Temporal variability of pyrethroid metabolite levels in bedtime, morning, and 24-hr urine samples for 50 adults in North Carolina

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ABSTRACT

Pyrethroid insecticides are widely used to control insects in both agricultural and residential settings worldwide. Few data are available on the temporal variability of pyrethroid metabolites in the urine of non-occupationally exposed adults. In this work, we describe the study design and sampling methodology for the Pilot Study to Estimate Human Exposures to Pyrethroids using an Exposure Reconstruction Approach (Ex-R study). Two major objectives were to quantify the concentrations of several pyrethroid metabolites in bedtime, first morning void (FMV), and 24-hr urine samples as concentration (wet weight), specific-gravity (SG) corrected, creatinine (CR) corrected, and excretion rate values for 50 Ex-R adults over a six-week monitoring period and to determine if these correction approaches for urine dilution reduced the variability of the biomarker levels. The Ex-R study was conducted at the United States Environmental Protection Agency's Human Studies Facility in Chapel Hill, North Carolina USA and at participants' homes within a 40-mile radius of this facility. Recruitment of participants and field activities occurred between October 2009 and May 2011. Participants, ages 19–50 years old, provided daily food, activity, and pesticide-use diaries and collected their own urine samples (bedtime, FMV, and 24-hr) during weeks 1, 2, and 6 of a six-week monitoring period. A total of 2503 urine samples were collected from the study participants. These samples were analyzed for the pyrethroid metabolites 3-phenoxybenzoic acid (3-PBA), *cis/trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*cis*/*trans*-DCCA), and 2-methyl-3-phenylbenzoic acid (MPA) using high performance liquid chromatography/tandem mass spectrometry. Only 3-PBA was frequently detected (> 50%) in the adult urine samples. Median urinary 3-PBA levels were 0.88 ng/mL, 0.96 ng/mL-SG, 1.04 ng/mg, and 1.04 ng/min for concentration, SG-corrected, CR-corrected, and excretion rate values, respectively, across all urine samples. The results showed that median urinary 3-PBA concentrations were consistently the lowest in FMV samples (0.77 ng/mL, 0.68 ng/mL-SG, 0.68 ng/mg, and 0.58 ng/min) and the highest in 24-hr samples (0.92 ng/mL, 1.06 ng/mL-SG, 1.18 ng/mg, and 1.19 ng/min) across all four methods. Intraclass correlation coefficient (ICC) estimates for 3-PBA indicated poor reproducibility (< 0.22) for all urine sample types and methods over a day, week, and six weeks. Correcting for urine sample dilution, based on either SG, CR or urine output, introduced additional measurement variability both between- and within-individuals. These results indicate that a single measure of urinary 3-PBA was not sufficient to characterize average exposure regardless of sample type, correction method, and time frame of collection. In addition, the study results can be used to inform the design of exposure characterization strategies in relevant environmental epidemiology studies in the future.

Keywords: Pyrethroids, adults, urine, homes, biomonitoring, variability

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Abbreviations

3-PBA, 3-phenoxybenzoic acid; CDC, Centers for Disease Control and Prevention; *cis/trans*-DCCA, *cis/trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid; EPA, Environmental Protection Agency; Ex-R study, Pilot Study to Estimate Human Exposures to Pyrethroids using an Exposure Reconstruction Approach; HSF, Human Studies Facility; FMV, first morning void; ICC, intraclass correlation coefficient; LOQ, limit of quantitation; NHANES, National Health and Nutritional Examination Survey; NC, North Carolina; MPA, 2-methyl-3-phenylbenzoic acid; RTP, Research Triangle Park; US, United States

1. Introduction

Pyrethroids are a class of insecticides that are widely and globally used to control a variety of insects at homes, on domestic pets, and on agricultural crops (US EPA, 2013; Saillenfait et al., 2015). These lipophilic insecticides have replaced many of the previous residential and some agricultural applications of the organophosphates because of their lower volatility and lower mammalian toxicity due to faster enzymatic detoxification (Elliot, 1976; Barr et al., 2010). However, recent research has raised concerns about potential adverse health effects (i.e., developmental and male reproductive) occurring from human exposure to pyrethroids at environmental levels (Saillenfait et al., 2015).

In the United States (US), at least 20 different pyrethroids are registered for commercial use in residential or agricultural settings (US EPA, 2011). However, no published data are currently available on the national retail sales or usage patterns of pyrethroid insecticides in these settings (Kuivila et al., 2012; Palmquist et al., 2012; Xue et al., 2014). Several US studies have detected a number of current-use pyrethroids (i.e., bifenthrin, cyfluthrin, cypermethrin, cyhalothrin, deltamethrin, and esfenvalerate) in dust, food, and/or wipes in residential environments (Morgan et al., 2007; Julien et al., 2008; Starr et al., 2008; Stout et al., 2009; Tulve et al., 2008; Chuang and Wilson, 2011; Trunnelle et al., 2014). Research has suggested that dietary ingestion is likely the major route of exposure to pyrethroids in the general US adult population (Reiderer et al., 2008; Barr et al., 2010).

After absorption into the body, pyrethroid insecticides are metabolized rapidly and primarily excreted in urine with an elimination half-life of less than 12-hr (Leng et al., 1997; Kuhn et al., 1999; Ratelle et al., 2015). Several cross-sectional studies have reported measureable concentrations of pyrethroid metabolites, including 3-phenoxybenzoic acid (3-PBA) and *cis/trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*cis*/*trans*-DCCA), in the spot urine samples of adults recruited from the general population (referred to subsequently as non-occupationally exposed) in the US (Berkowitz et al., 2003; Barr et al., 2010; McKelvey et al., 2013; Young et al., 2013; Trunnelle et al., 2014; CDC, 2015; Morgan, 2015). In the US National Health and Nutritional Examination Survey (NHANES, 2001-2002 cycle), which was a population-based survey that included 1128 adults, ages 20–59 years, 3-PBA, *cis*-DCCA, and *trans*-DCCA were detected in 76%, 33%, and 25% of the urine samples, respectively (Barr et al., 2010). 3-PBA is a nonspecific

urinary biomarker of at least 18 different pyrethroid insecticides including cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, and permethrin (CDC, 2009; Barr et al., 2010). The isomers, *cis*-DCCA and *trans*-DCCA, are also the nonspecific urinary biomarkers of cyfluthrin, cypermethrin, and permethrin (CDC, 2009).

