Factors Affecting Initial Cholinesterase Levels from a Pilot Study of Central Wisconsin Farmers

D. Stueland, A. R. Greenlee

Abstract

Measurement of cholinesterase levels can serve as an indirect estimate of exposure to organophosphate or carbamate insecticides. In addition to individual variability, there are potential predictable confounding factors that may affect the result. In a convenience sample of producers that were being evaluated for general health concerns, we performed a pilot study to evaluate parameters that might affect the group cholinesterase levels. Cholinesterase levels were found to be related to the type of grower, even though all claimed not to be exposed at the time of the tests. The plasma and red cell cholinesterase levels did not vary in the same direction between commodity groups. Plasma cholinesterase levels were higher for males than females, but red cell cholinesterase levels were not affected by gender. The plasma cholinesterase levels correlated positively with several tests of liver function whereas the red cell cholinesterase results were negatively correlated with albumin but positively correlated with age. This study underscores the fact that several parameters systematically affect cholinesterase levels. These parameters do not affect both the red cell and plasma cholinesterase similarly. These factors need to be considered when cholinesterase levels are used to compare groups of potentially exposed persons.

Keywords. Agriculture, Occupational health, Insecticides, Cholinesterase levels.

It is often not possible to measure direct chemical exposure and difficult to measure the indirect effects of chemical exposure. For organophosphate and carbamate insecticides, direct measurement of these compounds is rarely performed. Although the physiologic effect of these insecticides can be measured by neuropsychological tests, the cholinesterase levels are more useful measurements of exposure (Coy et al., 1987). Cholinesterase levels may be affected by individual parameters (Tafurri and Roberts, 1987) such as gender, age or concomitant medical conditions such as pregnancy (Rumenjak, 1998) or metabolism rates (Leng and Lewalter, 1999).

This study was undertaken to evaluate the factors that systematically affect cholinesterase levels. An understanding of these factors can help to make group evaluation of cholinesterase levels more easily understood.

Presented, in part, at the Fourth International Symposium "Rural Health and Safety in a Changing World" held 18-22 October 1998 in Saskatoon, Saskatchewan, Canada.

The authors are Dean Stueland, MD, MPH, Medical Director, National Farm Medicine Center, and Anne R. Greenlee, PhD, Associate Scientist, Marshfield Medical Research Foundation, Marshfield Clinic, Marshfield, Wis. Corresponding author: Dr. Dean Stueland, National Farm Medicine Center, Marshfield Clinic, Marshfield, WI 54449, voice: (715) 387-5497, fax: (715) 387-5240, e-mail: stueland@mfldclin.edu.

Methods

This study was part of a pilot study (Stueland and Ault, 1996; Stueland et al., 1997) undertaken to evaluate the willingness of producers to do brief health screening, including questionnaires and laboratory studies at meetings away from a medical center. The testing was done at previously scheduled commodity meetings. In the spring of 1994, farmers were asked to participate in laboratory studies and answer questions regarding their health and utilization of medical care. Each participant signed a consent form approved by the Institutional Review Board of the Marshfield Medical Research Foundation. The only incentive offered to the participants was the fact that the results were reported back to them personally by mail. In mid summer, after presumed exposure, the producers were re-evaluated (Stueland and Ault, 1996).

Laboratory specimens were obtained by a registered nurse and transported to the clinical laboratory within three hours. For each participant, a whole blood and a serum specimen were obtained. Specimens were chilled during the transportation to the clinical section of Marshfield Laboratories where analysis of the plasma cholinesterase was measured by an automated method (Dupont, 1995) and red cell cholinesterase was measured by manual method (George and Abernathy, 1983). The specimens were handled along with other clinical specimens so the laboratory maintained quality assurance. Results of the cholinesterase levels and other laboratory studies as well as answers to the questionnaire were entered into a computer data base. The data analysis was done using the Number Crunching Statistical System (NCSS) (Hintze, 1982-1992) for simple counts, percentages, and measurements of association. The 0.05 level of significance was adopted.

