Biological Monitoring of Organophosphorus Pesticide Exposure among Children of Agricultural Workers in Central Washington State

Carrie Loewenherz, Richard A. Fenske, Nancy J. Simcox, Garland Bellamy, and David Kalman
Department of Environmental Health, University of Washington, Seattle, WA 98195 USA

Children up to 6 years of age who lived with pesticide applicators were monitored for increased risk of pesticide exposure: 48 pesticide applicator and 14 reference families were recruited from an agricultural region of Washington State in June 1995. A total of 160 spot urine samples were collected from 88 children, including repeated measures 3–7 days apart. Samples were assayed by gas chromatography–flame photometric detector for dimethylphosphate metabolites. Dimethylthiophosphate (DMTP) was the dominant metabolite. DMTP levels were significantly higher in applicator children than in reference children (p = 0.015), with median concentrations of 0.021 and 0.005 μg/ml, respectively; maximum concentrations were 0.44 and 0.10 μg/ml, respectively. Percentages of detectable samples were 47% for applicator children and 27% for reference children. A marginally significant trend of increasing concentration was observed with increasing age among applicator children (p = 0.060), and younger children within these families had significantly higher concentrations when compared to their older siblings (p = 0.040). Applicator children living less than 200 feet from an orchard were associated with higher frequency of detectable DMTP levels than nonproximal applicator children (p = 0.036). These results indicate that applicator children experienced higher organophosphorus pesticide exposures than did reference children in the same community and that proximity to spraying is an important contributor to such exposures. Trends related to age suggest that child activity is an important variable for exposure. It is unlikely that any of the observed exposures posed a hazard of acute intoxication. This study points to the need for a more detailed understanding of pesticide exposure pathways for children of agricultural workers.

Key words: agriculture, applicator, azinphos-methyl, biological monitoring, children, exposure, insecticides, metabolites, organophosphates, organophosphorous, pesticides, phosmet, urine.

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The nonacute health consequences of children's exposure to pesticides is a subject of great uncertainty and increased public health concern. Reported associations between childhood leukemia and parental exposures to pesticides and/or residential applications of pesticides have been reported in the epidemiologic literature (1–4). A number of studies have investigated potential exposures following residential pesticide applications (5–7). Dietary pesticide exposure among infants and children was the focus of a recent report of the National Research Council (8). Passage of the Food Quality Protection Act of 1996 has led the EPA to consider childhood pesticide exposure in aggregate; i.e., total exposure to specific compounds from multiple sources and exposure pathways and total exposure to compounds with a common mechanism of toxicity (9). However, few studies to date have evaluated exposures in potential high risk populations such as children living with farmers, farmworkers, or near a farm. Studies on farm-proximal populations have most often followed a misapplication event, with no specific focus on children.

The exposure potential for children of agricultural families may be higher than for other child populations because concentrated formulations of pesticides are used in high volume near the home. Pesticides used during work also may be introduced into the home inadvertently via various take-home pathways. This type of exposure, often referred to as paraoccupational exposure, has been well documented for a number of industrial chemicals and was the subject of a recent report of the U.S. National Institute for Occupational Safety and Health (10). Poor hygienic practices among pesticide formulators have been associated with measurable blood levels of pesticides (chlordecone or kepone) in family members (11). Classic organophosphorus (OP) pesticide exposure symptoms in spouses and children of greenhouse workers have been reported (12). Several studies have also shown that agricultural workers bring contaminated clothing into the home (13,14).

A recent study by our group found evidence that children living with agricultural workers and in proximity to tree fruit orchards may have more opportunity for exposure than children living in homes without such risk factors (15). A recent pilot study in California's Central Valley reported generally higher pesticide house-dust concentrations in homes of farmworkers as compared to nonfarmworker homes (16). Improved analytical methods now allow measurement of a broad range of pesticide metabolites in the urine of agricultural workers and their families (17). The work presented here is part of a continuing effort to better characterize children's pesticide exposure in agricultural settings. This paper focuses on the use of biological monitoring to determine the extent to which children of pesticide applicators are exposed to OP pesticides. Specific aims were 1) to measure urinary metabolite levels of OP pesticides in children living with occupationally exposed parents and compare these with a reference population, and 2) to evaluate the relative importance of paraoccupational exposure pathways. A subsequent report will address the relationship between biological levels, environmental residues, and dermal exposures, with the purpose of identifying strategies to prevent or reduce such exposures.

Methods

Study design and population recruitment. The study design was cross-sectional with repeated measures. The agricultural region selected for the study is in central Washington State (Douglas and Chelan counties) and was the site of our previous investigation (15). The primary industry for the region is tree fruit, with a substantial portion of acreage in small family orchards. The area was chosen in part because participants were likely to represent both owner/operator

Address correspondence to R.A. Fenske, Department of Environmental Health, Box 357234, University of Washington, Seattle, WA 98195 USA.

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and nonseasonal farmworker populations and because of the opportunity to sample many of the homes included in the earlier study. The study took place in June and July of 1995 and involved two visits to each participating family.

