Biological Monitoring of Agricultural Workers Exposed to Pesticides: I. Cholinesterase Activity Determinations

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An estimated 4 to 5 million persons work in agriculture; within this sector, mixers, loaders, applicators, and other workers who directly handle agricultural chemicals are at highest risk for pesticide-related illnesses. Among field workers, seasonal workers engaged in certain cultivation and harvesting operations are also at risk of exposure to pesticide residues. Biological screening for agricultural workers is limited at present to pesticide mixers, loaders, and applicators, and consists almost exclusively of measurements of cholinesterase activity to estimate inhibition by organophosphate and carbamate compounds. We review here the measurement and interpretation of cholinesterase activity in occupationally exposed populations and the correlation between cholinesterase activity and symptoms of acute and chronic illness among agricultural workers. The only state to mandate medical surveillance for agricultural workers is California: criteria for surveillance, baseline cholinesterase determination, periodicity of testing, and criteria for removal from and return to work are described, and alternative approaches for screening and clinical evaluations are discussed.

Although estimates of the total labor force vary in agricultural work more than in any other sector, 4 to 5 million persons probably work in agriculture as their primary means of earning a living.[1] Within this sector, agricultural workers at particularly high risk for pesticide-related illness can be identified. By job category, these are primarily the mixers, loaders, applicators, and other workers who handle agricultural chemicals directly.

Among field workers, the approximately 2 million seasonal workers are at greatest risk of pesticide residue exposure because they are concentrated in high-risk crops and activities.[2] Of the 1 billion pounds of pesticides used annually in agriculture in the US, 800 million pounds are applied to approximately 20% of the total crop acreage[3]; most of these crops involve seasonal field labor. More than 50% of seasonal workers are hired for harvesting operations, which involve contact with foliage during or shortly after high pesticide application periods. Of the 27% who work in the cultivation of crops, more than one-third work in cotton, a crop requiring a very high rate of pesticide applications.[2]

There is also a geographic concentration of the work force at highest risk: more than 50% of hired farm workers on farms employing more than ten workers are found in just two states, California and Florida, and 65% are employed in production of vegetables, fruits, nuts, tobacco, or sugar. Two-thirds of all vegetables are produced in California, Idaho, Michigan, Texas, and Washington; California produces almost 50% of all fruit in the US, whereas Florida produces another 20% (unpublished data, 1983). This pattern of seasonal and geographic concentration is paralleled to a lesser extent by agricultural services (applicators, machinery operators, and other nonfield-production workers).

Biological monitoring of pesticide applicators and field workers was first performed for research purposes in the 1950s when organophosphates were introduced into commercial use and the risk of severe worker poisoning was recognized.[4] As organochlorine use declined and organophosphate use rose rapidly in the 1960s, however, increasing reports of applicator poisonings and of field harvester crew poisonings drew attention to the need for preventive programs.[2] Such pro-

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0096-1736/86/2808-619$02.00/0

grams, as instituted by the Environmental Protection Agency and the states, have largely depended upon the regulation of work practices and protective equipment for the protection of applicators and upon the establishment of reentry intervals after crop treatment to protect field workers.

Biological screening for agricultural workers at present is limited to pesticide mixers, loaders, and applicators, and consists almost exclusively of cholinesterase activity measurements to estimate inhibition by organophosphate and, less frequently, carbamate compounds. Medical supervision of workers in these job categories is required by regulation in only one state, California; baseline cholinesterase testing is mandated and further testing must be performed if requested by the supervising physician.[5] Clinic-based screening of field worker exposure to pesticides was conducted for 2 years by the California State Department of Agriculture (CDFA) in 1975–76.[6] In 1982, a US Public Health Service memorandum directed federally funded migrant health clinics to develop pesticide health hazard management programs, including some elements of biological surveillance.[1]

In Part I of this paper, we review the measurement and interpretation of cholinesterase activity in occupationally exposed populations and the correlation between cholinesterase activity and symptoms of acute and chronic illness. In Part II, we discuss the biologic monitoring of exposed workers for intact pesticides and their metabolites. In both sections, we have summarized current biological monitoring practices and presented some thoughts on potential developments in biological surveillance programs for agricultural workers.

