Monitoring Peach Harvest Workers Exposed to Azinphosmethyl Residues in Sutter County, California, 1991

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Peach harvest workers were evaluated for exposure to azinphosmethyl residues by measuring foliar residues, urinary alkylphosphate metabolites, butyrylcholinesterase (BChE), acetylcholinesterase (AChE), and dermal residues using clothing and skin washes. Workers entered orchards 51 days after application and worked in treated fields for 10 of the next 17 days. Dislodgeable foliar residues ranged from 0.82 to 1.72 μg/cm² and did not change significantly over the study period. Combined mean dermal exposure for the 3 consecutive monitoring days was 32 mg and ranged from 17.9 to 60.5 mg. Overall mean excretion levels for the 5 monitoring days were 1.7 mg dimethylphosphate and 1.9 mg dimethylphosphonate. There was no significant difference in BChE between the exposed harvesters and minimally exposed sorters. The exposed group had significantly lower AChE values than the sorters for 2 post-exposure blood draws by three testing methods, while no significant difference was found for the pre-exposure blood draw. The AChE values for the post-exposure blood samples for the exposed workers decreased significantly about 10–20% over the 3-week exposure period but increased or remained constant for the sorters. Urinary metabolite excretion increased with continuous exposure and was inversely correlated with both AChE and BChE but was not correlated with dermal exposure measurements. High correlations were generally observed between AChE measurements in the field using a new spectrophotometric kit and laboratory AChE measurements. Key words: Alkyl phosphates, azinphosmethyl, cholinesterase, exposure monitoring. Environ Health Perspect 102:580–585 (1994)

We conducted a study to evaluate field worker exposure to azinphosmethyl during peach harvest. Monitoring was conducted using dislodgeable foliar residues, dermal dosimeters, urinary metabolites, butyrylcholinesterase, and acetylcholinesterase. Harvest monitoring had historically been difficult to measure because dermal exposure monitoring provides only an exposure index, and although it is typically the primary exposure route, it may not reflect the important sources of exposure. The traditional method of using dermal dosimeters to measure potential dermal exposure, whether whole-body clothing or gauze pads, gives data that are inaccurate predictors of absorbed dose in humans (1,2).

Biological monitoring provides a means of indicating absorption or effect of the compound under study. Azinphosmethyl has been a suitable candidate for assessment using biological monitoring because of background information on dermal exposure and dermal absorption in humans and animals under laboratory conditions (3–5). Franklin and co-workers reported a strong linear correlation between urinary alkylphosphate levels and both dermal doses of azinphosmethyl in rats and amount of pesticide sprayed for orchard applicators (4,5). The assessment of azinphosmethyl exposure via biological monitoring has been successful in previous studies (6–8).

Materials and Methods

Orchard workers were monitored for exposure to azinphosmethyl residues while picking peaches for processing in Sutter County, California. Sampling consisted of dermal dosimeters, 24-hr urine collections, and blood draws for cholinesterase effects. Table 1 presents the study outline and summary of the monitoring schedule. Orchards were treated once with azinphosmethyl (0,0-Dimethyl-S-[4-oxo-1,2,3-benzotriazin-3-(4H)-yl-methyl]-phosphorodithioate; CAS 86-50-0), using Guthion 50WP (Mobay Corporation), 50% active ingredient at the rate of 1.5 lb active ingredient in 100 gallons of water per acre. No measurable rainfall was recorded over the study period. The orchards were flood irrigated. Trees harvested ranged from 6–20 years old. The younger trees had a much denser foliage and more of the fruit was obscured within the foliage. The older trees had a very open canopy. The sequential dermal and urinary monitoring took place while the workers were picking in the younger trees. The last blood draw and urine collection took place when the workers were in the older trees at the end of the season.

