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Farmworker Exposure to Organophosphorus Pesticide Residues During Apple Thinning in Central Washington State

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The purpose of this study was to characterize worker exposure to azinphos-methyl (Guthion™) over an entire 4–6 week apple-thinning season. Twenty workers from three work sites in the Chelan-Douglas County region of Washington state were recruited for the study. Exposure potential was estimated by dislodgeable foliar residue measurements, and individual exposures were estimated by biological monitoring through urinary metabolites. Measureable azinphos-methyl residues were found on apple foliage at all sites throughout the six-week sampling period, indicating continuous exposure potential (median residue level of 0.5 μg/cm²). Measurable levels of the urinary dialkylphosphate metabolite, DMTP, were found in virtually all urine samples (limit of detection = 0.04 μg/mL). Mean DMTP concentrations differed significantly across sites (0.53, 0.29, and 0.90 μg/mL for Sites 1–3, respectively; analysis of variance, $p<.002$), and intraindividual variability was much greater than interindividual differences. Group mean DMTP concentrations at each site fluctuated according to foliar residue levels. Measurable DMTP concentrations were found in 9% of reference workers, ranging from 0.04–0.18 μg/mL. Cholinesterase activity levels monitored with a field test kit were not considered reliable due to temperature changes of the instrument.

Keywords: azinphos-methyl, farmworkers, pesticide exposure

Labor, industry, and regulatory groups in the state of Washington have all shown concern for potential health risks associated with occupational pesticide exposures. In particular, apple thinning with its exposure to organophosphorus (OP) pesticides residues is viewed as a relatively high-exposure activity. Several comprehensive studies were undertaken by state researchers in the 1970s to address this issue, but none were ever published.[1] Thus, there are no well-documented studies of apple thinner exposure to OP pesticides in the Northwest. A number of studies of field-worker pesticide exposure in tree fruit have been conducted in California.[2-7]

Previous field-worker studies typically have employed a controlled field experimental design, monitoring workers in a single orchard over a limited time period (e.g., 1–5 days). Exposure measurements have included dislodgeable foliar residues, skin patches, handwashes, urine samples, and cholinesterase (ChE) measurements. In contrast, the study reported here was designed to follow groups of workers at multiple work sites throughout an entire thinning season and to conduct extensive biological monitoring of these workers.

The pesticide most commonly used in the Washington tree fruit industry during apple thinning is azinphos-methyl, O,O-Dimethyl S-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl)methyl] phosphorodithioate (Guthion™). Azinphos-methyl is effective against the coddling moth and is also compatible with many integrated pest management programs. The most commonly used formulations in Washington are Guthion 35 WP, applied at 2–3 lbs active ingredient per acre, and Guthion 50 WP, with an application rate of 1.5–2 lbs active ingredient per acre. Annual statistical information regarding the crop use and pounds

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per acre in Washington state is not available. In 1991 the Washington Department of Agriculture estimated use of azinphos-methyl at 345,000 lbs for apples, 29,900 lbs for pears, and 6,800 lbs for sweet cherries.[8]

Azinphos-methyl is activated *in vivo* to gutoxon, which is a potent inhibitor of the nervous system enzyme, acetylcholinesterase. It has high acute toxicity, with an LD_{50} ranging from 3–5 mg/kg (mouse, ip) to 220 mg/kg (rat, dermal).[9] It is categorized as a Toxicity I compound by the U.S. Environmental Protection Agency. Azinphos-methyl is readily absorbed through the skin, a particular concern for apple thinners who have extensive contact with foliage. The restricted re-entry interval for azinphos-methyl products in this part of Washington state at the time of the study was 48 hours.[10]

The pharmacokinetics of azinphos-methyl are not well known; however, estimates of the clearance rate range from 30 to 36 hours depending on the route of exposure.[11] Measuring urinary alkylphosphates in urine is considered to be the most sensitive and specific method for estimating internal dose in humans.[12] Blood ChE measures also have been used as a primary biological monitoring tool for workers exposed to OP pesticides.[13] A portable ChE kit has been developed recently for monitoring workers in the field.[14]

The goal of this study was to characterize worker exposure to azinphos-methyl over an entire 4–6 week apple thinning season. OP insecticides were targeted because of their potential high acute toxicity, widespread use, known mechanism of action, and because their metabolites can be detected in urine. This study design was unique in that crews of workers were followed daily over an entire thinning season at three different farms. Exposure potential was estimated by dislodgeable foliar residue measurements, and urine metabolites were used as indicators of dose.

MATERIALS AND METHODS

Field Conditions

In Washington state it is typical for an orchard to be divided into geographic blocks, which may or may not be contiguous. These blocks receive different scheduled spray applications throughout the season based on insect infestation, crop age, and variety. Workers may thin in several blocks in one week, all of which likely have different OP application schedules and foliar residue levels. Little information is available regarding pesticide use, work practices, and potential exposures as workers move from orchard to orchard and block to block throughout the thinning season.

Apple thinning is a task in which workers remove a number of immature fruit from the trees to encourage proper growth of the remaining fruit. Thinning requires substantial physical contact with fruit, leaves, twigs, and branches.

Subject and Site Selection

The study population consisted of apple orchard workers employed by three different farms during the spring of 1994. Orchard operations ranged from 40 to 304 acres. The orchards on each farm were divided into blocks that varied in size and geographic location. Table I provides descriptors of each study site.