Cholinesterase Activity in Populations Studied

Intraindividual Variation

There is less variation between serially drawn samples from the same person than between samples taken from different persons. As early as 1939, Jellinek and Looney[7] had followed serum cholinesterase in 20 subjects over a 1-week period, reporting an intraindividual correlation coefficient of 0.97. In 1957, Rider et al[8] published the first major study of cholinesterase values in 800 normal subjects, using the Michel method. The SE of both the erythrocyte and serum enzymes for individuals—either in triplicate split samples or repeated over several weeks—were negligible in comparison to variations between individuals.

In 1965, Wetstone and LaMotta[9] followed 82 subjects for up to 250 weeks, finding an intraindividual correlation of 0.98; there was a strong correlation between first and second tests ($r = .80$ for females and $r = .94$ for males), and the average difference between any two succeeding samples was only 8.6%. The coefficient of variation for the 82 subjects in 373 determinations was 8.4.[9] Despite these findings, normal workers who are not exposed to cholinesterase inhibitors may unpredictably show a striking fluctuation from one sample to the next; such fluctuations may be as great as 13 to 25% for RBC and 20 to 23% for serum activity.[10]

Intraindividual variation over a period of time is not related to initial or mean values, and there is no correlation between erythrocyte and serum enzyme activities in the absence of cholinesterase inhibition. The laboratory itself may be responsible for a significant proportion of the measured intraindividual variation in cholinesterase activity. In the study by Rider et al[8], three aliquots from each sample were run separately in the same laboratory on the same equipment. The SE for both red blood cell and plasma measurements was only 0.22 ± 0.002 ΔpH units.[8] In a later study by Serat and Mengle,[11] sample pairs were tested on separate equipment in the same laboratory; variation greater than 5% was found in 61% of the pairs, and even greater variation was found for assay values from two laboratories using the same technique and instruments. Autoanalytical procedures have since been developed for the Michel and Ellman methods, with a reduction of variability in commercial testing.[6]

Yager et al[12] analyzed the sources of variability in plasma and erythrocyte cholinesterase in a single laboratory. Intraindividual variation of the red cell enzyme activity was 10%, and that of the plasma enzyme activity was 14.4%. Laboratory variation contributed 40% of the variation in red blood cell values and 24% of the variation of plasma values even in this research laboratory setting.

Interindividual Variation

Interindividual variation was also reported in Rider's series. Mean red cell cholinesterase activity was 0.766 ΔpH units (male) and 0.750 (female), with SDs of $\pm$ 0.081 and $\pm$0.082 and ranges of 0.58 to 0.95 and 0.56 to 0.94, respectively. Mean serum cholinesterase activity was 0.95 (male) and 0.750 (female), with SDs of $\pm$0.187 (both) and ranges of 0.52 to 1.39 and 0.38 to 1.25, respectively.[8] Because of the wide range of activity levels among normal subjects, the importance of obtaining preexposure baseline measurements for the surveillance of potentially exposed workers is readily apparent.

When baseline values are available, the decline in activity required to diagnose inhibition with statistical confidence depends upon the number of baseline values. With a single baseline value, a 20% decline in plasma and 15% decline in erythrocyte activity is significant ($P < .05$); with ten preexposure values, a 15% fall in plasma and 11% fall in erythrocyte activity is significant.[10]

Many applicators and most field workers do not have baseline cholinesterase measurements. In the absence of a baseline, the interpretation of reported cholinesterase activity is difficult. Hayes[10] states that plasma cholinesterase levels 30% or more below the laboratory normal range, and erythrocyte levels 20% or more below this range, are required for a diagnosis of cholinesterase inhibition. Unfortunately, the upper limit of normal for a laboratory may be fully 225% of the lower limit value,

and workers suffering substantial declines from their individual baselines may still have cholinesterase levels well within the laboratory normal range. Knaak[15] suggests that knowledge of the distribution of cholinesterase activity within a control population, such as nonexposed workers analyzed at the same laboratory, is more helpful for evaluation of a single postexposure value than the lower limit of that laboratory normal range. For example, a postexposure value lying close to two SDs below the mean—even if 0.10 ΔpH units above the lower limit of normal—could be interpreted as probable inhibition using this approach.[15]