Results

The initial goal of this study was to have 50 participants in each of four commodity groups. In table 1, the participation by commodity group and gender is shown. Vegetable growers were willing to participate; however, the meeting was poorly attended. Therefore, only 26 vegetables growers participated. Gender varied according to the commodity group: 17 of 59 dairy producers were female and 13 out of 48 ginseng growers were female. In contrast, one of 26 vegetable growers and two of 48 cranberry growers were female.

Laboratory studies such as white blood count, hemoglobin, red cell indices, platelet count, thyroxine, liver function assays were shown to be unrelated to the commodity group. The total red blood cell count was found to be low for seven participants which included five ginseng growers, one cranberry grower, and one dairy producer. The red cell count was elevated for 13 producers which included seven from cranberry growers, four from dairy, and one each from the ginseng and vegetable groups. Chi-square was used to measure for independence of elevated red

Table 1. Participants by commodity type and gender*

Commodity Type	Total Number	N (%)† Males	N (%) Females
Vegetable	26	25 (96.2)	1 (3.8)
Ginseng	48	35 (72.9)	13 (27.1)
Cranberry	48	46 (95.8)	2 (4.2)
Dairy	59	42 (71.2)	17 (28.8)

* Chi-square of association between commodity type and gender = 16.93; p < 0 .01.
† The percent is the percent of row (commodity type).

cell count and group. The chi-square was found to be barely significant (Chi-square Score = 13.64; 3 degrees of freedom; P = 0.03) suggesting that the red cell count was marginally related to commodity group.

The relationship of the plasma and red blood cell (RBC) cholinesterase to gender is shown in table 2. The mean plasma cholinesterase was significantly higher for males than female farmers (17.43 and 14.63 U/mL, respectively; P < 0.01). There was no significant relationship between the red blood cell cholinesterase and gender.

In table 3 the relationship between plasma and red cell cholinesterase levels and commodity group is shown. The plasma cholinesterase levels were highest among cranberry growers (mean = 18.09 U/mL), and lowest among the ginseng growers (mean = 15.83 U/mL). The red cell cholinesterase levels were highest in the dairy farmers (mean = 40.39 U/gm) and lowest in the cranberry growers (mean = 35.52 U/gm).

The correlation (table 4) with plasma cholinesterase values was positive for six out of 11 items. There were no significant negative correlations. Age was not significantly related to plasma cholinesterase. RBC cholinesterase values were shown to be negatively correlated with albumin and positively correlated with LDH and age.

Discussion

There are certain limitations that should be noted in this study. It is a convenience sample in which numerous potential relationships were evaluated. Therefore, the probability of finding spurious, significant relationships is increased. Since it is a convenience study, it is not possible to effectively stratify for variables such as age, gender and exposure which are potentially representative of a larger group. However, in the analysis, only group statistics were used.

Table 2. Relationship of cholinesterase levels to gender

	Male (N = 148) Mean (95% CI)	Female (N = 33) Mean (95% CI)	T*	P
Plasma† cholinesterase (Unit/mL)	17.43 (16.85,18.0)	14.63 (13.56,15.70)	4.20	< 0.01
RBC‡ cholinesterase (Unit/gm Hgb)	38.11 (37.16, 39.06)	38.52 (36.88, 40.15)	0.37	0.71

* Mean and the 95th percent conference interval is shown with T test relationship.
† Normal range of plasma cholinesterase is 8.3 to 22.1 U/mL.
‡ Normal range of RBC cholinesterase is 30 to 53 U/gm hemoglobin.

Table 3. Relationship of cholinesterase level to commodity group*

Commodity Group	Plasma Mean	RBC Mean
Vegetable	17.04	37.58
Ginseng	15.83	38.46
Cranberries	18.09	35.52
Dairy	16.79	40.39
F Ratio	3.31	7.46
P	0.02	< 0.01

* Scheffes Multiple Comparison Test was done (critical value = 2.82) with a plasma mean square error (MSE) = 12.56, indicating a difference between the ginseng and cranberry groups. The red cell MSE was 28.86 with the difference between cranberry and dairy groups.