Workers initially were contacted through their primary employer prior to thinning season. Worker eligibility criteria included (1) performing apple thinning activities in treated orchards for at least 4 hours daily, (2) working full-time on no more than two farms, and (3) having English or Spanish as primary language.

TABLE I. Characteristics of Study Work Sites

Site	Acres	Apple Variety	Guthion 50WP Application Rate (lbs/acre formulation)	Range of Worker Re-entry Times (days)[A]	Number of Workers Recruited
1	304	Fuji, Red and Golden Delicious	1.75–2	5–25	8
2	37	San Rose, Gala, Fuji, Red and Golden Delicious	4	11–49	5
3	85	Gala, Spur, Starking, Red and Golden Delicious	2	2–20	7

[A]This column represents the range of the number of days between pesticide application and worker reentry into the orchard.

Twenty-seven workers met study criteria and indicated willingness to participate in the project. The study procedures were approved by the University of Washington Human Subjects Committee and included informed consent.

Thinning activity started May 9 (Site 2) and May 31 (Sites 1 and 3). Farm walkthroughs were conducted by study staff to observe work practices and farm activities. Differences in availability of washing and toilet facilities was noted across the sites. All workers used 10-foot ladders to thin the trees for most apple varieties with the exception of fuji trees. Workers thinned with their hands, and this activity required substantial whole-body contact with the tree canopy. Most workers wore long pants, long-sleeved shirts, a cap, and leather work boots or tennis shoes while thinning (Figure 1). Study staff returned to the farm sites in November to collect post-thinning blood and urine samples.

Site 1 was the largest operation. At this site, thinning quotas (100 trees/day) were given to the workers, and their thinning performance was monitored each day by management. A 5-day work week with an 8-hour workday was typical. Workers returned home for lunch. This farm had a number of experimental pheromone-treated blocks; for a few days workers were thinning blocks that received no Guthion pesticide treatments.

Workers at Sites 2 and 3 worked 6 days per week, 10 hours each day. Workers usually took a 30-min lunch break and ate lunch in the orchard. Limited washing facilities were provided at these work sites. Workers from both of these sites picked cherries at nonstudy farms for approximately 1 to 2 weeks, usually working an additional 3 hours after apple thinning each day.

A reference "occupationally unexposed" population was recruited from a small manufacturing factory in the same geographic region. Ten subjects were recruited during late May.

Exposure Assessment

Dislodgeable Foliar Residue

Foliar residue samples were collected using the standard leaf-punch method described by Iwata et al.[15] A sample consisted of 40 leaf punches collected from 10 trees (4 punches/tree, each punch from a different leaf). Each sampled tree was divided into four quadrants. One leaf punch was collected within 2 m of the ground in each quadrant. The leaf punching device (Birkestrand

FIGURE 1. Workers commonly wore long pants, long-sleeved shirts, a cap, and leather work boots while thinning.

Co., El Monte, Calif.) cuts a disk of 1.8 centimeters diameter (2.53 cm^2) from the leaf. The total leaf area per punch was 5.06 cm^2 including both sides of the punched leaf.

Three samples were collected from each block just before workers started to thin for the day (approximately between 5:30 am and 7:00 am). Two additional leaf samples were collected for quality control: (1) a repeat sample taken from the first set of trees sampled before thinning and (2) a post-thinning sample collected from the same initial set of trees at the end of the thinning day. All quality assurance samples were handled, stored, and transported in the same manner as field samples.

Clear glass collection jars were capped with a paper-lined phenolic lid. Samples were frozen (−10°C) within 2 hours of sampling and stored for up to 5 months at −20°C until laboratory analysis. A total of 114 field samples were collected from the 3 farms (48 on 10 different days from Site 1, 35 on 7 different days from Site 2, and 31 on 7 different days from Site 3). Forty quality assurance and control samples (20 repeats and 20 post-thinning) were collected to assess the precision and accuracy of the method. No significant difference in residue levels was found between the first morning samples averaged (0.82 $\mu g/cm^2$, range=0.17–3.92) and first morning repeat samples averaged (0.61 $\mu g/cm^2$, range=0.2–1.62) (Wilcoxon signed rank; $p=0.09$). The results of the first morning and its repeat sample were averaged to represent one sample. The mean coefficient of variation (CV) for the averaged samples was 20% (7–59%). A trend of higher pre-thinning residues as compared with post-thinning residues was found, but was not statistically significant (Wilcoxon signed rank; $p=0.19$).

A modified version of the California Environmental Protection Agency[16] and Gunther[17] methods was used to analyze leaf

samples for dislodgeable Guthion residues. Each sample jar was shaken with 50 mL of a 0.006% sodium dioctylsulfonsuccinate solution for 20 min. Ten milliliters of the surfactant rinse solution was then placed in a culture tube and the remaining solution poured off as waste. Two milliliters of the pooled rinse solution was supplemented with a recovery surrogate, tributyl phosphate (TBP), at 4.8 μg/mL rinse solution. After addition of excess sodium chloride (about 1.2 g) this aliquot was equilibrated with an equal volume of ethyl acetate by vortex mixing for 4 minutes. Following 5-minute centrifugation at 1000 × g, a 0.95-mL portion of the organic phase was brought to 1.00-mL final volume by addition of 0.05 mL of a triphenyl phosphate solution to provide a gas chromatography injection standard at 2.50 μg/mL final concentration. The resulting sample was analyzed by gas chromatography on a Hewlett Packard 5890 series II instrument equipped with a Restek Rtx-5 column (0.25 × 15 m, 0.25 μm film thickness) with helium carrier flow set at 25 cm/sec.

