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Adult and children's exposure to 2,4-D from multiple sources and pathways

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In this study, we investigated the 2,4-dichlorophenoxyacetic acid (2,4-D) herbicide exposures of 135 preschool-aged children and their adult caregivers at 135 homes in North Carolina (NC) and Ohio (OH). Participants were randomly recruited from six NC and six OH counties. Monitoring was performed over a 48-h period at the participants' homes. Environmental samples included soil, outdoor air, indoor air, and carpet dust. Personal samples collected by the adult caregivers concerning themselves and their children consisted of solid food, liquid food, hand wipe, and spot urine samples. All samples were analyzed for 2,4-D (free acid form) by gas chromatography/mass spectrometry. 2,4-D was detected in all types of environmental samples but most often in carpet dust samples, with detection frequencies of 83% and 98% in NC and OH, respectively. The median level of 2,4-D in the carpet dust samples was about three times higher in OH homes compared to NC homes (156 *vs.* 47.5 ng/g, $P<0.0002$). For personal samples, 2,4-D was more frequently detected in the hand wipe samples from OH participants (>48%) than from NC participants (<9%). Hand wipe levels at the 95th percentile were about five times higher for OH children (0.1 ng/cm^2) and adults (0.03 ng/cm^2) than for the NC children (0.02 ng/cm^2) and adults (<0.005 ng/cm^2). 2,4-D was detected in more than 85% of the child and adult urine samples in both states. The median urinary 2,4-D concentration was more than twice as high for OH children compared to NC children (1.2 *vs.* 0.5 ng/ml, $P<0.0001$); however, the median concentration was identical at 0.7 ng/ml for both NC and OH adults. The intraclass correlation coefficient of reliability for an individual's urinary 2,4-D measurements, estimated from the unadjusted (0.31–0.62) and specific gravity-adjusted (0.37–0.73) values, were somewhat low for each group in this study. The variability in urinary 2,4-D measurements over the 48-h period for both children and adults in NC and OH suggests that several spot samples were needed to adequately assess these participants' exposures to 2,4-D in residential settings. Results from this study showed that children and their adult caregivers in NC and OH were likely exposed to 2,4-D through several pathways at their homes. In addition, our findings suggest that the OH children might have been exposed to higher levels of 2,4-D through the dermal and nondietary routes of exposure than the NC children and the NC and OH adults.
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Introduction

2,4-Dichlorophenoxyacetic acid (2,4-D) is a herbicide that is commonly used to control broadleaf weeds in agricultural and residential settings in the United States (US; Baker et al., 2000; Arbuckle and Ritter, 2005; CDC (Centers for Disease Control and Prevention), 2005; Chuang et al., 2005; USEPA, 2005). Over 40 million pounds of 2,4-D are applied annually in the United States, mainly in agriculture (~70%) and to a lesser extent at residences (~25%) on lawns and gardens (USEPA, 2005). This herbicide is used predominantly on agricultural crops in the Northwestern, Midwest, and Great Plain regions of this country (USEPA, 2004).

Little information exists on the regional use patterns for 2,4-D in residential settings. In a 2001 survey of Ohio commercial turfgrass pesticide applicators, 2,4-D was the most frequently reported herbicide and was used by 89% of the applicators (Ohio Agricultural Research and Development Center, 2001). It was also the most heavily used herbicide, comprising 33% of all pounds of herbicides applied in this Midwestern state.

Few data exist on the nonoccupational exposures of children and adults to herbicides such as 2,4-D at their homes. There is concern that young children may be exposed to higher amounts of herbicides than older children and adults living in the same households. This may be due to differences in the children's activity patterns, higher body surface to volume ratios, and greater water and food consumption per kilogram body weight compared to adults (Bearer, 1995; Chance and Harmsen, 1998).

A few studies have recently reported measurable levels of 2,4-D in several media at children's homes in the United States (Nishioka et al., 2001; Wilson et al., 2003). Wilson et al. (2003) showed that 2,4-D residues were measurable in

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soil, air, carpet dust, and food samples collected at the homes of nine preschool children in North Carolina. Nishioka et al. (2001) reported measurable concentrations of 2,4-D in carpet dust and surface wipe (floors, tabletops, and window sills) samples at several children's homes in the Midwest. This information suggests that these families were potentially exposed to measurable levels of 2,4-D through several sources at their residences.

