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Nondietary ingestion of pesticides by children in an agricultural community on the US/Mexico border: Preliminary results

STUART L. SHALAT,[a] KIRBY C. DONNELLY,[b] NATALIE C.G. FREEMAN,[a] JAMES A. CALVIN,[b] SOWMYA RAMESH,[b] MARTA JIMENEZ,[a] KATHLEEN BLACK,[a] CATRIONA COUTINHO,[b] LARRY L. NEEDHAM,[c] DANA B. BARR[c] AND JUAN RAMIREZ[d]

[a]*Environmental and Occupational Health Sciences Institute (a jointly sponsored institute of Rutgers the State University of New Jersey and the University of Medicine and Dentistry of New Jersey), Robert Wood Johnson Medical School, Piscataway, New Jersey, USA*
[b]*Center for Environmental and Rural Health, Texas A&M University, College Station, Texas, USA*
[c]*National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA*
[d]*TDI-Brooks International, College Station, Texas, USA*

An environmental measurement and correlation study of nondietary ingestion of pesticides was carried out in a *colonia* in south Texas. The purpose of the study was to evaluate young children's exposure to environmental levels of organophosphate (OP) pesticides in the household. Samples were collected to measure levels of OP pesticides in housedust and on children's hands. These, in turn, were compared to levels of OP pesticide metabolites in urine. A total of 52 children, 25 boys and 27 girls, participated in the spring and summer of 2000. The children were 7–53 months of age at the time of recruitment. Univariate and multivariate regression analyses were carried out using SAS statistical software. Seventy-six percent of housedust samples and 50% of hand rinse samples contained OP pesticides. All urine samples had at least one metabolite and over 95% had at least two metabolites above the limit of detection (LOD). Total OP loadings in the housedust ranged from nondetectable (nd) to 78.03 nmol/100 cm^2 (mean=0.15 nmol/100 cm^2; median=0.07 nmol/100 cm^2); total OP loadings on the children's hands ranged from nd to 13.40 nmol/100 cm^2 (mean=1.21 nmol/100 cm^2; median=1.41 nmol/100 cm^2), and creatinine corrected urinary levels (nmol/mol creatinine) of total OP metabolites ranged from 3.2 to 257 nmol/mol creatinine (mean=42.6; median 27.4 nmol/mol creatinine). Urinary metabolites were inversely associated with the age of the child (in months) with the parameter estimate (pe)= −2.11, P=0.0070, and 95% confidence interval −3.60 to −0.61. The multivariate analysis observed a weak association between concentrations of OP pesticides in housedust, loadings in housedust, and concentration on hands, hand surface area, and urinary levels of OP metabolites. However, hand loadings of OP pesticides were more strongly associated (r^2=0.28; P=0.0156) with urinary levels of OP metabolites (pe=6.39; 95% CI 0.98–11.80). This study's preliminary findings suggest that surface loadings of pesticides, on hands, are more highly correlated with urinary bioassays and, therefore, may be more useful for estimation of exposure in epidemiologic studies than levels of pesticides in housedust.
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Introduction

In agricultural communities, the potential for exposure to pesticides is greater than for the general population (Shalat et al., 2002). Numerous studies have shown that children are exposed to environmental chemicals, particularly pesticides, through different mechanisms and often in greater quantities than adults (Fenske et al., 1990, 2000; Simcox et al., 1995; Bradman et al., 1997; Loewenherz et al., 1997; Lu and Fenske, 1999; Lu et al., 2000). The question of how vulnerable children are to the potential toxic effects of pesticides is clearly dependent upon the dose they receive. A major route of exposure that has long been appreciated is nondietary ingestion of pesticides through the mouthing behaviors of children. These exposures result from hand contact with floors, surfaces, and soil contaminated with pesticides and the subsequent ingestion, when hands inevitably end up in the child's mouth. These activities may also result in secondary contamination of food items with pesticides that have gotten on children's hands and/or food handling or preparation areas in the home. In addition, they occur when children's toys or other objects become contaminated with pesticides and the child puts those objects in their mouth. Regular use of pesticides to control household pests is widespread (Savage et al., 1981; Davis et al., 1992). The US EPA Nonoccupational Pesticide Exposure Study found elevated levels of pesticide residues, from household use, in nearly all houses sampled (Whitemore et al., 1994). Estimates of soil ingestion by children

1. Address all correspondence to: Dr. Stuart L. Shalat, EOHSI, 170 Frelinghuysen Road, Piscataway, NJ 08854, USA. Tel.: +1-732-445-1295. Fax: +1-732-445-0116. E-mail: shalat@eohsi.rutgers.edu
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range from 40 to 100 mg/day (van Wijnen et al., 1990). While dust ingestion has not been adequately assessed, it is assumed that most soil and dust ingestion will come directly from hand-to-mouth activities or object-to-mouth activities. The greatest frequency is assumed to occur among toddlers, slightly lower in infants, and the least among school age children. However, detailed models are scarce and data to validate them are limited.

