



Pesticide exposure and creatinine variation among young children

MARY KAY O'ROURKE,^a PATRICIA SÁNCHEZ LIZARDI,^a SÉUMAS P. ROGAN,^a NATALIE C. FREEMAN,^b AMANDA AGUIRRE^c AND CHRISTOPHER G. SAINT^d

^aEOH, College of Public Health, The University of Arizona, Tucson, Arizona 85721-0468

^bExposure Assessment and Measurement Division, EOHHSI, Rutgers University and the University of Medicine and Dentistry of New Jersey, Piscataway, New Jersey 08855-1179

^cWAHEC, 202 North Second Avenue, Suite 102, Yuma, Arizona 85366

^dUSEPA, R8723, 1401 M St. SW, Washington, District of Columbia 20460

Pesticide exposure may differentially impact young children; they live closer to the ground and take in greater amounts of food relative to body mass than older children or adults. We are using an organophosphate (OP) urinary biomarker screen (gas chromatography with flame photometric detection, GC/FPD) to evaluate pesticide exposure among 154 children ≤ 6 years of age living in a heavily farmed border (US–Mexico) community. The screen detects diethylphosphates (DEPs) and dimethylphosphates (DMPs) above a reference range of 1000 non-occupationally exposed individuals ($DL=25 \mu\text{g/g}$ creatinine, Cr). At least one metabolite was detected for 33% of the subjects; many samples contained multiple biomarkers. DEP was detected in 5% of the subjects. DMP and DMTP were frequently measured (25% and 26%, respectively). Biomarker concentrations are adjusted by the body's metabolism of Cr as an indicator of urine dilution. Cr concentrations were examined separately to evaluate their effect on internal dose measures. Cr concentrations were significantly different by season ($K-W=0.83$, $P=0.022$). Significant differences exist between the autumn:spring ($P=0.038$) Cr concentrations and between summer:autumn ($P=0.041$) Cr concentrations based on Mann–Whitney $U=1070.5$, $z=-2.041$, ($P=0.041$). Our analysis of NHANES III data did not reflect seasonal Cr differences for 6 year olds. No younger children were included. Absorbed daily dose (ADD) estimates were calculated for children with the highest concentrations of metabolite. Calculations are theoretical values assuming that the entirety of a given metabolite was metabolized from a single pesticide. Several class-appropriate pesticides were evaluated. For the children with the highest levels, almost all estimated ADDs exceeded the RfD. Although the actual metabolite concentrations dropped appreciably, ADD were still exceeded RfDs at the 95th percentile. The urinary OP screen was effective in identifying subjects with atypical internal doses. Daily Cr yield is a critical component in ADD calculations. Cr variability produces differences in internal dose measurement and estimates of ADD independent of exposure. Cr variability among young children needs to be examined, and caution should be applied when evaluating Cr adjusted internal doses for children. *Journal of Exposure Analysis and Environmental Epidemiology* (2000) **10**, 672–681.

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Introduction

Residents in agricultural communities, like Yuma County, AZ, have great awareness of pesticide exposure risks. Aerial application and real fear of pesticide drift enhance concerns of accidental pesticide exposure. In Yuma County, agriculture is a year-round activity. The greatest amount of pesticide is applied during the month of August when insect pests are the most numerous (Merrigan and Baker, 1995). For community members uninvolved with agriculture, late summer may be the season of greatest exposure risk. Worker exposure, excluding pesticide mixers and handlers, is limited during the summer because most of the fieldworks are mechanized. Excluding the risk of aerial drift, the

greatest potential for human contact with pesticide occurs during the late fall and winter months when crops are tended by seasonal and migrant laborers.

Agricultural worker residents receive job-related pesticide safety training. The Worker Protection Standard of 1992 (40 CFR Part 170) addresses worker exposure to pesticides used in farming, forestry, and nursery or greenhouse operations (EPA, 1994). The standard requires employers to prevent and mitigate exposure of agricultural workers to pesticides. Provisions require notification of application, safety training of the workers and handlers, availability of decontamination facilities, transportation to medical treatment, and provision of medical treatment for workers accidentally exposed. Training programs focus on issues related to worker safety. Some information address transfer of pesticide from the field to the home environment (e.g., separate laundering of field clothes). “Paraoccupational” or secondary exposure to family members living in or near the fields remains largely unevaluated (NIOSH, 1995;

Address all correspondence to: Dr. Mary Kay O'Rourke, PhD, Community, Environment and Policy, COPH, PO Box 210468, 1435 North Fremont Avenue, The University of Arizona, Tucson, AZ 85721-0468.

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Fenske, 1997), but initial evaluation shows evidence of these exposures (Simcox et al., 1995; Loewenherz et al., 1997).

Organophosphate (OP) pesticides are used widely in the fields against agricultural pests (methyl parathion, oxydemeton methyl) and in homes to combat termites and roaches (chlorpyrifos, diazinon). Exposure can occur through all routes, and children are particularly vulnerable. Children are not small adults; they have a high body surface to body volume ratio resulting in greater inhalation, ingestion, and dermal absorption than adults. Further, rates of body processes like excretion and metabolism are greater. These rates vary by developmental stage and among individuals within a given stage (Bearer, 1995). Behaviors of children (e.g., mouthing surfaces and crawling) further promote exposure (Zartarian et al., 1995, 1997; Reed et al., 1999). Collectively, these differences leave children more susceptible to exposures (Goldman, 1995). Proximity to source places children of farm workers at a greater risk for routine exposure (Fenske, 1997). Demonstrating these principles with field data, recent studies by Loewenherz et al. (1997) and Azaroff (1999) report detectable urinary metabolites (dialkylphosphates) among children living in agricultural regions.