Families living in the Chelan–Douglas County area with at least one child no older than 6 years of age were recruited on the basis of distance of the home from an orchard and on parental occupation. Participants were enrolled in the study in an effort to create two study populations differing in proximity to orchards and a reference population. Criteria for these populations were as follows: Population 1 had at least one household member working regularly as a pesticide applicator and the residence was within 200 ft (61 m) of a regularly treated orchard; Population 2 had at least one household member working regularly as a pesticide applicator, but the residence was more than 200 ft from an orchard or crop; and Population 3 (reference) had no family member working in the agricultural industry, and the residence was more than 200 ft from an orchard or crop. The proximity criterion for study groups (<200 ft or >200 ft) was based on our previous study of pesticide residues in this same region (15) and on corroborative findings in the literature (18). Recruitment goals were 25 families for each of the study populations and 15 families for the reference population. A population of pesticide applicators living >200 ft from farmland (Population 2) could not be identified, so the study design was reduced to a single study population and a reference population.

Agricultural families were identified in several ways. In March 1995, a request for participants was sent to 424 applicants registered with the Washington State University Cooperative Extension. An invitation to participate in the study was also placed in the April 1995 newsletter of the Washington Growers’ Clearinghouse in Chelan and Douglas counties, reaching approximately 3,000 members. Flyers were posted in the Washington State Department of Health’s Pesticide Laboratory, the site of other similar projects. Families were also recruited from the 59 subjects who had participated in a previous study by our group and from local organizations serving the farmworker population, including the Community Action and Columbia Valley Community Health Services—First Steps Program. Another pool of potential subjects resulted from follow-up calls made to the subjects who were asked to refer us to likely participants. Reference families were contacted through the same service organizations mentioned above (staff members and their neighbors), referrals made by existing subjects, and participants of a study conducted at the Health Department laboratory. Procedures associated with this study were approved by the University of Washington Human Subjects Review Committee, and informed consent was obtained from all subjects.

Urine sample collection. Two spot urine samples were collected within a week of each other from every willing child up to 6 years of age. In the event that a sample was not obtained during the home visit, collection apparatus and instructions were left with the parent. Specimens collected in this manner were picked up within 24 hr of the void. Samples were obtained using either a urine collection bag (Lil’Katch; General Medical Corp., Richmond, VA) for the non-pesticide applicator children or a commode insert (Specipan; Baxter Scientific, McGaw Park, IL) for the pesticide applicator children. Urine collection bag specimens were immediately transferred to freezer-safe polypropylene jars while the commode inserts were sealed using the provided snap top; all containers were then placed in plastic ziplock bags for transport in an ice chest.

All specimens were transported to the field lab where they were processed and stored at -10°C. All samples were stored at -20°C or at the analytical lab until analysis within 7 months from the date of sample collection.

Interviews. Interviews were administered in either Spanish or English, as necessary, and included questions regarding frequency and extent of occupational and residential pesticide use, cleaning activities, laundry practices, protective equipment use, proximity to spray sites, and child activity. Second visits included questions regarding child activity and pesticide use (residential and occupational) since the first visit.

Dialkylphosphate metabolite analysis. Analytes were limited to the dialkylphosphate metabolites that result from organophosphate compounds with dimethyl moieties. The three analytes were dimethylphosphate (DMP), dimethyly phosphoryl (DMTP), and dimethyldithiophosphate (DMDTP). This decision was based on prior knowledge that the only OP pesticides likely to be used in the region during the May to July portion of the season were azinphos-methyl, O,O-dimethyl S-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]methyl phosphorodithioate, also known as guthion, and phosphet, O,O-dimethyl phosphorodithioate S-ester with N-methylphosphoramidate, also known as imidan. Dialkylphosphate metabolite standards were provided by Miles Inc. (Kansas City, MO) and American Cyanamid (Wayne, NJ).

Solvent-based calibrants (0.01–1.00 µg/ml) were prepared by spiking acetonitrile with DMP, DMTP, and DMDTP. Benchmark samples were prepared by spiking an unexposed urine pool. Samples were prepared in batches for gas chromatographic analysis using the methods of Nutley and Cocker (19) and Fenske and Leffingwell (20), modified to minimize the presence of water during derivatization. Urine was stored at -10°C until preparation, which was as follows: azeotropic distillation was performed by adding 4.0 ml acetonitrile to a 1-ml aliquot of the sample, centrifuging at 2,500 RPM, and evaporating the supernatant twice under N₂ stream at 90°C (first evaporation to near dryness and second evaporation to complete dryness); samples were then reconstituted in acetonitrile. The samples were derivatized with 25 µl pentafluorobenzyl bromide (PFBB) and heated (50°C) for 16 hr to convert the phosphate acids to esters.

Quantification of the target metabolites was performed using a Hewlett-Packard gas chromatograph (HP5890) with a flame photometric detector (FPD). Injections of 1 µl were made in the splitless mode, and quantifications were achieved using internal injection of tributyl phosphate (TBP); i.e., metabolite concentrations were calculated by converting sample response (ratio of metabolite peak to TBP peak area) using a linear calibration curve. Conditions were as follows: helium was used as the carrier gas (column velocity -25 cm/sec); the oven temperature was 50°C initially for 1 min, with a 15°C increase per minute to 115°C, a 5°C increase per minute to 175°C, a 10°C increase per minute to 270°C, and then temperature was held at 270°C for 4 min (31 min total); an FPD was used in phosphorus mode at 230°C with hydrogen flow at 75 ± 1 ml/min, air flow at 100 ± 2 ml/min, and nitrogen make-up flow at 30 ± 1 ml/min.

