Journal of Exposure Science and Environmental Epidemiology (2009) 19, 69–78

www.nature.com/jes

The attribution of urban and suburban children's exposure to synthetic pyrethroid insecticides: a longitudinal assessment

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Despite the widespread use of synthetic pyrethroid insecticides that led to common exposure in the population, very few studies have been conducted to quantitatively assess human, particularly, children's, long-term exposures to pyrethroid insecticides. The objective of the Children Pesticide Exposure Study – Washington (CPES-WA) was to establish the longitudinal exposure profiles for pyrethroid insecticides in a cohort of children living in an urban and suburban community using urinary pyrethroid metabolites as exposure biomarkers. The results from this analysis will allow us to examine potential risk factors in relation to the elevated pyrethroid insecticide exposure in children. A total of 23 children, aged 3–11 years, who only consumed conventional diets were enrolled in this 1-year study. We provided organic food items to children for 5 consecutive days in the summer and fall sampling seasons. We measured urinary metabolites for the synthetic pyrethroid insecticides in urine samples that were collected twice daily during each of the four sampling seasons. 3-phenoxybenzoic acid was frequently detected in the urine samples with mean and median daily volume-weighted average levels of 1.5 and 1.2 μg/l, followed by *trans*-2,2-(dichloro)-2-dimethylvinylcyclopropane carboxylic acid (1.4 and 1.0 μg/l) and *cis*-2,2-(dichloro)-2-dimethylvinylcyclopropane carboxylic acid (0.5 μg/l, and non-detected). When we took into account season, age, sex, diet, and self-reported residential use of pyrethroid insecticides in a linear mixed-effects model, the results suggested that the combination of the use of pyrethroid insecticides in the household, dietary intake, and seasonal differences play a significant role in predicting children's exposure to synthetic pyrethroid insecticides. We found CPES-WA children were continuously exposed to pyrethroid insecticides through their diets all year long, and this chronic exposure pattern was periodically modified by episodes of relatively high exposures from residential uses. Future research should be devoted to enhancing our understanding of the complexity of pyrethroid insecticide exposure patterns.
Journal of Exposure Science and Environmental Epidemiology (2009) **19,** 69–78; doi:10.1038/jes.2008.49; published online 3 September 2008

Keywords: *children's pesticide exposure, longitudinal exposure, PBA, pyrethroid insecticides, pyrethroid metabolites, urinary biomarker.*

Introduction

Pyrethroid insecticides, a group of synthetic insecticides, are widely used in agriculture and public health programs worldwide (Heudorf and Angerer, 2001; Lothrop et al., 2007). Their availability for consumer use in the household has increased since the early 2000s (US Environmental Protection Agency, 2005) when restriction on use of organophosphorus pesticides (OPs) in residential environments in the United States was imposed (US Environmental Protection Agency, 1998). Unlike OPs, the application of pyrethroid insecticides by the public does not require professional licensing. The synthetic pyrethroid insecticides share a similar insecticidal function with the natural pyrethrins, which can be extracted from chrysanthemums, but are superior to pyrethrins in their resistance to environmental degradation and their relative potency. Substantial information about the toxicity of pyrethroid insecticides exists; however, unlike OPs, pyrethroid insecticides as a group do not impose a common toxicological mechanism in humans except for their primary mode of action on the voltage-sensitive channels in neurons. The synthetic pyrethroid insecticides are subject for review as probable developmental neurotoxicants (Shafer et al., 2005). Pyrethroid insecticides also reportedly have a suppressive effect on the immune system and the potential to cause lymph

1. Abbreviations: ATSDR, Agency for Toxic Substances and Diseases Registry; CDC, Center for Disease Control and Prevention; CPES, Children's Pesticide Exposure Study; CPES-WA, Children's Pesticide Exposure Study – Washington; CPES-GA, Children's Pesticide Exposure Study – Georgia; DBCA, *cis*-2,2-(dibromo)-2-dimethylvinylcyclopropane carboxylic acid; *cis*-DCCA, *cis*-2,2-(dichloro)-2-dimethylvinylcyclopropane carboxylic acid; *trans*-DCCA, *trans*-2,2-(dichloro)-2-dimethylvinylcyclopropane carboxylic acid; DVWA concentration, daily volume-weighted average concentration; FPBA, 4-fluoro-3-phenoxybenzoic acid; FQPA, Food Quality Protection Act; LOD, limit of detection; NHANES, National Human Nutrition and Health Survey; OP, organophosphorus pesticide; PBA, 3-phenoxybenzoic acid; US EPA United States Environmental Protection Agency

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Received 6 May 2008; accepted 30 June 2008; published online 3 September 2008

node and spleen damage (Repetto and Baliga, 1996). In addition, permethrin, the most widely used pyrethroid insecticide, is suspected as an endocrine disrupting chemical (Chen et al., 2002; Kakko et al., 2004; Kim et al., 2005) and, along with fenvalerate, has been classified as a potential carcinogen at high exposure levels (US Environmental Protection Agency, 1989). However, there are also studies that have reported little or no toxicity for pyrethroid insecticides (Kunimatsu et al., 2002; Presibella et al., 2005).

