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Assessment of Azinphosmethyl Exposure in California Peach Harvest Workers

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ABSTRACT. We compared measurements of urinary alkylphoshate metabolites and oxime-induced reactivation of plasma cholinesterase (P-ChE) and erythrocyte acetylcholinesterase (RBC-AChE) with measurements of foliar residues, skin and clothing contamination, and P-ChE and RBC-AChE activities among 20 Northern California peach orchard workers exposed to the organophosphate agent azinphosmethyl (Guthion®). Subjects entered orchards treated 30 d previously with azinphosmethyl and worked 21 d in treated fields during the ensuing 6 wk. Dislodgeable foliar residues ranged from 0.32–0.96 μg/cm². Median reduction in RBC-AChE activity was 7% ($p < .001$) over the initial 3-d period of exposure and 19% ($p < .01$) over the 6-wk season. Urinary metabolites were the most sensitive indicator of recent exposure and correlated moderately with dermal and clothing levels ($r_s = +0.31$–$+0.55$); urinary metabolites correlated well with RBC-AChE drawn 3 d after exposure began ($r_s = -0.77$). No significant oxime-induced reactivation was found.

AGRICULTURE has undergone dramatic changes in production methods in the past 40 y. Increased use of machinery and chemical compounds, including synthetic pesticide agents, has played an important role in achieving high levels of production. Azinphosmethyl, an *O,O*-dimethyl organophosphate (OP) compound, is one of the more common pesticide agents; in 1988, more than 500 000 lbs were sold in California to be used chiefly on nut and fruit crops.[1]

High-level exposure to OPs may cause acute and

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chronic health effects, whereas possible consequences of chronic low-level exposures are less well characterized.[2] Efforts to protect agricultural workers from exposures include personal protective equipment for persons directly handling the agents (mixers, loaders, and applicators) and re-entry intervals to prevent field workers from entering treated fields before environmental concentrations have decreased to a presumably safe level.

Measurements of residual environmental levels (e.g., dislodgeable foliar residues [DFR]) and of dermal or clothing contamination (e.g., levels measured from hand washings, patch dosimeters, or clothing) are commonly used to evaluate potential dermal exposures.[3] Although these methods may provide a useful index of exposure, they do not indicate the amount of the agent absorbed systemically.

Biomarkers (measurements of an agent or metabolite in body fluids or its effect on a target system such as cholinesterase activity) offer a superior means of evaluating exposure because they indicate absorption of the agent under study.[4,5] Plasma cholinesterase (P-ChE) and erythrocyte acetylcholinesterase (RBC-AChE) enzyme activities are commonly used biomarkers for exposure to OPs. However, absence of baseline values for an individual subject makes it difficult to know if an observed level of P-ChE or RBC-AChE activity represents a depression, indicating exposure to an OP, or it the value is normal for the subject. Nutritional status and acute or chronic health conditions may also affect cholinesterase, especially P-ChE, values.[2] Increases in enzyme activity after incubation of the sample with pyridine 2-aldoxime methochloride (2-PAM Cl), a cholinesterase-regenerating compound, may indicate that exposure to OPs has occurred, even if the pre-regeneration activity was in the normal range. Regeneration may not be observed, however, in cases where P-ChE or RBC-AChE molecules have spontaneously regenerated or have "aged," i.e., become irreversibly inhibited.[6]

Alkylphosphate metabolites of some OPs may be conveniently measured in urine collected before excretion is complete, usually within several days of exposure. The hydration state of the subject may affect the metabolite concentration in urine, however, and error from this source may be prevented by measuring total excretion over a specified time period or by standardizing with urinary creatinine concentration.

We conducted a cross-sectional study of peach workers exposed to azinphosmethyl to evaluate the utility of several measures of exposure. We measured P-ChE and RBC-AChE activities, their reactivation after treatment with 2-PAM Cl, and urinary azinphosmethyl metabolites. These biomarkers were correlated with each subject's work activities, measurements of azinphosmethyl and its oxon metabolite from environmental samples, and levels collected from dermal exposure matrices.

