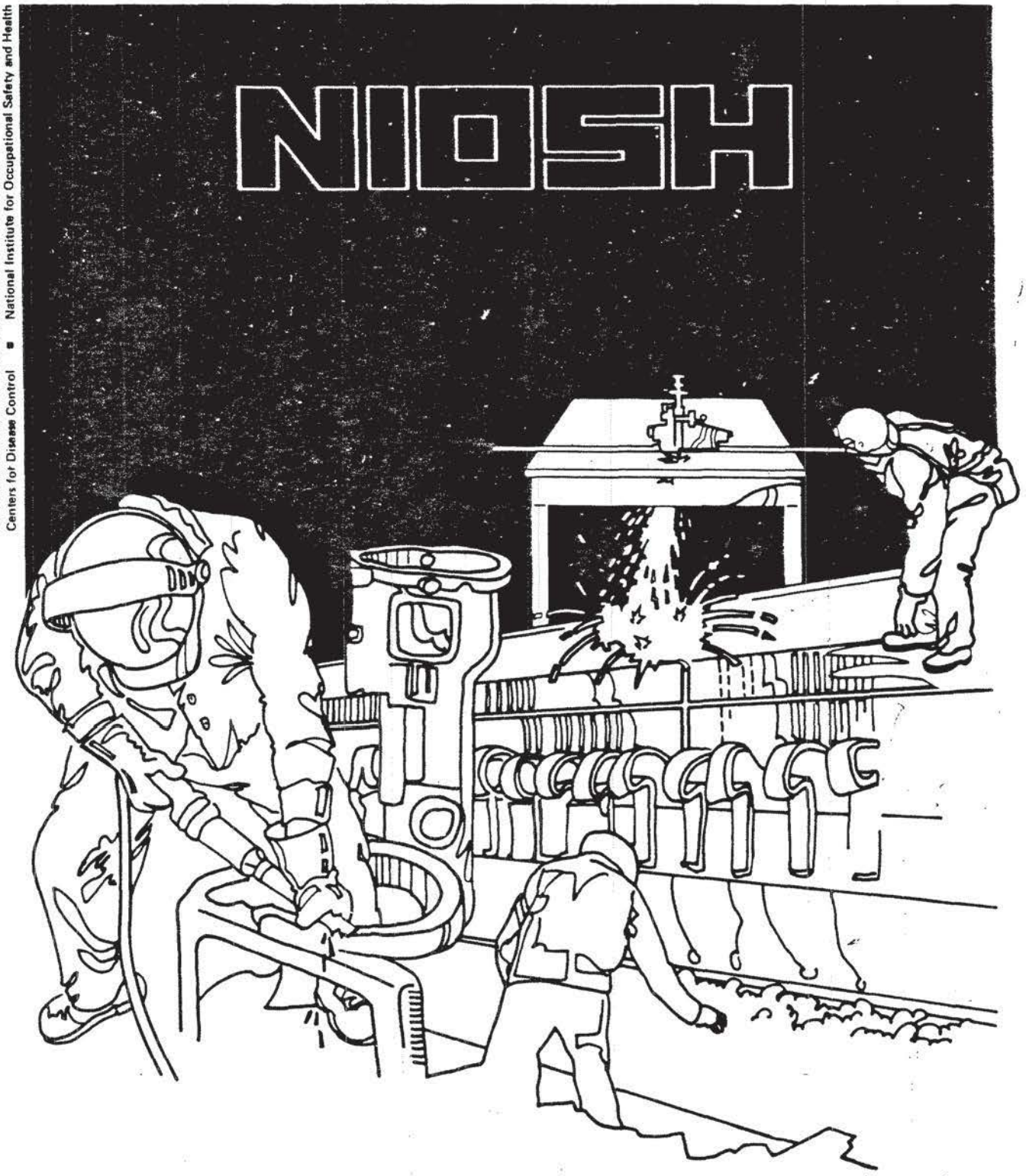
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES ■ Public Health Service
Centers for Disease Control ■ National Institute for Occupational Safety and Health

NIOSH



Health Hazard Evaluation Report

HETA 82-248-1472
PESTICIDE APPLICATORS
FRESNO, CALIFORNIA

PREFACE

The Hazard Evaluations and Technical Assistance Branch of NIOSH conducts field investigations of possible health hazards in the workplace. These investigations are conducted under the authority of Section 20(a)(6) of the Occupational Safety and Health Act of 1970, 29 U.S.C. 669(a)(6) which authorizes the Secretary of Health and Human Services, following a written request from any employer or authorized representative of employees, to determine whether any substance normally found in the place of employment has potentially toxic effects in such concentrations as used or found.