Recent studies have examined in detail the exposure and uptake of pesticides in children who reside in agricultural communities (Loewenherz et al., 1997; Fenske et al., 2000; Lu et al., 2000; O'Rourke et al., 2000; Sumner and Langley, 2000; McCauley et al., 2001). One of the findings of these studies is that children who live in agricultural communities had five times higher pesticide metabolites in their urine than children who resided in nonagricultural communities (Lu et al., 2000). In particular, they examined children who resided near orchards and found both environmental as well as urinary levels of pesticides to be statistically significantly higher in these children. When compared to EPA chronic dietary reference doses, 56% of those whose parents worked in agricultural settings exceeded these levels (Fenske et al., 2000).

Biological markers have been previously used to assess exposure to pesticides. Urine samples have been used to examine exposure to a suite of pesticides, through measurement of pesticides or their metabolites (Griffith and Duncan, 1985; McCurdy et al., 1994; Hill et al., 1995; Fenske et al., 2000; O'Rourke et al., 2000). In the most recent NHANES III report, 82% of individuals had measurable (>1 μg/l) trichloropyridinal (TCPY), the primary metabolite of chlorpyrifos, in their urine (Hill et al., 1995). Urinary pesticide metabolites have been found to correlate well with erythrocyte acetylcholinesterase (McCurdy et al., 1994). However, elevated levels of pesticide metabolites have been found in urine following organophosphate (OP) pesticide exposure, even when cholinesterase inhibition was not found in blood serum (Richter et al., 1992).

A study of a border population in Yuma county, AZ, observed OP pesticide metabolites in the urine of 33% of children 6 years of age or under (O'Rourke et al., 2000). Another study observed a very large and statistically significant difference between levels of exposure to OP pesticides in children of agricultural families and a nonagricultural comparison group (Lu et al., 2000). That study examined soil, housedust, and urine samples. While not surprising that children in these communities were highly exposed, the fact that 67% of the urine samples from the children had detectable levels (limit of detection, LOD, 1–10 μg/l) of dialkyl phosphates should be viewed as alarming. Mean and median levels for these metabolites were 20 and 5.0 μg/l, respectively. When comparing the percentage of children with detectable levels of pesticide, it is of course important to take into account the study's level of detection. The level of detection in the O'Rourke et al. (2000) study was 25 μg/l, potentially explaining the difference in the proportion in these two studies.

The purpose of this study was to examine an important aspect of the relationship between environmental exposures to OP pesticides in infants and young children who live in border agricultural communities (*colonias*) and dose levels of OP pesticides as measured by six of their metabolites in urine. Environmental media (soil and dust) were utilized to characterize the environmental exposures. The removable quantities of pesticides on floor surfaces and children's hands, known as loadings, were examined as well. By providing an alternative model for estimating pesticide exposure, a better understanding may be achieved of the possible risks pesticides may represent to children's health.

Methods

A 3-year environmental measurement and correlation study was conducted in the mid-Rio Grande Valley. Public meetings were held with several community groups, in order to identify communities with which to partner this study. Because of their history of activities in this community and their willingness to take an active role in the planning and conducting of the study, the Sisters of Mercy, a religious order, was the primary community study partner. The sisters make broad use of *promotoras* for lay health education in the community. For this reason, we chose to train the *promotoras* and employ them for contacting households, and administering questionnaires and sample collection.

As a result of the community meetings, a small *colonia* of approximately 5000 residents, located on the US/Mexico border, was selected for the study. More than 98% of the residents are Mexican/American. A census was conducted utilizing the *promotoras*, to identify all households having children between 6 and 48 months of age. Households were selected for possible participation on the basis of the census results. A total of 920 residences were identified; of these, 870 were occupied at the time of the census (as determined by direct observation and discussions with neighbors). Six hundred forty-three of these were contacted, in-person, by study personnel, with 91 households having at least one child under 3 years of age. Initially, only homes with two or more children were invited to participate.