Materials and methods

Population and exposure setting. The study population consisted of peach orchard workers employed by a grower in Sutter County, California. Initial contact with the grower was arranged through the California Department of Food and Agriculture (CDFA). After obtaining permission of the grower, the study team described the study in Spanish to the workers. Twenty workers agreed to participate (approximate participation rate = 80%) and gave written informed consent in accordance with requirements of the University of California, Davis, Human Subjects Review Committee and the CDFA. Participants received $20 for each day's participation.

Thirty days prior to data collection, azinphosmethyl (*O,O*-dimethyl *S*-[4-oxo-1,2,3,-benzotriazin-3(4H)-ylmethyl]phosphodithioate; CAS #86-500) had been applied to the study orchards at a rate of 1.5 lb (0.68 kg) active ingredient per acre (Fig. 1). The re-entry period for azinphosmethyl in California is 14 d.[7] The most intense work exposures and monitoring activities occurred on Days 1, 2, and 3. On Day 1 (July 26, 1989), all subjects were in a 19-acre block of 19-y-old Rand peaches for 8 h. The workers were in a 13-y-old orchard of Andross peaches for 7 h on Day 2 (July 27, 1989) and for 7.5 h on

FLOW CHART OF ACTIVITIES

	Day -30 6/26/89		Day -6 7/20/89		Day 1 7/26/89	Day 2 7/27/89	Day 3 7/28/89		Day 44 9/7/89
WORK TASKS									
Azinphosmethyl application	•								
Propping and thinning					•				
Harvesting						•	•	•	•
Irrigating					•	•	•	•	•
MONITORING									
Questionnaire administration					•				
Foliar sampling					•	•	•		
RBC-ChE and P-ChE			•				•		•
Urine sampling			•		•	•	•		•
Dermal and clothing sampling					•	•	•		

Fig. 1. Flow chart of activities, by participating agricultural workers.

Day 3 (July 28, 1989). During the ensuing 6 wk, subjects worked 18 additional d in treated peach orchards owned by the same grower. None of the fields in which they worked during this period was treated with azinphosmethyl or other OPs, apart from the initial application described above. Final monitoring activities occurred on Day 44 (September 7, 1989), i.e., 74 d after application of azinphosmethyl.

Major work activities included propping, thinning, and harvesting. Propping required the workers to walk between the rows of trees and position 1" × 6" × 14' boards against a tension wire strung around the tree near its top. Thinning involved the removal of excess fruit from the trees to allow the remaining fruit to grow larger. This activity may be done from the ground, using long sticks with hooks on the end to remove the undesired fruit. Harvesting involved using ladders to climb the trees and collection of the the fruit, by hand.

Questionnaire. A questionnaire that addressed general health, occupational history, and recent work activities and exposures was administered in Spanish by bilingual interviewers on the first day of the study.

Dermal and environmental exposure assessment. At the beginning of a shift, 10 subjects were selected on a convenience basis and given sampling garments, including a long-sleeved, 100%-cotton-knit undershirt worn under the subject's own button-up long-sleeved shirt and a pair of knee-length, cotton-acrylic blend athletic socks. The garments were collected for analysis at the end of the shift, and the subjects had wipe samples taken from their face, neck, and hands with premoistened towelettes. Hand wash samples, using 0.05% Surten® rinse, were also taken.

Foliage samples were taken for measurement of DFR levels of azinphosmethyl in three to four locations within each field on each of the 3 exposure d. Samples were prepared for analysis for azinphosmethyl and its oxon metabolite, using gas chromatography according to Gunther et al.[8] The dermal dosimetry matrices (socks, shirts, hand and face/neck wipes) were analyzed in a similar fashion by ethyl acetate extraction. Chromatography was done on a Hewlett-Packard 5880A chromatograph equipped with a nitrogen-phosphorus detector.

P-ChE/RBC-AChE measurements and oxime reactivation. Measurement of P-ChE and RBC-AChE activity was performed at the University of California, Davis, using an assay based on that of Ellman et al.[9,10] Blood samples were drawn from all subjects 6 d prior to exposure (July 20, 1989) and on Day 3 of exposure. Eleven subjects also had blood drawn on Day 44 of exposure. Blood was transported to the laboratory on ice. The sample tubes were kept at ambient temperature for a short period after the blood was drawn. The effect of this on spontaneous reactivation of the blood enzymes is not known.