For spot urine measurements, a major concern is that volume-based concentrations (e.g., ng/mL) can be affected by variable urine dilutions in humans from factors such as fluid intake, perspiration, gender, age, and disease status (e.g., kidney and heart) (Boeniger et al., 1993; Mage et al., 2004; Barr et al., 2005; Fortin et al., 2008). To adjust for variable urine dilutions in spot samples, correction methods involving specific gravity (SG) and creatinine (CR) measurements or timed excretion rates (e.g., ng/min) have been used to normalize pesticide biomarker measurements across and within individuals (Hwang et al., 1997; Fortin et al., 2008; Christensen et al., 2012). CR-correction of urine sample volumes is the most widely used method in studies, and assumes that CR is eliminated at a constant rate by the kidneys into urine in humans (Hwang et al., 1997; Barr et al., 2005). But, this correction method may also bias study results because CR excretion can also vary due to many factors such as age, gender, dietary intake, muscle mass, physical activity, and diurnal patterns (Boeniger et al., 1993; Hwang et al., 1997; Mage et al., 2004). Further complicating interpretation of these data, concentrations in spot samples likely only reflect recent exposures because these chemicals have short elimination half-lives (< 12-hr). Continual exposures to pyrethroid insecticides would likely result in relatively constant urinary metabolite levels over time (e.g., day, week or month) (NRC, 2006; Wielgomas 2013a). In contrast, episodic exposures to these insecticides would probably cause significant fluctuations in urinary biomarker levels over time (NRC, 2006). Little information, however, is currently known on the temporal variability of pyrethroid metabolites in non-occupationally exposed adults. Temporal variability is useful for understanding the patterns and magnitude of a person's exposures to pyrethroid insecticides over time using urinary biomonitoring. When relying on only one spot urine measurement, this unknown variability in an individual's biomarker level could lead to exposure misclassification in epidemiological studies (Lassen et al., 2013). Wielgomas (2013a) recently examined the temporal variability of 3-PBA concentrations in the urine samples of seven, non-occupationally exposed adults (ages 24–71 years old) over a week

in an urban area of Poland in 2011. In this study, the intraclass correlation coefficient (ICC), which quantifies reliability, were estimated for 3-PBA levels by urine sample type (first morning void [FMV], spot, and 24-hr) as concentration, CR-corrected, and excretion rate values. The ICCs were the highest for the CR-corrected spot urine samples (ICC=0.846). Based on this ICC of 0.846, a single spot sample appeared representative of average exposure over a single week, and that these adults were likely continually exposed to similar levels of pyrethroids, likely from the same sources, in their urban environments (Wielgomas, 2013a).

The "Pilot Study to Estimate Human Exposures to Pyrethroids using an Exposure Reconstruction Approach" (Ex-R study) investigated longitudinal exposures (over six weeks) of adults to pyrethroid insecticides commonly found in residential settings. The first study objective was to assess the variability of 3-PBA, *cis*-DCCA, *trans*-DCCA, and 2-methyl-3-phenylbenzoic acid (MPA; specific metabolite of bifenthrin) in the urine samples of 50 adults at home over a six-week monitoring period. The second study objective was to estimate the dietary and non-dietary exposures of these adults to selected current-use pyrethroids and to their preformed metabolites (e.g., 3-PBA) in media using an exposure reconstruction approach. Here we report the study design and sampling methodology for the Ex-R study, and the study results related to objective 1 above. Specifically for objective 1, we quantified the distributions of 3-PBA, *cis*-DCCA, *trans*-DCCA, and MPA by urine sample type (bedtime, FMV, and 24-hr) as concentration, SG-corrected, CR-corrected, and excretion rate values over a six-week monitoring period. We also determined if these three correction approaches for urine dilution reduced the variability of the observed biomarker levels (3-PBA, only). In addition, we calculated the number of urine samples needed to adequately assess the exposures of Ex-R adults to pyrethroids insecticides over a day, week, and six weeks.

2. Materials and methods

The design strategy, recruitment of participants, field sampling procedures, and analytical methodology (urine, only) for the Ex-R study are discussed below.

2.1. Study cohort

The Ex-R study investigated the longitudinal exposures of 50 adults to pyrethroid insecticides at their residences over a six-week monitoring period. The study was conducted at the US Environmental Protection Agency's (EPA's) Human Studies Facility (HSF) in Chapel Hill, North Carolina (NC) and at participants' homes within a 40-mile radius of the HSF. From October 2009 to March 2011, adults were recruited by an on-site US EPA contractor (WESTAT) at the HSF via their in-house database of volunteers or by word of mouth (i.e., US EPA researchers or prior participants). Interested individuals were asked several questions (e.g., "Are you exposed at work to products that contain pyrethroid insecticides [yes or no]?)" over the phone by a WESTAT technician to determine their eligibility to participate. To be eligible, a participant had to be a healthy, non-pregnant adult (18-50 years old), had no pre-existing medical conditions (e.g., kidney disease) affecting urine output, was currently living in a residential home or apartment, had no occupational exposures to pyrethroids, could provide their own transportation to and from the HSF, and could speak, read, and use English fluently. A total of 56 adults who contacted WESTAT were eligible to participate in the study; only two of these adults declined to provide consent to participate (e.g., work conflict). Before beginning the study, the adult participants were trained individually (~2 hrs) by US EPA researchers at the HSF on the procedures for filling out diaries (activity, food, and pesticide-use), collecting their own samples (urine, solid food, drinking water, surface wipes, and vacuum dust), and storing the samples in portable thermoelectric coolers until collected by study personnel.

2.2 Human subjects protection

The study protocol and procedures to acquire informed consent from the adult participants followed EPA policies and the guidelines set forth by the Scientific and Ethical Approaches for Observational Exposure Studies report (US EPA, 2008) and were reviewed and approved by the US EPA's Human Subjects Research Review Official and by the University of North Carolina's Institutional Review Board (study number 09-0741). Adult volunteers read and signed informed consent documents prior to beginning the study.

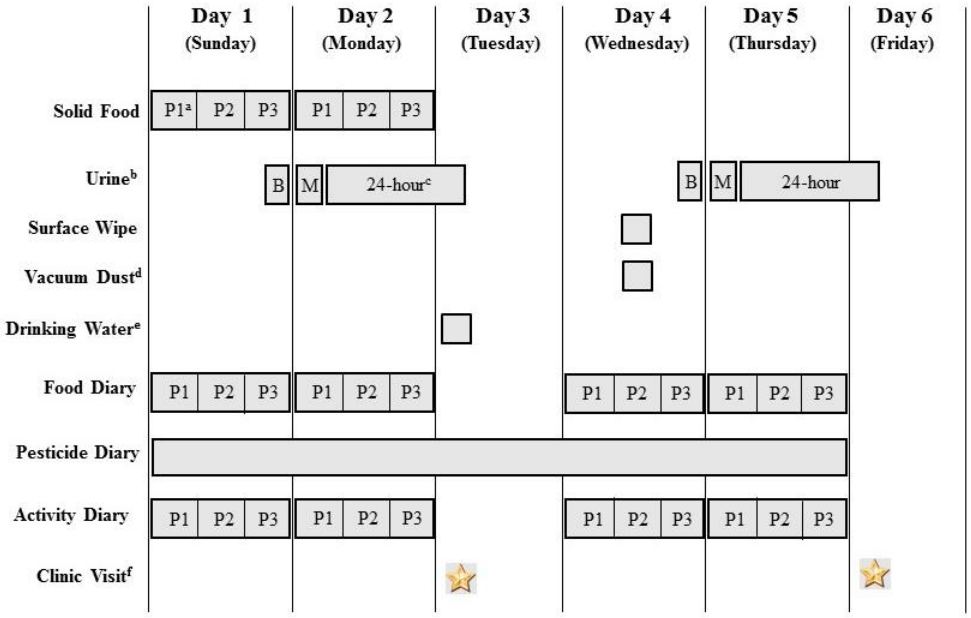
2.3. Field sampling

Field activities were performed between November 2009 and May 2011. A total of 11 consecutive, six-week monitoring periods were performed in this study. During each monitoring period, up to six adult participants filled out diaries and collected their own samples during weeks 1, 2 and 6. Fig. 1 presents the sampling schedule that the participants followed for each sampling week. Each sampling week began in the morning of day 1 (Sunday) and ended in the morning of day 6 (Friday). There were two sampling intervals, days 1-3 (Sunday to Tuesday) and days 4-6 (Wednesday to Friday), per sampling week.

