Urinary concentrations of insecticide metabolites in northern California families and their relationship to indoor home insecticide levels, part of SUPERB.

Kelly J. Trunnelle[1], Deborah H. Bennett[2], Nicolle S. Tulve[3], Matthew Scott Clifton[3], Mark D. Davis[4], Antonia M. Calafat[4], Rebecca Moran[2], Daniel J. Tancredi[5], Irva Hertz-Picciotto[2]*

[1]Agricultural and Environmental Chemistry, [2] Public Health Sciences, University of California, Davis, CA, USA
[3]Human Exposure and Atmospheric Sciences, National Exposure Research Laboratory, Office of Research and Development, US EPA, Research Triangle Park, NC, USA
[4] Division of Laboratory Sciences, National Center for Environmental Health, CDC, Atlanta, GA, USA
[5]Department of Pediatrics, University of California, Davis, School of Medicine, Sacramento, CA, USA

Corresponding Author:
Kelly J. Trunnelle, PhD, Department of Environmental Toxicology, 1 Shields Avenue, University of California, Davis, CA 95616 USA; Email: kjtrunnelle@ucdavis.edu;
Cell: (925) 408-5177

Running Title: Urinary metabolites and indoor insecticide levels.

Key words: insecticide, floor wipe, organophosphate, pyrethroid, urine

Acknowledgments:

The US EPA through its Office of Research and Development funded this research through a Science to Achieve Results (STAR) grant #RD-83154001. The US EPA provided in-kind support for analysis of wipe samples. Analysis of urinary metabolites was provided through in-kind support by CDC. This study would not have been possible without the participants who patiently gave their time. We acknowledge the technical assistance of C. Chambers, P. Olive, P. Restrepo, W. Roman, and E. Wade in measuring the urinary concentrations.

We have no competing financial interests for this project.

Abbreviations:

SUPERB - Study of Use of Products and Exposure Related Behavior

NHANES – National Health and Nutrition Examination Survey

OP - organophosphate

SRS - surrogate recovery standard

IS - internal standard

%D - detection frequencies

LOD - limit of detection

MDL - method detection limit

SD - standard deviation

CI - confidence intervals

r_s - Spearman rank correlation coefficient

ND - non-detect

agmr - adjusted geometric mean ratio

Abstract

Since the 2001 U.S. federally mandated phase-out of residential uses of organophosphate (OP) insecticides, the use of and potential for human exposure to pyrethroid insecticides in the indoor residential environment increases, while that for OPs decreases. Here we report indoor concentrations of several common pyrethroids, pyrethroid metabolites, and chlorpyrifos based on floor wipe samples collected from 81 northern California households. We report urinary concentrations of pyrethroid metabolites and the chlorpyrifos metabolite TCPy in samples collected in 2007–2009 from 83 children and 90 adults who lived in these 81 households and took part in the Study of Use of Products and Exposure Related Behavior (SUPERB). We examined correlations between concentrations in floor wipe and urine samples. The most frequently detected urinary pyrethroid metabolite was 3PBA (62.4%, median concentration of 0.79 ng/mL). TCPy was detected at a similar frequency (64.7%, median concentration of 1.47 ng/mL). Compared to the National Health and Nutrition Examination Survey (NHANES) in 1999-2002, this population had substantially higher pyrethroid metabolite and lower TCPy urinary concentrations than the general population in the U.S. This may be related to the increased residential use of pyrethroids after the phase-out of OPs. Chlorpyrifos (98.7%), *cis*- and *trans*-permethrin (97.5%), bifenthrin (59.3%), 3PBA (98.7%) and 4F3PBA (34.2%) were frequently detected in the floor wipes. Floor wipe concentrations for pyrethroid insecticides were found to be significant predictors of child urinary metabolite concentrations (*p*-values = 0.0004 - 0.049) suggesting that indoor residential exposure to pyrethroid insecticides is an important exposure route for children.

Introduction

Insecticides are commonly used both in and around residential homes in the United States. Since the 2001 U.S. federally mandated phase-out of residential uses of the organophosphate (OP) insecticides chlorpyrifos and diazinon, the usage of synthetic pyrethroids indoors has increased (Horton et al. 2011; USEPA 2000, 2012; Williams et al. 2008).

Although pyrethroids have relatively low mammalian toxicity, there is still caution with regard to human exposure. Studies have shown that exposure to high levels of pyrethroids may cause significant toxicity and health effects, including acute neurotoxic effects (Costa et al. 2008), immunotoxic effects (Emara and Draz 2007) and negative effects on mammalian reproduction (Ji et al. 2011). They also are possible human carcinogens (USEPA 2006).

Pyrethroids metabolize quickly in the body and most also degrade rapidly in sunlight. 3-Phenoxybenzoic acid (3PBA) is a non-specific metabolite of multiple pyrethroids including cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, permethrin and sumithrin. The metabolite 4-fluoro-3-phenoxybenzoic acid (4F3PBA) is specifically produced from cyfluthrin. 3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (DCCA) is a metabolite of cyfluthrin, cypermethrin and permethrin, and 3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (DBCA) is a specific metabolite of deltamethrin. 3PBA has been commonly used as a non-specific biomarker for evaluating human exposure to multiple pyrethroid insecticides (Barr et al. 2010), and has also been measured in the indoor environment (Starr et al. 2008).

Exposure to insecticides may result from ingestion of food; ingestion of drinking water; inhalation, dermal contact and non-dietary ingestion resulting from residential applications. Once insecticides have entered the home, carpets and cushioned furniture can act as repositories for the parent compounds and their metabolites (Starr et al. 2008). Children have an increased risk of exposure to environmental contaminants partly because of behaviors leading to higher non-dietary ingestion than adults (Cohen Hubal et al. 2000). Thus, indoor sources pose a special risk to children (Arcury et al. 2007; Bradman et al. 2007). The indoor residential environment is a more important route of exposure to pyrethroids than dietary ingestion, unlike OP insecticides (C. Lu et al. 2006; Morgan 2012; Naeher et al. 2010; Tulve et al. 2011; Zartarian et al. 2012).