Since the analysis included both a surfactant rinse and an organic solvent extraction, the recovery of each operation was independently evaluated. The relative recovery of the rinse step was determined by rinsing nine leaf samples (collected in the field) twice with the surfactant solution as described above. The first and second rinses were separately extracted with ethyl acetate. Of the total azinphosmethyl recovered, 92% (±1.2%) was in the first rinse and 8% (±1.2%) in the second. When the first rinse solution was extracted a second time with ethyl acetate, 97% (±1.3%) of the total pesticide recovered in the two extractions was present in the first extract. The adequacy of a single extraction was confirmed by an absolute recovery experiment in which 2 mL portions of blank rinse solution (N=6) were fortified with Guthion 50WP at a final concentration of 3.2 μg/mL azinphos-methyl; 98% (±0.13%) of the added pesticide was removed by a single extraction.

Blood ChE

Assessment of worker and control blood ChE activities was conducted according to the guidelines outlined by the California Department of Health Services, as described by Fillmore and Lessenger.[18] Baseline ChE determinations were made for most workers at least 2 weeks prior to when they engaged in thinning activities. Two measures of ChE activities were made at least 3 days apart (there was no more than 15% difference between any enzyme measures for any individual, so a third baseline measure was deemed unnecessary). The average of these two measures served as the individual's personal baseline measurement. The collection of baseline measures allowed each worker to serve as his or her own control.

Blood samples were collected at Site 1 prior to thinning and during the first and last week of thinning only. Blood samples at all other sites were collected prior to thinning and then biweekly for 6 weeks until thinning ended. Blood samples at the reference site were collected during the 6-week thinning period. Blood samples (approximately 200 μL/sample) were analyzed on-site with the EQM Test Mate Kit (EQM Research, Inc.; Cincinnati, Ohio) for red blood cell (RBC) and plasma ChE activity. Tests were performed by the same two technicians using the same two testing devices according to the method of McConnell et al.[14] Enzyme activity measures obtained included RBC ChE, hemoglobin-adjusted RBC ChE, and plasma ChE. All tests were performed at the worker and control work sites at ambient temperatures.

Urine

Urine was collected from each participant at least 2 weeks prior to the thinning season for baseline measurements (pre-thinning). Baseline urine pH, glucose, and protein were determined with color indicator sticks and results given to the worker during the first week of the study. Spot urine samples were then collected daily, normally at the end of the shift, throughout the thinning period. Reference subject spot urine samples were collected at the nonagricultural work site during the lunch hour twice each week. Workers were asked to provide specimens in clean sample collection bottles supplied by the field staff; samples were refrigerated until pick-up at the end of the day. Urine collection was continued through 1 week after cessation of thinning activities. Urine samples were assessed for total voided volume, specific gravity, and refractive index; the total voided volume was recorded and 25 mL were decanted and frozen until transport to the Department of Environmental Health laboratory at the University of Washington. All urines were transported on ice and stored at −20°C until analysis.

Three types of field quality assurance samples were used: field blanks, field spikes, and sample duplicates. All quality assurance samples were handled, stored, and transported in the same manner as field samples. Reference urine was collected from the University of Washington Environmental Health laboratory staff and pooled together for preparation of field blanks and spikes. The reference urine contained mean background levels of DMTP at concentrations of 0.04 μg/mL. Duplicate samples were prepared by decanting an extra 25-mL sample from each worker urine void whenever possible.

The Environmental Health Laboratory analyzed all samples for dimethyl alkylphosphate metabolites using a modification of the method of Nutley and Cocker: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), and dimethyldithiophosphate (DMDTP).[19] Solvent-based calibrants (0.04–3.00 μg/mL) were prepared by fortifying acetonitrile with DMP, DMTP, DMDTP, and dibutylphosphate (DBP, a semiquantitative recovery internal standard). One-milliliter urine samples were prepared for analysis by azeotropic distillation with acetonitrile after addition of DBP. Calibrants and urine distillates were esterified by derivatization with pentafluorobenzylbromide for 16 hours at 50°C.

Derivatized samples were supplemented with TBP as an additional internal standard, and analyzed by capillary gas chromatography on a Hewlett-Packard HP5890 series II instrument, equipped with a flame photometric detector. Sample response, expressed as the ratio of metabolite peak area to TBP peak area, was converted to concentration by reference to a linear calibration curve based on the analysis of a derivatized calibrant set prepared in each analytical batch. The maximum acceptable calibration regression fit error was set at 15%. Concentrations were reported by the laboratory without correction for extraction efficiency. Mean extraction efficiencies of DMDTP and DMTP were 62 and 80%, respectively, and the coefficients of variation were good, less than 15%. DMP values were considered semiquantitative because calibration curve fit errors frequently exceeded 15% and extraction recovery was low (39% ± 17). The limit of detection for the method was 0.04 μg/mL.

Field blanks were nondetectable or contained the expected DMTP background concentrations found in the reference urine. Mean recovery efficiencies from field spikes were 89% (63–109) and 71% (56–81) for DMTP and DMDTP, respectively. These values indicated consistent recovery of primary metabolites, but were not used to adjust field sampling data. Coefficients of variation for duplicates ranged from 1–27% (mean = 0.10), indicating acceptable analytical precision. Some of the variability found among duplicates may have been from batch-to-batch interprecision variability.