The participants used portable thermoelectric coolers (13.5"D x 12" W x 14.5"H Princess International or Vinotemp®) to store their samples and diaries (in an outside pocket) for each sampling interval. Each cooler contained a notebook with sampling instructions, calendar and schedule, a notebook containing food, activity, and/or pesticide-use diaries, sampling equipment and containers, gloves, ballpoint pens, and an electric power adaptor (Koolatron® Model AC-15). Barcodes were attached to all diaries and sampling containers. Temperature data recorders (Easy Log EL-USB-LITE or EL-USB-1, Lascar Electronic, Ltd) were placed inside each cooler to record internal temperatures. The participants kept the coolers at reduced temperatures by plugging them into their vehicle (using a car adaptor) or an electric wall outlet at home or other location (e.g., work).

The participants were provided with two coolers for the first sampling interval (days 1-3) on the Friday before each sampling week. These two coolers were returned by the participants to the HSF between 8:00 am–11:00 am on day 3 of each sampling week. After arriving at the HSF, US EPA researchers checked-in all study items in the two coolers with each participant. Afterwards, the participants were provided with two new coolers for the next sampling interval (days 4-6) of each sampling week. These two coolers were returned to the HSF between 8:00 am–11:00 am on day 6 of each sampling week, and all study items were checked-in by US EPA researchers. After each sampling interval, the used coolers containing collected study items with blue ice were then transported (~20 miles) by US EPA researchers in a van to the US EPA laboratory in Research Triangle Park (RTP), NC. The participants came to the HSF a total of eight times, including the training session, during the six-week monitoring period.

2.4. Collection of diaries



[a] Period 1 (P1) = 4:00 am–11:00 am; Period 2 (P2) = 11:00 am–5:00 pm; Period 3 (P3) = 5:00 pm–4:00 am
[b] Bedtime void (B), first morning void (M), and 24-hr sample
[c] Individual urine voids were collected over the 24-hr period and analyzed separately
[d] Vacuum dust sample was collected only on day 4 of week 6
[e] Drinking water sample was collected only on day 3 of week 6
[f] Study items were dropped off at the HSF between 8:00 am – 11:00 am

Fig. 1. Field sampling activities that the Ex-R adult participants performed during each sampling week (1, 2, or 6).

The participants filled out three different types of diaries (food, activity, and pesticide-use) during sampling weeks 1, 2, and 6 (Fig. 1). For the food and activity diaries, information was collected during three consecutive time periods on days 1-2 and days 4-5 of each sampling week. These time periods were period 1 (4:00 am -11:00 am), period 2 (11:00 am - 5:00 pm), and period 3 (5:00 pm to 4:00 am). In the food diary, the participants recorded the types and amount of foods and beverages they consumed during each time period. In the activity diary, the participants recorded their primary location (i.e., inside home, outside home, away from home, or transit) and primary activity (i.e., sleeping, low activity [e.g., sitting], medium activity [e.g., walking] or high activity [e.g., running]) in 30-minute time intervals for each time period. They also recorded when they ate, drank, or urinated during these 30-minute time intervals. For the pesticide-use diary, information was collected on day 5 of each sampling week. In this diary, the participants recorded the

types of insecticides that were used at their home over a period of 30 days prior to and during the six-week monitoring period. They also recorded their pesticide usage patterns including type, location, frequency, and day of insecticide application during each sampling week.

2.5. Collection of environmental samples

Environmental samples including solid food, drinking water, surface wipes, and vacuum dust were collected by the adult participants at their residences during sampling weeks 1, 2, and/or 6 (Fig. 1). Detailed information on the collection of these media by the participants can be found in the Supplemental Information section.

2.6. Collection of urine samples

Spot urine voids (bedtime and FMV) and 24-hr urine samples were collected by the participants during each sampling week (Fig. 1). Bedtime voids were collected before going to sleep on days 1 and 4 of each sampling week. FMV's were collected after waking up on days 2 and 5 of each sampling week. For the 24-hr samples, individual urine voids for a person were collected starting after the FMV on day 2 or 5 until collection of the FMV the next day (e.g., day 3 or 6) (Fig. 1). Up to 11 individual voids were collected by a participant over a 24-hr sampling period.

All urine voids were collected by the participants in separate 1 L polypropylene containers. The entire amount of urine was collected for each type of urine void. The time of sample collection for each void was recorded on a label affixed to the lid of the container. At the HSF, US EPA researchers recorded the volume of each urine void using a transparent, graduated plastic cylinder that was placed over the container. A total of 2503 urine voids (includes bedtime, FMV, and 24-h) were collected by the participants during the study.

At the US EPA laboratory, up to eight, 8 mL aliquots of urine from each void were placed into separate 10 mL cryogenic vials with lids. All aliquots were stored in laboratory freezers (-80^{o}C) until analysis.

2.7 Analysis of urine samples

In 2010–2011, US EPA laboratory technicians shipped 2503 urine aliquots (8 mL each) in coolers containing dry ice overnight by UPS to Emory University's Rollins School of Public Health laboratory in Atlanta, Georgia. The urine aliquots were stored in -80^{o}C freezers until analyzed (2013–2014). For the 24-hr urine samples, each void was analyzed separately. All urine samples were extracted and levels of 3-PBA, *cis*-DCCA, *trans*-DCCA, and MPA were measured using a slight modification of the method by Baker et al. (2004). Briefly, 0.5-mL aliquot of urine from each sample was hydrolyzed with β-glucuronidase/sulfatase for ~16 hrs. Next, the target analytes were extracted using solid phase extraction (3cc-60 mg OASIS HLB cartridge) and then evaporated to dryness using a TurboVap LV Evaporator set at 45^{o}C and 15 psi N_2. Finally, the extract was reconstituted with 100 µL of a 30% methanol/70% deionized water solution prior to chemical analysis. The urine extracts were quantified for the target pyrethroid metabolites using a high performance liquid chromatography/tandem mass spectrometer (Agilent Tech, Waldbronn, Germany) equipped with a negative mode jet-stream electrospray chemical ionization interface. Separation was performed using a Betasil C_{18}, 100 x 2.1 mm - 3 µm particle size, column (Thermo Scientific, Waltham, MA, USA) with a gradient elution using 0.1% acetic acid in deionized water and 0.1% acetic acid in methanol. The column temperature was maintained at 45^{o}C, and the total run time was seven minutes. The estimated limits of quantitation (LOQ) were 0.25 ng/mL for 3-PBA, *cis*-DCCA, and *trans*-DCCA and 0.5 ng/mL for MPA.

All urine samples were also analyzed for SG and CR levels. At the US EPA laboratory in 2012, another identical set of urine aliquots (8 mL each) was thawed overnight in a refrigerator (~4^{o}C). Each aliquot of urine was vortex mixed (~5 seconds) and then transferred into a 50 mL vial and sonicated for 15 minutes. A 50 µL aliquot of urine was removed from each 50 mL vial and analyzed for CR levels using a spectrophotometer (Biotek® ELx800 or Elx808ui), equipped with a 96-well plate at a wavelength at 490 nm using a modified version of the Jaffé method (Andersen et al., 2014). SG was also measured by dipping the tip of a hand-held refractometer (Atago® model no. 3741) into each 50 mL vial of remaining urine (~7.9 mL) using water as a calibrant.

