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Comparison of organochlorine compound concentrations in colostrum and mature milk

Zhiwei Yu [a], Lubica Palkovicova [b], Beata Drobna [c], Jan Petrik [c], Anton Kocan [c], Tomas Trnovec [c], Irva Hertz-Picciotto [a,*]

[a] *University of California, Davis, Department of Public Health Sciences, TB #168, Davis, CA 95616, United States*
[b] *Slovak Medical University, Department of Environmental Medicine, Slovakia*
[c] *Slovak Medical University, Department of Toxic Organic Pollutants, Slovakia*

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Abstract

Human breast milk represents the best choice for the nutrition of infants. It is often used for monitoring human exposures to environmental chemicals. Uniquely suited to meet human biological needs, breast milk composition, especially fat content, changes significantly with time from delivery. With the aim to compare the concentration of organochlorine compounds (OCs) in colostrum versus mature milk, we obtained samples of fourth–fifth day postpartum milk (colostrum) and day-14 postpartum milk (mature milk) from 12 women enrolled in the project ''Early Childhood Development and PCB Exposure in Slovakia''. The concentrations of selected organochlorine pesticides and congeners of polychlorinated biphenyls (PCBs) were measured using gas chromatography with electron capture detection and reported on lipid adjusted basis. No significant differences were found between organochlorine levels in colostrum and those in mature milk samples. A very close correlation was found between the concentrations of organochlorine compounds in colostrum and mature milk (Spearman correlation coefficient $r = 0.94$–0.98 for PCBs, and $r = 0.85$–0.99 for organochlorine pesticides, $p < 0.001$ for all compounds). The present study concludes that the use of colostrum samples provides a close estimate of the child's exposure to OCs in the early neonatal period and demonstrates that, despite the lower fat content, colostrum specimens are adequate to conduct OC analyses.

Keywords: Breast milk; Organochlorine compounds; Colostrum; Mature milk; Postnatal exposure; Milk sampling

1. Introduction

Breast milk provides all the necessary nutrients, growth factors and immunological components a healthy term infant needs (Newton, 2004; American Academy of Pediatrics (AAP), 2005). Breast milk composition changes during lactation, with fat concentrations being the most variable of the energy components of breast milk—the others being lactose and proteins (Mitoulas et al., 2002; Mandel et al., 2005).

Human colostrum is the first milk produced after birth. This secretion gradually changes to mature milk, with the transition complete by 14 days (Lawrence, 1994). As compared with the composition of mature milk, colostrum has higher protein, lower fat, and a lactose solution rich in immunoglobulins and other important immune factors and mediators (Playford et al., 2000; Ogawa et al., 2004; Issacs, 2005).

Human milk fat, present in the form of milk fat globules, is composed of 98% triglycerides, followed by phospholipids—0.7%, and cholesterol—0.5% (Newton, 2004). There is considerable within and between woman variability, but on average, with the increase of the milk fat content during the first weeks of lactation, the ratio

* Corresponding author. Tel.: +530 752 3025; fax: +530 752 3239.
E-mail address: ihp@ucdavis.edu (I. Hertz-Picciotto).

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of phospholipids and cholesterol to triglycerides decreases (Koletzko et al., 2001). Breast milk composition and lipid quality/quantity may also vary according to maternal BMI, dietary intake, smoking habits, intensive exercise and weight loss during lactation (Villalpando and del Prado, 1999; Agostoni et al., 2003; Bopp et al., 2005).

Polychlorinated biphenyls (PCBs) and other OCs such as organochlorine pesticides, polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) are toxic synthetic compounds formerly used widely for industrial purposes and consumer products and now ubiquitous in the environment. Their lipophilic properties together with high persistence in the environment allow them to bio-accumulate through the food chain, despite the fact that their production and usage were banned in most industrial countries more than 20 years ago (Breivik et al., 2004; Ross, 2004). OCs may elicit dysfunction of endocrine, immune, nervous and reproductive systems and jeopardize cognitive development and growth (Agency for Toxic Substances and Disease Registry (ATSDR), 2004; Langer et al., 2005). OCs are stored mainly in the adipose tissue of humans as well as animals. Food intake, mainly fish, meat and dairy products, accounts for more than 90% of the human body burden of PCBs and related compounds (Patandin et al., 1999; WHO, 2003).