We report results from 83 child and 90 adult northern California residents who participated in the Study of Use of Products and Exposure Related Behavior (SUPERB). Specifically, we report urinary concentrations of 3PBA, *trans*-DCCA, *cis*-DBCA and 4F3PBA, as well as the chlorpyrifos metabolite 3,5,6-trichloro-2-pyridinol (TCPy). We report the indoor environmental concentrations (as measured from floor wipes samples of 81 of the participating households) of allethrin, bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, λ-cyhalothrin, *cis*-permethrin, *trans*-permethrin, sumithrin, tetramethrin, chlorpyrifos, 3PBA, 4F3PBA, DCCA and DBCA. Finally we evaluate whether measurements in floor wipe samples are correlated with urinary concentrations of metabolites.

Methods

Study Population

SUPERB investigated human behaviors that could influence exposure to environmental pollutants by studying food consumption patterns, household product use, and daily activities. One cohort consisted of families (one parent and one child, n = 499) from northern California counties. Households with young children born between 2000 and 2005 were identified through birth certificate records and randomly selected with details previously published (Hertz-Picciotto et al. 2010). Ninety households were enrolled in a sub-study to collect environmental and biological samples and this paper reports pyrethroid and OP insecticide results. At the time of enrollment to this sub-study all children were aged two through eight years. All recruitment and data collection protocols were approved by the Institutional Review Boards at the University of California, Davis (UC Davis) and the Centers for Disease Control and Prevention (CDC). Informed consent for participation was obtained upon enrollment.

Sample collection

Samples were collected December 2007-November 2009. An end of day spot urine sample was collected from 90 adults and 83 children. Participants were provided specimen collection “hats” (Commode Specimen Collector, Fisher Scientific P/N 22-363-149) to be placed under the toilet seat (Barr et al. 2010) and standard plastic urine cups to store the collected sample overnight in the refrigerator. Adult participants were instructed to record the volume and time of both the collected and previous void for themselves and their child. Samples were collected and transported on ice packs to UC Davis the following day, and were frozen and stored at -80^{o}C until shipment to the CDC facility in Atlanta, GA for analysis.

On the day urine samples were retrieved, UC Davis staff also collected floor wipe samples from kitchen floors in 88 out of the 90 homes using two 4” X 4” pre-cleaned cotton Twillwipe cloths (M.G. Chemicals, P/N 829-50) dampened with 6mL isopropanol (pesticide residue analysis grade, Fisher Scientific, Fair Lawn, NJ). Wipes were pre-cleaned via Soxhlet extraction with isopropanol, followed by extraction with hexane, dried in a vacuum oven, and ultimately placed in a sealed clean glass jar until use (Clifton et al. 2013). Floor wipe samples were collected from approximately 930 cm^2 of the kitchen floor. The section was marked with painter's masking tape, wiped uniformly in one direction then wiped again perpendicular to the first direction. The procedure was repeated with the second wipe. Immediately after use, each wipe was placed in a single pre-cleaned 60 mL amber jar. Samples were transported back to UC Davis on ice packs, frozen and stored at -20^{o}C until shipment to the U.S. EPA (Research Triangle Park, NC) and stored at −20 °C until extracted.

Sample Analysis

Urine samples were analyzed at CDC following a modification of a previously reported method (Davis et al. 2013; Olsson et al. 2004). Briefly, 1 mL of urine was fortified with a standard solution of isotopically labeled 3PBA, 4F3PBA, *cis*-DBCA and TCPy, and incubated with β-glucuronidase/sulfatase (Sigma-Aldrich, Co., St. Louis, MO) to hydrolyze conjugated metabolites. Metabolites were extracted using the Quadra 3 (Tomtec Inc. Hamden, CT) 96-well plate technology with an OASIS HLB mixed-mode solid-phase extraction 96-well plate (Waters Corp., Milford, MA). The plate was washed with a 25% methanol in 0.1% acetic acid solution, and the metabolites were eluted with acetone. Target insecticide metabolites in the eluates were separated and quantified using high-performance liquid chromatography (Agilent Technologies, Santa Clara, CA) coupled with heated electrospray ionization tandem mass spectrometry (Thermo Scientific, West Palm Beach, FL) operating in the negative ion mode. 3PBA, 4F3PBA, *cis*-DBCA and TCPy were quantified using isotope dilution calibration, *trans*-DCCA was quantified using the labeled 3PBA as the internal standard. Because calibrators were prepared in diluted urine, corrections were made to account for these endogenous compounds in the calibrator matrix. Positive and negative control samples represented 10% of the samples analyzed and were used as laboratory controls to ensure proper method operation. The limits of detection (LODs) for 3PBA, *trans*-DCCA, *cis*-DBCA and TCPy was the lowest detectable standard in each analytical run. The LOD for 4F3PBA was calculated as three times the standard deviation (SD) of the blank concentrations (Taylor 1987). Urinary creatinine concentrations were determined (Barr et al. 2010) and metabolite concentrations were adjusted using creatinine concentrations in order to correct for variable urine dilutions in the "spot" samples.

Wipe samples were analyzed by U.S. EPA with a published method (Clifton et al. 2013). Briefly, after being spiked with 50 µL of the surrogate recovery solution (SRS) of the insecticide surrogates diazinon-d_{10} and *trans*-permethrin-$^{13}C_6$ (Cambridge Isotope Labs), which were prepared together in a solution at 2.4 µg/mL in hexane, the wipes were extracted with an acetone:hexane solution (1:1 by volume) in an ultrasonic cleaner for 30 minutes. Extracts were solvent exchanged into hexane and partitioned with aqueous NaOH with a total of three portions of hexane. The hexane layers containing the insecticides were passed through ~10g of Na_2SO_4 before being volume reduced and purified using Bond Elut NH_2 SPE cartridges (Agilent Technologies, Palo Alto, CA). The cartridges were washed with 25% acetone in hexane followed by hexane, and the pyrethroids were eluted using hexane followed by a 25% acetone in hexane solution. Eluates were concentrated, an internal standard (IS) solution of phenanthrene-d_{10} and *cis*-permethrin-$^{13}C_6$ was added, and samples were analyzed using electron impact gas chromatography–mass spectrometry.