2.8 Quality assurance and quality control

A total of 150 field blanks and 150 field spikes were prepared prior to the onset of sampling activities by laboratory technicians at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia in 2009. These quality control (QC) samples consisted of pooled urine samples that were collected from non-occupationally exposed adult volunteers at CDC. The pooled urine samples were prescreened for 3-PBA, *cis*-DCCA, and *trans*-DCCA and diluted, when necessary, to reduce background levels. Each field blank or field spike consisted of 8 mL of urine in a 10-mL polypropylene cryovial. For the field spikes, 3-PBA, *cis*-DCCA, and *trans*-DCCA standards (25 ng/mL) were individually added to each cryovial; the MPA standard was not obtained until after preparation of the field spikes at CDC. These QC samples were shipped in coolers on dry ice overnight by Fed-Ex from the CDC laboratory to the US EPA laboratory in 2009 and kept frozen in freezers (-80^{o}C) until field sampling.

A single field blank and field spike were assigned to each study participant during days 4-6 of each sampling week. These QC samples were thawed overnight in an US EPA refrigerator and then placed unopened in a (plugged-in) portable thermoelectric cooler in a study room at the HSF. After each sampling interval, the field blanks and field spikes were transported in separate coolers along with study sample coolers to the US EPA laboratory and kept frozen (-80^{o}C) in freezers.

In 2010–2011, 150 field blanks, 150 field spikes, and 150 duplicate urine samples were shipped in coolers on dry ice by UPS from the US EPA laboratory to the Emory University laboratory in Atlanta, GA. These QC samples were analyzed for the target pyrethroid metabolites following the same analytical procedures described in section 2.7 above. For the field blanks and field spikes, a value of $\mathrm{LOQ}/\sqrt{2}$ was first imputed for all target analyte measurements that were below the LOQ (Verbovsek, 2011). Precision estimates, expressed as percent relative standard deviations (RSDs), were then calculated for the field blanks and field spikes. Measures of field blanks were all below the LOQ for *cis*-DCCA, *trans*-DCCA, and MPA. However, all field blanks had low 3-PBA background levels (likely stemming from true background levels in the pooled urine samples, and not sample contamination), and yielded an RSD estimate of 2.5%. For the field spikes, estimates of percent relative standard deviation across the samples were between 6% and 7% for 3-

PBA, *cis*-DCCA, and *trans*-DCCA. Precision across duplicate urine samples was evaluated using relative percent difference estimates at both the metabolite and sample levels. Not including zero values (where the initial and replicate measures were identical – generally a consequence of single value imputation for measurements <LOQ), median relative percent differences across duplicate sample measures were estimated to be 17% for 3-PBA (based on 129 samples), 26% for *cis*-DCCA (based on 64 samples), 18% for *trans*-DCCA (based on 80 samples), and 41% for MPA (based on 4 samples).

In addition to field QCs, laboratory QC samples (~15% of the samples tested) were used to evaluate the quality of each analytic run. A minimum of eight calibrants, 2 QC materials, 2 blank samples, and 1 replicate sample were analyzed concurrently with 30-40 unknown samples in each analytic run. QC samples had to be within 20% of the spiked concentration for the run to be considered valid. Replicate samples were not used to determine run validity but were rather used as a quality check. Replicate samples were generally within 20% of their respective duplicate.

2.9. Statistical analysis

All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA). For each pyrethroid metabolite, all urine data values below the LOQ were assigned the value of $LOQ/\sqrt{2}$ (Verbovsek, 2011**)**. Descriptive statistics including geometric mean, range, and selected percentiles (25^{th}, 50^{th}, 75^{th}, 95^{th}, and 99^{th}) were calculated for the concentrations of 3-PBA, *cis*-DCCA, and *trans*-DCCA by urine sample type (bedtime, FMV, 24-hr, and overall) as concentration (ng/mL), SG-corrected (ng/mL-SG), CR-corrected (ng/mg), and excretion rate (ng/min) values. These descriptive statistics were not calculated for MPA as it was detected in only 2% of the urine samples. SG-corrected values were computed using the following equation (Morgan et al., 2008): SG-corrected value (ng/mL-SG) = urine concentration (ng/mL) x $(SG_{target} - 1.000)/(SG_{urine\ sample} - 1.000)$, where SG_{target} was selected to be the median SG value (1.014) across all study measurements. SG values ranged from 1.0018–1.0372 ng/mL-SG. CR-corrected values were calculated using the following equation (Morgan et al., 2008): CR-corrected values (ng/mg) = 100 mL/dL x urine concentration (ng/mL)/CR concentration (mg/dL). CR concentration values ranged from 7.0 – 442 mg/dL. The metabolite excretion rate was calculated as follows: Excretion

rate (ng/min) = urine concentration (ng/mL) x volume of urine void (mL)/time since previous urine void (min). Urine void volumes ranged from 5 mL to 1000 mL (limited by container size of 1 L).

Pairwise associations between urinary 3-PBA, *cis*-DCCA, and *trans*-DCCA levels were evaluated using Spearman correlation coefficients. Since repeated measures from individuals are not independent, the results of these analyses were used only to screen for general trends between the urinary metabolites.

Urinary 3-PBA was the only frequently detected analyte (>50%), and therefore the only analyte on which parametric analyses were performed. Based on the Shapiro-Wilks normality test, the distribution of urinary 3-PBA was found to be log-normal, and therefore all urine values were log-transformed (ln) for further statistical analyses. For the frequently detected 3-PBA, one-way random effects models (Proc Mixed) were used to estimate within- and between-person variance components for ln-transformed 3-PBA. These estimates were then used to estimate ICCs and 95% fold-ranges ($R_{0.95}$) according to Rappaport and Kupper, 2008. The ICC represents the ratio of between-person variance to total variance. ICC values can range from 0 to 1; values nearer to 0 signify low reliability, and values nearer to 1 signify high reliability (Fleiss, 1985). ICCs values can be used to indicate poor reproducibility (<0.4), good reproducibility (0.4 to 0.75) or excellent reproducibility (> 0.75) of a spot urine measurement (Rosner, 2006). Traditionally, an ICC value of 0.80 or greater indicates that a random spot urine measurement would accurately represent the average biomarker value over a defined period of time (Fleiss, 1985). The between-person fold-range ($_{b}R_{0.95}$) represents the fold-range containing the middle 95% of individual-specific mean biomarker levels. The within-person fold-range ($_{w}R_{0.95}$) represents the fold-range containing the middle 95% of biomarker levels for any individual. ICC and fold-range estimates for ln(urinary 3-PBA levels) were calculated separately by urine sample type as concentration, SG-corrected, CR-corrected, and excretion rate measures corresponding to a day, week, and six-weeks. Lastly, we estimated the number of random spot urine voids per adult that would be required to provide a reliable biomarker estimate (ICC = 0.80) by sample type and method for each time period using the following equation (Fleiss, 1985): $m = (p_{r,m}(1 - p_r)) / (p_r(1 - p_{r,m}))$. In this equation, m is the number of random spot urine measurements per adult required to correctly rank individuals within

a cohort, $p_{r,m}$ is the specified reliability of the mean (ICC= 0.80), and p_r is the ICC value for each urine sample type and method.

