

Blood Cholinesterases from Washington State Orchard Workers

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Abstract Court-ordered monitoring of blood cholinesterases (ChEs) from orchard workers in Washington State is underway. In 2008, the mean red blood cell acetylcholinesterase (AChE, EC 3.1.1.7) activity was 9.65 ± 1.11 $\mu\text{moles/min/ml}$ ($n = 1,793$) and the mean serum (BChE, 3.1.1.6) activity was 5.19 ± 0.90 $\mu\text{moles/min/ml}$ ($n = 1,811$). Determinations were made using the Ellman assay and automated equipment of Pathology Associates Medical Laboratories (PAML), Spokane, Washington.

Keywords Blood cholinesterases · Pesticide exposures

Inhibition of ChEs in human blood is commonly used to identify possible exposures to many neurochemicals used as pesticides and military agents. Following WW II, the release of classified information about nerve agents and the increased use of neurochemicals in agriculture (Wilson 2001) resulted in setting regulations based largely upon animal studies and ChE activities of human cells such as erythrocytes.

Examples of human ChE data include a large pH blood assay database of the Department of Defense Cholinesterase Reference Laboratory (CRL) at the U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM), compilations of data from blood of agricultural workers (Wiedemann et al. 1995) and a comparison of serum and RBC values from 400 male and 400 female blood donors (Rider et al. 1957). Monitoring of agricultural workers usually is performed with small numbers of samples ordered by physicians under state regulated programs or individual samples taken when exposures to pesticides are suspected. These results are ordinarily not stored in databases accessible for epidemiological analysis.

The findings presented here were obtained from a court ordered monitoring program conducted under the auspices of the Department of Labor and Industries of the State of Washington (Harrington 2002). They represent base line data of blood ChEs from a sample of presumably unexposed orchard workers.

They are presented here to show the distribution of enzyme activity in blood of a large population of orchard workers in Washington State.

Materials and Methods

Informed consent of the participants was obtained. Results are available upon request from the Washington State Department of Labor and Industries. Blood was obtained by venipuncture in Washington by licensed phlebotomists from presumably unexposed pesticide mixers, loaders and applicators enrolled in the monitoring program. The blood was collected in EDTA-containing vacutainers, and shipped cold for analysis by Pathology Associates Medical Laboratories (PAML). There, the temperatures of the

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samples were recorded, and hematocrits obtained. Acetylthiocholine hydrolysis was determined for sera and whole blood using an automated instrument (Olympus) and the Ellman assay (Ellman et al. 1961). Sera and red blood cell activities were calculated and the data stored for further processing using the averages of duplicate measurements. A bovine red blood cell ghost AChE preparation from the Wilson laboratory (Wilson et al. 2005) and a commercial human serum ChE preparation, Precipath (Roche Diagnostics), were included as standards. Separation of the data into categories such as age and gender were not germane to the purposes of the study.

Results and Discussion

The data were from a large sample of agricultural workers. Red blood cell AChE activities averaged 9.65 ± 1.11 ($\mu\text{mol}/\text{min}/\text{ml}$ blood, $n = 1,793$, mean \pm SD) and serum ChE activities averaged 5.19 ± 0.90 ($\mu\text{mol}/\text{min}/\text{ml}$ serum, $n = 1,811$) at 37°C , as represented in the unimodal distributions shown in Figs. 1 and 2.

There have been few studies of ChE levels in blood of large populations. One of the most recent is that of Arrieta et al. (2009), which presents a unimodal distribution of red blood cell AChE from the CHPPM database (determined by pH assays). RBC AChE activities of 991 subjects averaged 8.11 ± 0.92 ($\mu\text{mol}/\text{min}/\text{ml}$ RBC; converted to Ellman units, Arrieta et al. 2004) at 25°C independent of age or gender, $\sim 85\%$ of the mean RBC value from PAML (assayed at 37°C). Another study is that of Rider et al. (1957) in which blood from 400 male and 400 female donors divided into 10 year age groups (12 males and 12 females per group) were assayed using the delta pH method of Michel (1949). They found no significant difference in RBC AChE values due to gender or age.

The data shown here were based on clinical laboratory monitoring rather than on a controlled research study.

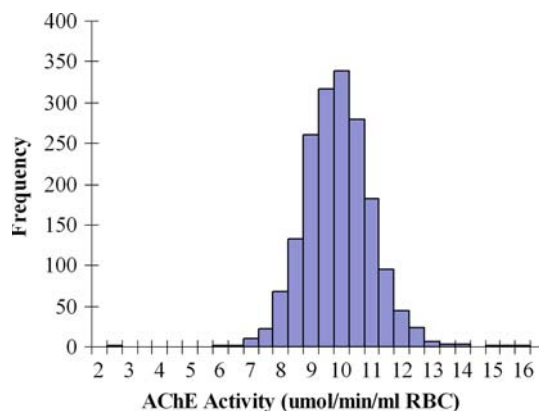


Fig. 1 Distribution of baseline RBC AChE activities in WA orchard workers

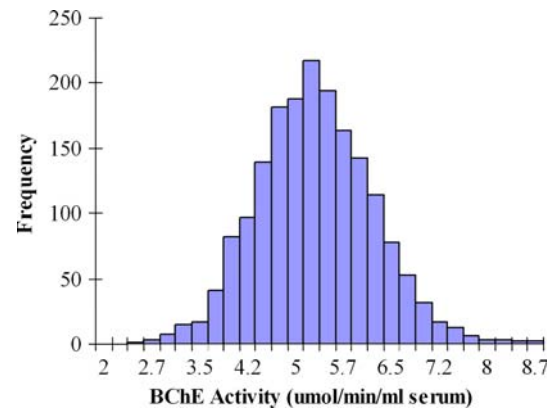


Fig. 2 Distribution of baseline serum ChE activities in WA orchard workers

PAML is to be complimented for taking the time to revise their procedures to meet the needs of this public health project. The next step in this project will focus on the blood ChEs of orchard workers during spray season before and after handling pesticides.

Long before this orchard worker monitoring study was initiated; the state of California chose a decrease from base line of 20% of blood ChE activity to signal investigations of possible exposures to organophosphorus and carbamate pesticides (Title 3, California Code of Regulations, section 6,728 (d), Medical Supervision). Perhaps comparing individual enzyme levels to base line population distributions can be used when individual base lines are not available. Although it is accepted that decreases of ChE activity of 20%–30% suggest a red flag of exposure (Wilson 2001), a solid no-adverse-effect-level is lacking to specify further damage.

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