In spite of the increasing annual use of pyrethroid insecticides ranging from several thousands to a million pounds in recent years (Agency for Toxic Substances and Disease Registry, 2003), very few studies have been conducted to quantitatively assess human, particularly children's, long-term exposures to them. Most of the relevant data were obtained from studies conducted in Germany or in occupational settings (Hardt and Angerer, 2003; Leng et al., 2003). Recently, the Center for Disease Control and Prevention (CDC) reported urinary pyrethroid metabolite levels for the US population aged 6–59 years in the Third National Report on Human Exposure to Environmental Chemicals, which is derived from samples collected as a part of the National Health and Nutrition Examination Survey (NHANES) conducted in 2001–2002 (Centers for Disease Control, 2005). All of these studies were conducted in a cross-sectional setting and therefore, those results only represent exposures over relatively short-time periods and are not comparable to the data collected from this study. The objective of this article is to establish the longitudinal exposure profiles for the synthetic pyrethroid insecticides, in a cohort of elementary school-aged children who participated in the Children's Pesticide Exposure Study (CPES) using urinary pyrethroid metabolites as exposure biomarkers. The results from this longitudinal analysis will allow us to examine potential exposure risk factors in relation to the elevated pyrethroid insecticides exposure in children participating in the CPES.

Materials and methods

Study Design

This study is part of the CPES that was first conducted in Seattle (the Children Pesticide Exposure Study – Washington, CPES-WA) from 2003 to 2004, and then repeated in Atlanta, GA (CPES-GA) from 2006 to 2007. The CPES was designed to assess urban and suburban children's longitudinal exposure to a variety of pesticides, including organophosphorus insecticides, pyrethroid insecticides, and herbicides that are commonly used in the environment. Details of the study design and methods have been published previously (Lu et al., 2006). In brief, 23 elementary school children ages 3–11 recruited from local public and Montessori schools were first enrolled in the CPES-WA that included multiple consecutive-day sampling periods in summer 2003, with repeated samplings in fall 2003, winter 2004, and spring 2004. Subject eligibility for enrollment included children exclusively consuming a conventional diet and spending most of their time in one residence. Household pesticide use information was obtained via an in-person interview during an in-home appointment prior to each of the sampling periods. Written consent was obtained from parents and older children, whereas oral assent was obtained from younger children before the initiation of this study. The University of Washington Human Subject Committee approved the use of human subjects in CPES-WA (no. 03-5899), and the Emory University Internal Review Board (no. 084-2005) approved continued analysis of this dataset with a collaborative agreement with the CDC.

Each child committed to a 15- and 12-consecutive-day sampling period in the summer (July/August) and fall (October/November) 2003, respectively, and a 7-consecutive-day sampling period in the winter (January/February) and spring (April/May) 2004. In the summer and fall sampling periods, an organic diet substitution phase (from days 4 to 8 for a total of 5 consecutive days) was incorporated into the study design with the aim of assessing the contribution of daily pesticide exposures resulting from dietary intake. We substituted most of children's conventional diet with organic items based on a grocery-shopping list provided by the parents or caregivers. Those substituted food items included fresh fruits and vegetables, juices, processed fruit or vegetables (e.g. salsa), and some wheat- or corn-based items (e.g. pasta, cereal, popcorn, or chips). Children otherwise consumed their regular conventional diet for the remaining sampling days. No organic diet substitution was made during the winter and spring sampling periods. We selected a portion of organic food items that were purchased to be analyzed in one of the United States Department of Agriculture Pesticide Data Program contracted laboratories in Yakima (WA, USA) to confirm that the food items were indeed free of pesticides. No pyrethroid insecticides or other pesticides were detected in any of the organic food items analyzed.

Urine Sample Collection and Analysis

For each sampling day, we instructed each participant to collect two spot urine samples, the last void before bedtime and the first void in the next morning. We also asked each participant to collect 24-h duplicate food samples, twice in the summer and once in the fall sampling period, as well as recording daily dietary consumption information throughout the four sampling periods. During the duplicate food sampling days (a total of 3 days), two additional spot urine samples, one after lunch and the other after dinner, were collected by the participants. All urine samples were refrigerated or maintained on ice in a cooler prior to daily collection and processing in the research laboratory where the

specific gravity and volume for each urine sample was measured. Urine samples were then aliquoted and stored at −20°C until pesticide metabolite analysis was performed at the National Center for Environmental Health at the CDC in Atlanta (GA, USA) using the same analytical method for urine samples collected for the NHANES study (Olsson et al., 2004). The quality assurance and the quality control information have been reported (Centers for Disease Control, 2005). The targeted metabolites for pyrethroid insecticides included 3-phenoxybenzoic acid (PBA), 4-fluoro-3-phenoxybenzoic acid (FPBA), *cis*-2,2-(dichloro)-2-dimethylvinylcyclopropane carboxylic acid (*cis*-DCCA), *trans*-2,2-(dichloro)-2-dimethylvinylcyclopropane carboxylic acid (*trans*-DCCA), and *cis*-2,2-(dibromo)-2-dimethylvinylcyclopropane carboxylic acid (DBCA). Kuhn et al. (1999) demonstrated the relationship of chemical structures between common pyrethroid insecticides and their urinary metabolites.

Data Analysis

The limits of detection (LOD) for the metabolites are constant for each urine sample analyzed and listed in Table 1. The LOD for each metabolite is a statistically derived value in some instances, values below the LOD could be calculated. Although some of those values have greater than 100% uncertainty associated with them, calculated values would likely have less uncertainty than the imputed values. For urinary pyrethroid metabolite concentrations that were reported as detectable (>LOD) or detectable but not quantifiable (<LOD but with a measured laboratory value that met quality control measurements), laboratory reported concentrations were used for data analysis. For urine samples for which no measured laboratory value could be calculated (usually referred as non-detectable), "0" was assigned for those samples. We calculated the daily volume-weighted average (DVWA) of pyrethroid metabolites (Eq. (1)) by averaging the metabolite concentrations in the morning sample (e.g. day 3) and the previous day's bedtime sample (e.g. day 2), and then normalizing for the total volume of these two urine samples. This DVWA concentration is then considered as the estimate of day 2 exposure to pyrethroid insecticides. In cases where only one of the two urine samples was collected (97 urine samples, or approximately 5% of the total of 1854 spot urine samples were not collected), the metabolite concentration of the collected sample was used as the DVWA concentration. Urinary concentrations of pyrethroid metabolites were not adjusted by creatinine or specific gravity.

$$\mathrm{DVWA}(\mu\mathrm{g/l}) = \sum [C_i(\mu\mathrm{g/l}) \times V_i(\mathrm{ml})] / \sum [V_i(\mathrm{ml})] \quad (1)$$

where C_i is individual urinary concentration, V_i, volume of the correspondent spot urine sample.