Oxime reactivation of RBC-AChE was performed at the University of California, Davis, using a method discussed in Wilson et al.[6] Reactivations were considered significant if they resulted in a 5% increase in activity after 2-PAM Cl treatment and statistical significance at the 95% confidence interval (one-tailed Student's *t* test for paired comparison). P-ChE and RBC-AChE activity depressions after exposure were expressed as the percentage decrease in activity relative to the subject's pre-exposure value.

Urine measurements. Urine was collected 6 d before the subjects began working in orchards where azinphosmethyl had been applied, on Days 1, 2, and 3 when work began in the treated orchards, and on the last day of harvesting, Day 44. Plastic 1-l jugs were given to the subjects at the start of the workday. All urine voids for 24 h were to be collected, and the containers were kept on ice throughout the collection period. At the beginning of the next workday, the samples were collected and subjects were given new containers. Pre-exposure samples were collected for less than a full 24 h, and the final Day 44 sample consisted of a single void. The volume of the samples was measured, and an aliquot was placed in a glass sample jar, placed on dry ice, and transported to the laboratory at the University of California, Davis, to be frozen at −40 °C prior to analysis. Creatinine determinations were performed with the Sigma procedure #555[11] for analysis, using microtiter plates and a UV plate reader. The urine samples were assayed for dimethylphosphate (DMP), dimethylthiophosphate (DMTP), and dimethyldithiophosphate (DMDTP), based on the chromatographic method of Weisskopf and Seiber.[12]

Data management and statistical methods. The questionnaires and laboratory data were coded, keypunched, and entered into a VAX 3100 computer. Distributions of continuous variables are described, using median and percentile scores. Except where otherwise indicated, comparisons between groups for continuous variables were made with the Kruskal-Wallis test, using rank score values, and Spearman rank correlation coefficients were calculated[13] after adjusting for the effect of repeated measurements where necessary. Comparison of P-ChE and RBC-AChE values with the individual subject's own baseline value employed the Wilcoxon one-sample test, a nonparametric equivalent to the paired *t* test. Analysis employed procedures available in the Statistical Analysis System (SAS) software library.[14]

Results

Study population. Twenty farm workers participated in the study. All were Hispanic males and completed the questionnaire in Spanish. On the first day of field work, 10 subjects worked as thinners, 6 worked as proppers, 3 worked as irrigators, and 1 worked as a supervisor. On the second and third day of field work, all subjects performed harvesting, except the irrigators. One new subject joined the crew, and 1 subject left the study on the second day of field work. Twelve study personnel who did not perform agricultural work served as controls for various portions of the study. Subjects ranged in age from 18 to 58 y (median = 28.5 y); 65% had a sixth-grade education or less. Employment duration in agriculture ranged from 2 to 35 y (median = 7.5 y); all worked full time in agriculture. None of the participants reported pesticide use in the 2 wk preceding field entrance.

Environmental exposure measurements. DFR values ranged from 0.32–0.96 μg/cm² during the 3-d exposure period. The proportion of compound as the oxon metab-

olite ranged from nondetectable to 2.3% of parent compound + oxon concentration.

Dermal and clothing exposures. For all groups, the highest levels of azinphosmethyl and its oxon were found in the shirts, on the hands, and in hand wash samples (Table 1). Harvesters exhibited the highest shirt levels. Thinners had lower shirt levels than harvesters, but their hand wipe and hand wash levels were comparable to the harvesters. Proppers had the lowest levels.

P-ChE and RBC-AChE activity and oxime reactivation. In comparison with the baseline median value, the median RBC-AChE activity for all agricultural workers combined (Fig. 2) decreased 7% over the initial 3-d period of field-work exposure (11.2 versus 12.1 μmole acetylthiocholine hydrolyzed/min • ml packed RBCs; $p < .001$) and 19% over the 6-wk season (9.81 versus 12.1 μmole AthCh hydrolyzed/min • ml packed RBCs; $p < .01$). The median P-ChE activity for all agricultural workers combined (Fig. 3) fell 9% over the 3-d initial work exposure period (1.97 versus 2.17 μmole AthCh hydrolyzed/min • ml plasma; $p < .01$) and 12% over the 6-wk session (1.91 versus 2.17 AthCh hydrolyzed/min •