Table 4. Correlation of selected laboratory parameters to plasma and RBC cholinesterase values

	Plasma Cholinesterase		RBC Cholinesterase	
	r	p	r	p
WBC	0.21	< 0.01	0.09	0.22
RBC	0.52	< 0.01	–0.06	0.40
Hemoglobin	0.52	< 0.01	–0.13	0.07
Hematocrit	0.48	< 0.01	–0.09	0.21
Total protein	0.02	0.75	–0.05	0.47
Albumin	0.38	< 0.01	–0.21	< 0.01
Total bilirubin	0.14	0.06	–0.16	0.03
AST*	0.01	0.84	–0.05	0.53
Alkaline phosphatase	0.19	0.01	0.08	0.85
GGT†	0.33	< 0.01	–0.05	0.55
LDH‡	0.00	0.97	0.15	0.04
AGE	–0.05	0.15	0.27	< 0.01

* Aspartate aminotransferase.
† Gamma glutamyl transferase.
‡ Lactate dehydrogenase.

The fact that the plasma cholinesterase was significantly affected by several laboratory studies has been previously noted (Tafurri and Roberts, 1987), and RBC cholinesterase levels have been used to monitor for subclinical exposure to organophosphate and carbamate insecticides (Karr et al., 1992). However, RBC activity has been shown to be elevated in pregnant women (Rumenjak, 1998). Furthermore, in this study we could not establish whether exposure to insecticides affected the laboratory parameters, although mice exposed to organophosphorus pesticides do develop hepatic injury (Gomes et al., 1999). Therefore, although plasma cholinesterase is a technically easier study to perform and potentially less expensive, its correlation to other parameters can limit its usefulness since a change in other conditions may potentially result in a change in the plasma cholinesterase level. The red cell cholinesterase level, which is expressed as activity per gram of hemoglobin, shows fewer relationships to other laboratory parameters. Although this is a more difficult test, this test is less likely to be affected by other laboratory parameters although it is correlated to age.

This pilot study stresses the problem that in practice, the plasma cholinesterase level, which is easier to perform, is affected by more parameters than the more reliable study (RBC cholinesterase). The clinical dilemma for monitoring is related to this observation plus the fact that the RBC cholinesterase level is more expensive. Both studies show variation by demographic and laboratory parameters. This observation underscores the recommendations to establish baseline for individuals who can be observed over time (Ames et al., 1989).

References

Ames, R. G., S. K. Brown, D. C. Mengle, E. Kahn, J. W. Stratton, and R. J. Jackson. 1989. Cholinesterase depression among California agricultural pesticide applicators. *Am J Ind Med* 15: 143-150.

Coy, M., P. Barnett, J. Midtling et al. 1987. Clinical confirmation of organophosphate poisoning by serial cholinesterase analysis. *Arch Int Med* 147: 438-442.

Dupont. 11/95. ACA Product Labeling for PCHE Method. No. 8-60029-02. Wilmington, Del.

George, P. M., and M. H. Abernathy. 1983. *Clin Chem* 29: 365-368.

Gomes, J., A. H. Dawodu, O. Lloyd, D. M. Revitt, and S. V. Anilal. 1999. Hepatic injury and disturbed amino acid metabolism in mice following prolonged exposure to organophosphorus pesticides. *Human & Experimental Toxicol* 18(1): 33-7.

Hintze, J. L. 1982-1992. Number Crunching Statistical System (NCSS). Kaysville, Utah.

Karr, C., P. Demers et al. 1992. Organophosphate pesticide exposure in a group of Washington State orchard applicators. *Environ Res* 59(1): 229-37.

Leng, G., and J. Lewalter. 1999. Role of individual susceptibility and risk assessment of pesticides. *Occup & Environ Med* 56(7): 449-53.

Rumenjak, V. 1998. Distribution of human erythrocyte acetylcholinesterase according to age, sex and pregnancy. *Acta Medica Croatica* 52(3): 187-189.

Stueland, D. T., and B. J. Ault. 1996. A preliminary analysis of health information obtained from agriculture producers at commodity meetings. *J Agromed* 3(3): 33-45.

Stueland, D. T., B. J. Ault, and P. D. Gundersen. 1997. Change in cholinesterase symptoms and self reported symptoms over two years. *J Agromed* 4(1/2): 151-156.

Tafurri, J., and J. Roberts. 1987. Organophosphate poisoning. *Annals Emergency Med* 16: 193-202.