We used a linear mixed-effects model in SPSS 13 (SPSS Inc., Chicago, IL, USA) with repeated urinary measurements to test the associations with residential pyrethroid insecticide use, diet (conventional *vs* organic diet), season, age, and sex of children to determine whether these factors would influence their longitudinal pyrethroid pesticide exposure. The children were divided into two age groups so the number of children in each age group would be

Table 1. Descriptive statistics of DVWA concentrations (μg/l) of urinary metabolites of pyrethroid insecticides measured in the CPES-WA children over a 12-month period, and the comparison to the NHANES (Centers for Disease Control, 2005) results.[a]

	Mean[b] (SD)	LOD	*N*[c]	Range[d]	Frequency[e] of detection	Percentile						
						5th	10th	25th	50th	75th	90th	95th
PBA (CPES-WA)	1.5 (3.1)	0.1	706	(0, 51.4)	94	0	0.2	0.5	1.2	1.5	2.6	4.1
PBA (NHANES)	0.33	0.1	580	NA	NA	NA	N/A	NA	0.3	0.8	1.8	3.3
FPBA (CPES-WA)	0.2 (0.4)	0.2	706	(0, 3.5)	19	0	0	0	0	0	0.9	1.2
FPBA (NHANES)	NA	0.2	580	NA	NA	NA	NA	NA	<LOD	<LOD	<LOD	<LOD
cis-DCCA (CPES-WA)	0.5 (2.0)	0.2	706	(0, 38.8)	44	0	0	0	0	0.7	1.1	1.4
cis-DCCA (NHANES)	NA	0.2	*580*	NA	NA	NA	NA	NA	<LOD	0.1	0.4	0.7
trans-DCCA (CPES-WA)	1.4 (4.1)	0.4	706	(0, 81.6)	83	0	0	0.3	1.0	1.5	2.3	3.7
trans-DCCA (CPES-WA)	NA	0.4	580	NA	NA	NA	NA	NA	<LOD	0.5	1.4	2.5
DBCA (CPES-WA)	0.007 (0.03)	0.1	706	(0, 0.2)	6	0	0	0	0	0	0	0.04
DBCA (CPES-WA)	NA	0.1	580	NA	NA	NA	NA	NA	NA	NA	NA	NA

Abbreviations: CPES-WA, Children Pesticide Exposure Study – Washington; DCCA, 2,2-(dichloro)-2-dimethylvinylcyclopropane carboxylic acid; DBCA, 2,2-(dibromo)-2-dimethylvinylcyclopropane carboxylic acid; FPBA, 4-fluoro-3-phenoxybenzoic acid; LOD, limits of detection; NA, not applicable; NHANES, National Health and Nutrition Examination Survey; PBA, 4-fluoro-3-phenoxybenzoic acid.

[a]NHANES data are cross-sectional collected from 6- to 11-year-old children, whereas CPES data represent longitudinal measurements of pyrethorids exposure.

[b]Mean values for NHANES data are geometric mean.

[c]Number of DVWA measurements. These numbers do not include the measurements from the days (5 days) when participants consumed organic diets.

[d](minimum, maximum) of concentrations.

[e]Percentage.

approximately equal (13 children, aged 3–7 and 10 children, aged 8–11).

Results

Children Pesticide Exposure Study – Washington started with 23 children, 13 males and 10 females, in August 2003 and ended with 19 children, 11 males and 8 females in May 2004. Subject attrition was due to either family reasons (moving to new locations) or the difficulty of collecting repeated specimen samples from young children. In total, we collected 1757 spot urine samples from the cohort over the 1-year period, or 724, 516, 260, and 257 urine samples from the summer, fall, winter, and spring periods, respectively. Missing samples were most obvious in the first sampling period (summer 2003) where 7% of total samples were not collected. As families became familiar with the study protocol, the loss of samples decreased to 5% for the following fall and winter sessions and 3% for the spring session.

The frequency of detection varied (Table 1) among the urinary pyrethroid metabolites. The most frequently detected was PBA, a common urinary metabolite for many pyrethroid insecticides (Heudorf and Angerer, 2001), including permethrin, cypermethrin, and deltamethrin, with 94% of urine samples containing detectable PBA levels. The second and the third most frequently detected were *trans*-DCCA (83% detection) and *cis*-DCCA (44% detection), two urinary metabolites for pyrethroid insecticides, such as permethrin, cypermethrin, and cyfluthrin, which contain the *cis*- and *trans*-isomer, respectively. Very few urine samples had detectable levels of FPBA and DCBA, the specific metabolites for cyfluthrin and deltamethrin, respectively.

Table 1 also shows the descriptive statistics of the DVWA concentrations for the five urinary pyrethroid metabolites measured in the CPES-WA children over a 1-year period. In parallel to the frequency of detection, PBA has the highest mean and median DVWA level of 1.5 and 1.2 μg/l, respectively, followed by *trans*-DCCA (1.4 and 1.0 μg/l) and *cis*-DCCA (0.5 μg/l, and non-detected). As the majority of urine samples did not contain detectable levels of FPBA, and DCBA, their data were not analyzed further. The seasonal variations of pyrethroid pesticide exposures among the CPES-WA children are shown in Table 2. The DVWA of PBA and *trans*-DCCA levels paralleled each other with lowest median concentrations for each measured in summer 2003. The DVWA concentrations of *cis*-DCCA were generally low throughout the four seasons; however, it is worth noting that the highest level of *cis*-DCCA coincided with the highest DVWA levels of both PBA and *trans*-DCCA measured in the fall season from the same child participant.