A recent investigation by the National Institute for Occupational Safety and Health (NIOSH) of workers exposed to the organophosphate pesticide diazinon in a mushroom farm in California illustrates the potential difficulties in evaluation of postexposure cholinesterase activity without baseline values for comparison. None of the workers had preexposure baselines. The normal range for the laboratory was 0.55 to 1.25 ΔpH units. Initial red cell activities averaged 0.61 ΔpH units, and only two of the workers would have been diagnosed as cholinesterase-inhibited by Hayes' suggested criteria. Three weeks later, the group's mean RBC values had risen by an average of 20 ΔpH units, or 29% of their original mean; the smallest increase was 0.16 ΔpH units.[14] This suggests that the workers had, in fact, suffered a marked cholinesterase inhibition. Clinical diagnosis was facilitated in this case by the fact that a group of workers had a common history of exposure and symptoms consistent with organophosphate poisoning. When an individual farm worker has a comparable history and symptoms, however, cholinesterase values in the low normal range are routinely used to exclude this diagnosis.

Age, Sex, and Race

The patterns of erythrocyte and plasma cholinesterase activity among persons not exposed to inhibitors have been studied extensively and are reviewed in Witter,[15] Shanor et al,[16] and Hayes.[10] Erythrocyte cholinesterase activity does not change with age in adults. Studies of serum cholinesterase changes with age are contradictory for both sexes, but when reported, the differences are very small. There is no difference in red cell enzyme activity associated with sex, whereas serum cholinesterase activity is significantly higher in males than in females. Reinhold et al[17] reported serum but not red cell cholinesterase activity to be lower in black persons than in white persons of the same sex; it is unknown whether this is the result of genetic or nutritional factors.

Disease

The effects on serum cholinesterase of many diseases which affect hepatic function are well known. Neoplasms, parenchymal liver disease, malnutrition, acute infections, and some anemias all depress serum cholinesterase values. Most of these conditions also prevent the individual from working, however, so they are rarely responsible for depressed cholinesterase in occupationally exposed populations. Certain chemicals such as organic mercury compounds, carbon disulfide, and benzalkonium salts can depress serum cholinesterase activity[10]; carbon disulfide has been used as a fumigant.

Erythrocyte cholinesterase activity, which is measured per unit volume of whole blood, may be decreased in the presence of anemia if the measurement is not corrected for the hematocrit. In addition, Baetjer[18] has documented a significantly greater erythrocyte cholinesterase inhibition in the presence of mild water deprivation and food restriction in rats, suggesting that persons subject to moderate nutritional deficiency and/or water deficit—as is frequently encountered in migrant and seasonal farm workers—may be particularly susceptible to organophosphate and carbamate exposures.

Genetic Variance

A small number of workers exhibit unusual sensitivity to some quaternary ammonium esterase inhibitors; such workers also have reduced serum cholinesterase activity. Four autosomal genes combine to produce seven detectable phenotypes, of which the three most commonly studied are the usual, intermediate, and atypical. The frequency of the atypical is estimated at 1 per 2,820 workers; the incidence of the heterozygote (intermediate) phenotype is approximately 3% among whites and less than 1% among blacks.[19, 20] The atypical enzyme is less reactive with certain inhibitors. One of these, dibucaine hydrochloride, is commonly used to detect the atypical and intermediate phenotypes by their inhibition of cholinesterase activity (16% and 62%, respectively, *v* 79% for the usual phenotype). This genetic variance is not associated with any increased susceptibility to acetylcholinesterase inhibition or to poisoning by organophosphates and carbamates.

There is also a genetic polymorphism in the plasma enzyme paraoxonase, which inactivates both parathion and its active metabolite paraoxon. The distribution of enzyme activity for paraoxonase is bimodal, with approximately equal proportions of the populations tested having either the high-activity or low-activity (ie, one-third to one-sixth the activity of the former) phenotypes. The practical implications for persons occupationally exposed to parathion remain to be studied.[21, 22]

Pregnancy

Pregnancy alters the concentrations of many blood proteins. Evans and Wroe[23] studied the serum cholinesterase activities of 941 women, distributed evenly over 40 weeks of gestation and the first postnatal week, and 119 nonpregnant control subjects who were not using birth control pills. An acute drop in cholinesterase val-

ues was found in the first trimester, with a slight apparent but nonsignificant rise in the third trimester, whereas the lowest values were observed during the second to seventh days postpartum.