Urinary biomarkers can be reported as concentration/unit volume or as concentrations relative to a urine concentration. Many factors influence daily urinary output both within and between individuals. For instance, water, urea, salt, specific gravity, and osmolality will increase or decrease depending on the amount of water consumed. Using a volumetric approach, toxicants or metabolites reported as units per liter of urine can vary independent of exposure (Elkins et al., 1974). Another approach employs a metabolic “by-product” generated by the human body, then the amount of metabolite (e.g., a dialkylphosphate) is expressed in relationship to the amount of “by-product” produced by the body (e.g., creatinine, Cr). The assumption is that both the metabolized toxicant and the metabolic by-product are responding to the same processes. Cr is a metabolite produced constantly in the body through the metabolism of tissue and protoplasmic molecules (i.e., creatine and phosphocreatine in muscle). Cr has little day-to-day and interindividual variability among healthy, working, aged adults. Urine samples from the elderly frequently show a decline in urinary Cr. Work evaluating glomerular filtration rates suggests that the decline is caused by insufficient dietary protein consumption and when values are adjusted for diet, no Cr excretion decline is found (Kimmel et al., 1996). The stability of Cr production and excretion among young children is assumed to be the same as for adults.

This paper reports initial results of Cr adjusted urinary metabolite frequency using an OP screen. We focused on children living in small agricultural communities south of Yuma, AZ; they were between the ages of 2 and 6 years and

potty-trained. In the process of evaluating results, we examined factors affecting urinary Cr concentrations and the influence of Cr concentrations on “exposure” as measured by the biomarkers. Since the dialkylphosphates represent aggregate exposure across multiple pesticides within a class, calculations of “theoretical” absorbed daily dose(s) (ADDs) are presented to explore the meaning of “high” laboratory results as they relate to locally used OP pesticides.

Methods

Study Area and Population Recruitment

Seven Yuma County census tracts (1990) were selected for the National Human Exposure Assessment Survey (NHEXAS) and the Arizona Border Survey (ABS) using a population proportional to size study design (Robertson et al., 1999). We designed the Children's Exposure to Pesticide Survey (CPS) to be performed in the same area. This enabled us to build CPS on a base of previously collected data. The community of interest extends from the south side of the city of Yuma to the United States border with Mexico. The community includes homes in unincorporated areas and the villages of Somerton, Gadsden, and San Luis, AZ (Figure 1). According to the 1990 census, this fastgrowing agricultural community was over 65% Hispanic.

Study subjects were recruited in two ways. (1) NHEXAS and ABS used a population-based probability design; 111 households were contacted in Yuma County. Of these, 11 families with children ≤ 6 years in age were enrolled in the NHEXAS and ABS. Through strict adherence to the study design, these families were not selected for NHEXAS or ABS environmental sampling (stage II or III). We recontacted these families and enrolled seven of them in the CPS. These families and families with eligible children sampled in NHEXAS and ABS provide a population base for bootstrap modeling. (2) We enrolled 143 additional families meeting study criteria by focusing on services catering to children with the desired characteristics ($2 - \leq 6$ years and possessing bladder control). Participants were enrolled through Head Start, Migrant Head Start, and Women, Infants, and Children (WIC) programs located in San Luis and Somerton, AZ, by “promotoras” from the Western Arizona Area Health Education Center (WAHEC). Using both methods, a total of 150 families were enrolled. Ten of the children celebrated birthdays prior to stage II sampling (see below). By the conclusion of the study, we sampled 140 homes with 154 children meeting the enrollment criteria to participate in the CPS.

Study Design

Our study of children's pesticide exposure in Yuma County involves five stages. This report will evaluate results from

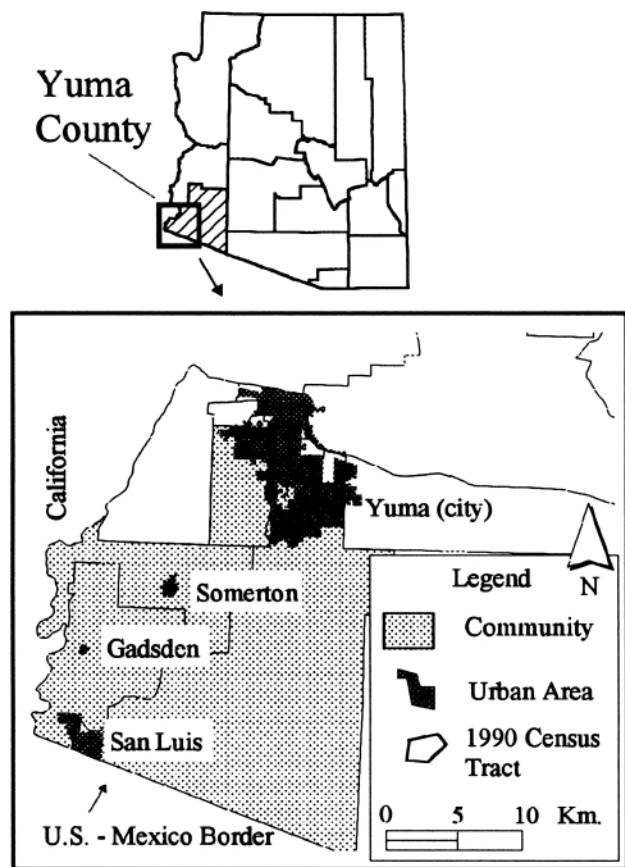


Figure 1. CPS community location within Yuma County and the state of Arizona. Urbanized areas are shaded grey; rural areas are stippled. 1990 Census tract boundaries are delineated.