The NaOH layer which still contained the metabolites was acidified using 2N HCl. This layer was partitioned three times with dichloromethane, passing through Na_2SO_4 prior to volume reduction and solvent exchanged into ethyl acetate. An internal standard containing 3PBA-$^{13}C_6$ was added before extracts were transferred to auto sampler vials and silylation with Sylon BFT (Supelco, Bellefonte, PA, USA) at 70 ºC for 10 minutes. The silylated extracts were analyzed by

EI GC/MS in selected ion monitoring mode. Method detection limits (MDLs) were determined using the guidelines from 40 CFR Part 136, Appendix B and were calculated based on a standard 930 cm^2 sampling area (Clifton et al. 2013).

Statistical analysis

All detectable floor wipe concentrations were reported by the EPA. To maximize this data, all concentrations, including those reported but below the MDL, were used with non-detected concentrations reported as 0. The CDC only reported urinary metabolite concentrations above the LOD. Concentrations below the LOD were set equal to LOD/$\sqrt{2}$. Summary statistics were calculated. A Spearman rank-order correlation coefficient (r_s) was calculated to determine intra-household correlations between parent and child urinary concentrations of 3PBA and TCPy, with significance set at $p < 0.05$. Confidence intervals (CI) for correlation coefficients were computed using Fisher's z-transformation.

Visual analysis of a normal quantile-quantile plot showed that urinary concentrations and floor wipe levels were not normally distributed, and were log-transformed. Bivariate analyses were conducted in which log-transformed volume-based adult or child urinary metabolite concentrations were regressed on log-transformed creatinine concentrations and log-transformed individual floor wipe levels of insecticides or metabolites using a general linear model (Barr et al. 2005b). To express associations on the original scale of measurement for the outcome, point estimates and confidence intervals for regression coefficients were inverse log-transformed and reported as adjusted geometric mean ratios. These ratios compare mean levels for the outcome associated with a 1-unit change in the log-transformed exposure (i.e. a multiplication of the exposure on the original scale by Euler's constant, *e*). All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

Results

Population Demographics

Demographic information is presented in Table 1. Participating adults were 90% female (mean age = 36 years; 91% having at least some college education). The proportions of stay-at-home (48%) and employed (42%) parents were almost equal, and 22% of participants were foreign born. With approximately 60% of this population having a bachelor's degree or higher compared to 30% of adults aged 25 or older in the U.S. (Ryan and Siebens 2012), this population is not representative of the general U.S. population.

Urinary Concentrations

Urinary concentrations of pyrethroid metabolites were detected in 63% of samples with a detectable range of 0.67–89.7 ng/mL 3PBA, 4.92-121 ng/mL *trans*-DCCA, 1.09-1.48 ng/mL *cis*-DBCA, and 0.43-0.77 ng/mL 4F3PBA. LODs varied by analytical batch and were 0.58–0.75 ng/mL for 3PBA, 1.11–2.54 ng/mL for *trans*-DCCA, 0.16–0.74 ng/mL for *cis*-DBCA, 0.32 ng/mL for 4F3PBA, and 0.62–1.17 ng/mL for TCPy. 3PBA was the most frequently detected pyrethroid metabolite with 50 children and 58 adult samples having detectable concentrations, corresponding to 39 households with detectable urinary concentrations in both the adult and child. Urinary concentrations of 3PBA between adults and children within a household were positively correlated for both volume- based (r_s=0.43, 95% CI: 0.23 – 0.59, $p < 0.0001$) and creatinine-adjusted concentrations (r_s=0.46, 95% CI: 0.27 – 0.62, $p < 0.0001$). Summary statistics are presented in Table 2.

The other pyrethroid metabolites were detected less frequently. *Trans*-DCCA was detected in 2 children and 8 adults, none of whom were from the same household. *Cis*-DBCA was detected in 2 children and 1 adult, with one household having both a child and an adult with detectable concentrations. 4F3PBA was detected in 3 children and 8 adults, with only 1 household having detectable concentrations in both the adult and child. A summary of *trans*-DCCA, *cis*-DBCA and 4F3PBA detection frequency and concentration ranges are in the Supplemental Material, Table S1.

Urinary concentrations of the chlorpyrifos metabolite TCPy were detected in 64.7% of samples with 61 children and 51 adult samples having concentrations ranging from 0.74–18.9 ng/mL, corresponding to 40 households with detectable levels in both the adult and child. Urinary concentrations of TCPy within a household were positively correlated for both volume- based (r_s=0.32, 95% CI: 0.11 – 0.50, $p = 0.003$) and creatinine-adjusted concentrations (r_s=0.34, 95% CI: 0.13 – 0.52, $p = 0.001$).

We looked at the co-occurrence of specific urinary pyrethroid metabolites within individual participants. All adult and child urine samples with detectable concentrations of *trans*-DCCA, *cis*-DBCA or 4F3PBA also had detectable concentrations of 3PBA, with the exception of one child's urine sample having a detectable concentration of 4F3PBA and no detectable concentration of any other pyrethroid metabolite. Of the 8 adults with detectable *trans*-DCCA urinary concentrations, 3 also had detectable 4F3PBA concentrations. Those 3 adult participants had 3 of the 4 highest urinary concentrations of 3PBA. Most notably was one adult who had the highest urinary concentrations of both *trans*-DCCA (121.4 ng/mL) and 4F3PBA (22.5 ng/mL) of the entire population (adults and children), and the highest urinary concentration of 3PBA (44.8 ng/mL) of the adults.

Floor Wipe Concentrations

The distribution of insecticides and metabolites in floor wipes from all homes, combining data from both homes with young children and homes with older adults, participating inSUPERB has been previously reported (Clifton et al. 2013). Measurements were available from 81 out of 88 household samples; and the distribution of pyrethroids and metabolites along with MDL values (0.0012 to 0.0038 ng/cm^2 for parent pyrethroids and 0.0059 to 0.011 ng/cm^2 for metabolites) are presented in Table 3. Chlorpyrifos (99%), *cis*- and *trans*-permethrin (97.5%) and bifenthrin (59.3%) were frequently detected, with the remaining compounds only detected in 2.5 – 14.8% of samples. Although chlorpyrifos was the most frequently detected, the mean concentration (0.0014 ng/cm^2) was over an order of magnitude lower than either *cis*- or *trans*-permethrin. 3PBA was the most frequently detected pyrethroid metabolite (98.7%). 4F3PBA was also detected with moderate frequency (34.2%). DCCA was detected in only 2.5% of samples, and DBCA was not detected in any samples.