The study protocol, questionnaires, and letter of consent were all reviewed and approved by UMDNJ-RWJMS Institutional Review Board (IRB no. 2708). The interviews and sampling were timed to coincide with one of the two growing seasons in the area. The initial round began in the spring of 2000. Households were contacted and invited to participate in the study. An appointment was scheduled for the administration of the questionnaire, collection of

environmental samples, and videotaping of the child. This was carried out on 1 day and urine samples were collected the following morning. Every attempt was made to videotape and obtain hand rinses of children residing in the same household on consecutive days. After a complete description of the study was provided, those who agreed to participate were asked to sign a letter of informed consent. To those parents who agreed to participate, a baseline questionnaire, which included medical and occupational information, time/activity questions, questions on hand-to-mouth activities, diet, and residential pesticide use, was administered in Spanish — the primary language of the residents of the community.

Because of the complexity of sources and conditions that effect environmental exposure to pesticides, and to better characterize the residential exposures of children from this agricultural area, four exposure metrics were used in this study. The metrics included: (1) pesticide concentration in soil; (2) pesticides in housedust; (3) pesticides on children's hands; and (4) urinary levels of pesticide metabolites. Metrics 1, 2, and 3 were assessed in combination with direct observation of hand/mouth activity and parental questionnaire and compared to actual bioassay measurements in metric 4. Environmental samples of housedust were taken from the floor inside the home, near the main entrance, which in most instances was also a major play area for the children. Dust was collected by a wipe sample from a square meter of floor. The samples were wrapped in aluminum foil, then placed into a polyethylene collection bag, and shipped to the laboratory for analysis. Hand rinses utilizing 225 ml of isopropyl alcohol were obtained from the child's hands. Tracings of the child's hands were also obtained in order to provide an estimate of surface area. This was used in concert with the pesticide levels measured in the alcohol rinses to compute pesticide loading on the child's hands. Urine samples were used to assess exposure to pesticides through the measurement of pesticide metabolites.

Soil samples were collected from the front yard of one house from each block of the neighborhood. This was done in order to reflect levels of pesticide drift from the nearby farm fields. One sample was collected from each of these houses. Surface soils were collected with a precleaned aluminum foil-wrapped, stainless steel trowel. The area to be sampled was first cleaned of any surface debris or vegetation. The trowel was then used to remove soil to a depth of approximately 4–15 cm. The soil was placed in a precleaned I-Chem® jar (I-Chem, New Castle, DE) with a Teflon-lined lid, labeled, and stored in a ziplock bag.

The housedust floor sample was obtained as close as possible to the front door, on tile or linoleum. One sample was collected from each house participating in the study. The area that was sampled was marked and was approximately 1 m^2. The exact area sampled was measured and recorded in the logbook. While wearing gloves, the technician removed a prepared glass fiber filter cloth out of the aluminum foil. The cloth was then wet with 30 ml of pesticide grade isopropyl alcohol. The area of the floor to be sampled was then cleaned with the cloth in the following way: starting at the top of the area and going from left to right, the cloth was passed in one straight line across the very top of the area to be cleaned. Next, going from right to left, the cloth was passed in one straight line back across the area, directly below the first swipe. This process was continued until the entire area was cleaned. The fiber cloth was then folded and returned to the aluminum foil.

Prior to the commencement of videotaping, the child's hands were rinsed as described below and the rinse discarded. The child was then videotaped by a technician while the child resumed his/her normal activities over the subsequent 4 h. One hand rinse was collected for analysis after the completion of the videotaping. Hand rinse samples were collected by washing the child's hands in 225 ml of reagent-grade isopropanol. The hands were initially rinsed in 150 ml of isopropanol in a clean ziploc bag. This was carried out by having the child place both hands in the bag and having the trained technician gently agitate the bag from the outside for approximately 30 s. After collection, the handwash sample was transferred to a clean pesticide-grade I-Chem® 250-ml amber glass jar. The ziploc bag was then washed one time with the remaining 75 ml of isopropanol to remove residues from the ziploc bag. The rinse was then added to the sample in the amber jar. The total volume of the isopropanol used for each rinse was 225 ml.

Pesticide extraction and analysis of all housedust, hand rinses, and soils were performed at a laboratory (TDI-Brooks International) located in College Station, TX. TDI-Brooks International has served as a contract laboratory on trace organics and metals analysis for the US Fish and Wildlife Services, Texas A&M University, and various government agencies, and has consistently been one of the top performers on the annual NIST/NOAA/EPA trace organic intercalibration exercises. Analysis was carried out for the presence of OP pesticides. The following pesticides were selected for analysis based upon information obtained from the local office of the Texas Agricultural Extension Service (TAEX): azinphos-methyl, chlorpyrifos, demoton O, demoton S, diazinon, disulfoton, ethion, fenithrothion, fonofos, malathion, ethyl parathion, and methyl parathion.

