3,5'	1	2
41	2,2',3,4	2	5
74	2,4,4',5	5	35

TABLE 2. (Continued)

PCB isomer No.	Chlorine substitution pattern	ng/g Milkfat	
		Monkey milk	Human milk
66*	2,3',4,4'	7	8
60	2,3,4,4'	2	4
99	2,2',4,4',5	3	44
110*	2,3,3',4',6	4	1
118	2,3',4,4',5	22	41
153	2,2',4,4',5,5'	19	60
105*	2,3,3',4,4'	9	12
141	2,2',3,4,5,5'	1	<1
138	2,2',3,4,4',5	17	59
187	2,2',3,4',5,5',6	3	15
183	2,2',3,4,4',5',6	2	7
156	2,3,3',4,4',5	5	13
157	2,3,3',4,4',5	1	4
180*	2,2',3,4,4',5,5'	15	41
193	2,3,3',4',5,5',6	2	4
191	2,3,3',4,4',5',6	<1	2
201	2,2',3,3',4',5,5',6	6	10
203	2,2',3,4,4',5,5',6	3	7
189	2,3,3',4,4',5,5'	<1	1
194	2,2',3,3',4,4',5,5'	5	8
206	2,2',3,3',4,4',5,5',6	5	2
209	2,2',3,3',4,4',5,5',6,6'	3	2

[a] Numbering system according to Ballschmiter and Zell (1980).
* A statistically significant difference was observed at a 99% confidence level.

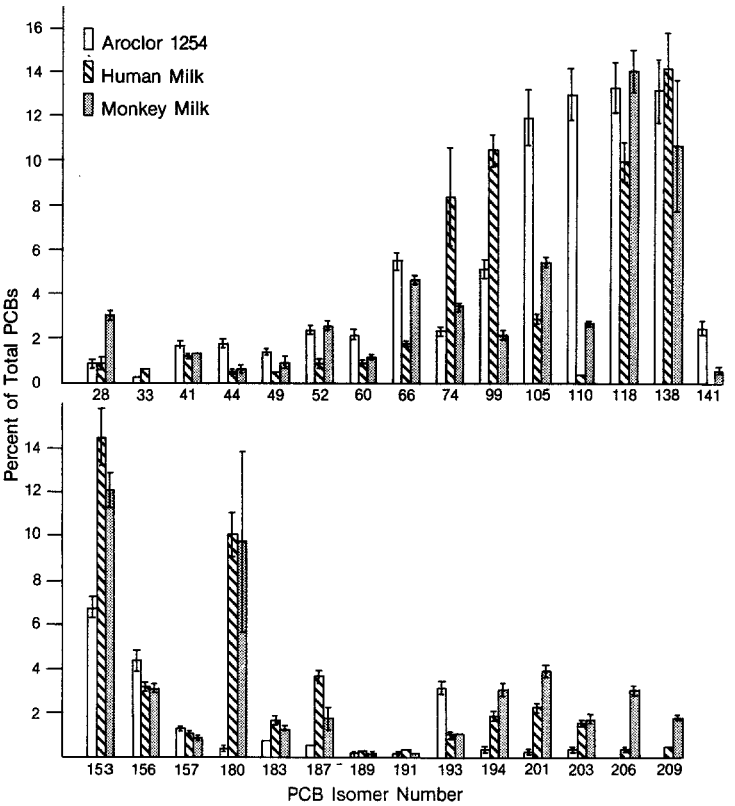

Figure 1. A histogram comparing PCB isomer distribution in human milk, monkey milk and Aroclor 1254 for selected isomers. The variations indicated at the top of the bars were obtained for triplicate determinations of the same extract.

Figure 1 illustrates the differences between monkey milk, human milk and Aroclor 1254 in terms of isomer distribution, based on the 29 selected PCB isomers. Aroclor 1254 was thereby treated as a sample and measured against the selected PCB isomers. Based on this measurement, the distribution of the selected isomers in Aroclor 1254 differed considerably from that of human and monkey milk samples, particularly with respect to isomer No. 110. In addition, marked differences were observed between the PCB isomer compositions of human and monkey milk. PCB isomers 28, 52, 66, 105, 110, 118, 180, 194, 200, 206 and 209 made up considerably higher percentages of the total measured PCBs of monkey milk than the same isomers in human milk. Human milk showed higher percentages of isomer numbers 74, 99, 153 and 187 in its total measured PCBs.

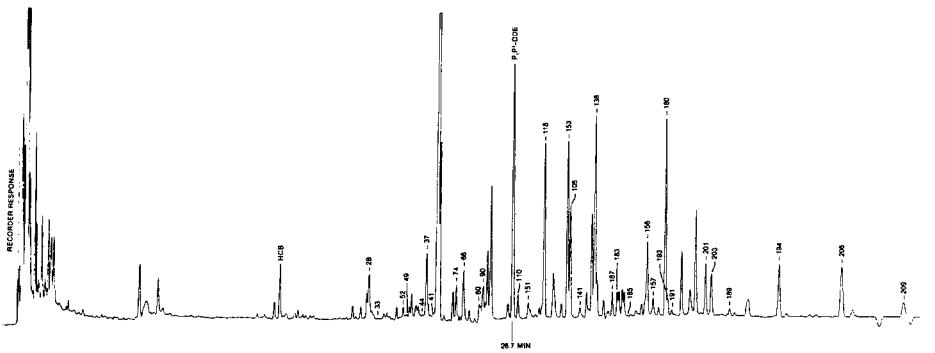

Figure 2. The GC elution pattern of the PCB fraction from monkey milk extract.

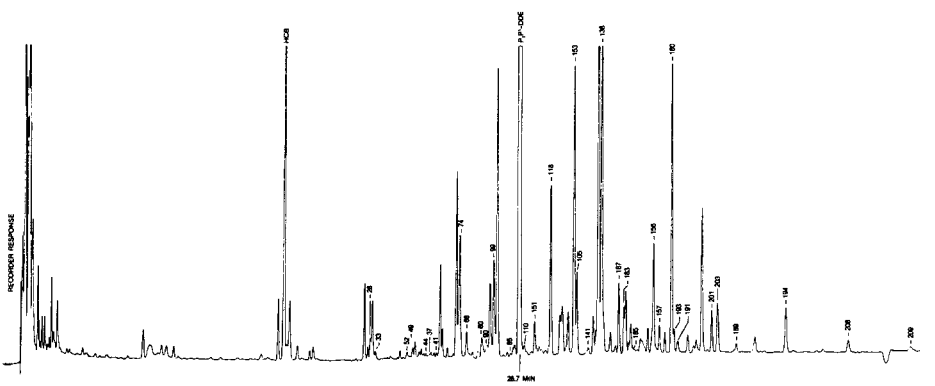

Figure 3. The GC elution pattern of the PCB fraction from human milk extract.

Figures 2 and 3 compare the GC elution patterns of PCB isomers in human and monkey milk and show that almost the same PCB isomers were observed in both. PCB isomer 101, although frequently reported in human milk (Safe et al. 1985; Mes et al. 1987) was not measured, since its retention time coincides with that of isomer 90, an impurity found in isomer 99 used in this study.

In summary, the results of this study indicate that most of the predominant PCB isomers found in human milk are also observed in monkey milk. Monkey milk had relatively low levels of PCB isomers No. 74 and 99.

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