the first two stages. Stage I — Enrollment. All families were administered a descriptive questionnaire to determine the characteristics of the participant population. Questions addressed household location, characteristics, and demographic information for each household resident. Stage II — Screening. This stage required two visits to each enrolled household. Interviews included a baseline questionnaire related to subject exposure, a 1-day record of typical activities and their duration, and a 1-day record of all food and beverages consumed. The interview was conducted in the language (Spanish or English) selected by the subject's parent/guardian. A first morning urine sample and a household dust sample were collected. The remaining three stages (intensive sampling, subject education, and community education) are just concluding and will be reported later.

Stage II Sampling, Handling, and Processing

Parents of all participating children were provided with a urine cup and basic written and verbal instructions on sample collection; they were asked to collect the first urine

void of the day for participating children. Parents were instructed to place all samples in their freezer until the field team returned to retrieve the sample (usually the same day of the urine sample collection). Each urine sample was labeled with the child's study identification number. Frozen urine samples were shipped to Tucson with cold packs by an overnight express service. Once received, samples were logged and held in a -20°C freezer. Samples were held and shipped in batches of 20 samples to Pacific Toxicology Laboratories on dry ice for dialkylphosphate metabolites analysis, with a detection limit of $25\text{ }\mu\text{g/l}$. Residual aliquots were archived at -18°C by Pacific Toxicology Laboratories. Freezing does not appear to affect metabolite concentrations (Ito et al., 1979). Urinary metabolites were evaluated and results were returned as raw values ($\mu\text{g/l}$) and Cr adjusted values ($\mu\text{g/g}$). Cr values were also reported separately in grams of Cr per liter of urine. Cautions regarding measurement validity were reported if urinary Cr levels were <0.5 or $>3.0\text{ g/l}$. Dust samples were collected, processed, and screened for pesticides. Those results will not be discussed in this paper.

Urine Sample Analysis A minimum of 25 ml of urine was placed in test tubes for sample analysis. The urine was freeze-dried and derivatized using benzyltolyltriazine reagent (Daughton et al., 1979; Takade et al., 1979). A saturated salt solution was added to the tubes and the benzyl derivatives were extracted using cyclohexane and analyzed by gas chromatography with flame photometric detection (GC/FPD) (Shafik and Enos, 1969; Lores and Bradway, 1977).

Laboratory Quality Assurance Procedures Calibration standards were prepared from pooled blank urine collected from laboratory personnel. Aliquots of urine were fortified on the first day of sample preparation from spiking solutions prepared from analytical grade salts of the dialkylphosphates. All standards were extracted and derivatized in the same manner as patient samples and expired after 12 months.

Controls were prepared by fortifying pooled blank urine (collected from laboratory personnel) with spiking solution using analytical grade material of a different lot than that used to prepare the calibration standard spiking material. After spiking, the urine was aliquoted (2 ml) in separate screw cap test tubes and frozen until use. Controls were prepared at two levels. The low control was prepared at the limit of detection. The high control was prepared at $40\times$ the low control.

One low control and one high control were extracted and analyzed with each batch of subject samples. The "control" recoveries were compared to a "QC range" set by evaluating 10–20 consecutive samples, determining the mean and standard deviation of those samples, and

establishing a QC range at ± 2 SD of the mean. An acceptable mean was considered anything between 70% and 120% of the amount fortified. Results were only valid when the controls were within the QC range. Control samples are tracked across all batches run at the laboratory and evaluated for systematic trends and errors using the Westgard Rules.

Data Management and Analysis

All questionnaires used for this project were produced in Teleform and were scanned into SPSS[™] portable databases (O'Rourke et al., 1999). Samples were labeled with barcodes and logged in into a computerized system. The results of the urinary dialkylphosphate metabolites were provided in paper and electronic formats. Data were hand-entered twice, compared, and errors corrected. The final database was 100% visually verified against the original laboratory reports.

Calculations

Data analysis was performed using SPSS[™] version 9.0 for Windows[™]. Exploratory values of ADD were calculated using the formula:

$$\text{ADD} = [(\mu\text{g biomarker/g Cr}) \times (\text{g Cr excreted/day}) \times (\text{compound wt/metabolite wt}) \times (\% \text{ cleared})] / \text{child's body weight in kg}$$

Empirical data available for the analysis included the biomarker concentration per gram of Cr, the compound (specific pesticide) and metabolite (DEP, DMP, DMTP) weights, the child's age and body weight as reported by the parent. To determine the grams of Cr excreted in a day, we took the product of the Cr yield of the analyzed urine sample (g/l) and the age adjusted daily urinary excretion rate as reported by Snyder et al. (1975). We assume no gender differences in excretion rates for children ≤ 6 years (Snyder et al., 1975) and 100% clearance.

