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Persistent organic pollutants in matched breast milk and infant faeces samples

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HIGHLIGHTS

- Persistent organic pollutants could be measured in faeces with reproducible results.
- Infant faecal concentrations are highly predicted by maternal milk concentrations.
- Two possible mechanisms are postulated for the correlation between milk and faeces.
- Using faeces as an external measure of internal exposure in infants looks promising.

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ABSTRACT

Assessing blood concentration of persistent organic pollutants (POPs) in infants is difficult due to the ethical and practical difficulties in obtaining sufficient quantities of blood. To determine whether measuring POPs in faeces might reflect blood concentration during infancy, we measured the concentrations of a range of POPs (i.e. polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs)) in a pilot study using matched breast milk and infant faecal samples obtained from ten mother–child pairs. All infants were breast fed, with 8 of them also receiving solid food at the time of faecal sampling. In this small dataset faecal concentrations (range 0.01–41 ng g^{-1} lipid) are strongly associated with milk concentrations (range 0.02–230 ng g^{-1} lipid). Associations with other factors generally could not be detected in this dataset, with the exception of a small effect of age or growth. Different sources (external or internal) of exposure appeared to directly influence faecal concentrations of different chemicals based on different inter-individual variability in the faeces-to-milk concentration ratio R_{fm}. Overall, the matrix of faeces as an external measure of internal exposure in infants looks promising for some chemicals and is worth assessing further in larger datasets.

1. Introduction

The link between exposure to some persistent organic pollutants (POPs) and endocrine disrupting/carcinogenic effects seems to be clear from laboratory studies on a range of biota (Tanabe, 2002; Li et al., 2006; Alonso-Magdalena et al., 2011; Bergman et al., 2013), and there is increasing evidence that exposure in early life is an important determinant of long-term disease risk (Nagayama et al., 2007; Ochiai et al., 2014). However, more large-scale epidemiological studies are needed for understanding the exposure window that can elicit effects and the extent of the contribution of lipophilic pollutants to the increased risk of incommunicable diseases in humans (Nickerson, 2006; Jorissen, 2007). Infancy is a critical stage of development and is associated with unique exposure pathways that can increase exposure to lipophilic pollutants (Solomon and Weiss, 2002). Nevertheless, exposure assessment in early life is challenging and complicated by reluctance of parents and investigators to take blood samples from infants. In addition, the small volume sample that may be obtained poses technical challenges for measuring analytes.

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To avoid sampling blood, physiologically based pharmacokinetic (PBPK) models have been used to generate individualized toxicokinetic profiles of lipophilic pollutants in infants. Such models usually contain many variables, such as: the initial value (e.g., the concentration in meconium or umbilical cord blood), ongoing exposure values (e.g., concentrations in mother's breast milk), intrinsic elimination values (estimated from assembled data from longitudinal monitoring data or estimated via measuring excretion rate) and infants' growth rate (Lorber and Phillips, 2002; Verner et al., 2013). Each of these variables is uncertain, and consequently the modelled exposure of the infant can also be associated with considerable uncertainty. In this context, finding a non-invasive matrix that has a direct relationship with the blood concentration and can be measured repeatedly throughout infancy would improve exposure assessment of lipophilic pollutants in infants.

Evidence in adults suggests that the faecal concentration of some lipophilic pollutants (polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB)) is directly related to the blood concentration. Schrey et al. reported that faecal excretion of most PCDD/Fs examined exceeded the daily intakes from food (Schrey et al., 1998). Moser and McLachlan also found that altering dietary exposure did not influence faecal excretion of PCDD/Fs, HCB, and PCBs (Moser and McLachlan, 2001), which means faecal concentration was not affected by the concentration of POPs in diet. Further, Rohde et al. found a correlation between levels in blood and levels in faeces for PCDD/Fs over a very broad concentration range (Rohde et al., 1999). To-Figueras and his team pointed out a strong correlation between HCB levels in faeces and in serum (To-Figueras et al., 2000).

In two breast fed children, faecal excretion of PCDD/Fs was found not to decrease during the weaning period to the same extent as the estimated decrease in exposure/uptake resulting from substituting breast milk with a solid diet (Abraham et al., 1996), implying that the faecal concentration in these two infants was at least partially determined by the blood concentration.

Maternal breast milk is considered the most accessible matrix that provides reliable information related to the intake of lipophilic pollutants in the corresponding infant (Verner et al., 2013) for those chemicals for which uptake via food is the primary exposure pathway for the infant (although dust is acknowledged as another important source for some POPs) (Toms et al., 2009; Lee et al., 2014). In addition, after a decline during the first month of lactation, concentrations of lipophilic pollutants in breast milk have been found to remain steady throughout the breastfeeding period, and to be close to the initial infant blood concentrations (Abraham et al., 1994; Lee et al., 2013; Verner et al., 2013; Vigh et al., 2013).