Creatinine analysis. Creatinine concentrations (mg%) were measured to identify adulterated or abnormal samples. Determinations were made using a Sigma 555-A colorimetric kit (Sigma, St. Louis, MO) and a Milton Roy Spectronic 301 spectrophotometer (Milton Roy, San Landro, CA). A total of 243 child urine samples from this and a companion study constituted a database from which a normal range could be estimated. The creatinine range for all samples was 3–197 mg % creatinine. The 5th and 95th percentiles were 10 and 124 mg % creatinine, respectively. Of the 17 study samples that fell outside this range, very dilute detectable samples were considered to be true positives, and very concentrated nondetectables or trace samples were considered true negatives. Very dilute nondetectables and very concentrated detectables (10
samples) were considered unreliable measures and were excluded from all enumerative and statistical analyses.

**Quality assurance.** Table 1 specifies the method limits of detection and quantitation, method extraction efficiencies, and field recovery efficiencies. Extraction efficiencies of DMTP averaged 80%; DMDTP was less efficiently extracted (62%); and extraction of DMP was poor (39%). DMTP was also the most frequent metabolite measured, followed by DMDTP. DMP concentrations are not reported or discussed due to their low percent recovery and the fact that calibration curve fit errors frequently exceeded 15%. Extraction efficiency values were based on laboratory spike and recovery studies conducted prior to sample analysis. Additionally, extraction efficiency was monitored on a sample-by-sample basis through use of dibutyl phosphate as a recovery surrogate and was found to be consistent with the above values. None of the metabolite concentrations from samples were corrected for extraction efficiency.

Field blank urine samples (n = 23), prepared at the University of Washington (UW) laboratory from pooled urine of unexposed children and stored at -20°C (UW lab) or -10°C (field lab), had no detectable DMTP attributable to field activities. Field urine spikes (n = 22) were prepared prior to field work from pooled urine of unexposed children. DMTP field spikes ranged between 0.062 µg/ml and 0.104 µg/ml, resulting in an average recovery efficiency of 116%, with a coefficient of variation (CV) of 14%. Field recoveries were, with one exception, always >94% for DMTP. Recoveries for DMDTP field spikes were very poor, averaging 47% with a CV of 46%.

Ten storage stability samples were also prepared: a pooled sample of urine from unexposed Seattle children was spiked with the dialkylphosphate metabolites at a 0.07 µg/ml spike level and then split into 10 aliquots. Three samples were analyzed at the beginning of the study, three with the first batch of field samples, and four with the final batch of samples. Results from these samples did not indicate a loss of metabolites over time from collection to analysis. DMTP recoveries averaged 124% with a CV of 14%. DMDTP recoveries were again less efficient, with an average of 42% recovered and a CV of 41%.

Intra-assay precision was assessed through replicate injections of a calibrant interspersed between unknown samples for each batch. Precision values (CV) were 5.0% for DMTP, 11.4% for DMP, and 3.2% for DMDTP. The average of these calibrants provided a measure of inter-assay precision over 10 batches.

| Table 1. Instrument limits of detection (LOD), method limit of quantitation (MLOQ), method extraction efficiencies, and field recovery efficiencies for analysis of dialkylphosphate metabolites in urine |
|-------------------|-----------------|-----------------|-----------------|
| Metabolite        | LOD a (µg/ml)   | MLOQ b (µg/ml)  | Method extraction efficiency c |
| DMTP              | 0.015           | 0.020           | 80% (11)         |
| DMDTP             | 0.013           | 0.040           | 62% (9)          |
| Field recovery efficiency d |
| DMTP              | 116% (13)       | 47% (43)        | |

Abbreviations: DMTP, dimethylphosphate; DMDTP, dimethyldithiophosphate.

a The instrument LOD was determined with analytical standards in solvent (no matrix effect) and is defined as the concentration at which the peak height is three times the baseline noise.

b The LOD was determined by spiking urine to account for matrix effects.

c Values are means, with coefficients of variation in parentheses. Twelve samples were spiked at 0.36 µg/ml for DMTP and at 0.28 µg/ml for DMDTP.

d Values are means, with coefficients of variation in parentheses. Twenty-three samples were spiked at 0.072 µg/ml for DMTP and at 0.070 µg/ml for DMDTP.

**Statistical analysis.** A majority of the samples was found to contain either trace or nondetectable concentrations of dialkyl urinary metabolites. Preliminary analyses were thus limited to enumerations of detectable, trace [detectable but with concentrations below the limit of detection (LOD)], and nondetectable samples and chi-square tests for homogeneity across groups. More sophisticated analyses entailed development of a standard procedure for assigning quantitative values for the trace samples. The most common treatment of such samples has been to assign them the value of one-half the LOD, especially when the majority of measurements fall below the LOD. This method was used to replace the trace (<LOD) values, while nondetectable samples were assigned values of zero.

Data were not normally or log-normally distributed; means, medians, concentration ranges, and nonparametric statistical tests were employed using the untransformed metabolite data. All participating children were sampled twice to provide an estimate of within-person variability. The Wilcoxon Signed Rank test was used to compare means associated with the two different sampling sessions and metabolite levels in siblings within a sampling session. The Mann-Whitney U test and Kruskal-Wallis analysis of variance (ANOVA) were used to compare metabolite means across groups within a sampling session. The statistical significance was set at the α = 0.05 level, while a level of α = 0.10 was used to indicate marginally significant relationships.

The analyses used for these data require an assumption of independence across samples. This proved problematic in that the data included two dependency structures: within-child due to repeated measures, and within-household due to sibling inclusion. In order to handle the potential within-child dependence, frequencies, means, and medians were compared across sampling visits (e.g., Visit 2 samples from one group compared with Visit 2 samples from another group). Removing the within-household dependence required that each household be associated with only one child for most analyses.