Statistical Methods

The foliar residue variability from all sites was described. Residue concentrations from each block were averaged to calculate a block mean. Four sampling days of data were excluded (three from Site 3 and one from Site 1) because field sampling protocols were not followed or samples were lost.

Urine metabolite concentrations were log-normally distributed; consequently, data was $\log_{10}$ transformed. Parametric statistical tests were used to analyze the data. The paired t-test was used for the pre-thinning and thinning data sets of the exposed groups. One way analysis of variance (ANOVA) was used to determine whether there were group differences between sites. The between- and within-worker variance components, designated as ${}_bS_y2$ and ${}_wS_y2$, respectively, were estimated from the log-transformed exposure concentrations using the ANOVA methods described extensively elsewhere by Heederick,[20] Boleij,[21] and Kromhout.[22] In summary, the mean squares value from an ANOVA analysis describes the within-worker (day-to-day) variance and is used in the following calculation to determine the between-worker variance component:

$$ {}_bS_y^2 = \frac{\text{Between Mean Squares} - \text{Within Mean Squares}}{k} $$

where k = the number of repeated measurements per worker. In this case, the data were unbalanced and, therefore, k can be approximated by the following:

$$ k = 1 + (\text{degrees of freedom between} \div \text{degrees of freedom within} + 1)^{[20]} $$

DMTP concentrations for all workers at each site were averaged for each day and compared with the dislodgeable foliar residues over time to show the daily fluctuations of these values over the thinning period.

RESULTS

Study Population

Twenty workers from three participating farms were included in the study. Seven of the 27 workers recruited for participation were eliminated from data analysis. One worker was ineligible because he was not a full-time thinner (e.g., applicator); others changed jobs during the first week of the study (e.g., two women started working in local fruit warehouses). Three workers moved out of the study site location during the study period. One worker did not want to participate after the first week. All subjects were Hispanic field-workers who had lived in central Washington, most for about 5 years. Four subjects had arrived in the area for the first time less than 2 weeks prior to recruitment. The average age of study subjects was 30 years (range 18–52 years). Nineteen were men. Ten participants reported living in the area all year long; others left during winter for 3 to 4 months. In November, study staff located six workers (at least one from each farm site) to conduct follow-up blood and urine testing.

Ten reference subjects were recruited. One reference subject dropped out of the study during the second week of monitoring. Four male and five female employees participated in the full 6 weeks of the study. Referent subjects had an average age of 31 years (range 24–52 years), reported living in Wenatchee on average for 12 years, and indicated that they did not leave the area for extended periods during any months of the year.

Dislodgeable Foliar Residues (DFR)

DFR levels by site, block, and day post-application are presented in Table II. Blocks in Sites 1 and 3 received two separate sprays, approximately 3 weeks apart; blocks in Site 2 were sprayed only once during the course of the study. Foliar residue samples were collected periodically at each site from blocks in which workers were thinning; however, thinning activities occurred daily, except for some weekend days.

Median dislodgeable Guthion foliar residues for all three sites during thinning activities were the same, approximately 0.5 μg/cm². Mean residue levels varied: 0.55 μg/cm² at Site 1, 0.76 μg/cm² at Site 2, and 1.26 μg/cm² at Site 3. The highest block average of Guthion DFR, 3.67 μg/cm², was found at Site 3, with a maximum value of 3.9 μg/cm². Variability of replicate samples within a block were generally low (<50% in most cases). An exception was Block 5C at Site 1, in which both treated and untreated trees were sampled.

Cholinesterase Monitoring

The Op Test-Mate Kit used for this study produced anomalous results in some cases. Recent evaluations of the Test-Mate kit have indicated that it can be very sensitive to changes in temperature.[23] Ambient temperatures changed significantly from preseason baseline sample collection (55–65°F) to mid- and late-season collection (75–85°F). Thus, the ChE measurements in this study were not considered reliable. Symptoms of ChE inhibition among the study population were not evaluated specifically. However, no such symptoms were reported to field staff, who were not aware of any worker seeking medical attention for OP-related illness during the study period.

Urinary Metabolites

A total of 710 urine samples were collected from exposed workers, 102 samples from the reference population, and 103 quality control samples. Of these, 648 were analyzed (464 worker urine samples, 81 reference, 103 quality control). The remaining 62 samples were not analyzed because of financial limitations. The following samples were given highest priority: all pre- and post-thinning samples, first and last week of thinning samples, and those collected during blood and leaf testing. Samples collected on other days were randomly selected for analysis (about 50% of the remaining days). Data analysis focused on DMTP, the primary metabolite found in the samples. DMDTP was detected in 5% of the exposed worker urine samples (0.04–0.290 μg/mL), and in 4% of the reference samples.

DMTP levels were at trace concentrations or nondetectable for the majority of workers at Sites 1 and 3 during the baseline pre-thinning period. Based on work histories, workers at these sites had not started thinning when baseline samples were collected. At Site 2, however, measurable levels of DMTP were found in every worker's baseline urine sample (Table III). Analysis of work histories indicated that workers at Site 2 started thinning prior to the study baseline sample collection. Pre-thinning DMTP concentrations for all workers combined were significantly lower than during thinning (paired t-test, $p<.0001$); however, a site-by-site comparison found that workers at Site 2 did not have a significant difference between pre-thinning and thinning DMTP concentrations (paired t-test, $p=0.644$).