Results

Demographics

Of the 140 households with stage II sampling, two children under the age of 6 years were recruited in 14 homes for a total of 154 subjects under the age of 6. The study design also called for evaluation of some older sibling for comparison; 38 older children were recruited. Forty-six percent of the population was recruited from San Luis, AZ, a town on the US–Mexico border; 49% of the population was recruited from Somerton, AZ, a town ~ 8 km northeast of San Luis. The remaining 5% was from Gadsden and Yuma (city). These are the mailing addresses. Many of

these homes are in non-urbanized settings proximal to these small towns.

All children were Hispanic and most households were bilingual. Families were asked their preferred language. Subsequent contacts were made in that language, including reports of study results. Only 3% of the families preferred to be interviewed in English. Spanish was the preferred language for questionnaire completion in 97% of the homes surveyed. About two thirds of the families spoke both languages to some extent. Fifty-one percent of the children was comprised of male and 49% female. The majority of the children ≤ 6 years of age were 5 years of age (47%) with near equal representation of 3 and 4 year olds (21% and 25%, respectively). Each age group had about the same gender split.

Cr Concentrations and Sample Validity

We collected 196 urine samples from children of all ages; four samples were lost to breakage during shipment. One hundred fifty-four samples were evaluated from children ≤ 6 years — the focus of this study. The mean Cr value for all 154 samples was 0.90 g/l (SD=0.11). Table 1 presents the distribution of urine samples for each Cr class (g/l). We considered the effects of a child's age and urinary output as potential variables (Snyder et al., 1975) and adjusted the grams per liter Cr measures (Table 1) to reflect daily excretion of Cr (g/day). Figure 2 illustrates the paired Cr concentration lines (g/l and g/day). The grams per liter values were sorted in ascending order and the case numbers were fixed. The corresponding values in grams per day were adjusted using urinary output constants by subject age. The trends of the two lines are similar. [The highest Cr value (7.5 g/l or 4.5 g/day) was excluded from Figure 2 for purposes of scaling.]

The analytical laboratory warned that metabolite results were questionable for samples with Cr less than 0.5 or greater than 3.0 g/l. Thirty-two of these samples were so designated by the laboratory. By most standards, the Cr levels appeared low, especially since the samples were represented as first morning voids (g/l). Of the 121 "valid" samples, the mean Cr level was 1.05 g/l (SD=0.68). Of the 32 samples with low Cr yield, the mean Cr level was 0.33 g/l (SD=0.11).

Table 1. Percent of urine samples from children ≤ 6 years of age ($n=154$) with Cr levels by volume (g/l) and adjusted for age and daily excretion rate (g/day).

Cr classes→	<0.5	≥ 0.5 to <0.7	≥ 0.7 to <2.0	≥ 2.0 to <3.0	≥ 3.0
Percent of samples (g/l)	20.8	20.8	55.9	1.9	0.6
Percent of samples (g/day)	48.1	25.3	26.0	—	0.6

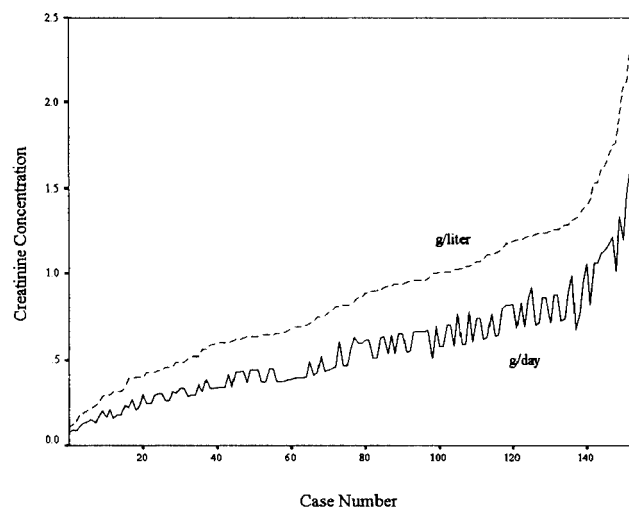


Figure 2. Cr concentrations for 153 urine samples from CPS. Concentrations are presented as grams per liter of urine and adjusted by age-specific daily excretion rates (g/day).

To determine whether the 32 samples with low Cr (g/l) were first morning voids, we contacted the parents and asked questions regarding the sampling time frame and the parent's recollection of the child's toilet habits. Results are presented in Table 2. Only one parent admitted to having no recollection of the sampling time frame. None of the children wore diapers to bed routinely. Sixteen percent of the parents reported that their child usually wet the bed; parents confirmed one bedwetting event and two other parents considered bedwetting a possibility. Half of the parents believed that the child may have used the toilet during the night without the parent's knowledge. If we assume that half of the children with unknown nighttime toilet use actually urinated, then about three quarters of the children with low Cr yield probably conformed to the protocol. Some other factors must account for the variable Cr yield in these children.

Table 2. Evaluation of potential nocturnal urination invalidating the first morning void protocol.

Questions asked	Yes	No	DK
Parent recalled sampling	97% (31)	3% (1)	
Child usually wore diapers to bed		100% (32)	
Child wore diapers the night before the sample collection	3% (1)	97% (31)	
Child usually "wet" the bed	16% (5)	84% (27)	
Child "wet" the bed on the night before sample collection	3% (1)	91% (29)	6% (2)
Child could have used the bathroom prior sample collection without parental notice	50% (16)	41% (13)	9% (3)

Table 3. Mean seasonal Cr content of urine among children ≤ 6 years of age.