The crew was Spanish speaking, and an interpreter explained the procedures and solicited the workers’ voluntary cooperation. Workers agreeing to participate gave written informed consent. The pickers were male and the sorters were male and female. All participants performed their work in the usual manner, and additional instructions were not given beyond explaining the study procedures. Some workers included with the sorters performed additional tasks including fruit hauling and supervising. Height, weight, and age were obtained for each study volunteer. Since detailed questionnaires were not administered, additional information regarding personal habits, diet, etc., was not available. The sorters were considered to have had minimal exposure to azinphosmethyl and were used as a control group for the pickers. The sorters lived in the area and had no exposure to organophosphates prior to the start of the harvest season. The sorters go through the fruit contained in the bins in the field, removing culls or fruit that is too green. They have minimal contact with tree foliage, occasionally picking off a few fruit that may have been left behind by one of the harvesters. The typical work attire for both pickers and sorters consisted of a long-sleeved, buttoned shirt worn over a short-sleeved T-shirt, long pants, shoes, socks, and a hat. Peach harvesting took place from mid-July to early September, spanning about 6 weeks. Workdays were approximately 8 hr. Picking began on August 1 in untreated fields. Workers were picking in treated fields from August 19 through 22, and pickers 27 to 29 and September 3 to 6. They worked in untreated fields from August 23 to 26 and August 30 to September 2.

The orchards were sampled for dislodgeable foliar residues (DFR) using the methods of Gunther et al. (9). Samples were taken from 10 trees in 3–5 locations in treated orchards, at a height of 3–6 feet and consisted of 40 leaf disks, 2.54 cm in diameter, cut with a leaf punch. Sample jars were sealed with aluminum foil, capped, and kept on ice or refrigerated until extraction.

Dermal Monitoring

Dermal exposure monitoring was conducted for harvesters the entire workday. Hand
residue samples were obtained by having the workers wash their hands. Each worker washed his hands in a 1-gallon plastic bag containing 500 ml of 1% sodium dioctyl sulfosuccinate for 2 min. Face and neck residues were obtained by wiping these regions with two premoistened disposable wipes, which were combined for analysis. Wipes were stored in 4-oz glass jars and hand wash solution in 0.5-l Nalgene bottles. Each worker was given a new, 100% cotton, long-sleeved white knit shirt each monitoring day. They wore the shirts next to the skin under a regular cotton work shirt. The shirts covered the hip region and were tucked into the workers’ trousers. At the end of the monitoring period the shirts were stored in separate, sealed 1-gallon plastic bags with a track seal. All dermal dosimeter samples were frozen until extraction. As a check on the effects of dermal monitoring and urinary metabolite excretion levels, not all the field workers participated in the dermal exposure portion of the study. Urinary metabolite levels of the two groups were compared.

**Sample Analysis**

Dermal samples and DFR were analyzed by California Department of Food and Agriculture Chemistry Laboratory Services. Leaf disks were shaken three times with 50 ml 0.05% sodium dioctyl sulfosuccinate solution, which was then back extracted three times using 50 ml ethyl acetate. The organic extract was then dried by adding anhydrous sodium sulfate. In all cases where a concentration of the sample was required for analytical analysis, 15 ml of the sample was placed into a graduated centrifuge test tube, placed in a water bath set at 30°C, and evaporated under a nitrogen stream to a final volume of 1.5 ml. After concentration the samples were analyzed by gas liquid chromatography. Hand washes were extracted using ethyl acetate, dried with anhydrous sodium sulfate, and diluted as necessary for analysis. Shirts and wipes were extracted and analyzed similarly. Azinphosmethyl was analyzed on a Hewlett-Packard 5880A chromatograph equipped with a phosphorus detector. The chromatographic conditions were: column, 10 m x 0.33 mm 50% phenyl methyl silicone; carrier gas (He), 20 ml/min; H2, 4 ml/min; air, 90 ml/min; injector and detector temperature, 250°C; oven temperature, 240°C, isothermal. Using these conditions, the retention time was 6.00 min for azinphosmethyl and 4.89 min for azinphosmethyl oxon. Standards were introduced periodically during the analysis. Minimum detectable levels for azinphosmethyl were 5, 1, 1, and 0.25 µg/sample, for the undershirts, wipes, hand washes, and dislodgable foliar residues, respectively. The corresponding minimum detectable levels for the oxon were 10, 1, 2, and 0.5 µg/sample.