OCs in pregnant women are transported to their babies trans-placentally and after delivery via breast milk. Several factors have been reported to be associated with the concentrations of OCs in human breast milk; e.g. parity (higher levels found in primiparas), maternal age (positive correlation), timing of sampling, and type of diet consumed by the mother (Furst et al., 1989; Chao et al., 2004; Uehara et al., 2006). The time of breast milk sampling for chemical analyses varies across studies. Proposals have been made by the World Health Organization (WHO) to standardize the methodology for breast milk collection and reporting of results for levels of contaminants with respect to the time of collection and use of lipid basis to express concentrations (WHO, 1985). Usually, mature milk is collected between second and eighth week postpartum; both individual and pooled samples are used for surveillance purposes (Larsen et al., 1994; Patandin et al., 1999; Ayotte et al., 2003).

This sub-study was conducted as the part of the large international collaborative project ''Early Childhood Development and PCB Exposure in Slovakia'' (Hertz-Picciotto et al., 2003), launched in 2001. The parent project is focused on the distribution of PCBs and their metabolites in biological samples of over 1100 woman/baby pairs and subsequent health effects in infants and children. Participants are from two selected districts of eastern Slovakia—Michalovce district as a high exposure area (PCB production in the past, improper PCB disposal) and Svidnik/Stropkov with background levels of PCBs in the environment (distance from Michalovce, approx. 70 km north).

Milk samples were collected on the fourth–fifth day postpartum while the mother was still in the hospital. To determine the validity of postnatal OC exposure estimates in breastfed infants based on these specimens, we also obtained a 14-day postpartum sample from a subgroup of participants, and compared these paired fourth–fifth and 14-day samples with respect to their OC concentrations. For logistical and cost reasons, it was not feasible to obtain 14-day samples on all women within the parent study.

For this study, we define colostrum as the milk produced on the fourth and fifth day postpartum and mature milk as that produced day-14 postpartum.

2. Material and methods

2.1. Subjects

The study subjects were mothers ($N = 14$) from the Michalovce district enrolled in the study ''Early Childhood Development and PCB Exposure in Slovakia''. This validation substudy focused on women who delivered between August 5th and September 17th, 2003. All women fulfilled enrollment criteria for the parent project—age higher than 18 years, no serious illnesses during this pregnancy, not a multiple pregnancy, parity between 0 and 3 and residency in the district for five or more years.

2.2. Sample collection

As part of PCBs and Early Childhood Development in Slovakia study protocol, breast milk samples were collected on day 4 or 5 postpartum, before the mother's discharge from the maternity ward. Milk was manually expressed by the mother into a 60 ml clear vial and then immediately frozen at −20 °C, and later transported frozen in thermoboxes from eastern Slovakia to Bratislava, in the west of the country, where they were analyzed at the Research Base of the Slovak Medical University, Department of Toxic Organic Pollutants. The milk specimens varied in volume from 10 to 50 ml.

Fourteen mothers were asked to donate a mature milk sample. The mother was provided with another vial at the time she left the hospital and instructed to collect and store a day-14 milk specimen in the same manner as above. Mature milk samples were collected by project staff and kept frozen until analyzed.

2.3. Analytical standards and methods

The milk sample of up to 60 ml was weighed and placed into a separatory funnel. Ten milliliter of 5% natrium oxalate solution, 50 ml ethanol and 20 ml diethyl ether were added to the milk sample. Once combined, the mixture was shaken vigorously for 1 min. Then, 30 ml hexane was added, and the mixture was shaken for an additional 5 min. The organic phase was transferred into a different separatory funnel. The aqueous phase was extracted twice with 30 ml hexane. The joint hexane phases were extracted

twice with 20 ml of distilled water, separated from aqueous solution, dried, concentrated, and weighed as lipid.

Lipid (ca. 100–200 mg) was spiked with recovery standard (50 μl, PCB174, $c = 500$ ng ml^{-1}), dissolved in 1 ml hexane and transferred onto a combined Florisil-Silica Gel column (0.5 g Florisil, 1 g 44% sulfuric acid on silica gel, 0.5 g Florisil, 1 g anhydrous sodium sulfate). Residues were eluted with 10 ml of 10% (v/v) dichloromethane in hexane. Eluate was collected to a 50 ml flask, rotary evaporated to the volume of ca 0.5–1 ml and blown up to dryness under a gentle N_2 flow. Prior to injection to the gas chromatograph, the residues were redissolved in known volume of the syringe standard to correct the volume of samples analyzed (*n*-heptane solution of PCB103, $c = 500$ ng ml^{-1}).