In summary, physiological factors are not likely to confound the interpretation of erythrocyte cholinesterase activity levels in healthy workers who have not been exposed to cholinesterase inhibitors. The genetic variation that reduces serum cholinesterase activity in 3% of the population can be identified with the incorporation of dibucaine in the usual laboratory test procedures, and pregnancy is clinically evident by the time that serum cholinesterase activity is significantly affected. Interpretation of cholinesterase activity levels is complicated, however, by issues of intraindividual variation, interindividual variation when baseline values are not available, and the competence of the laboratory performing the test.

NIOSH researchers have investigated the use of an immunological assay for total serum cholinesterase protein to determine "pseudo" baselines against which cholinesterase activity can be compared for workers who lack preexposure baselines. The ratio of cholinesterase activity to total enzyme protein, in in vivo and in vitro testing, was a sensitive method of detecting organophosphate-inhibited samples. Although this method is still experimental, it suggests a possible solution to the problem of evaluating cholinesterase activity in the absence of baseline values in field workers who have low-normal or below-normal range cholinesterase activity, whether in field monitoring or in clinical diagnosis.[24]

Laboratory Methods of Cholinesterase Determination

In this comparative evaluation of laboratory methods for cholinesterase activity measurement, we have been primarily concerned with the characteristics of each method that determine the settings in which it is most useful. Factors of equipment cost and complexity, technical skills required, accuracy, and current usage divide the methods into two general groups: (1) The electrometric (Michel) and colorimetric (Ellman) methods are useful in surveillance and field research within developed countries and for more limited research purposes or for reference laboratories in developing countries. (2) The tintometric and field spectrophotometric methods are useful in surveillance and field research within developing countries and are potentially useful for field worker surveillance in developed countries.

Electrometric Method

Developed by Michel at the Army Chemical Center Biochemistry Section, the electrometric method is still the most widely used for clinical and surveillance purposes, and the most extensive data on human cholinesterase values have been accumulated in studies using this method.[25] Like earlier methods, the Michel method quantifies the acid produced by hydrolysis of the ester acetylcholine, measured as the change in pH per unit time. In the Michel method, the pH change is detected by a glass electrode, the potentiometer. Because cholinesterase activity decreases markedly with a fall in pH over the physiological range of pH 8 to 6, a phosphate barbital buffer, which has a parallel decrease in buffer capacity over the same pH range, is used to maintain a straight-line relationship between the fall in pH and time during the incubation period.

The Michel method is relatively simple, and the equipment (a pH meter and an incubator bath) is available in most clinical laboratories. Results are given in ΔpH units, or the fall in pH produced by 0.1 mL of whole blood in one hour multiplied by 100. A rate curve with multiple data points over the reaction cannot be achieved with this method. Its precision is adequate for field research, although it is not as sensitive as the Ellman method because of the buffered system, and it is not as absolutely accurate as the titrimetric (pH stat) method. The Michel method is less satisfactory for estimation of the effect of carbamate exposure than for that of organophosphates because carbamate-induced inhibition may be largely reversed during the period of incubation with the buffer. A period of one hour is still required for this incubation, although automation has increased the number of samples that may be analyzed at one time.

Approximately 48% of commercial laboratories certified by the state of California for cholinesterase testing use the Michel method. Although Ellman's colorimetric method has replaced the Michel method to a significant extent over the past 10 years, many laboratories continue to use the Michel method because physicians and agricultural employers who contract for surveillance of pesticide applicators are accustomed to evaluating results in Michel units, and because baseline data using the Michel method have already been accumulated on many workers.

Colorimetric Method

In 1961, Ellman published a spectrophotometric method that measured the activity of cholinesterases on acetylthiocholine or butyrylthiocholine as substrates.[26] Upon hydrolysis, thiocholine is liberated and reacts with dithiobis nitrobenzoic acid (DTNB) to release a nitrobenzoate anion, which is yellow and is measured with a spectrophotometer. Unlike the Michel test, the Ellman measures thiocholine rather than the hydrolysis product, but the method is more sensitive than that of Michel, and multiple data points over the course of the reaction can be produced. The heme in erythrocytes can interfere with the spectrophotometric measurement of thiocholine. Although a blood blank is subtracted from the measurement, hemolysis still may present a problem. Under optimal conditions, each sample should provide its own blank. An automated version of the Ellman method has been developed which separates the erythrocytes from the thiocholine prior to the reaction.[13]

The Ellman method is rapid, convenient, and depend-

able for screening and research purposes. The only equipment required is a standard spectrophotometer, present in all clinical laboratories. Reagent kits are available at a cost of approximately $1 per test. Like the Michel method, the Ellman method has been successfully automated, and more than half of the state-certified commercial laboratories in California currently use the Ellman method for cholinesterase determinations.