Table 1.—Azinphosmethyl Concentration (μg/Sample) on Skin and Clothing by Day and Working Activity

Activity	Face wipe	Neck wipe	Hand wipe	Hand wash	Socks	Shirt
			Day 1			
Propping (n = 6)						
Median	6.5	5.5	16.5	5.5	5.5	324.5
Range	3-8	3-32	11-62	3-11	3-9	191-1 437
Thinning (n = 4)						
Median	121	129.0	4 159.5	1 712	68	4 497.5
Range	95-193	98-240	2 306-8 315	608-2 325	30-169	3 305- 7 853
			Day 2			
Harvesting (n = 10)						
Median	120	93.0	3 544	1 635	113	7 808.5
Range	66-195	31-160	3 200-4 812	933-2 140	28-398	4 071-13 764
			Day 3			
Harvesting (n = 10)						
Median	131	86.0	2 290	1 301	217	9 570
Range	75-1 560	33-148	117-3 626	260-1 934	15-273	2 953-14 498

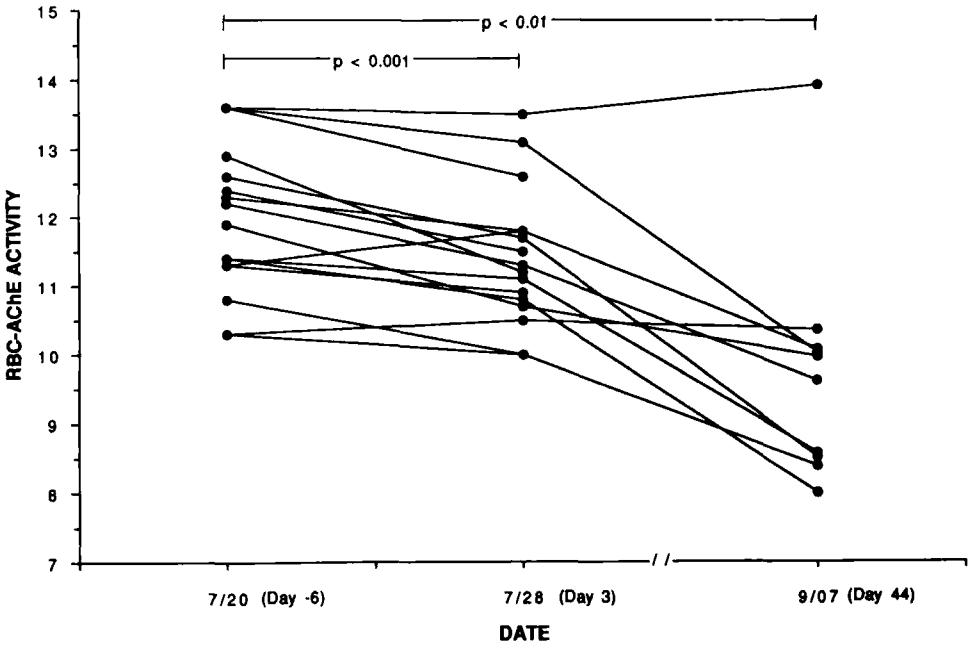

Fig. 2. RBC-AChE activity among participating agricultural workers. RBC-AChE activity is measured as μmoles acetylthiocholine hydrolyzed/min · ml packed erythrocytes.

