Predictors of urinary levels of 2,4-dichlorophenoxyacetic acid, 3,5,6-trichloro-2-pyridinol, 3-phenoxybenzoic acid, and pentachlorophenol in 121 adults in Ohio

Marsha K. Morgan*

United States Environmental Protection Agency, National Exposure Research Laboratory, 109 T.W. Alexander Drive, Research Triangle Park North Carolina, 27713, USA

ABSTRACT

Limited data exist on the driving factors that influence the non-occupational exposures of adults to pesticides using urinary biomonitoring. In this work, the objectives were to quantify the urinary levels of 2,4-dichlorophenoxyacetic acid (2,4-D), 3,5,6-trichloro-2-pyridinol (TCP), 3-phenoxybenzoic acid (3-PBA), and pentachlorophenol (PCP) in 121 adults over a 48-hour monitoring period and to examine the associations between selected sociodemographic and lifestyle factors and urinary levels of each pesticide biomarker. Adults, ages 20-49 years old, were recruited from six counties in Ohio (OH) in 2001. The participants collected 4-6 spot urine samples and completed questionnaires and diaries at home over a 48-hour monitoring period. Urine samples were analyzed for 2,4-D, TCP, 3-PBA, and PCP by gas chromatography/mass spectrometry. Multiple regression modeling was used to determine the impact of selected sociodemographic and lifestyle factors on the log-transformed (ln) levels of each pesticide biomarker in adults. The pesticide biomarkers were detected in $\geq$ 89% of the urine samples, except for 3-PBA (66%). Median urinary levels of 2,4-D, TCP, 3-PBA, and PCP were 0.7, 3.4, 0.3, and 0.5 ng/mL, respectively. Results showed that 48-hour sweet/salty snack consumption, 48-hour time spend outside at home, and ln(creatinine) levels were significant predictors ($p<0.05$), and race was a marginally significant predictor ($p=0.093$) of the adults' ln(urinary 2,4-D) concentrations. Strong predictors ($p<0.05$) of the adults' ln(urinary TCP) concentrations were urbanicity, employment status, sampling season, and ln(creatinine) levels. For 3-PBA, sampling season, pet ownership and removal of shoes before entering the home were significant predictors ($p<0.05$) of the adults' ln(urinary 3-PBA) levels. Finally for PCP, removal of shoes before entering the home and ln(creatinine) levels were significant predictors ($p<0.05$), and pet ownership was a marginally significant predictor ($p=0.056$) of the adults' ln(urinary PCP) concentrations. In conclusion, specific sociodemographic and lifestyle factors were identified that increased the exposures of these adults to several different pesticides in their daily environments.

Keywords: Biomarkers, urine, pesticides, adult caregivers, exposure, determinants

Corresponding author. Tel.: +1-919-541-2598
E-mail address: morgan.marsha@epa.gov (M.K. Morgan)

Introduction

Pesticides are used to control a variety of insects, weeds, and fungi in residential and agricultural settings worldwide. In the United States (US), it is estimated that approximately 750 million pounds of pesticides are applied in these settings each year (US EPA, 2013a). About ~10% and ~90% of these pesticides are used in residential and agricultural environments, respectively (US EPA, 2013a). A number of these pesticides (i.e., acid herbicides, organophosphates, pyrethroids, and organochlorines) have been detected in food and other media including air, dust, soil, and/or wipes collected at US residences (Colt et al., 2004; Stout et al., 2009; Wilson et al., 2010; Morgan et al., 2014, Trunnelle et al., 2014). In addition, studies across the US have reported measureable concentrations of several different pesticide biomarkers including 2,4-dichlorophenoxyacetic acid (2,4-D), 3,5,6-trichloro-2-pyridinol (TCP), 3-phenoxybenzoic acid (3-PBA), and pentachlorophenol (PCP) in the urine of the general adult population (Berkowitz et al., 2003; Barr et al., 2005a; Meeker et al., 2005; Morgan et al., 2008a; CDC, 2009; Barr et al., 2010; McKelvey et al., 2013; Trunnelle et al., 2014) (Fig. 1).

2,4-D is a urinary biomarker of the acid herbicide 2,4-D which is extensively used to kill broadleaf weeds on home lawns and agricultural fields (US EPA, 2005). TCP is a urinary metabolite of the organophosphorus insecticides, chlorpyrifos and chlorpyrifos-methyl (CDC, 2009). Chlorpyrifos is commonly used to control insects on food and non-food crops, but was often applied at residences until 2001 (US EPA, 2014). Chlorpyrifos-methyl is primarily sprayed on stored grains or into empty grain bins to eliminate damaging insects (e.g., beetles, weevils, and moths) (US EPA, 2000a). 3-PBA is a urinary metabolite of at least 10 different pyrethroid insecticides (e.g., permethrin, cypermethrin, and cyfluthrin) (Olsson et al., 2004). The pyrethroid insecticides are frequently used to control insects in and around homes, on pets, and on agricultural crops (US EPA, 2013b). Lastly, PCP is a urinary biomarker of the phenol, PCP, and the organochlorine hexachlorobenzene (CDC, 2009). PCP was a widely used herbicide, insecticide, fungicide, and microbial agent in residential and agricultural settings until the 1980's (USEPA, 2008), and is now only used as a wood preservative in limited applications (i.e., telephone poles) (Fisher, 1991; USEPA, 2008). Hexachlorobenzene was primarily applied as a fungicide on grain seeds until the mid-1980's, and is no longer commercially available (US EPA, 2000b).

Despite evidence of widespread exposure among the US general population to these above

pesticides, limited data are available on the important sociodemographic and lifestyle factors that influence the urinary levels of TCP and 3-PBA in adults, and no data exist for 2,4-D and PCP in non-occupational settings (Berkowitz et al., 2003; Egeghy et al., 2005; Meeker et al., 2005; Reiderer et al., 2008; McKelvey et al., 2013). Egeghy et al. (2005) showed that education level was a significant predictor ($p=0.0006$) of urinary TCP concentrations in 80 individuals in Baltimore, Maryland in 1995-1996. In another study, Meeker et al. (2005) showed that urinary TCP concentrations were significantly associated ($p<0.05$) with sampling season and 24-hour consumption of cheese and grapes in 10 adult males in the New England area in 2001-2003. In addition, Berkowitz et al. (2003) reported that education level was a significant predictor ($p<0.05$) of urinary TCP concentrations in 386 pregnant women in New York City (NYC), New York, in 1998-2001. The authors also showed that sampling season, marital status, education level, and residential ownership were significantly associated ($p<0.05$) with the women's urinary 3-PBA levels. In a much larger study, Reiderer et al. (2000) showed that employment status and dietary consumption of bacon, spinach, broccoli, lettuce, salty snacks, biscuits, salsa, rice, peanut butter, and orange juice were all strong predictors ($p<0.05$) of urinary 3-PBA concentrations in 1087 adults in the 1999-2002 NHANES. More recently, McKelvey et al. (2013) showed that sex, race, and weekly consumption of green vegetables were significantly associated ($p<0.05$) with the urinary 3-PBA concentrations in 1452 adults in NYC, New York in 2004. These above studies provide evidence that certain sociodemographic and lifestyle factors likely increase the exposures of adults to some pesticides in their daily environments. As data are limited, more research is necessary to understand the driving factors that impact adult exposures to pesticides in non-occupational settings.

In previous publications from the Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) study, we examined the influence of selected sociodemographic and/or lifestyle factors on the urinary levels of 2,4-D, TCP, 3-PBA, or PCP in a large set of preschool children in North Carolina (NC) and/or Ohio (OH) (Morgan and Jones, 2013; Morgan et al., 2015). However for the CTEPP adult caregivers (mostly parents), no published data are available on the sociodemographic and lifestyle factors that may substantially impact their pesticide biomarkers levels in urine. Therefore for this current work, the objectives were to quantify the urinary levels of 2,4-D, TCP, 3-PBA, and PCP levels in 121 CTEPP adults in OH over a 48-hour monitoring period and to examine associations between selected sociodemographic and lifestyle factors and urinary levels of each biomarker.

