Bull. Environ. Contam. Toxicol. (1991) 47:491–498
© 1991 Springer-Verlag New York Inc.

Environmental Contamination and Toxicology

Coplanar PCBs in Human Milk in the Province of Québec, Canada: Are They More Toxic than Dioxin for Breast Fed Infants?

Éric Dewailly,[1] Jean-Philippe Weber,[2] Suzanne Gingras,[1] and Claire Laliberté[1]

[1]Community Health Department—CHUL, 2050, boul. St-Cyrille Ouest and [2]Québec Toxicology Center—CHUL, Ste-Foy, Québec, G1V 2K8, Canada

Organochlorinated compounds have been found in the milk of women from the general population in numerous countries (Jensen 1987). Most of these surveys focused on chlorinated pesticides and polychlorinated biphenyls (PCBs). More recently, dioxins (PCDDs) and furans (PCDFs) have also been recovered in human milk fat at levels ranging from 8 ppt to 23 ppt (Schecter et al. 1990) of 2,3,7,8-TCDD Toxic Equivalent Quantity (TEQ) using the International Toxic Equivalency Factors (I-TEF) (NATO 1988). Under such conditions, a breast-fed baby receives daily between 50 and 200 pg per kg of body weight (bw) which is several times more than the Acceptable Daily Intakes of various countries (0.1 to 10 pg/kg bw/day). Some epidemiologic studies have found neurologic and developmental effects related to prenatal exposure but the impact of nursing was less associated with these health effects (Jacobson et al. 1990, Rogan et al. 1986, Fein et al. 1984, Gladen et al. 1988).

Despite the presence of such compounds in the breast milk and because present knowledge is limited, WHO recommended that breast-feeding should be encouraged and promoted on the basis of evidence of the benefits of breast-feeding to the overall health of infants (WHO 1987).

Different experimental studies have increased our understanding of toxic mechanisms of PCBs, PCDDs and PCDFs. Recently, coplanar PCBs which are approximate stereoisomers of 2,3,7,8-TCDD have been found to be the most toxic congeners of PCBs with a toxicity relative to that of 2,3,7,8 TCDD ranging from 0.001 for mono-ortho coplanars to 0.01, 0.1 and 0.05 respectively for tetra, penta and hexa non-ortho coplanar congeners (IUPAC No: 77: 3,3',4,4'; 126: 3,3',4,4',5 and 169: 3,3',4,4',5,5'). These congeners are present in different biological specimens at very high levels when compared with levels of TCDD.

Therefore, coplanar PCBs determination is useful in evaluating the toxic potential of breast milk for infants.

Send reprint requests to Eric Dewailly at the above address.

Moreover, the assessment of the relative importance of these compounds compared with other PCB congeners and PCDDs/PCDFs could be helpful in choosing the most suitable toxic parameters for the biomonitoring of human populations.

This paper presents and discusses the levels of coplanar PCBs in the breast milk of a representative sample of 96 women from the general population of the province of Québec - Canada.

MATERIALS AND METHODS

Milk samples were collected from 96 volunteer women who had delivered in 10 different hospitals between December 1988 and February 1989. All 40 ml samples were collected at home between the 14^{th} and the 21^{st} day after delivery. Milk was collected by a nurse over a 24 hours period, frozen at -20°C in 60 ml polycarbonate vials, and sent frozen to the laboratory for analyses.

These women were the first 96 included within a provincial monitoring program (600 women, 22 hospitals) for the presence of organochlorines in breast milk. The mean age of the study population was 28.4 years. The average number of previous living babies was 1.0. The 10 hospitals where milk samples were collected are mostly situated in rural regions. None of the samples came from urban areas (Montréal or Québec City).

In order to obtain a sufficient quantity of milk (≈ 60 ml) for the different analyses, 10 ml milk samples were pooled and 16 pools of six samples each were so constituted. In order to minimize the intrapool variations and to obtain ranges of levels which allowed us to perform correlation analysis between congeners, milk samples were pooled according to their previously measured PCB levels (Aroclor 1260).

Milk samples, 25 to 40 g, were fortified with nine $^{13}C_{12}$-labeled PCDD and PCDF and three $^{13}C_{12}$-PCB internal quantitation standards. These internal standards represented each of the PCDD and PCDF homologs and the three coplanar PCBs of interest (IUPAC No 77, 126 and 169). The milk samples were mixed with an aqueous solution of sodium oxalate, ethyl ether and ethanol, and then extracted with hexane. The hexane extracts were washed with reagent water, the hexane was then concentrated to constant weight, and the percent lipid was determined gravimetrically.