Humans can be exposed to 2,4-D through inhalation, ingestion, and dermal routes of exposure. Once 2,4-D is absorbed into the body, it is rapidly eliminated unchanged in the urine (Knopp and Glass, 1991; Garabrant and Philbert, 2002; Chuang et al., 2005). The biological half-life of 2,4-D through oral ingestion ranges from 10 to 33 h in humans (Kohli et al., 1974; Sauerhoff et al., 1977; CDC (Centers for Disease Control and Prevention), 2005). A few studies have recently reported measuring 2,4-D (<3.5 ng/ml) in urine samples of nonoccupationally exposed adults and children in the United States (Wilson et al., 2003; CDC (Centers for Disease Control and Prevention), 2005; Curwin et al., 2005).

The temporal variability of pesticides or their metabolites measured in urine for children or adults is not well characterized (Kissel et al., 2005; Meeker et al., 2005; Scher et al., 2006). Kissel et al. (2005) showed high variability in urinary organophosphate metabolite concentrations for young children over a 24-h period at their homes in Washington state. In addition, Scher et al. (2006) recently reported that single urine voids over a 24-h period may either over- or underestimate exposures of farm families to pesticides (i.e., 2,4-D or chlorpyrifos), particularly after recent applications. This research has suggested that 24-h voids or several spot urine samples collected over the course of a day may be needed to adequately assess short-term human exposures to current-use pesticides at residences.

In the work reported here, the objectives were to quantify the distributions of 2,4-D in environmental media and personal samples for a large set of preschool-aged children and their adult caregivers at homes in NC and OH, to assess the variability of urinary 2,4-D concentrations for a subset of these children and adults over a 48-h period, and to identify the important sources (media) and pathways of the participants' exposure to this herbicide.

Methods

Study Design

The Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) study investigated the exposures of 257 preschool children and their adult caregivers to chemicals commonly found in their everyday environments in NC and OH. The participants included 135 children who stayed home during the day with their adult caregivers and 122 children who attended daycare during the day while their adult caregivers worked outside the home. The study design for the CTEPP study has been previously described by Wilson et al. (2004).

One hypothesis in CTEPP study was that children's exposures to targeted pollutants were significantly greater than those of adults living in the same household. In this work, we compare the potential exposures of 135 children (66 in NC and 69 in OH) who did not attend daycare, with their 135 adult caregivers, to 2,4-D at their homes. Briefly, preschool children, with ages 2–5 years, were randomly recruited from homes in six NC and six OH counties using a probability-based, multistage-stratified random sampling plan. The children were recruited by field staff between February 2000 and February 2001 in NC and January 2001 and November 2001 in OH. The mean ($\pm$SD) ages of the children were 42.8 ± 11.3 and 45.7 ± 10.4 months in NC and OH, respectively. The mean ages of the adults were 32.8 ± 6.0 and 33.1 ± 5.8 years in NC and OH, respectively.

This was an "observational research" study, as defined in 40 CFR Part 26.402. "Observational research" investigates the magnitude, pathways, and routes of exposure that occur as people go about their normal, everyday lives with no added exposure or manipulation of their behavior. The study protocol and the procedures to obtain the assent of the children and informed consent of their parents or guardians were reviewed and approved by an independent Institutional Review Board and complied with all applicable requirements of the Common Rule regarding additional protections for children. In addition, the research was reviewed and approved by EPA's Human Subjects Research Review Official prior to its start.

Field Sampling

A detailed description of the sampling methodology in the CTEPP study has been described earlier by Wilson et al. (2004). Field sampling occurred between July 2000 and March 2001 in NC and April 2001 and November 2001 in OH. Environmental and personal samples were collected over a 48-h period at residences. Environmental samples that were collected by field staff at the homes (one per location) included soil, outdoor air, indoor air, drinking water, and carpet dust. Drinking water samples were not analyzed for 2,4-D due to analytical constraints in the study. Personal samples collected by the adult caregivers from themselves and their children consisted of solid food, liquid food, hand wipe, and spot urine samples. Up to six spot urine voids (i.e., morning, after lunch, and before bedtime) were collected from each participant over the 48-h sampling period. Additional environmental samples were collected if any pesticide had been applied inside or outside a home within 7 days of the field monitoring. These additional samples consisted of transferable residues, hard floor surface wipes, and food preparation surface wipes. The researchers and the field team had no prior knowledge about the use of pesticides

or other chemicals in homes at the time of scheduling and any recent uses were coincidental to the scheduled visits to the home.

A total of 18 and 16 NC and OH homeowners applied a pesticide at their own discretion within 7 days of field monitoring at the residences. Only one homeowner reported using a product containing 2,4-D; the granular product was used in a garden and landscaping at the residence 3 days before field monitoring. Other data that were collected from the participants consisted of household observations, pre- and post-monitoring questionnaires, children's activity diaries, and food diaries.

