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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