**Results**

Ninety-seven potential applicator families were contacted, and 48 (50%) were ultimately enrolled. Of those who were not enrolled in the study, 11 declined to participate, 35 completed the screening process but were not eligible, and 3 completed the sampling sessions but had incomplete or invalid samples. Forty potential reference families were contacted and 14 (35%) were ultimately enrolled. The families not enrolled included 1 who declined to participate and 25 who were ineligible either based on the reference criteria (i.e., no child in the proper age group or residence in proximity to an orchard) or because the family members worked in the fields or packing houses. An additional 3 families (7.5%) met the study criteria initially, but were ruled ineligible during the course of the study: one was found to be living too close to an orchard, another lived with a second family that included fieldworkers, and a third did not have a primary parent available at the time of interview.

The majority of participating households were Hispanic (71% for applicator and 64% for reference families). Twenty-three households included more than one participating child. To eliminate potential within-house dependence between siblings, one child from each household was identified as a focus child. Focus children were chosen on the following grounds: completeness of sampling (two samples) and quality of samples (acceptable creatinine measurement). Random selection was then made for households with more than one child meeting these criteria. Applicator
families included a total of 70 children with a mean age of 3.45 years. Reference families had a total of 18 children with a mean age of 3.48 years. The male/female distribution was nearly equal for the applicator children: 51% of all applicator children were males, as were 52% of focus children. Reference children were more heavily represented by males: 67% of all children and 79% of focus children.

**Agricultural pesticide use.** The 48 applicators were surveyed regarding occupational use of pesticides, use of personal protective equipment, and personal hygiene practices. During the 1995 spray period (January 1–July 1), 37/48 (77%) applicators reported using at least one dimethyl OP pesticide and 18/48 (38%) reported using more than one dimethyl OP pesticide. The most commonly used OP pesticide was azinphos-methyl (guthion); 75% (36/48) of applicators reported its use. The next most commonly used OP pesticide was chlorpyrifos (Lorsban, a diethylphosphate compound), with 67% reporting its use. Phosmet (imidazal) was the second most commonly dimethyl OP pesticide used [16/48 (33%)]. Six applicators (13%) reported spraying ethyl parathion. Nearly two-thirds (63%) of the applicators had sprayed within 200 ft of their homes at least once during the season.

The number of days since an applicator’s last spray was either recorded directly during interview or inferred from survey data. The most recently sprayed dimethyl OP pesticide was azinphos-methyl, with applications ranging from the day of sampling to a maximum of 80 days before initial sampling (Visit 1). Phosmet was applied from 6 days to a maximum of 109 days before the first visit. Frequency of OP application was difficult to determine. A review of the surveys indicated that participants may have interpreted this question differently: some provided the total number of days spent spraying while others reported total number of application events, which usually included several spray days. During the second sampling (Visit 2), 11 applicators (23%) reported using an OP pesticide since the time of the first visit, with all reporting azinphos-methyl use. Applications of azinphos-methyl occurred a maximum of 3–8 days before the second sampling.

About 21% of the households were found to include more than one person employed in agriculture: types of work included thinning and pruning trees and picking and bagging fruit. Only one family had more than one applicator living at the house.

**Residential pesticide use.** Participants were asked about any residential pesticide use (including herbicides, insecticides, and fungicides) over the last 6 months. Very few were found to have used dimethyl OP pesticides residentially. Of the 29 applicator families with pets, 9 reported treating them at home or commercially with flea powders or collars and/or shampoos. These products could have contained OP pesticides, but participants were unable to provide product names. Fourteen applicator households (29%) had used a pesticide product in their homes in the last 6 months, and 13 households (27%) reported using one on their lawn. One of these, an orchard owner/applicator, reported applying chlorpyrifos to his lawn.

**Applicator and reference children comparisons.** A total of 177 samples were analyzed for dialkyl urinary metabolites. Seventeen samples were excluded from statistical analyses, 11 due to abnormal creatinine measurements and 6 due to the child not meeting the age criterion of ≤6 years old. DMTP was detected with far greater frequency than was its di-sulfur counterpart DMDTP. For DMTP, 40% of all samples were detectable, 17% were trace (<LOD), and 43% were nondetectable; for DMDTP, 6.3% were detectable, 13.4% of samples were trace (<LOD), and 80.3% were nondetectable. Thus, DMTP was chosen as the most appropriate biomarker of exposure for this population, and data presented here are limited to DMTP concentrations only.

Differences in frequency of DMTP detectability among applicator and reference focus children for the two sampling visits are illustrated in Figure 1. These differences were not found to be statistically significant for Visit 1, but were significant for Visit 2 (chi-square, p = 0.022). The difference in frequency of nondetectable samples was nearly twofold for reference and applicator children during Visit 2 (60% and 33%, respectively). Tables 2–4 provide statistical descriptors of DMTP concentrations for all children and for focus children by study group, visit, and age group. The median DMTP concentration in applicator focus children was 0.021 µg/ml, four times that of reference children (Mann-Whitney U test, p = 0.015). This difference was particularly evident for Visit 2. These same statistical tests were also performed on creatinine-adjusted urine concentrations, with similar results (creatine-adjusted data is presented in the Appendix).

Sampling sessions occurred over a 5-week period, with first and second visits to different households often scheduled for the same day. DMTP median concentrations appeared to rise over the final 3 weeks for the applicator children, but this trend
was not statistically significant. There was, however, a notable increase in the variability in sample concentrations as sampling progressed, with the highest concentrations collected in the final 2 weeks. DMTP concentrations in reference children were slightly higher in the fourth week compared to the first week, but the highest median value was observed in the second week.