Sample Analysis

Extraction and analytical procedures for 2,4-D are described in Morgan et al. (2004). The same laboratory and technical staff were responsible for the extraction and analysis of all samples. The acid form of 2,4-D was analyzed in each environmental and personal medium. The surrogate recovery standard (SRS) was ^{13}C-labeled 2,4-dichlorophenoxyacetic acid (2,4-D-^{13}C) and the internal standard was dicamba-d_3. Matrix spikes were used for 2,4-D in all media. Samples were analyzed by a gas chromatograph/mass selective detector (GC/MSD; Hewlett-Packard 6890/5973A) in the selected ion monitoring mode, equipped with an autosampler. We estimated the limit of quantitation (LOQ) based on the lowest calibration standard with a signal-to-noise ratio above 2, which was 2 ng/ml. The estimated LOQs for 2,4-D were 0.8 ng/g in soil, 8.0 ng/g in dust, 0.3 ng/m^3 in air, 0.4 ng/ml in liquid food, 0.5 ng/g in solid food, 0.01 ng/cm^2 in hand wipes, and 0.4 ng/ml in urine. The estimated instrument limit of detection (LOD) was about $\frac{1}{2}$ the reported LOQ.

Quality Assurance/Quality Control

Quality control samples ($\sim$10%) were used to assess the overall quality of sample collection, extraction, and analysis. Field blanks were collected for wipes, dust/soil, liquid food, solid food, air, and urine samples. The field blanks were below the detection limits in all the media in both states, except for one wipe and one air sample in OH; therefore, no background correction was made. The laboratory blanks were all below the method detection limit in all the media in NC and OH. The matrix spikes for 2,4-D had mean recoveries between 67% and 92% in all media from both states. The SRS for 2,4-D had percent recoveries between 50 and 142% for all media in both states. Duplicate samples (aliquots of the same sample) were analyzed for 2,4-D in dust, soil, solid food, liquid food, and urine. Relative percent differences between duplicate samples were less than 15% in all media except for two solid food samples ($<$27%) in NC. For OH, the relative percent differences were less than 13% in all media, except for one dust sample (24%). Analytical duplicates (repeat analysis of the same extract) were analyzed for 2,4-D in air, dust/soil, wipes, solid food, liquid food, and urine. Relative percent differences between analytical duplicate samples were less than 23% in all media in both states.

Statistical Analysis

We assigned all data values that were less than the LOD with the value of LOD divided by the square root of two, except for the liquid food data values, which had less than 7% detects for participants in both states (Hornung and Reed, 1990). Because of the very low detection frequency for liquid food, results below the LOD were replaced by the value of LOD divided by 10. Descriptive statistics (median, range, and select percentiles (25th, 75th, and 95th)) were calculated for each medium at the participants' homes in NC and OH. Descriptive statistics were also computed for urine samples as unadjusted (ng/ml), creatinine-adjusted (ng/mg), and specific gravity-adjusted (ng/ml) values for children and their adult caregivers. Two types of urine samples were collected: composite and noncomposite. The urine samples collected over a 48-h period were composited at the laboratory before analysis for all participants who did not have a recent pesticide application at their homes. For participants who had a recent pesticide application, their urine samples were not composited and each urine void was analyzed separately. The total volume collected for each urine sample (individual or pooled) was not recorded in this study. All composited 48-h urine samples were analyzed for creatinine and specific gravity. Noncomposited samples were analyzed only for specific gravity since these spot samples often lacked sufficient volume for creatinine analysis. In addition, we used the average urinary 2,4-D concentration for each participant who had a recent pesticide application at their home in the statistical analyses. A total of 13/18 and 15/16 participant pairs in NC and OH correctly reported the days and times they collected their urine samples. The Wilcoxon (two-sample) test was used to determine if there were statistically significant differences in distributions of concentrations for media between the two states.

The relationship between a single urinary metabolite measurement and the mean of a series of measurements from the same participant was evaluated among noncomposited samples (Fleiss, 1985). The reliability of a single measurement (i.e., a urinary 2,4-D concentration) in representing a true longer term average is a function of the variability of a series of measurements. More specifically, it is a function of the magnitude of the intraperson component of variance with respect to the total variance, or the intraclass correlation coefficient (ρ_r). A ρ_r of at least 0.80 is generally considered to indicate good reliability (Fleiss, 1985), signifying that the values are consistent from sample to sample and that a single measurement sufficiently represents the average of the series of measurements over a specific time period.