Season→	Spring	Summer	Autumn	Winter
Number of all 154 children ≥ 6 years of age	23	48	58	25
Mean Cr concentration (g/l)	0.97	0.92	0.79	1.05
Mean Cr concentration (g/day)	0.64	0.60	0.51	0.62
121 Children ≥ 6 years of age ("valid")	20	39	41	21
Mean Cr concentration (g/l)	1.06	1.07	0.97	1.17
Number of NHANES III children ≤ 6 years ($n=477$)	139	88	124	126
Mean NHANES Cr concentration (g/l)	0.86	0.82	0.88	0.79

These results are derived from the 154 children sampled.

Next, we considered whether Cr content of urine varied seasonally as found by Freeman et al. (1995). The maximum daily temperature in Yuma County varies between warm and hot. We divided the year into two seasons: April 15–October 15 (hot) and October 16 through April 14 (warm). No difference was seen in urinary Cr levels during these seasons (0.90 g/l with an n of 104 and 0.91 g/l with an n of 48, respectively). When results were divided into locally relevant seasons, the observed Cr concentrations were significantly different by season (K–W median test: median=0.83, $\chi^2=9.613$, $df=3$, $P=0.022$). Significant differences exist between the autumn and spring Cr concentrations (Mann–Whitney $U=469$; $z=-2.04$, $P=0.038$); another significant difference was found between summer and autumn Cr concentrations (Mann–Whitney $U=1070.5$, $z=-2.041$, $P=0.041$). No other differences among seasonal Cr values were significant. Table 3 presents the seasonal values by both grams per liter and grams per day of Cr in first morning voids across all age groups. The seasonal distribution of 121 valid samples (excluding those with Cr <0.5 g/l) shows the same, albeit less pronounced, trend.

For further comparison, we examined the Cr results for 6 year olds from the NHANES III survey (DHHS, 1996). The range of Cr difference was under 0.1 g/l across all seasons and there was no statistically significant seasonal variability ($P=0.05$). The NHANES III Cr data presented other interesting opportunities. By race, Cr values are significantly greater for African–Americans (mean=0.94 g Cr/l urine; $n=173$) than whites (mean=0.78 g Cr/l urine; $n=285$) with a Mann–Whitney $U=19,241$ ($z=-3.94$; $P=0.0000$). Between ethnic groups, non-Hispanics had significantly greater Cr concentrations (mean=0.89 g Cr/l urine; $n=287$) than Hispanics (mean=0.77 g Cr/l urine; $n=190$) with a Mann–Whitney $U=23,102.5$ ($z=-2.85$; $P=0.005$). These results for 6-

year-old children suggest that Cr variability needs to be considered not only in terms of age, but also in terms of race and ethnicity.

Urinary Metabolites Indicative of Pesticide Exposure

The dialkylphosphates were measured in the urine of 121 children 6 years or younger who were able to urinate into the specimen cup and whose Cr yield was sufficient for laboratory analysis. Overall, at least one metabolite was detected for 33% of the subjects. Many samples contained multiple biomarkers. Only 5% of the subjects produced any of the diethylphosphates (DEPs). None of the other DEPs [diethylphosphorothioate (DETP) or diethylphosphorodithioate (DEDTP)] was reported from the urine samples. Most of the detectable measures were from the dimethylphosphates (DMPs). DMP and DMTP (dimethylphosphorothioate) were frequently reported (25% and 26%, respectively). DMDTP (dimethylphosphorodithioate) was found in only 3% of the samples. Hayes et al. (1980) indicate that DMTP had the greatest recovery among the dialkylphosphates. Thus, in most studies, DMTP appears most commonly. These values and results from other comparable studies are presented in Table 4. DMTP was the most frequently detected metabolite by Azaroff (1999) and Loewenherz et al. (1997). The highest concentrations of dialkylphosphates were reported among citrus growers by Griffith and Duncan (1985); results were compared with those of NHANES II.

Table 4 contains the percent of dialkylphosphate concentrations elevated beyond the reference range of the specific analyte (DEP, DMP, DMTP). The laboratory defined the reference range as the level experienced by the 95th percentile individual in a survey of 1000 non-occupationally exposed individuals for that analyte. Since

detection was unexpected among non-occupationally exposed individuals, the reference range is defined as zero at the limit of detection for the method (25 ppb). Therefore, anything above 25 ppb was considered above the reference range and is a detect. Viewing all detectable levels as "high" only serves to describe result levels relative to other individuals — a typical method used in clinical analyses. Detection does not imply any toxicological impact or risk. The non-specificity of the results limits the utility of the test; there are no published levels of OP residues associated with any kind of toxicological effect.

ADD is usually calculated for a single pesticide with a specific metabolite. We have identified pesticides commonly used in the Yuma area. Metabolism of each pesticide would result in a class of metabolite (DEP, DMP, DMTP). Table 5 contains the ADD based on actual biomarkers collected from this population of children. Three children are presented for each pesticide example, and each child excreted the greatest concentration of biomarker for his/her age class. For these calculations, we assume that a single pesticide was metabolized, it produced a single metabolite, and all of the metabolites were cleared.

The measured DEP could be derived from either chlorpyrifos or diazinon, among other pesticides not discussed. Both pesticides are reportedly used in the Yuma area. For the highest DEP report, the ADD exceeds the permissible reference dose by 126 times, in the worst case — diazinon. Levels of DMP and DMTP as associated with methyl parathion exceed the reference dose by 385 and 61 times, respectively. In most cases, there is a considerable difference between the greatest metabolite concentration and the (still elevated) concentration at the 95th percentile of the distribution. Using the same subject parameters (i.e., g Cr/day and subject

Table 4. Percentage of the population with specific urinary metabolites above the detection limit.

