Bull Environ Contam Toxicol (2011) 87:21–25
DOI 10.1007/s00128-011-0262-7

Human Milk as a Vector and an Indicator of Exposure to PCBs and PBDEs: Temporal Trend of Samples Collected in Rome

S. Alivernini · C. L. Battistelli · L. Turrio-Baldassarri

Received: 19 November 2010 / Accepted: 22 March 2011 / Published online: 5 April 2011

Abstract Thirty-seven polychlorobiphenyl (PCB) congeners and seven polybromodiphenylether (PBDE) congeners were measured in human milk samples collected in Rome between 2005 and 2007. The comparison of results with two previous studies performed in Rome in 1984 and in 2000–2001 indicates a 64% decrease of PCB levels, still in progress; profile differences with time were also evident as lighter congeners are less relevant now; data are in good agreement with recent European studies. PBDE contamination profiles were different in individual samples and a similar variability was observed in data from different countries, suggesting different exposure pathways and profiles.

Keywords PCB · PBDE · Breast milk · Temporal trend

Polychlorobiphenyls (PCBs) and polybromodiphenylethers (PBDEs) are two classes of compounds roughly sharing both structure and environmental behaviour and with opposite production history: PCB production ends roughly when PBDE production begins. It is then relevant to study the temporal trends of the levels in human milk of both classes of compounds. Human milk is a particularly significant indicator for both mother and infant: for the mother, as it accounts for a lifelong exposure; for the infant, as it is the vehicle of the contamination. The level of organohalogen compounds in human milk is higher than in the adult diet (Fürst 2006) and this implies that, excluding specific contamination accidents, breast-feeding represents the most relevant form of human exposure for general population. In fact, human milk has a high organohalogen content and is the exclusive food of infants.

For these reasons, in the late 80s, the World Health Organization (WHO) promoted international studies to investigate the presence of "dioxins" (polychlorinated dibenzo-*p*-dioxins [PCDDs] and polychlorinated dibenzo furans [PCDFs]) in human milk worldwide (WHO 2007). Many studies demonstrated that levels of such substances were decreasing in time (Fürst 2006). However they pointed almost exclusively at dioxins, micropollutants unintentionally produced in combustions and industrial processes, having different sources and diffusion pathways from the industrial products PCBs and PBDEs. It is not certain whether PCBs have decreasing rates similar to dioxins or not.

Finally, PBDE production volume is increasing and it was shown that their levels in both the environment and biota are increasing (Frederiksena et al. 2009).

The studies of temporal trends of PCBs and PBDEs in human milk are less systematic than those on dioxins and the data available are scarcely useful for time comparison as they differ for location, collection protocol and analytical target.

The present study, conceived to be comparable to two previous studies on Rome mother milk (Larsen et al. 1994; Ingelido et al. 2007), aims at observing temporal trends of PCBs and PBDEs.

Materials and Methods

Thirteen milk samples (about 50 mL each) were collected from primiparae women living in Rome for at least the last

S. Alivernini · C. L. Battistelli · L. Turrio-Baldassarri (✉)
Istituto Superiore di Sanità, Viale Regina Elena 299,
00161 Rome, Italy
e-mail: luigi.turrio@iss.it

10 years, during the 3rd month after delivery, between 2005 and 2007. Detailed instructions on sample collection and storage, together with specially cleaned containers and a questionnaire (about age, place of birth, residence, occupation, dietary habits, exposure) were given to the participants. Donors were selected in agreement, as much as possible, with the WHO protocol (WHO 2007). The mean age of the donors was 34 ± 3 years; the mean birth weight of the infants was 3.39 ± 0.34 kg; the mean lipid content of breast milk was 2.80 ± 1.53%. Milk samples were kept at 4°C during collection, and stored at −20°C until analysis.

PCB and PBDE congeners were provided by Cambridge Isotope Laboratories (Andover, MA, USA), Ultra Scientific Inc. (North Kingstown, RI, USA), AccuStandard Inc. (New Haven, CT, USA) or Wellington Laboratories Inc. (Wellington, Ontario, Canada).