The Hazard Evaluations and Technical Assistance Branch also provides, upon request, medical, nursing, and industrial hygiene technical and consultative assistance (TA) to Federal, state, and local agencies; labor; industry and other groups or individuals to control occupational health hazards and to prevent related trauma and disease.

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I. SUMMARY

On May 2, 1982, the National Institute for Occupational Safety and Health (NIOSH) received a request for technical assistance from the California State Department of Industrial Relations, Division of Occupational Safety and Health in the evaluation of a new method for the estimation of cholinesterase inhibition in pesticide applicators developed at the University of Michigan Department of Pharmacology laboratories.

The purpose of this evaluation was to test whether organophosphate exposure could be quantitated by measuring the ratio of serum cholinesterase (butyrylcholinesterase, BCHE, also referred to as plasma cholinesterase) activity to the quantity of total BCHE protein determined by immunodiffusion. This method would normalize for individual variation in BCHE levels, and provide a means of estimating BCHE inhibition in individuals who do not have a pre-exposure baseline BCHE measurement.

Cooperation was provided by the University of Michigan Department of Pharmacology, a commercial laboratory, and three pesticide application companies in Fresno, California. Sera from healthy volunteers were spiked in vitro with an organophosphate (dichlorvos or paraoxon). Reduction of activity by the organophosphate did not reduce the quantity of BCHE protein, and the ratio of BCHE activity/protein fell in the treated samples.

The ratio was then quantitated in the serum of 37 pesticide applicators (crop dusting crews). Three had low BCHE activity; two also had a low ratio of BCHE activity/protein. These three individuals and four others were reanalyzed two to four weeks later. The two with a low ratio had increased BCHE activity and ratio (presumably representing recovery from exposure-related inhibition), and the one with low activity and ratio was unchanged (probably a constitutively low level of BCHE). The ratio and activity of the four others were unchanged. These results suggest a combination of BCHE activity and immunoassay could quantitate individual exposure to organophosphates even if pre-exposure BCHE activity is not known.

Because fewer applicators than expected manifested a significant cholinesterase inhibition, NIOSH recommended that further testing of this method with sera from severely cholinesterase-inhibited individuals be carried out, together with in vitro analysis of serum samples spiked with a wide range of organophosphates in order to investigate variation of effect. With further validation and development of a commercially feasible technique, this method may offer a practical approach to the laboratory evaluation of organophosphate exposures in workers without pre-exposure baseline cholinesterase levels.

NIOSH evaluated whether organophosphate exposure could be quantitated by measuring the ratio of serum cholinesterase (BCHE) activity to the quantity of BCHE protein determined by immunodiffusion, in vitro testing and in pesticide applicators. Due to the absence of severe cholinesterase inhibition among the applicators, further testing of this method is recommended. The method may offer a means of laboratory confirmation of organophosphate exposures in workers without pre-exposure baseline cholinesterase levels.

KEYWORDS: SIC 0721 (Crop Planting, Cultivation and Protection) - pesticides, cholinesterase, immunodiffusion, dichlorvos and paraoxon.

II. INTRODUCTION AND BACKGROUND

The standard laboratory method for diagnosis of organophosphate pesticide exposure and/or poisoning is measurement of erythrocyte and plasma cholinesterase activity. Initial diagnosis and treatment depends upon the history of exposure, presenting signs and symptoms, and response to trial doses of atropine, rather than awaiting laboratory measurements of cholinesterase activity. As these measurements become available they are used to confirm the diagnosis of organophosphate (or carbamate) exposure to plan subsequent clinical management of the patient, and to determine the appropriate point for return to work in potentially organophosphate-exposed areas.

As an analog of the enzyme found in nevous system tissues, erythrocyte (RBC) cholinesterase is a better indicator of biologic effect than serum cholinesterase. Serum cholinesterase, however, is in general more sensitive to organophosphates, manifesting a more rapid and a greater total depression than RBC cholinesterase. For this reason it is particularly valuable in the initial confirmation of organophosphate exposure. The optimal method of confirming organophosphate exposure is to compare pre-exposure baseline levels for the individual with post-exposure levels, because cholinesterase activity varies significantly among individuals. Many pesticide applicators and most farmworkers do not have baseline cholinesterase measurements. In the absence of baseline levels, post-exposure activity levels are compared with the range of normal activity for that laboratory. This range is quite large: the upper limit of normal may be 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 (1).