Floor Wipe vs. Urine Concentrations

Multiple regression analyses were performed, using data from children and adults, in which log-transformed volume-based 3PBA urinary concentrations were regressed on log-transformed creatinine concentrations and log-transformed individual floor wipe concentrations of individual pyrethroid and pyrethroid metabolites. Parent pyrethroids that metabolize to 3PBA and some metabolites in floor wipes were included as independent variables in this analysis. Because several commercially available residential insecticide products contain a mixture of pyrethroids, correlations between 3PBA and the other metabolites (from the household floor wipes) were investigated. A similar regression analysis in which log-transformed volume-based TCPy urinary concentrations were regressed on log-transformed creatinine concentrations and log-transformed chlorpyrifos floor wipe concentrations was also performed for adults and children.

Floor wipe concentrations of *cis*-permethrin (p = 0.0004), *trans*-permethrin (p = 0.0003), and 4F3PBA (p = 0.049) were significant predictors of urinary concentrations of 3PBA in children. The summed total of pyrethroid concentrations (p = 0.001), as well as the summed total of parent pyrethroids plus 3PBA concentrations found in the floor wipes (p = 0.009) were also significant predictors of urinary concentrations of 3PBA in children. The linear regression coefficients can be seen in Table 4. When data from the one child with the highest concentration of 3PBA (89.7 ng/mL, almost 20 times higher than the 95th percentile for the SUPERB population) was removed from the analysis there was no longer a significant effect of 4F3PBA from floor wipe samples on 3PBA urinary concentrations, but relationships with parent compounds *cis*-permethrin (adjusted geometric mean ratio (agmr) = 1.30, 95% CI: 1.09 – 1.55, p = 0.004), *trans*-permethrin (agmr = 1.32 , 95% CI: 1.10 – 1.60, p = 0.004), and the total summed concentrations of pyrethroids that metabolize to 3PBA (agmr = 1.26 , 95% CI: 1.06 – 1.50, p = 0.01) remained significant. In contrast, concentrations of chlorpyrifos in the floor wipes were not significantly predictors of urinary concentrations of TCPy for children (95% CI: -0.46-0.21, p = 0.47).

None of the parent pyrethroids detected in floor wipes were significant predictors of urinary concentrations of 3PBA in adult participants. Only 4F3PBA in floor wipe samples had a marginal effect on adult urinary concentrations of 3PBA (agmr = 1.13, 95% CI: 0.99 – 1.28, p =

0.07). Concentrations of chlorpyrifos in floor wipes were also not significant predictors of log-transformed urinary concentrations of TCPy for adults (95% CI: -0.37-0.31, $p = 0.87$).

Due to low detection frequencies of *trans*-DCCA, *cis*-DBCA and 4F3PBA in urine samples, we did not report the full results of regression analyses comparing these concentrations to floor wipe levels. In the homes of the 8 adults with detectable levels of *trans*-DCCA, a metabolite of permethrin, cypermethrin and cyfluthrin, two homes had detectable levels of cypermethrin and seven homes had detectable levels of permethrin in the floor wipes. Creatinine-adjusted geometric mean concentrations of *trans*-DCCA were marginally (but not statistically significantly) higher in adults whose homes had detectable levels of cypermethrin (agmr = 1.15, 95% CI: 0.99-1.32, $p = 0.06$) in the floor wipes and in adults whose homes had detectable levels of permethrin (agmr = 1.14, 95% CI: 0.94-1.37, $p = 0.18$) in the floor wipes. These data suggest that these adult participants were possibly exposed to pyrethroids in their home. The child with the highest urinary concentration of *trans*-DCCA (111.3 ng/mL) lived in the home with the highest floor wipe concentration of total permethrin (2.7 ng/cm^2), suggesting the possibility that this child's exposure came from the home.

The metabolite *cis*-DBCA, specific to deltamethrin, was detected in the urine of only one adult and two children. However, neither deltamethrin nor *cis*-DBCA was detected in the floor wipe samples from these households, suggesting that exposure came from other sources.

Five of the eight adults and two of the three children with detectable levels of 4F3PBA, the specific metabolite of cyfluthrin, resided in the 27 homes with detectable levels of 4F3PBA in the floor wipes. Creatinine-adjusted geometric mean concentrations of 4F3PBA, were higher, but not significantly higher, in adults (agmr = 1.07, 95% CI: 0.99-1.15, $p = 0.0996$) and children (agmr = 1.03, 95% CI: 0.99-1.07, $p = 0.17$) whose homes had detectable levels of 4F3PBA.

Cyfluthrin was detected in only three homes, one of which had a child with detectable urinary 4F3PBA. Creatinine-adjusted geometric mean concentrations of 4F3PBA were significantly higher in children (agmr = 1.10, 95% CI: 1.03-1.17, p = 0.006) whose homes had detectable levels of cyfluthrin. These data suggest that some, but not all, of the participants with evidence of cyfluthrin exposure may have been exposed to it and/or its metabolite in their home environment.

Discussion

Measurements from 90 northern California households show pyrethroid exposure occurs commonly, based on the frequent detection of several pyrethroid metabolites in participants' urine. A portion of this exposure is likely to result from indoor residential applications or track-in from outdoor residential applications, as bifenthrin, *cis*-permethrin and *trans*-permethrin were measured in the majority of floor wipe samples. The detection of the non-specific pyrethroid metabolite 3PBA in urine may also be a result of exposure to 3PBA in the home environment, as this metabolite was measured in the majority of floor wipe samples.

The median creatinine-adjusted urinary concentrations of 3PBA in the 2001–2002 National Health and Nutrition Examination Survey (NHANES), a population based sample, were 0.33 and 0.30 µg/g for children (aged 6-11 years) and adults (aged 20-59 years), respectively (Table 2) (Barr et al. 2010). The median creatinine-adjusted urinary 3PBA concentrations in the SUPERB participants, recruited in 2007-2009, were over twice as high at 0.80 and 0.61µg/g creatinine for children and adults, respectively. Similarly, the SUPERB population has higher 75th and 95th percentiles urinary concentrations of 3PBA than the 2001-2002 NHANES population (Table 2). The higher urinary concentrations of 3PBA in SUPERB participants are potentially due to the well-documented increased use of pyrethroids for indoor and outdoor residential applications since the 2001 federally mandated phase-out of residential uses of the OP insecticides chlorpyrifos and diazinon (Horton et al. 2011; USEPA 2000, 2012; Williams et al. 2008).