When we took into account season, age, sex, diet, and self-reported residential use of pyrethroid insecticides, together in a linear mixed-effects model using the DVWA of metabolite concentrations for each individual child as the repeated measurements, the results (Table 3) suggested that seasonality is a significant contributor to PBA and, to a lesser degree, *trans*-DCCA, levels in urine. The lowest median urinary PBA and *trans*-DCCA levels were measured in summer 2003, and

Table 2. Descriptive statistics of seasonal DVWA concentrations (μg/l) of PBA, *cis*-DCCA, and *trans*-DCCA measured in the CPES-WA children.

	Mean (SD)	N[a]	Range[b]	Percentile						
				5th	10th	25th	50th	75th	90th	95th
PBA										
Summer	1.2 (2.4)	246	(0, 25.3)	0	0.03	0.3	0.5	1.2	3.1	5.0
Fall	1.6 (4.2)	157	(0, 51.4)	0.04	0.3	0.7	1.2	1.5	2.1	3.7
Winter	2.0 (3.8)	157	(0, 41.5)	0.5	0.8	1.1	1.3	1.6	2.7	4.7
Spring	1.5 (0.8)	146	(0, 6.1)	0.5	0.8	1.1	1.3	1.6	2.4	3.0
cis-DCCA										
Summer	0.4 (1.1)	246	(0, 15.3)	0	0	0	0.03	0.4	1.0	1.4
Fall	0.7 (3.1)	157	(0, 38.8)	0	0	0	0.3	0.9	1.1	1.3
Winter	0.7 (2.4)	157	(0, 26.1)	0	0	0	0	0.9	1.2	2.1
Spring	0.3 (0.5)	146	(0, 2.0)	0	0	0	0	0.2	1.2	1.4
trans-DCCA										
Summer	1.3 (2.5)	246	(0, 25.1)	0	0.01	0.2	0.4	1.1	3.5	5.3
Fall	1.7 (6.5)	157	(0, 81.6)	0	0.1	0.8	1.2	1.4	2.0	2.5
Winter	1.8 (4.6)	157	(0, 47.2)	0	0	0.3	1.2	1.5	2.2	4.2
Spring	1.0 (0.9)	146	(0, 4.9)	0	0	0	1.2	1.6	2.0	2.4

Abbreviations: DCCA, 2,2-(dichloro)-2-dimethylvinylcyclopropane carboxylic acid; PBA, 4-fluoro-3-phenoxybenzoic acid.
[a]Number of DVWA measurements. These numbers do not include the measurements from the days (5 days) when participants consumed organic diets.
[b](minimum, maximum) of concentrations.

Table 3. Selected SPSS results of a linear mixed-effects model with the repeated measurement of DVWA of urinary PBA, *cis*-DCCA and *trans*-DCCA concentrations (μg/l) collected from the CPES-WA children over a 12-month period.

Source	Numerator d.f.	PBA		*cis*-DCCA		*trans*-DCCA	
		Denominator d.f.	F-value (Pr > F)	Denominator d.f.	F-value (Pr > F)	Denominator d.f.	F-value (Pr > F)
Intercept	1	98	0.05 (0.83)	180	<0.01 (0.97)	145	<0.01 (0.95)
Season	3	672	3.7 (0.01)[a]	643	1.7 (0.16)	654	2.5 (0.06)[b]
Age[c]	1	19	0.3 (0.57)	21	0.2 (0.71)	20	0.4 (0.56)
Sex	1	19	0.2 (0.65)	22	0.02 (0.89)	20	0.2 (0.64)
Residential							
Use (RU)	1	469	2.2 (0.14)	316	1.5 (0.22)	355	4.5 (0.04)[a]
Diet	1	673	<0.01 (0.99)	677	<0.01 (0.96)	676	<0.01 (0.93)
Age × sex	1	19	1.4 (0.26)	22	0.7 (0.41)	20	1.5 (0.24)
RU × season	3	670	2.3 (0.08)	639	1.7 (0.17)	652	2.2 (0.09)

Abbreviations: d.f., degree of freedom; DCCA, 2,2-(dichloro)-2-dimethylvinylcyclopropane carboxylic acid; Pr, probability; PBA, 4-fluoro-3-phenoxybenzoic acid.
[a]Significantly different (linear mixed-effects model using repeated measurements in SPSS).
[b]Marginal significantly different (linear mixed-effects model using repeated measurements in SPSS).
[c]Age grouped by 3- to 7- and 8- to 11-year old.