**Cholinesterase Monitoring**

Blood draws for cholinesterase (ChE) measurements in the volunteers were performed by a phlebotomist on the three sampling dates specified in Table 1. Workers were considered to be in one of two groups: the exposed (harvesters) and the control group or minimally exposed group (sorters). Butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) activities were determined using the Ellman method (10). Two 5-ml blood aliquots were drawn from each worker at each sampling period. The phlebotomist transported one set of tubes in a cooler containing Blue Ice to laboratory 1. The second set of tubes were iced as the samples were drawn and transported by study staff to laboratory 2. Transportation time was about 45 min. Results for laboratory 1 are reported in international enzyme units per liter (IU), defined as the activity of enzyme which converts 1 µmol/l of acetylthiocholine in 1 min at standard conditions. Laboratory 2 units are µmol/min/ml.

Additionally, a finger-prick sample was taken at the time of the last two blood draws and analyzed in the field by an AChE method using the Test Mate OP Kit (11,12). This kit uses an Ellman-like spectrophotometric method adapted for field studies. Procedural modifications were 1) automatic micropipettes were used to add the 120 µl buffer and 160 µl distilled water, and 2) a micropipette was used to transfer the dissolved ChE reagent to the spectrophotometer cuvette. Results were automatically temperature adjusted and electronically displayed for hemoglobin-adjusted AChE (AChE divided by hemoglobin = IU/g hemoglobin).

**Urine Monitoring**

Each worker was provided with 1-l polyethylene urine collection bottles as needed each day. Workers were instructed to collect all urine for a 24-hr period that began with the start of the work day and ended after the next morning’s void. At the end of every 24 hr the volumes were recorded and a 100-ml aliquot was stored in a 250-ml polyethylene bottle. Samples were stored and shipped frozen to the Pacific Toxicology Laboratories (Los Angeles, California), where analyses for dimethyldithiophosphate (DMDTP), dimethylphosphate (DMP) and dimethylthio phosphosphate (DMTP) measurements were completed using the method of Takade et al. (13). Creatinine measurements were used to adjust results of dialkylyphosphates to milligrams per gram creatinine. Creatinine was measured by an alkaline picrate method performed on a Beckman CX5 automated analyzer.

**Data Analysis**

DFR results for azinphosmethyl and azinphosmethyl oxon were summed for each study day. We calculated individual daily dermal exposures by summing the contribution of the thion and oxon for each dermal medium. Daily means and their standard errors are reported.

We analyzed continuous variables using Pearson correlation coefficients and t-tests. For urinary metabolites and ChE measurements, nonparametric tests and measures (Wilcoxon rank sum, Wilcoxon signed rank, and Spearman correlation coefficient) were calculated in addition to the parametric ones; there were no differences, and only parametric results are reported.

Multivariate analyses were used for analyzing cross-sectional comparisons of ChE levels between exposed harvesters and minimally exposed sorters, while simultaneously considering longitudinal changes in ChE over time in both groups. These analyses were conducted using the SAS procedure PROC MIXED (14). PROC MIXED as used here is analogous to the SAS PROC GLM multivariate analyses using the REPEATED feature, although
PROC MIXED is more flexible. These analyses took into account the correlation between repeat measurements over time on the same person (an exchangeable correlation matrix was assumed). Only fixed effects were considered. PROC MIXED uses maximum likelihood estimation assuming multivariate normal data, an assumption reasonably well met for these data. However, large sample size assumptions for maximum likelihood estimates may not be fully justified in these data given the relatively limited sample size.

**Results**

DFR values for azinphosmethyl ranged from 0.82 to 1.72 μg/cm² over the 3-week study period. Azinphosmethyl levels did not vary significantly during the study, with means of 1.34 ± 0.04 μg/cm² (n = 19), 1.18 ± 0.10 μg/cm² (n = 6), and 1.36 ± 0.03 μg/cm² (n = 6) for weeks 1, 2, and 3, respectively. Oxon levels were never above the detection limit of 0.01 μg/cm². DFR levels for August 19, 20, and 21, when dermal exposure monitoring was conducted, ranged from 1.1 to 1.7 μg/cm² and were not significantly different (p = 0.05).

The mean daily dermal exposure for the 3 monitoring days was 32 ± 1.6 mg (n = 41) and ranged from 17.9 to 60.5 mg per person per day. Mean results were similar for each day with levels of 31.2 ± 1.3 mg (n = 15); 35.6 ± 3.2 mg (n = 13); and 31.1 ± 3.2 mg (n = 13) for August 19, 20, and 21, respectively. The long-sleeved undershirts, the hands, and the face/neck wipes accounted for 82%, 16%, and 2% of the total body exposure, respectively. Oxon levels were detected on all sampling media and ranged from 0.6 to 3.7 mg on the shirts, 0.1 to 0.4 mg on the hands, and 0.002 to 0.05 mg for the face/neck wipes. The oxon residues accounted for 6% of the dermal exposure.

