

Monitoring Organophosphate Insecticide-Exposed Workers for Cholinesterase Depression

New Technology for Office or Field Use

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A serious limitation to the diagnosis of mild organophosphate poisoning and to the preventive screening of organophosphate-exposed workers has been the large interindividual variability in erythrocyte cholinesterase. This makes it necessary to obtain a pre-exposure baseline measurement of enzyme activity as a basis for evaluating subsequent declines. To evaluate a new battery-operated colorimetric erythrocyte cholinesterase kit, 23 workers at a Mexican pesticide formulation plant were examined. All workers had normal cholinesterase, and exposed and unexposed workers were found to have similar mean cholinesterase levels. Although erythrocyte cholinesterase was found to have a coefficient of variation of 12% (similar to that reported in previous studies), hemoglobin-adjusted erythrocyte cholinesterase had a markedly reduced coefficient of variation (7.4%). The 90% confidence interval (24.9–31.7 IU/g hemoglobin) resulted in a lower normal limit that is 78% of the upper limit. Even if a pre-exposure baseline were high normal but unknown at the time of examination, the supervising clinician can be confident that any person with a normal result will be no less than 78% of baseline. The kit is moderately priced, easy to use in the field setting, and the low variability to the assay should allow improvement in diagnosis, screening, and in the epidemiologic evaluation of exposure.

Poisoning with organophosphate insecticides is common among pesticide mixers and loaders and occurs sporadically among other insecticide-exposed individuals.¹ Depression of neuronal cholinesterase results in headache, lightheadedness, nausea, vomiting, profuse sweating, and (in severe cases) coma, respiratory depression, and death.² Measurement of erythrocyte and plasma cholinesterase are useful for the diagnosis of organophosphate poisoning. In addition, the routine monitoring of organophosphate-exposed workers for plasma or erythrocyte cholinesterase depression has been used for many years for identifying workers at risk for poisoning before the appearance of clinical symptoms.³ Poisoning can thus be prevented by improving working conditions and removing from exposure those workers with low cholinesterase activity. In California, workers exposed to US Environmental Protection Agency category I (most hazardous) organophosphate or carbamate insecticides are required by law to be provided with pre-exposure and periodic cholinesterase measurements.⁴ Any worker whose erythrocyte cholinesterase falls to 70% of baseline or less must be removed from exposure.

Unfortunately, except for certain categories of pesticide-exposed workers in California, persons exposed to cholinesterase inhibitors

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often have not had pre-exposure baseline measurements. The inter-individual normal range for cholinesterase is wide: over twofold in the case of plasma and almost twofold in the case of erythrocyte cholinesterase (see Table 1). Therefore, a marked depression in cholinesterase in a person with high normal (baseline) cholinesterase activity may result in postexposure activity within the low normal (interindividual) range. This presents a diagnostic dilemma for the clinician, who is often faced with a patient without pre-exposure cholinesterase assays. If this patient has mild symptoms compatible with organophosphate poisoning (headache, nausea, lightheadedness) and low normal cholinesterase activity, the diagnosis may be difficult to make. In addition, clinicians supervising cholinesterase screening programs for workers exposed to organophosphates who have no baseline measurements are faced with the dilemma of allowing overexposed workers at risk of poisoning to continue to be exposed to organophosphates.

This study evaluates the precision and field performance of a new kit (which is based on the Ellman assay⁵) for measuring erythrocyte cholinesterase. The battery-operated colorimeter makes use of a novel application of a light-emitting diode (the first application of this technology to medical diagnostics). By adjusting cholinesterase for hemoglobin concentration, it is possible to markedly reduce interindividual variability and the associated false-negative cholinesterase tests common to other assays.

Methods

The study population consisted of 23 pesticide formulators exposed to hexachlorobenzene and DDT (not cholinesterase inhibitors) and to methyl parathion during the 2 weeks before evaluation. All 13 formulators (12 men and 1 woman) on the first two shifts were sampled for erythrocyte cholinesterase in conjunction with a follow-up inspection of working conditions conducted by the Mexican federal health authority.

Ten unexposed workers (all men from the day shift) assigned to preparation of inert ingredients or working as chauffeurs or office staff also were sampled. A questionnaire was administered to all participants asking about history of poisoning and of exposure.

Subsequent to the study of the formulation plant workers, erythrocyte cholinesterase activity was measured in a Nicaraguan field worker poisoned with organophosphates.