	MDL ($\mu\text{g/l}$)	<i>n</i>	DEPs ($\mu\text{g/g}$)			DMPs ($\mu\text{g/g}$)		
			DEP	DETP	DEDTP	DMP	DMTP	DMDTP
Percent detects (this study)	25	121	5%	0%	0%	25%	26%	3%
Percent detects NHANES ^a	20–40	6895	6.2%	5.2%	0.1%	10.2%	5.3%	0.3%
Percent detects — citrus workers ^a	20–40	597	57.8%	30.2%	15.2%	29.5%	11.5%	6.92%
Percent detects — farm workers, El Salvador ^b	20–30	358	*	*	2%	*	28%	0%
Percent detects — children of applicators ^c	13–15	70	*	*	*	*	40%	6.3%
Range (this study)			ND–263	ND	ND	ND–981	ND–369	ND–37
95th percentile (this study)			22	ND	ND	124	141	1

These values exceed the 95th percentile of non-occupationally exposed reference range of 1000 people.

^aGriffith and Duncan (1985).

^bAzaroff (1999).

^cLoewenherz et al. (1997).

*Metabolite not measured.

Table 5. Theoretical ADD calculated for “example pesticides” based on children with the most elevated biomarker measure for their age class and a reduction of the biomarker yield to the 95th percentile.

Example pesticide	Reference dose ^a (mg kg ⁻¹ day ⁻¹)	Example child with “high” biomarker sample by age in years	ADD in mg kg ⁻¹ day ⁻¹ for example pesticide based on maximum metabolite yield			ADD in mg kg ⁻¹ day ⁻¹ for example pesticide based on metabolite yield 95th percentile		
			DEP	DMP	DMTP	DEP	DMP	DMTP
Chlorpyrifos	0.003	3	0.00259	NA	NA	0.00248	NA	NA
		4	0.00243 ^b	NA	NA	0.00172 ^b	NA	NA
		5	0.01300	NA	NA	0.00108	NA	NA
Diazinon	0.00009	3	0.00226	NA	NA	0.00216	NA	NA
		4	0.00211 ^b	NA	NA	0.00150 ^b	NA	NA
		5	0.01133	NA	NA	0.00094	NA	NA
Malathion	0.02	3	NA	0.00798	0.01676	NA	0.00343	0.00850
		4	NA	0.01313	0.01846	NA	0.00909	0.00688
		5	NA	0.12137	0.01872	NA	0.01534	0.00715
Methyl parathion	0.00025	3	NA	0.00625	0.01337	NA	0.00273	0.00678
		4	NA	0.01067	0.01472	NA	0.00722	0.00549
		5	NA	0.09635	0.01535	NA	0.01217	0.00570

^aEPA (1994).

^bEstimated weight.

weight), theoretical values were calculated for the 95th percentile (Table 5).

Discussion

Evaluating pesticide exposure and health effects is a difficult undertaking. Clearly, children face greater risks to non-occupational pesticide exposure than adults. Pound for pound of body weight, children eat more, breath more, and have a faster metabolism. Behaviorally, they play on the floor or lawn where pesticides are commonly applied and have more frequent hand-to-mouth contacts (Zartarian et al., 1995, 1997; Bradman et al., 1997; Eskenazi et al., 1999; Reed et al., 1999). Based on the laboratory reference range procedure, 5% exposure was expected. DEP measures met expectations. DMP and DMTP exceeded expectations by 20% and 21%, respectively.

Several factors may account for these numbers. (1) Subjects experience paraoccupational exposure as children of farm workers. Pesticides are used routinely near their homes, and their parent's occupation promotes transport of pesticides from the field to the home. (2) As children, their behaviors and biology place them at greater risk. (3) The reference population may not be comparable to the study population in terms of age, activities, and temperature regimes experienced (region of the country).

Further, results from the NHEXAS studies indicate a gradual increase of chlorpyrifos exposure when compared with NHANES III data. More people appear exposed to low levels of pesticide as time progresses. If the laboratory reference population for CPS was evaluated more than 10 years ago, its relevance today may be questionable.

Few studies have focused on children's exposure to pesticides, making comparisons difficult. Guille et al. (1998) conducted a study in Mexico with 4–5-year-old Yaqui children with assumed low level chronic exposure to agricultural pesticides and compared them with children living in a nearby community perceived as having less pesticide use. They found that children who lived in the agricultural area had significant deficits in tests of stamina, eye–hand coordination, short-term memory, and ability to draw a person when compared with the presumably non-exposed children. There were no direct measures of pesticide dose from the children and environmental measures were few. The paper shows trends, but has no evaluation, of metabolites indicating internal dose.

Azaroff (1999) conducted a study with farm workers from rural El Salvador. She found that nearly half of 358 samples had detectable levels of OP metabolites. Detectable metabolite levels were found among 30% of the non-occupationally exposed subjects. Similar rates were found

among children (any age younger than 18) and adults. Among subjects, excretion of OP metabolites was predicted by performing fieldwork during the past 2 weeks, having the head of household applying OP pesticides during the past 2 years, and reporting the use of OP pesticides in the house or yard.