Materials and methods

Study cohort

In the CTEPP study, we originally examined the aggregate exposures of 256 preschool children, ages 2-5 years old, and their primary adult caregivers to over 40 chemicals commonly found in their everyday surroundings (Morgan et al., 2004). The primary adult caregiver was defined as either a parent or other adult that lived in the same household with the selected child (Wilson et al., 2004). A detailed description of the CTEPP study design and sampling methodology that was performed in NC and OH can be found in Wilson et al. (2004). Briefly in OH, the study cohort consisted of 127 preschool children and their 127 primary adult caregivers that were recruited from six counties including Cuyahoga, Defiance, Fayette, Franklin, Hamilton, and Licking. In this cohort, 69 adult caregivers stayed-at-home with their children during the day while 58 adult caregivers went-to-work and left their children at licensed daycare centers during the day. For the adults, field sampling activities occurred at their homes over a 48-hour monitoring period between April 2001 and November 2001. The adults collected their own spot urine samples and completed study questionnaires and diaries during the monitoring period.

In this current work, database records were examined for the subset of 127 adult caregivers that participated in the OH part of the CTEPP study. Six of these adults were excluded from this dataset because they had either missing urinary biomarker data or questionnaire/diary data. The final dataset consisted of a total of 121 adults (67 stay-at-home and 54 went-to-work). There were a total of 111 females and 10 males, and their ages ranged from 20-49 years old.

Human subjects protection

The study protocol and procedures to obtain informed consent from the adult participants were reviewed by and received approved from the EPA's Human Subjects Approving Official and the Battelle Centers for Public Health Research and Evaluation Institutional Review Board (study number - FG823319-02). The adults read and signed an informed consent document prior to participating in the CTEPP study. In addition, they were given new identification numbers in the publically, accessible study database http://www.epa.gov/heds/study_75973.html) to protect their privacy and personal information.

Collection of questionnaires and diaries

The CTEPP adults filled out several different types of questionnaires and diaries at home during the 48-hour monitoring period. The pre-monitoring and post-monitoring questionnaires were used to collect a variety of data about the participants including their personal information (i.e., age, gender, race, and education level), occupation, residential pesticide use, pet ownership, and household characteristics (e.g., age of home). The activity and food diaries were used to record data on the participants' normal activity patterns (i.e., time spent outside) and eating habits over the 48-hour monitoring period.

Collection of spot urine samples

The adult participants were trained by Battelle field technicians to collect their own spot urine samples at home over the 48-hour monitoring period. Adults that stay-at-home during the day collected a spot urine sample in the morning, after lunch, and before bedtime each sampling day (6 total). For adults that went to work during the day, they collected a spot urine sample in the morning and before bedtime each sampling day (4 total). The participants collected each spot urine sample by urinating into a provided 120 mL polypropylene jar and recapping it with a lid. The adults placed the spot urine samples into insulated coolers with blue ice at home until they were picked up by field technicians at the end of the monitoring period. Field technicians then transported the coolers containing the spot urine samples by motor vehicle to the Battelle analytical laboratory located in Columbus, OH. All urine samples were kept frozen (< -20^{o}C) in freezers at the laboratory until analysis.

Chemical analysis of spot urine samples

The analytical methods used for the preparation, extraction, and analysis of 2,4-D, TCP, 3-PBA, and PCP in the urine samples have been described previously in Morgan et al., 2004. Briefly in 2002, Battelle laboratory technicians removed a total of 606 spot urine samples from the freezers and thawed them to room temperature. The spot urine samples for each adult were then pooled into one sample, except for 15 participants that had a recent pesticide application at home (< 7 days) prior to field sampling. The spot urine samples (n=80) for these 15 participants were not pooled into one sample, but were each analyzed separately. For the analysis of 2,4-D, 3-

PBA, and PCP, a 10 mL aliquot of urine was pipetted from each sample into a centrifuge tube. Each aliquot was hydrolyzed with 500 µL of concentrated hydrochloric acid (HCL) and 1 mL of chlorobutane, and then heated at 80^{o}C in an oven for about one hour. Next, each extract was rinsed with 10 mL of a 20% sodium chloride (NaCl) solution followed by 10 mL of dichloromethane (three times). The extract was concentrated to 1 mL using a Kuderna-Danish evaporator and transferred into a vial. For the analysis of TCP, a 1 mL aliquot of urine from each sample was pipetted into a centrifuge tube. Each urine aliquot was hydrolyzed with 100 µL of concentrated HCL and heated at 80^{o}C in an oven for about one hour. Next, 1 mL of a 20% NaCl solution and 1 mL of chlorobutane was added to the extract and then it was vortexed for 10 minutes. Afterwards, 800 µL of each extract was pipetted into a new centrifuge tube, silylated with 100 µL of N-(tert-butyldimethylsilyl)-N-methyltrifluoro-acetamide, and finally it was transferred into a vial. The urine extracts were quantified for the levels of each target biomarker using a gas chromatograph/mass selective detector (Hewlett-Packard 6890/5973A, Agilent Technologies, Golden, CO, USA) equipped with an autosampler (Morgan et al., 2004). The estimated limit of quantification (LOQ) was 0.4 ng/mL for 2,4-D, 3-PBA, and PCP in urine, and 2.0 ng/mL for TCP in urine. The estimated limit of detection (LOD) for each pesticide biomarker was 0.2 ng/mL in urine, except for TCP (1.0 ng/mL).

In this study, creatinine levels were only measured in the pooled urine samples of the adult participants. At the laboratory, Battelle technicians pipetted an additional 10 mL aliquot of urine from each pooled sample into a 15 mL centrifuge tube. The urine aliquots were shipped in coolers with dry ice to the nearby Ohio State University Clinical Laboratory (Columbus, OH, USA). The Jaffe picric colorimetric method was used to quantify the levels of creatinine in each urine aliquot (Morgan et al., 2004).

Quality assurance procedures

Quality control samples consisting of field blanks, laboratory blanks, matrix spikes, field duplicates, and analytical duplicates were used to determine the overall quality of collection and analysis of the urine samples. All field and laboratory blanks were below the LOD for each pesticide biomarker in urine. Relative percent differences between duplicates samples (field and analytical) were less than 12% for all pesticide biomarkers in urine, except one duplicate field sample for TCP (22%). All matrix spike recoveries ranged from 83-132% after excluding one sample for 3-PBA that had a low percent recovery (< 50%) due to a sample matrix effect.

Statistical analysis of data

Descriptive statistics (JMP version 11.1.1, SAS, Cary, NC, USA) including arithmetic mean, range, and selected percentiles (25^{th}, 50^{th}, 75^{th}, and 95^{th}) were calculated for the levels of 2,4-D, TCP, 3-PBA, and PCP in the urine of the adults as unadjusted (ng/mL) and creatinine-adjusted (ng/mg) values. All pesticide biomarker values below the LOD in urine were assigned the value of $LOD/\sqrt{2}$ (Verbovsek, 2011). For the 15 adults that had a recent pesticide application at home, the mean urinary biomarker value of their spot urine samples was used in the descriptive statistics. Creatinine–adjusted values were computed for the pooled urine samples using the proceeding equation (Morgan et al., 2008a): Creatinine-adjusted value (ng/mg) = 100 mL/dL x urinary biomarker concentration (ng/mL)/creatinine level (mg/dL).

In GraphPad Prism 5.04 (GraphPad Software, San Diego, CA, USA), an unpaired t-test or analysis of variance (ANOVA) was used to assess the bivariate associations between selected sociodemographic and lifestyle factors and urinary levels of each pesticide biomarker (log-transformed). Sociodemographic and lifestyle factors were selected from the available data in the study questionnaires and diaries. Sociodemographic factors included age, race, body mass index, income status, urbanicity, employment status, education level, and sampling season. The sociodemographic factor, sex, was excluded in the analysis because of the small sample size (n=10) of the adult, male participants. For employment status, none of the participants reported working in positions (e.g., farmers) that may have occupationally-exposed them to pesticides. Lifestyle factors included 48-hour food frequency consumption (fruits, vegetables, meats, dairy, grains, and sweet/salty snacks), insecticide use, herbicide use, 48-hour time spent outside at home, age of home, removal of shoes before entering home, and pet ownership (dog or cat). In addition, the unadjusted urinary 2,4-D, TCP, 3-PBA, and PCP concentrations in adults were log-transformed (ln) because all the distributions were found to be non-normal using the Shapiro-Wilk normality test (GraphPad Prism Software, San Diego, CA, USA).