Titrimetric Method

The titrimetric assay for esterase activity determinations was introduced by Stedman in 1932, used for in vitro agricultural research by Knaak et al,[27] and developed as a readily practicable biological screening method by Nabb and Whitfield at the Centers for Disease Control in the mid-1960s.[28] The acetic acid liberated on hydrolysis of the cholinesterase is titrated by a base, usually sodium hydroxide, whereas the pH is held constant with a potentiometer. Automatic recording titratographs are now used and achieve excellent precision and accuracy. The equipment is moderately complex, and more expensive than that for other laboratory methods, although the procedure itself is relatively simple. Only one sample can be analyzed at a time, and each sample requires five to ten minutes to run; multiple vessels are available to make the method somewhat more efficient.

For research purposes, the substrate and enzyme concentrations can be maintained at levels allowing optimal enzyme activity, or they can be varied independently; a rate curve is produced, and the reaction is followed over a longer time period and with greater accuracy and sensitivity than would be possible by either the Michel or Ellman methods. These differences are generally significant only for laboratory (nonfield) research, however, and for reasons of cost and technical complexity, the Michel and Ellman methods are preferred.

Each of the three methods described above is acceptable for field research, medical surveillance, or clinical diagnosis. Particularly when laboratories perform the test infrequently, quality control may be inadequate for any method used. Problems encountered by commercial laboratories in California have included insufficient substrate, hemolysis of samples before centrifugation, failure to control for temperature, failure to use heparin as the anticoagulant, problems of transport and storage, and problems of reagent preparation and storage. The American College of Pathologists and the Centers for Disease Control, which develop proficiency testing programs for laboratories in the United States, have not yet developed a program for laboratories performing cholinesterase determinations.

Tintometric Method

The tintometric method was first described by Edson in 1958. Fingerstick whole blood samples from exposed subjects and from a control (nonexposed) person are allowed to incubate with acetylcholine and the indicator bromothymol blue. Changes in color, reflecting acid produced by hydrolysis of the acetylcholine, are measured by comparison with colored glass standards. The time required to reach 100% of acetylcholinesterase activity is established with the control sample, and then compared to that for the exposed subject. Values from separate days are not directly comparable.[29]

As a field test for screening workers potentially exposed to cholinesterase inhibitors, the tintometric method has been widely used for many years. It is easily learned, the equipment is inexpensive and completely portable with no base laboratory facilities required, premeasured reagents are available, and 20 tests can be performed by a single operator within approximately two hours. The method has been shown to have good reproducibility and to correlate well with both the Michel and Ellman methods. Miller and Mukhtar[30] recently reported a high degree of correlation between the tintometric method and the Michel method in field trials as well as in measurement of in vitro inhibition of cholinesterase by organophosphates and an oxon analog.

Field Spectrophotometric Method

In the early 1970s, the World Health Organization also sponsored the development of a field method based upon the Ellman spectrophotometric method, using a portable minispectrophotometer and manual microcentrifuge. The method requires a base laboratory equipped with electricity, water, and a refrigerator, but is otherwise relatively simple and can be taught to persons with little technical training. With an assistant to draw the fingerstick blood samples, the operator can perform 40 tests per day.

The method is quite precise, with good reproducibility. The initial cost of the minispectrophotometer is approximately double that of the tintometer, but subsequent reagent costs are low. At present, this method is still not widely used, but has been well tested and shows promise as a more precise field method than the tintometer; in particular, baseline data can be compared with later values for the same subject.[31]

Cholinesterase Activity Among Agricultural Workers and Correlation With Signs and Symptoms of Illness

Cholinesterase determinations have been used since the 1950s to document acute poisonings and patterns of chronic exposure among pesticide applicators. In general, the acute cholinergic effects of severe organophosphate poisoning correlate well with cholinesterase inhibition. Chronic moderate exposure results in a cumulative inhibition of the RBC and plasma enzymes. The appearance of symptoms depends more upon the rate of fall in cholinesterase activity than the absolute level of activity reached. Workers may exhibit 70% to 80% inhibition of both cholinesterase enzymes after several weeks of moderate exposure without manifesting cholin-

ergic symptoms. On the other hand, a previously unexposed individual may develop symptoms after sudden exposure and a rapid drop in cholinesterase activity of less than 30%.