Two microliters of treated sample solution were injected. Analyses were performed on an Agilent Technologies 6890N gas chromatograph (GC) equipped with ^{63}Ni electron capture detection. The GC was equipped with DB-5 capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, USA). Operating conditions: injection mode, splitless for 1 min; column temperature held at 110 °C for 1.5 min and then raised to 200 °C at 30 °C min^{-1}, to 305 °C at 2.5 °C min^{-1}; carrier gas, He—constant flow; makeup gas, N_2 at 50 ml min^{-1}.

Each GC peak in the sample chromatogram was identified by accurate comparison with retention time of an authentic standard. Identified peaks were checked by HRGC/LRMS-SIM (GC HP 5890 in combination with MSD HP5790B) in problematic cases. External standards were used for quantitation. Each analytical batch consisted of a blank sample (solvent blank), one recovery sample and 7–10 milk samples. The blank was not allowed to exceed 1/20 of the level of compounds analyzed in the sample. To confirm recovery of the analytical method, a sample of pure olive oil (internal reference material—spiked) with known amount of PCB congeners was used. The samples were diluted so that the peak area of the determined compound was in the middle of the calibration curve. An individual congener was quantitated if the retention time fit the retention time in standard solution ±6 s.

For purposes of this analysis, we present the seven most abundant PCB congeners (CB-IUPAC# 118, 138, 153, 156 + 171, 167, 170 and 180) and four organochlorine pesticides (hexachlorobenzene—HCB; beta-hexachlorocyclohexane—β-HCH; dichlorodiphenyltrichloroethane—*p*,*p*′-DDT and dichlorodiphenyldichloroethylene—*p*,*p*′-DDE). The limits of detections (LODs) were evaluated from the ratio of noise/peak height (peak of analyte should be at least three times higher than the noise). LODs for PCBs were 0.062–0.597 ng g^{-1} lipid for colostrum and 0.114–0.547 ng g^{-1} lipid for mature milk samples (depending on the volume of sample and concentration of lipids); LODs for selected pesticides were 0.055–0.747 ng g^{-1} lipid for colostrum and 0.101–0.683 ng g^{-1} lipid for mature milk samples in the present study.

2.4. Statistical analysis

Analyses were conducted on the seven most abundant congeners (IUPAC #118, 138, 153, 156 + 171, 167, 170 and 180), their sum, and four organochlorine insecticides: HCB, β-CH, *p*,*p*′-DDE and *p*,*p*′-DDT. Levels of all OCs were above the LOD. Shapiro–Wilk W test was used for the assessment of Normality of the data. Lipid levels in breast milk were normally distributed, so for comparison of lipid levels between colostrum and day-14 milk, paired *t*-test and Pearson correlations were used. Since the majority of OCs were not Normally distributed, besides arithmetic mean ± SD, we also present medians and range of the values. Spearman correlations were utilized to assess correlations in OCs levels between colostrum and mature milk samples in individual mothers and the Wilcoxon ranksum test was used for the comparison of OCs levels between colostrum and day-14 milk.

3. Results

Fourteen mothers participated in this study. Due to inadequate volume of colostrum received from two participants, complete analysis was conducted on twelve paired specimens.

Maternal age ranged from 20 to 33 years (mean 26.2 years); body mass index (BMI) from 18 to 25 (mean 21). Average number of years of education was 11.9 years with 67% ($N = 8$) being high school graduates. Additionally, 50% ($N = 6$) were primiparas, 33% ($N = 4$) smoked before pregnancy and only 1 woman smoked during this pregnancy. No difference with regard to these characteristics was found between the subset of 12 women and the whole cohort.

The fat content in colostrum was compared with that in 14-day milk specimens (Fig. 1). Average fat content was lower in colostrum than in mature milk, with means and SDs 1.95% ± 0.95% for colostrum versus 2.70% ± 1.17% for day-14 milk, although the difference was not statistically significant. The lipid content of the colostrum and that of mature milk were not correlated.