**Cholinesterase Monitoring**

Figures 1 and 2 show the results for AChE and BChE for both exposed (harvesters) and controls (sorters), for both labs. Pre-exposure values were within the range of laboratory norms. For AChE, harvesters have lower values than sorters for the two post-exposure blood draws, and harvesters, but not sorters, show a decrease over time after exposure begins. BChE shows little difference between exposure groups. Both groups show a downward shift in comparison to their baseline values. The shift in BChE values suggests the occurrence of laboratory drift (15).

Means for BChE and AChE for harvesters and sorters are given in Table 2, as well as p-values for t-tests comparing harvesters to sorters at each test date. For laboratories 1 and 2 on August 26 and September 5, t-tests compare the means in each group after subtracting the baseline value for each individual. There were no significant differences in BChE between exposed and controls at any testing date. The harvesters showed lower AChE (but not BChE) compared to the control sorters after exposure, but not at baseline before exposure. No symptoms of organophosphate poisoning were reported by any of the workers.

Table 3 presents longitudinal analyses over time (mean differences and p-values for t-tests) for the AChE and BChE data. For the exposed group, three of four comparisons, (two post-exposure tests at two labs) for AChE showed a decrease in AChE values post-exposure versus baseline pre-exposure values (two were significant). The

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**Figure 1.** Acetylcholinesterase levels for harvesters (exposed) and sorters (controls) found by two different laboratories.

**Figure 2.** Butyrylcholinesterase levels for harvesters (exposed) and sorters (controls) found by two different laboratories.

**Table 2.** Mean cholinesterase results for harvesters (exposed) and sorters (controls) found by two different laboratories.

| Analysis/lab/group | Day 0 (8/12) | Day 14 (8/26) | Day 23 (9/5) |
|--------------------|--------------|--------------|--------------|
| BChE/Lab 1/S       | 2853 ± 203 (9) | 2565 ± 208 (9) | 2339 ± 276 (6) |
| BChE/Lab 1/H       | 2651 ± 61 (24) | 2511 ± 69 (24) | 2363 ± 74 (17) |
| BChE/Lab 2/S       | 1.9 ± 0.2 (10) | 1.9 ± 0.2 (9) | 1.6 ± 0.2 (6) |
| BChE/Lab 2/H       | 2.1 ± 0.1 (24) | 1.9 ± 0.05 (24) | 1.7 ± 0.1 (17) |
| AChE/Lab 1/S       | 6893 ± 202 (9) | 6553 ± 158 (9) | 7062 ± 250 (6) |
| AChE/Lab 1/H       | 6515 ± 143 (24) | 5495 ± 104 (24) | 5983 ± 300 (17) |
| AChE/Lab 2/S       | 9.8 ± 0.3 (10) | 11.3 ± 0.3 (9) | 10.5 ± 0.3 (6) |
| AChE/Lab 2/H       | 9.5 ± 0.2 (24) | 9.8 ± 0.4 (24) | 8.1 ± 0.4 (17) |
| AChE/Kit/S         | NA            | 30.4 ± 1.1 (8) | 33.4 ± 1.4 (6) |
| AChE/Kit/H         | NA            | 28.8 ± 1.0 (23) | 25.7 ± 1.2 (22) |

**Abbreviations:** BChE, butyrylcholinesterase; AChE, acetylcholinesterase; H, harvesters; S, sorters.

*Data are presented as means ± SE. The p-values for labs 1 and 2 for cross-sectional comparisons of exposed versus controls at day 14 and day 23 are for t-tests comparing mean levels for each group after subtracting the baseline value for each individual. No baseline was available for the kit.

*Sorter values were summinly and served as controls for expanded harvesters. Lab 1 units are IU. Lab 2 units are μmol/min/ml. Kit (Test Mate OP Kit) units are IU/g hemoglobin.*
harvesters’ BChE showed a significant decrease over time for all four comparisons. For the controls, AChE increased in three of four comparisons, while all the BChE values decreased, but not significantly. The kit data could not be evaluated longitudinally for lack of a baseline. No pattern of decreases was observed for the controls.