Loewenherz et al. (1997) measured urinary metabolites from two groups of children in Washington State. Children (44%), with parents who were pesticide applicators had urinary metabolites above the detection level, while only 27% of the non-applicator's children had detectable levels. The children of non-applicators also lived in agricultural environments. The method used by Loewenherz et al. (1997) was about twice as sensitive as those of other studies (Table 4). The increased sensitivity levels may influence the percentage reported. Non-applicator children may reflect the enhanced "background" measures. If lower detection levels were available to CPS, we would expect an increased percentage of the Yuma population to respond.

When assessing a urine sample for OP concentration, it is logical to obtain the greatest accumulation of urine over a specified time as is possible. The first morning void meets these needs. The investigator must rely on the child and parent to make sure the sample is a first morning void. Age adjusted Cr concentrations are one approach to evaluating compliance to the protocol. We found no relationship between low Cr levels and bedwetting or diaper-wearing. The potential for children to use the toilet unbeknown to the parent appeared evenly split and remains a possibility for about 10% of the population. In addition to protocol non-compliance, elevated Cr concentrations in first morning voids may impede the chemical assay. Cr values exceeding 3.0 g/l were only found in one sample from the CPS. Other studies counterbalanced Cr by collecting spot urine samples. Researchers measure Cr and rely on it being more or less constant.

Freeman et al. (1995) found highly variable Cr levels among children under the age of 6 years living in a Jersey city (NJ) neighborhood near a chromium waste site. Overall, Cr levels appeared low. Further, summer mean Cr levels were 0.716 g/l (17 children) and autumn values were 0.395 g/l (14 children). Our results confirm seasonal variability in Cr concentration among young children.

A variety of explanations abound for the seasonal trend in these two studies. Foremost among these is limited knowledge of norms for healthy children. Activity and increased perspiration may play a role. Yet, CPS children spent 8 h sleeping prior to sample collection. Some differences might be caused by changes in household air cooling method (air conditioning versus open windows). Another important factor is dietary intake. Elders with reduced dietary protein have a decline in urinary Cr

clearance (Kimmel et al., 1996). Children may have a diet with greater seasonal variation than adults. Ethnic differences in diet may relate to the racial and ethnic Cr differences in the NHANES III data. Lower Cr concentrations yield increased adjusted biomarker measures for a toxicant. What is the impact of these adjustments on concepts like environmental justice if different groups of children metabolize Cr at different rates or dietary issues confound the Cr yield?

The classic paper by Elkins et al. (1974) clearly demonstrates the need for some method of standardizing toxicants in urine. Investigators carefully seek the laboratory with the best detection limit for the analyte of interest along with quality assurance procedures; less thought is provided to the Cr standard. Our evaluation of the CDC Cr concentrations among 6 year olds in NHANES III showed a total spread of only 0.09 g/l across all seasons. Six years of age marks a major change for children. They enter school for a complete day and activities change. These changes may affect Cr yield along with racial and ethnic factors. An alternative is the caliber of laboratory work done at CDC. They may yield consistently better Cr measurements than commercial laboratories.

Cr concentration is fundamental to the calculation of internal dose and ADD. We calculated the ADD for a 4-year-old child using daily Cr concentrations measured in the field and using those reported by Snyder et al. (1975). Assuming exposure to methyl parathion of 179 $\mu\text{g/g Cr}$ and using the greater measures found for our population (0.68 g/day as opposed to a reference value of 0.25 g/day), the ADDs were 0.01687 and 0.00620 $\text{mg kg}^{-1} \text{day}^{-1}$. Both measures exceed the RfD; the value based on our actual Cr measure is almost three times greater. Laboratory errors on Cr measures may result in erroneous conclusions regarding pesticide safety. Further, our results and those of Freeman et al. (1995) suggest that Cr yield may be more variable among children. NHANES IV will evaluate children under the age of 6 years across all seasons. Results may better characterize Cr production in young children across a variety of temporal and spatial regimes.

The OP screen provides interesting, although non-specific, results. It enables investigators to evaluate a "class" of pesticides and not miss an important exposure by a poor selection of specific metabolites. In our future stage III evaluations of CPS, we can examine the relationships between the OP screen and metabolites specific to a pesticide.

Conclusions

In Yuma County, one or more urinary metabolites to OP pesticides were observed in 33% of the children ≤ 6 years

of age. Paraoccupational exposure is a likely source. Bedwetting or wearing of diapers did not appear to be a problem in obtaining a first morning void. There may be a seasonal variation in Cr yield in young children. Cr yield per day is a critical component of ADD calculations. Variability in Cr measures will produce differences in internal dose and estimates of ADD independent of exposure.