3. Results

Table 1 presents the physical characteristics including gender, age, weight, height, and race of the 50 adult participants in the Ex-R study. There were a total of 30 females and 20 males, and their ages ranged from 19-50 years old. All of the participants completed the six-week monitoring period, except for one female who dropped out of the study due to a family emergency during the last sampling interval (days 4-6) of week six (Fig. 1.). Four additional participants were dropped from the study before completing the six-week monitoring period because they did not properly follow sampling procedures (e.g., missed collection of several solid food and/or urine samples).

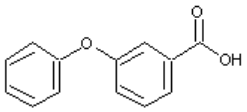
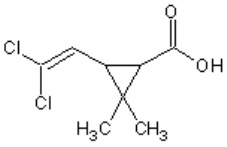
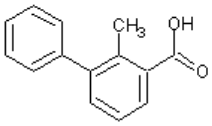
During sampling weeks 1, 2, and 6 a total of 12%, 14%, and 12% of the participants, respectively, reported that a product containing a pyrethroid was applied to kill insect pests at their residences. In addition, during non-sampling weeks, 10% of the participants reported that pyrethroid products had been used for insect control at their homes. Targeted insects included ants, cockroaches, crickets, fleas, mosquitoes, silverfish, spiders, and/or ticks. Pyrethroids contained in these applied products at the homes were allethrin, bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, cyphenothrin, deltamethrin, esfenvalerate, imiprothrin, metofluthrin, permethrin, phenothrin, prallethrin and tralomethrin. Based on this information, at least 70% of the applied pyrethroid insecticides at the participants' homes (within a month and during the six-week monitoring period) can be metabolized to form 3-PBA, *cis*-DCCA, *trans*-DCCA, and/or MPA in the body (Fig. 2).

The participants collected on average 8 urine voids (range = 3–11) per sampling day. The pyrethroid metabolites, 3-PBA, *cis*-DCCA, *trans*-DCCA, and MPA were detected in 74%, 32%, 39%, and 2% of the urine samples, respectively. Table 2 provides the descriptive statistics for the urinary levels of 3-PBA, *cis*-DCCA, and *trans*-DCCA by urine sample type and method. In all of the urine samples at the 75^{th} and 95^{th} percentiles, urinary levels of 3-PBA were consistently higher compared to urinary levels of *cis*-DCCA and *trans*-DCCA for all methods. For 3-PBA (the only metabolite with measurements >LOQ at the 50^{th} percentile), median urinary levels were consistently the lowest in FMV samples

Table 1
Physical characteristics of the adult participants in the Ex-R study.

Characteristic	All	Females	Males
Sex (number)	50	30	20
Age (years)	33.4±9.1 (19-50)[a]	34.8±9.4 (21-50)	31.3±8.5 (19-48)
Weight (kilogram)	82.2±18.9 (48.1-130)	75.6±17.9 (48.1-130)	92.0±16.2 (57.6-130)
Height (cm)	170±8.5 (149-191)	165±5.8 (149-191)	178±5.3 (166-188)
Race[b]			
Non-Hispanic White	25 (56%)[c]	14 (31%)	11 (25%)
Non-Hispanic Black	11 (25%)	9 (20%)	2 (4%)
Hispanic	6 (13%)	3 (7%)	3 (7%)
Asian	2 (4%)	1 (2%)	1 (2%)
Native American	1 (2%)	0 (0%)	1 (2%)

[a] Mean ± standard deviation and range
[b] Race data were collected ~ 4 months after the study was completed. These data were collected from 45 out of 50 of the participants
[c] Number and percentage of total

Urinary Metabolite	**3-PBA**	***cis/trans*-DCCA**	**MPA**
Chemical Structure			
Corresponding Pyrethroid Insecticide			
Allethrin			
Bifenthrin			●
Cyfluthrin		●	
Cyhalothrin	●		
Cypermethrin	●	●	
Cyphenothrin	●		
Deltamethrin	●		
Esfenvalerate	●		
Imiprothrin			
Metofluthrin			
Permethrin	●	●	
Phenothrin	●		
Prallethrin			
Tralomethrin	●		

[a] 3-phenoxybenzoic acid (3-PBA), *cis/trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*cis*-DCCA), and 2-methyl-3-phenylbenzoic acid (MPA)

Fig. 2. Urinary metabolites measured in Ex-R adults and their corresponding parent pyrethroid(s) used at study homes one month before and during the six-week monitoring period [a]

Table 2
Urinary 3-PBA, *cis*-DCCA, and *trans*-DCCA levels in 50 Ex-R adults over a six-week monitoring period[ab]

Type of Urine Sample	N[c]	%[d]	Unit[e]	GM[h]	GSD[i]	Percentiles					Max.
						25th	50th	75th	95th	99th	
Bedtime											
3-PBA	291	77	ng/mL	0.96	4.02	0.35	0.82	2.15	16.3	37.6	101.9
	288		ng/mL-SG[f]	0.88	4.33	0.31	0.81	2.38	14.9	31.2	43.9
	288		ng/mg[f]	0.94	4.67	0.29	0.80	2.52	17.2	33.3	50.6
	285		ng/min[g]	0.90	5.13	0.25	0.83	2.87	19.3	46.4	46.8
cis-DCCA	291	27	ng/mL	----[j]	----	<	<	0.38	3.25	11.4	17.0
	288		ng/ml-SG	----	----	<	<	0.50	2.34	9.00	14.9
	288		ng/mg	----	----	<	<	0.59	3.51	8.85	17.0
	285		ng/min	----	----	<	<	0.59	3.47	13.7	22.8
trans-DCCA	290	33	ng/mL	----	----	<	<	0.74	5.90	26.7	36.6
	287		ng/ml-SG	----	----	<	<	0.67	4.87	22.4	27.4
	287		ng/mg	----	----	<	<	0.83	6.69	27.7	36.4
	284		ng/min	----	----	<	<	0.84	7.33	28.9	31.9
FMV											
3-PBA	296	70	ng/mL	0.83	3.99	<	0.77	2.34	7.54	92.3	180.6
	295		ng/mL-SG	0.76	4.13	<	0.68	2.16	8.35	56.1	115.1
	295		ng/mg	0.74	4.29	<	0.68	2.05	9.78	45.6	116.4
	293		ng/min	0.62	4.25	<	0.58	1.64	7.69	54.8	134.6
cis-DCCA	298	32	ng/mL	<	<	<	<	0.50	2.56	15.3	39.4
	297		ng/mL-SG	<	<	<	<	0.45	2.23	13.8	24.0
	297		ng/mg	<	<	<	<	0.46	2.71	13.5	19.5
	295		ng/min	<	<	<	<	0.37	2.09	12.4	23.4
trans-DCCA	297	38	ng/mL	<	<	<	<	0.99	5.62	17.8	67.1
	296		ng/mL-SG	<	<	<	<	0.84	4.71	14.9	40.8
	296		ng/mg	<	<	<	<	0.86	4.54	14.6	40.2
	294		ng/min	<	<	<	<	0.62	4.24	15.3	39.8
24-hr											
3-PBA	1877	74	ng/mL	0.98	4.29	<	0.92	2.39	12.3	64.7	3154.9
	1856		ng/mL-SG	1.17	4.80	<	1.06	3.24	18.7	80.4	1983.9
	1856		ng/mg	1.30	5.28	<	1.18	3.75	24.3	115.5	1784.5
	1864		ng/min	1.32	5.38	<	1.19	3.95	23.7	106.1	2035.4
cis-DCCA	1888	33	ng/mL	<	<	<	<	0.54	3.28	18.2	702.5
	1866		ng/mL-SG	<	<	<	<	0.71	4.45	25.0	441.8
	1866		ng/mg	<	<	<	<	0.90	5.62	30.3	397.3
	1875		ng/min	<	<	<	<	0.95	5.33	35.0	453.2
trans-DCCA	1887	41	ng/mL	<	<	<	<	1.03	7.54	42.3	197.7
	1865		ng/mL-SG	<	<	<	<	1.20	9.73	39.6	291.4
	1865		ng/mg	<	<	<	<	1.40	12.3	48.2	516.1
	1874		ng/min	<	<	<	<	1.53	12.3	62.5	563.8
All											
3-PBA	2472	74	ng/mL	0.96	4.22	<	0.88	2.33	12.2	56.4	3154.9
	2446		ng/mL-SG	1.07	4.70	<	0.96	2.88	16.5	72.6	1983.9
	2446		ng/mg	1.17	5.13	<	1.04	3.30	20.6	93.2	1784.5
	2449		ng/min	1.15	5.31	<	1.04	3.47	21.3	93.9	2035.4
cis-DCCA	2485	32	ng/mL	<	<	<	<	0.50	3.16	16.9	702.5
	2458		ng/mL-SG	<	<	<	<	0.64	3.84	20.2	441.8
	2458		ng/mg	<	<	<	<	0.81	4.85	24.9	397.3
	2462		ng/min	<	<	<	<	0.84	4.61	30.7	453.2
trans-DCCA	2482	39	ng/mL	<	<	<	<	0.99	7.15	32.9	197.7