When regression lines for AChE were fit separately for exposed and control groups over time (each lab separately), the exposed group showed a significantly negative (downward) slope over time (-0.041 mol/min/ml per day, p = 0.003, at lab 2 and -32.87 IU per day, p = 0.0004, at lab 1). Although expressed in different units, these downward slopes were similar in magnitude. In contrast, the control group showed a flat (lab 1) or upward (lab 2) slope over time. For both laboratories, the slopes of the regression lines for exposed and controls differed significantly.

Intercepts at baseline did not differ for either laboratory. Table 4 presents correlation coefficients and significance levels between AChE methods by date for the exposed group. All correlations were highly significant for all test days.

Multivariate analyses of the AChE data showed no effect of age, weight, height, or sex. A cell mean model (ANOVA) showed a significant difference between the average of the two post-exposure AChE means, with exposed lower than controls, for both laboratories (p = 0.0007 for lab 1 and p = 0.0002 for lab 2).

### Urinary Monitoring

The results of the analyses for the presence of DMP and DMTP are reported in Table 5. Values for DMDTP are not reported because it was not detected in 90% of the samples. Mean creatinine values were 1.4 g/l with 90% of the 24-hr urine collection having volumes greater than 700 ml. Both sorters and harvesters generally showed increasing levels of metabolite excretion over sequential monitoring days (8/20–8/23). Metabolite excretion for sorters averaged 4% of that for the harvesters. While both 8/22 and 9/6 represent a third sequential exposure day, the urinary metabolite means for 9/6 are 30–40% of those collected the morning of 8/23. Overall means of DMP and of DMTP and their associated standard errors for the 5 urine monitoring days using creatinine-adjusted (mg/g creatinine) values were 1.8 ± 0.17 and 2.0 ± 0.14, respectively, for the harvesters (n = 119); and 0.06 ± 0.009 and 0.08 ± 0.01, respectively, for the sorters (n = 41).

Dermal exposure on day 1 correlated with total metabolites (DMP + DMTP) collected the following morning (r = 0.58, p = 0.04); total metabolites collected after 48 hr were less well correlated (r = 0.47, p = 0.08). Other comparisons of dermal exposure to urinary metabolites had associated p-values >0.10. There was no difference in urinary metabolite levels between those pickers monitored for dermal exposure and pickers providing only 24-hour voids.

To assess the correlation between urinary phosphates and ChE among the exposed group, we averaged the five urinary phosphate levels (August 20, 21, 22, 23, and September 6), and then correlated these means with the average post-exposure (August 25 and September 5) AChE and BChE (after subtraction off the baseline value). The results (Table 6) indicate that AChE was significantly inversely correlated with urinary phosphates at both laboratories for each of the two urinary phosphates (DMP and DMTP). BChE was inversely correlated as well, but the correlation was not statistically significant. Although the inverse correlation between average urinary phosphate and average post-exposure BChE was not significant, on the one occasion when both urine and cholinesterase data were obtained at approximately the same time (September 5 ChE and September 6 urine), there was a significant inverse correlation at lab 1, although not at lab 2. Both BChE and urinary phosphates should reflect exposure during the previous 1–2 days.
Discussion

DFR levels were twice those found during the 2 previous years at this location in 1989 (7) (0.59 \( \mu g/cm^2 \)) and 1990 (8) (0.46 \( \mu g/cm^2 \)), although application rates were unchanged over the 3 years. This is similar to the observations of other researchers (16,17), where DFR levels varied from 0.43 to 2.2 \( \mu g/cm^2 \) days after application at the same locale over 2 consecutive years. DFR samples were not taken at the time of application, but the half-life for azinphosmethyl at this locale was previously calculated to be 32 ± 7 days (8). Knaak et al. (18) calculated a safe level for azinphosmethyl on foliage of 1.6 \( \mu g/cm^2 \) using dermal dose–response curves for cholinesterase inhibition, developed from animal models, and field exposure data. Knaak's method took into account the higher toxicity levels of the oxon residues, which were not detected in this study. DFR levels remained unchanged over the study period, and any trend reflecting changes in dermal exposure or urinary metabolite levels could not be evaluated in its relationship to foliage residues.