Multiple regression models were constructed separately to collectively examine the relationships between the ln 2,4-D, TCP, 3-PBA, or PCP levels in adults (dependent variable) and selected sociodemographic/lifestyle factors (independent variables) that had a p-value of $\leq$0.200 in the above bivariate analyses. In each model, creatinine levels were ln-transformed and included as an additional independent variable to adjust for variable dilutions in spot urine volumes (Barr et al., 2005b). The multiple regression analyses were performed using a step-wise,

backward elimination process using PROC GLM in SAS version 9.3.

Results

Descriptive statistics for the unadjusted (ng/mL) and creatinine-adjusted (ng/mg) concentrations of urinary 2,4-D, TCP, 3-PBA, and PCP in the CTEPP adult participants are provided in Table 1. The pesticide biomarkers were detected in ≥ 89% of the unadjusted urine samples, except for 3-PBA (66%). At least one pesticide biomarker was detected in each urine sample. Also, 55% of the adult urine samples had detectable levels of all four pesticide biomarkers. The results in Table 1 show that the median urinary TCP levels (3.4 ng/mL) were at least four times higher compared to median urinary levels of 2,4-D, 3-PBA, and PCP (≤0.7 ng/mL). The maximum values of urinary 2,4-D, TCP, 3-PBA, and PCP in these adults were 8.1, 30.5, 4.9, and 3.7 ng/mL, respectively. For the creatinine-adjusted urine samples, median urinary TCP concentrations (2.6 ng/mL) were also at least five times greater than median urinary 2,4-D, 3-PBA, and PCP concentrations (≤0.5 ng/mg).

Table 2 provides the bivariate associations between selected sociodemographic or lifestyle factors and ln urinary 2,4-D, TCP, 3-PBA, and PCP concentrations in the CTEPP adults. Urinary 2,4-D concentrations were significantly higher (p=0.025) in younger adults (GM=0.80 ng/mL) compared to older adults (GM=0.54 ng/mL). Urinary TCP levels were significantly greater (p=0.018) in participants residing in urban counties (GM=3.9 ng/mL) compared to rural counties (GM=2.4 ng/mL). The adults' urinary TCP concentrations were also significantly different (p=0.005) across the three sampling seasons (spring, summer, and fall), with the summer season having the highest biomarker levels (GM=4.3 ng/mL). For 3-PBA, the adults' urinary biomarker concentrations were statistically different (p=0.002) among the three sampling seasons, with the greatest biomarker levels occurring in the spring season (GM=0.51 ng/mL). For PCP, the adults' urinary PCP concentrations were also statistically different (p=0.012) across the three sampling seasons, with the lowest levels occurring in the spring season. In addition, urinary PCP levels were significantly higher (p=0.046) in participants consuming foods containing grains (i.e., breads, cereals, and pastas) < 4 times compared to ≥ 4 times during the 48-hour monitoring period.

Table 3 presents the results of the final reduced regression models of the sociodemographic and lifestyle factors influencing the ln urinary concentrations of 2,4-D, TCP, 3-PBA, or PCP in the CTEPP adults. The results showed that 48-hour sweet/salty snack consumption, 48-hour time

spent outside at home, and ln(creatinine) levels were significant predictors ($p<0.05$), and race was a marginally significant predictor ($p=0.093$) of the adults' ln(urinary 2,4-D) concentrations. In particular, ln(urinary 2,4-D) levels were significantly greater ($p=0.043$) in participants that consumed sweet/salty snacks $\geq$ 2 times compared to < 2 times during the 48-hour monitoring period. The adults also had significantly higher ($p=0.038$) ln(urinary 2,4-D) levels for those that spent < 3 hours compared to $\geq$ 3 hours outside at home during the monitoring period. In addition, ln(urinary 2,4-D) levels were marginally higher ($p=0.093$) in black participants compared white participants. For TCP, urbanicity, employment status, sampling season, and ln(creatinine) levels were all strong predictors ($p<0.05$) of the adults' ln(urinary TCP) concentrations. Specifically, ln(urinary TCP) levels were significantly higher ($p=0.032$) for participants living in urban counties compared to rural counties. Also, adults that were not employed had significantly higher ($p=0.018$) ln(urinary TCP) levels compared to those that were employed. However, this result may be biased by the sampling design as the adults that went-to-work during the day did not collect a mid-day spot urine sample. In addition, the adults' ln (urinary TCP) concentrations were also significantly different ($p=0.043$) across the three sampling seasons with the highest levels occurring in the summer season. For 3-PBA, sampling season was a highly significant predictor ($p<0.0001$) and pet ownership and removal of shoes before entering the home were significant predictors ($p<0.05$) of the adults' ln(urinary 3-PBA) levels. These three factors collectively explained 25% of the variability of 3-PBA in the adult urine samples. In particular, the adults' ln(urinary 3-PBA) levels were highly significantly different ($p<0.0001$) across the sampling seasons with the highest concentrations occurring in the spring season. The ln(urinary 3-PBA) levels were also significantly higher ($p=0.025$) in adults that did not own a dog/cat compared to those that did own a dog/cat. In addition, ln(urinary 3-PBA) levels were significantly higher ($p=0.020$) in adults that did not remove their shoes compared to those that did removed their shoes before entering their homes. Finally for PCP, removal of shoes before entering the home and ln(creatinine) levels were significant predictors ($p<0.05$), and pet ownership was a marginally significant predictor ($p=0.056$) of the adult's ln(urinary PCP) levels. The ln(urinary PCP) levels were significantly higher ($p=0.041$) in adults that did not removed their shoes compared to those that did remove their shoes before entering the home.

Discussion

Based on the urinary biomonitoring data, 95% of the CTEPP adults were exposed to at least

three different pesticides over the 48-hour monitoring period in OH in 2001. The median urinary levels of 2,4-D, TCP, 3-PBA, and PCP in the adult participants were 0.7, 3.4, 0.3, and 0.5 ng/mL, respectively. In comparison, median urinary levels of 2,4-D were at least three times higher in the CTEPP adults (0.7 ng/mL) compared to the NHANES adults (<0.2 ng/mL) in 2001-2002 (CDC, 2009). In addition, median urinary TCP concentrations were higher in the CTEPP adults (3.4 ng/mL) than in the NHANES adults (1.9 ng/mL). This information suggests that there is probably geographical differences in the exposures of US adults to some pesticides (i.e., 2,4-D and chlorpyrifos/chlorpyrifos-methyl). This information is supported by a previous article (Morgan et al., 2008a) that also reported median urinary 2,4-D levels of 0.7 ng/mL in 66 CTEPP adults in NC in 2000-2001. In addition, Berokowitz et al. (2003) reported even higher median urinary levels of TCP (7.5 ng/mL) in 386 pregnant women in New York State between 1998 and 2001. Interestingly, median urinary levels of 2,4-D, TCP, and PCP have not generally increased in NHANES adults from 1999 to 2008 (CDC, 2014). However for 3-PBA, median urinary levels were about twice as high in 2007-2008 (0.42 ng/mL) compared to 1999-2000 (0.23 ng/mL) in NHANES adults. This is likely due to the increased use of pyrethroid insecticides in residential settings and some agricultural settings (Barr et al., 2010). Research suggests that mainly permethrin and cypermethrin contributed to the majority of the observed urinary 3-PBA levels in the US general population in the last decade (Morgan et al., 2012; Xue et al., 2014).

