Science of the Total Environment 512–513 (2015) 337–344

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Distribution and determinants of urinary biomarkers of exposure to organophosphate insecticides in Puerto Rican pregnant women

Ryan C. Lewis [a], David E. Cantonwine [b], Liza V. Anzalota Del Toro [c], Antonia M. Calafat [d], Liza Valentin-Blasini [d], Mark D. Davis [d], M. Angela Montesano [d], Akram N. Alshawabkeh [e], José F. Cordero [c], John D. Meeker [a,*]

[a] University of Michigan School of Public Health, Department of Environmental Health Sciences, 1415 Washington Heights, Ann Arbor, MI 48109, United States
[b] Brigham and Women's Hospital, Department of Obstetrics and Gynecology, 75 Francis Street, Boston, MA 02115, United States
[c] University of Puerto Rico Graduate School of Public Health, Medical Sciences Campus, San Juan 00935, Puerto Rico
[d] Centers for Disease Control and Prevention, National Center for Environmental Health, Division of Laboratory Sciences, 4770 Buford Highway, Atlanta, GA 30341, United States
[e] Northeastern University, College of Engineering, 360 Huntington Avenue, Boston, MA 02115, United States

HIGHLIGHTS

- We studied repeated urinary levels of OP insecticide metabolites during pregnancy.
- Detection frequencies for all 10 urinary metabolites ranged from 3–90%.
- Repeated measures of TCPY, PNP, DETP, and DMTP had poor reproducibility.
- Certain demographic and lifestyle variables are determinants of exposure.

ARTICLE INFO

Article history:
Received 6 December 2014
Received in revised form 13 January 2015
Accepted 19 January 2015
Available online 27 January 2015

Editor: Adrian Covaci

Keywords:
Biomarker
Pesticides
Pregnancy
Urine
Women

ABSTRACT

Globally, human exposures to organophosphate (OP) insecticides may pose a significant burden to the health of mothers and their developing fetuses. Unfortunately, relevant data is limited in certain areas of the world concerning sources of exposure to OP insecticides in pregnant populations. To begin to address this gap in information for Puerto Rico, we studied repeated measures of urinary concentrations of 10 OP insecticide metabolites among 54 pregnant women from the northern karst region of the island. We also collected demographic data and self-reported information on the consumption of fruits, vegetables, and legumes in the past 48 h before urine collection and home pest-related issues. We calculated the distributions of the urinary biomarkers and compared them to women of reproductive age from the general U.S. population. We also used statistical models accounting for correlated data to assess within-subject temporal variability of the urinary biomarkers and to identify predictors of exposure. We found that for all but two metabolites (*para*-nitrophenol [PNP], diethylthiophosphate [DETP]), 50th or 95th percentile urinary concentrations (the metric that was used for comparison was based on the biomarker's detection frequency) of the other eight metabolites (3,5,6-trichloro-2-pyridinol [TCPY], 2-isopropyl-4-methyl-6-hydroxy-pyrimidine, malathion dicarboxylic acid, diethylphosphate, diethyldithiophosphate, dimethylphosphate, dimethylthiophosphate [DMTP], dimethyldithiophosphate) were somewhat lower in our cohort compared with similarly aged women from the continental United States. TCPY, PNP, DETP, and DMTP, which were the only urinary metabolites detected in greater than 50% of the samples, had poor reproducibility (intraclass correlation coefficient range: 0.19–0.28) during pregnancy. Positive predictors of OP insecticide exposure included: age; marital or employment status; consumption of cherries, grape juice, peanuts, peanut butter, or raisins; and residential application of pesticides. Further research is needed to understand what aspects of the predictors identified influence OP insecticide exposure during pregnancy.

Abbreviations: CDC, Centers for Disease Control and Prevention; CI, confidence interval; DEDTP, diethyldithiophosphate; DEP, diethylphosphate; DETP, diethylthiophosphate; DMDTP, dimethyldithiophosphate; DMP, dimethylphosphate; DMTP, dimethylthiophosphate; GM, geometric mean; ICC, intraclass correlation coefficient; IMPY, 2-Isopropyl-4-methyl-6-hydroxy-pyrimidine; LOD, limit of detection; MDA, malathion dicarboxylic acid; NHANES, National Health and Nutrition Examination Survey; OP, organophosphate; PNP, *para*-nitrophenol; PROTECT, Puerto Rico Test site for Exploring Contamination Threats; SG, specific gravity; SPE-HPLC–MS/MS, solid phase extraction and high-performance liquid chromatography–isotope dilution tandem mass spectrometry; TCPY, 3,5,6-trichloro-2-pyridinol.