2455	ng/mL-SG	<	<	<	<	1.08	8.66	35.4	291.4
2455	ng/mg	<	<	<	<	1.19	10.3	43.2	516.1
2459	ng/min	<	<	<	<	1.29	9.64	52.3	563.8

[a] 3-Phenoxybenzoic acid (3-PBA), *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*cis*-DCCA), and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (*trans*-DCCA).
[b] 2-methyl-3-phenylbenzoic acid (MPA) values are not reported because it was detected in 2% of the urine samples.
[c] Number of urine samples (note: not all 2503 samples could be analyzed due to limited sample volume)
[d] Percentage of urine samples at or above the limit of quantitation (LOQ)
[e] Data are provided as concentration (ng/mL), specific gravity-corrected (ng/mL-SG), creatinine-corrected (ng/mg), and excretion rate (ng/min) values
[f] 26 urine samples were excluded because of missing creatinine and specific gravity data due to laboratory error
[g] 17 urine samples were excluded due to missing or partial voids provided by the adult participants
[h] Geometric mean
[i] Geometric standard deviation (unitless)
[j] The geometric mean and geometric standard deviation are not reported for pyrethroid metabolites that were detected in less than 50% of the urine samples.
[k] Below the LOQ (<0.25 ng/mL) for a pyrethroid metabolite in the urine samples.

(0.77 ng/mL, 0.68 ng/mL-SG, 0.68 ng/mg, and 0.58 ng/min) and the highest in 24-hr samples (0.92 ng/mL, 1.06 ng/mL-SG, 1.18 ng/mg, and 1.19 ng/min) across all four methods.

Based on Spearman correlation coefficients, *cis*-DCCA and *trans*-DCCA levels (ng/mL) were highly correlated ($r = 0.86$, $p<0.0001$) with each other in the adult urine samples. In addition, levels of *cis*-DCCA ($r = 0.64$, $p<0.0001$) and *trans*-DCCA ($r = 0.69$, $p<0.0001$) were strongly correlated with 3-PBA levels.

Table 3 presents ICC and fold-range estimates for repeated 3-PBA measurements by urine sample type and method for the Ex-R adults over a day, week, and six weeks. ICCs indicated poor reproducibility (< 0.22) for all urine sample types and methods over each time period. In general, ICCs for SG-corrected, CR-corrected, and excretion rate values were only slightly higher than those based on unadjusted concentration values (suggesting a larger proportion of between-person variance to total variance). All of the ICCs indicated a low level of reliability of repeated 3-PBA measurements in spot urine samples for each method over a day, week, or six weeks. The number of random spot urine samples required to provide a reliable 3-PBA biomarker estimate for an individual ranged from 15 to 800 samples depending on the sample type and method used. CR-corrected FMV urine samples had the lowest number of urine samples (n=15) needed to provide a reliable 3-PBA biomarker estimate (over a week). Regardless of the sample type and method, an unreasonably high number of spot urine samples would be required to provide a reliable estimate of the average 3-PBA concentration for the Ex-R participants over a day or longer.

Table 3

Variance components and other related statistics for 3-PBA levels in adults by urine sample type and method over a day, week, and six-weeks[a]

Urine sample type	ng/mL				ng/mL-SG				ng/mg				ng/min			
	${}_{b}R_{0.95}$ [b]	${}_{w}R_{0.95}$ [c]	ICC[d]	m[e]	${}_{b}R_{0.95}$	${}_{w}R_{0.95}$	ICC	m	${}_{b}R_{0.95}$	${}_{w}R_{0.95}$	ICC	m	${}_{b}R_{0.95}$	${}_{w}R_{0.95}$	ICC	m
One Day																
24-hr[f]	2.1	182	0.02	204	4.9	243	0.08	47	9.6	325	0.13	26	7.9	341	0.11	32
One Week																
Bedtime	1.0	360	0.00	---[h]	1.0	441	0.00	---	2.8	500	0.03	144	6.8	514	0.09	43
FMV[g]	4.0	210	0.06	60	9.3	175	0.16	22	13.9	163	0.21	15	10.6	220	0.16	21
24-hr	3.7	214	0.06	66	6.1	306	0.09	40	9.8	393	0.13	27	8.4	449	0.11	33
Six Weeks																
Bedtime	1.5	232	0.01	800	3.6	271	0.05	77	5.9	322	0.09	42	6.5	460	0.09	43
FMV	3.0	204	0.04	95	5.9	196	0.10	35	7.1	212	0.12	30	5.2	228	0.08	43
24-hr	2.7	278	0.03	128	7.6	333	0.11	33	12.5	412	0.15	23	9.6	490	0.12	30

[a] Urine data (log-transformed) are provided as concentration (ng/mL), specific gravity-corrected (ng/mL-SG), creatinine-corrected (ng/mg), and excretion rate (ng/min) values.
[b] Between-person fold range
[c] Within-person fold range
[d] Intraclass correlation coefficient
[e] Number of random spot urine samples per adult likely needed to have a reliable biomarker estimate (ICC = 0.80) (Fleiss, 1985).
[f] Results are limited to the first 24-hr interval of week one as the completion rates were the highest for this interval (88%) compared to all intervals (6 total).
[g] First morning void
[h] The between-person variance was zero resulting in an ICC of 0.00, therefore, no sample size could be estimated.

Between- and within-person fold-range estimates in Table 3 support ICC estimates and further indicate that 3-PBA measurements were far more variable within individuals than between individuals. In addition to producing the smallest ICC estimates, 3-PBA concentration values also produced the smallest between- and within-person fold-ranges. Results for 3-PBA concentrations suggest that the central 95% of individual-specific mean levels were observed across a 1 to 4–fold range, whereas the central 95% of individual measures for a given person were observed across a 182 to 360-fold range, depending on sample type and evaluation period. In contrast to the concentration values, 3-PBA excretion rate values had the highest within-person fold ranges, and the second highest between-person fold ranges. The highest between-person fold ranges were observed for CR-corrected values. As shown in Table 3, the central 95% of mean CR-corrected levels were observed across a 2.8 to 13.9-fold range, and the central 95% of individual excretion rate measures for a given person were observed across a 220 to 514–fold range. These results in Table 3 indicate that correcting for urine sample dilution, based on either SG, CR, or urine output, actually introduced measurement variability, both between- and within-individuals, when evaluating urinary 3-PBA levels.

