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Exposure to organophosphorus pesticides in Norwegian mothers and their children: Diurnal variability in concentrations of their biomarkers and associations with food consumption

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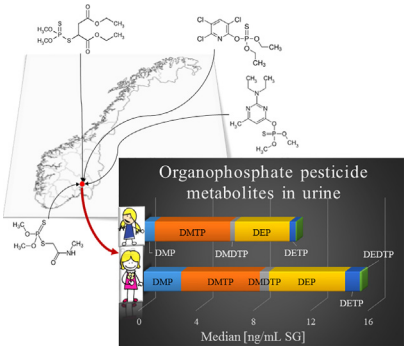
HIGHLIGHTS

- Monitoring of organophosphate (OP) pesticides was performed using urine from mother-child pairs.
- OP pesticide metabolite median concentrations in urine were higher for mothers than for children.
- The diurnal variation in OP pesticide metabolite levels for mothers resulted in 0.49 < ICCs < 0.68.
- Fruit consumption was associated with urinary OP pesticide metabolite levels.
- Estimated daily intakes for 3 EU approved pesticides showed no risk for adverse health effects.

GRAPHICAL ABSTRACT



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ABSTRACT

Several studies have suggested that exposure to organophosphorus (OP) pesticides is detrimental for health, and in particular for children where moderate doses may have a negative impact on the neurodevelopment. This study surveys levels of the 6 non-specific urinary metabolites (dialkyl phosphates (DAPs)) of OP pesticides in Norwegian mothers (n = 48) and their children (n = 54), and examines the diurnal variation in concentrations as well as associations with consumption of specific food products. The highest median concentration measured in urine was found for dimethyl thiophosphate (5.3 and 5.5 ng/mL$_{SG}$; specific gravity corrected) for both children and mothers, respectively, followed by diethyl phosphate (3.8 and 5.3 ng/mL$_{SG}$, respectively). The intra-class correlation coefficients of DAPs among mothers were moderate (0.49–0.68), and consumption of fruit explained between 8% and 55% of the variations in the mothers' and their children's urinary DAP concentrations.

Abbreviations: ADI, acceptable daily intake; BMI, body mass index; CI, confidence interval; DF, detection frequency; DAPs, dialkyl phosphates; DEDTP, diethyl dithiophosphate; DEP, diethyl phosphate; DETP, diethyl thiophosphate; DMDTP, dimethyl dithiophosphate; DMP, dimethyl phosphate; DMTP, dimethyl thiophosphate; EFSA, European Food Safety Authority; EU, European Union; FMV, first morning void; ICC, intra-class correlation coefficients; MS, mass spectrometry; MDL, method detection limit; NBEs, no observed adverse effect biomarker equivalents; OP, organophosphate; RSD, relative standard deviation; R, Spearman's rank correlation coefficient; SG, specific gravity; ∑DEAP, sum of diethyl alkyl phosphates; ∑DMAP, sum of dimethyl alkyl phosphates; TOF, time-of-flight; UPLC, ultra-performance liquid chromatography; TCPy, 3,5,6-trichloro-2-pyridinol.

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1. Introduction

Organophosphorus (OP) pesticides are substances used mainly for insect control in agriculture and homes. The beneficial use of such chemicals is undermined by the many associations with adverse health outcomes reported in the general population (e.g., impaired neurobehavioral functioning (Mackenzie Ross et al., 2010), prostate cancer (Koutros et al., 2013), and reduced fertility in men (Swan et al., 2003)) and with especial concerns for foetus and children at a vulnerable age (e.g., reduced birth weight (Rauch et al., 2012), impaired child neurodevelopment (Bouchard et al., 2010; Fortenberry et al., 2014), and childhood obesity (Garza et al., 2011)). Deziel et al. (2015) have reviewed the human exposure pathways to pesticides for non-occupationally exposed subjects. There are indications that the exposure to OP pesticides depends on the agricultural activities in the area or country (Berman et al., 2013; Lewis et al., 2015). Diet has been identified as the primary source of exposure to OP pesticides for the general population (Becker et al., 2006; Berman et al., 2013). Many studies have recently investigated the influence of various foods and food groups to such exposure (Holme et al., 2016; Lewis et al., 2015; Melnyk et al., 2014; Sokoloff et al., 2016), finding fruit, and in some cases vegetables, as major contributors.

Due to the high latitude and a relatively short growing season in Norway, the pest population is limited compared to the middle and southern Europe. However, the concentrations of urinary biomarkers of exposure to OP pesticides in non-occupationally exposed Norwegian mothers and children were in the range to concentrations seen in other countries with more agricultural activities (Cequier et al., 2016). Therefore, imported food products seem to play an important role to the exposure to OP pesticides (Ye et al., 2009).

In the EU, there are almost 500 approved pesticides for plants, from which 105 are insecticides. Only 7 approved insecticides belong to the class of OP pesticides (i.e., "methyl-pesticides": methyl-pimiriphos/chlorpyrifos, malathion, dimethoate, and phosmet, "ethyl-pesticides": chlorpyrifos, and ethoprophos, which contains ethyl and propyl side chains) (EU Pesticides Database, 2016) (Table S1).