DMTP concentrations in exposed workers ranged from nondetectable to 3.96 μg/mL (Figure 2). Most (>90%) were above the limit of detection during the thinning season. Workers from

TABLE II. Summary Statistics of DFR in Micrograms per Square Centimeter by Site

Block No.	Spray Date	Sample Date	Post-Application Day[A]	Number of Samples	DFR Mean (μg/cm²)	DFR CV[B] (%)	DFR Range (μg/cm²)
Site 1							
6A	May 26	June 2	7	3	0.29	61	0.17–0.50
5C[C]	May 26	July 10	5	3	0.28	105	0.004–0.6
9A	May 26	June 14	19	3	0.50	15	0.42–0.55
7A	May 26 June 15	June 21	6	3	1.49	12	1.29–1.60
7A	May 26 June 15	June 24	9	3	1.02	26	0.75–1.27
7B	May 26 June 15	June 28	13	3	1.25	4	1.22–1.30
8	May 26 June 15	July 1	16	2	1.21	2	1.20–1.23
2	May 26 June 16	July 5	19	3	0.42	48	0.23–0.63
3	May 26 June 16	July 8	21	3	0.35	22	0.26–0.40
Site 2							
1A	May 27	June 15	19	3	0.75	25	0.64–0.96
1	May 27	June 17	21	3	0.52	42	0.36–0.78
1	May 27	June 21	25	3	0.77	28	0.61–1.02
2	May 27	June 28	32	3	0.63	6	0.59–0.66
2	May 27	July 1	35	3	0.50	11	0.46–0.56
3	May 27	July 5	39	3	0.37	33	0.27–0.50
4	May 27	July 8	42	3	0.30	18	0.24–0.34
Site 3							
15	May 17	June 3	17	3	0.35	57	0.31–0.56
14	May 18	June 6	19	2	0.31	10	0.29–0.33
10	May 18 June 7	June 9	2	2	3.67	10	3.42–3.92
2	May 21 June 7	June 20	13	3	0.71	26	0.53–0.90

[A]Post-Application Day is the number of days between pesticide application and sampling.
[B]CV is the coefficient of variation.
[C]This block was an experimental block that did not receive Guthion applications as other blocks; only four rows were sprayed in the entire block. Two of three samples were collected from sprayed rows.

Site 2 had the lowest mean DMTP concentrations. Measurable DMTP concentrations were found in only 9% of reference workers (0.04 μg/mL–0.18 μg/mL).

ANOVA of $\log_{10}$-transformed DMTP data revealed a significant difference between DMTP mean concentrations across the three work sites ($p<.002$), with significant differences between Sites 1 and 3, and Sites 2 and 3 ($p<.04$ and $p<.002$, respectively). For all workers the difference in average DMTP concentrations between workers was small (${}_bS_y2=.14$) compared with those concentrations from day to day(${}_wS_y2=0.85$). The day-to-day variability was almost tenfold higher than the between worker variability at Sites 1 (${}_wS_y2=0.95$ vs. ${}_bS_y2=.07$) and 3 (${}_wS_y2=0.94$ vs. ${}_bS_y2=.10$), but the component values were virtually identical for the two sites. At Site 2 day-to-day variability was eighty-five-fold greater than between worker variability (${}_wS_y2=0.60$ vs. ${}_bS_y2=0.007$), and both of these values were markedly lower than the corresponding values for Sites 1 and 3.

Only six workers (at least one from each site) were available for monitoring during the post-thinning period. All DMTP concentrations were below the limit of detection 16 weeks post-thinning.

Comparison of Urinary and DFR Measurements

Urine DMTP mean concentrations generally decreased with lower potential exposure to Guthion as measured by DFR. Workers entered an orchard block at least 10 days after a pesticide application during most thinning days (15 of 20 days). One of the primary goals of this study was to understand the nature of exposures during a thinning season, as the worker progressed through blocks with different post-application times. Measurable levels of urine DMTP were found on all post-application days, indicating a chronic low-level exposure profile. A comparison between average DMTP concentrations and foliar residue measurements at Sites 1–3 over the entire thinning season illustrates the typical day-to-day pattern observed in this study (Figures 3–5). For all workers exposures were highly variable, and some peak concentrations were observed throughout the season, often following pesticide applications. For example, the highest peak DMTP concentrations and leaf residues occurred at Site 3, where workers entered an orchard block 2 days after an application (Figure 3).

DISCUSSION

Measurable levels of Guthion residues were found on apple foliage throughout the 6-week sampling period, indicating the potential for continuous exposure for workers conducting hand labor activities such as apple thinning. Measurable concentrations of the OP metabolite DMTP also were found in virtually all urine