We used the following equation to estimate the "intraclass correlation coefficient of reliability" (ρ_r):

$$\rho_r = \hat{\sigma}_B^2/(\hat{\sigma}_B^2 + \hat{\sigma}_W^2),$$

where the interperson variance σ_B^2 and the intraperson variance σ_W^2 were estimated from a random effects model using mixed effects regression. Once ρ_r was estimated, the number of random measurements per person (m) necessary to rank individuals correctly within a population with a desired reliability of the mean ($\rho_{r,m}$) of 0.80 was determined by the following equation (Fleiss, 1985):

$$m = (\rho_{r,m}(1-\rho_r))/(\rho_r(1-\rho_{r,m})).$$

Variance estimates were repeated with urine values adjusted for specific gravity of the sample (SG_{sample}) using a target value (SG_{target}) of 1.022 for both children and adults (Salita et al., 1998; Carrieri et al., 2001) and the following equation (Miller et al., 2004):

$$\text{SG-adjusted value} = \text{value} \times (SG_{target} - 1.000)/(SG_{sample} - 1.000).$$

All statistical analyses were performed using SAS version 8.02.

Results

The participants in both states spent the majority of their time (~70%) indoors when at home (Morgan et al., 2004). Table 1 presents the distributions of 2,4-D in environmental media at the participants' homes. This herbicide was detected in all environmental media. 2,4-D was detected most often in the carpet dust samples (>82%). However, median levels of 2,4-D in the carpet dust samples were three times higher in OH than in NC homes, and the distributions were significantly different (156 *vs.* 47.5 ng/g, $P<0.0002$).

A total of 64 and 46% of OH and NC participants, respectively, reported past uses of herbicides at their homes. Over 97% of those using herbicides reported that the most recent use was during the current or previous year and 89% of these applications were by a household member. Many product names reported by participants were not specific enough to ascertain the active ingredients, but product types and lawn care uses that might involve broadleaf weed control agents such as 2,4-D were reported more often in OH than in NC. In addition, 2,4-D residues were detected more frequently in residential soil samples from OH (45%) than that from NC (17%).

Only one participant in OH had recently applied 2,4-D granules to their garden and landscaping 3 days before field monitoring occurred. Before this application, the homeowner had reported not using any herbicides at this residence for the past 4 years. This home had the highest levels of 2,4-D in carpet dust (21,700 ng/g) compared to all homes in this study. This carpet dust value was at least three times higher than the next highest dust value of 7390 ng/g at a home in NC. Table 2 shows the distributions of 2,4-D in personal samples of children and adults over a 48-h period in NC and OH. 2,4-D was detected at low levels in all personal media for children and adults. 2,4-D was detected in lesser than 7% of the participants' liquid food samples, and the maximum level was 0.8 ng/ml for one adult in OH. In contrast, this herbicide was detected in greater than 43% of the participants' solid food samples. The levels of 2,4-D in the solid food samples were low for all participants and the maximum value for one OH child was 20.2 ng/g. The herbicide was detected in 8% compared to 49% of the hand

Table 1. Concentrations of 2,4-D in environmental media collected at the participants' homes in North Carolina and Ohio.

Medium	State	Descriptive statistics							
		N	%[a]	Minimum	25th[b]	50th	75th	95th	Maximum
Indoor air (ng/m^3)	NC	66	38	<[c]	<	<	0.8	1.7	3.7
	OH	67	49	<	<	<	0.8	1.6	2.0
Outdoor air (ng/m^3)	NC	65	19	<	<	<	<	0.8	1.7
	OH	65	34	<	<	<	0.3	1.6	3.2
Soil (ng/g)	NC	65	17	<	<	<	<	1.8	30.5
	OH	69	45	<	<	<	1.2	4.6	13.3
Carpet dust (ng/g)	NC	66	83	<	22.2	47.5	155	863	7390
	OH	62	98	<	66.3	156	451	1730	21,700
Carpet dust (ng/cm^2)	NC	66	83	<	0.002	0.01	0.02	0.06	0.6
	OH	62	98	<	0.005	0.01	0.04	0.5	1.9

[a]Percentage of samples with detectable levels of 2,4-D.
[b]Percentiles (25th, 50th, 75th, and 95th).
[c]The '<' symbol was assigned to samples that were below the detection limit.

Table 2. Concentrations of 2,4-D in personal exposure samples collected from children and their primary caregivers over a 48-h period in North Carolina and Ohio.