The inhibition of red cell cholinesterase is a better indicator of biological effect than serum cholinesterase, because it is analogous to the enzyme found in nervous system tissues. Most compounds preferentially inhibit either the serum or the red cell enzyme, and exposure to two or more compounds may have a marked synergistic effect. Both the preferential inhibition of cholinesterases and synergistic effects of specific compounds are summarized by Hayes.[10]

Recovery from organophosphate-induced cholinesterase inhibition is much more prolonged than that following carbamate exposure, because phosphorylation results in a chemical bond between the organophosphate compound and the cholinesterase enzyme. Carbamates achieve only a physical blockage of active sites on the enzyme; carbamylation is therefore easily and rapidly reversed, and cannot be detected by periodic cholinesterase monitoring unless the workers are exposed very frequently to substantial amounts of carbamates or the samples are drawn and analyzed immediately after a substantial exposure. For this reason, the following discussion will primarily concern the assessment of organophosphate exposure.

Both plasma and erythrocyte cholinesterase have been used as indices of exposure for assessments of low-level chronic residue exposure among fieldworkers. In the study by Quinones et al[32] of migrant farm workers in New Jersey, the farm workers were found to have significantly lower plasma cholinesterase activity than either Puerto Rican or white control subjects. Levin and Rodnitzky[33] also found that farm workers had significantly lower plasma cholinesterase activity than control subjects. When red cell cholinesterase was used as an index of exposure by Brown et al[34] in a study of vegetable farmers in Ontario, Canada, fluctuations in individual activity levels were found to correspond with seasonal organophosphate applications. Spigiel et al[35] also found that approximately one third of 98 farmers and commercial applicators experienced a significant decline in cholinesterase activity during the course of the application season in Nebraska. It should be noted that the absolute inhibition measured was moderate in all cases, and that the populations studied were continuing to work.

In addition to the acute symptoms of cholinergic excess, constellations of nonspecific CNS symptoms are often seen as sequelae to cases of acute poisoning, and in association with chronic exposures to low levels of organophosphates. When these nonspecific symptoms were investigated in the farm worker studies mentioned above, group correlations were found between the prevalence of symptoms and the moderate levels of cholinesterase inhibition encountered, presumably reflecting the association between inhibition as an index of exposure and this effect. Neither the presence nor the severity of these symptoms are well correlated with cholinesterase inhibition for individuals.[36, 37] This is consistent with the lack of such correlation reported by researchers employing volunteers in laboratory experiments studying ingested doses of organophosphates,[36] and suggests a major limitation in the use of cholinesterase screening to predict the health effects of chronic low-level residue exposure.

Surveillance of Organophosphate-Exposed Agricultural Workers

Nationally and in California, agricultural workers suffer among the highest reported occupational illness rates of all major economic sectors.[38, 39] The California State Department of Health Services has estimated that only 1% of pesticide-related illness among farm workers is reported, despite a strong state regulation requiring physicians to report all cases of suspected pesticide exposure illness to the county health officer within 24 hours of diagnosis.[40] Approximately 250 cases among farm workers are reported to the CDFA each year; this suggests an actual total of 25,000 cases among farm workers in California. Based on conservative Occupational Safety and Health Administration (OSHA) estimates of approximately 1 million farm workers in the highest risk crops and activities, the national total would be an estimated 80,000 cases annually.

Because California is the only state in the nation to mandate medical surveillance for agricultural workers, we describe that program in some detail. The program, established by the CDFA in 1977, requires medical surveillance for all pesticide applicators, mixers, loaders, flaggers, and other workers handling category I organophosphate compounds for 30 hours or more in a 30-day period.[5] (This will soon be changed to require testing of those handling such compounds for four or more days in a 30-day period.) EPA category I pesticides are those of highest toxicity by oral and/or dermal LD_{50}. Medical surveillance should include baseline cholinesterase determinations and subsequent periodic testing at the discretion of the physician providing surveillance.

The baseline must be established when the worker has not been exposed to organophosphates for at least 30 days; a minimum of two preexposure tests should be performed at least three days, but not more than 14 days apart. If these two tests differ by as much as 20%, a third sample must be tested. The baseline is obtained by averaging these tests.