The milk samples were analysed for the 37 PCB congeners and the 7 PBDE congeners listed in the result tables. An internal standard solution containing the ^{13}C-labelled congeners PCB 105, PCB 114, PCB 118, PCB 123, PCB 156, PCB 157, PCB 167, PCB 189, PBDE 28, PBDE 47, PBDE 99, PBDE 153 and PBDE 154 was used to spike the samples. PCB mean recovery yields ranged between 49% and 92%, PBDE between 41% and 66%.

Milk samples were spiked with the ^{13}C-labelled standard solution, added with sodium oxalate, and subjected to a liquid–liquid extraction with, in sequence, methanol, diethyl ether and n-hexane. The extracts were dried, concentrated and the fat content was gravimetrically determined. Clean-up was carried out by column elution with n-hexane over concentrated sulphuric acid coated on Extrelut from Merck (Darmstadt, Germany). The concentrated eluate was further purified using the automated multi-column Power Prep system (Fluid Managment Systems, Waltham, MA, USA) slightly modifying a previous method (Turrio-Baldassarri et al. 2009) to include PBDEs, in the following way: the eluate was passed on the multi-layer column; the PCB congeners aliquot was eluted through an alumina column with n-hexane/dichloromethane (98/2 v/v); the PBDE aliquot was eluted with n-hexane/dichloromethane (50/50 v/v).

A procedural blank was associated with each three-sample batch and processed in the same manner.

The determination of PCB and PBDE was carried out on a gas chromatograph-mass spectrometer (GC–MS) Trace MS DSQ (Thermo Scientific, Waltham, MA, USA) as previously described (Turrio-Baldassarri et al. 2009).

QA/QC: our laboratory regularly uses certified reference materials and routinely employs laboratory reference materials; moreover, it participated since the year 2000 to the international "Dioxin in food" interlaboratory exercises organized by the Norwegian Folkehelsa Institute, determining PCDDs, PCDFs, dioxin-like PCBs, non-dioxin-like PCBs and lately PBDEs in three different food matrices each year. The results were always satisfactory and were described elsewhere (Turrio-Baldassarri et al. 2008).

Results and Discussion

In Table 1 the mean values of the 37 PCB congeners analysed in the 13 samples, together with their limit of quantification (LOQ) are reported; in the last two lines the sum of the analysed congeners and the sum of the six indicator congeners (28, 52, 101, 138, 153, 180) are shown. In Table 1 some congeners are marked with one or two asterisks, as a function of the blank incidence: one asterisk indicates a blank incidence on the signal of the analyte from 5% to 25%; two asterisks mark an incidence between 25% and 75%; in the first case the blank is not subtracted, in the second it is.

The main purpose of this study was to compare actual results with two previous studies conducted in Rome with the same protocol in terms of sample origin, donor eligibility, sample collection, analytical methods and choice of analysed congeners. Table 2 reports the mean values and

Table 1 Mean concentration (ng/g lipid) of PCB congeners in Rome human milk samples and their LOQ (ng/g lipid)

PCB congener	Mean	LOQ	PCB congener	Mean	LOQ
18	<0.05**	0.05	146	4.64	0.08
28	0.80*	0.05	149	0.30**	0.08
49	<0.05**	0.05	151	0.22	0.08
52	0.15**	0.05	153	53.50	0.08
66 + 80	0.69	0.05	156	7.36	0.08
70	<0.05*	0.05	157	1.16*	0.08
74	6.10	0.05	167	1.63	0.08
91	<0.06	0.06	170	16.19	0.1
95	0.20**	0.06	174	<0.1	0.1
99	8.00	0.06	177	1.80	0.1
101	0.42**	0.06	180	32.05	0.1
105	2.23	0.06	183	3.03	0.1
110	0.18**	0.06	187	6.37	0.1
114	0.98	0.06	189	0.48	0.1
118	10.62	0.06	194	4.55	0.2
123	0.13	0.06	196 + 203	3.31	0.2
128	0.41	0.08	Sum PCBs	200	
138 + 163	32.51	0.08	Sum 6 PCBs	119	
141	<0.08	0.08			

* Blank incidence on the signal of the analyte from 5% to 25%

** Blank incidence on the signal of the analyte between 25% and 75%

Table 2 Mean concentration (ng/g fat) and ratio to PCB 153 of selected PCB congeners in human milk samples from Rome at different times

