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Evaluation of pyrethroid exposures in pregnant women from 10 Caribbean countries

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ABSTRACT

Pyrethroid pesticides are commonly used in tropical regions such as the Caribbean as household insecticides, pet sprays, and where malaria is endemic, impregnated into mosquito-repellent nets. Of particular concern is exposure during pregnancy, as these compounds have the potential to cross the placental barrier and interfere with fetal development, as was shown in limited animal studies. The objective of this study was to evaluate exposure to pyrethroids to pregnant women residing in 10 English-speaking Caribbean countries. Pyrethroid exposures were determined by analyzing five pyrethroid metabolites in urine samples from 295 pregnant women: *cis*-DBCA, *cis*-DCCA, *trans*-DCCA, 3-PBA, and 4-F-3-PBA. Pyrethroid metabolite concentrations in Caribbean pregnant women were generally higher in the 10 Caribbean countries than levels reported for Canadian and U.S. women. In Antigua & Barbuda and Jamaica participants the geometric mean concentrations of *cis*-DBCA was significantly higher than in the other nine countries together ($p < 0.0001$ and <0.0012 respectively). For *cis*- and *trans*-DCCA, only Antigua & Barbuda women differed significantly from participants of the other nine Caribbean countries ($p < 0.0001$). Urinary 4-F-3-PBA and 3-PBA levels were significantly higher in Antigua & Barbuda ($p < 0.0028$ and $p < 0.0001$ respectively) as well as in Grenada ($p < 0.0001$ and $p < 0.007$ respectively). These results indicate extensive use of pyrethroid compounds such as permethrin and cypermethrin in Caribbean households. In Antigua & Barbuda, the data reveals a greater use of deltamethrin. This study underscores the need for Caribbean public health authorities to encourage their populations, and in particular pregnant women, to utilize this class of pesticides more judiciously given the potentially adverse effects of exposure on fetuses and infants.

1. Introduction

Pyrethroid insecticides, synthetic versions of the natural compound pyrethrin produced by the *Chrysanthemum* flower, have been extensively used in agricultural and home formulations for more than 30 years and account for approximately one-fourth of the worldwide insecticide market (Casida and Quistad, 1998). In malaria-endemic zones, pyrethroid insecticides are used to impregnate mosquito nets and clothing for the prevention of malaria (Health-Canada, 2004). Common human exposure to pyrethroids occurs primarily through the use of pyrethroid containing household insecticides and pet sprays, and through the ingestion of food and drinking water contaminated with pyrethroid residues (ATSDR, 2003; US-EPA, 2006).

In tropical regions, such as the Caribbean, the use of pyrethroid containing household sprays is common and widespread due to their broad availability and the need to control domesticated vectors of diseases such as mosquitoes, or to control nuisance infestation of living spaces such as cockroaches and ants. Additionally, seasonal upsurges in the incidence of dengue fever result in preventative and reactive measures of extensive spraying of homes to control elevated *Aedes aegypti* populations and the nuisance *Culex* sp. mosquito. The widespread use of pyrethroid pesticides in the Caribbean as well as other parts of the world is also due in part to avoid the use of other more toxic classes of insecticides such as organophosphate pesticides (chlorpyrifos and diazinon), which are no longer registered for indoor use.

Pyrethroid insecticides are considered less toxic to humans compared to other classes of insecticides such as organophosphates (Snodgrass,

Abbreviations: ANOVA, analysis of variance; CHMS, Canadian Health Measure Survey; CHUQ, Centre Hospitalier universitaire de Québec; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid; CTQ, Centre de toxicologie du Québec; EPA, Environmental Health Agency; IDRC, International Development Research Centre; INSPQ, Institut national de santé publique du Québec; LOD, Limit of detection; MS, Mass spectrometer; NHANES, National Health and Nutritional Examination Survey; POPs, Persistent Organic Pollutants; QA/QC, Quality assurance/Quality control; *trans*-DCCA, *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid; 4-F-3-PBA, 4-fluoro-3-phenoxybenzoic acid; 3-PBA, 3-phenoxybenzoic acid.