The medians, range, arithmetic means and standard deviations of organochlorine compounds expressed on milk fat basis (ng g^{-1} lipid) in colostrum and mature milk

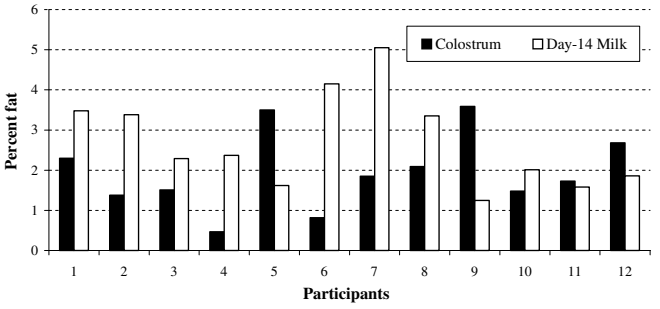

Fig. 1. Fat content (%) in the samples of colostrum and day-14 milk in mothers from Michalovce, Slovakia, 2003.

Table 1
Concentrations of selected organochlorine compounds in human colostrum and day-14 milk (ng g^{-1} fat) in mothers from Michalovce, Slovakia, 2003

Compound	Colostrum				Day-14 milk			
	Med	Min–Max	Mean	SD	Med	Min–Max	Mean	SD
PCBSum	541	310–1303	651	344	496	205–1288	599	373
CB-118	18	5–47	18	12	17	4–49	17	12
CB-138	121	43–279	136	80	116	34–285	130	85
CB-153	184	47–394	205	118	175	38–404	196	127
CB-156 + 171	19	11–49	22	12	17	9–51	21	13
CB-167	4	1–15	5	4	4	1–15	5	4
CB-170	70	38–187	82	44	55	28–182	73	46
CB-180	153	81–409	182	95	118	65–396	158	101
HCB	80	37–373	98	89	79	37–435	102	108
β-HCH	15	6–52	19	13	16	6–57	20	14
p,p′-DDE	537	143–1778	659	485	543	142–1677	632	453
p,p′-DDT	27	19–74	36	18	26	14–68	33	19

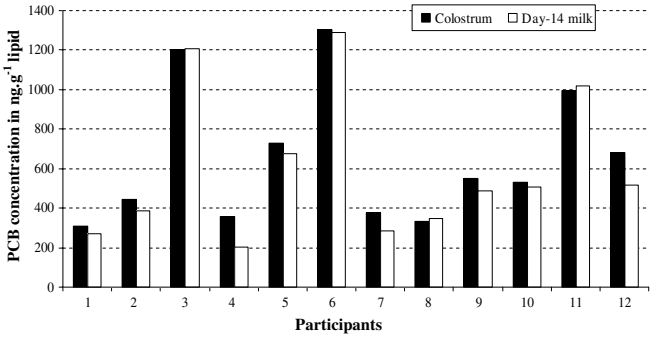

Fig. 2. Total Sum of CB-118, 138, 153, 156 + 171, 167, 170 and 180 in individual samples of colostrum and day-14 milk in mothers from Michalovce, Slovakia, 2003.

are presented in Table 1. CB#153 was the most abundant, followed by the CB#180 and CB#138; CB#167 was the least abundant in both colostrum and mature milk samples. The highest organochlorine pesticide concentrations were for *p,p′*-DDE in both colostrum and mature milk samples.

Colostrum concentrations and mature milk concentrations of OCs were highly correlated for both classes of compounds: PCB congeners ($r = 0.94$–0.98, $p < 0.001$) and organochlorine pesticides ($r = 0.85$–0.99, $p < 0.001$).

Despite the lower lipid concentration in colostrum as compared to day-14 milk, no significant difference was found in either PCB or organochlorine pesticide lipid-based concentrations (Figs. 2 and 3).