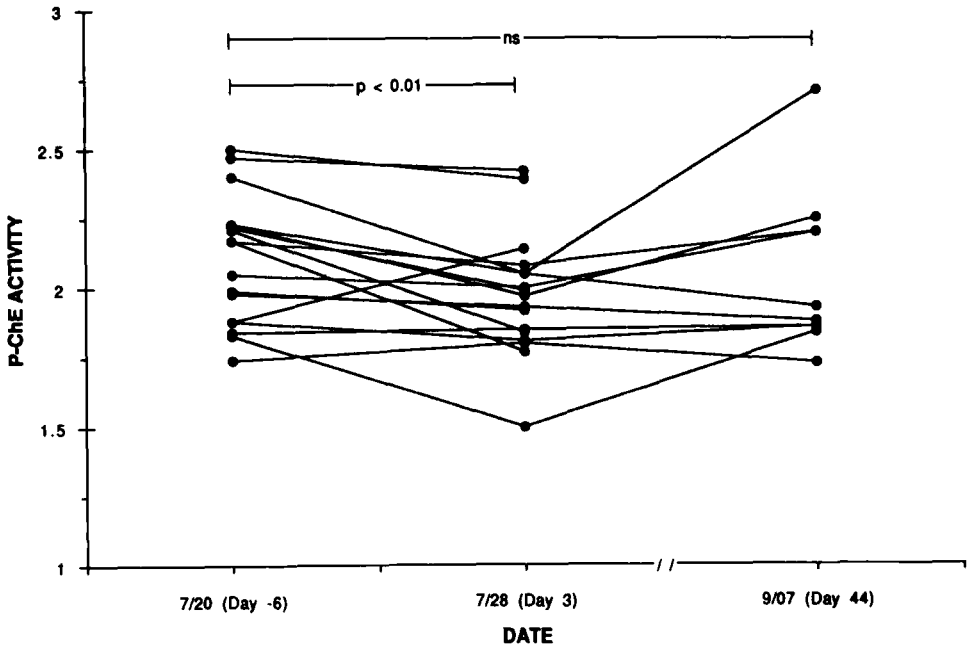

Fig. 3. P-ChE activity among participating agricultural workers. P-ChE activity is measured as μmoles acetylthiocholine hydrolyzed/min · ml plasma.

ml plasma [not significant]). No subject had a positive oxime reactivation test.

Urinary alkylphosphate levels. Initial analysis included correction for urinary creatinine concentration and evaluated urinary alkylphosphate levels of DMP, DMTP, and DMDTP separately. Similar patterns were seen for corrected and uncorrected values of the metabolites, however, and these were subsequently summed and expressed as μmoles/d excreted (Fig. 4). The agricultural workers showed steadily increasing levels of urinary azinphosmethyl metabolites throughout the period of exposure. Thinners and proppers demonstrated higher levels than irrigators, and proppers had lower levels than thinners on Day 1 (median = 1.21 versus 3.84 μmoles/d, $p < .01$). We note that one irrigator (subject #0013) showed a pattern of increasing levels of urinary alkylphosphates over the initial 3 exposure d. This subject also manifested a reduction in RBC-AChE activity. There was a discrepancy in the work records of this individual, and it is probable that he was engaged in thinning as well as irrigating.

Correlation between measures of exposure. Dermal and clothing exposure measurements (azinphosmethyl levels on the hands, wash samples, and shirt) correlated moderately with each other (r_s = +0.26–+0.65). Correlations were not calculated for neck wipes, face wipes, or sock concentrations because of the low levels observed. The levels of the various urinary azinphosmethyl metabolites correlated well with each other (r_s = +0.60–+0.77).

The dermal or clothing measures appeared comparable with respect to correlation with urinary metabolite levels for azinphosmethyl (r_s = +0.31–+0.55). The DFR levels correlated best with the levels measured on skin or clothing (r_s = +0.50–+0.57). Correlation between urinary metabolites (all metabolites summed over the 3 d of exposure to provide an index of total exposure) was poor for P-ChE (expressed as percentage of baseline) drawn on Day 3 (r_s = 0.09 [not significant (NS)]) and for P-ChE drawn on Day 44 (r_s = −0.39; NS). Better correlation was observed with RBC-AChE (expressed as percentage of baseline) drawn on Day 3 (r_s = −0.77; $p < .0001$) and RBC-AChE drawn on Day 44 (r_s = −0.51 [not significant]).

Discussion

We studied a population of peach harvest workers who worked in fields treated with azinphosmethyl and evaluated exposure to the agent, using measurements of environmental exposure, contamination of skin and clothing, and biomarkers. We observed a reduction of RBC-AChE activity and appearance of urinary metabolites of azinphosmethyl in subjects entering treated orchards after the re-entry interval had elapsed and in which measured DFR levels were less than 1 μg/cm². RBC-AChE activity values were more likely to show an exposure-related effect than P-ChE, and the urinary metabolites showed a clear pattern of increase with exposure and good correlation with the RBC-AChE levels drawn on Day 3.