Extraction of dust filters employed an automated extraction apparatus, Dionex ASE 200 Accelerated Solvent Extractor (Dionex, Sunnyvale, CA) (Richter et al., 1994, 2001; EPA, 1997; Schantz et al., 1997; Ezzel, 1998; Zuloaga et al., 2000). This was used to extract organic analytes from 15 g of dried sediment and dust filter samples. Triphenyl phosphate was used as a surrogate and was added to the samples before extraction and used to assess the extraction and concentration efficiency of the procedure. The surrogate compound was resolved from — but eluted

in close proximity to — the analytes of interest. The extractions were performed with dichloromethane:acetone (50:50) inside stainless steel extraction cells held at elevated temperature (100°C) and solvent pressure (2000 psi). The analytes dissolved in the solvent were transferred from the heated extraction cells to a 60-ml glass collection vial. Extracts were then concentrated to a final volume of 1 ml (hexane:acetone 50:50) using an evaporative solvent reduction apparatus (Zymark TurboVap II; Zymark, Hopkinton, MA) (Patnaik, 1997; Wade et al., 1986; EPA, 1997a; Zuloaga et al., 2000; Richter et al., 2001; Schantz et al., 1997; Richter et al., 1994; Ezzel, 1998).

Extraction of hand rinses was carried out as follows. The surrogate compound (triphenyl phosphate) was added to the dermal rinse samples (collected in 100% isopropyl alcohol) prior to concentration and was used to assess the efficiency of the procedure. The surrogate compound was resolved from — but eluted in close proximity to — the analytes of interest. Extracts were concentrated to a final volume of 1 ml of hexane:acetone (50:50) using the Zymark TurboVap II (Zymark) set at an initial temperature of 70°C.

A capillary gas chromatographic method was used to determine the concentration of OP compounds. Fused silica, open-tubular columns were used in this method as they offered improved resolution, selectivity, increased sensitivity, and decreased time over packed columns. Quantification of pesticides was carried out with a HP5890 gas chromatograph (GC; Hewlett-Packard, Palo Alto, CA) with a nitrogen phosphorous detector (GC-NPD) (Richter et al., 1994, 2001; EPA, 1997). The internal standard solution of 1-bromo-2-nitrobenzene was added to the sample extracts prior to instrument analysis for the determination.

The GC-NPD was temperature-programmed and operated in the split with a DB-5 (30 m, 0.25 mm ID, and 0.25 μm film thickness; J&W Scientific, Folsom, CA). Carrier flow was by conventional pressure control. The autosampler is capable of making 1–5 μl of injections. Single high-resolution capillary column and NPD were used. The data acquisition system was by HP Chemstation software, capable of acquiring and processing GC data. Instruments were calibrated using commercially purchased standards. Calibration solutions for pesticides were prepared at five concentrations, ranging from 0.5 to 10 μg/ml, by diluting a commercially available solution containing the analytes of interest. A calibration curve was established by analyzing each of the five calibration standards (0.5, 1.0, 5.0, 8.0, and 10.0 μg/ml), and fitting the data to a linear equation.

Urine collection is a relatively easy and noninvasive method for biological monitoring. Urine samples were used to examine exposure to a suite of OP pesticides through measurement of nonspecific dialkyl phosphate metabolites of the pesticide. A complete void was collected on the morning following the collection of the housedust and hand rinse samples, so as to better represent the exposure period.

Collection of urine samples from children who are not toilet-trained has been considered problematic and, thus, 2-year-olds are typically not used in studies where urine sampling is conducted. Because of difficulties in extracting the urine from the gel formed in commercially available disposable diapers, these are generally not considered useful for urine collection. Some investigators have utilized cotton gauze for this purpose (Hu et al., 2000). A similar methodology was employed; however, in place of cotton gauze, cotton terry cloth diaper inserts (Organic Diaper Doublers; Ecobaby Organics, El Cajon, CA) were employed. These are larger, more absorbent, and, in tests we conducted, exhibited no breakthrough with up to 500 ml of liquid. Prior to use, each insert was washed once in a mild detergent and rinsed five times, with the final rinse containing vinegar. Diapers were dried following washing. The inserts were provided to the parents along with a disposable outer covering (water-proof laminate), purchased from the same source. Children who were toilet-trained had their urine sample collected either directly from lined ''potty chairs'' or they were allowed to urinate directly into a cup. The urine samples were collected the morning after the hand rinse and placed in a plastic bag and stored on ice. Diaper inserts were similarly stored and sent to our laboratory at TAMU for extraction. Inserts were extracted with a needleless 20-ml syringe. Urine was recovered from the diaper insert by placing the syringe firmly on the insert and withdrawing the plunger. After collection, samples were shipped, on dry ice, to the Centers for Disease Control and Prevention (CDC) in Atlanta.