A comparison of the available published studies that have reported the median 2,4-D, TCP, 3-PBA, and/or PCP levels (ng/mL) in the urine of the general adult population worldwide are presented in Table 4. The median urinary TCP levels of the CTEPP adults (3.4 ng/mL) were almost two times higher compared to the median urinary TCP levels of study adults (1.2-1.8 ng/mL) from other countries (Germany, Mexico, and the Netherlands). This information supports the above evidence that there were likely geographical differences in the exposures of adults to chlorpyrifos and chlorpyrifos-methyl in the US in the early 2000's. For 3-PBA, the CTEPP adults (0.3 ng/mL) had similar median urinary metabolite levels as study adults (0.2 – 0.4 ng/mL) in Canada, Germany, Japan, and Poland. However in China (Qi et al., 2012), the median adult urinary 3-PBA concentrations (1.0 ng/mL) were at least two times higher compared to study adults in the US and in other countries. The authors speculate that the higher urinary 3-PBA levels observed in these adults (pregnant women) may be due to the markedly increased use of pyrethroid insecticides in residential and agricultural settings in China (Qi et al., 2012).

Only one published study was found that has assessed the relationship between any

sociodemographic/lifestyle factor and urinary 2,4-D levels in adults in non-occupational settings, globally (Lewis et al., 2014). Recently, Lewis et al. (2014) reported that marital status and the 48-hour consumption of collards and spinach were significantly associated ($p<0.05$) with ln(urinary 2,4-D) levels in 54 pregnant women in Puerto Rico in 2010-2012; however these data are limited as this biomarker was only detected in 12% of the urine samples. In comparison to this study, results showed that 48-hour time spent outside at home, 48-hour sweet/salty snack consumption, and ln(creatinine) levels were significant predictors ($p<0.05$) and race was a marginally significant predictor ($p=0.093$) of the ln(urinary 2,4-D) levels in the CTEPP adults, collectively explaining 20% of the variability of 2,4-D in the urine samples. An important result was that the CTEPP adults' urinary 2,4-D levels were significantly higher ($p=0.038$) for those that spent $<$ 3 hours compared to $\geq$ 3 hours outside at home during the 48-hour monitoring period. This suggests that the more time the CTEPP adults spent indoors at home likely increased their exposures to 2,4-D residues. This is supported by earlier research showing that 98% of the carpet dust samples had detectable levels of 2,4-D at the CTEPP homes in OH in 2001 (Morgan et al., 2008a). This is concerning since 2,4-D is restricted for outdoor residential use (US EPA, 2005). Research has suggested that 2,4-D residues can be substantially tracked into homes by occupants and their pet dogs, particularly after lawn applications (Nishioka et al., 2001). Another important result was that urinary 2,4-D concentrations were significantly higher ($p=0.043$) in CTEPP OH adults that consumed sweet/salty snacks $\geq$ 2 times versus $<$ 2 times over the 48-hour monitoring period. Sweet and salty snacks were defined as highly processed food items like pretzels, popcorn, potato chips, rice cakes, crackers, cookies, crackers, and cereal/granola bars. Dietary ingestion is considered a major exposure route of 2,4-D in the general population in the US (Nishioka et al., 2001; Morgan et al., 2004; Wilson et al., 2010). However, scant data are available on the specific foods consumed by adults that contain 2,4-D residues (US FDA, 2014). In the US FDA's Total Diet Study (2004-2005), 2,4-D residues were found in only a few samples of cereals, breads, and rice purchased from grocery stores in four different geographical areas of the US. As data are very limited, more research is necessary on the specific foods or food categories that contain measureable residues of 2,4-D in the US.

A limited number of studies have examined the influence of sociodemographic or lifestyle factors on the urinary concentrations of TCP or 3-PBA in non-occupationally exposed adults worldwide (Kieskaz et al., 2002; Berkowitz et al., 2003; Egeghy et al., 2005; Meeker et al., 2005; Reiderer et al., 2008; Ye et al., 2008; Kimata et al., 2009; Fortes et al., 2013; McKelvey et al.,

2013; Wielgomas and Piskunowicz, 2013). In this current study, modeling results showed that urbanicity, employment status, sampling season, and ln(creatinine) levels were significant predictors ($p<0.05$) of the CTEPP adults' ln(urinary TCP) concentrations, together explaining 20% of the variability of TCP in the adult urine samples. In particular, the CTEPP adults had significantly higher ($p=0.032$) urinary TCP levels for those living in urban counties compared to rural counties in OH. Saieva et al. (2004) also reported that urbanicity was a strong predictor ($p=0.01$) in the 24-hour urinary excretion of TCP in 69 non-occupationally-exposed adults in Italy (1993-1998). Italian adults living in the urban area of Florence had significantly higher urinary TCP concentrations compared to those living in the rural area of Ragusa. This information suggests that the usage patterns for chlorpyrifos were probably markedly different in urban environments compared to rural environments (McKinlay et al., 2008). One plausible explanation is that chlorpyrifos was commonly used in public places (i.e., parks, recreational fields, and gardens) in urban environments which may have contributed to the higher biomarker levels observed in these urban residents (McKinlay et al., 2008; US EPA, 2014). Another important result was that the CTEPP adults' urinary TCP concentrations were significantly different ($p=0.043$) across the three sampling seasons (spring, summer, and fall). Similarly, Meeker et al. (2005) also reported seasonal differences ($p<0.01$) in urinary TCP concentrations in 10 healthy males in the New England area in 2001-2003. Research has suggested that the variability of adult exposures to pesticides (i.e., chlorpyrifos) over time is likely influenced by several factors such as diet, activity patterns, pesticide-use, and geographical area (Colt et al., 2004; Meeker et al., 2005). For 3-PBA, the modeling results showed that sampling season, pet ownership, and removal of shoes before entering the home were all strong predictors ($p<0.05$) of the CTEPP adults' ln(urinary 3-PBA) levels, together explaining 25% of the variability of 3-PBA in the urine samples. A major result was that the adult's urinary 3-PBA levels were highly significantly different ($p<0.0001$) across the three sampling seasons in OH. Berkowitz et al. (2004) also found seasonal differences ($p<0.001$) in urinary 3-PBA concentrations in 386 pregnant women in New York State (1998-2001). These findings are supported by Weston et al. (2009) that reported seasonal differences in residential runoff of pyrethroids, including permethrin and cypermethrin, into urban creeks in California in 2006-2007. Lastly, the urinary 3-PBA levels were significantly higher ($p=0.020$) in CTEPP adults that did not remove their shoes compared to those that did removed their shoes before entering their homes. Studies have shown that families can track-in significant amounts of pesticide residues indoors, particularly after

lawn applications (Nishioka et al., 2001; Morgan et al., 2008b). This information suggests that this lifestyle factor (removing shoes) may substantially reduce the track-in and potential (nondietary) exposures of occupants to pyrethroids used outdoors at residences.

Few studies have assessed the relationship between any sociodemographic or lifestyle factor and urinary PCP concentrations in adults in non-occupational settings around the world (Kieskaz et al., 2002; Berkowitz et al., 2003). In a study by Kieszak et al. (2002), no associations were found between the self-reported monthly consumption of foods including fruits, vegetables, and bread products and urinary PCP levels in 978 US adults in the 1988-1994 NHANES. In a later study conducted by Berkowitz et al. (2003), the authors also did not find any associations between age of residence, type of dwelling, or residential ownership and urinary PCP levels in 386 pregnant women in New York State in 1998-2001. In agreement with these two prior studies, the current modeling results showed that the frequent consumption of foods (fruits, vegetables, meats, dairy, or grains) or age of residence were not significant predictors of the CTEPP adults ln(urinary PCP) levels in OH. However, removing shoes before entering the home and ln(creatinine) levels were significant predictors ($p<0.05$) and owning a cat or dog was a marginally significant predictor ($p=0.056$) of the CTEPP adults' ln(urinary PCP) concentrations. Nevertheless, these two lifestyle factors only explained a small percentage (6%) of the variability of PCP in the adult urine samples. As PCP is a persistent and ubiquitous environmental pollutant globally and a probable human carcinogen (US EPA, 2008; Zheng et al., 2011), more research is needed to identify the major factors that significantly influence the urinary PCP levels in adults.

Conclusions

Based on the urinary biomonitoring data, most of the CTEPP adults (95%) were exposed to several different pesticides over the 48-hour monitoring period in OH. Specific risk-modifying factors were identified that increased the exposures of the adults to these pesticides in their daily environments. However, these factors tended to vary by pesticide for this cohort of adults. Important sociodemographic or lifestyle factors were urbanicity (TCP), employment status (TCP), sampling season (TCP and 3-PBA), 48-hour sweet/salty snack consumption (2,4-D), 48-hour time spent outside at home (2,4-D), pet ownership (3-PBA), and removal of shoes before entering the home (3-PBA and PCP).