Age and exposure. A marginally significant trend of increasing concentration was observed with decreasing age within the applicator children (Mann-Whitney U test, \( p = 0.060 \)), and DMTP concentrations in 3-4 year olds were significantly greater than those of 5-6 year olds during Visit 2. Paired analysis of the 21 sibling pairs in the study found that the younger child in each pair had a significantly higher metabolite level than the elder child (Wilcoxon Signed Rank test, \( p = 0.040 \)).

Proximity to spraying. The 48 applicator households were categorized by distance from a nearby orchard, with 29 (60%) living within 50 ft, 8 (17%) living between 50 and 200 ft, 4 (8%) living 200 ft to one-fourth mile away, and 7 (15%) living farther than one-fourth mile. Sample size was insufficient for analysis across these four categories, so households falling into the first two and the last two categories were combined into two groups (<200 ft and >200 ft). Table 5 presents statistical descriptors for each visit, grouped by proximity. DMTP concentrations were highly variable within each group (all coefficients of variation >100%). Median DMTP concentrations did not differ between these two groups of children during Visit 1, but a marginally significant difference was observed during Visit 2, with proximal child concentrations higher than those of children living more distant from orchard spraying (Mann-Whitney U test, \( p = 0.062 \)). An effect of proximity on urinary metabolite concentrations during Visit 2 was also observed when we compared the frequency of detectable, trace, and nondetectable samples across these two groups in focus children, as illustrated in Figure 2. The frequency of detectable or trace samples was significantly higher in the proximal children (Fisher Exact test, \( p = 0.036 \)).

Exposures within applicator households. Twenty-one applicator households included at least two study children. Table 6 indicates that for Visit 1 the majority of sibling pairs (62%) had nondetectable DMTP levels for each child. Frequency of detectable samples increased significantly during Visit 2 (chi-square, \( p = 0.018 \)). Both siblings had detectable levels for nearly half of the pairs (48%) during Visit 2.

The 10 households having sibling pairs with detectable samples during Visit 2 were examined for common characteristics and compared to the 6 households with

### Table 2. Dimethylthiophosphate (DMTP) concentrations in urine (µg/ml) of applicator and reference children for both visits combined

|                      | Applicator children | Reference children |
|----------------------|---------------------|-------------------|
| All children         |                     |                   |
| Mean \(^b\)          | 0.039               | 0.018             |
| Median               | 0.015               | 0.000             |
| CV \(^c\)            | 162%                | 172%              |
| Range                | ND–0.435            | ND–0.104          |
| Number               | 127                 | 33                |
| Focus children (one per household) |       |                   |
| Mean                 | 0.042               | 0.016             |
| Median               | 0.021*              | 0.006*            |
| CV                   | 164%                | 203%              |
| Range                | ND–0.435            | ND–0.098          |
| Number               | 90                  | 25                |

Abbreviations: CV, coefficient of variation; ND, not detectable; LOD, limit of detection.

\(^a\)Tests for statistical significance were applied to data for focus children only (see Methods).

\(^b\)Mean and other univariate statistics were calculated by estimating trace samples as 1/2 LOD.

\(^c\)CV = standard deviation/mean × 100

\(^*\)Significant difference across applicator and reference children: \( p = 0.015 \) (Mann-Whitney U test).

### Table 3. Dimethylthiophosphate (DMTP) concentrations in urine (µg/ml) of applicator and reference children for each separate visit

|                      | Applicator children | Reference children |
|----------------------|---------------------|-------------------|
| All children         |                     |                   |
| Mean \(^b\)          | 0.027               | 0.048             |
| Median               | 0.009               | 0.023             |
| CV \(^c\)            | 163%                | 152%              |
| Range                | ND–0.196            | ND–0.435          |
| Number               | 63                  | 64                |
| Focus children (one per household) |       |                   |
| Mean                 | 0.033               | 0.049             |
| Median               | 0.015               | 0.019*            |
| CV                   | 162%                | 167%              |
| Range                | ND–0.196            | ND–0.435          |
| Number               | 46                  | 46                |

Abbreviations: CV, coefficient of variation; ND, not detectable; LOD, limit of detection.

\(^a\)Tests for statistical significance were applied to data for focus children only (see Methods).

\(^b\)Mean and other univariate statistics were calculated by estimating trace samples as 1/2 LOD.

\(^c\)CV = standard deviation/mean × 100

\(^*\)Significant difference across applicator and reference children: \( p = 0.036 \) (Mann-Whitney U test).

### Table 4. Dimethylthiophosphate (DMTP) concentrations in urine (µg/ml) of applicator and reference children by age of child

|                      | Applicator children | Reference children |
|----------------------|---------------------|-------------------|
| Age (years)          | Visit 1             | Visit 2           |
|                      |                     |                   |
| 0–2                  | 0.038               | 0.045             |
| Mean \(^b\)          | 0.017               | 0.026             |
| Median               | 0.015               | 0.023             |
| CV \(^c\)            | 91.1%               | 91.1%             |
| Range                | ND–0.140            | ND–0.126          |
| Number               | 19                  | 20                |
| 3–4                  | 0.039               | 0.059             |
| Mean \(^b\)          | 0.020               | 0.015             |
| Median               | 0.009               | 0.033*            |
| CV                   | 168%                | 168%              |
| Range                | ND–0.196            | ND–0.435          |
| Number               | 25                  | 25                |
| 5–6                  | 0.025               | 0.035             |
| Mean \(^b\)          | 0.004               | 0.021             |
| Median               | 0.009               | 0.009             |
| CV                   | 171%                | 171%              |
| Range                | ND–0.176            | ND–0.189          |
| Number               | 19                  | 20                |

Abbreviations: CV, coefficient of variation; ND, not detectable; LOD, limit of detection.