The urine samples were analyzed for the presence metabolites of OP pesticides. All OPs have the same general structure and mode of toxicity. They are composed of a phosphate (or phosphorothioate or phosphorodithioate) moiety — which, in most cases, is *O*,*O*-dialkyl-substituted, where the alkyl groups are either dimethyl or diethyl — and an organic group, which is specific to each pesticide. For instance, chlorpyrifos is composed of an *O*,*O*-diethyl phosphorothioate to which a 3,5,6-trichloropyridinyl group is attached. After a given OP pesticide enters the body, it is usually metabolized by enzymatic hydrolysis to form one or more of six common dialkyl phosphate metabolites and the more specific ''organic'' moiety. All of these metabolites are excreted in urine. However, only 28 of 39 commercially used OP pesticides metabolize to these six metabolites. At the CDC, the common alkyl phosphate metabolites are measured *via* codistillation of 4 ml of urine, chemical derivatization of the metabolites to chloropropyl phosphate esters, and analysis using gas chromatography tandem mass spectrometry (GC-MS/MS) with quantification by the isotope dilution technique (Bravo et al., 2002). Matrix-based pools were used for quality control.

The use of the stable isotope of each of these metabolites allows for the highest degree of accuracy and precision. The

Table 1. CDC reference OP pesticide analysis summary.

Analyte	Parent compound(s)	Analytical LOD[a]	Reference range[b,c] μg/l (μg/g)
DMP	Any dimethyl OP (e.g., methyl parathion, azinphos methyl)	1.0	Median=1.8 (1.5); 95th=12 (18)
DMTP	Any dimethyl OP with one or two sulfurs (e.g., methyl parathion, chlorpyrifos methyl)	1.0	Median=4.8 (4.0); 95th=67 (59)
DMDTP	Any dimethyl OP with two sulfurs (e.g., azinphos methyl, dimethoate)	0.50	Median=0.55 (0.39); 95th=18 (18)
DEP	Any diethyl OP (e.g., fonofos, chlopyrifos)	1.0	Median=4.5 (3.5); 95th=25 (34)
DETP	Any diethyl OP with one or two sulfurs (e.g., chlorpyrifos, parathion)	0.50	Median=1.2 (1.1); 95th=23 (23)
DEDTP	Any diethyl OP with two sulfurs (e.g., fonofos, terbufos)	0.50	Median=nd (0.05); 95th=3.1 (2.3)

[a]Calculated for each study. Results nearer to the LOD are subject to greater uncertainty. The LOD is determined for the entire measurement system, not just the instrument.
[b]CDC, unpublished data, NHANES III (1988–1994).
[c]95th=95th percentile; ND means not detected above the LOD; values are expressed in micrograms per liter urine and micrograms per gram creatinine in parentheses.

LODs of the method are in the low to mid picograms-per-milliliter range (parts per trillion) with coefficients of variation (CV) of about 10–15% for most of the analytes. These six metabolites are dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). *O*,*O*-dimethyl pesticides can metabolize to DMP, DMTP, and/or DMDTP. For example, methyl malathion, which is an *O*,*O*-dimethyl phosphorothioate, can metabolize to DMP and DMTP. *O*,*O*-diethyl pesticides can metabolize to DEP, DETP, and/or DMDTP. For example, chlorpyrifos can metabolize to form DEP and DETP. The LOD and the general information on metabolites analyzed at CDC are presented in Table 1. All laboratory procedures were performed in accordance with the Clinical Laboratory Improvement Act of 1988.

Statistical analysis was carried out on the results of the environmental and bioassay sampling. Both univariate and multivariate analyses were carried out by utilizing SAS statistical software (version 8.0). For environmental samples, the recorded value, even if less than the method detection limit (MDL), was used in the calculations. All urine samples under the LOD were assumed to be 0.00. The statistics computed included the mean, median, range, distribution, and standard error of the environmental and bioassay samples. Multiple linear regression analyses were computed, as well as correlation matrices. This analysis was used to examine the potential association between the environmental metrics (housedust and hand loading of pesticides) and the urinary bioassay (pesticide metabolites) as the outcome variable. Total OP levels were compared for these three metrics. Other variables included gender, age (months), and hand surface area.

