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Organophosphorus and pyrethroid insecticide urinary metabolite concentrations in young children living in a southeastern United States city

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ABSTRACT

Pesticide metabolites are routinely measured in the urine of children in the United States. Although the sources of these metabolites are believed to include residues in food from agricultural applications and residues from applications in everyday environments (e.g., homes), few studies have been able to demonstrate an association between indoor residential pesticide applications and pesticide metabolite concentrations. To better quantify the effects of potential risk factors related to demographics, household characteristics, occupation, and pesticide use practices on urinary biomarker levels, we performed a study in a city (Jacksonville, Florida) previously determined to have elevated rates of pesticide use. We enrolled a convenience sample of 203 children ranging in age from 4 to 6 years; their caregivers completed a questionnaire and the children provided a urine sample, which was analyzed for a series of organophosphorus and pyrethroid insecticide metabolites. The questionnaire responses substantiated much higher pesticide use for the study participants as compared to other studies. Urinary metabolite concentrations were approximately an order of magnitude higher than concentrations reported for young children in other studies. Few statistically significant differences (at the $p<0.05$ level) were observed, however, several trends are worth noting. In general, mean urinary pesticide metabolite concentrations were higher for males, Caucasians, and those children living in homes with an indoor pesticide application occurring within the past four weeks. Comparing the urinary pesticide metabolite concentrations in this study to those reported in the NHANES and GerES studies showed that the children living in Jacksonville had substantially higher pyrethroid pesticide exposures than the general populations of the United States and Germany. Further research is needed in communities where routine pesticide use has been documented to obtain information on the most important routes and pathways of exposure and to develop the most effective strategies for reducing pesticide exposures for children.

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1. Introduction

Seventeen million pounds of home and garden insecticide active ingredient was applied by homeowners to homes, gardens, and lawns in single- and multiple-unit housing in the United States; this comprised 16% of the total pesticide use in 2001 (US EPA, 2004). Few data exist on regional pesticide use patterns in the United States; however, in 1981 the US EPA's National Urban Pesticide Applicator Survey identified Jacksonville, Florida, a city in the southeastern United States, as being representative of relatively high pesticide use and Springfield and Chicopee, Massachusetts, in the northeastern United States, as a region of the country representative of relatively low to moderate pesticide use based on a questionnaire survey of professional applicators (US EPA, 1984). The US EPA's Non-Occupational Pesticide Exposure Study (NOPES) later showed that airborne pesticide concentrations were higher in Jacksonville, Florida, as compared to Springfield and Chicopee, Massachusetts, suggesting that the likelihood of routine pesticide use in the Southeast is greater than in the Northeast (Whitmore et al., 1994).

Routine pesticide use on indoor and outdoor surfaces in the residential environment may result in human exposure; this exposure can be assessed by biomonitoring, which involves measuring the pesticides or their metabolites in biological samples such as urine, blood,

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breath, hair, or nail clippings (CDC, 2005). Observational measurement studies are undertaken to collect samples, including biological samples, which are then analyzed for the chemicals of interest.

Few national biomonitoring surveys exist. In the United States, the *National Report on Human Exposure to Environmental Chemicals* (CDC, 2005) is published by the Centers for Disease Control and Prevention (CDC) based on the results from the National Health and Nutrition Examination Survey (NHANES; http://www.cdc.gov/nchs/nhanes.htm). NHANES is a program of studies designed to describe the health and nutritional status of adults and children in the United States through interviews and physical examinations. The *National Report on Human Exposure to Environmental Chemicals* provides an on-going assessment of biomarker concentrations for the civilian, non-institutionalized U.S. population; the *Third Report* provided data on 148 environmental chemicals in various biological samples (CDC, 2005). Similarly, the German Environmental Survey (GerES) is a large-scale, representative population study conducted approximately every 7 years that measures the biomarker concentrations of chemicals to which residents of Germany may be exposed (Seifert et al., 2000a,b; Becker et al., 2006).

Many biomonitoring studies targeting children have been conducted in the United States and Europe, including: A Pilot Study of Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP Study; http://www.epa.gov/heasd/ctepp/index.htm; Morgan et al., 2004); the Minnesota Children's Pesticide Exposure Study (Adgate et al., 2001); an evaluation of the exposure of young children living in the Salinas Valley, California as part of the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study (Bradman et al., 2007); decades of work determining children's exposures to pesticides in various regions within Washington State (Fenske et al., 2005); and research to determine the exposures of young children living in Italy (Aprea et al., 2000). All of these studies have provided valuable information on which pesticides children may be coming into contact with in their everyday environments and what their urinary metabolite concentrations may be; however, these studies have not definitively indicated that using pesticides in the residential environment may lead to increases in pesticide urinary metabolite concentrations in occupants living in treated residences. As a result, we undertook a biomonitoring study in Jacksonville, Florida to measure young children's urinary metabolite concentrations in a city that had been previously determined to have higher environmental pesticide concentrations than other selected locations.