4. Discussion

The Ex-R study was designed to be a participatory-based, exposure measurements study that investigated the longitudinal exposures of 50 adults to pyrethroid insecticides and to their preformed metabolites in media at their homes in NC in 2009-2011. We found that participatory-based sampling was quite an effective way to engage and actively involve the adult volunteers in the Ex-R study. The 50 participants filled out a total of 299 (99%) food, 299 (99%) activity, and 149 (99%) pesticide-use diaries. They also collected a total of 783 (99%) solid food, 50 (100%) drinking water, 149 (99%) surface wipe, 48 (96%) vacuum dust, and 2502 (98%) urine samples during the study. The 100% and 98% completeness for the recruitment of 50 participants and field samples/diaries surpassed our study completeness goals of $\geq$85% and $\geq$95%, respectively. In addition, despite the subject burden (~10-hr per person a week), participatory-based sampling resulted in few problems in the field. The most frequently encountered problems by study participants included malfunctioning temperature data loggers due to low batteries, moisture occurring in the

temperature data loggers due to cooler condensation, loose electric power adaptor/cooler connections, broken cooler adaptors, and broken cooler zippers. We quickly corrected the temperature data logger problems by purchasing a new model (EL-USB-1) that had a longer battery life and by placing the new loggers into 10 mL cryogenic vials with lids to eliminate condensation when inside the coolers. The loose power adaptor problem was easily fixed by using an inexpensive ($5) Velco strap (Speedtech International Inc., Racine, Wisconsin) that snuggly connected the power adaptor with the cooler plug.

We believe this is the first study in the literature to report the urinary levels of MPA, the specific metabolite of bifenthrin, in non-occupationally exposed adults in the US. Bifenthrin is currently registered by the US EPA for use in both agricultural and residential settings (NPIC, 2011). In our study, conducted in 2009–2011, MPA was detected in less than 3% of the Ex-R adult urine samples (maximum=8.9 ng/mL). This information suggests that the majority of the participants were probably not exposed to measureable levels of bifenthrin residues in food or residential media during the six-week monitoring period in NC. These results are supported by a recent study (Tao et al., 2013) showing that MPA was not detected (<0.04 ng/mL) in the spot urine samples of 10 US children, ages 3-11 years old, in Massachusetts, USA.

Our biomonitoring results, however, did show that 3-PBA was frequently detected (74%) in the Ex-R adult urine samples. This is in agreement with other published US studies that have also found 3-PBA detected often (≥55%) in the urine of non-occupationally exposed adults (Berkowitz et al., 2003; Barr et al., 2010; McKelvey et al., 2013; Young et al., 2013; Trunnelle et al., 2014; Morgan, 2015). Interestingly, the geometric mean 3-PBA levels (0.96 ng/mL) in Ex-R participants were twice as high compared to the geometric mean 3-PBA levels (0.42 ng/mL) in 2009-2010 NHANES adults (CDC, 2015). Trunnelle et al. (2014) also recently reported two times higher median urinary 3-PBA concentrations (0.82 ng/mL) in 90 Californian adults in 2007-2009 in comparison to the 2009–2010 NHANES adults (0.39 ng/mL). This information suggests that there is probably geographical differences in US adult exposures to pyrethroid insecticides that are metabolized to form 3-PBA in the body. Studies conducted outside the contiguous US have also reported 3-PBA being frequently detected (≥70%) in the urine of the general adult population, except for one in Puerto Rico (46%) (Table 4). In these

Table 4
Comparison of urinary 3-PBA levels in non-occupationally exposed adults worldwide.

Country	Study Year	N[a]	Age	%[b]	GM[c]	95[th d]	Reference
ng/mL							
Canada	2005	120	18–64	99	----[e]	4.23	Fortin et al., 2008
Caribbean[f]	2008-2011	297	20–39	100	0.54	3.51	Dewailly et al., 2014
China	2009-2010	1149	17–45	99	0.97	5.39	Qi et al., 2012
Germany	2000[g]	46	17–61	70	----	0.67	Schettgen et al., 2002
Japan	2005	448	39–85	98	0.29	1.96	Ueyama et al., 2009
Poland	2010-2011	132	5–77[g]	80	0.26	1.15	Wielgomas et al., 2013b
Puerto Rico	2010-2012	54	18–40	46	0.20	2.30	Lewis et al., 2014
USA (NHANES)[h]	2007-2008	1110	20–59	--	0.43	6.65	CDC, 2015
	2009-2010	1296	20–59	--	0.42	6.95	CDC, 2015
This study	2009-2011	50	19–50	74	0.96	12.2	----
ng/mg							
China	2009-2010	1149	17–45	99	1.53	8.11	Qi et al., 2012
Japan	2005	448	39–85	98	0.40	2.33	Ueyama et al., 2009
Poland	2010-2011	132	5–77	80	0.22	0.95	Wielgomas et al., 2013b
USA (NHANES)	2007-2008	1110	20–59	--	0.44	6.29	CDC, 2015
	2009-2010	1296	20–59	--	0.42	4.72	CDC, 2015
This study	2009-2011	50	19–50	74	1.17	20.6	----

[a] Number of study participants
[b] Percentage of urine samples with detectable levels of 3-PBA
[c] Geometric mean
[d] Percentile
[e] Not provided
[f] Includes ten different countries
[g] Eight of the participants were children below the age of 14 years old
[h] NHANES adults between 20-59 years old, only

available studies, geometric mean urinary 3-PBA concentrations were the lowest for Puerto Rico, Poland, and Japan (<0.30 ng/mL) and the highest for China (0.97 ng/mL). Our study results (GM=0.96 ng/mL) were similar to the reported geometric mean 3-PBA levels of 0.97 ng/mL reported in 1149 women from China in 2009-2010 (Qi et al., 2012). This likely reflects the increased usage of pyrethroid insecticides in residential settings and some agricultural settings in the US and China (Barr et al., 2010; Qi et al., 2012).

We found that urinary *cis*-DCCA and *trans*-DCCA levels were highly correlated with each other ($r = 0.86$, $p<0.0001$) and with urinary 3-PBA levels (*cis*-DCCA: $r = 0.64$, $p<0.0001$; *trans*-DCCA: $r = 0.69$, $p<0.0001$) in the Ex-R adult urine samples. Similar results were observed by Barr et al. (2010) showing strong correlations occurring between the concentrations of these two isomers ($r= 0.89$, $p<0.001$) and 3-PBA ($r=0.77$, $p=0.02$) in the urine samples of 1999–2002 NHANES adults. Only the current-use pyrethroids, permethrin and cypermethrin, are known to breakdown to form both 3-PBA and DCCA in the body (Tao et al., 2013; CDC, 2009). The strong correlations occurring between these

urinary biomarkers suggest that the Ex-R adults were likely primarily exposed to these two pyrethroids in their residential environments (Barr et al., 2010). This is in agreement with recent research by Xue et al. (2014) indicating that permethrin and cypermethrin likely contributed to about 80% of the observed urinary levels of 3-PBA and DCCA in the general US population.