Medium	State	Children								Adults							
		N	%[a]	Minimum	25th[b]	50th	75th	95th	Maximum	*N*	%	Minimum	25th	50th	75th	95th	Maximum
Hand wipe (ng/cm^2)	NC	66	8	<[c]	<	<	<	0.02	0.04	66	5	<	<	<	<	<	0.02
	OH	67	49	<	<	<	0.03	0.1	0.1	68	63	<	<	0.01	0.01	0.03	0.1
Solid food (ng/g)	NC	66	47	<	<	<	0.9	1.8	4.4	66	50	<	<	<	0.8	2.3	4.0
	OH	69	44	<	<	<	0.4	1.9	20.2	69	48	<	<	<	0.6	2.1	3.7
Liquid food (ng/ml)	NC	64	2	<	<	<	<	<	0.2	64	3	<	<	<	<	<	0.3
	OH	69	3	<	<	<	<	<	0.3	67	6	<	<	<	<	0.2	0.8

[a]Percentage of samples with detectable levels of 2,4-D.
[b]Percentiles (25th, 50th, 75th, and 95th).
[c]The '<' symbol was assigned to samples that were below the detection limit.

Table 3. Urinary 2,4-D concentrations in children and their primary caregivers from the same households over a 48-h period in North Carolina and Ohio.

Urine	State	Children								Adults							
		N	%[a]	Minimum	25th[b]	50th	75th	95th	Maximum	*N*	%	Minimum	25th	50th	75th	95th	Maximum
ng/ml	NC	66	88	<[c]	0.3	0.5	1.1	1.9	3.3	66	86	<	0.3	0.7	1.2	2.8	5.1
	OH	69	97	<	0.7	1.2	1.5	4.3	12.5	69	87	<	0.4	0.7	1.2	3.3	8.1
ng/mg-creatinine[d]	NC	55	87	<	0.5	1.0	1.4	3.4	3.9	55	85	<	0.3	0.6	0.9	2.3	3.9
	OH	59	97	<	1.0	1.5	2.2	5.1	15.4	59	85	<	0.3	0.5	0.9	3.3	4.1
ng/ml-specific gravity[e]	NC	66	88	<	0.3	0.6	1.1	2.1	6.3	66	86	<	0.3	0.7	1.2	3.4	6.1
	OH	69	97	<	0.8	1.2	1.8	3.2	12.8	68	87	<	0.3	0.7	1.1	4.0	7.6

[a]Percentage of samples with detectable levels of 2,4-D.
[b]Percentiles (25th, 50th, 75th, and 95th).
[c]The '<' symbol indicates that values were below the detection limit.
[d]Creatinine was not analyzed in the urine of participants who reported recent pesticide applications at their homes. Creatinine-adjusted values were calculated using the following equation: Creatinine-adjusted value (ng/mg) = 100 ml/dl × urine concentration (ng/ml)/creatinine concentration (mg/dl).
[e]Measurements adjusted for specific gravity using a target urine specific gravity of 1.022 for both children and adults. Specific gravity values were calculated using the following equation: SG-adjusted value (ng/ml) = value × $(SG_{target}-1.000)/(SG_{sample}-1.000)$.

wipe samples for NC children and OH children, respectively. Similarly, 2,4-D was detected in 5% compared to 63% of the hand wipe samples from NC adults and OH adults, respectively. Among OH participants, the level was about three times higher for children as compared to adults. This information suggests that OH children were probably exposed to higher levels of 2,4-D through the dermal and nondietary routes of exposure in comparison to the other participants.

Table 3 presents the distribution of unadjusted (ng/ml), creatinine-adjusted (ng/mg-c), and specific gravity-adjusted (ng/ml-sp) urinary 2,4-D concentrations for both children and adults from each state. The children's unadjusted urinary 2,4-D concentrations were not significantly different by gender or age group (<48 months *vs.* >48 months) within each state. However, the median urinary 2,4-D concentrations were two times higher for OH children as compared to NC children (1.2 *vs.* 0.5 ng/ml) and the distributions were significantly different ($P<0.0001$). In contrast, the median urinary 2,4-D concentrations were identical at 0.7 ng/ml for both NC and OH adults. The maximum levels of 2,4-D were 12.5 and 8.1 ng/ml for one OH child and adult, respectively, from different households. The results from biomonitoring showed that most preschool children and their adult caregivers in this study had measurable concentrations of 2,4-D in their urine samples. They also suggest that the OH children might have been exposed to higher levels of 2,4-D in their environments compared to the other groups in this study.

The estimated inter- and intrapersonal variability for the unadjusted and the specific gravity-adjusted urinary

Table 4. Components of variance and other associated statistics for unadjusted urinary 2,4-D concentrations collected over a 48-h period (specific gravity-adjusted values in parentheses).