Although SUPERB participants show increased levels of 3PBA as compared to NHANES, they are still lower than other populations reported in the literature. A 2001 biomonitoring study in Jacksonville, FL, a city previously determined to have elevated rates of pesticide use, showed median urinary 3PBA levels of 2.5 ug/g in children aged 4-5 years (Naeher et al. 2010), triple the median urinary 3PBA levels found SUPERB children. The Children's Environmental Health Study following a multiethnic urban cohort in New York City from 1998 to 2001 reported median urinary 3PBA levels of 19.3 ug/g in pregnant adult women (Berkowitz et al. 2003), although it was suspected that sumithrin sprayed in the area during sampling may have contributed to these findings.

We saw similar creatinine-adjusted urinary TCPy concentrations in SUPERB adults (median = 0.88 µg/g, 75^{th} percentile = 2.34 µg/g, 95^{th} percentile = 3.63 µg/g) as in adult NHANES participants in 2001-2002 (median = 1.33 µg/g, 75^{th} percentile = 2.37 µg/g, 95^{th} percentile = 6.42 µg/g) (Barr et al. 2005a). OP insecticide use is still present in agriculture thus exposure to these insecticides through the food pathway continues. The creatinine-adjusted urinary TCPy concentrations in SUPERB children were lower (median = 2.53 µg/g, 75^{th} percentile = 4.81 µg/g, 95^{th} percentile = 7.72 µg/g) than in children from NHANES in 2001-2002 (median = 3.20 µg/g, 75^{th} percentile = 6.37 µg/g, 95^{th} percentile = 14.0 µg/g) (Barr et al. 2005a). The level in children could have decreased over time due to reduced agricultural use for foods highly consumed by children (USEPA 2000). Another potential cause for this reduction is that a greater percentage of a child's exposure is through the indoor home pathway than for adults and exposure through this pathway has likely been reduced since the 2001–2002 NHANES samples were collected. This coincides with the almost ten-fold difference of the mean concentration of chlorpyrifos in floor wipes collected from SUPERB homes of 0.0014 ng/cm^2, than that measured in the American Healthy Homes Survey's national study of residential pesticides, measured from floor wipes from 1131 homes in 2005-2006, of 0.01 ng/cm^2 (Stout et al. 2009), although other factors, such as differences in insecticide use practices between the populations, may have also contributed to the lower concentrations found in SUPERB homes. Still, chlorpyrifos was detected in nearly 100% of SUPERB homes, indicating that residential exposure to this insecticide that persists in the indoor environment will continue (Shin et al. 2012).

Urinary concentrations of 3PBA from adults and children within a household were positively correlated. There were also a few cases of overlap with other urinary metabolites, specifically one household with both parent and child having detectable urinary concentrations of *cis*-DBCA, and another household with both parent and child having detectable urinary concentrations of 4F3PBA. These data suggest that parents and children in these households are likely being exposed to common sources of pyrethroids, either from the home or a common diet, although discrepancies in other homes may point to additional sources of exposure, or from differences in diet.

Floor wipe pyrethroid concentrations were correlated with urinary concentrations of 3PBA in children, but not adults. This finding suggests that children may be receiving a higher portion of their exposure to pyrethroids from their home environment than adults. Once pyrethroid insecticides have entered the home, the carpets and cushioned furniture act as sources and sinks for insecticides (Starr et al. 2008). The presence of insecticides in carpet dust is a particular concern for young children who, due to their continual exploration of their environments, spend a large amount of time on the floor and have extensive hand-to-mouth activity, resulting in increased exposure to pollutants through dermal and non-dietary ingestion routes (Cohen Hubal et al. 2000). Even after children have reached an age where hand-to-mouth activity has significantly reduced, they still spend a large amount of time doing activities on the floor, and are therefore still susceptible to higher insecticide exposure than adults.

Urinary concentrations of TCPy from adults and children within a household were positively correlated. In contrast to pyrethroids, floor wipe OP concentrations were not correlated with urinary concentrations of TCPY for children. These data suggest that at the current time parents and children in these households are likely being exposed to common sources of chlorpyrifos which are not from the home environment, most likely a common diet (C. Lu et al. 2006).

Although many studies have measured both indoor environmental concentrations of insecticides and urinary concentrations of metabolites, only a few have looked at correlations between those measurements, and those have focused on OP insecticides with studies conducted both when OPs were still applied indoors (Bradman et al. 2007; Rothlein et al. 2006), and after the federally mandated phase-out of residential OP use (Quirós-Alcalá et al. 2012). The total urinary concentrations of diethyl phosphate metabolites (nmol/L), the common metabolite of OP insecticides, measured in overnight urine collected from diapers of 20 farm worker children aged 5-27 months in Salinas Valley, CA from June to September, 2002, were significantly correlated with diazinon ($p < 0.05$) measured in house dust and chlorpyrifos ($p < 0.05$) measured in toy wipe samples (Bradman et al. 2007). A study looking at OP insecticide exposure among 93 Hispanic workers in the agricultural community of Hood River, Oregon in the summer and fall of 1999 reported a moderate but significant correlation between methyl OP insecticides measured in house dust and their urinary metabolites (Rothlein et al. 2006). In contrast the OP insecticides chlorpyrifos and diazinon levels measured in the house dust of 40 urban (Oakland, CA) and farmworker (Salinas, CA) homes from July to September of 2006 were not significantly correlated to urinary concentrations of the metabolites in children aged 3-6 years in a study looking at OP breakdown products in house dust and children's urine (Quirós-Alcalá et al. 2012). Levels of OP insecticides are expected to be higher in the dust of farm worker homes than in homes of the general population due to drift from agricultural applications (Harnly et al. 2009). As OPs are applied agriculturally in large quantities, there is exposure through dietary ingestion as well. Due to higher indoor levels in farm worker homes, a greater portion of the exposure results from the indoor environment than for the general population, potentially explaining why previous reports of correlations between indoor OP levels and urinary concentrations of OP metabolites were limited to agricultural regions. We expect higher and more consistent correlations between pyrethroid levels within these CA homes and urinary pyrethroid metabolites, as less pyrethroids are used in CA agriculture than chlorpyrifos (CDPR 2011), lessening the exposure from dietary ingestion. Results from the present study and multiple other reports for a wide range of communities support this hypothesis: data on indoor levels of pyrethroid insecticides and urinary concentrations of metabolites indicate residential insecticide use to be one of the most important contributors to pyrethroid exposures (C. Lu et al. 2006; Naeher et al. 2010; Tulve et al. 2011; Zartarian et al. 2012).