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References

- Azaroff L. Biomarkers of exposure to organophosphorous insecticides among farmers' families in rural El Salvador: factors associated with exposure. *Environ Res Sect A* 1999; 80: 138–147.
- Bearer C.F. How are children different from adults? *Environ Health Perspect* 1995; 103 (suppl 6): 7–12.
- Bradman M.A., Harnley M.E., Draper W., Seidel S., Teran S., Wakeham D., and Neutra R. Pesticide exposures to children from California's Central Valley: results of a pilot study. *J Expos Anal Environ Epidemiol* 1997; 7: 217–234.
- Daughton C.G., Cook A.M., and Alexander M. Gas chromatographic determination of phosphorus containing pesticide metabolites via benzoylation. *Anal Chem* 1979; 51: 1949–1953.
- DHHS (United States Department of Health and Human Services). National Center for Health Statistics. Third National Health and Nutrition Examination Survey, 1988–1994, NHANES III Laboratory Data File (CD-ROM). Public Use Data File Documentation Number 76200. Centers for Disease Control and Prevention, Hyattsville, MD, 1996.
- Elkins H.B., Pagnotto L.D., and Smith H.L. Concentration adjustments in urinalysis. *Am Ind Hyg Assoc J* 1974; 35: 559–565.
- EPA (Environmental Protection Agency). Integrated Risk Information System Database. Washington, DC, 1994.
- Eskenazi B., Bradman A., and Castorina R. Exposure of children to organophosphate pesticides and their potential adverse health effects. *Environ Health Perspect* 1999; 107 (suppl 3): 409–419.
- Fenske R.A. Pesticide exposure assessment of workers and their families. *Occup Med: State Art Rev* 1997; 12: 221–237.
- Freeman N.C.G., Wainman T., Lioy P.J., Stern A.H., and Shupack S.I. The effects of remediation of chromium waste sites on chromium levels in urine of children living in the surrounding neighborhood. *J Air Waste Manage Assoc* 1995; 45: 604–614.
- Goldman L.R. Children — unique and vulnerable. Environmental risks facing children and recommendations for response. *Environ Health Perspect* 1995; 103 (suppl 6): 13–18.
- Griffith J.G., and Duncan R.C. Dialkyl phosphate residue values in the urine of Florida citrus fieldworkers compared to the National Health and Nutrition Examination Survey (HANES) sample. *Bull Environ Contam Toxicol* 1985; 34: 210–215.
- Guillette E.A., Meza M.M., Aquilar M.G., Soto A.D., and García I.E. An anthropological approach to the evaluation of preschool children exposed to pesticides in Mexico. *Environ Health Perspect* 1998; 106: 347–353.
- Hayes A.L., Wise R.A., and Wier F.W. Assessment of occupational exposure to organophosphates in pest control operators. *Am Ind Hyg Assoc* 1980; 41: 568–575.
- Ito G., Kilgore W.W., and Seabury J.J. Effect of freezer storage on alkyl phosphate metabolites in urine. *Bull Environ Contam Toxicol* 1979; 22: 530–535.
- Kimmel P.L., Lew S.Q., and Bosch J.P. Nutrition, ageing and GFR: is age associated decline inevitable? *Nephrol Dial Transplant* 1996; 11 (suppl 9): 85–88.
- Loewenherz C., Fenske R.A., Simcox N.J., Bellamy G., and Kalman D. Biological monitoring of organophosphorous pesticide exposure among children of agricultural workers in central Washington state. *Environ Health Perspect* 1997; 105: 1344–1353.
- Lores F.M., and Bradway D.F. Extraction and recovery of organophosphorus metabolites from urine using an anion exchange resin. *J Agric Food Chem* 1977; 25: 75–79.
- Merrigan S., and Baker P. Results of the 1993 pesticide sales survey. Prepared by the Pesticide Coordinators Office, College of Agriculture. The University of Arizona, 1995, pp. 1–9.
- NIOSH (National Institute for Occupational Safety and Health). Report to Congress on Workers' Home Contamination Study conducted under the Workers' Family Protection Act. Cincinnati U.S. Department of Health and Human Services, NIOSH, 1995.
- O'Rourke M.K., Fernandez L.M., Bittel C.N., Sherrill J.L., and Robbins D.R. Mass data massage: a automated data processing system used for NHEXAS, Arizona. *J Expos Anal Environ Epidemiol* 1999; 9: 471–484.
- Reed K.J., Jimenez M., Freeman N.C., and Lioy P.J. Quantification of children's hand and mouthing activities through a videotaping methodology. *J Expos Anal Environ Epidemiol* 1999; 9: 513–520.
- Robertson G.L., Lebowitz M.D., O'Rourke, Gordon S., and Moschandreas D. National Human Exposure Assessment Survey (NHEXAS) study in Arizona — introduction and preliminary results. *J Expos Anal Environ Epidemiol* 1999; 9: 427–434.
- Shafik M.T., and Enos H.F. Determination of metabolic and hydrolytic products of organophosphorous pesticide chemicals in human blood and urine. *J Agric Food Chem* 1969; 17: 1186–1189.
- Simcox N.J., Fenske R.A., Wolz S.A., Lee I.C., and Kalman D.A. Pesticides in house dust and soil: exposure pathways for children

- of agricultural families. *Environ Health Perspect* 1995; 103: 1126–1134.
- Snyder W.S., Cook M.J., Karhausen L.R., Nasset E.S., Parry Howells G., and Tipton I.H. Report of the Task Group on Reference Man. International Commission on Radiological Protection No. 23. Pergamon Press, Oxford, 1975, pp. 354–357.
- Takade D.Y., Renyolds J.M., and Nelson J.H. 1-(4-Nitrobenzyl)-3-(4-tolyl)triazine as derivatizing reagent for the analysis of urinary dialkyl phosphate metabolites of organophosphorous pesticides by gas chromatography. *J Agric Food Chem* 1979; 27: 746–753.
- Zartarian V., Streicker J., Rivera A., Cornejo C., Molina S., Valedex O., and Leckie J.O. A pilot study to collect microactivity data on two- to four-year-old farm labor children in Salinas Valley, California. *J Expos Anal Environ Epidemiol* 1995; 5: 21–34.
- Zartarian V., Ferguson A.C., and Leckie J.O. Quantified dermal activity data from a four-child pilot field study. *J Expos Anal Environ Epidemiol* 1997; 7: 543–553.