A prototype of the Testmate® Cholinesterase Kit (EQM Research, Cincinnati, Ohio) was used to analyze capillary blood samples for erythrocyte cholinesterase: (1) The skin of the right lateral thumb of each worker was wiped twice with commercial ethanol swabs. Ten μ L of blood were drawn from a finger stick and immediately transferred to a 2 mL solution of phosphate buffer containing detergent (to lyse cells) and quinidine (to inhibit plasma cholinesterase) in a cuvette. (2) Oxyhemoglobin concentration was measured in the kit's colorimeter.

(3) Four drops of distilled water were added to dissolve a premeasured single test lyophilized reagent. Reagent included substrate (acetylthiocholine) and indicator (dithionitrobenzoic acid). The reagent solution was transferred to the cuvette containing buffer and blood, and the change in absorbance (reflecting cholinesterase activity) was read.

Results were automatically temperature adjusted and electronically displayed as erythrocyte cholinesterase (in international units [IU]/ μ L of blood), oxyhemoglobin (in g/dL of blood), and hemoglobin-adjusted cholinesterase (cholinesterase divided by hemoglobin—in IU/g hemoglobin). The biochemistry of this assay has been described more fully in a previous publication.⁶

Each worker was sampled twice (to evaluate repeated sample variability), and each worker was given the averaged result of these measurements. Except as indicated, the averaged result was used for all the following calculations.

One of the authors (R.M.) was sampled in the field and four other times over the subsequent 4 weeks. On four occasions a lyophilized cholinesterase standard available with the kit was reconstituted with buffer. On each occasion, two standards were assayed and results were averaged to obtain a result for that day.

Results

Formulation Plant Workers

The mean age of the workers was 36, and duration of employment

TABLE 1

Previously Published Interindividual Ranges for Plasma and Erythrocyte Cholinesterase in Normal Persons*

	Mean	Standard Deviation	Coefficient of Variation %	Normal Range	Lower Normal (as % of Upper Normal)
Plasma cholinesterase ⁷ (N = 10)	1.8	0.41	22.8	1.1–2.5	45
Erythrocyte cholinesterase ⁸ (N = 116)	6.0	0.89	15.0	4.5–7.5	60

* Results in IU/mL blood; normal range = mean \pm 1.645 SD.

ranged from 3 months to 14 years. Thirteen (60%) of the workers reported poisoning at some time in the past, although no one had required treatment in the 8 months before this study.

Mean cholinesterase among exposed formulators (4.1 IU/mL blood; SD 0.52; N = 13) was almost identical to results among plant workers unexposed to pesticides (4.2 IU/mL; SD 0.45; N = 10). Hemoglobin (Hgb)-adjusted cholinesterase was also very similar in the two groups (28.5 IU/g Hgb; SD 1.98 among exposed workers compared with 28.0 IU/g Hgb; SD 2.37 among unexposed workers). In addition, no person had clinically abnormal Hgb-adjusted cholinesterase. Therefore, both groups were considered unexposed, and results of both groups were pooled (see Table 2). Although the coefficient of variation for erythrocyte cholinesterase (12%) was similar to that reported previously,^{6,8} Hgb-adjusted cholinesterase had a markedly reduced variability (coefficient of variation) com-

pared with erythrocyte cholinesterase. The resulting normal range should allow detection of anyone who might drop to 78% or less of baseline, even if no pre-exposure baseline was available.

The repeated sample variance (for the same person) was also less (as a proportion of interindividual variance) for Hgb-adjusted cholinesterase (Table 3), confirming the importance of Hgb adjustment, presumably because most of the error to the assay was introduced in the first step (pipetting blood).

One worker (the female formulator) had a low cholinesterase level (3.0 IU/mL blood). However, she also had low Hgb (10.6 g/dL). Hemoglobin-adjusted cholinesterase was normal (28.8 IU/g Hgb). One worker had grossly contaminated his hand with 3% methyl parathion dust immediately before sampling. Cholinesterase from blood sampled from washed skin was within the normal range (26.0 IU/g Hgb). However, cholinesterase in the blood from the same worker al-

lowed to drip onto grossly contaminated skin before sampling was artifactually low (23.6 IU/gm Hgb).

Results of repeated sampling over 4 weeks from the same person demonstrated a coefficient of variation of 3.7% for Hgb-adjusted cholinesterase (mean 26.1 IU/g Hgb; SD 0.96; n = 5). Repeated samples of the lyophilized standard demonstrated a coefficient of variation of 3.0% (mean 16.8 IU/g Hgb; SD = 0.50; n = 4), suggesting that this standard is a useful addition to the kit to assure quality control.