Our ICC results indicate poor reproducibility (<0.22) of repeated 3-PBA measurements in the spot urine samples of the Ex-R adults regardless of the sample type (bedtime, FMV, or 24-hr) and method (non-corrected or corrected) over a day, week, and six weeks. This ICC information suggests that a random spot urine sample did not provide a reliable estimate of the average 3-PBA concentration for the adults in this study. In addition, we found regardless of sample type or method used an unreasonably high number of random spot urine samples (15-800) would likely be required per person to provide a reliable biomarker estimate over a day or longer--which is unrealistic for most exposure and epidemiological studies due to budget constraints and participant burden. Our results also imply that the Ex-R adults were likely not continually exposed to low levels of pyrethroid residues in consumed foods and/or other residential media (i.e., dust and surfaces) over the six-week monitoring period. Our study results are in contrast to Wielgomas (2013a) that reported much higher ICCs for 3-PBA measurements in FMV, spot, and 24-hr urine samples by concentration, CR-corrected, and excretion rate values for seven non-occupationally exposed adults over a week in an urban area of Poland in 2011. In that study, the highest ICCs occurred in the CR-corrected spot (ICC=0.846) and the CR-corrected 24-hr urine samples (ICC=0.796). Based on these ICCs, the authors concluded that a random spot urine sample would adequately represent the average 3-PBA biomarker concentration for an individual over a week, and that the Polish adults were likely continually exposed to similar levels of pyrethroid residues in their urban environments. This above research suggests that the temporal usage patterns and/or exposure patterns of adults to pyrethroids may be quite different in Poland compared to the US and more research is needed worldwide.

In our current study, we found that comparisons of biomarker measurement variance and ICC estimates across concentration, SG-corrected, CR-corrected, and excretion rate measures yielded unexpected results. It is generally assumed that, for urinary biomarkers

eliminated via renal filtration, measurement error will decrease after correcting for SG, CR, or urine output. However, the opposite trend was observed in the Ex-R study. Specifically, measurement variability was the lowest both between- and within-individuals for concentration-based measures. Correcting for CR, SG, and urine output increased fold-range estimates in most instances. Furthermore, these correction approaches increased between-person measurement variability more so than within-person measurement variability, leading to elevated ICC estimates for corrected values. While higher ICCs are beneficial from the standpoint of measurement reliability, they are meant to reflect true exposure differences between individuals. In this study, it is conceivable that the observed variability between subjects in urine output, CR excretion, and SG levels, generally resulting from differences in hydration status, diet, body mass, etc., are responsible for the heightened ICCs. Given these sources of measurement variation, (reflecting physiological and behavioral factors and not pyrethroid exposure) researchers are urged to use caution when selecting urinary biomarker-based exposure metrics for 3-PBA as part of exposure and epidemiology studies in the future.

At the moment, it is unclear whether preformed metabolites of pyrethroid insecticides (e.g., 3-PBA, *cis*-DCCA, and *trans*-DCCA) in media are substantially contributing to the measured urinary pesticide metabolite levels in humans. Few studies have measured for the concurrent levels of pyrethroids and their preformed metabolites in foods or other environmental media (Starr et al., 2008; Chen et al., 2012; Trunnelle et al., 2014). Starr et al. (2008) showed that permethrin, DCCA, and 3-PBA, were frequently detected ($\geq$67%) in 85 vacuum dust samples collected at homes and childcare centers in NC and Ohio in 2000–2001. Chen et al. (2012) also reported detectable levels of several different pyrethroids and 3-PBA in 23 different produce samples including blackberries, blueberries, cherries, strawberries, and green onions in California in 2010. More recently, Trunnelle et al. (2014) reported that permethrin and 3-PBA were both frequently detected (>97%) in 79 kitchen floor wipe samples collected at homes in California in 2007–2009. This above research suggests that humans can be exposed to measureable levels of preformed pyrethroid metabolites in several different media (i.e., food, dust, and surface wipes) at residences. Therefore, more research is necessary to determine if preformed metabolites in media are overinflating measured urinary pyrethroid biomarker levels in non-

occupationally exposed adults. Future work is planned to quantify the distributions of the target pyrethroids and their preformed metabolites in solid food, drinking water, vacuum dust, and surface wipe samples collected at the participant's residences in the Ex-R study.

5. Conclusions

Based on the urinary biomonitoring data, the majority of the Ex-R adults were likely temporally exposed to one or more pyrethroid insecticides at their homes during the six-week monitoring period in NC in 2009–2011. For the frequently detected 3-PBA (>50%), there was poor reproducibility of repeated measurements of this metabolite in the urine samples of Ex-R adults regardless of sample type (bedtime, FMV, and 24-hr) and method (concentration, SG-corrected, CR-corrected, and excretion rate) over a day, week, and six weeks. Correcting for urine sample dilution, based on either SG, CR, or urine output, was found to increase measurement variability between- and within-individuals. Given these findings, scientists should use caution when selecting urinary biomarker-based exposure metrics for 3-PBA in their future exposure and environmental epidemiology studies.

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Supplemental Information

The adult participant's collected their own solid food, drinking water, surface wipes, and vacuum dust samples at their homes over the six-week monitoring period. Duplicate amounts of solid foods were collected by the participants on days 1-2 of each sampling week. Liquid food samples were not collected as several pyrethroid insecticides were detected at low levels in beverages (i.e., juices, sodas, and milk) in a previous study (Morgan et al., 2007). For each sampling day, solid food samples were collected during three consecutive time periods (*as described above*). Food samples for each time period were placed into a pre-labeled 31 x 31 cm re-sealable polyethylene bag (Uline Shipping Supply Specialists) and then into a larger 41 x 41 cm re-sealable polyethylene bag for secondary containment.

Drinking water samples (> 500 mL) were collected in a 1 L polypropylene container on the morning of day 3 of the last sampling week at home from the participant's primary source of drinking water (e.g., well or municipal). The source of drinking water (well-unfiltered, well-filtered, city-unfiltered, city-filtered, or bottled) was recorded on the sample lid.

Surface wipe samples were collected in two high traffic areas (e.g., sink, stove, or refrigerator) of the kitchen on day 4 of each sampling week. A 100% pre-cleaned cotton pad (Twillwipes 100 cm^2 - 1 ply) wetted with 10 mL of isopropanol was used to wipe (left to right) a 960 cm^2 area (using an aluminum template) of the kitchen floor. The cotton pad then was folded in half (soiled side in) and the entire surface area was wiped (left to right) again. The pad was then placed into a pre-cleaned 60 mL amber glass jar with lid. The participant repeated the same procedure in another high traffic area of their kitchen floor.

Dust samples were collected from carpets and/or hard floors (i.e., hardwood, tile, and vinyl) of the main activity areas of the participants' homes using their own vacuum cleaners on day 4 of the last sampling week. Before sampling, participants with bag-style vacuum cleaners removed their existing bag and replaced it with a new identical one. Individuals with canister-style vacuum cleaners removed their existing filter and replaced it with a new identical one and removed the existing dust sweepings. After vacuuming the main activity areas, the bag or the filter/contents of the canister (> 2 cups) were placed into a 31 x 38 cm re-sealable, polyethylene bag.

All environmental samples were stored in $\leq$ -20^{o}C freezers at the US EPA laboratory until analyses.