TABLE III. DMTP Urinary Levels (μg/mL) During Entire Study Period by Subject

Worker ID	Pre-Thinning DMTP Levels (μg/mL)		Thinning DMTP Levels (μg/mL)						Post-Thinning DMTP Levels (μg/mL)
	N	AM	N	AM	Median	Range	GM	GSD	
Site 1									
62	2	0.06	18	0.43	0.28	0.04–1.42	0.29	2.6	na
63	2	0.03	13	0.50	0.36	0.04–1.89	0.28	3.3	na
64	1	0.02	17	0.29	0.17	0.04–0.99	0.20	2.4	na
65	2	0.19	20	0.88	0.57	0.02–2.88	0.65	2.4	na
66	2	0.02	23	0.58	0.34	0.02–2.42	0.38	2.7	na
68	2	0.08	21	0.46	0.26	0.02–2.32	0.32	2.6	0.05
70	2	0.05	22	0.48	0.29	0.02–1.85	0.33	2.7	0.02
76	na	na	19	0.60	0.37	0.07–1.73	0.41	2.6	0.03
Site 2									
50	2	0.22	21	0.31	0.26	0.02–0.73	0.29	1.8	na
52	2	0.24	21	0.32	0.25	0.08–0.82	0.26	2.0	0.02
73	2	0.28	18	0.32	0.21	0.02–1.19	0.19	3.1	na
74	2	0.27	14	0.21	0.21	0.02–0.77	0.18	2.1	na
75	2	0.21	14	0.30	0.24	0.14–0.58	0.26	1.6	na
Site 3									
54	2	0.05	18	0.52	0.31	0.04–1.86	0.34	2.8	na
55	2	0.02	13	0.56	0.28	0.02–1.07	0.35	2.6	na
56	2	0.09	15	1.13	1.04	0.02–3.45	0.84	2.6	na
57	1	0.10	15	0.57	0.41	0.07–1.60	0.39	2.5	na
59	1	0.02	18	1.13	0.74	0.08–3.96	0.69	2.9	na
88	na	na	5	1.58	1.60	0.13–2.53	0.98	2.9	0.02
89	na	na	8	0.78	0.71	0.02–1.35	0.63	1.8	0.09
Reference									
77	na	na	7	0.03	0.04	0.02–0.06	0.03	1.6	0.05
78	na	na	5	0.02	0.04	0.02	0.02	1.0	na
79	na	na	10	0.02	0.02	0.02	0.02	1.0	na
80	na	na	9	0.02	0.02	0.02	0.02	1.0	0.02
81	na	na	10	0.02	0.02	0.02–0.18	0.03	1.9	0.02
82	na	na	9	0.03	0.02	0.02–0.11	0.03	2.1	na
83	na	na	8	0.01	0.02	0.02–0.10	0.02	1.7	0.02
84	na	na	10	0.02	0.02	0.02–0.10	0.03	1.7	0.02
85	na	na	8	0.03	0.02	0.02–0.15	0.03	2.0	na

Note: Nondetectable concentrations were assigned one-half the limit of detection (0.02μg/mL) for statistical purposes. na = not available, AM = arithmetic mean, GM = geometric mean, and GSD = geometric standard deviation.

samples collected from workers at the three study sites. The group DMTP concentration profiles were consistent across the three sites and tracked the rise and fall of Guthion foliar residue levels. These findings support the conclusion that workers absorbed Guthion daily due to their contact with pesticide-treated foliage during apple thinning. DMTP metabolites also were found in the reference population in some instances, but the measured values were similar to other nonoccupationally exposed populations reported in the literature.[24]

Variance component analysis for all samples indicated that differences in mean exposures within workers (within worker variance, ${}_{w}S^{2}_{y}$) were more prominent than differences between workers (between worker variance, ${}_{b}S^{2}_{y}$). However, when stratified by site, the variance components varied considerably. This may reflect the differences in farm site size, management style, worker practices, and pesticide applications among the three sites examined in this study. The between- and within-worker variance components for Sites 1 and 3 were proportionally similar, and the values themselves were comparable, as was the total variance.

Site 2 had much greater day-to-day variability as compared with between-worker variability, and the between-worker variability for this site was an order of magnitude lower than the other sites. The total variability for this site was also much lower than for the other two sites. A number of factors may explain this difference: (1) foliar residue variability was much lower at Site 2 than at Sites 1 or 3 (average coefficient of variation for DFR levels was 33% at Site 2, compared with 64 and 128% for Sites 1 and 3, respectively); (2) workers entered the Site 2 orchard at a much later point in the pesticide residue decay process (no earlier than 11 days postapplication at Site 2 as compared with 2 or 5 days at Sites 3 and 1); and (3) only one pesticide application was made to the orchard.

Agricultural workers and their workplaces are unique. The workers are a mobile group, activities are performed outdoors, peak exposures may occur with repeat chemical applications, and work sites establish irregular worker reentry times. Analysis of the components of variance among agricultural workers has not been published previously and is deserving of further study.

It is important to keep in mind several limitations regarding foliar residue and biological monitoring in this study. First, daily foliar residue samples could not be collected due to the duration and multisite nature of the study. Second, it was not possible to measure the oxon derivative of Guthion in the foliar residue samples. Thus, the extent to which this transformation product was present in the work environment is not known. Third, spot urine