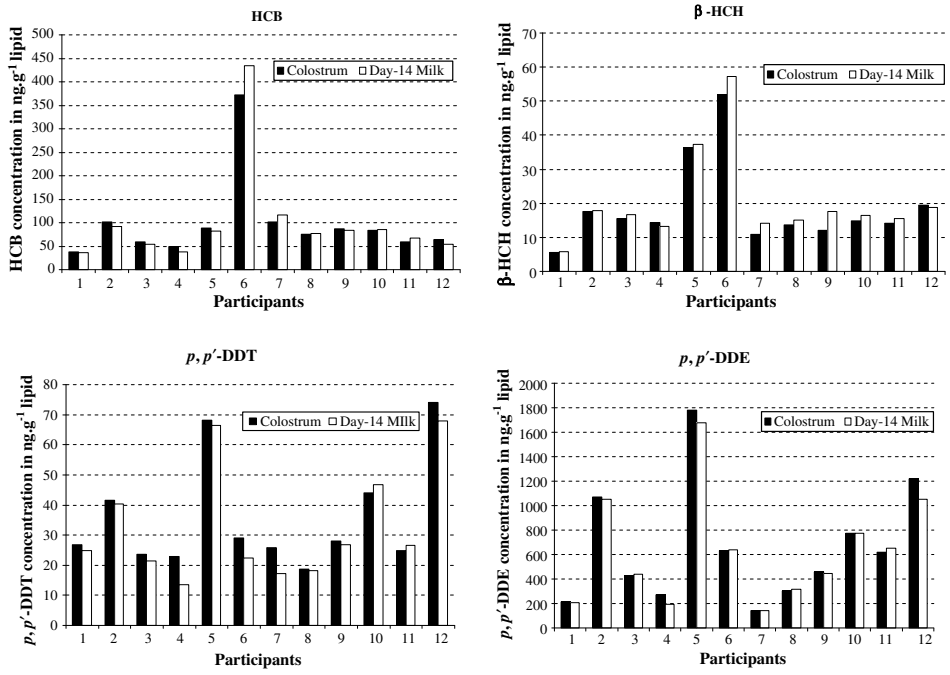

Fig. 3. Concentrations of organochlorine pesticides (HCB, β-HCH, *p,p′*-DDT and *p,p′*-DDE) in individual samples of colostrum and day-14 milk in mothers from Michalovce, Slovakia, 2003.

In general, slight decreasing trends of PCB concentrations were observed from colostrum to mature milk, with a median decrease for the PCB sum of 9.6%. Levels of the sum of PCB congeners ranged from 310 to 1304 ng g^{-1} lipid with a median of 541 ng g^{-1} lipid in colostrum and from 204 to 1288 ng g^{-1} lipid with a median of 495 ng g^{-1} lipid in mature milk. Fig. 2 shows the paired values for the sum of the seven PCB congeners for each of the 12 women.

Concentrations of organochlorine pesticides basically followed the same pattern, except for β-HCH, which showed a slight increase from colostrum to mature milk concentrations.

Lipid-based OC concentrations did not correlate with the percentage of fat, either in colostrum or in mature milk, although a negative association was implied in the majority of cases.

4. Discussion

The aim of our study was to compare the levels of organochlorine compounds in colostrum with those in day-14 milk and to evaluate if the lower amount of fat in colostrum adversely influences the quantitation of OC concentrations. In particular, we sought to determine whether colostrum is an adequate medium for estimation of early postnatal exposure to OCs.

We found that on average, lipid concentration in colostrum was lower than in mature milk, in accordance with other authors (Agostoni et al., 2001; Agostoni et al., 2003). Nevertheless, 3 samples out of 12 in our study had higher lipid levels in colostrum than in mature milk. This finding might be partially explained by extreme variability of lipid content in breast milk, with variations reported during the same day, within the same suckling period, or even between breasts within mothers (Villalpando and del Prado, 1999; LaKind et al., 2002a; Mitoulas et al., 2002).

Guvenius et al. (2003) reported a median lipid content of 1.9% (0.8–4.9%) for breast milk samples collected within the wide interval of 2–77 days after delivery. Studies collecting breast milk samples at least 30 days after delivery showed average lipid content to be 3.90% with the range 3.67–4.16% and 4% with the range 0.7–10.5% (Takekuma et al., 2004; Arcus-Arth et al., 2005). Differences in fat content measured in breast milk could be ascribed to different time period of collection after delivery and different collection protocols (LaKind and Berlin, 2002b). Our finding of no association between lipid concentration in colostrum and mature milk samples supports results by Agostoni et al. (2001), who did not find significant correlation of milk lipid content between colostrum and mature milk collected at the end of the first month.