	Children			Adults		
	NC	OH	Both	NC	OH	Both
N [a]	13	15	28	13	15	28
σ_B^2 [b]	0.59 (0.98)	0.73 (0.82)	0.81 (1.03)	0.25 (0.28)	0.66 (0.62)	0.55 (0.55)
σ_W^2 [c]	0.41 (0.37)	0.76 (0.91)	0.61 (0.68)	0.56 (0.48)	0.41 (0.38)	0.47 (0.42)
ρ_r [d]	0.59 (0.73)	0.49 (0.48)	0.57 (0.60)	0.31 (0.37)	0.62 (0.62)	0.54 (0.57)
m [e]	3 (2)	4 (4)	3 (3)	9 (7)	2 (2)	3 (3)

[a]A total of 13 and 15 participant pairs in NC and OH reported the days and times they collected their urine samples.
[b]Between-person component of variance of logged values.
[c]Within-person component of variance of logged values.
[d]Intraclass correlation coefficient of reliability.
[e]Number of random measurements per subject necessary to achieve a reliable estimate over the 48-h period.

concentration measurements collected over the 48-h monitoring period are presented in Table 4. Additional statistics derived from these values, such as the intraclass ρ_r are also presented. Reliability describes the degree to which a randomly selected single measurement represents the average of a set of measurements taken from an individual over a specific time period (Pang et al., 2002). It is a function of both the temporal variability observed in a given individual's measurements and the interpersonal variability observed among the averages for the individuals in the population. The intraclass correlation coefficient of reliability can range from 0 to 1, with values near zero indicating low reliability and values near one indicating high reliability. A value of 0.80 is typically considered the benchmark above which a single measurement for an individual adequately represents his or her average. The urinary 2,4-D concentrations (ng/ml) were more consistent over time for NC children compared to OH children, as evidenced by the lower intraperson variability and a higher ρ_r. In contrast, the urinary 2,4-D concentrations were far less consistent for NC adults than for OH adults. The within- and between-person variability in urinary 2,4-D (ng/ml) levels in our sample of children and adults in NC and OH is illustrated in Figure 1 in the form of profile plots with all measurements from a given participant connected by a line. Estimates of ρ_r based on our sample of children and adults suggest that urinary 2,4-D concentrations do not exhibit sufficient temporal stability within the monitoring period to adequately rank (with a $\rho_{r,m}$ of 0.80) individuals with a single measurement. Assuming that these participants represent a random sample of all children and adults in NC and OH, three to four measurements would be necessary for a reliable estimate among the children, and two to nine samples would be necessary among the adults.

We examined the association between 2,4-D concentrations in urine and each matrix (i.e., solid food, dust, air, and hand wipes) for children and adults. No statistically significant associations existed between urine and any of the matrices, except for solid food. In this case, we found a significant, positive association for concentrations in solid food *vs.* urine samples for OH adults ($r^2 = 0.27$, $P < 0.0001$), but not for the other groups in this study. This above finding remains unclear since the levels of 2,4-D in the solid food and urine samples were fairly similar among the groups. In addition, we examined the correlations between urinary 2,4-D concentrations for children and adults in each state. Urinary 2,4-D concentrations were statistically significant and correlated for OH children and adults (Pearson correlation; $r = 0.39$, $P < 0.001$), but correlated only marginally for NC children and adults ($r = 0.24$, $P < 0.053$).

On the basis of the participants' urinary concentrations (ng/ml), their exposures were low as compared to established reference doses (IRIS, US Integrated Risk Information System, 2006). The highest unadjusted urinary concentration in this study was 12.5 ng/ml for one OH child. This concentration represents an exposure of 280 ng/kg/day during the study period, assuming a daily urine excretion of 22.4 ml/kg body weight for children (Miller and Stapleton, 1989; Szabo and Fegyverneki, 1995; Wilson et al., 2003). Assuming a steady-state absorption of 2,4-D, this estimated exposure level is at least 35 times lower than established oral reference dose of 0.01 mg/kg/day in the USEPA Integrated Risk Information System (IRIS, US Integrated Risk Information System, 2006).