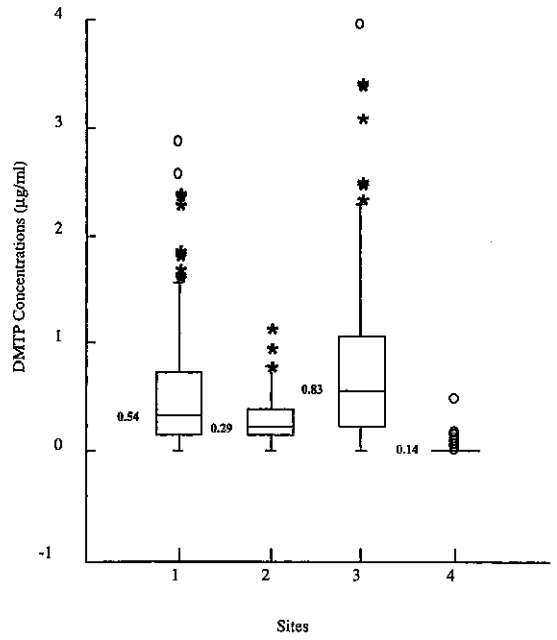

FIGURE 2. Box-and-whisker plots comparing DMTP concentrations in urine samples from apple thinners (Sites 1–3) and reference workers (Site 4). The box shows the lower and upper quartiles, and the central line is the median. Median DMTP values are also provided in the figure. The points at the end of the whiskers are the 2.5 and 97.5% values. The symbols shown outside of the whiskers indicate the extreme values of the data. Outside values as defined by Systat (5.2 version) statistical software package are plotted with asterisks and far outside values are plotted with empty circles.

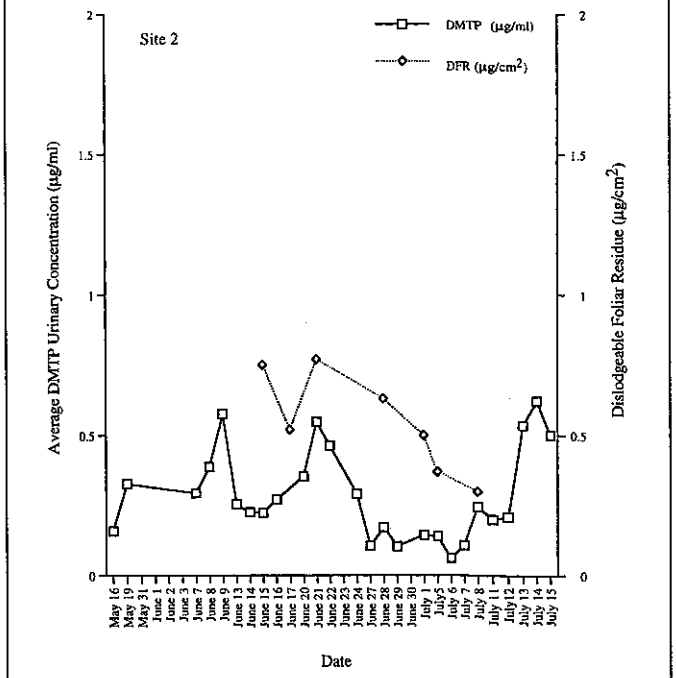

FIGURE 4. Mean DMTP concentration and leaf residue profiles over the thinning period for selected workers Site 2

samples were collected rather than 24-hour urines for logistical reasons. Concentration of metabolites in spot urine samples may vary with hydration.[2]

Other studies have described detectable dialkylphosphate metabolites and/or reduced cholinesterase levels following worker exposure to Guthion during tree-fruit thinning (Table IV). In

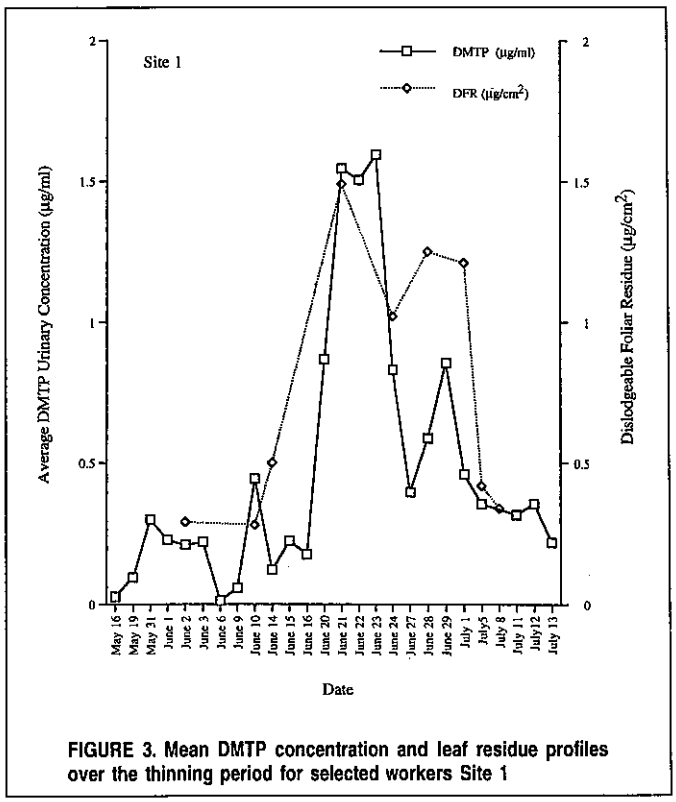

FIGURE 3. Mean DMTP concentration and leaf residue profiles over the thinning period for selected workers Site 1