We expected the greatest inhibition of cholinesterase activity to occur on Day 3. However, we observed a median decrease of 7% for RBC-AChE on Day 3 and a 19% median depression on Day 44. The observed inhibition for Day 3 may have been lessened artifactually because the samples were not placed on ice immediately following collection, and some spontaneous regeneration of activity may have occurred. The enzyme activity depression observed at the end of the season was apparently

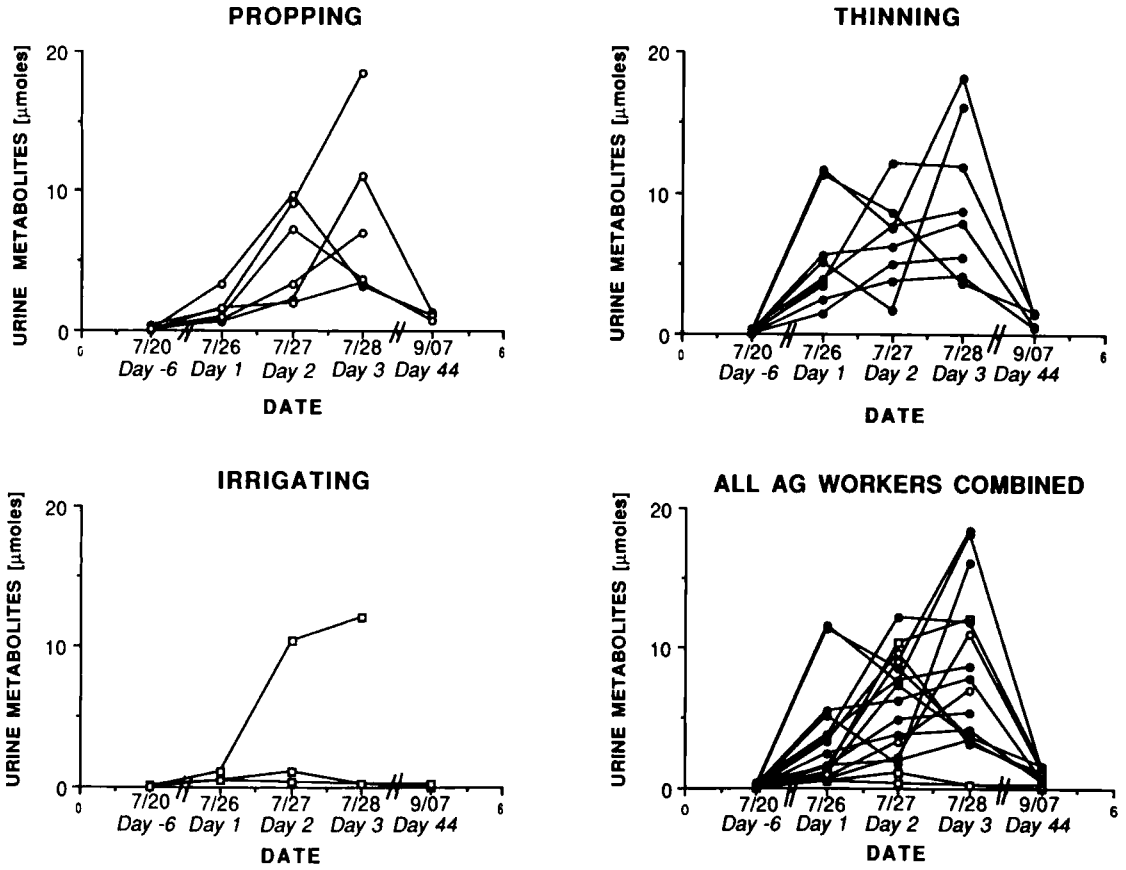

Fig. 4. Urinary alkylphosphate metabolites among participating agricultural workers. Urine metabolites represent the sum of DMP, DMTP, and DMDTP per day. Persons engaged in propping and thinning only performed these activities on Day 1; on the following workdays, these subjects engaged in harvesting.

the result of cumulative exposures relating to the original application 1 mo before work began. Although it is possible that the subjects had other undocumented exposures to OPs, we feel this is unlikely, and the absence of urinary alkylphosphate residues at the end of the season indicates that exposures to azinphosmethyl in the days prior to the final collection were negligible.