\(^a\)Tests for statistical significance were applied to data for focus children only.

\(^b\)Mean and other univariate statistics were calculated by estimating trace samples as 1/2 LOD.

\(^c\)CV = standard deviation/mean × 100

\(^*\)Marginally significant difference for 3–4 year old and 5–6 year old applicator children: \( p = 0.060 \) (Mann-Whitney U test).

\(^\)Significant differences across visits: \( p = 0.047 \) (Wilcoxon Signed Rank test).
nondetectable pairs (Table 7). Households with 9 of 10 detectable sibling pairs were within 50 ft of an orchard, while only 2 of 6 households with nondetectable pairs were so near, resulting in a 57% relative difference. A review of survey data also indicated that 9 of 10 of the sibling pairs with detectable samples lived with applicators who reported spraying within 200 ft of their home—a 40% difference from sibling pairs with nondetectable levels only. Seventy percent of the applicators that lived with the sibling pairs with detectable samples reported wearing work shoes inside the home compared to 33% of the siblings with nondetectables samples. No other striking differences were found between the two household types.

**Discussion**

This study is, to our knowledge, the first to document exposures to OP pesticides among children of agricultural workers and to compare these exposures to those of a reference population in the same region. In evaluating these findings, it is important to note several study limitations. First, this study does not represent a true probability sample of pesticide applicator households. Most registered pesticide applicators in the region were invited to participate in the study, but recruitment ultimately relied on voluntary participation. Also, some participants heard of the study by word of mouth, which may have resulted in interaction among participants. However, there is no evidence to suggest that the final study groups were atypical of the region’s population or that participant interaction affected the survey. Our work in this region and with this population over the past 5 years leads us to believe that the study participants were representative of the local population.

Second, it is important to consider that only a single biomarker was used for quantitative analysis. Organophosphorous pesticides such as azinphos-methyl and phosmet can be metabolized to several excretion products: DMP, DMTP, and DMDTP. Because the method for DMP analysis was variable and DMTP was found in very few samples, no attempt was made to calculate a pesticide equivalency value based on molar proportions of metabolites, as has been done in studies where higher concentrations were observed (21). Instead, DMTP was selected as the most reliable biomarker for comparison across study populations and for analysis of exposure pathway variables. Reliance on this single metabolite could result in some error due to variability in metabolism of these compounds, and molar equivalency values would be higher than those reported here.

**Table 5.** Dimethylthiophosphate (DMTP) concentrations (μg/ml) by proximity for applicator children only

| Proximity | All children (n = 64) | Focus children (n = 46) |
|-----------|-----------------------|------------------------|
|           | Visit 1 | Visit 2 | Visit 1 | Visit 2 |
| <200 ft   | 0.028 | 0.053  | 0.034  | 0.056  |
| Mean      | 0.009  | 0.028  | 0.015  | 0.023* |
| Median    | 171%   | 147%   | 159%   | 159%   |
| CV        | ND-0.196| ND-0.435| ND-0.196| ND-0.435|
| Range     | 19 (37)| 32 (63)| 16 (44)| 21 (58)|
| Frequency | 51     | 51     | 36     | 36     |
| Number    | 12     | 13     | 10     | 9      |

Abbreviations: CV, coefficient of variation; ND, not detectable; LOD, limit of detection.

*Marginally significant differences across proximity groups: p = 0.062 (Mann-Whitney U test). Statistical analysis conducted on focus children data only.

**Table 6.** Frequency of agreement in dimethylthiophosphate (DMTP) detectability between samples from applicator children of the same household, with percents shown in parentheses

|          | 1st Visit | 2nd Visit |
|----------|-----------|-----------|
| (child pairs = 21) | (child pairs = 21) |
| Both detectables | 2 (9.5)** | 10 (47.6)** |
| Two nondetectables | 13 (61.9)** | 6 (28.6)** |
| Disagreement between samples | 6 (26.8)** | 5 (23.8)** |

*Includes only those sample pairs where both children had detectable DMTP levels.
**Includes sample pairs where one child had nondetectable and/or trace DMTP levels; one sibling pair in this category included an 8-year-old child.
*Includes same pairs where one child had a detectable DMTP level while the other had either a nondetectable or trace level.
**Significant difference between children in the same household for Visit 2; p = 0.040 (Wilcoxon Signed Rank test).
**Significant differences across visits; p = 0.018 (chi-square).

**Table 7.** Trends in households with between-sibling agreement in dimethylthiophosphate (DMTP) detectability (Visit 2 only)

|          | Both detectable | Both nondetectable | Percent difference |
|----------|-----------------|--------------------|--------------------|
| Home -50 ft from orchard | 90% | 33% | 57% |
| Spray -200 ft from home | 90% | 50% | 40% |
| Applicator wore work shoes inside home | 70% | 33% | 37% |
Third, it is important to recognize that exposures to diethyl OP pesticides were not documented in this study, although these compounds were in use during the spray season. It was our original intention to measure both the dimethyl and diethyl alkyl phosphate metabolites, but our laboratory was unable to obtain reliable analytical standards for the diethyl compounds. Thus, total OP pesticide exposures may have been higher than those reported here.