Table 2. Results of the environmental sampling for OP pesticides (nmol).

Pesticide	Housedust (n=29)			Hand rinses (n=41)		
	Mean	Median	SD	Mean	Median	SD
Demeton O	1.33	0.00	3.67	0.08	0.08	0.42
Demeton S	2.22	0.62	6.56	0.28	0.00	1.05
Fonofos	0.08	0.04	0.11	0.03	0.00	0.11
Diazinon	4.03	0.20	16.31	0.11	0.00	0.31
Disulfoton	0.17	0.11	0.24	0.10	0.00	0.14
Parathion methyl	0.49	0.15	0.64	0.50	0.00	1.66
Fenithrothion	0.36	0.11	0.77	0.01	0.00	0.06
Malathion	0.06	0.00	0.11	0.07	0.00	0.23
Chlorpyrifos	0.87	0.06	3.11	0.08	0.00	0.27
Parathion ethyl	0.00	0.00	0.00	0.13	0.00	0.36
Ethion	0.34	0.13	0.56	0.02	0.00	0.06
Azonphos methyl	0.94	0.16	1.61	0.11	0.00	0.34
Total OP (levels)	10.88	5.47	17.24	1.43	0.30	2.86
Total OP (loadings) (nmol/100 cm^2)	0.15	0.07	0.23	1.33	0.27	2.69

Results

The total number of households enrolled was 29 (target 30) and the total number of children enrolled was 52, composed of 25 boys and 27 girls (target 30 boys and 30 girls). The children were 7–53 months of age at time of testing in the spring/summer of 2000.

The fathers or adult male members of the household worked in a variety of occupations: truck driver (13.3%), carpenter (13.3%), electrician (13.3%), construction worker (10%), and farmworker (13.3%) were the most common. None of the men worked directly in mixing or loading of pesticides, with one driving farm equipment. Only three women in the study households (10%) worked outside the home, with one working in a farm-related activity (packer).

Indoor use of pesticides within the last 6 months was reported by 82.8% of the families. One-third of respondents did not know what pesticide was used. The most frequently reported pesticides were Raid (six) and Green Light (four). Also reported were Combat, Ray, Ray Max, Roachbox, Baygon, Hotshot/Gis, Tat, D-Con, and Max (one each). Parents were asked where they used pesticides. Most of the families reported using the pesticides in the kitchen. More than half of the families reported using pesticides in other rooms as well.

The most common areas in rooms where the pesticides were used were the floor (48.3%), cupboards where dishes were stored (41.4%), and cabinets used for storage (31%). All applications were performed by the resident, and none done by professional exterminators. Most of the users reported using pesticides three to six times in the last 6 months (61.1%). Most reported that they used pesticides on an ''as needed'' basis (87%).

Outdoor use of pesticides was reported by 58.6% of the families, although no specific products were reported. Outdoor use was usually two to three times in the last 6 months (45.5% of users). Treatment of lawns was infrequently reported (10.3%). Only about 30% of the homes has lawns. The majority of participants reported that they purchased pesticides for their house and yard at the supermarket (76%). Pesticides were also purchased at hardware stores (10.1%) and farm supply stores (10.1%). We also examined whether children might be exposed to pesticides through the pets or other residential animals. Forty-two percent of families reported having at least one dog. Flea collars were used by half of the families with dogs. Other commonly owned animals were birds (25.9%) and chickens (11.1%).

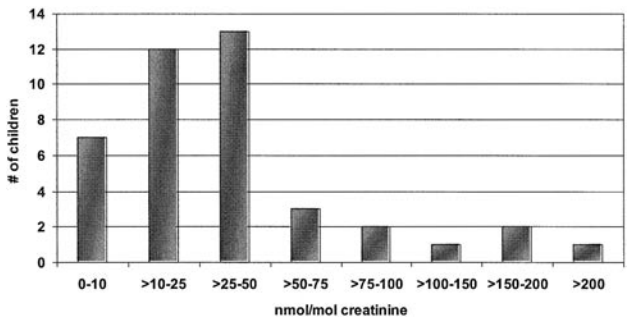

Figure 1. Distribution of levels (nmol/mol creatinine) of urinary metabolites for total OP pesticides in 41 children residing in Rio Bravo, TX.