Conflict of interest

The author declares no conflict of interests related to this manuscript.

Acknowledgements

I would like to sincerely thank the adult caregivers that participated in the CTEPP study. In addition, I would like to thank Jon R. Sobus and Paul A. Jones for their technical guidance on the statistical analyses of these data. *Disclaimer:* The United States Environmental Protection Agency through its Office of Research and Development funded and managed the research described here under Contract #68-D-99-011 to Battelle. It has been subjected to Agency review and approved for publication.

References

Barr, D.B., Allen, R., Olsson, A.O., Bravo, R., Caltabiano, L.M., Montesano, A., et al., 2005a. Concentrations of selective metabolites of organophosphorus pesticides in the United States population. Environ. Res. 99, 314–326.

Barr, D.B., Wilder, L.C., Caudill, S.P., Gonzalez, A.J., Needham, L.L, Pirkle, J.L., 2005b. Urinary creatinine concentrations in the US population: implications for urinary biologic monitoring measurements. Environ. Health Perspect. 113,192–200.

Barr, D.B., Olsson, A.O., Wong, L.Y., Udunka, S.O., Baker, S.E., Whitehead, R.D., et al., 2010. Urinary concentrations of metabolites of pyrethroid insecticides in the general US population: National Health and Nutrition Examination Survey 1999-2002. Environ. Health Perspect. 118, 742–748.

Berkowitz, G.S., Obel, J., Deych, E., Lapinski, R., Godbold, J., Liu, Z., et al., 2003. Exposure to indoor pesticides during pregnancy in a multiethnic, urban cohort. Environ. Health Perspect. 111, 79–84.

CDC (Centers for Disease Control and Prevention), 2009. The fourth national report on human exposures to environmental chemicals. Available from: http://www.cdc.gov/exposurereport/. (accessed 02.11.15).

CDC (Centers for Disease Control and Prevention), 2014. National report on human exposure to Environmental Chemicals, updated tables (August). Available from: http://www.cdc.gov/exposurereport/. (accessed 12.04.14).

CHMS (Canadian Health Measures Survey), 2010. Summary of the biomonitoring results and government actions. Available from http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/chms-ecms/index-eng.php. (accessed 03.25.15).

Chen, M., Tang, R., Fu, G., Xu, B., Zhu, P., Qiao, S., et al., 2013. Association of exposure to phenols and idiopathic male infertility. J Hazard. Mater. 250/251, 115–121.

Colt, J.S., Lubin, J., Camann, D., Davis, S., Cerhan, J., Severson, R.K., et al., 2004. Comparison of pesticide levels in carpet dust and self-reported pest treatment practices in four US sites. J. Expo. Anal. Environ. Epidemiol. 14, 74–83.

Egeghy, P.P., Quackenboss, J.J., Catlin, S., Ryan, P.B., 2005. Determinants of temporal variability in NHEXAS-Maryland environmental concentrations, exposures, and biomarkers. J. Expo. Anal. Environ. Epidemiol. 15, 388–397.

Fisher, B., 1991. Pentachlorophenol: Toxicology and environmental fate. J. Pesticide Reform 11, 2–5.

Fortenberry, G.Z., Meeker, J.D., Sanchez, B.N., Boyd Barr, D., Panuwet, P., Bellinger, D et al., 2014. Urinary 3,5,6-trichloro-2-pyridinol (TCPy) in pregnant women from Mexico City: Distribution, temporal variability, and relationship with child attention and hyperactivity. Int. J. Hyg Environ Health 217, 405–412.

Fortes, C., Mastroeni, S., Pilla, M.A., Antonelli, G., Aprea, C., 2013. The relation between dietary habits and urinary levels of 3-phenoxybenzoic acid, a pyrethroid metabolite. Food Chem. Toxicol. 52, 91-96.

Kieszak, S.M., Naeher, L.P., Rubin, C.S., Needham, L.L., Backer, L., Barr, D. et al., 2002. Investigation of the relation between self-reported food consumption and household chemical exposures with urinary levels of selected nonpersistent pesticides. J. Expo. Anal. Environ. Epidemiol. 12, 404–408.

Kimata, A. Kondo, T., Ueyama, J., Yamamoto, K., Kamijima, M., Suzuki, K., 2009. Relationship between dietary habits and urinary concentrations 3-phenoxybenzoic acid in a middle-aged and elderly general population in Japan. Environ. Health Prev. Med. 14, 173–179.

Koch, H.M., Hardt, J., Angerer J., 2001. Biological monitoring of exposure of the general population to the organophosphorus pesticides chlorpyrifos and chlorpyrifos-methyl by determination of their specific metabolite 3,5,6-trichloro-2-pyridinol. Int. J. Hyg. Environ. Health 204, 175–180.

Lewis, R.C., Cantonwine, D.E., Anzalota Del Toro, L.V., Calafat, A.M., Valentin-Blasini, L., Davis, M.D., et al., 2014. Urinary biomarkers of exposure to insecticides, herbicides, and one insect repellent among pregnant women in Puerto Rico. Environ. Health (*ePub ahead of print*).

McKelvey, W., Jacobson, J.B., Kass, D., Barr, D.B., Davis, M., Calafat, A.M., et al., 2013. Population-based biomonitoring of exposure to organophosphate and pyrethroid pesticides in New York City. Environ. Health Perspect. 121, 1349–1356.

McKinlay, R., Plant J.A., Bell, J.N.B., Voulvoulis, N., 2008. Calculating human exposure to endocrine disrupting pesticides via agricultural and non-agricultural exposure routes. Sci. Total Environ. 398, 1–12.

Meeker, J.D., Barr, D.B., Ryan, L., Herrick, R.F., Bennett, D.H., Bravo, R., et al., 2005. Temporal variability of urinary levels of nonpersistent insecticides in adult men. J Expos Anal

Environ. Epidemiol. 15, 271–281.

Morgan, M.K., Sheldon, L.S., Croghan, C.W., Chuang, J.C., Lordo, R., Wilson, N.K., et al., 2004. A pilot study of children's total exposure to persistent pesticides and other persistent organic pollutants (CTEPP). EPA/600/R-041/193. Available from: http://www.epa.gov/heasd/research/ctepp.html (accessed 12.20.14).

Morgan, M.K., Sheldon, L.S., Thomas, K.W., Egeghy, P.P., Croghan, C.W., Jones, P.A., et al., 2008a. Adult and children's exposure to 2,4-D from multiple sources and pathways. J. Expo. Sci. Environ. Epidemiol. 18, 486–494.

Morgan, M.K., Stout, D.M., Jones, P.A., Barr, D.B., 2008. An observational study of the potential for human exposure to pet-borne diazinon residues following lawn applications. Environ. Res. 107, 336–342.

Morgan, M.K., 2012. Children's exposures to pyrethroid insecticides at home: A review of available exposure measurement studies conducted in the United States. Int. J. Environ. Res. Public Health 9, 2964–2985.

Morgan, M.K., Jones, P.A., 2013. Dietary predictors of young children's exposures to current-use pesticides using urinary biomonitoring. Food Chem. Toxicol. 62, 131–141.

Morgan, M.K., Wilson, N.K., Chuang, J.C., 2014. Exposures of 129 preschool children to organochlorines, organophosphates, pyrethroids, and acid herbicides at their homes and daycares in North Carolina. Int. J. Environ. Res. Public Health 11, 3743–3764.

Morgan, M.K., Jones, P.A., Sobus, J., 2015. Short-term variability and predictors of urinary pentachlorophenol concentrations in preschool children in Ohio. Int. J. Environ. Res. Public Health 12, 800–815.

Nishioka, M.G., Lewis, R.G., Brinkman, M.C., Burkholder, H.M., Hines, C.E., Menkedick, J.R., 2001. Distribution of 2,4-D in air and on surfaces inside residences after lawn applications: Comparing exposure estimates from various media for young children. Environ. Health Perspect. 109, 1185–1191.