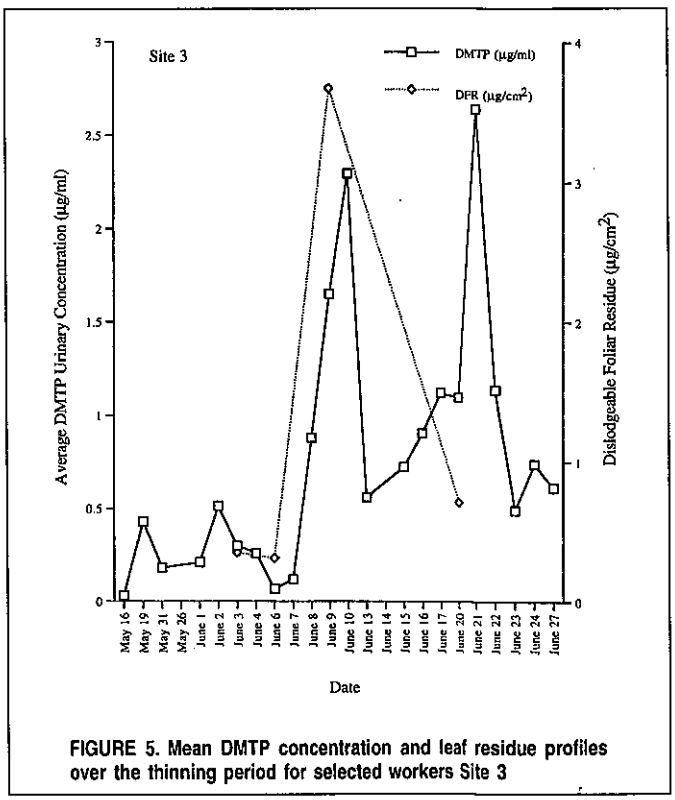

FIGURE 5. Mean DMTP concentration and leaf residue profiles over the thinning period for selected workers Site 3

TABLE IV. Comparison of Foliar Residue, Urinary Metabolite, and ChE Depression Values from Previous Studies of Peach or Apple Thinners with Current Study Values

Study and Region	Activity	Foliar Residues		Urinary Metabolites		Mean RBC ChE Inhibition (%)
		N	Mean DFR (μg/cm²)	N	Mean DMTP (μm/mL)	
Foster 1973 Washington	peach and apple thinning	5	2.62	8	0.83	4
Kraus et al. 1977 California	peach thinning	3	2.10	16	2.09	15
Richards et al. 1978 California	peach thinning	10	2.63	8	14.1	12
McCurdy et al. 1994 California	peach thinning	—	0.64	9	0.72[A]	7
Simcox et al. 1997 Washington	apple thinning Site 1	9	0.76	8	0.53	—
Simcox et al. 1997 Washington	apple thinning Site 2	7	0.55	5	0.29	—
Simcox et al. 1997 Washington	apple thinning Site 3	4	1.26	7	0.90	—

[A]Calculated from original data provided by author.

most of these studies, only a single orchard block was used for thinning, and the re-entry times were well-defined. In contrast, the present study design focused on following workers as they moved through orchard blocks, and reentry times were documented rather than controlled. This primary difference in design is important to consider in comparing these studies, since each worker's DMTP level for any given day depends primarily on the previous day's exposure, which in turn is related to the orchard block and worker re-entry interval.

Average Guthion foliar residue levels were substantially lower in the current study than in the first three studies,[1–3] since in those studies all samples were collected the first few days following application. DFR levels from the 1994 California study[7] were comparable with the current study; in that study workers did not enter treated fields until 30 days after Guthion application.

With regard to urinary metabolite excretion (DMTP), the earlier California studies found substantially higher concentrations than did the current study. However, the 1994 California study[7] found levels similar to those reported here. It is not clear why Foster[1] found relatively low DMTP concentrations, similar to those of the current study, when the foliar residue levels were approximately 2–4 times greater. Since the Foster data were not formally documented, it is not possible to identify the source of this discrepancy.

All of the previous studies reported some inhibition of group mean RBC ChE. The earlier California studies, in which metabolite levels were elevated, found inhibition greater than 10%. The earlier Washington studies found approximately 4% average inhibition, but this difference was not statistically significant. McCurdy et al. found a 7% inhibition during the study period, and later found a 19% inhibition at the end of the thinning season.[7] The McCurdy et al. study is the most comparable with the current study in terms of dislodgeable residue levels and urinary metabolite levels, and the method used for ChE activity measurements is considered to be both precise and accurate.[7] The California group found measurable Guthion-oxon levels on foliage; in some samples the oxon component represented more than 2% of total dislodgeable foliar residue. Although the oxon derivative of Guthion was not measured in the present study, it has not been found in significant amounts in foliar residue studies in the Northwest tree-fruit region.[25] Since the oxon derivative of Guthion is approximately 10 times more toxic than its parent compound, even small differences in oxon exposures among workers could explain differences in measured ChE inhibition across previous studies.[26]

CONCLUSION

This study demonstrated that measurable Guthion residues are present on pesticide-treated foliage in central Washington apple orchards throughout a typical thinning season, and that contact with foliar residues results in daily pesticide absorption for workers conducting thinning activities. Worker exposure varies across the thinning season and across work sites, as workers may enter fields at very different times postapplication. Also, the organization of work activities can differ considerably across work sites, introducing further variability in worker exposure measurements. It was encouraging to find reduced DMTP concentrations 16 weeks after thinning among the few workers monitored.

Continued elucidation of the health significance of low-level OP exposure among these workers via collaboration of industry, labor, regulatory agencies, and academic groups is encouraged. Future research also should confirm the presence or absence of oxon derivatives of OP pesticides on foliage. In addition, the authors encourage the inclusion and evaluation of industrial hygiene control measures in future agricultural studies.

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