Reference	Larsen et al. (1994)		Ingelido et al. (2007)		This study	
Year of collection	1987		2000–2001		2005–2007	
PCB congener	ng/g fat	Ratio to 153	ng/g fat	Ratio to 153	ng/g fat	Ratio to 153
28	–	–	3.5	0.05	0.80*	0.01
52	–	–	0.3	0.004	0.15**	0.003
74	24.3	0.22	–	–	6.1	0.11
101	–	–	0.6	0.01	0.4	0.01
118	32.7	0.29	14.1[a]	0.18	10. 6	0.20
128	–	–	0.7	0.01	0.4	0.01
138 + 163	60.4[b]	0.54[b]	58	0.75	32.5	0.61
153	111.2	1.00	77	1.00	53.5	1.00
170	26.0	0.23	21	0.27	16.2	0.30
180	53.9	0.48	56	0.73	32.1	0.60
183	11.7	0.11	6.1	0.08	3.0	0.06
187	20.8	0.19	15	0.19	6.4	0.12
194	–	–	6.3	0.08	4.6	0.09
Sum PCBs	550	4.95	–	–	200	3.74
Sum 6 PCBs	–	–	195.4	2.54	119	2.23
Sum 7 PCBs	–	–	209.5	2.72	130	2.43

* Blank incidence on the signal of the analyte from 5% to 25%; ** Blank incidence on the signal of the analyte between 25% and 75%

[a] Abballe et al. 2008

[b] The GC determination was performed using two different columns: it was then possible to separate congener 138 from congener 163; the reported datum refers to congener 138 only

the ratios, relative to the most abundant congener (PCB 153), of the PCB congeners measured in this study and in the two previous ones.

The first study was performed on nine samples collected in 1987 in Rome, the results were published in 1994 (Larsen et al. 1994). The main differences with the present study are the presence among the donors of five mothers not primiparae and the detection technique used, electron-capture (EC) instead of MS. The analytical differences are negligible, as a check with GC–MS was also performed on some samples and GC–MS data were in good agreement with GC-ECD data; moreover, the actual method is derived from the one used in 1987 and, for each modification introduced, an internal validation was performed; the same kind of chromatographic column is still used. The difference due to the presence of milk from pluriparae in 1987 samples may be relevant; it should however lead to an underestimation of the PCB levels present in Rome samples in 1987 and consequently, to a underestimation of the decrease trend eventually observed.

The second study, published in 2007 (Ingelido et al. 2007), reports results on both PCBs and PBDEs on a pool of ten samples collected in Rome between 2000 and 2001. Analytical procedure, eligibility and collection protocols were almost identical to the present study.

The comparison of the present results with the 1987 data is based on the total PCB value, as using single congeners may be misleading due to the profile differences discussed later: total PCB decrease from 550 ng/g in 1987 to 200 ng/g in 2005–2007.

Although the selection of the congeners is not identical in the two studies, the difference can be considered negligible as in both cases more than 30 congeners, including all the relevant ones, are quantified.

PCB levels found in the present study indicate that the decreasing trend is still continuing in the years form 2000–2001 to 2005–2007: the data reported in Table 2 are self evident.

There are also differences in the contamination profiles, that is the relative abundance of each congener with respect to the most relevant one (PCB 153). Table 2 shows that, although the absolute concentration of each congener decreases in time for all of the congeners considered, their ratio to PCB 153 may decrease or increase. This may depend on the fact that the congener is, respectively, less or more resistant to degradation than 153 and less or more capable of bioaccumulation. Light congeners generally had higher ratios to PCB 153 in 1987 than in 2005–2007.

In Table 3, data from the present study are compared to other European studies of the same period. The following

Table 3 Mean values (ng/g) of selected PCB congeners in human milk samples of this study, in comparison with recent European studies

Country (Year of collection) Reference	Italy (2005–2007) This study	Belgium (2006) Colles et al. (2008)	Germany (2005) Raab et al. (2008)	Norway (2002–2006) Polder et al. (2008)	Sweden (1996–2006) Lignell et al. (2009)	UK (2001–2003) Kalantzi et al. (2004)
PCB congener						
28	0.8*	1.02	–	2.4	2.8	–
52	0.15**	0.36	–	0.3	1.3	–
118	10.6	9.05	–	14	11	–
138	32.5[a]	21.9	43	39	29	41
153	53.5	38.4	65	52	58	53
180	32.1	18.4	32	26	28	27