Discussion

Participants were exposed to low levels of 2,4-D from several sources and pathways at their homes. Residues of 2,4-D were found in over 80% of the carpet dust samples at all homes in this study. However, the median 2,4-D levels in the dust samples were at least three times higher in OH homes (156 ng/g) than in NC homes (47.5 ng/g). Because 2,4-D is not used indoors, the residential dust measurement results

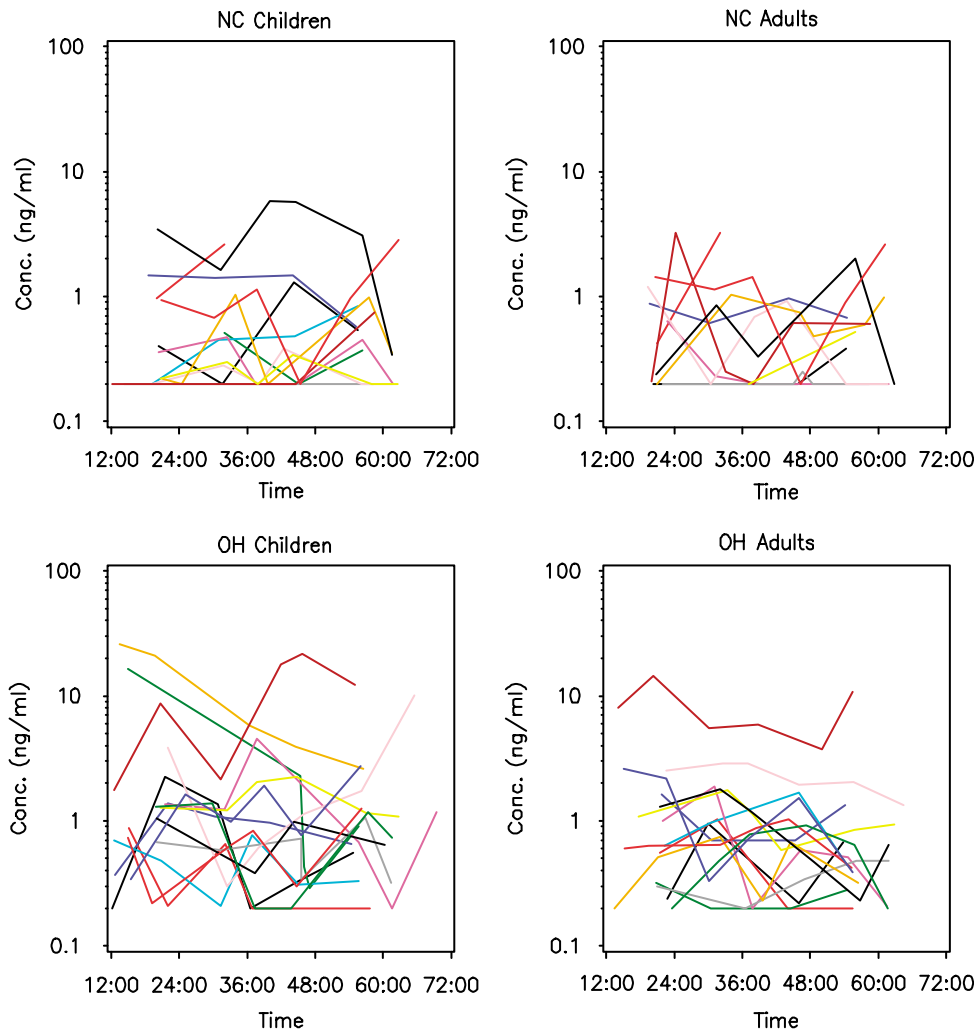

Figure 1. Profile plots showing unadjusted urinary 2,4-D values over time for all participants with noncomposited urine samples. (a) NC and OH participants began collecting their first urine sample after dinner and after lunch, respectively, over a 48-h study period. (b) The estimated LOD was 0.2 ng/ml for urine.

suggest that 2,4-D is being tracked in by occupants or by migrating by air infiltration indoors after outdoor applications at or near their residences. Nishioka et al. (2001) showed that 2,4-D can be tracked indoors onto floors and/or transported through particle infiltration into the home. In addition, this information suggests that 2,4-D may be more commonly used in certain regions of this country. Colt et al. (2004) showed that geometric mean 2,4-D concentrations in dust samples from over 500 homes varied by region. The geometric mean concentrations were 606, 1512, 87, and 374 ng/g in Michigan, Iowa, California, and Washington, respectively. More research is needed to determine if there are geographical differences in application of 2,4-D and other pesticides at or near homes throughout the United States.