* Corresponding author.
E-mail addresses: rclewis@umich.edu (R.C. Lewis), dcantonwine@partners.org (D.E. Cantonwine), liza.anzalota@upr.edu (L.V. Anzalota Del Toro), aic7@cdc.gov (A.M. Calafat), lvb5@cdc.gov (L. Valentin-Blasini), msd7@cdc.gov (M.D. Davis), ahm2@cdc.gov (M.A. Montesano), aalsha@coe.neu.edu (A.N. Alshawabkeh), jose.cordero6@upr.edu (J.F. Cordero), meekerj@umich.edu (J.D. Meeker).

http://dx.doi.org/10.1016/j.scitotenv.2015.01.059
0048-9697/

1. Introduction

The annual global use of pesticides in agricultural and non-agricultural (e.g., home, garden, commercial, government) settings is an estimated 5.2 billion pounds of active ingredients (EPA, 2011). On a weight basis, insecticides account for 17% of all pesticides (e.g., herbicides, fungicides, nematicides) used around the world (EPA, 2011). Organophosphate (OP) insecticides, such as chlorpyrifos, malathion, and diazinon, are one class of insecticide characterized by their potent acetylcholinesterase inhibitor activity (Clune et al., 2012). Use of OP insecticides in the United States has generally decreased over time (EPA, 2011). While this decrease is due in part to regulatory efforts (Clune et al., 2012), OP insecticides still account for 35% of all insecticides used in the U.S., which is equivalent to 33 million pounds of OP insecticides on an annual basis (EPA, 2011).

Due to widespread use, humans can be exposed to OP insecticides through multiple routes, including inhalation from spray drift, ingestion of residues on foods, dust, and soil, and dermal absorption from skin contact (Fortenberry et al., 2014). Human exposure to OP insecticides has been most notably associated with detrimental child neurodevelopment (Bouchard et al., 2010; Engel et al., 2011; Fortenberry et al., 2014; Marks et al., 2010). In addition, human exposure to OP insecticides has been linked with a wide variety of other adverse human health effects, including decreased gestational age (Eskenazi et al., 2004; Rauch et al., 2012; Wang et al., 2012), reduced birth weight (Rauch et al., 2012), altered serum hormone concentrations (Meeker et al., 2006a,b, 2008), reduced semen quality (Swan et al., 2003), wheeze (Hoppin et al., 2006), and lung cancer (Lee et al., 2004).

The rates of preterm birth (Martin et al., 2011; March of Dimes, 2012) and many other adverse human health conditions with potential environmental influences, such as childhood obesity and asthma (Garza et al., 2011; Otero-González and García-Fragoso, 2008; Rivera-Soto et al., 2010), and adult obesity, metabolic syndrome, and diabetes (CDC, 2012; Pérez et al., 2008) are higher in Puerto Rico compared to the mainland U.S. and in some cases (e.g., preterm birth) most other parts of the world (Blencowe et al., 2012). Puerto Rico also has a history of pesticide drift, illegal use and applications of pesticides, and pesticide-contaminated land (EPA, 2001, 2003, 2004, 2008), including a Superfund hazardous waste site with elevated soil levels of OP insecticides (EPA, 2012). However, little is known regarding human exposures to environmental chemicals in Puerto Rico, including OP insecticides.

Our study had three primary objectives: to 1) describe distributions, 2) assess within-subject temporal variability, and 3) identify predictors of urinary concentrations of 10 OP insecticide metabolites in pregnant women from Puerto Rico. Studies on these aspects of OP insecticide exposure in pregnant women have been limited to date (especially #2 and #3), and may also directly assist with understanding the potential burden of OP insecticide exposure globally, identifying sources of exposure, and designing exposure characterization components of future epidemiology studies.