For this reason, diagnosis of moderate organophosphate poisoning is often difficult in agricultural populations. This study evaluated the use of a new method for quantifying individual levels of serum cholinesterase, and the derivation of a ratio of cholinesterase activity/total protein, as a means of estimating cholinesterase inhibition more accurately in individuals without established pre-exposure cholinesterase levels.

III. EVALUATION DESIGN AND METHODS

A. Laboratory Methods: Measurement of Serum Cholinesterase Activity and Quantification of Cholinesterase Protein

Serum cholinesterase activity was measured using benzoylcholine as the substrate according to the method of Kalow and Lindsay (2). One unit of activity equals one (1) micromole of benzoylcholine hydrolyzed per minute.

Serum cholinesterase was quantified by immunodiffusion (3), utilizing the method of Eckerson and La Du, which uses immunologic reactivity to measure total enzymatic protein. In quantification of serum cholinesterase (BCHE), purified human BCHE is used to induce immunity in experimental animals (rabbits), antiserum is harvested and immunodiffusion plates prepared. The reactivity of aliquots of the study subject's serum are compared with standardized samples of BCHE. The combined individual and laboratory variation of the combined individual and laboratory variation of the immunodiffusion method is 7.5%.(4) One unit of enzyme protein (1U) is that quantity of purified cholinesterase which hydrolyzes one unit of benzoylcholine.

B. In Vitro Tests

Serum samples were obtained from local healthy volunteers for the in vitro inhibition tests at the University of Michigan Department of Pharmacology laboratories. 0.9 umol paraoxon (diethyl p-nitrophenyl phosphate) or dichlorvos (0,0-dimethyl 0-(2,2-dichlorovinyl) phosphate; DDVP) were incubated with serum for 90 minutes at 25C, for 18 hours at 4C and then frozen at - 20C. Varying levels of inhibition were obtained by mixing treated and control serum after the 90 minute incubation. The samples were randomly assigned code numbers and assayed blindly. The code was not broken until the assays were completed.

C. Pesticide Applicator Tests

A commercial laboratory conducting cholinesterase measurements as part of a medical surveillance program for certified pesticide applicators in Fresno County provided split samples for all applicators employed by three local application companies, after informed consent was obtained from the applicators. The applicators were engaged in aerial crop dusting. The samples were drawn in heparinized tubes and maintained on ice until reaching the commercial laboratory. Aliquots of five (5) cc were then drawn and shipped on ice to the University of Michigan laboratories within 24 hours, where they were frozen at -20C upon arrival. Serum cholinesterase activity and the quantity of cholinesterase protein were measured as indicated in (A) above.

IV. RESULTS AND DISCUSSION

To test whether organophosphate inhibited cholinesterase reacted immunologically, serum of normal healthy volunteers was incubated in vitro with an organophosphate. Paraoxon and DDVP were used as they do not require metabolic activation for cholinesterase inhibition. Serum from two individuals was used, one having a relatively high (#1) and the other (#2) a low level of cholinesterase.

The immunoassay for cholinesterase was able to detect the inhibited cholinesterase. Even when 0.93 of 1.15 U/ml cholinesterase were inhibited 1.21 of 1.35 IU/ml were found. For one unit of inhibited enzyme 0.7 units were found. Since the immunodiffusion assay utilizes cholinesterase activity to mark the extent of reaction, it is not surprising that samples with low activity appear to have slightly less enzyme protein.

The degree of inhibition of samples treated in vitro with paraoxon or DDVP (Fig. 1.) was very well correlated with the ratio of serum cholinesterase activity/enzyme protein measured immunologically (Fig. 2; r=0.981).

After organophosphate treatment these in vitro samples were handled in a manner similar to those of the pesticide applicators. Therefore, it appears to be possible to use the ratio of cholinesterase activity/enzyme protein to estimate the degree of exposure of the latter individuals to a long acting organophosphate insecticide.

Serum from pesticide applicators was sampled on a regular basis and analyzed for cholinesterase activity. We measured both cholinesterase activity, cholinesterase enzyme protein and the ratio of cholinesterase activity/enzyme protein, 32 individuals were studied. Of the 32, seven were sampled twice (39 samples total). None of the individuals were reported as exhibiting clinical signs of exposure.