We found strong correlations in the concentrations of organochlorine compounds between colostrum and mature milk for individual selected PCB congeners and organochlorine pesticides, in accordance with Waliszewski et al. (2001). In contrast, Ribas-Fito et al. (2005) found significant correlation between colostrum and mature milk (collected during the third week postpartum) only for HCB, with no correlation for *p,p′*-DDE and PCBs. Both authors presented data with lipid-adjusted OC concentrations.

In our results, the sum of PCB concentrations differed only slightly between the two samples. Despite the lower percentage of lipids, the colostrum concentrations of PCBs and the majority of organochlorine pesticides were higher than that of day-14 milk, indicating a small reduction in maternal body burden over the 10-day period, but this difference was not statistically significant. Other researchers have found more dramatic differences in OCs levels in breast milk collected at different times. Furst et al. (1989) reported significant reduction in levels of PCDDs, PCDFs, PCBs and organochlorines pesticides in breast milk during lactation with the strongest decline in the OC levels between the first and fifth weeks after delivery. Ramos et al. (1997) analyzed breast milk samples gathered at intervals of 1–2 weeks during four lactating periods from three individual mothers. They observed maximum variation in PCB content during the first weeks of lactation with more stable levels after two months. Waliszewski et al. (2001) compared the contents of HCB, β-HCH, *p,p′*-DDE and *p,p′*-DDT among various body compartments of 60 mothers, including colostrum collected within the fifth day and mature milk, collected the 30th day postpartum. The most predominant compound was *p,p′*-DDE, with a significant decrease of 26% from colostrum to mature milk. On average, they found a 36% decrease in the levels of HCB, β-HCH, *p,p′*-DDE and the sum of DDT in mature milk compared to colostrum. Ribas-Fito et al. (2005) measured organochlorine concentrations (DDE, HCB and PCBs) in the samples of colostrum (collected the third day postpartum) and mature milk (collected on third week postpartum). PCB levels were presented as the sum of congeners CB-28, 52, 101, 118, 138, 153, and 180. Like us, they found higher concentrations in the colostrum than in mature milk, but greater decreases: 22% for *p,p′*-DDE, 31% for HCB and 36% for the sum of PCBs. We found the average decline in concentrations to be 11% for the sum of PCBs and even lower for HCB, *p,p′*-DDE and *p,p′*-DDT (2%, 4% and 10%, respectively). Levels of β-HCH varied, with tendency to increase from colostrum to day-14 milk. The lower decrease in the OCs concentrations found in our study is likely the result of the relatively short period between the collections of milk milk samples: approximately 10 days vs. 18 in the comparison by Ribas-Fito.

Fängström et al. (2005) published results on congener-specific PCB levels in pooled samples of human colostrum (collected third–fifth day postpartum) from the highly exposed population of the Faroe Islands; on average, levels detected in their cohort exceeded results found in our study by a factor above 3. Similar to many other studies, we found CB-153, CB-180 and CB-138 were the most abundant congeners in breast milk (Ramos et al., 1997; Fängström et al., 2005). Schoula et al. (1996) reported the median PCB levels in breast milk to be 817 ng g^{-1} lipid

(CB118 + CB138 + CB153 + CB180) in the Czech women within 1993–1994; these levels were higher than those found in mature milk in our study (median 424 ng g^{-1} lipid). The most dominant congener was CB-153, which constituted approximately 30% of PCB content. This PCB congener represented 34% of the total PCB sum in our study.

Given the variable fat content of breast milk, the timing of sampling is critical in relation to interpretation of findings across studies (Harris et al., 2001). Detailed protocols on breast milk collection with characteristics of participating mothers should be implemented in every study, in order to permit interstudy comparisons, to explore trends and determinants of levels in human milk, and to reach valid conclusions about infant exposures via breastmilk (Solomon and Weiss, 2002; LaKind and Berlin, 2002b). In general, breast milk for analytical purposes is collected between second and eighth week after delivery, after lactation has been established (Lovelady et al., 2002). When using human colostrum, its high nutritional value and limited volume must be taken into consideration with the aim not to compromise the nutritional status of infant, which takes priority over the need for satisfactory volume of a milk sample to conduct valid laboratory assays.

In conclusion, our investigation confirms that quantitation of PCBs in colostrum samples provides a close estimate of the breastfed child's exposure to PCBs in the early neonatal period and thus, despite the lower fat content, colostrum specimens are adequate to conduct PCB analyses. Timing of breast milk sampling must be taken into consideration when comparisons to other studies are performed.

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