The key aims of this study were: to assess the concentration of a range of POPs (PCBs, polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs)) in matched infant faeces and breast milk samples with reproducible results; to evaluate whether concentrations in the two media are correlated; and to evaluate whether faecal concentrations can potentially be used as biomarkers of the blood concentration of POPs in infants.

2. Materials and methods

2.1. Chemicals

Standard solutions of native PCBs, PBDEs and OCPs were purchased from AccuStandard Inc. Isotopic carbon-labelled PCB standard solutions and PBDE standard solutions were purchased from Wellington Laboratories Inc., and isotopic carbon-labelled OCP standards were ordered from Cambridge Isotope Laboratories Inc. n-Hexane (SupraSolv®), dichloromethane (DCM, SupraSolv®), granulated anhydrous sodium sulphate (Scharlau Chemie S.A.), silica (Davisil Grade 633, Sigma–Aldrich®) and Florisil® (Fluka, Sigma–Aldrich®) were used for extraction and clean-up. Silica and Florisil were activated at 140 °C for at least 12 h before use. Sodium sulphate was baked at 400 °C for at least 12 h before use.

2.2. Recruitment and sample collection

This project received ethics approval by The University of Queensland Ethics Committee (approval number H/308 NRCET). Ten mothers were recruited opportunistically when they were pregnant, from Sydney (7) and Brisbane (3), Australia. Breast milk samples were collected in August, 2013. A minimum of 50 mL of breast milk was collected from each mother over multiple collections over a one-month period into a clean glass jar that was provided. These mothers then collected faecal samples from their infants twice a week for two weeks, beginning 12–20 weeks after breast milk sampling. Only faecal material that had not been in contact with the inner liner of the diaper was collected (Supporting Information Instruction for Sampling Faeces). Questionnaires requesting demographic information about each mother–infant pair were completed by the mothers (Supporting Information Tables S1, S2). Breast milk and faecal samples were stored in a freezer at the participants' homes until transportation (on ice, in a cool bag) to the laboratory. In the laboratory, samples were stored at −20 °C until analysis.

2.3. Sample analysis

Lipid content in milk samples was determined gravimetrically in an aliquot based on a liquid–liquid extraction protocol. Approximately 1 g of homogenized milk sample was weighed in a 15 mL centrifuge tube. Then 2 mL of methanol, 1 mL of methyl tert-butyl ether (MTBE) and 1 mL of hexane were added consecutively. The centrifuge tube was shaken vigorously (10 min), sonicated (10 min) and centrifuged at 2000 rpm (10 min). The upper layer was transferred to a pre-weighed tube. Again 1 mL MTBE and 1 mL hexane were added to the original tube which then was shaken (10 min), sonicated (20 min) and centrifuged at 4000 rpm (10 min). The upper layers were combined and blown down to dryness. The tube was put into a 103 °C oven and weighed periodically until a stable reading was obtained.

For contaminant analysis, the homogenized milk sample (~6 g) was transferred to a centrifuge tube and spiked with labeled standard solution (see Table S3 in the Supporting Information). The milk was extracted as described above for lipid content measurement. After concentrating the extract to about 1 mL, it was applied onto the top of a glass column (30 cm × 1.0 cm i.d.) containing 1 cm of anhydrous $NaSO_4$, 14 cm of alumina, 14 cm of florisil and 1 cm of $NaSO_4$ from top to bottom. The sample was then eluted with 100 mL of a mixture of hexane and DCM (1:1 V:V). The eluate was concentrated to 0.5 mL and applied onto the top of another column (17 cm × 0.8 cm i.d.) containing 0.5 cm of $NaSO_4$ and 15 cm of silica gel from top to bottom. The second column was then eluted with 30 mL of a mixture of hexane and DCM (1:1 V:V). The eluate was concentrated to 25 μl and 25 μl isooctane containing 200 pg ^{13}C-PCB141 was added as the recovery standard right before instrumental analysis.

The homogenized, freeze-dried faecal samples (~3 g) from each infant were placed into 100 mL ASE cells with 10 g silica gel, spiked with internal standards and then extracted using hexane and DCM (1:1 V:V) on a Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor system. The program was: 100 °C, 1500 psi, 3 static cycles of 7 min and purge time of 120 s. The extracts were transferred to a column (1.2 cm i.d.) containing 20 cm silica, and eluted with 100 mL of a hexane/DCM mixture (1:1 V:V). The extract was