This biomonitoring study was one component of a large collaborative research study conducted by the CDC, the United States Environmental Protection Agency (EPA), and the Duval County Health Department (DCHD) to evaluate young children's (4 to 6 years) exposures to current-use pesticides in their everyday environments. The CDC component of the study involved the collection of a urine sample from the child, completion of a questionnaire by the child's primary caregiver, and the analyses of the urine samples for pesticide metabolites and creatinine. This paper describes the results of this biomonitoring study.

2. Materials and methods

2.1. Study participants

Participants were recruited for the biomonitoring study when they visited a health care center operated by the DCHD for routine care (e.g., wellness visits) during the summer and fall of 2001. The six health care centers that participated in this study encompassed urban, suburban, and rural geographic segments in Duval County and were openly accessible. A convenience sample of 203 children ranging in age from 4 to 6 years was enrolled into the biomonitoring study. DCHD staff identified potential study participants by age when the child and accompanying adult visited the clinic. Each accompanying adult was provided written materials describing the purpose, methods, and risks of the study. If the accompanying adult was interested in participating in the study and was able to consent for the child to participate, then a DCHD staff member administered the informed consent to the adult and obtained assent from the child. This was an observational research study, as defined in 40 CFR Part 26.402. The study protocol and procedures to obtain the assent of the children and informed consent of their parents or guardians were reviewed and approved by three independent institutional review boards representing each collaborating agency and complied with all applicable requirements of the Common Rule regarding additional protections for children (Subpart D).

2.2. Field sample collection

2.2.1. Questionnaire

While at the health care center, each caregiver completed a survey questionnaire. The questionnaire collected demographic information about the child (e.g., age, sex, race/ethnicity), pesticide usage information (e.g., frequency of application, recent applications, and types of products used), household characteristics (e.g., type of dwelling), household habits, and occupational information for each adult living in the home.

2.2.2. Urine samples

Urine samples were collected from all children during the clinic visit, unless the child was unable to produce a sample; in which case, the parent was asked to collect a sample from the child at home and the sample was retrieved by DCHD staff. If the urine sample needed to be collected at home, instructions and a standard plastic urine collection cup (BD Medical Supplies, Franklin Lakes, NJ) were sent home with the caregivers. Parents and children were instructed to wash their hands before the child voided into the cup and to not touch the inside of the cap or cup. In an attempt to collect at least 30 mL of urine, children were asked to fill the collection cup as much as they could.

DCHD staff separated an aliquot of urine from the collection cup into a 30 mL glass bottle (Qorpak, Bridgeville, PA) with Teflon-lined screw cap and pre-labeled with a study-specific bar-coded label. Urine aliquots were stored at the health care centers, either in a dedicated sample refrigerator or in a cooler. Once a day, DCHD staff collected all urine samples from the health care centers and delivered them to the state laboratory for refrigerated storage until they were shipped on ice to CDC, where they were stored in a −70 °C freezer until sample extraction and analysis. All collection materials were tested prior to use to ensure they were not contaminated with the target analytes.

Urine samples were analyzed for organophosphorus (OP) and pyrethroid insecticide metabolites using published methods (Baker et al., 2004; Bravo et al., 2004). Specifically, six common dialkylphosphate (DAP) metabolites of OP insecticides [i.e., dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP)] and four metabolites of pyrethroid insecticides [i.e., 3-phenoxybenzoic acid (3-PBA), a common metabolite of many pyrethroids; 4-fluoro-3-phenoxybenzoic acid (4F-3-PBA), a metabolite of cyfluthrin; and *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (*cis*- and *trans*-DCCA), metabolites of permethrin, cypermethrin, and cyfluthrin] were measured. Table 1 shows the relationship between selected commonly detected OP and pyrethroid pesticides and their non-specific metabolites, as well as common uses for these pesticides. Brief descriptions of the analytical methods follow. For the dialkylphosphates, 2 mL urine aliquots were lyophilized, derivatized to their respective chloropropyl phosphate esters, and analyzed using gas chromatography-tandem mass spectrometry (Bravo et al., 2004). The detection limits ranged from 0.1 to 0.6 ng/mL. For the pyrethroid metabolites, 2 mL urine aliquots were extracted using solid phase extraction cartridges. The extracts were analyzed using high-performance liquid chromatography-atmospheric