There were several limitations to this study. For this exploratory analysis, we evaluated multiple candidate exposure-outcome associations simultaneously because each comparison was of independent scientific interest. This allowed us to control the type 1 error rate at 5% on a per-comparison basis, but confirmation of our results in other studies is necessary to support the associations we report. Although our sample size was moderately large, our study was not powered to detect more modest exposure-outcome associations that may still be clinically significant. We had relatively low detection frequencies of several target analytes: allethrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, λ-cyhalothrin, sumithrin, tetramethrin, DCCA and DBCA in the floor wipes; and *trans*-DCCA, *cis*-DBCA and 4F3PBA in urine samples; making it difficult to achieve precise estimates of correlation parameters. This population was highly educated, 90% of the adults reported having a high school diploma, and 60% reported having a 4-year college degree or higher, making them less than representative of a more diverse population. Also, there is often variability in urinary concentrations of metabolites of non-persistent compounds like pyrethroids when only single spot urine samples are collected. Further, the use urinary biomarkers to estimate children's exposures to pesticides in their environment may be questionable (Morgan et al. 2010).

Despite its limitations, this study contributes to existing research in multiple ways. Results presented here provide further evidence that children are being exposed to pyrethroid insecticides in their home. Additionally, a comparison of measured levels in this study vs. those reported for samples collected 2001-2002 is consistent with increased use of pyrethroids and decreased use of OPs for residential applications since the 2001 federally mandated phase-out of residential uses of chlorpyrifos and diazinon. Further research is warranted to investigate the sources of these exposures to pyrethroid insecticides.

Disclaimer

The use of trade names is for identification only and does not constitute endorsement by the US Department of Health and Human Services or the CDC. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the CDC. This work has not, as of yet, been subjected to US EPA administrative review, and is therefore not currently approved for publication.

References

Arcury TA, Grzywacz JG, Barr DB, Tapia J, Chen H, Quandt SA. 2007. Pesticide urinary metabolite levels of children in eastern North Carolina farmworker households. Environ Health Perspect 115: 1254-1260.

Barr DB, Allen R, Olsson AO, Bravo R, Caltabiano LM, Montesano A, et al. 2005a. Concentrations of selective metabolites of organophosphorus pesticides in the United States population. Environ Res 99: 314-326.

Barr DB, Olsson AO, Wong L-Y, Udunka S, Baker SE, Whitehead RD, Jr., et al. 2010. Urinary Concentrations of Metabolites of Pyrethroid Insecticides in the General U.S. Population: National Health and Nutrition Examination Survey 1999-2002. Environ Health Perspect 118: 742-748.

Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. 2005b. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect 113: 192-200.

Berkowitz G, 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.

Bradman A, Whitaker D, Quiros L, Castorina R, Henn BC, Nishioka M, et al. 2007. Pesticides and their metabolites in the homes and urine of farmworker children living in the Salinas Valley, CA. J Expo Sci Environ Epidemiol 17: 331-349.

CDPR. 2011. The top 100 pesticides used pounds of active ingredients statewide in 2010 (all sites combined). Sacramento, CA.

Clifton MS, Wargo JP, Weathers WS, Colon M, Bennett DH, Tulve NS. 2013. Quantitative Analysis of Organophosphate and Pyrethroid Insecticides, Pyrethroid Transformation Products, Polybrominated Diphenyl Ethers and Bisphenol A in Residential Surface Wipe Samples. Journal of Chromatography A 1273: 1-11.

Cohen Hubal EA, Sheldon LS, Burke JM, McCurdy TR, Berry MR, Rigas ML, et al. 2000. Children's Exposure Assessment: A Review of Factors Influencing Children's Exposure, and the Data Available to Characterize and Assess That Exposure. Environ Health Perspect 108: 475-486.

Costa L, Giordano G, Guizzetti M, Vitalone A. 2008. Neurotoxicity of pesticides: a brief review. Front Biosci 13: 1240-1249.

Davis MD, Wade EL, Restrepo PR, Roman-Esteva W, Bravo R, Kuklenyik P, et al. 2013. Semi-Automated Solid Phase Extraction Method for the Mass Spectrometric Quantification of 12 Specific Metabolites of Organophosphorus Pesticides, Synthetic Pyrethroids, and Select Herbicides in Human Urine. Journal of Chromatography B.

Emara AM, Draz EI. 2007. Immunotoxicological study of one of the most common over-the-counter pyrethroid insecticide products in Egypt. Inhal Toxicol 19: 997-1009.

Harnly ME, Bradman A, Nishioka M, McKone TE, Smith D, McLaughlin R, et al. 2009. Pesticides in dust from homes in an agricultural area. Environ Sci Technol 43: 8767-8774.

Hertz-Picciotto I, Cassady D, Lee K, Bennett D, Ritz B, Vogt R. 2010. Study of Use of Products and Exposure-Related Behaviors (SUPERB): study design, methods, and demographic characteristics of cohorts. Environ Health 9.

Horton MK, Jacobson JB, McKelvey W, Holmes D, Fincher B, Quantano A, et al. 2011. Characterization of residential pest control products used in inner city communities in New York City. J Expo Sci Environ Epidemiol 21: 291-301.

Ji G, Xia Y, Gu A, Shi X, Long Y, Song L, et al. 2011. Effects of non-occupational environmental exposure to pyrethroids on semen quality and sperm DNA integrity in Chinese men. Reprod Toxicol 31: 171-176.