The lipid residue was diluted in hexane and was cleaned using a sulfuric acid-silica gel slurry followed by elution through a neutral/acid-silica gel chromatographic column. Subsequent cleanup steps included separation

Table 1. Non-ortho coplanar PCBs in human milk fat

CONGENER	IUPAC No	N	MEAN pg/g	RANGE	SE
3,3',4,4'	77	16	8.07	4.1 - 13.4	0.68
3,3',4,4',5	126	16	80.46	44 - 143	8.01
3,3',4,4',5,5'	169	16	32.71	16.2 - 62	3.04

of the PCDDs/PCDFs and coplanar PCBs from interferences using neutral alumina 22 and Carbopack C/Celite columns. The eluent from the Carbopack C/Celite was concentrated to 5 μL. The final extracts were analyzed using a VG 70250S HRMS at a mass resolution of 10,000. Separation was achieved using a 60 m DB-5 column. Two ions characteristic of each PCDD and PCDF homolog, the coplanar PCBs and the respective internal quantitation standards were monitored for each analysis. Identification of the PCDDs, PCDFs, and coplanar PCBs was based on retention time information and the comparison of the ratios of the characteristic ions with theoretical values.

The sample analysis for total PCBs and spectific PCB congeners was based on 5 g samples extracted as described above. However, the extract cleanup was based on the neutral/acidic silica gel column only and the final volume was adjusted to 250 μL. The analysis was achieved using a 60 m DB-5 column and an electron capture detector. Identification and quantitation of the specific congeners was achieved by comparing responses and retention times to calibration standards for each analyte of interest. Total PCBs (as Aroclor 1260) were similarly measured by electron capture capillary gas chromatography, as described previously (Dewailly et al. 1989).

Statistical analyses were performed using the SAS statistical package (SAS 1985). Student's t-test was used for arithmetic mean comparisons. Pearson's correlation coefficient was used to measure association between congeners. For these correlations we included the data from the 9 mother's milk samples which had been analyzed following the St-Basile PCB fire. We have previously reported that the exposure to PCBs, PCDDs and PCDFs was negligible (Dewailly et al. 1990). For this reason, correlation coefficients are based on 25 observations.

RESULTS AND DISCUSSION

Non-ortho coplanar PCBs were detected in each of the 16 milk samples. Results are presented in Table 1. Level of congener 126 is the most important and is three times and 10 times more than that of congeners 169

and 77 respectively. Moreover this penta congener is also the most toxic of the non-ortho coplanar PCBs.

We present in Table 2 the results for non-ortho coplanar PCBs, mono-ortho coplanar PCBs, PCDDs and PCDFs measured on these 16 pools of breast milk samples. Because this group was used as control group to assess the St-Basile PCB fire, PCDDs and PCDFs levels have been presented previously and are not discussed in this paper.

The Toxic Equivalency Factors used for PCDDs and PCDFs are from NATO (1990). For non and mono-ortho coplanar PCBs we used TEFs recently proposed by Safe (Safe et al. 1990). In Toxic Equivalent Quantity (TEQ), the sum of non-ortho coplanar PCBs is 9.76 pg/g, the sum of mono-ortho coplanar PCBs is 28 pg/g and the sum of PCDDs and PCDFs is 13.26 pg/g.

The associations between non-ortho coplanar PCBs and others PCB congeners are presented in Table 3. Correlation between coplanar PCB 77 and other congeners is poor and the significance at the 0.05 statistical level is mainly observed for tetra and penta PCB congeners (IUPAC No 74, 105, 118).

Coplanar PCB 126 is well predicted by different PCB congeners especially penta congeners 105 and 118 at the 0.01 statistical level. The sum of PCB congeners and total PCBs expressed as Aroclor 1260 have respective correlation coefficients of 0.48 and 0.49 ($P < 0.05$). Coplanar PCB 169 is strongly associated with hexa and hepta PCB congeners ($R = 0.59$ to 0.84, $P < 0.01$) and with the sum of PCB congeners ($R = 0.83$, $P < 0.01$) and total PCB Aroclor 1260 ($R = 0.80$, $P < 0.01$).

Levels of coplanar PCBs in human milk are similar but slightly lower than those found recently in Sweden. For seven human milk samples taken in 1988 and 1989, Norén reported levels of 27, 98 and 47 pg/g fat for 77, 126 and 169 non-ortho coplanar PCBs respectively (Norén and Lundén 1990). The pattern is also similar i.e. 126 > 169 > 77. Congener 126 is therefore the most abundant among these three congeners. Because it is also the most toxic compound (TEQ of 0.1), this congener is the most important non-ortho coplanar PCB in human milk of women living in the province of Québec. The lower levels found in this study when compared with the Swedish data are possibly attributable in part to the rural origin of these 96 women vs the urban origin of the Swedish women (Stockholm region). Moreover body burdens of organochlorines are generally higher in Europe than in North America.