Olsson, A.O., Baker, S.E., Nguyen, J.V., Romanoff, L.C., Udunka, S.O., Walker, R.D., et al., 2004. A liquid chromatography-tandem mass spectrometry multiresidue method for quantification of specific metabolites of organophosphorus pesticides, synthetic pyrethroids, selected herbicides, and DEET in human urine. Anal. Chem. 76, 2453–2461.

Qi, X., Zheng, M., Wu, C., Wang, G., Feng, C., Zhou, Z., 2012. Urinary pyrethroid metabolites among pregnant women in an agricultural area of the Province of Jiangsu, China. Int. J. Hyg. Environ. Health 215, 487–495.

Riederer, A.M., Bartell, S.M., Barr, D.B., Ryan, P.B., 2008. Diet and nondiet predictors of urinary 3-phenoxybenzoic acid in NHANES 1999-2002. Environ. Health Perspect. 116, 1015–1022.

Saieva, C., Aprea, C., Tumino, R., Masala, G., Salvini, S., Frasca, G., et al., 2004. Twenty-four-hour urinary excretion of ten pesticide metabolites in healthy adults in two different areas of Italy (Florence and Ragusa). Sci. Total Environ. 332, 71–80.

Schettgen, T., Koch, H.M., Drexler, H., Angerer, J., 2002. New gas-chromatographic mass spectrometric method for the determination of urinary pyrethroid metabolites in environmental medicine. J. Chromatogr. B. 778, 121–130.

Schulz, C., Conrad, A., Becker, K., Kolossa-Gehring, M, Seiwert, M., Seifert, B., 2007. Twenty years of the German Environmental Survey (GerES): Human biomonitoring – temporal and spatial (West Germany/East Germany) differences in population exposure. Int. J. Hyg Environ Health 210, 271–297.

Stout, D.M., Bradham, K.D., Egeghy, P.P., Jones, P.A., Croghan, C.W., Ashley, P.A., et al., 2009. American Healthy Homes Survey: National study of residential pesticides measured from floor wipes. Environ. Sci. Technol. 43, 4294–4300.

Treble, R.G., Thompson, T.S., 1996. Normal values for pentachlorophenol in urine samples collected from the general population. J. Anal. Toxicol. 20, 313–317.

Trunnelle, K.J., Bennett, D.H., Tulve, N.S., Clifton, M.S., Davis, M.D., Calafat, A.M., et al., 2014. Urinary pyrethroid and chlorpyrifos metabolite concentrations in northern California families and their relationship to indoor residential insecticide levels, part of the Study of the Use of Products and Exposure Related Behavior (SUPERB). Environ. Sci. Technol. 48, 1931–1939.

US EPA (United States Environmental Protection Agency), 2000a. Chlorpyrifos-methyl facts. Available from: http://www.epa.gov/opp00001/chem_search/reg_actions/reregistration/fs_PC-059102_1-Oct-00.pdf (accessed 01.25.15).

US EPA (United States Environmental Protection Agency), 2000b. Draft PBT National action plan for hexachlorobenzene (HCB) for public review. Available from: http://www.epa.gov/pbt/pubs/hcbactionplan.pdf (accessed 01.26.15).

US EPA (United States Environmental Protection Agency), 2005. Reregistration eligibility decision for 2,4-D. Available from: http://www.epa.gov/pesticides/reregistration/24d/ (accessed 01.27.15).

US EPA (Unites States Environmental Protection Agency), 2008. Reregistration eligibility decision for pentachlorophenol. Available from: http://www.epa.gov/oppsrrd1/reregistration/pentachlorophenol/ (accessed 01.25.15).

US EPA (United States Environmental Protection Agency), 2013a. 2006-2007 Pesticide market estimates. Available from: http://www.epa.gov/opp00001/pestsales/07pestsales/usage2007.htm (accessed 01.22.15).

US EPA (United States Environmental Protection Agency), 2013b. Pyrethroids and Pyrethrins. Available from: http://www.epa.gov/oppsrrd1/reevaluation/pyrethroids-pyrethrins.html (accessed 01.22.15).

US EPA (United States Environmental Protection Agency), 2014. Chlorpyrifos: Revised human health risk assessment for registration review. Available from: http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2008-0850-0195 (accessed 01.27.15).

US FDA (United States Food and Drug Administration), 2014. Total Diet Study – Analytical results. Available from: http://www.fda.gov/Food/FoodScienceResearch/TotalDietStudy/ucm184293.htm (accessed 02.05.15).

Verbovsek, T., 2011. A comparison of parameters below the limit of detection in geochemical analyses by substitution methods. Materials and Geoenvironment. 58, 393–404.

Weston, D.P., Holmes, R.W., Lydy, M.J., 2009. Residential runoff as a source of pyrethroid pesticides to urban creeks. Environ. Pollut. 157, 287–294.

Wielgomas, B., Piskunowicz, M., 2013. Biomonitoring of pyrethroid exposure among rural and urban populations in northern Poland. Chemosphere 93, 2547–2553.

Wielgomas, B., Nahorski, W., Czarnowski, W., 2013. Urinary concentrations of pyrethroid metabolites in the convenience sample of urban population of northern Poland. Int. J. Hyg. Environ. Health 216, 295–300.

Wilson, N.K., Chuang, J.C., Iachan, R., Lyu, C., Gordon, S.M., Morgan, M.K., et al., 2004. Design and sampling methodology for a large study of preschool children's aggregate exposures to persistent organic pollutants in their everyday environments. J. Expo. Anal. Environ. Epidemiol. 14, 260–274.

Wilson, N.K., Strauss, W.J., Iroz-Elardo, N., Chuang, J.C., 2010. Exposures of preschool children to chlorpyrifos, diazinon, pentachlorophenol, and 2,4-dichlorophenoxyacetic acid over 3 years from 2003 to 2005: A longitudinal model. J. Expo. Sci. Environ. Epidemiol. 20, 546–558.

Xue, J., Zartarian, V.G., Tornero-Velez, R., Tulve N.S., 2014. EPA's SHEDS-multimedia model: Children's cumulative pyrethroid exposure estimates and evaluation against NHANES biomarker data. Environ. Int. 73, 304–311.

Ye, X., Pierik, F.H., Hauser, R., Duty, S., Angerer, J., Park, M.M., et al., 2008. Urinary metabolite concentrations of organophosphorous pesticides, bisphenol A, and phthalates among pregnant women in Rotterdam, the Netherlands: The Generation R study. Environ. Res. 108, 260–267.

Zhang, J., Hisada, A., Yoshinaga, J., Shiraishi, H., Shimodaira, K., Okai, T., et al, 2013. Exposure to pyrethroids insecticides and serum levels of thyroid-related measures in pregnant women. Environ. Res. 127, 16–21.

Zheng, W.; Wang, X.; Yu, H.; Tao, X.; Zhou, Y.; Qu, W., 2011. Global trends and diversity in pentachlorophenol levels in the environment and in humans: A meta-analysis. Environ. Sci. Technol. 45, 4668-4675.

Table 1

Pesticide biomarker levels in the unadjusted (ng/mL) and creatinine-adjusted (ng/mg) urine of CTEPP adults over a 48-hour monitoring period

Urinary Biomarker	N[a]	%[b]	Mean±SD[c]	Min.	Percentiles				Max.
					25th	50th	75th	95th	
2,4-D[d]									
ng/mL	121	89	1.0±1.1	<0.2	0.4	0.7	1.3	3.1	8.1
ng/mg[e]	106	88	0.8±0.9	<0.2	0.3	0.5	0.8	2.6	6.2
TCP									
ng/mL	121	98	4.9±4.8	<1.0	2.0	3.4	6.0	13.3	30.5
ng/mg	106	97	3.6±4.0	<1.0	1.6	2.6	4.2	12.5	25.4
3-PBA									
ng/mL	121	66	0.6±0.8	<0.2	<0.2	0.3	0.8	2.0	4.9
ng/mg	106	64	0.5±0.7	<0.2	<0.2	0.2	0.6	1.7	5.1
PCP									
ng/mL	121	96	0.8±0.7	<0.2	0.4	0.5	0.9	2.7	3.7
ng/mg	106	96	0.7±0.9	<0.2	0.2	0.4	0.6	2.4	6.4

[a] Number of adult caregivers

[b] Percentage of urine samples detected at or above the limit of detection.