Using activity level alone, four sample results were unusually low. In contrast, using the ratio of cholinesterase activity/enzyme protein only two samples had a low ratio. One individual who was sampled twice had low activity and low enzyme protein levels on both occasions (Table 1. describes all individuals who were sampled twice). Certainly, the scatter of cholinesterase activity (SD=0.353) is greater than that of the ratio of cholinesterase activity/enzyme protein (SD=0.235) but the average of each is similar, 0.96 U/ml and 0.93 U/IU, respectively. If those individuals are excluded who have low activity then the SD are 0.242 U/ml and 0.123 U/IU, respectively.

The cholinesterase activity and ratio of activity/enzyme protein are presented for those pesticide applicators who were sampled twice. Three different patterns are evident: one in which activity and ratio were of average level on both samplings (top group); another in which activity was low but ratio normal on both occasions (individual #5); in the third group the activity and ratio were low only at the first sampling (botton group).

We have previously reported that the ratio of cholinesterase activity/enzyme protein is a stable characteristic of an individual (Eckerson and La Du, 1983) as seen in groups one and two. We suggest that the top groups of applicators were not exposed to significant levels of insecticide and that MM has a constitutively low level of enzyme. In contrast workers #6 and #7 have a pattern which is characteristic of exposure and recovery .

What should be noted is that individual #5 does not appear to have been exposed, a normal ratio, but by activity alone this individual should have been labelled as exposed. Of more importance is that the ratio of activity/protein should detect individuals who were exposed but still had activity in the "normal" range, but none were found in this small study.

V. CONCLUSIONS

These results show that immunoassay of cholinesterase can detect organophosphate poisoned enzyme. Using the ratio of cholinesterase activity/enzyme protein as an index of organophosphate insecticide exposure appears to be advantageous over activity alone since it more precisely identifies poisoned persons. This would be especially so for individuals whose normal level of activity is unknown.

VI. RECOMMENDATIONS

Further testing of this method with sera from severely cholinesterase-inhibited individuals should be carried out, together with in vitro analysis of serum samples spiked with a wide range of organophosphates in order to investigate variation of effect.

IX. REFERENCES

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Figure I: Immunoassay of Cholinesterase Detects DDVP or Parathion Inhibited Enzyme