Dietary intake represents another potential route of exposure to 2,4-D. The levels of 2,4-D in the solid food samples were below 21 ng/g for all participants in this study. Wilson et al. (2003) has reported somewhat lower levels of 2,4-D in solid food samples, ranging from <0.5 to 2.2 ng/g, collected from nine preschool children at their homes in NC. In the FDA's Total Diet Study of 2003, 2,4-D residues were measurable at ≤ 10 ng/g in only a few types of cereals and breads purchased from local grocery stores and supermarkets throughout the country (Food and Drug Administration (FDA), 2003). Because we analyzed only composited solid food samples for each participant, we do not know if some participants' individual foods (i.e., grains) might have contained higher residues of 2,4-D than other types of foods.

In our study, the urinary measurements indicated that most of these participants were exposed to low levels of 2,4-D. A few studies have also recently reported somewhat lower levels of 2,4-D in nonoccupationally exposed adults (<1.6 ng/ml) and children (<3.5 ng/ml) in the United States (Wilson et al., 2003; CDC (Centers for Disease Control and Prevention), 2005; Curwin et al., 2005). The National Health and Nutrition Examination Survey (NHANES,

2001–2002), a general population study that included measurement results of 546 children, aged 6–11 years, reported that over 25% of them had measurable levels of 2,4-D in their urine samples (1.6 ng/ml (95th percentile)).

Despite the short monitoring period of 48 h, the intraclass correlation coefficient estimated from the unadjusted urinary 2,4-D measurements was fairly low for each group in this study, ranging from 0.31 to 0.62. These coefficients (except the one for NC adults) are, however, higher than the 0.40 and 0.48 reported for the urinary pesticide metabolites TCPy and 1-naphthol, respectively, measured among adults over a much longer (1 year) period (Egeghy et al., 2005). The exceptionally low ρ_r for NC adults relative to the values for the other groups is driven by a relatively low estimated between-person variance and may be an artifact of sample size rather than evidence of a true difference among populations. The small sample of 13 individuals might not have adequately captured the full range of urinary 2,4-D levels in NC adults. When generalizing to a larger population, it may be more appropriate to aggregate the urine samples for children or adults across the two states. Combining measurements from NC and OH yields an estimate of three repeated measurements (Table 4) for either children or adults to obtain reliable values over a 48-h period. These results support the CTEPP study design decision to collect multiple urinary metabolite measurements.

The observed variability of urinary 2,4-D concentrations over 48-h for these participants may be due to differences in such factors as fluid intake, toxicokinetics (e.g., kidney excretion rates), and urine output. In addition, the variability likely includes short-term changes in exposure concentrations by different routes. More research is needed to understand the important factors that affect the temporal variability of pesticides in urine for both children and adults.

Currently, there are several approaches that can be used to reduce the impact of variability in urine output. Collection of 24-h samples can smoothen some short-term fluctuations in urine output, but relatively large differences in urine volume may still be encountered from day to day for an individual. Corrections such as creatinine concentration, osmolality, and specific gravity have been used to adjust for variable dilutions in spot urine samples, however, research suggests that these methods may not be reliable adjustment measures for children and some adults (O'Rourke et al., 2000; Barr et al., 2005; Meeker et al., 2005). For instance, there can be considerable intra- and interindividual variability in creatinine excretion within and across days resulting from differences in dietary intake, physical activity, muscle mass, age, and diurnal variability (Boeniger et al., 1993; O'Rourke et al., 2000; Barr et al., 2005). It may be possible to adjust for some of these differences between individuals using age and body composition information. Another approach is to determine the amount of pesticide or metabolite excreted during the measurement period; this requires knowledge of the previous void time and collection of the entire urine void (for spot samples) or all of the urine excreted during a given time period (i.e., for composite or 24-h samples). A metabolite excretion rate can then be calculated that is largely independent of the urine volume.

There are several forms of 2,4-D (acid, salt, or ester) that are commonly used in commercial products for residential and agricultural use. One limitation of this study is that only the acid and salt (through dissociation) forms of 2,4-D were quantifiable in the environmental and personal media (except urine). Once absorbed into the body, the ester forms of 2,4-D are degraded into the acid form and primarily eliminated in the urine. Therefore, the measured 2,4-D concentrations in the urine samples could have been from the participants' exposures to the acid, salt, and/or ester forms (Garabrant and Philbert, 2002).

In conclusion, many participants in this study were exposed to 2,4-D from several sources and pathways at their homes. The urinary biomarker of exposure confirmed that most of the children and adults in this study absorbed low levels of 2,4-D into their bodies. In addition, this information suggests that the OH children might have been exposed to higher levels of 2,4-D through the dermal and nondietary routes of exposure than all other groups. Finally, the variability in urinary 2,4-D measurements for the participants in both states suggests that several spot urine voids are needed on a daily basis to adequately assess exposures to 2,4-D in residential settings.

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