[c] Arithmetric mean and standard deviation

[d] Urinary 2,4-D levels in adult caregivers that stayed-at-home with their preschool children during the day were previously reported in Morgan et al., 2008a.

[e] Creatinine levels were not measured in the urine samples of 15 adults that had a recent pesticide application (< 7 days) at their residences.

.

Table 2
Levels of urinary pesticide biomarkers (ng/mL) in adults by selected sociodemographic or lifestyle factor

Variables	n (%)[a]	2,4-D		TCP		3-PBA		PCP	
		GM (95% CI)[b]	p-Value[c]	GM (95% CI)	p-Value	GM (95% CI)	p-Value	GM (95% CI)	p-Value
Sociodemographic factors[d]									
Age									
20-35 years	83 (69)	0.80 (0.67, 0.96)	**0.025**	3.4 (2.9, 3.9)	0.131	0.37 (0.29, 0.47)	0.118	0.60 (0.51, 0.71)	0.577
36-49 years	38 (31)	0.54 (0.40, 0.74)		4.2 (3.2, 5.5)		0.26 (0.20, 0.35)		0.55 (0.44, 0.69)	
Race[e]									
Black	29 (25)	0.93 (0.67, 1.3)	0.060	3.6 (2.8, 4.8)	0.909	0.38 (0.26, 0.55)	0.485	0.60 (0.44, 0.81)	0.643
White	87 (75)	0.65 (0.54, 0.78)		3.6 (3.0, 4.2)		0.32 (0.26, 0.40)		0.56 (0.48, 0.64)	
Body mass index[f]									
Normal (18.5-24.9)	55 (47)	0.66 (0.53, 0.82)	0.510	3.5 (2.8, 4.3)	0.902	0.32 (0.24, 0.42)	0.488	0.55 (0.45, 0.67)	0.604
Overweight (25.0-29.9)	30 (25)	0.70 (0.49, 0.98)		3.7 (2.8, 5.0)		0.42 (0.29, 0.59)		0.57 (0.45, 0.74)	
Obese ($\geq$ 30)	33 (28)	0.83 (0.61, 1.1)		3.7 (3.0, 4.5)		0.32 (0.22, 0.46)		0.65 (0.49, 0.85)	
Income status[g]									
Low-income	39 (36)	0.74 (0.56, 0.98)	0.728	3.6 (2.8, 4.5)	0.733	0.35 (0.25, 0.49)	0.390	0.59 (0.46, 0.66)	0.664
Middle/high-income	69 (64)	0.70 (0.56, 0.86)		3.4 (2.9, 4.0)		0.29 (0.23, 0.38)		0.55 (0.48, 0.73)	
Urbanicity (*county-level*)[h]									
Urban	104 (86)	0.69 (0.59, 0.82)	0.476	3.9 (3.3, 4.4)	**0.018**	0.35 (0.28, 0.43)	0.202	0.56 (0.49, 0.65)	0.097
Rural	17 (14)	0.82 (0.54, 1.2)		2.4 (1.7, 3.4)		0.24 (0.16, 0.37)		0.77 (0.55, 1.1)	
Employment status[i]									
Employed	77 (64)	0.72 (0.60, 0.87)	0.763	3.3 (2.8, 3.9)	0.104	0.31 (0.24, 0.40)	0.428	0.56 (0.48, 0.66)	0.458
Unemployed	44 (36)	0.69 (0.52, 0.91)		4.2 (3.4, 5.2)		0.37 (0.20, 0.36)		0.63 (0.50, 0.79)	
Education level									
$\leq$ High school	32 (26)	0.77 (0.57, 1.0)	0.336	3.7 (2.9, 4.6)	0.816	0.45 (0.32, 0.63)	0.130	0.68 (0.52. 0.87)	0.298
Some college/post-training	32 (26)	0.82 (0.61, 1.1)		3.7 (2.8, 4.7)		0.27 (0.18, 0.40)		0.61 (0.48, 0.77)	
College degree	57 (48)	0.63 (0.50, 0.80)		3.3 (2.7, 4.1)		0.31 (0.23, 0.40)		0.53 (0.43, 0.64)	
Sampling season[j]									
Spring	41 (34)	0.74 (0.56, 0.97)	0.875	3.5 (3.0, 4.2)	**0.005**	0.51 (0.35, 0.75)	**0.002**	0.44 (0.37, 0.53)	**0.012**
Summer	56 (46)	0.72 (0.57, 0.90)		4.3 (3.5, 5.4)		0.29 (0.23, 0.37)		0.67 (0.56, 0.81)	
Fall	24 (20)	0.66 (0.46, 0.94)		2.4 (1.8, 3.2)		0.21 (0.16, 0.27)		0.68 (0.47, 0.98)	
Lifestyle factors									
48-h fruit consumption									
None	64 (53)	0.74 (0.59, 0.93)	0.571	3.4 (2.9, 4.0)	0.447	0.34 (0.27, 0.44)	0.728	0.62 (0.52, 0.75)	0.346
$\geq$ 1 times	57 (47)	0.68 (0.54, 0.84)		3.8 (3.1, 4.8)		0.32 (0.24, 0.43)		0.55 (0.45, 0.66)	
48-h vegetable consumption									
< 2 times	59 (49)	0.68 (0.55, 0.84)	0.551	3.9 (3.3, 4.8)	0.205	0.36 (0.28, 0.47)	0.365	0.63 (0.52, 0.75)	0.353

≥ 2 times	62 (51)	0.75 (0.59, 0.94)		3.3 (2.7, 4.0)		0.30 (0.23, 0.40)		0.55 (0.46, 0.66)	
48-h meat consumption									
< 2 times	51 (42)	0.80 (0.66, 0.98)	0.205	3.3 (2.7, 3.9)	0.232	0.34 (0.24, 0.46)	0.903	0.54 (0.44, 0.66)	0.282
≥ 2 times	70 (58)	0.65 (0.52, 0.82)		3.9 (3.2, 4.7)		0.33 (0.26, 0.41)		0.62 (0.53, 0.74)	
48-h dairy consumption									
< 2 times	78 (64)	0.70 (0.58, 0.85)	0.864	3.6 (3.0, 4.3)	0.924	0.36 (0.28, 0.46)	0.228	0.63 (0.53, 0.74)	0.185
≥ 2 times	43 (36)	0.72 (0.54, 0.96)		3.6 (2.8, 4.5)		0.28 (0.21, 0.38)		0.52 (0.42, 0.65)	
48-h grain consumption									
< 4 times	76 (63)	0.71 (0.59, 0.86)	0.985	3.8 (3.2, 4.4)	0.448	0.36 (0.28, 0.45)	0.284	0.65 (0.55, 0.76)	**0.046**
≥ 4 times	45 (37)	0.71 (0.54, 0.95)		3.4 (2.7, 4.2)		0.29 (0.21, 0.39)		0.49 (0.40, 0.61)	
48-h snack consumption[k]									
< 2 times	66 (55)	0.64 (0.52, 0.79)	0.159	3.3 (2.7, 3.9)	0.144	0.36 (0.28, 0.47)	0.294	0.63 (0.53, 0.77)	0.201
≥ 2 times	55 (45)	0.81 (0.63, 1.0)		4.0 (3.3, 5.0)		0.30 (0.23, 0.39)		0.53 (0.45, 0.64)	
Insecticide use at home[l]									
Yes	63 (53)	0.75 (0.61, 0.92)	0.478	3.9 (3.1, 4.8)	0.277	0.30 (0.24, 0.39)	0.392	0.57 (0.47, 0.68)	0.787
No	56 (47)	0.67 (0.52, 0.85)		3.3 (2.8, 3.9)		0.36 (0.27, 0.49)		0.59 (0.49, 0.71)	
Herbicide use at home[m]									
Yes	62 (52)	0.77 (0.61, 0.96)	0.260	3.7 (3.1, 4.5)	0.628	0.32 (0.25, 0.41)	0.689	0.54 (0.45, 0.65)	0.299
No	58 (48)	0.64 (0.51, 0.80)		3.5 (2.9, 4.2)		0.34 (0.26, 0.46)		0.63 (0.52, 0.76)	
48-h time spent outside at home									
< 3 hours	74 (61)	0.80 (0.66, 0.96)	0.074	3.7 (3.1, 4.3)	0.801	0.31 (0.25, 0.39)	0.453	0.59 (0.50, 0.70)	0.876
≥ 3 hours	47 (39)	0.59 (0.45, 0.78)		3.5 (2.8, 4.4)		0.36 (0.26, 0.50)		0.58 (0.47, 0.71)	
Age of home									
≤ 20 years	38 (31)	0.79 (0.62, 1.0)	0.364	4.1 (3.1, 5.3)	0.229	0.34 (0.25, 0.47)	0.801	0.50 (0.39, 0.64)	0.108
> 20 years	83 (69)	0.68 (0.55, 0.83)		3.4 (2.9, 4.0)		0.33 (0.26, 0.41)		0.63 (0.54, 0.73)	
Removal of shoes inside home									
Yes	43 (36)	0.61 (0.47, 0.80)	0.178	3.4 (2.7, 4.2)	0.451	0.26 (0.19, 0.35)	0.063	0.49 (0.41, 0.59)	0.051
No	78 (64)	0.77 (0.63, 0.94)		3.7 (3.2, 4.4)		0.38 (0.30, 0.48)		0.65 (0.54, 0.77)	
Own a dog or cat									
Yes	60 (50)	0.62 (0.50, 0.78)	0.103	3.3 (2.8, 4.0)	0.261	0.28 (0.22, 0.36)[l]	0.090	0.64 (0.53, 0.78)	0.196
No	61 (50)	0.81 (0.65, 1.0)		3.9 (3.2, 4.8)		0.39 (0.30, 0.51)		0.54 (0.45, 0.64)	