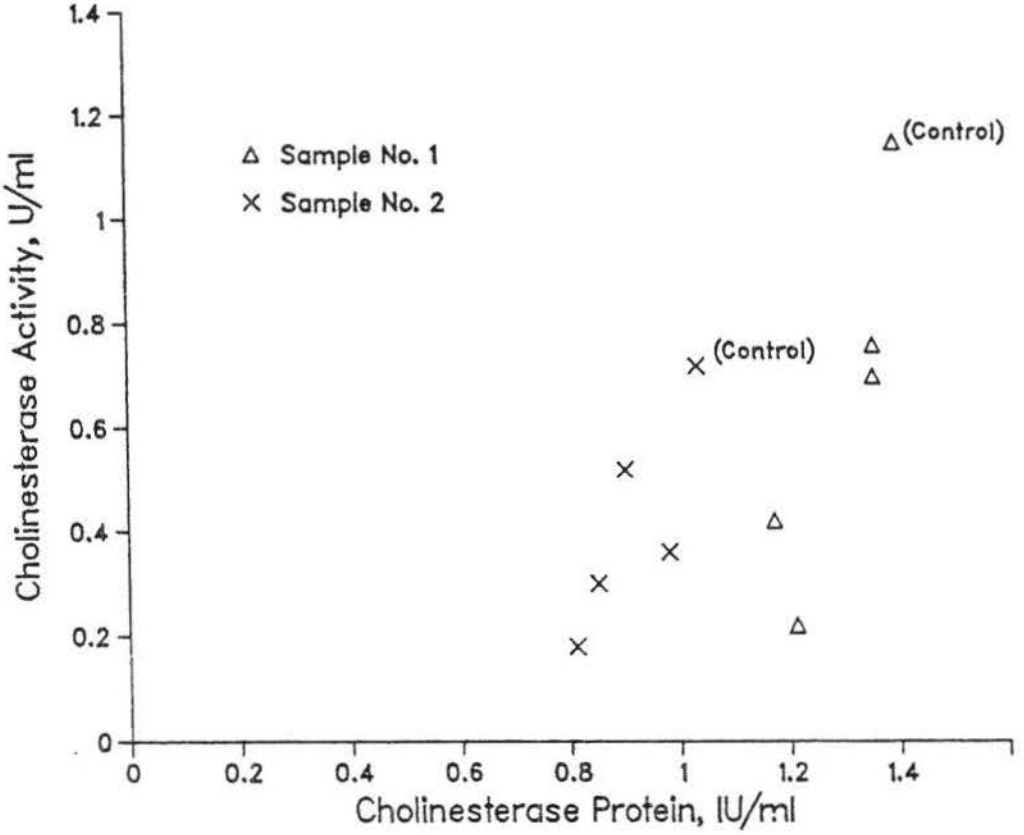

Figure II: Percent Inhibition of Cholinesterase is Well Predicted by the Ratio of Cholinesterase Activity/ Enzyme Protein

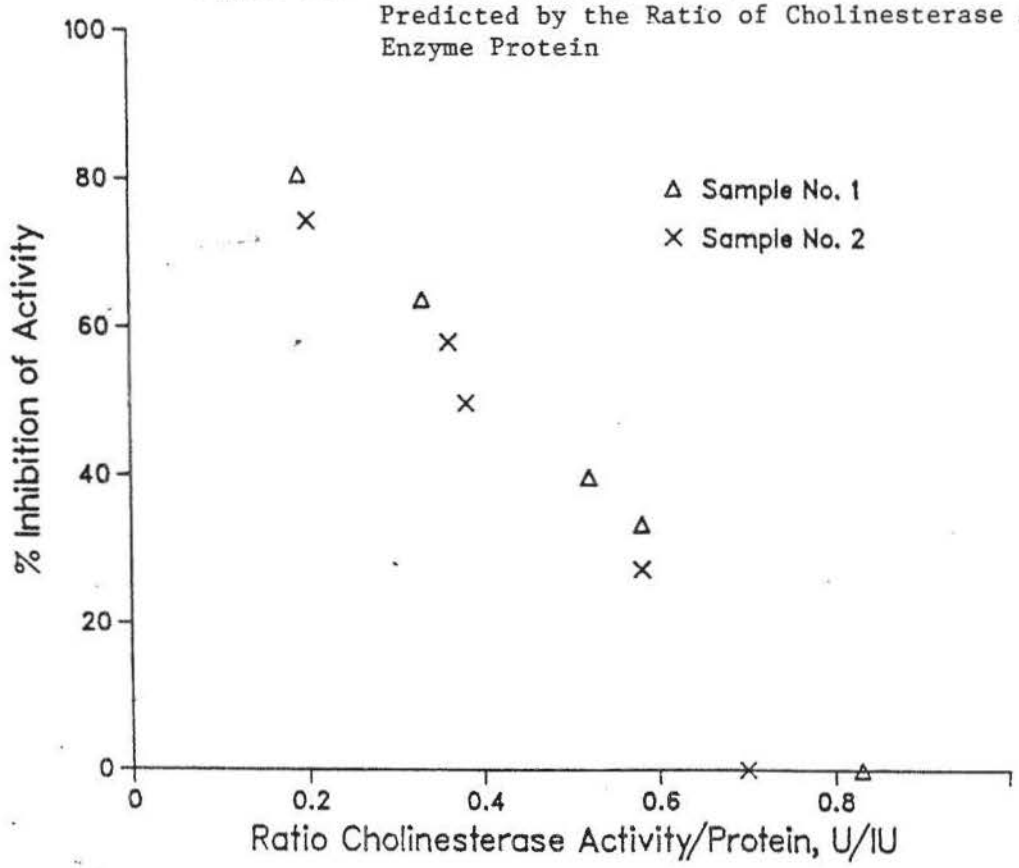

TABLE 1

Measures of OP Exposure with Repeated

Sampling of Pesticide Applicators

Worker (Job)	Date	Cholinesterase Activity U/ml	Ratio U/IU
1	7-8	1.02	0.95
(P)	8-5	1.07	1.09
2	7-14	1.27	1.00
(F)	8-4	1.15	1.04
3	7-14	1.17	0.93
(L)	8-4	0.98	0.93
4	7-14	1.12	1.04
(F)	8-4	1.14	1.09
5	7-8	0.27	0.83
(L/F)	8-5	0.34	0.86
6	7-8	<.01	<.01
(L)	8-5	0.61	0.72
7	7-8	0.12	0.14
(L)	8-5	0.99	0.92

Job Description: P = Pilot, F = Flagman, L = Loade