Case Report

A 29-year-old Nicaraguan pesticide applicator sought medical attention for headache, lightheadedness, increasing malaise, insomnia, anorexia, and decreased sexual drive during the month before examination. During this period he had mixed methyl parathion and mephosfolan concentrate on 10 occasions for a crew of backpack sprayers with whom he also worked as an applicator. Erythrocyte cho-

TABLE 2
Interindividual Means and Normal Range for Erythrocyte Cholinesterase, Hemoglobin, and Hemoglobin-Adjusted Erythrocyte Cholinesterase among Formulation Plant Workers*

	Mean	Standard Deviation	Coefficient of Variation (%)	Normal Range	Lower Normal (as % of Upper Normal)
Hemoglobin (g/dL)	14.7	1.3	8.8	12.6-16.9	74
Erythrocyte cholinesterase	4.1	0.49	12	3.4-5.0	67
Hemoglobin-adjusted erythrocyte cholinesterase	28.3	2.1	7.4	24.9-31.7	78

* Results in IU/mL blood or IU/g Hgb; normal range = mean \pm 1.645 SD; N = 23.

TABLE 3
Mean Interindividual Findings* Compared with the Mean of the Difference between Two Repeated Assays from Each Participant†

	Mean (Standard Deviation)	Mean Difference (Standard Deviation)	Ratio of Variances
Hemoglobin (g/dL)	14.7 (1.3)	0.67 (0.53)	0.24
Erythrocyte cholinesterase	4.1 (0.49)	0.17 (0.23)	0.22
Hemoglobin-adjusted erythrocyte cholinesterase	28.3 (2.1)	0.66 (0.51)	0.06

* From Table 2.

† Results in IU/mL blood or IU/g Hgb; N = 23.

linesterase activity was 0.44 IU/g Hgb one day after his last exposure.

Discussion

The testing kit performed well under field conditions. Workers from the formulation plant were found to have minimal exposure to organophosphates at the time of the field visit, as determined by cholinesterase measurements. Markedly inhibited cholinesterase activity was demonstrated in a pesticide applicator poisoned with organophosphates. The procedure was simple; the technician was trained in 2 hours. No external power source was needed. One sample should be adequate for routine diagnosis or screening (based on the low repeated sample variability). Cost is moderate (\$850) for the kit, including reagents for 96 assays. Subsequent assays cost \$1/assay (with discounts to \$0.80 for bulk purchases). Twelve assays hourly could be performed comfortably by a single technician.

Because the procedure used with the testing kit allows for hemoglobin measurement and assay of erythrocyte cholinesterase activity on the same dilution of blood, the Hgb-adjusted cholinesterase is highly reproducible and corrects for blood sampling error and anemia (a common cause of false positive [artificially low] cholinesterase results, as in the example of the anemic woman at the formulation plant). The exciting implication of reducing the interindividual variability to 7.4% for Hgb-adjusted erythrocyte cholinesterase is that any organophosphate-exposed person whose measurement drops by as little as

22% (to 78% of baseline) will be identified as abnormal by this procedure, even if a pre-exposure measurement is not available. With the kit the likelihood of overlooking subacute organophosphate poisoning (false negative) also is reduced.

The low cholinesterase measurements from the worker whose blood sample was grossly contaminated with methyl parathion demonstrates that artifactual depression of cholinesterase can occur in the occupational setting. This has been reported previously in the experimental setting.⁹ Although blood sampled from cleaned skin was normal in this worker, we cannot rule out a small depression from residual methyl parathion contamination of skin. Until more data are available on this problem, if artifactual depression of cholinesterase from skin organophosphate residues is suspected, a low cholinesterase level could be confirmed by drawing antecubital venous blood (which is less likely to come into contact with contaminated skin).

The lyophilized standard for quality control and the high accuracy of the testing kit make it a useful tool not only for routine screening and diagnostic work but also for epidemiologic work where subtle shifts in population means are to be evaluated as an index of exposure. If pre-exposure measurements are available, it should be possible with this kit to identify small decreases from baseline, thus increasing the statistical power available for epidemiologic investigations that rely on erythrocyte cholinesterase as a measure of exposure

to pesticides. The kit (which weighs 7.5 pounds) comes in a portable carrying case for field use.

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