Dermal exposure measurements of 32 mg were similarly twice the levels measured the previous 2 years at 15.5 mg in 1989 and 13 mg in 1990 (7,8). The contribution of the dermal exposure measured from the long-sleeved shirts was greater than in the 2 previous years, (82% versus 66% and 57% in 1989–90) but was similar to the exposure distribution measured at three other locations (8). The mean cumulative dermal exposure for the 3 days of dermal monitoring was 98.4 mg per person. The cumulative urinary equivalents (the ratio of the molecular weight of azinphosmethyl to the molecular weight of the various metabolites) was 37.8 mg for the 4 consecutive days that correspond to the dermal monitoring. An indirect estimate of 28% dermal absorption was calculated by dividing the urinary equivalents by the sum of dermal exposure and urinary equivalents. This estimate is similar to previous estimates for dermal absorption ranging from 17% to 35% azinphosmethyl (8) and is within the range of 16–42%, depending on regional variation in absorption, found in laboratory studies (4,19).

Feldman and Maibach (3) showed urinary metabolite excretion for azinphosmethyl to be 5.5, 5, 3, 1.4, and 1%, respectively, for days 1–5 after a single topical application. Our study showed an increase in urinary phosphates levels for consecutive exposure days due to the additive effects of previous exposure days. This additive effect may account for the poor correlations that were seen between dermal exposure and urinary metabolites after the first exposure day. Dermal exposure is comparatively easier to monitor and in some situations one might prefer to use dermal exposure as a predictor of absorbed dose in humans. Dialkylyphosphate results for September 5–6 were lower than expected because harvesters had been exposed for 3 consecutive days to DFR levels similar to those of August 26. This decline could be related to differences in growth habit that were observed between tree varieties. The trees harvested earlier in the season had denser foliage, and the later variety grew more vaselike, with an open canopy, allowing the worker to contact less foliage during picking.

Urinary phosphates are an indicator of exposure. Because exposure conditions are likely to be relatively uniform for all workers on a given day, urinary phosphate levels on a given day may vary principally due to different work practices by different workers, although unique absorption and excretion biology may also account for some interindividual variability. Urinary phosphates were measured on 5 separate days, and pairwise correlations for exposed workers between these different days were consistently highly positive and highly significant, probably indicating that some workers consistently engaged in work practices which led to higher exposures than other workers. When we averaged all five urine measurements taken during the entire exposure period and compared them to the average of the two post-exposure cholinesterase measurements (after subtracting the baseline ChE), we found a significant inverse correlation at both labs for AChE, but not for BChE. This finding is in accord with our findings for cholinesterase, which indicate that pesticide exposure among these workers resulted in some measurable inhibition of AChE, but no BChE inhibition. In a previous study (7) where monitoring was conducted under similar circumstances, the correlation with urinary phosphates was poor for both BChE and AChE values, but DFR values were half those in this study. Richards et al. (17) studied workers thinning peaches and correlated dialkylyphosphate metabolites with percent decline in AChE (r = -0.581 and -0.598 for DMP and DMTP, respectively) where azinphosmethyl DFR levels were 2.2 \( \mu g/cm^2 \), results similar to our findings.

Our findings for AChE indicated that exposed workers experienced a 10–20% decrease over the 3-week exposure period, while the minimally exposed controls did not. BChE was not affected. These findings are consistent with the literature showing an AChE but not BChE effect of azinphosmethyl, as is typical for methylated organophosphates (17). Biologically, it is plausible that workers exposed at a relatively constant dose would exhibit constantly decreasing AChE levels over time (as opposed to an initial inhibition, which remains constant over the exposure period). However, this depends on a complicated interaction between the strength of the organophosphate cholinesterase bond, the regeneration rate of red cells, and the level of dose.

The Test-Mate OP Kit was easy to use in the field and the workers preferred the finger prick to the drawing of a venous blood sample. McConnell et al. (20) found that the kit performed well in the field with interindividual variability of 7.4% for a nonexposed group for hemoglobin-adjusted AChE. In this study the coefficient of variation was approximately 10% for the control group hemoglobin-adjusted values. Although there were positive correlations with the other two laboratories and the kit, the lack of a "gold standard" for cholinesterase prevents any conclusions regarding the relative validity of the kit and laboratory for cholinesterase measurements.

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