Table 4. Descriptive statistics of seasonal and year long DVWA concentrations (μg/l) of PBA, *cis*-DCCA, and *trans*-DCCA measured in the CPES-WA children whose parents reported uses of pyrethorid insecticides in the households.[a]

	Mean (SD)	*N*[b]	Min	Max	Percentile						
					5th	10th	25th	50th	75th	90th	95th
PBA											
Summer	1.9 (3.3)	77	0	25.3	0.1	0.2	0.4	0.7	2.5	4.5	5.8
Fall	2.7 (7.2)	52	0	51.4	0.1	0.2	0.5	1.3	1.8	4.4	6.2
Winter	2.0 (2.4)	56	0	12.9	0.6	0.8	1.2	1.3	2.0	2.7	5.4
Spring	1.3 (0.9)	46	0	6.1	0.3	0.6	1.0	1.2	1.5	2.0	2.2
1 year	2.0 (4.1)	231	0	51.4	0.1	0.3	0.6	1.2	1.9	3.7	5.4
cis-DCCA											
Summer	0.6 (1.9)	76	0	15.3	0	0	0	0	0.7	1.2	1.6
Fall	1.4 (5.3)	52	0	38.8	0	0	0	0.8	1.1	1.3	1.4
Winter	0.8 (1.3)	56	0	7.3	0	0	0	0.7	1.1	1.3	2.4
Spring	0.4 (0.5)	46	0	1.9	0	0	0	0	0.7	1.2	1.3
1 year	0.8 (2.8)	230	0	38.8	0	0	0	0.2	1.0	1.3	1.6
trans-DCCA											
Summer	2.0 (3.4)	77	0	25.1	0	0	0.3	0.6	2.8	4.4	7.2
Fall	2.9 (11.3)	52	0	81.6	0	0.1	0.5	1.2	1.8	2.3	2.9
Winter	1.9 (3.0)	56	0	17.3	0	0	0.6	1.3	1.7	2.9	5.6
Spring	1.3 (0.8)	46	0	3.2	0	0	0.8	1.3	1.7	2.2	2.6
1 year	2.0 (5.9)	231	0	81.6	0	0	0.4	1.2	1.9	3.3	5.3
FPBA (1 year)	0.2 (0.4)	230	0	1.4	0	0	0	0	0	0.8	1.2
DBCA (1 year)	0.02 (0.05)	231	0	0.2	0	0	0	0	0	0.1	0.2

Abbreviations: DBCA, 2,2-(dibromo)-2-dimethylvinylcyclopropane carboxylic acid; DCCA, 2,2-(dichloro)-2-dimethylvinylcyclopropane carboxylic acid; FPBA, 4-fluoro-3-phenoxybenzoic acid; PBA, 4-fluoro-3-phenoxybenzoic acid.
[a]Seven families reported uses of pyrethroid pesticides in the households.
[b]Number of DVWA measurements. These numbers do not include the measurements from the days (5 days) when participants consumed organic diets in summer and fall seasons.

as the season proceeded to fall, those levels increased accordingly (Figure 1a). Both PBA and *trans*-DCCA levels were not significantly different among fall, winter, and spring seasons. The self-reported residential use of pyrethroid pesticides continues to be a significant contributor to urinary *trans*-DCCA levels. The interaction between residential use

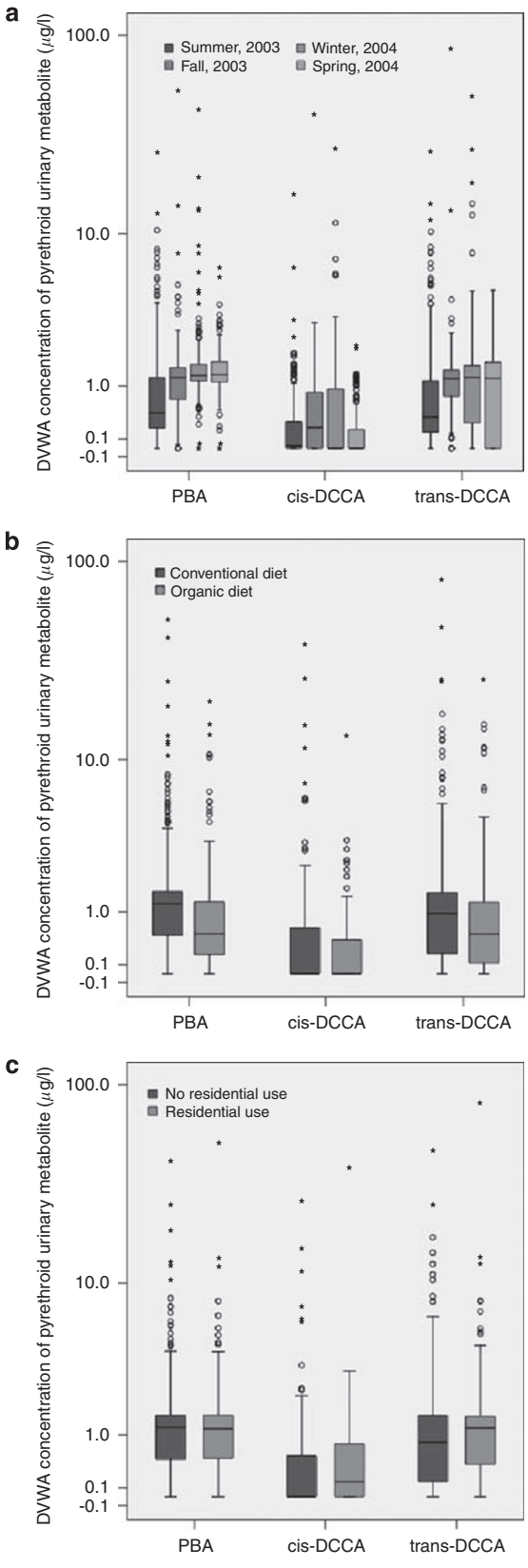
and season approaches significance as shown in Table 3. Table 4 includes the descriptive statistics for seasonal and year long DVWA concentrations of PBA, *cis*-, and *trans*-DCCA measured in children whose parents reported residential use of pyrethroid insecticides. Compared to the data presented in Tables 1 and 2, it is apparent that children living in households where pyrethroid insecticides have been used are exposed to higher levels of pyrethroid insecticides, and that the highest levels occurred during the fall, a season in which pesticides are likely to be used for the purpose of pest control (Table 4). Although the overall detection of FPBA and DBCA were very low (19% and 6%, respectively, Table 1), the majority of urine samples (44% and 95%, respectively) containing detectable FPBA and DBCA concentrations were collected from children whose parents reported use of pyrethroid insecticides in their homes (Table 4). Neither sex nor age was a significant predictor for any of the pyrethroid metabolite concentrations in urine.