[a] Number and percentage of adult caregivers; [b] Geometric mean and 95% confidence interval; [c] Statistically significant associations ($p<0.05$) are in bold text; [d] Sex was excluded from the bivariate analysis due to the small sample size (n=10) of male adult caregivers; [e] Two other race categories (3 Hispanic and 2 Asian/Pacific Island adults) were excluded in the bivariate analysis due to their small sample size; [f] Underweight was not included as a category due to the small sample size (n=3) of adults; [g] Missing data on income-class for 13 adult caregivers; [h] Urban counties (Cuyahoga, Licking, Franklin, and Hamilton) and rural counties (Defiance and Fayette); [i] In this cohort, 46% of the adult caregivers that stayed-at- home with their children reported working during the week (i.e., nights or weekends), and 85% of the adult caregivers that left their children at licensed daycare centers reported working during the week; [j] Field sampling activities were performed between April 2001 and November 2001; [k] Sweet and salty snacks (i.e., pretzels, popcorn, potato chips, crackers, cakes, and candies); [l] Missing data on insecticide use for two adult caregivers; [m] Missing data on herbicide use for one adult caregiver

Table 3
Final reduced regression models of factors influencing ln urinary levels of 2,4-D, TCP, 3-PBA, or PCP in adults[a]

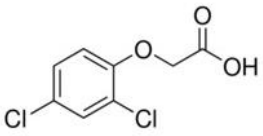
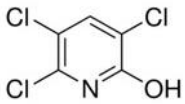
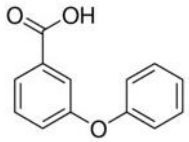
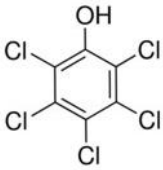
Factors	β coefficient	SE[b]	P-value[c]
2,4-D (r^2 = 0.20)			
Intercept	-3.34	0.850	**0.0002**
Race			0.093
White	-0.312	0.184	
Black	0 (ref.)	----	
48-Hour sweet/salty snack consumption			**0.043**
< 2 times	-0.350	0.171	
≥ 2 times	0 (ref.)	----	
48-Hour time spent outside at home			**0.038**
< 3 hours	0.365	0.173	
≥ 3 hours	0 (ref.)	----	
Creatinine level[d]	0.657	0.175	**0.0003**
TCP (r^2 = 0.20)			
Intercept	-0.994	0.704	0.161
Urbanicity			**0.032**
Urban county	0.419	0.193	
Rural county	0 (ref.)	----	
Employment status			**0.018**
Employed	-0.327	0.136	
Not employed	0 (ref.)	----	
Sampling season			**0.043**
Spring	0.395	0.186	
Summer	0.410	0.169	
Fall	0 (ref.)	----	
Creatinine level	0.359	0.139	**0.011**
3-PBA (r^2 = 0.25)			
Intercept	-1.34	0.221	**<0.0001**
Sampling season			**<0.0001**
Spring	1.15	0.257	
Summer	0.400	0.245	
Fall	0 (ref.)	----	
Own a dog or cat			**0.025**
Yes	-0.424	0.187	
No	0 (ref.)	----	
Remove shoes before entering home			**0.020**
Yes	-0.465	0.197	
No	0 (ref.)	----	
PCP (r^2 = 0.11)			
Intercept	-2.28	0.754	**0.003**
Own a dog or cat			0.056
Yes	0.271	0.140	
No	0 (ref.)	----	
Remove shoes before entering home			**0.041**
Yes	-0.300	0.145	
No	0 (ref.)	----	
Creatinine level	0.353	0.150	**0.020**

[a] All models had 106 adult caregivers, except for the 2,4-D model (101 adults). The 2,4-D model only included adults from racial categories (black and white) that had sufficient sample sizes for this analysis
[b] Standard error
[c] Statistically significant variables ($p < 0.05$) are in bold text
[d] Continuous variable (log-transformed); units are mg/dL

Table 4
Comparison of the median 2,4-D, TCP, 3-PBA, and PCP concentrations (ng/mL) measured in urine of the general adult population worldwide

Country	Year	N[a]	Urinary Pesticide Biomarker				Reference
			2,4-D	TCP	3-PBA	PCP	
This study	2001	121	0.7	3.4	0.3	0.5	-----
USA (NHANES)	2001-2002	1100[b]	<0.2	1.9	0.3	<0.5	CDC, 2009
Canada	1994[c]	69[d]	----	----	----	0.5	Treble and Thompson, 1996
	2007-2009	2380[b]	<1.0	----	0.2	----	CHMS, 2010
China	2005-2010	713	----	----	----	<0.4	Chen et al., 2013
	2009-2010	1149	----	----	1.0	----	Qi et al., 2012
Germany	1998	600	----	----	----	1.0	Schulz et al., 2007
	2000	50	----	1.4	----	----	Koch et al., 2001
	2002	46	----	----	0.2	----	Schettgen et al., 2002
Japan	2009-2011	231	----	----	0.4	----	Zhang et al., 2013
Mexico	1994-2005	187	----	1.8	----	----	Fortenberry et al., 2014
Poland	2010-2011	132[e]	----	----	0.3	----	Wielgomas et al., 2013
Puerto Rico	2010-2012	54	<0.4	----	<0.1	----	Lewis et al., 2014
The Netherlands	2004	100	----	1.2	----	----	Ye et al., 2008

[a]Number of adults
[b]Estimated number of adults as sample size varies by analyte
[c]Estimated study date (not provided in article)
[d]The participants ages ranged from 6-87 years of age
[e]Eight of the participants were children less than 14 years of age

Urinary Pesticide Biomarker[a]			
2,4-Dichlorophenoxyacetic acid (2,4-D) 	3,5,6-Trichloro-2-pyridinol (TCP) 	3-Phenoxybenzoic acid (3-PBA) 	Pentachlorophenol (PCP) 
Parent Chemical			
2,4-Dichlorophenoxyacetic acid	Chlorpyrifos Chlorpyrifos-methyl	Cyfluthrin, Cyhalothrin, Cypermethrin, Deltamethrin, Esfenvalerate, Fenpropathrin, Permethrin, Tralomethrin	Pentachlorophenol Hexachlorobenzene

[a]Chemical structures (Sigma-Aldrich, http://www.sigmaaldrich.com)

Fig. 1. Urinary pesticide biomarkers and their corresponding parent chemicals