As shown in Table 2, if Toxic Equivalency Factors recently proposed for PCBs are substantiated, it is then likely that non-ortho coplanar PCBs

Table 2. Mean levels and relative toxicity of coplanar PCBs, PCDDs and PCDFs in human milk fat.

PCBs	MEAN (pg/g)	TEF*	TEQ (pg/g)
Non-ortho COPLANAR			
3,3',4,4'-tetra CB	8.1	0.01	0.08
3,3',4,4',5-penta CB	80.5	0.10	8.05
3,3',4,4',5,5'-hexa CB	32.7	0.05	1.63
Mono-ortho COPLANAR			
2,3,3',4,4'-penta CB	4 400	0.001	4.4
2,3,4,4',5-penta CB	17 400	0.001	17.4
2,3,3',4,4',5-hexa CB	6 200	0.001	6.2
PCDFs and PCDDs	MEAN (pg/g)	TEF**	TEQ (pg/g)
DIBENZOFURANS			
2,3,7,8-TCDF	6.1	0.1	0.60
2,3,4,7,8-PECDF	5.2	0.5	2.60
1,2,3,4,7,8-HXCDF	3.3	0.1	0.33
1,2,3,6,7,8-HXCDF	2.3	0.1	0.23
2,3,4,6,7,8-HXCDF	1.1	0.1	0.11
1,2,3,4,6,7,8-HPCDF	4.5	0.01	0.05
DIOXINS			
2,3,7,8-TCDD	2.3	1	2.30
1,2,3,7,8-PECDD	4.8	0.5	2.40
1,2,3,(4+6)7,8-HXCDDs	34.6	0.1	3.46
1,2,3,7,8,9-HXCDD	6.4	0.1	0.64
1,2,3,4,6,7,8-HPCDD	40.5	0.01	0.41
OCDD	131.7	0.001	0.13

* Safe, 1990 ** NATO, 1988

Table 3. Correlation coefficients between PCB congeners and non-ortho coplanar PCBs in 25 human milk samples.

PCBs (IUPAC No)	COPLANAR PCBs (IUPAC No)		
	77	126	169
28	0.08	0.18	0.54**
74	0.38*	0.52**	0.69**
99	0.03	0.15	0.61**
105	0.59**	0.70**	0.47*
118	0.43*	0.77**	0.64**
128	0.33	0.36	0.59**
138	0.22	0.47*	0.78**
153	0.11	0.38	0.81**
156	0.37	0.46*	0.74**
170	0.19	0.35	0.84**
180	- 0.06	0.19	0.82**
183	0.43*	0.42*	0.50*
187	0.38	0.35	0.64**
PCB[a]	0.25	0.48*	0.83**
Aro1260[b]	- 0.006	0.49*	0.80*

[a] Sum of the above congeners [b] Total PCBs expressed as Aroclor 1260
* $P < 0.05$ ** $P < 0.01$

represent a more important factor of risk for infants than PCDDs and PCDFs. Moreover, the presence of a mono-ortho coplanar PCB such as 2,3',4,4',5-pentaCB (IUPAC No 118) found in human milk fat at a mean level of 17.4 ng/g with a TEF of 0.001, represents a TEQ of 17.4 pg/g which is 7.5 times more than that of 2,3,7,8-TCDD (2.3 pg/g). A baby receiving 120 ml/kg bw of milk per day with 3.5 % of fat, consumes 4.2 g of milk fat per kg bw/day (WHO 1988). Under these conditions, the total daily intakes for non-ortho coplanar PCBs, mono-ortho coplanar PCBs and dioxins and furans are respectively 41, 117.6 and 55.7 pg/kg bw. When we consider only the toxic responses mediated by the Ah receptor, total PCB represents 158.6 pg/kg TEF which is 3 times more than for dioxins and furans and which is 159 times more than the ADI of 1 pg/kg bw/day proposed by ATSDR (1989).

Because coplanar PCBs are helpful in assessing the toxic potential of human milk and because the determination of these congeners is difficult and costly (HRMS), we explored the associations between PCBs and coplanar PCBs. Because of their toxicity, one should include mono-ortho coplanar PCBs (IUPAC No 105, 118, 156) in HRGC analyses of breast milk samples. Furthermore, once the specific population pattern has been established for non-ortho coplanar PCBs, it is possible to accurately predict the level of coplanar 169 from the sum of PCB congeners ($R = 0.83$, $P < 0.0001$). For coplanar PCB 126, the best predictors are the levels of PCBs 105 and 118 (two mono-ortho coplanar PCBs).