The physician responsible for medical supervision decides how often the cholinesterase tests are to be performed, depending upon the conditions of the workplace, the workers' own attention to hygiene, the toxicity of the pesticides used, and the actual duration of exposure. The department suggests weekly testing if individuals work regularly with compounds labeled "Danger." All evaluations should include both RBC and plasma cholinesterase activity determinations; generally, the RBC value is more important, especially in cases of long-term exposure. After the baseline value is determined, subsequent tests should be performed in the same laboratory using the same method.

If a 30% drop from baseline in plasma or RBC levels is measured, the worker must be retested immediately.

If this fall in activity is confirmed, the employer is directed to investigate the work situation and work practices. If a 40% decline in RBC or 50% decline in plasma activity is measured, the employer should expect the physician to order that all exposures to organophosphates or carbamates for that person be stopped and not resumed until both cholinesterase levels return to the preexposure baseline range. The results of this monitoring must be maintained in the medical records of the supervising physician, but are not collected centrally by the CDFA. Collection and analysis of the results of this surveillance would provide better information on the prevalence of cholinesterase inhibition among pesticide applicators.

Based on our experience in California, and in accord with general principles of occupational health surveillance, we suggest that biological monitoring programs for agricultural workers exposed to organophosphate pesticides should include the following components.

1. Identification of High Risk Populations Requiring Surveillance. Mixers, loaders, applicators, flaggers, and others who directly handle pesticides should be provided with a full surveillance program as outlined here. When a review of local field labor patterns by crop and by periods of pesticides application identifies farm workers at significant risk of residue exposures, studies comparing the cholinesterase activity of these workers with their preseason levels (or with other workers in untreated fields) will allow assessment of actual exposures and of the need for periodic surveillance. The Rural Health and NIOSH units in Region IX of the Public Health Service have produced a county-based model for the development of crop and pesticide profiles in the identification of high-risk field exposures.[41] In addition, reports of individual or crew poisonings should trigger investigations of applicaton or field work activities which have not been previously identified as high risk.

2. Baseline Cholinesterase Determinations. Optimally, both red cell and plasma cholinesterase activity should be determined as a baseline for all high-risk workers, ie, those who will handle EPA category I and possibly some category II organophosphate pesticides. When only one test is performed, red cell cholinesterase activity determinations are preferable. In routine surveillance of field workers' exposures or the surveillance of applicators in developing nations where resources are extremely limited, the whole-blood field methods may be employed.

3. Periodic Surveillance. At least one subsequent cholinesterase activity measurement should be required for all high-risk workers each season, to be drawn at the peak of the application period. Additional tests should be required at the discretion of the supervising physician or responsible agencies. These data should be collected by or referred to the appropriate public health agency for analysis of the prevalence of cholinesterase activity inhibition, and to assess the adequacy of worker protection programs.

4. Criteria for Removal From Work. Criteria for the retesting of workers and for their eventual removal from work should be established. If red cell cholinesterase measurements are required, as recommended above, a decline of greater than 15% from the worker's baseline value may be regarded as significant ($P < .05$), and might be used as the criterion for retesting of cholinesterase activity. Hayes' suggested criteria for removal from work—a 15% decline from the red cell baseline and a 20% decline from the plasma baseline—may be difficult to implement in field situations, but they do reflect the association between moderate cholinesterase inhibition and disabling symptoms more accurately than the current CDFA criteria of 40% and 50% inhibition, respectively. The CDFA is currently revising these criteria in consultation with the California State Department of Health Services and NIOSH.

Workers identified as cholinesterase-inhibited on routine surveillance should not be returned to work involving potential reexposure to organophosphates or carbamates until their cholinesterase activity has returned to the previously established baseline. If a baseline is not available, however, decisions regarding return to work are complicated by the fact that the patterns of recovery for cholinesterase activity are not well understood.

The recovery rates characterized by Grob et al[42] in 1950 and commonly reported in most texts are derived from experimental observation of severe acute inhibition induced in laboratory animals. The more rapid recovery of plasma cholinesterase activity is produced by dissociation of the inhibitor from the enzyme, whereas the recovery of erythrocyte cholinesterase activity depends primarily upon replacement of the erythrocytes themselves (leading to the generally assumed recovery rate of approximately 1% per day). In fact, reticulocytes have much less activity than adult cells, and reach full cholinesterase activity only at 30 days.[43] Recovery rates for both enzymes differ quite substantially with the specific compound to which the experimental animal or human is exposed, and recovery rates in cases of mild or moderate agricultural worker exposure have varied markedly.