Discussion

Owing to the restrictive use of OPs in residential environments imposed by the US EPA (US Environmental Protection Agency, 1998) in the early 2000s, the availability of synthetic pyrethroid insecticides to the public has increased substantially in the retail markets. However, the assessment of pyrethroid exposure using the biomarker approach in the US population, particularly, for children, is limited to a handful of cross-sectional studies, including the NHANES (Centers for Disease Control, 2005). The data that we report here were collected from the ongoing CPES, which aims to assess children's longitudinal exposures to a variety of pesticides, including pyrethroid insecticides that are commonly used in children's daily environments. The results from this longitudinal dataset provide a complete characterization of urban and suburban children's exposure to pyrethroid insecticides in over a period of 1 year and allow the examination of potential risk factors in relation to high exposure levels.

In many aspects, the findings from the analysis of the longitudinal data are consistent with those in our previous report containing data only from the summer sampling season (Lu et al., 2006). The PBA and *trans*-DCCA continue to be the most frequently detected urinary metabolites from exposures to pyrethroid insecticides (such as permethrin, cypermethrin, and deltamethrin) and pyrethroid insecticides containing *trans*-isomer of the chlorinated pyrethorids (permethrin, cypermethrin, and cyfluthrin), respectively. Either one of these two urinary metabolites is therefore considered as a good biomarker for assessing total pyrethroid insecticides exposure. If exposure to specific pyrethroid insecticides such as deltamethrin or cyfluthrin is the concern, the assessment should be focus on measuring urinary metabolite of DBCA or FPBA, respectively. Although *cis*-DCCA has a lower detection frequency comparing to its *trans*-isomer counterpart, it is still valuable to quantify its level periodically to validate the *trans*-DCCA results measured in the same urine samples. The concentration ratios of *trans*- and *cis*-DCCA should correspond to the *trans*- and *cis*-isomer content of pyrethroid pesticides (such as permethrin) in most consumer products, which range from 1.67 to 3.34. Any concentration ratio of *trans*- and *cis*-DCCA deviating from this range requires further attention for validating the results. A previous study (Leng et al., 1997) suggested that the pathway for permethrin exposure could be elucidated from the concentration ratio of *trans*- and *cis*-DCCA (ratio of 2 is from inhalation or ingestion route, whereas ratio of 1 is from dermal exposure); however, it is not possible to attribute sources of exposure for pyrethoids containing chlorinated isomers based on this suggestion when multipathway of pyrethorid exposures are fairly common.

The association of self-reported pyrethroid insecticides use by the parents and the elevated pyrethroid insecticides metabolite levels measured in their children's urine remain significant across seasons (Figure 1b). Such significance is particularly true for *trans*-DCCA but less for PBA. The close proximity between where the children lived and where the pesticides were applied, the probable frequent contact of pesticide-treated surfaces by the children, and the likelihood that pyrethroid applications were conducted by household members instead of professionals may have resulted in higher exposure for the children. This finding is echoed by an earlier report regarding the use of OPs in urban and suburban homes with children living in the same region (Lu et al., 2001). Several self-reported pyrethroid insecticides use cases in the CPES-WA households support this conclusion. For instance, the highest urinary concentrations of PBA in the summer, fall, and spring seasons and of *trans*-DCCA in all seasons (except for the second highest in the spring) were found in a 4-year-old child whose parents used several pyrethroid products containing permethrin on the child's bedding and to treat a head lice problem. The seasonal average levels of PBA and *trans*-DCCA measured in this child were approximately two to four times higher than the average concentrations measured in the other CPES-WA children whose parents reported residential use of pyrethroid insecticides and two to seven times higher than the CPES-WA children as a group. Another case involved a child who was the only participant in the CPES-WA cohort consistently exposed to deltamethrin throughout the year. Forty of the forty-two urine samples containing detectable levels of DBCA, the specific urinary metabolite for deltamethrin, were collected from this child whose parents reportedly used gardening products containing deltamethrin. Although the DBCA levels were relatively low compared to other