Table 3. Results of OP metabolite in urine testing (nmol/mol creatinine corrected); $N = 41$.

Analyte	Mean[a]	Median[a]	Range	% Detects
DMP	20.0	8.8	nd–143.5	95.7
DMTP	5.0	0.0	nd–79.6	19.6
DMDTP	0.04	0.0	nd–1.5	4.4
DEP	9.8	1.8	nd–64.8	56.5
DETP	7.6	4.6	0.9–40.6	100.0
DEDTP	0.6	0.0	nd–12.8	26.1
Total OPs	42.6	27.4	3.2–257.0	100.0

nd = nondetect.

[a]Nondetects were assumed as 0.00 for purposes of computing means and medians.

Analysis of housedust and hand rinses was conducted for OP pesticides. The pesticides that were detected, either in housedust or hand rinses were: azinphos-methyl, chlorpyrifos, demoton O, demoton S, diazinon, ethion, fenithrothion, ethyl parathion, and methyl parathion. Actual LODs for total quantity of sample were between 1 and 10 pg. MDL for OPs was 0.5 μg/g for housedust and 0.5 μg/ml for hand rinses. The means, medians, and standard deviations for the individual OP pesticides detected in the housedust and hand rinses are presented in Table 2. A univariate statistical analysis was carried out on the levels of pesticide in the housedust, hand rinse, and urine samples. Approximately three-quarters (76%) of the housedust samples and half of the hand rinse samples and none of the soil samples contained OP pesticides. In addition to concentrations, OP loadings were calculated for floor surfaces and children's hands. These were computed by multiplying the detected concentration by the quantity of sample and dividing by the measured surface area.

The results of the CDC analysis on six urinary metabolites of OP pesticides for 41 urine samples are presented in Table 3 and the distribution of levels of total OP pesticide metabolites is presented in Figure 1. The most commonly detected metabolites were DMP and DETP, which were found in 100% and 95.7% of the subjects, respectively. Additionally, DMP had the highest mean and median levels of metabolite detected. This metabolite is consistent with the pesticides methyl parathion and azinphos-methyl, both of which were detected in the hand rinses and housedust samples. Either methyl parathion or

Table 4. Multivariate regression analysis — final model.

Parameter	Estimate	Pr>|t| 95%	Confidence limits
Age (months)	−2.11	0.0070	−3.60 to −0.61
Gender (male=1, female=0)	−14.82	0.3101	−44.02 to 14.38
Hand area (cm^2)	1.27	0.49	0.28 to 2.28
Hand load (ng/cm^2)	6.39	0.0219	0.98 to 11.80

azinphos methyl was detected in approximately 83% of the dust samples and 24% of the hand rinses. Total OP loadings in the housedust ranged from nondetectable (nd) to 78.03 nmol/100 cm^2 (mean=0.15 nmol/100 cm^2; median=0.07 nmol/100 cm^2); total OP loadings on the children's hands ranged from nd to13.40 nmol/100 cm^2 (mean=1.21 nmol/100 cm^2; median=1.41 nmol/100 cm^2), and creatinine corrected urinary levels (nmol/mol creatinine) of total OP metabolites ranged from 3.2 to 257 (mean=42.6; median 27.4 nmol/mol creatinine).

Multivariate linear regression analysis was carried out to evaluate the association of pesticide levels and loadings of total OPs in housedust and on hands with total urinary OP metabolite levels. Age in months and gender (male=1, female=0) were also included in the model, as well as hand surface area. The latter term was included as an additional surrogate of physical development in addition to age, as age and growth can be effected by premature birth. Only those children with all environmental variables were included in the analysis. A total of 41 children were included in the multivariate analyses (19 boys and 22 girls). Separate analyses were carried out using levels and loadings. Models that included both were somewhat unreliable in their parameter estimates because of the high degree of colinearity between levels and loadings both for housedust and hand rinses. For the former, the correlation coefficient was 0.99 and for the latter 0.90. In the final model (Table 4), age was inversely associated with urinary levels of OP metabolites parameter estimate, with total OP levels in urine declining by 2.1 nmol/mol creatinine/month of age (95% CI −3.60 to −0.61). Gender was not statistically significantly predictive for OP metabolite level. The overall fit of the complete model with regard to housedust and hand pesticide loadings just missed statistical significance at the 0.05 level (r^2=0.26; P=0.0758). When housedust was dropped from the model, the fit improved (r^2=0.28; P=0.0156). Individual terms included in the model were hand surface area (pe=1.28, 95% CI 0.28–2.28) and the hand loading term (pe=6.39, 95% CI 0.98–11.80). Levels of correlation between dependent variables did not exhibit a high degree of colinearity. The highest correlation coefficient observed was between age (months) and hand surface area (r=0.6581). Somewhat surprisingly, hand surface area and hand loadings of OPs were not highly correlated at all (r=0.0300). Separate analyses utilizing transforms to adjust the data to compensate for lack of normality in the distribution of the dependent and independent variables did not improve the fit of the model.