In an investigation of 16 cauliflower workers poisoned by mevinphos and phosphamidon in the Salinas Valley in 1980, Midtling et al[44] followed the workers' RBC and plasma values weekly for 126 days after exposure. Because the workers did not have baselines, normal cholinesterase activities were estimated for each subject by averaging the last three values before release to work. Cholinesterase activities regenerated to 95% of this plateau value within an average of 57 days for plasma and 66 days for RBC enzyme activity. Symptoms commonly associated with cholinergic excess (nausea, dizziness, vomiting, abdominal pain, and ataxia) were reported by many patients initially, but abated within 28 days. Symptoms of headache, weakness, and anorexia persisted much longer in many patients; blurred vision was one of the most common initial complaints and persisted throughout the 126 days of the study.

Based on these findings, and a NIOSH investigation of a similar field-poisoning incident,[45] the use of sequential postexposure cholinesterase determinations to confirm cholinesterase inhibition appears to offer a feasible

and useful alternative to reliance on the laboratory normal range in the absence of baseline values and when the clinical presentation does not require the use of atropine, which would confirm the diagnosis more definitively. Plasma cholinesterase should increase 15% to 20% between the initial test at the time of exposure and retesting two to three days later if a significant organophosphate-induced cholinesterase inhibition has occurred. Regeneration of plasma activity is more likely to be seen during this short period because of its more rapid rate of recovery. Further increases on subsequent determinations would confirm the diagnosis.

Affected workers should be kept from work involving exposure to these chemicals until their red cell cholinesterase has regenerated. Erythrocyte rather than plasma values are recommended as the end point because the former better reflect physiological effects on the nervous system. In determining when regeneration has been completed, consideration should be given to the fact that the red cell cholinesterase activity of a healthy person may normally vary by 10% upon retesting. If a value increases by more than 10% over a value drawn ten days previously (using the conservative estimate of 1% per day for red cell cholinesterase recovery), the baseline may not yet have been reached. In most cases, agricultural workers are forced to return to work for economic reasons long before their red cell cholinesterase can be demonstrated to have completed regeneration and very often before their symptoms have completely resolved.

In addition to monitoring worker exposure in routine surveillance programs, cholinesterase determinations are also extensively used to establish reentry intervals, to assess changes in application or field work practices, to evaluate personal protective equipment and field application equipment, to establish exposure limits, and to monitor compliance with crop treatment and reentry schedules.[46, 47]

Acknowledgments

Dr James Knaak, Dr Jon Rosenberg, and Mr Michael Gee provided assistance in the preparation of this manuscript.

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Privileges of the Physician

I have been dean for student affairs at Harvard Medical School for eight years. It is my responsibility and privilege to listen to medical students and to support their personal and professional development. What they have been telling me this year troubles me. It is exemplified by a recent encounter with a third-year student.

The student was distressed. What had sustained him through the preclinical years was the anticipation of learning patient care. Now he had earned the right to wear a white coat and enter the wards. What he had encountered had discouraged him profoundly. The problem was not the work; that was as exciting as he had hoped it would be. It was his interaction with his teachers. Once the formal teaching rounds were over, they talked only about the problems they faced. For some, the talk was about the malpractice crisis, the freeze on Medicare fees, the impact of diagnosis related groups, and shrinking incomes. For others, it was the endless paper work in applying for research funding, the competition for the declining number of grants, and the uncertainties of sustaining a research career. Medicine, they said, was no fun any more. . . .

How absurd! It stands the world on its head to suggest that the liabilities of a career in medicine outweigh the assets. Of course there are major problems in the delivery of medical care, and we ought to be in the vanguard of those seeking solutions to them. But to lose sight of just how lucky we are to have a profession in which we do well for ourselves by doing well for others reflects a puzzling loss of perspective. The satisfaction of being able to relieve pain and restore function, the intellectual challenge of solving clinical problems, and the variety of human issues we confront in daily clinical practice will remain the essence of doctoring, whatever the changes in the organizational and economic structure of medicine.

—From "Sounding Board: It Is Still A Privilege To Be A Doctor" by Carola Eisenberg, MD, in *The New England Journal of Medicine*, April 24, 1986.