2. Materials and methods

2.1. Study participants

This analysis involved 54 pregnant women participating in the Puerto Rico Test site for Exploring Contamination Threats (PROTECT) project. PROTECT is an ongoing prospective birth cohort in the northern karst region of Puerto Rico designed to assess the potential relationship between environmental exposures and risk of preterm birth and other adverse pregnancy outcomes (Cantonwine et al., 2014; Meeker et al., 2013). Participants were recruited at approximately 14 ± 2 weeks of gestation at seven prenatal clinics and hospitals during 2010–2012. Pregnant women were eligible if they were 18–40 years of age, resided in a municipality within the northern karst region, received their first prenatal visit by the 20th week of pregnancy, did not use oral contraceptives three months prior to pregnancy or had in vitro fertilization as a method of assisted reproductive technology, and were free of known medical/obstetrics complications. The participants provided spot urine samples during three study visits at approximately 20 ± 2 weeks, 24 ± 2 weeks, and 28 ± 2 weeks of gestation. Questionnaires were also administered at each visit prior to collecting the urine to obtain information on demographics and self-reported consumption of fruits, vegetables, and legumes in the 48 h prior to urine collection, and home pest-related issues. The study was described in detail to all the participants who then gave informed consent. The Ethics and Research Committees of the University of Puerto Rico, the University of Michigan, and Northeastern University approved the research protocol. The involvement of the Centers for Disease Control and Prevention (CDC) did not constitute engagement in human subject research.

2.2. Urinary biomarkers of pesticide exposure

At each study visit, the participants provided one spot urine sample, which was collected and processed using procedures that were comparable to those that the CDC has developed for the National Health and Nutrition Examination Survey (NHANES) and other studies. Urine samples were analyzed within about two years after collection at the National Center for Environmental Health of the CDC (Atlanta, GA, USA). The urine was analyzed for the following four metabolites of specific OP insecticides: 3,5,6-trichloro-2-pyridinol (TCPY), a metabolite of chlorpyrifos and chlorpyrifos-methyl; 2-isopropyl-4-methyl-6-hydroxy-pyrimidine (IMPY), a metabolite of diazinon; malathion dicarboxylic acid (MDA), a metabolite of malathion; and *para*-nitrophenol (PNP), a metabolite of parathion and methyl parathion. The urine was also analyzed for the following six metabolites that are common to many OP insecticides, not just the ones mentioned in the preceding sentence: diethylphosphate (DEP), diethylthiophosphate (DETP), diethyldithiophosphate (DEDTP), dimethylphosphate (DMP), dimethylthiophosphate (DMTP), and dimethyldithiophosphate (DMDTP). Quantification of TCPY, IMPY, MDA and PNP used solid phase extraction and high-performance liquid chromatography–isotope dilution tandem mass spectrometry (SPE-HPLC–MS/MS) as described previously (Davis et al., 2013). The six dialkyl substituted OP metabolites were measured using a modification of the analytical method of Odetokun et al. (2010) that also employs SPE-HPLC–MS/MS with isotope dilution calibration. Accuracy and precision for each analytical run were monitored through the use of calibration standards, reagent blanks, and quality control materials of high and low concentrations. Where applicable (Tables 1 and 2 and Fig. 1), concentrations below the limit of detection (LOD) were assigned a value of LOD divided by the square root of 2. Where adjustment for urinary output was necessary (Table 2 and Fig. 1), urinary concentrations were corrected for specific gravity (SG), which was measured at the University of Puerto Rico using a digital handheld refractometer (Atago Co., Ltd., Tokyo, Japan), using the following formula: $P_c = P_m[(SG_p - 1) / (SG_m - 1)]$, where P_c is the SG-corrected urinary concentration (ng/ml), P_m is the measured urinary concentration (ng/ml), SG_p is the median of the urinary SGs for the population (1.019), and SG_m is the measured urinary SG.

2.3. Statistical analysis

Statistical analysis was performed using SAS version 9.3 for Windows (SAS Institute, Cary, NC, USA). Distributions of urinary concentrations were calculated and compared to those measured most recently (either the 2007–2008 or 2009–2010 cycles) in U.S. women 18–40 years of age from NHANES (www.cdc.gov/nchs/nhanes.htm). Comparisons of urinary biomarker concentrations were made using the 50th percentile value for biomarkers with a sufficiently high frequency of detects (i.e., TCPY, PNP, and DMTP). For all other