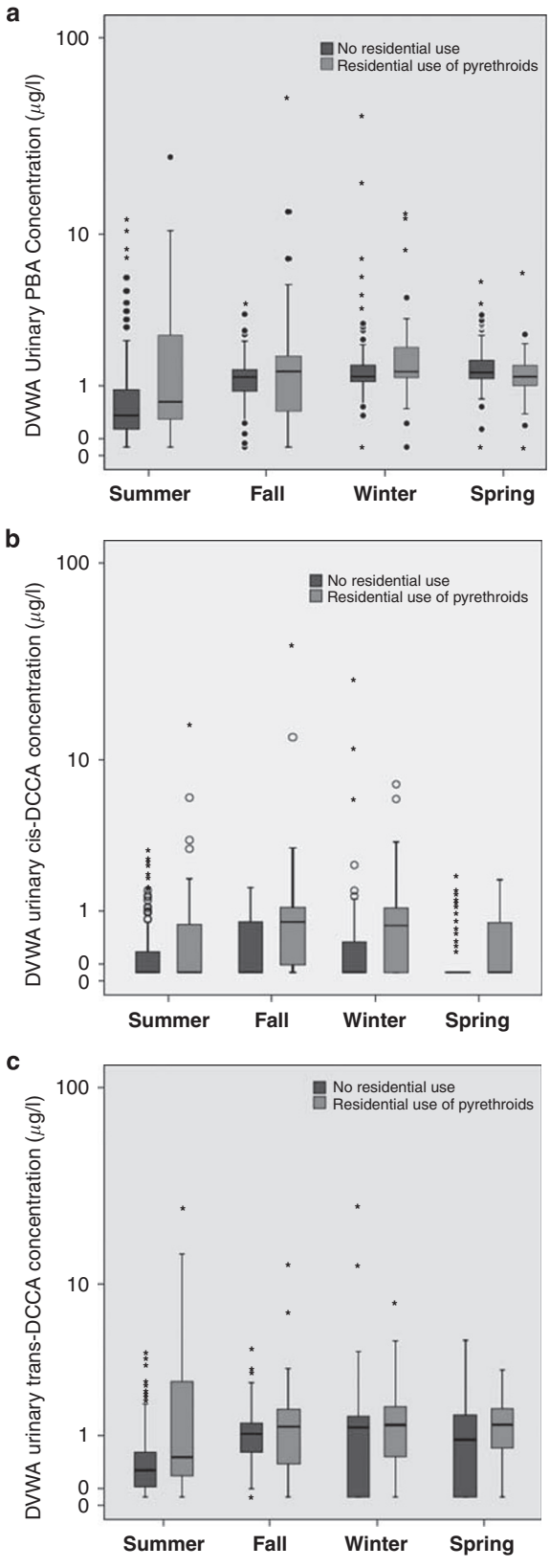


pyrethroid urinary metabolites, its presence in this child's urine was persistent.

The exposure to pyrethroid insecticides through dietary intake also represents an important pathway as evident by the boxplots in Figure 1c. However, switching children's diets to mostly organic food items alone is not sufficient to lower their pyrethroid insecticides exposure to non-detectable levels, as is the case for OPs (Lu et al., 2006, 2008). Despite that pyrethroid metabolites were still prevalent in the urine samples collected during the five organic diet days, there was an approximate 50% reduction of pyrethroid exposure during this organic diet intervention. Although it is not possible to attribute exposure to specific pyrethroid insecticides from a single pathway using common urinary biomarkers such as PBA or *trans*-DCCA, the implementation of organic food substitution in the study design, coupled with the knowledge of pyrethroid insecticides use in the homes, allows us to draw the conclusion that CPES-WA children are simultaneously exposed to pyrethroid insecticides found in foods and from the use of pyrethroid insecticides in their daily environments. Although we did not measure environmental pyrethroid insecticides levels in this study, children's exposures to pyrethroid insecticides via dietary intake (Heudorf and Angerer, 2001; Schettgen et al., 2002; Ortiz-Perez et al., 2005; Becker et al., 2006), as well as from application of pyrethroid insecticides in the residential environment (Becker et al., 2006; Morgan et al., 2007; Tulve et al., 2007) have been reported previously. Pyrethroid insecticides and OP residues in the 24-h duplicate food samples that were collected in this study are being analyzed currently and will provide better characterizations of the dietary source of pyrethroid insecticides exposure.

In other aspects, however, the current findings from the analysis of this longitudinal dataset reveal new insights that our previous report on the summer season data were not able to demonstrate or explain (Lu et al., 2006). For example, when we incorporated age with other factors in the linear mixed-effect model, its significance in predicting pyrethroid exposure, as was reported previously (Lu et al., 2006), no longer existed. Instead, seasonality became a significant predictor for urinary PBA and *trans*-DCCA levels (Table 3) in which exposures to pyrethroid insecticides were higher in the fall than the summer, with the levels remaining elevated

Figure 1. The distribution of the daily volume-weighted average concentration (DVWA) of urinary 3-phenoxybenzoic acid (PBA), *cis*-DCCA and *trans*-DCCA concentrations (μg/l) in the the Children Pesticide Exposure Study – Washington (CPES-WA) children grouped by (a) four seasons; switch (b) consumption of conventional and organic diets and (c) self-reported of residential use of pyrethroid insecticides. Boxplot: the horizontal lines in each plot represent 10th, 25th, 50th, 75th, and 90th percentiles, bottom to top. The concentration data on the *y* axis is on the log scale. "o" and "*" symbols represent outliers and the extreme values, respectively.



during the following winter and spring seasons (Figure 2a–c). The significant increase of pyrethroid insecticides exposure in the fall season is likely due to the seven CPES-WA children whose parents reported use of pyrethroid insecticides in their homes (Table 4); whereas the elevated pyrethroid insecticides exposures in the winter and spring seasons may have resulted from dietary sources. The descriptive statistics of DVWA concentrations of PBA and *trans*-DCCA for winter and spring seasons presented in Table 4 are similar to those in Table 1, suggesting residential use of pyrethroid insecticides does not contribute additional exposures to these seven children whose parents reported use of pyrethroid insecticides at home. We found a similar seasonal elevation of OP exposures in the winter and spring seasons measured in the same group of CPES-WA children (Lu et al., 2008). On the basis of daily dietary consumption information collected during the four seasons, some of the fresh produce consumed by the CPES-WA children during the winter and spring seasons, such as cantaloupe, grapes, lettuce, strawberries, tomatoes, and watermelon, may have come from imported sources. Although the evidence to support this conclusion came from a US EPA Office of Inspector General report (US Environmental Protection Agency, 2006) focusing on OPs, it is plausible to suspect that the majority of fresh produce imported to the United States during the winter and early spring seasons may also contain higher pyrethroid insecticides residues than the domestically grown produce that are generally available for consumption in the summer and fall seasons. The combination of intensive use of pyrethroid insecticides in the households in the fall season and the consumption of imported produce during the winter and spring seasons may all contribute to the seasonality of pyrethroid insecticides exposure among the CPES-WA children.