Fourth, the use of a general biomarker such as DMTP has the advantage of integrating exposure from several OP pesticides simultaneously, thus providing a better estimate of total body burden among these children. This approach is in keeping with the spirit of the recent National Research Council report (8), which called for child assessments that evaluate exposures for chemicals with a common mechanism of toxicity. However, these OP pesticides have different toxicities in regard to the primary acute health endpoint—cholinesterase inhibition—and may have very different modes of action in regard to chronic health endpoints (if any are so identified). While it is possible to infer specific chemical exposures for particular children based on pesticide use and questionnaire data, the DMTP measurements reported here cannot be considered direct measures of toxicologic potential.

Finally, this study did not address sources of exposure other than residential or occupational. Exposures from air, dietary intake, and from pesticide use in nonresidential settings (e.g., day care centers or homes of extended family members) may also have contributed to the observed urinary metabolite concentrations.

Despite these limitations, this study produced several findings of value from a public health perspective. First, children of pesticide applicator workers were found to have higher urinary biomarker levels of OP pesticides than reference children. These results were consistent with those reported in our earlier study (15), in which OP pesticide concentrations in soil and house dust were found to be significantly higher in the homes of agricultural workers when compared to reference homes.

Second, variability in biomarker concentrations for the applicator children was observed to increase over the sampling period, regardless of the time of individual visits. This study was conducted shortly after spraying for codling moth had begun and lasted nearly to the end of this spray cycle. As the season progressed, it seems plausible to infer that the children’s environments were becoming increasingly contaminated, leading to an increase in daily exposure. The biological half-life of azinphos-methyl is estimated to be between 30 and 36 hr (22), so repeated exposures could have produced a buildup of this compound over time; one result would have been more children having detectable or increased levels during later sampling, as was observed in this study. This interpretation is limited by the fact that spot urines used to estimate individual body burdens. The point that each of these samples represents on the individual’s clearance profile is unknown and increases the uncertainty of the data. A few studies on adults using urinary metabolites and cholinesterase measurements confirm that sustained or increasing body burdens can occur among applicators and fieldworkers as the spray season progresses (23–25).

Third, age appeared to play a role in exposure. A marginally significant trend of decreasing concentration with increasing age was observed, and children of 3–4 years of age were found to have significantly higher exposures than those of 5–6 years of age during Visit 2. Furthermore, younger children had higher exposures than their older siblings. These trends may be due to behavioral differences (e.g., greater hand-to-mouth behavior among younger children), although an analysis of general time–activity patterns across these ages did not explain the observed patterns.

Fourth, this study provided an opportunity to examine the relationship between exposure and proximity to farmland regularly treated with pesticides. As anticipated, the closer a child lived to an orchard the greater the exposure. These findings are consistent with recent studies indicating that drift from airblast (speed sprayer) applications typical in orchards is most significant up to 200 ft (18). Less certain is whether proximity was the primary determinant of children’s exposure, with paracoccidental factors playing a secondary role. In the initial stages of the study, it was hoped that these two factors could be separated; however, the number of applicators living distant from treated orchards (>200 ft) was very small. Also, the scope of the study did not permit identification of specific attributes of living near an orchard, which might have helped to predict exposures (e.g., effect of pesticide drift, location of children during and immediately after applications).

Fifth, it appears that residential pesticide use in this population was much less than the nationwide average. It has been estimated that 90% of all households in the United States use pesticides (26–27), whereas only about one-half of the study population reported such use.

Sixth, use of azinphos-methyl by workers for residential pest control and the finding of several elevated metabolite levels in association with this use raises an important issue for public health education. This is take home pesticide exposure in the literal sense of the term. Azinphos-methyl is an...
Appendix

Creatinine-adjusted dimethylthiophosphate (DMTP) levels in applicator and reference children

| Table A.1. Creatinine-adjusted dimethylthiophosphate (DMTP) levels (µg/g creatinine) in applicator and reference children for both visits combined |
|--------------------------------------------------|
| All children                                     |
| Mean (µg/g)                                      | 0.097 | 0.043 |
| Median                                          | 0.030 | 0.000 |
| CV                                              | 231%  | 221%  |
| Range                                           | ND--2.006 | ND--0.493 |
| Frequency                                       | 58 (48) | 9 (28) |
| Number                                          | 121   | 32    |
| Focus child (one per household)                 |
| Mean                                            | 0.094 | 0.040 |
| Median                                          | 0.037* | 0.000* |
| CV                                              | 166%  | 253%  |
| Range                                           | ND--0.768 | ND--0.493 |
| Frequency                                       | 45 (51)* | 7 (28)* |
| Number                                          | 89    | 25    |

Abbreviations: CV, coefficient of variation; ND, not detectable; LOD, limit of detection.

*Tests for statistical significance applied to focus children data only (see Methods).

*Mean and other univariate statistics were calculated by estimating trace samples as 1/2 LOD.

CV = standard deviation/mean × 100.

*Percent is shown in parentheses.

*Significant difference across applicator and reference children: p = 0.011 (Mann-Whitney U test); p = 0.041 (chi-square test).

EPA Toxicity I compound and has not been registered for residential use because of its high acute toxicity. It is critical that workers understand that this type of restriction is designed to protect them and their families and that risks are substantial when such compounds are brought into the home. A precaution regarding this behavior has been included in the follow-up materials distributed to the participants of this study.