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2001). They are rapidly metabolized and eliminated from the body through hydrolysis, oxidation, and conjugation. Following oral ingestion, inhalation, or dermal intake, pyrethroids are metabolized into carboxylic and phenoxybenzoic acids and then excreted in the urine. Pyrethroid metabolites can be measured in blood and urine and concentrations are reflective of recent (the previous few days) exposure to the parent compound or the metabolite in the environment (ATSDR, 2003; Kuhn et al., 1999; Narahashi, 2001; Starr et al., 2008).

In mammals, several pyrethroid metabolites have been identified. 3-phenoxybenzoic acid (3-PBA) is formed from the oxidation of most pyrethroids. The metabolite 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA) is a specific metabolite of cyfluthrin and the metabolite *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic (*cis*-DBCA) is specific to deltamethrin. *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (*cis*-DCCA) and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (*trans*-DCCA) are both metabolites of chlorinated pyrethroids such as permethrin, cypermethrin, and cyfluthrin.

Like many other classes of pesticides, pyrethroids are neurotoxicants (Shafer et al., 2005). Pyrethroid compounds can cross the placental barrier and are known to interfere with hormonal and neurological development, the immune system and other physiological functions (Chanda and Pope, 1996; Doucet et al., 2009; Gupta et al., 1985; Muto et al., 1992). To date, there are a limited number of human studies that have examined the effects of synthetic pyrethroids on developmental outcomes. Bell et al. (2001) reported an increased risk of fetal death due to congenital anomalies when synthetic pyrethroids were used in the same township, range, or section during the 3rd to 8th week of pregnancy. Hanke et al. (2003) found a significant reduction in birth weight among the offspring of mothers potentially exposed to synthetic pyrethroids during the three months prior to conception and the first trimester of pregnancy. Conversely Dabrowski et al. (2003) found no significant reduction in birth weight after reported farm use of synthetic pyrethroids during the first or second trimester of pregnancy. Furthermore, the only study that measured biomarkers of exposure in maternal urine during the third trimester of pregnancy found no association with birth weight, birth length, or head circumference (Berkowitz et al., 2004). Also, Horton et al. (2011) did not observe a significant association between prenatal exposure to permethrin and adverse neurodevelopment. Finally, a study of 113 women using a pyrethroid cream to treat head lice did not show an increased risk for birth defects or pregnancy complications (Kennedy et al., 2005).

In laboratory animals, one study showed that cypermethrin was able to induce oxidative stress and produce apoptosis through the involvement of caspases in zebrafish embryos (Shi et al., 2011). Another study conducted with rats demonstrated that deltamethrin increased early embryonic deaths and caused growth retardation (Abdel-Khalik et al., 1993).

In the Caribbean region, pregnant and delivering mothers' fetuses are being exposed to various chemical substances. This study was part of a larger research project designed to address the question of prenatal exposure to persistent organic pollutants (POPs), heavy metals such as mercury and lead, and other classes of pesticides such as organophosphates, carbamates, and pyrethroids. The main objective of this component of the study was to evaluate exposure of pregnant women to pyrethroids by measuring five metabolites in urine samples (Fig. 1) and identifying which chemicals are of prime concern at national and regional levels.

2. Materials and methods

2.1. Ethics and sampling

Between August 2008 and April 2011, 442 pregnant or delivering women from 10 Caribbean countries were recruited to participate in this study. Applications were made to the institutional review boards or ethics committee (whichever existed) in each participating country to obtain ethical approval for the implementation of the research project. Ethics approvals were also obtained from the ethics boards of this study's principal investigator's institutions—Laval University, Canada, and St. George's University, Grenada. Once granted, governmental approval was then sought and obtained through the Ministry of Health within each country where this study was executed. Once governmental and ethical approvals were secured, local nurses and laboratory technicians were identified and trained to collect the samples in their respective country. Thus, in each of the 10 countries where this study was executed, locally trained personnel recruited the women to participate in this study, obtained their informed consent, collected the samples, and then prepared for shipment to the laboratories for analysis.