* Blank incidence on the signal of the analyte from 5% to 25%; ** Blank incidence on the signal of the 175 analyte between 25% and 75%

[a] The reported datum of PCB 138 of this study refers to congener 138 + 163

Table 4 Mean concentration, results on pooled extracts and LOQ (ng/g lipid) of PBDE congeners in breast milk obtained in this study; results from one previous Italian study and from recent European studies (ng/g lipid)

Country (Year of collection)	Italy (2005–2007)			Italy (2000–2001)	Belgium (2006)	Germany (2005)	Norway (2002–2006)	Sweden (1996–2006)	UK (2001–2003)
Reference	This study			Ingelido et al. (2007)	Colles et al. (2008)	Raab et al. (2008)	Polder et al. (2008)	Lignell et al. (2009)	Kalantzi et al. (2004)
PBDE	Mean	Pooled	LOQ	Pooled	Pooled	Mean	Mean	Mean	Mean
28	<0.3	0.065	0.3	0.082	0.065	–	0.12		
47	0.82*	0.75	0.3	1.9	0.893	0.67	1.74	–	–
66	<0.3	<0.03	0.3	0.019	0.0045	–	–	1.9	3.9
85	<0.3	<0.03	0.3	0.074	0.017	–	–	–	–
100	<0.4	<0.03	0.4	0.48	0.212	0.17	0.38	–	–
153	0.5	0.48	0.4	0.47	0.492	0.64	0.77	0.36	0.6
154	<0.4	<0.03	0.4	0.070	0.021	–	0.07	0.64	1.4*

* Blank incidence on the signal of the analyte from 5% to 25%

studies, in which both PCBs and PBDEs were determined, were selected: Colles et al. (2008), Raab et al. (2008), Polder et al. (2008), Lignell et al. (2009) and Kalantzi et al. (2004).

Not all of these studies reported all of the six indicator congeners, but Table 3 shows quite clearly that all data agree on both contamination level and profile, meaning that contamination levels throughout Europe are comparable.

Exposure to PCBs through breastfeeding is significantly decreased in the last decades: based on the analyses of 2032 samples collected in Germany between 1984 and 2003, it was found that the intake of PCB due exclusively to breastfeeding decreased by 80% in this period. Similar results were reported also by other European countries such as The Netherlands, Norway and Sweden (EFSA 2005).

The comparison of the three studies reported in Table 2 may give useful indications on the same issue: the total PCB value of 2005–2007, compared to the 1987 one, suggests that infant exposure to PCB through breastfeeding decreased considerably in Rome in 20 years; the three studies also suggest that the decrease is gradual and still in progress.

A previous study on PCDD and PCDF (Abballe et al. 2008) reported a decrease of about 60% of the equivalent toxicity between 1987 and 2001 in Italy; we now report that human milk in Rome shows a similar decrease (64%) in the period 1987–2007, suggesting that PCBs display decrease rates comparable to dioxins.

In Table 4 the level of PBDE congeners and their LOQ in the present study are reported together with the results of one previous Italian study and of some recent European studies. The concentration of most of the congeners obtained in this study was lower than the LOQ of the method, so in an attempt to get more information, we pooled all the extracts and injected the resulting pooled extract after reducing the volume. The results obtained on the pooled sample are reported in the second column of Table 4: we reduced the LOQ of about one order of magnitude and could so determine congener 28.

The comparison with the previous 2000–2001 study from Rome (Ingelido et al. 2007) indicates that PBDE seem to be slightly decreasing.

Data from the same European studies, reported in Table 3 for PCBs and in Table 4 for PBDEs, suggest that differences in the contamination levels of PBDEs in different countries are much higher than for PCBs.

The coefficients of variation of the concentrations of the three major PCB congeners among different countries are, respectively, 16%, 24% and 18% for PCBs 153, 138 and 180, while the coefficients of variation of the 3 major PBDE congeners 47, 100 and 153 are respectively 70%, 47% and again 47%. This shows that even the mean values from a representative number of individual samples display contamination levels of single congeners widely different in different countries, and suggests that the contamination profile of PBDEs in human milk is not well defined.