We were able to establish a chronic exposure profile based on the CPES-WA longitudinal dataset. We found CPES-WA children were exposed continuously to low levels of pyrethroid insecticides through their diets, and that this chronic exposure is periodically modified by episodes of relatively high exposures from environmental pathways, especially, when pyrethroid insecticides were used in and around the children's homes. This pattern of chronic and fluctuating exposure repeats itself in many CPES-WA children, with differences between high and low daily

Figure 2. The distribution of the seasonal daily volume-weighted average concentration (DVWA) of urinary (a) 3-phenoxybenzoic acid (PBA); (b) *cis*-DCCA; and (c) *trans*-DCCA concentrations (μg/l) in the the Children Pesticide Exposure Study – Washington (CPES-WA) children grouped by the self-reported of residential use of pyrethroid insecticides. Boxplot: the horizontal lines in each plot represent 10th, 25th, 50th, 75th, and 90th percentiles, bottom to top. The concentration data on the *y* axis is on the log scale. "o" and "*" symbols represent outliers and the extreme values, respectively.

exposures reaching as much as an 80-fold difference. Although there are abundant animal toxicological data for exposures to various pyrethroid pesticides, most of the toxicological end points or adverse health outcomes resulting from pyrethroid exposure were obtained from a single bolus dose regime. There are essentially no pyrethroid toxicological data from experiments mimicking real-life exposures, such as the fluctuating chronic exposures experienced by the CPES-WA children. A study published recently has demonstrated persistent cognitive impairments in rats following chronic dietary exposure to the common OP, chlorpyrifos, accompanied by acute doses that were sufficient to induce symptoms of toxicity (Samsam et al., 2005). Although the children in this study did not report any symptoms of acute toxicity, the exposure scenario in this animal study loosely simulates the fluctuating daily pyrethroid exposure in the CPES-WA children. The finding of the fluctuating daily pyrethroid insecticides exposure in children and the implication of this animal study emphasize the importance of assessing pesticide exposures repeatedly, in a longitudinal manner, and highlight the need to acquire toxicological data under a real-life exposure scenario to gain knowledge of links between exposures and adverse health outcomes in children.

Assessing pyrethroid insecticides exposure in a population-based setting remains a very challenging task for the following reasons. First of all, due to fewer regulatory restrictions imposed on the use of pyrethroid insecticides, this group of insecticides is routinely being applied in agriculture, as well as by consumers, for pest control purposes. Such use pattern will no doubt result in a complex multipathway exposure for pyrethroid insecticides. Therefore, a cross-sectional study attempting to characterize aggregate exposure to pyrethroid insecticides from environmental and dietary sampling may generate stochastic values, instead of measures that would reflect the true exposure. Apparently, a longitudinal exposure assessment incorporating an extensive environmental sampling scheme could overcome this limitation, and the resulting aggregate exposure data are greatly needed for evaluations required by the Food Quality Protection Act (FQPA; US Food and Drug Administration, 1996). Assessing aggregate exposure as well as the cumulative risks for pyrethroid insecticides, both required by the FQPA, using a biological monitoring approach could also be problematic. Very few pyrethroid insecticides have specific urinary biomarkers (such as DBCA for deltamethrin) that are suitable for aggregate exposure assessment, and unlike OPs, only a handful of pyrethroid pesticides share generic urinary biomarkers, such as PBA or *trans*-DCCA, that can be used for the purpose of measuring cumulative risks for pyrethroid exposures. Although PBA is considered a good urinary biomarker for the majority of pyrethroid insecticides, several commonly used pyrethroid insecticides, such as allethrin and fenvalerate, do not metabolize to PBA. Also, unlike OP or carbamate pesticides, pyrethroid insecticides, as a group, do not appear to exhibit a single common toxicological mechanism in humans. These challenges will directly impact the implementation of the FQPA for pyrethroid insecticides.

Conclusion

This report is one of the first to systematically assess young children's longitudinal exposure to synthetic pyrethroid insecticides using repeated urine sample collection over a 1-year period. We found children in the CPES-WA study continuously exposed to pyrethroid insecticides through their diets all year long, and this chronic low-level exposure pattern was periodically modified by episodes of relatively high exposures from environmental pathways – especially in children who lived in homes where pesticides were applied seasonally. Although we saw a reduction of pyrethroid insecticides exposure by changing children's diets from conventional to organic foods, the combination of the use of pyrethroid insecticides in the household, dietary intake, and seasonal differences play a significant role in predicting children's exposure to synthetic pyrethroid insecticides. The increasing use of pyrethroid insecticides both in agriculture and in the residential setting and their diverse toxicological end points require thorough regulatory reviews to safeguard public health. Future research should be devoted to enhance our knowledge in understanding the complexity of pyrethroid insecticides exposure patterns and to develop new tools, such as the analytical ability and capacity, biomarker of exposure, and physiologically based pharmacokinetic models, in assessing pyrethroid insecticides exposures at the population-based level.

Acknowledgements

This study was supported by the US Environmental Protection Agency, Science to Achieve Results program (RD-829364) and the National Center for Environmental Health in the Centers for Disease Control and Prevention, Atlanta, GA. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of US EPA or CDC. We express our sincere appreciation to the children who participated and to their parents who greatly assisted in this study. We also thank Rene Irish, Kathryn Toepel, Patrick Sande, and Richard Fenske at the University of Washington, Seattle, WA, for their assistance in conducting this study, and Paula Restrepo, Jessica Norrgran, Robert Walker, and Charles Chambers at the NCEH/CDC for their help with sample analysis and data management.

Conflict of interest

The authors declare no competing financial interests.

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