Using recruitment and sampling protocols that were comparable to those employed in a similar exposure assessment program carried out in circumpolar countries (Van Oostdam et al., 2004), a goal of recruiting 50 pregnant women within a narrow age range in each country was set. For budgetary reasons, we were able to meet this goal only for Dominica ($n = 48$), Grenada ($n = 50$), Jamaica ($n = 45$), and St Vincent ($n = 50$). For the other six countries, we had to restrict analyses to between 15 and 22 participants (randomly selected from the 50 participants) with the exception of Montserrat where all 15 participant samples were analyzed. The total number of pyrethroid analyses performed was therefore 295.

Urine samples were collected from pregnant women coming to the main hospital or health clinics during their last prenatal visits, just before delivery (last trimester). In the event it was not possible, the sampling was done within two weeks after their discharge from the Hospital. Women who gave consent to participate in the study were provided with an information sheet and given the opportunity to sign a written consent form.

Urine samples were poured into 10 mL vials and stored at −80 °C prior to shipping on dry ice to Québec's Toxicology Center (CTQ) of the Institut national de santé publique du Québec (INSPQ) toxicology laboratory located in Quebec City, Canada, for analysis. This facility is the reference laboratory for human toxicology in the Province of Quebec, Canada, and also participates in the quality assurance/quality control (QA/QC) program of the Canadian Northern Contaminants Program.

2.2. Analysis

2.2.1. Pyrethroid metabolites in urine

Five main urinary pyrethroid metabolites were measured: 4-F-3-PBA; *cis*-DBCA; *cis*-DCCA; *trans*-DCCA; and 3-PBA. A 5-mL aliquot of urine collected was first enriched with isotopically labeled internal standards (trans-DCCA-$^{13}C_4$-D_3, 3-PBA-$^{13}C_6$ and 4-F-3PBA-$^{13}C_6$) and then mixed with an acetate buffer at pH 5. The urinary metabolites were then hydrolyzed with ß-glucuronidase enzyme and the resulting mixture was acidified with HCl and extracted 9 mL of hexane. The extracts were evaporated to dryness and derivatized with 30 μL hexafluoro-2-propanol plus 20 μL of diisopropylcarbodiimide and extracted a second time with 2 mL of isooctane:hexane (2:98). Evaporated extracts were dissolved in hexane and analyzed by gas chromatography–mass spectrometry (GC–MS) on an Agilent 6890 Series Plus gas chromatograph equipped with an Agilent 7683 series automatic injector and an Agilent 5973 Network mass spectrometer (Agilent Technologies; Mississauga, Ontario, Canada) operated in the single ion monitoring mode following negative chemical ionization with methane as the reagent gas. Quantification and qualifier ions were respectively 322 (m/z) and 358 (m/z) for *cis*-DCCA, 322 (m/z) and 324 (m/z) for *trans*-DCCA, 366 (m/z) and 79 (m/z) for *cis*-DCBA, 364 (m/z) and 213 (m/z) for 3-PBA and 231 (m/z) and 382 (m/z) for 4-F-3-PBA. Helium was used as the carrier gas and all injections were 1 μL in splitless mode. The analytical column used was an Agilent 60 m DB-XLB (0.25 mm i.d., 0.25 μm film thickness) (Agilent Technologies). Limits of detection (LODs) for each metabolite were as follows: 0.006 μg/L for *cis*-DBCA; 0.007 μg/L for *cis*-DCCA; 0.01 μg/L for *trans*-DCCA; 0.01 μg/L for 3-PBA, and 0.008 μg/L for 4-F-