The underlying reason for monitoring exposure to OPs is to maintain exposures below levels potentially injurious to health. Monitoring helps evaluate the effectiveness of exposure mitigation strategies. In addition, exposure monitoring may confirm the etiology and source of acute pesticide illnesses when overexposure has occurred. Exposure may be inferred by collecting pesticide residues on skin or clothing. Recently, fluorescent tracers have been used in experimental settings to study deposition patterns.[15,16] However, measurement of skin or clothing residues only indicates pesticide collected in the area of the patch or collection garment, rather than over the entire absorptive surface area, and does not indicate amount absorbed or measure a biologic effect.[17,18] In addition, solvents used to collect skin wash samples may cause irritation or interfere with analysis.[19]

Exposure to OP agents may also be assessed by measuring P-ChE and RBC-AChE activity. P-ChE represents a mixture of enzymes and is a less specific measure of OP exposure than is RBC-AChE. In contrast, RBC-AChE is a single enzyme analogous to that found in nerve tissue; depression of RBC-AChE activity correlates better with clinical toxicity in the nervous system.[20] An acute OP exposure will typically depress both P-ChE and RBC-AChE; P-ChE will then regenerate to normal or supranormal levels (a transient "rebound effect"[20]) in 1 to 8 wk, whereas RBC-AChE will remain depressed for up to 3 mo.[2]

In this study, we observed that RBC-AChE activity was superior to P-ChE activity in detecting exposure to azinphosmethyl. The major problem with using P-ChE or RBC-AChE activity, however, is high intersubject variability.[21] Thus, a low value may be normal in an individual subject, and a normal value may actually represent a reduction from a previously higher level. Establishment of pre-exposure baseline values may be helpful in interpretation, and in California, removal from exposure is required for pesticide workers exhibiting a 40% depression in P-ChE activity or a 30% depression in RBC-AChE activity.[22] Baseline values from which to evaluate depression of activity are not always available; however, when they are available, uncertainty is still not removed entirely.

The oxime reactivation reaction offers a method for evaluating exposure in the absence of baseline values. A positive reaction signifies the ability to regenerate cholinesterase activity and implies that suppression had oc-

curred. In this study, we found no significant reactivation, indicating that the test was relatively insensitive under the conditions of this study. Exposure occurred for days and weeks, and it is likely that a combination of spontaneous regeneration and "aging" led to lack of response to 2-PAM Cl. In particular, dimethyl-substituted organophosphate compounds, such as azinphosmethyl, age and spontaneously regenerate quickly compared with diethyl-substituted agents.[23]

Urinary metabolites offer several advantages for exposure monitoring.[4,5] First, sample collection is noninvasive. Second, the elimination half-life of the substance and metabolites is relatively short, so the method is well suited for monitoring acute exposures. Third, the assay is sensitive and specific, indicating actual absorption of the pesticide from all sources, in contrast to measures focusing on environmental levels or body contamination. Urinary metabolites may detect exposures that do not result in recognized cholinesterase depression and are considered the definitive measure for recent exposures.[24-27] Urinary alkylphosphate metabolites will not indicate cumulative exposures, however, which suggests that combined measurements of metabolites and RBC-AChE activity would be most informative. The usefulness of urinary metabolites may also be limited by lack of availability of assays for certain agents, expense, and delay between sample collection and provision of results.

These data confirm and expand upon studies conducted by other investigators. Weisskopf and co-workers studied a group of subjects applying diazinon in California and measured urinary metabolites, dermal exposures, and exposure by personal air sampling.[3] These investigators found good correlation ($r^2 = 0.65$) between respiratory exposure to diazinon and levels of urinary metabolite.

Drevenkar and co-workers studied 97 orchard workers exposed to azinphosmethyl over two seasons.[28] Subjects underwent testing for P-ChE and urinary alkylphosphate metabolites. Samples were taken approximately 1 mo before the beginning of the first season, at the end of the first season, and at the end of the second season. P-ChE values were depressed up to 48%. Urinary metabolites proved to be a more sensitive indicator of exposure, but poor correlation was noted between urinary metabolite levels and P-ChE values.