Biomonitoring of OP pesticides is usually carried out by determining concentrations of dialkyl phosphates (DAPs) in urine samples (i.e., dimethyl phosphate (DMP), diethyl phosphate (DEP), dimethyl thiophosphate (DMTP), diethyl thiophosphate (DETP), dimethyl dithiophosphate (DMDTP), and diethyl dithiophosphate (DEDTP)). DAPs are non-specific metabolites of OP pesticides (insecticides), and their concentrations in urine reflect a particular time window of exposure. Due to the short half-lives of the OP pesticides in humans, the metabolites are excreted within 24-h (Needham, 2005), and therefore the levels measured in urine are highly dependent on the time of the sample collection. Some studies have assessed the variability of the DAP concentrations in human urine in a time frame from one week to one year (Attfield et al., 2014; Aylward et al., 2012; Bradman et al., 2005, 2013; Spaan et al., 2015). These studies concluded that there is high within person variability in OP pesticide exposure, which results in major challenges for conducting optimal epidemiological studies where usually just one spot-urine has been collected. On the other hand, it seems that a shorter assessment of 48-h reduces the within person variability (Egeghy et al., 2011).

To the best of our knowledge, there are only two studies assessing the occurrence of OP pesticide metabolites in Norwegians. A study conducted in 2004, where DAPs were measured in 10 pools of urine from 110 women (Ye et al., 2009), and our previous work, where we presented a novel analytical method for the determination of DAPs in urine together with the concentrations in Norwegian mothers and children (n = 104) (Cequier et al., 2016). This work continues the exposure assessment of this Norwegian study group by (i) evaluating the diurnal variation of DAP concentrations in urine (ii) identifying foods and food groups which contribute to OP pesticides exposure, and (iii) estimating the daily intake of OP pesticides.

2. Materials and methods

2.1. Study population and urine collection

A mother-child study group (48 mothers and their offspring, 54 children in total) was established in 2012 in the greater Oslo area. Informed consent was obtained from all the participants and the project was approved by the Regional Committee for Medical Research Ethics. The mothers' age ranged from 32 to 56 years (median 41 years) and children's age (both genders) ranged from 6 to 12 years (median 10 years). Mothers were encouraged to provide urine during 24-h (2–8 samples; total n = 254), and children were asked to provide the first morning void (FMV) and one afternoon urine sample (total n = 112). All urine samples were stored at −20 °C until analysis. Further details about the enrolment and sample collection have been published previously (Cequier et al., 2015).

The participants' diet was reported using a 24-h food recall method, which has been described in our previous study (Cequier et al., 2015). Food was categorized as follows: bread, cereal based products, potatoes, vegetables, fruit, meat, fish, eggs, cheese, milk, drinks (e.g., water, coffee, soda, etc), sugar, cakes, and butter and oils. The units used were grams of food item consumed per day (g/day). In total, one hundred and two 24-h food recalls were collected.

2.2. Analysis of urine samples

The analytical methodology used to determine the 6 DAPs in urine has been published earlier (Cequier et al., 2016). Briefly, the samples were centrifuged and 300 μL of urine was diluted with water to 600 μL, acidified, and spiked with isotopically labelled internal standards (4–24 ng). A clean-up was performed on Strata-X-AW 96-well plates, and DAPs were subsequently eluted with 0.5 mL of acetone containing 5% of triethylamine. The eluate was evaporated to some few microliters and adjusted to approximately 100 μL with a solution containing 40 mM of tripropylammonium formate (ion-pair agent). Twenty microliters of this solution was injected into a UPLC-TOF-MS system. The ion-pair chromatography was performed on an Acquity® C_{18} BEH column (100 mm × 2.1 mm × 1.7 μm) from Waters (Milford, MA, USA) using a mobile phase containing 0.5 mM of tripropylammonium formate. Identification and quantification of the DAPs was carried out using a Xevo® G2-S QTOF from Waters. Levels of DAPs in the blanks (same treatment as samples without urine) were lower than the method detection limit (MDL). Matrix effects were overcome using ^{13}C internal standards. Accuracies were assessed by spiking a control sample at 4 different concentrations (from 1.3 to 100 ng/mL). DAP accuracies ranged from 67 to 134% (relative standard deviation (RSD) 1–19%). For quality assurance, a quality control sample spiked at 5 ng/mL was monitored throughout the study (n > 30). The inter-day precision of the quality control sample for the DAPs was between 16% and 23%, except for DEDTP whose RSD was 40%. Specific gravity (SG) was measured in all samples and spanned from 1.003 to 1.032 (mean = 1.015) in mothers and from 1.009 to 1.032 (mean = 1.024) in children. The urinary concentrations were calculated according to the equation proposed by Boeniger et al. (1993) and using the SG values of the participants in the calculation.

2.3. Calculation of DAP daily intakes

In this study, daily intakes were estimated for some authorized pesticides in the European Union (EU) (i.e., chlorpyrifos, dimethoate, and methyl-chlorpyrifos) (EU Pesticides Database, 2016) based on the molar sum of urinary levels of their metabolites (Fenske et al., 2000).

The worst-case scenarios for mothers and children, were estimated using (i) the 95 percentile DAP concentrations, (ii) average body weight from our study (67 kg for mothers and 37 kg for children), (iii) 24-h urine volume of 1.6 L for mothers (Daudon et al., 2005) and 0.82 L for

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