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Pesticides in human milk of Western Australian women and their influence on infant growth outcomes: A cross-sectional study

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HIGHLIGHTS

- Cross-sectional study of 88 POPs in human milk over first year of lactation.
- *p,p′*-DDE was detected in 87.5% of the human milk samples.
- No significant associations between *p,p′*-DDE and infant growth outcomes.
- Estimated daily intake overestimates human milk POP concentration.
- Human milk infant intake of DDTs is below the recommended daily intake guidelines.

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ABSTRACT

Persistent organic pollutants in human milk (HM) at high levels are considered to be detrimental to the breastfed infant. To determine the pesticide concentration in HM, a pilot cross-sectional study of 40 Western Australian (WA) women was carried out. Gas chromatography-tandem mass spectrometry (GC-MS/MS) with a validated QuEChERS was used for the analysis of 88 pesticides in HM. *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) with a mean concentration of 62.8 ± 54.5 ng/g fat was found, whereas other organochlorines, organophosphates, carbamates and pyrethroids were not detected in HM. Overall, no association was observed between HM *p,p′*-DDE concentrations and maternal age, parity, body mass index and percentage fat mass. Furthermore, for the first time no significant association was found between *p,p′*-DDE concentrations in HM and infant growth outcomes such as weight, length, head circumference and percentage fat mass. The calculated daily intake was significantly different to the estimated daily intake of total DDTs and was well below the guideline proposed by WHO. The DDTs levels in WA have also significantly decreased by 42 - fold since the 1970s and are currently the lowest in Australia.

1. Introduction

Persistent organic pollutants (POPs) such as organochlorine pesticides (OCPs), organophosphate pesticides (OPPs), pyrethroids and carbamate pesticides are widely used in agricultural practice (Köhler and Triebskorn, 2013). Many of these pesticides are resistant to chemical, physical and biological degradation, thus they are ubiquitously found in the environment despite restrictions on their use. Besides being effective in eradicating pests, many of the pesticides are also harmful to human health. Thus, there is widespread concern about the potential health effects of POPs in HM on infant growth and development. To date, most studies have used prenatal pesticides exposure and infant anthropometrical size at birth as a proxy measure of *in utero* development, and have failed to yield consistent results (Mazdai et al., 2003; Wu et al., 2010; Stasinska et al., 2014). To the best of our knowledge, little is known about the effects of the pesticides in HM on the infant

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growth outcomes during breastfeeding.

In Australia, dichlorodiphenyltrichloroethane (DDT) was first introduced in the 1940s and other pesticides such as heptachlor, aldrin, dieldrin, chlordane, hexachlorocyclohexane (HCH) and hexachlorobenzene (HCB) were registered for use in the 1970s (Reid et al., 2013). All new houses in Western Australia (WA) were treated with pesticides during the early stages of construction in order to eradicate termites (Stacey and Tatum, 1985). Since the 1970s, production and application of DDT and most pesticides were restricted and prohibited in WA (Stacey et al., 1985). However, as many of these POPs have long half-lives and high fat solubility properties, they tend to bio-magnify in wildlife, especially in species at the top of food chain, such as animals (e.g. seals and dolphins) and humans (Radcliffe, 2002; Tanabe, 2002). As the presence of POPs can interfere with the function of normal endocrine system, the immature defense mechanism of the developing fetus and infants makes them more vulnerable to these pesticides than adults (Bruckner, 2000). POPs exposure prenatally via the placenta and postnatally via breastfeeding may result in delayed development, immune deficiency, abnormal behavior and growth retardation (Eskenazi et al., 2006; Chao et al., 2007; Koureas et al., 2012). Surprisingly there has been no investigation into the relationship between POP concentrations in HM and the breastfed infant growth outcomes. As HM is uniquely tailored for human infant and constitute a major portion of the infants' diet particularly in early life, studies about the possible influence of POPs in HM on infant growth outcomes are warranted. Previous studies have detected POPs in HM from WA, and have observed a decline in POP concentrations in HM (Stacey and Thomas, 1975; Stacey et al., 1985; Stevens et al., 1993). The most recent study of POP concentrations in WA was based on a single pooled HM sample from 11 women in 2003, which could not represent the POP concentrations for individual mothers and the current concentration in the general population (Mueller et al., 2008). Since then, no further studies of these levels have been reported. Based on the public concern about the current status of the contamination and safety of HM, it is essential to continue monitoring the POPs in WA.

The aims of this study were to (1) describe the current pesticide concentrations in HM from WA mothers and the changes in these pesticide concentrations during the first year of lactation in a cross-sectional cohort; (2) investigate associations between the detected pesticides and maternal and infant characteristics and anthropometrics; (3) calculate the daily intake of the pesticides by individual infants and evaluate the risk to the infant.