Lu C, Barr D, Pearson M, Bartell S, Bravo R. 2006. A longitudinal approach of assessing urban and suburban children's exposure to pyrethroid pesticides. Epidemiology 17: S406-S407.

Lu C, Toepel K, Irish R, Fenske RA, Barr DB, Bravo R. 2006. Organic diets significantly lower children's dietary exposure to organophosphorus pesticides. Environ Health Perspect 116: 260-263.

Morgan MK. 2012. Children’s Exposures to Pyrethroid Insecticides at Home: A Review of Data Collected in Published Exposure Measurement Studies Conducted in the United States. Int J Environ Res Public Health 9: 2964-2985.

Morgan MK, Sheldon LS, Jones PA, Croghan CW, Chuang JC, Wilson NK. 2010. The reliability of using urinary biomarkers to estimate children's exposures to chlorpyrifos and diazinon. J Expo Sci Environ Epidemiol 21: 289-290.

Naeher LP, Tulve NS, Egeghy PP, Barr DB, Adetona O, Fortmann RC, et al. 2010. Organophosphorus and pyrethroid insecticide urinary metabolite concentrations in young children living in a southeastern United States city. Sci Total Environ 408: 1145-1153.

Olsson AO, Baker SE, Nguyen JV, Romanoff LC, Udunka SO, Walker RD, 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.

Quirós-Alcalá L, Bradman A, Smith K, Weerasekera G, Odetokun M, Barr DB, et al. 2012. Organophosphorous pesticide breakdown products in house dust and children's urine. J Expo Sci Environ Epidemiol 22: 559-568.

Rothlein J, Rohlman D, Lasarev M, Phillips J, Muniz J, McCauley L. 2006. Organophosphate Pesticide Exposure and Neurobehavioral Performance in Agricultural and Nonagricultural Hispanic Workers. Environ Health Perspect 114: 691-696.

Ryan CL, Siebens J. 2012. Educational Attainment in the United States: 2009. Available: http://www.census.gov/prod/2012pubs/p20-566.pdf [accessed April 2013].

Shin HM, McKone TE, Tulve NS, Clifton MS, Bennett DH. 2012. Indoor Residence Times of Semivolatile Organic Compounds: Model Estimation and Field Evaluation. . Environ Sci Technol Provisionally accepted with minor revision in ES&T.

Starr J, Graham S, Stout D, Andrews K, Nishioka M. 2008. Pyrethroid pesticides and their metabolites in vacuum cleaner dust collected from homes and day-care centers. Environ Res 108: 271-279.

Stout DMn, Bradham KD, Egeghy PP, Jones PA, Croghan CW, Ashley PA, et al. 2009. American Healthy Homes Survey: a national study of residential pesticides measured from floor wipes. Environ Sci Technol 43: 4294-4300.

Taylor JK. 1987. Quality Assurance of Chemical Measurements. Chelsea, MI,: Lewis Publishers:.

Tulve NS, Egeghy PP, Fortmann RC, Xue JP, Evans J, Whitaker DA, et al. 2011. Methodologies for estimating cumulative human exposures to current-use pyrethroid pesticides. Journal of Exposure Science and Environmental Epidemiology 21: 317-327.

USEPA. 2000. Chlorpyrifos Revised Risk Assessment and Agreement with Registrants. Available: http://www.ibiblio.org/london/NAFEX/message-archives/old/pdf00000.pdf [accessed May 2013].

USEPA. 2006. Permethrin Facts (Reregistration Eligibility Decision (RED) Fact Sheet). Available: http://www.epa.gov/oppsrrd1/REDs/factsheets/permethrin_fs.htm#health [accessed October 2011].

USEPA. 2012. Pesticides: Regulating Pesticides: Pyrethroids & Pyrethrins Available: http://www.epa.gov/oppsrrd1/reevaluation/pyrethroids-pyrethrins.html July 9, 2012].

Williams MK, Rundle A, Holmes D, Reyes M, Hoepner LA, Barr DB, et al. 2008. Changes in pest infestation levels, self-reported pesticide use, and permethrin exposure during pregnancy after the 2000-2001 U.S. Environmental Protection Agency restriction of organophosphates. Environ Health Perspect 116: 1681-1688.

Zartarian V, Xue JP, Glen G, Smith L, Tulve N, Tornero-Velez R. 2012. Quantifying children's aggregate (dietary and residential) exposure and dose to permethrin: application and evaluation of EPA's probabilistic SHEDS-Multimedia model. Journal of Exposure Science and Environmental Epidemiology 22: 267-273.

Table 1. Socio-demographic characteristics of participants, SUPERB 2007–2009.

Adult Characteristics (N = 90)	**N (%)**
Sex	
Female	81 (90)
Male	9 (10)
Age Group	
18-34 years	26 (29)
35-54 years	62 (69)
55+ years	2 (2)
Mean Age of Primary Adult participant	36.6
Education Level	
0-11 years	0 (0)
12 years	8 (9)
13-15 years	27 (30)
16 years	31 (34.4)
>16 years	23 (26)
Missing	1 (1)
Employment Status	
Employed	38 (43)
Stay at home parent	43 (48)
Unemployed	1 (1)
Other	6 (7)
Missing	1 (1)
Race/Ethnicity	
Non-Latino White	60 (67)
Latino (of any race)	13 (14)
African American	2 (2)
Asian	7 (8)
Other	4 (4)
Multiple	3 (3)
Missing	1 (1)
Foreign Born	
Yes	20 (22)

No	69 (77)
Missing	1 (1)
Marital Status	
Married/ Living together	89 (99)
Widowed/Divorced/Separated/Single	0 (0)
Missing	1 (1)
Homeowner	
Yes	71 (79)
No	18 (20)
Missing	1 (1)
Number of Children in the family/ living in home	
1 - 2	67 (74)
3 - 4	20 (22)
5 - 6	2 (2)
Missing	1 (1)
Child Characteristics (N = 90)	
Sex	
Female	45 (50)
Male	45 (50)
Age Group	
2-3.9 years	18 (20)
4-5.9 years	38 (42)
6-7.9 years	31 (34)
8-8.1 years	3 (3)
Mean Age of Child Participant (years)	5.5

Table 2. Volume based (ng/mL) and creatinine-adjusted (μg/g) 3PBA and TCPy urinary concentrations from SUPERB (2009) and NHANES (2001-2002).