Discussion

Seventy-six percent of the homes sampled contained detectable OP pesticides, while half the rinses from children's hands had detectable levels of OP pesticides. The soil samples contained no OP pesticides above the MDL. The absence of OP pesticides in soils was somewhat surprising, but may be the result of the unusually warm weather in the area during the first round of sampling in the spring/summer of 2000. These hot, sunny conditions may have increased the rate at which OP pesticides degraded outdoors, while these same compounds may not have degraded as readily in dust samples from within the dwellings and, therefore, out of direct sunlight. At the same time, detectable levels of OP metabolites were present in the urine samples from all the children tested in the first round of this study.

In the present study, detectable levels of OP metabolites were observed in urine samples from all of the children tested. However, only three out of four of the homes had detectable levels of OPs in the housedust. It also is worth noting that over 21% of the urine samples had OP metabolite levels greater than 50 nmol/mol creatinine, with 9.8% over 100 nmol/mol creatinine. This suggests that the cross-sectional environmental sampling conducted in this study was not able to identify all of the children's sources of exposure. Even though many of the current OP compounds are readily metabolized and excreted, a detailed understanding of the relationship between observed environmental levels of pesticides and urinary metabolites clearly requires more study.

Another interesting factor about the population in the current study is that the level of OP pesticide metabolites found in the urine samples of these children was relatively high, given that these were not "farm" households. When compared to NHANES (1999) data for the general population, which are available for 6- to 59-year-olds, the levels of the six metabolites in these infants and young children ranged from approximately 3 1/2 to almost 13 times that observed in NHANES (CDC, 1992). Since our findings suggest that younger children have higher levels of OP metabolites in their urine than older children, and the NHANES population is considerably older than ours, perhaps this finding should not be considered surprising.

The preliminary data presented found virtually no correlation between loadings of OPs in housedust on floor surfaces and levels of OP metabolites measured in urines.

This may be explained by the fact that exposures of these very young children are likely to be multifactorial, including ingestion of soil and dietary ingestion. In our limited study of soils in this area, we were, however, surprised by the fact that in general, soils had lower levels and fewer detectable pesticides than housedust. Environmental factors including the high spring and summer ambient temperatures and intense ultraviolet levels from sunlight may have effected these findings. The most unexpected factor in the analysis, and therefore one that should be viewed guardedly, is the apparent importance of hand surface area as a separate predictor of dose. While it cannot be excluded that this is solely a chance finding, it is possible that hand size is acting as a surrogate for some factor or factors associated with the child's developmental stage. This will be evaluated in the later rounds of testing in the current study.

The study observed an apparent association between loadings of OPs on children's hands and levels of urinary metabolites of OPs. The current model explains about one-quarter of the association at best. Clearly, child behavior is a critical factor in the relationship between hand loadings and nondietary ingestion of OPs. Currently, quantitative analysis of hand-to-mouth and object-to-mouth behaviors of these children is proceeding. This quantitative behavioral analysis has the potential for providing important information on the effect modification of an individual child's behavior on nondietary ingestion and, therefore, on dose. It is also likely that some general characteristics associated with age- and gender-specific behavior patterns will clarify this association. Given the broad range of interindividual variation in the frequencies of these behaviors that have been observed in the preliminary analysis of the data, more data will be required to develop meaningful models of exposure for epidemiologic purposes. It is only through a better understanding of the interaction between children and their environment that accurate dose estimations will become possible.

One conclusion that clearly should emerge from this preliminary evaluation is that young children, in these border communities, have elevated levels of OPs in their urine. We also observed a monotonic decline in urinary OP levels from 6 months of age, suggesting need for study of even younger infants. Additionally, given the youthful demographics of US/Mexico border communities and the high birth rate, the development of a more comprehensive understanding of the human health risks these environmental contaminants present is essential. Little is known about the potential for chronic health hazards long-term exposure to these chemicals may represent. For these reasons, the importance of continued study of environmental pesticide exposure and its possible role in the etiology of chronic illness among infants and children in communities along the US/Mexico border must not be overlooked.

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