Finally for PCB 77, the least toxic of coplanar PCBs, a strong correlation has been found only between this congener and the coplanar 3,3',4,4',5-pentaCB (No 126) ($R = 0.63$, $P < 0.001$). However the correlation coefficient with congener 105 is 0.59 ($P < 0.01$). Ratios of non-ortho coplanar PCBs on the sum of PCB congeners were 1/2330 for the penta coplanar (IUPAC No 126) and 1/5740 for the hexa coplanar (IUPAC No 169).

In conclusion, coplanar PCBs are excreted in human milk fat at high levels, non-ortho coplanar congeners, the most toxic compounds, at ppt levels and mono-ortho coplanar congeners at ppb levels. Using recently published TEFs, we conclude that in our study population, PCBs represent a higher risk than PCDDs and PCDFs. Mono-ortho coplanar PCBs such as IUPAC No 105, 118 and 156 represent an important part of the toxicity of PCB and PCDD/PCDF mixtures. Therefore, it is important to measure these congeners for human body burden assessment. It is also possible using these congeners, to predict, for a specific population, the levels of the most toxic congeners i.e. the non-ortho coplanar PCBs.

Acknowledgments. This study was made possible through a grant from the Ministry of Health of Québec. We thank Kathy E. Boggess of Midwest Research Institute for analysis support for PCDDs, PCDFs and PCB congeners analysis, Evelyne Pelletier and Liliane Ferron of Québec Toxicology Center for total PCB analysis and Lise Côté for technical support.

REFERENCES

ATSDR (1989) Agency for Toxic Substances and Disease Registry. Toxicological profile for 2,3,7,8-Tetrachloro-Dibenzo-p-Dioxin. US-PHS. ATSDR/TP-88/23.

Dewailly E, Nantel AJ, Weber J-P, Meyer F (1989) High levels of PCBs in breast milk of Inuit women from arctic Québec - Bull Environ Contam Toxicol 43: 641-646.

Dewailly E, Tremblay-Rousseau H, Carrier G et al. (1990) PCDDs, PCDFs and PCBs in human milk of women exposed to a PCB fire and of women from the general population of the province of Québec-Canada. In: Organohalogen Compounds, Vol. 1: Dioxin'90 - EPRI - Seminar. Ed by O. Hutzinger and H. Fielder - Bayreuth: Ecoinforma Press p. 227-230.

Fein GG, Jacobson JL, Jacobson SW, Schwartz PM, Dowler JK (1984) Prenatal exposure to polychlorinated biphenyls: effect on birth size and gestational age. J Pediatr 105;2: 315-320.

Gladen BC, Rogan WJ, Hardy P, Thullen J, Tingelstad J, Tully M (1988) Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene transplacentally and through human milk. J Pediatr 113; 6: 991-995.

Jacobson JL, Jacobson SW, Humphrey HEB (1990) Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. J Pediatr 116; 1: 38-45.

Jensen AA (1987) Polychlorobiphenyls (PCBs), Polychlorodibenzo-p Dioxins (PCDDs) and Polychlorodibenzofurans (PCDFs) in human milk, blood and adipose tissue. Sci Total Environ 64:59-293.

NATO:North Atlantic Treaty Organization Committee on the Challenges of Modern Society (1988) Pilot study on international information exchange on dioxins and related compounds. Scientific basis for the development of the International Toxicity Equivalency Factor (I-TEF). Method of risk assessment for complex mixtures of dioxins and related compounds. #178.

Norén K and Lundén A (1990) Trend studies of polychlorinated biphenyls, Dibenzo-p-dioxins and Dibenzofurans in human milk. In: Organohalogen Compounds, Vol. 1: Dioxin'90-EPRI-Seminar. Ed by O. Hutzinger and H. Fielder. Bayreuth: Ecoinforma Press p. 263-266.

Rogan WJ, Gladen BC, McKinney JD et al. (1986) Neonatal effects of transplacental exposure to PCBs and DDE. J Pediatr 109;2: 335-341.

Safe S, Yao C, Davis D (1990) Development of Toxic Equivalency Factors for polychlorinated biphenyls (PCBs). In: Organohalogen Compounds Vol. 2: Dioxin'90 -EPRI-Seminar. Ed. by O. Hutzinger and H. Fielder - Bayreuth: Ecoinforma Press. p. 55-59.

Schecter A, Startin JR, Rose M et al (1990) Chlorinated dioxin and dibenzofuran levels in human milk from Africa, Pakistan, Southern Vietnam, the Southern U.S. and England. Chemosphere 20; 7-9: 919-925.

Statistical Analysis System (1985)SAS Institute Inc., Cary N.C. User's Guide.

WHO. Regional Office for Europe (1987) Assessment of health risks in infants associated with exposure to PCBs, PCDDs and PCDFs in breast milk. Report on a WHO Working Group - Albano/Terme February 1987.

Received February 6, 1991; accepted May 1, 1991.