		3PBA					TCPy				
				Selected Percentiles					Selected Percentiles		
		n	**% detection frequency**	**50th**	**75th**	**95th**	**n**	**% detection frequency**	**50th**	**75th**	**95th**
Total Population	SUPERB*										
	Volume-based	173	62	0.79	1.56	5.34	173	65	1.47	2.88	5.86
	Creatinine-adjusted			0.76	1.77	9.04			1.51	0.30	0.06
	NHANES**										
	volume based	1,994	75	0.27	0.70	2.54	1,994	91	1.70	3.50	9.90
	creatinine adjusted			0.29	0.60	3.35			1.47	2.85	5.43
Children	SUPERB*, aged 2-8										
	Volume-based	83	60	0.75	1.56	4.69	83	74	1.70	3.19	5.70
	Creatinine-adjusted			0.80	2.34	6.26			2.53	0.048	0.077
	NHANES**, aged 6-11										
	Volume-based	580	75	0.30	0.76	3.38	481	97	2.70	6.90	16.0
	Creatinine-adjusted			0.33	0.66	3.04			3.20	6.37	14.0
Adults	SUPERB*, aged 18-57										
	Volume-based	90	64	0.82	1.58	9.44	90	57	1.22	2.57	6.08
	Creatinine-adjusted			0.61	1.39	12.61			0.88	0.02	0.036
	NHANES**, aged 20-59										
	Volume- based	1,128	76	0.27	0.67	3.25	832	89	1.50	2.80	8.60
	Creatinine-adjusted			0.30	0.60	3.43			1.33	2.37	6.40

* The limits of detection (LODs) were 0.58–0.75 ng/mL (3PBA), and 0.62–1.17 ng/mL (TCPy)
** The LODs were 0.1 ng/mL (3PBA), and 0.4 ng/mL (TCPy)

Table 3. Insecticide and metabolite concentrations (ng/cm^2) in floor wipes, SUPERB 2009.

Compound	**N**	**MDL**	**% D**	**Mean**	**Median**	**75th**	**95th**	**Max**
Chlorpyrifos	81	0.000087	98.7	0.0014	0.00048	0.00088	0.0038	0.033
cis-Permethrin	81	0.0016	97.5	0.048	0.0074	0.024	0.21	0.8
trans-Permethrin	81	0.002	97.5	0.072	0.01	0.041	0.3	1.95
Bifenthrin	81	0.0012	59.3	0.0068	0.00058	0.0029	0.035	0.15
Cypermethrin	81	0.0038	14.8	-	ND	ND	0.14	0.54
Tetramethrin	81	0.0012	9.9	-	ND	ND	0.0029	0.018
Allethrin	81	0.0034	7.4	-	ND	ND	0.0093	0.29
Sumithrin	81	0.0012	6.2	-	ND	ND	0.004	0.19
Cyfluthrin	81	0.0021	3.7	-	ND	ND	ND	0.13
Deltamethrin	81	0.0029	2.5	-	ND	ND	ND	0.043
Esfenvalerate	81	0.0028	2.5	-	ND	ND	ND	0.0081
λ-Cyhalothrin	81	0.0031	2.5	-	ND		ND	0.062
3PBA	79	0.0059	98.7	-	0.0014	0.0025	0.0047	0.0076
4F3PBA	79	0.0068	34.2	-	ND	0.00033	0.00066	0.0017
DCCA	79	0.0085	2.5	-	ND	ND	ND	0.0055
DBCA	79	0.011	0	-	-	-	-	-

N=Number; MDL=Method Detection Limit; %D=Detection Frequency; Max=Maximum; ND=non-detect

Table 4. Estimated coefficients from multiple linear model analyses showing the relationship between child log-transformed volume-based urinary concentrations of 3PBA (dependent variable) and log-transformed floor wipe concentrations.*

Compound	N	Estimate	Standard Error	t Value	Pr > |t|
Creatinine	76	1.07	0.25	4.35	**<.0001**
Cypermethrin	76	0.06	0.07	0.91	0.47
Deltamethrin	76	-0.07	0.21	-0.35	0.66
Esfenvalerate	76	0.26	0.25	1.05	0.34
λ-Cyhalothrin	76	-0.04	0.21	-0.2	0.79
cis-Permethrin	76	0.26	0.09	3.02	**0.0004**
trans-Permethrin	76	0.28	0.09	2.98	**0.0003**
Sumithrin	76	0.09	0.12	0.72	0.50
Pyrethroid Total	76	0.29	0.09	3.34	**0.001**
Pyrethroid Total	75	0.26	0.10	2.70	**0.009**
trans-DCCA	75	0.26	0.36	0.74	0.43
3PBA	75	0.15	0.2	0.79	0.41
4F3PBA	75	0.1	0.06	1.59	**0.049**
Metabolite Total	75	0.22	0.19	1.14	0.20

*Separate linear regression models were fit for each of the listed compounds, each contained two independent variables: log-transformed creatinine and the listed log-transformed compound.

Supplemental Material

Table S1. Number of samples with detectable urinary concentrations of t*rans*-DCCA, *cis*-DBCA and 4F3PBA and range of concentrations both volume-based (ng/mL) and creatinine-adjusted (μg/g) from a subset of SUPERB participants recruited in 2009.

Category (n)	**# D**		**Min**	**Max**
Children (83)				
trans-DCCA	2	Volume-based	10.0	111
		Creatinine-adjusted	26.2	77.2
cis-DBCA	2	Volume-based	1.09	1.48
		Creatinine-adjusted	0.87	0.60
4F3PBA	3	Volume-volume based	0.49	0.77
		Creatinine-adjusted	0.99	0.77
Adults (90)				
trans-DCCA	8	Volume-based	4.99	121
		Creatinine-adjusted	2.79	119
cis-DBCA	1	Volume-based		1.32
		Creatinine-adjusted		1.03
4F3PBA	8	Volume-based	0.43	22.4
		Creatinine-adjusted	0.13	22.1