Kraus et al.[29] studied a group of 21 Northern California peach orchard harvest workers who entered an orchard to perform thinning 12–18 h after spraying with 2 lbs a. i. azinphosmethyl per 100 gallons of water. A similar application to the orchard had been made 2 wk earlier. Initial azinphosmethyl residues were 2.58 μg/cm², dropping to 1.70 μg/cm² at the end of the 5-d exposure period. A group mean reduction in whole blood cholinesterase activity of 15% was noted over this period. Alkylphosphate metabolites in the urine were higher among thinners than foremen or irrigators. Urinary metabolite levels were not corrected for urinary creatinine to adjust for hydration state of the subject, however, and the results of the urinary measurements were not formally correlated with other indices of exposure.

Kraus et al. performed a follow-up survey[30] in 15 peach orchard workers participating in a 5-d thinning operation. Eight men worked in an orchard treated with azinphosmethyl, and 7 men worked in an orchard treated with chlordimeform, a noncholinesterase-depressing pesticide. Mean DFR levels for azinphosmethyl in the treated orchard were 2.4 μg/cm² on the day on which the workers entered the orchard. The investigators found a mean reduction of 8.3% in RBC-AChE activity through the exposure period for the workers exposed to azinphosmethyl. P-ChE activity showed no clear pattern. Urinary alkylphosphate metabolites were found only among workers in the azinphosmethyl-exposed group. Urinary metabolite levels correlated moderately well ($r = -0.581, -0.598$) with percentage decline in RBC-AChE activity. In the study we report here, exposure to azinphosmethyl residues was lower than observed in the two studies cited above, as indicated by DFR levels. Accordingly, we observed a smaller mean percentage decrease in RBC-AChE activity during the 3-d course of exposure.

Although our report describes the relationships between various methods of measuring exposure to azinphosmethyl, its most important limitation is that it did not examine health outcomes and their relation to pesticide exposure. Acute symptomatic OP poisoning results from cholinergic overactivity in the parasympathetic, central, and peripheral nervous systems.[31] Symptoms in severe cases may include salivation, lacrimation, vomiting, bradycardia, bronchorrhea, diarrhea, urinary incontinence, sweating, mental status changes, and muscular weakness.[2,20] Acute symptoms are typically associated with 50% or greater depression of cholinesterase activity, but may be seen with lesser degrees of depression, especially if depression occurs rapidly.[20,24,32] Data from Israeli kibbutz residents with environmental exposures to OPs suggest that chronic low-level OP exposures can be documented with urinary metabolite assays in the absence of evident cholinesterase depression and be associated with signs and symptoms.[27] In this regard, further work is required to examine exposure-health outcome relationships and implications for protecting persons with occupational or environmental exposures.

Ultimately, the choice of modalities for measuring exposure to OPs requires consideration of the circumstances of exposure and the underlying monitoring purpose. Environmental measures are useful for studying degradation of these agents, identifying sources of exposure in disease outbreaks, and determining re-entry intervals. For evaluating acute and subacute health risks in workers, urinary metabolites are definitive if samples can be obtained before excretion is complete, usually within 1 to 2 d. These assays offer a sensitive and specific measure of exposure and may detect exposures not evident with cholinesterase assays. Measurements of cholinesterase activity are helpful in that they are currently incorporated in the regulatory framework and are useful for longer periods following an acute exposure than are urinary metabolites. Recently, a kit has become available for performing field testing of RBC-AChE levels[21] and is undergoing testing at the University of California, Davis, and elsewhere. In addition to providing convenient field testing, the kit adjusts for the subject's hemoglobin, reducing test variability. Oxime regeneration ap-

pears promising, but may be less helpful in the setting of cumulative exposures because of spontaneous regeneration and "aging."

With respect to studies of outcomes related to chronic exposure to OPs, assessment of aggregate lifetime exposure or exposure occurring more than several months prior to study remains problematic. Ideally, assessment of chronic or cumulative exposures would be based on sensitive and specific biomarkers. Unfortunately, such indices are not generally available, and studies of outcomes related to chronic exposures must continue to rely on epidemiologic methods such as work histories or prospective surveillance. Epidemiologic measures are hampered by the general lack of well-documented individual exposure records and validated questionnaire instruments. Further research is needed to develop and validate such instruments for research into chronic effects of OPs.

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