The high variability of the environmental contamination profiles and the different incidences of dietary and inhalatory human exposure are among the most probable causes of PBDE profile differences.

However, it is evident that, unlike PCBs, the time trend of PBDE in human milk needs more studies to be clearly outlined.

References

Abballe A, Ballard TJ, Dellatte E, di Domenico A, Ferri F, Fulgenzi AR, Grisanti G, Iacovella N, Ingelido AM, Malisch R, Miniero R, Porpora MG, Risica S, Ziemacki G, De Felip E (2008) Persistent environmental contaminants in human milk: concentration levels and time trends in Italy. Chemosphere 73:S220–S227

Colles A, Koppen G, Hanot V, Nelen V, Dewolf MC, Noël E, Malisch R, Kotz A, Kypke K, Biot P, Vinkx C, Schoeters G (2008) Fourth WHO-coordinated survey of human milk for persistent organic pollutants (POPs): Belgian results. Chemosphere 73(6):907–914

EFSA (2005) Opinion of the Scientific Panel on Contaminants in the Food Chain on the presence of non dioxin-like polychlorinated biphenyls (PCB) in feed and food. EFSA J 284:1–137 (plus annexes). http://www.efsa.europa.eu/en/efsajournal/doc/s284.pdf

Frederiksena M, Vorkamp A, Thomsenc M, Knudsena LE (2009) Human internal and external exposure to PBDEs–A review of levels and sources. Int J Hyg Environ Health 212:109–134

Fürst P (2006) Dioxins, polychlorinated biphenyls and other organo halogen compounds in human milk. Levels, correlations, trends and exposure through breastfeeding. Mol Nutr Food Res 50:922–933

WHO (2007) Fourth WHO-Coordinated Survey of Human Milk for Persistent Organic Pollutants in Cooperation with UNEP. WHO Guidelines for Developing a National Protocol., Geneva

Ingelido AM, Ballard T, Dellatte E, di Domenico A, Ferri F, Fulgenzi AR, Herrmann T, Iacovella N, Miniero R, Päpke O, Porpora MG, De Felip E (2007) Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in milk from Italian women living in Rome and Venice. Chemosphere 67(9):S301–S306

Kalantzi OI, Martin F, Thomas GO, Alcock RE, Tang HR, Drury SC, Carmichael PL, Nicholson JK, Jones KC (2004) Different Levels of Polibrominated Diphenil Ethers (PBDEs) and Chlorinated Compounds in Breast Milk from two U.K. Regions. Environ Health Perspect 112(10):1085–1091

Larsen BR, Turrio-Baldassarri L, Nilsson T, Iacovella N, di Domenico A, Montagna M, Facchetti S (1994) Toxic PCB congeners and organochlorine pesticides in italian human milk. Ecotoxicol. Environ Safety 28:1–13

Lignell S, Aune M, Darnerud PO, Cnattingius S, Glynn A (2009) Persistent organochlorine and organobromine compounds in mother's milk from Sweden 1996–2006: compounds-specific temporal trends. Environ Res 109(6):760–767

Polder A, Thomsen C, Lindström G, Løken KB, Skaare JU (2008) Levels and temporal trends of chlorinated pesticides, polychlorinated biphenyls and brominated flame retardants in individual human breast milk samples from Northern and Southern Norway. Chemosphere 73:14–23

Raab U, Preiss U, Albrecht M, Shahin N, Parlar H, Fromme H (2008) Concentration of polybrominated diphenil ethers, organochlorine compounds and nitro musk in mother's milk from Germany. Chemosphere 72:87–94

Turrio-Baldassarri L, Abate V, Battistelli CL, Carasi S, Casella M, Iacovella N, Indelicato A, La Rocca C, Scarcella C, Alivernini S (2008) PCDD/F and PCB in human serum of differently exposed population groups of an Italian city. Chemosphere 73(1):S228–S234

Turrio-Baldassarri L, Alivernini S, Carasi S, Casella M, Fuselli S, Iacovella N, Iamiceli AL, La Rocca C, Scarcella C, Battistelli CL (2009) PCDD and PCDF contamination of food of animal origin as the effect of soil pollution and the cause of human exposure in Brescia Chemosphere. PCB 76(2):278–285