2. Materials and methods

2.1. Chemicals

The pesticide standard solutions (100 μg/mL) at 95% or higher purity were obtained from Ultra Scientific (North Kingstown, RI, USA). The pesticide standard solutions (100 μg/mL) were mixed and diluted with acetonitrile (ACN) to prepare a stock standard solution (1 μg/mL) of all the pesticides. LC-MS grade acetonitrile (ACN) and water were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Ethylglycerol (98%), gulonolactone (95%) and D-sorbitol (≥98%) were from Sigma-Aldrich (St. Louis, MO, USA). Sodium acetate (>99.0%) and magnesium sulfate (99.5%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Octadecylsilyl (C18) and primary secondary amine (PSA) were obtained from Agilent (Little Falls, DE, USA). Isotopic labeled quality control (QC) standards, acenaphthene-D_{10}, phenathrene-D_{10} and chrysene-D_{12} were purchased from Restek (Bellefonte, PA, USA), and the internal standard (IS), triphenylphosphate (TPP) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Study population and sample collection

The study was approved by the Human Research Ethics Committee of The University of Western Australia. Breastfeeding dyads (n = 40) were recruited in metropolitan WA between 2013 and 2015. The mothers whom volunteered were predominantly of Caucasian background, married and with a college degree and provided a milk sample in one of the following months of lactation: 2 (n = 11), 5 (n = 9), 9 (n = 10) or 12 months (n = 10). Milk samples (1–5 mL) were collected in glass containers before and after feeding from each breast. The fat content of HM was measured immediately using the Creamatocrit method (Meier et al., 2002), and the remaining milk was stored at −20 °C. All participants provided informed written consent and completed a questionnaire including relevant demographic data.

2.3. HM sample treatment

HM samples (n = 40) were thawed at room temperature for 3 h and then homogenized with a mixer (ELMI Ltd., Riga, Latvia) for 15 s 1 mL of HM was placed into a 15 mL centrifuge tube and 1 mL ACN containing 1% HAc and 100 ng/mL QC standards (acenaphthene-D_{10}, phenathrene-D_{10} and chrysene-D_{12}) was added. A validated acetate buffered QuEChERS method was employed to extract the HM (Lehotay et al., 2005a, 2005b). Extraction reagents (0.4 g $MgSO_4$ and 0.1 g NaAc) were added and shaken immediately, and the tube was then placed in an ice bath. The extraction tube was centrifuged at 3993 *g* for 10 min. 0.6 mL of the supernatant was transferred into a clean 15 mL centrifuge tube and stored in −20 °C for 2 h. Freezing is critical for fatty samples to remove coextractives with limited solubility in ACN (e.g. lipid, wax and sugars). The supernatant was then centrifuged at 3993 *g* (0 °C) for 10 min and 0.5 mL was transferred to a cleanup tube (157 mg $MgSO_4$, 9 mg C18 and 9 mg PSA). The tube was vortexed and centrifuged at 3993 *g* for 10 min. The final extract was transferred into a screw cap amber vial and kept at −80 °C until analysis.

2.4. Working standard solutions preparation

The working standard solutions (0.5, 1, 2, 5, 10, 20, 50 and 100 ng/mL) were prepared by appropriate dilution of the stock standard solution (1 μg/mL) with ACN. A combination of analyte protectants (APs) mixture (ethylglycerol, gulonolactone and D-sorbitol) containing internal standard TPP (IS) was added to the final extract of each HM sample and to all working standard solutions for GC-MS/MS analysis. The final concentrations of ethylglycerol, gulonolactone and D-sorbitol were 20, 2 and 2 mg/mL, respectively and the IS was 100 ng/mL. As demonstrated in our previous study, the addition of APs mixture can equalize the enhancement difference between the pesticides in pure solvent and in HM extract, which can then be used for the quantification of the pesticides in HM.

2.5. GC-MS/MS analysis

Chromatographic separation and determination of the pesticides were carried on a Bruker Daltonics 450 gas chromatography (GC) with a Bruker Daltonics Scion TQ triple quadrupole mass spectrometer (MS) (Billerica, MA, USA), a Bruker 1177 Split/Splitless injector and a PAL COMBI autosampler (CTC Analytics AG, Switzerland). Sky 4.0 ID precision inlet liners with wool from Restek (Bellefonte, PA, USA) were used. Injection was performed at pulsed splitless mode (head pressure: 44 psi) with an injection volume of 2 μL. For GC separation, a Rtx-5MS with Integra-Guard column (10 m + 30 m × 0.25 mm x 0.25 μm) (Bellefonte, PA,