Finally, DMTP levels measured in applicator children could be viewed as relatively low when compared to agricultural worker exposures, as they never exceeded 0.5 µg/ml (ppm). Recent investigations by our group in this region have involved biological monitoring of apple thinners entering orchards 1–49 days postapplication (28) and of tree fruit orchard applicators who had sprayed within 6 days of sampling (29). The OP pesticide used most often in both cases was azinphos-methyl. Figure 3 compares median DMTP concentrations for these populations. The median DMTP level for the apple thinners during the thinning season was about eight times higher than that of the applicator children monitored in this study, while the levels for applicators at peak season were about two to three times higher. Symptoms typically associated with cholinesterase inhibition were not present in either of these worker populations, and plasma and red blood cell cholinesterase did not appear to be inhibited by these exposures. Based on these comparisons, it appears unlikely that the exposures experienced by the applicator children in this study were sufficient to produce acute health effects, i.e., significant cholinesterase inhibition. Several points of caution should be noted with this interpretation, however: 1) the proportion of total metabolite excretion represented by a single metabolite such as DMTP may vary considerably across groups; and 2) virtually no data exist to assist in interpreting such data relative to other health endpoints, and little is known regarding the metabolism of these compounds in children as compared to adults.

The complex nature of this data set limited the focus of this paper to assessing the relationship between DMTP concentrations and single variables using nonparametric tests. The association of numerous factors such as child activity and location, hygienic practices, occupational pesticide use, and application schedule could not satisfactorily be analyzed using these methods. Other techniques, such as multiple linear regression and analysis of repeated measures, may prove useful in understanding exposure pathways. Environmental and personal samples (i.e., house dust, surface wipes, hand wipes) collected simultaneously with the urine specimens will be incorporated into such an analysis, and the results will be reported in a separate paper.

Conclusions

Children living in households with pesticide applicators and in proximity to pesticide-treated orchards experienced greater OP pesticide exposures than did children of families with no occupational connection to agriculture who resided at a distance from agricultural spraying. Younger children tended to have higher exposures than older children within the 0–6 year age category.

The exposures measured did not appear to pose an acute health risk for children in the study population. However, exposures to only one category of cholinesterase inhibiting compounds, the dimethyl OP pesticides, were evaluated. These same children were undoubtedly exposed to deethyl OP compounds and carbamate pesticides during the course of the spraying season, though not necessarily simultaneous with the exposures reported here. An assessment of total exposure to all cholinesterase inhibitors would require more extensive analytical work than was possible in this study. Health risks associated with chronic exposures to these pesticides have not been well characterized, particularly for children in this age range. Additional studies that address cumulative exposures to multiple anticholinergic pesticides appear warranted.

The interaction of paraoccupational characteristics and proximity to orchards did not allow conclusions to be drawn regarding the relative importance of these factors for children’s exposures. Knowledge of the temporal nature of pesticide concentrations in other microenvironments, such as outdoor play areas and nonresidential indoor environments, as well as more detailed time-location data for these children, would greatly assist in an analysis of exposure pathways.

Agricultural workers should be cautioned regarding the dangers inherent in the use of acutely toxic pesticides in residential environments. A program aimed at reducing this practice should be considered by appropriate public health agencies.
Table A. 2. Creatinine-adjusted dimethylphosphatate (DMTP) levels (μg/g creatinine) in applicator and reference children for each separate visit

| Age (years) | Visit 1 | Visit 2 | Visit 1 | Visit 2 |
|-------------|---------|---------|---------|---------|
| 0–2         |         |         |         |         |
| Mean a      | 0.087   | 0.086   | 0.087   | 0.086   |
| Median      | 0.029   | 0.029   | 0.029   | 0.029   |
| CV b        | 0.182   | 0.207   | 0.173   | 0.173   |
| Range       | ND–0.90 | ND–0.90 | ND–0.90 | ND–0.90 |
| Frequency   | 7       | 12      | 7       | 12      |
| Number      | 25      | 24      | 25      | 24      |
| 3–4         |         |         |         |         |
| Mean a      | 0.089   | 0.089   | 0.089   | 0.089   |
| Median      | 0.013   | 0.013   | 0.013   | 0.013   |
| CV b        | 0.216   | 0.216   | 0.216   | 0.216   |
| Range       | ND–0.90 | ND–0.90 | ND–0.90 | ND–0.90 |
| Frequency   | 9       | 9       | 9       | 9       |
| Number      | 25      | 24      | 25      | 24      |
| 5–6         |         |         |         |         |
| Mean a      | 0.084   | 0.086   | 0.084   | 0.086   |
| Median      | 0.017   | 0.017   | 0.017   | 0.017   |
| CV b        | 0.156   | 0.194   | 0.154   | 0.194   |
| Range       | ND–0.90 | ND–0.90 | ND–0.90 | ND–0.90 |
| Frequency   | 7       | 8       | 7       | 8       |
| Number      | 19      | 17      | 19      | 17      |

Abbreviations: CV, coefficient of variation; ND, not detectable; LOD, limit of detection.

* Tests for statistical significance applied to focus children data only. (see Methods).

** Mean and other univariate statistics were calculated by estimating trace samples as 1/2 LOD.

# Percent is shown in parentheses.

* Significant difference across applicator and reference children: p = 0.011 (Mann-Whitney U test); p = 0.015 (chi-square test).

** Significant difference across applicator and reference children: p = 0.002 (Mann-Whitney U test); marginally significant difference across groups: p = 0.078 (chi-square test).

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