Acetaminophen Interference in Organophosphate Pesticide Testing

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Background

In Washington State, organophosphate pesticide exposure is monitored by evaluating serum cholinesterase activity in the blood of pesticide handlers. Depressed serum cholinesterase activity of 20% or more below the handlers' baseline requires an investigation of work practices.

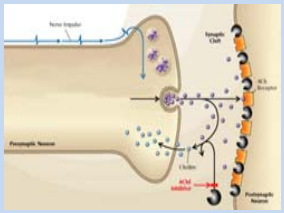

Figure 1:. An AChE inhibitor blocks acetylcholine hydrolysis, and decreases neuron excitability.

Organophosphates inhibit cholinesterases such as acetylcholinesterase, which are located at cholergenic neuron synapses (Figure 1). The organophosphate covalently attaches to the enzyme's active site and extends the enzyme regeneration time from milliseconds to hours. Cholergenic neurons depend heavily on acetylcholinesterase to maintain excitability. Overexposure to organophosphate pesticides causes physiological complications ranging from muscle fasciculation, to muscle paralysis at higher doses [1].

Questions have arisen in the pesticide exposure monitoring program about how food and over-the-counter drugs can affect the results of the test for cholinesterase activity (Figure 2). Serum cholinesterase activity may be indirectly depressed by acetaminophen. Acetaminophen is toxic to the liver and reduces its synthetic function, potentially reducing the amount of serum cholinesterase secreted by the liver. This would lower enzyme activity in the blood [2]. To quantify the amount of decreased cholinesterase activity from regular acetaminophen consumption in this study, healthy human subjects were administered acetaminophen at 75% of the maximum recommended dose over an extended length of time. Serum samples were taken daily, and pseudocholinesterase activity was assayed. Activity of serum aminotransferase enzymes ALT and AST was measured as an indicator of liver health. Furthermore, previous studies indicated an increase in ALT and AST during the a two week course of the maximum recommended acetaminophen dosage [3].

Figure 2: Field test kit for serum cholinesterase activity.

Methods:

Treatment Group: 15 healthy adults in Table 1 were administered acetaminophen at doses of 1g 3X daily, for 6 days. Serum samples from each subject were collected daily. Liver enzyme activity was monitored on Day 0 and Days 4-6 to verify that subjects were within a healthy range of aspartate aminotransferase (AST) and aspartate aminotransferase (ALT). One subject was monitored for Days 0-6.

Table 1: Demographics of human subjects.

	Gender, (%)		Age	Ethnicity, (%)		
	Male	Female	Mean (Range)	Caucasian	Asian	African American
Participants	9(60%)	6(40%)	37(23-51)	11 (73%)	2 (13%)	2 (13%)

DTNB 5-5'-Dithiobis (2-nitro-benzoic acid) is the chromagen utilized to monitor enzyme activity in the Ellman method [4] for pseudocholinesterase (PChE) analysis. When PChE reacts with butylthiolcholine, thiocholine is liberated through hydrolysis. Thiocholine can then react with DTNB (Figure 3), producing a mixed disulphide and TNB (yellow).

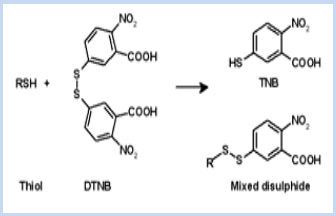

Figure 3: As thiocholine is released from butyrylthiocholine, it reacts with DTNB to produce a mixed disulfide, and TNB. The rate of TNB formation correlates to enzyme activity.

Sample Analysis: Samples were analyzed using a microplate spectrophotometer. The reaction took place in a 96 well microplate. Samples were added to the odd numbered wells, along with DTNB. Enzyme substrate (butyrylthiocholine) was added to the even numbered wells. The odd and even columns were then mixed, and the rate of TNB formation was recorded by the spectrophotometer.

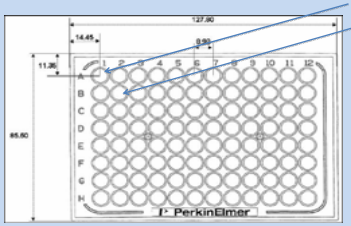

Figure 4: Diagram of 96 well microplate. Prior to analysis with a spectrophotometer, the contents of the odd wells were mixed into the even wells. Each plate was devoted to 1 subject, and each treatment day was randomized and analyzed in quadruplicate.

Figure 5: Enzyme activity was measured on a plate reader spectrophotometer, by monitoring the change in absorbance as DNTB reacted with enzyme products to form TNB.

Table 2: Percent Change in baseline Values for PChE, AST, and ALT

Day	PChE	AST	ALT
0	0%	0%	0%
1	0.06%±6.02%		
2	1.39%±8.08%		
3	4.14%±7.99%		
4	1.16%±8.89%	0.06%±6.02%	1.39%±8.08%
5	2.33%±7.83%	13.13%±26.60%	20.65%±20.86%
6	3.68%±8.20%	28.73%±33.2%	25.37%±29.24%

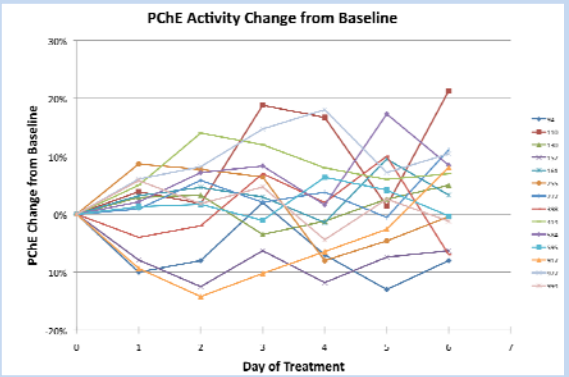

Figure 6: Combined PChE depression values for all 15 subjects.

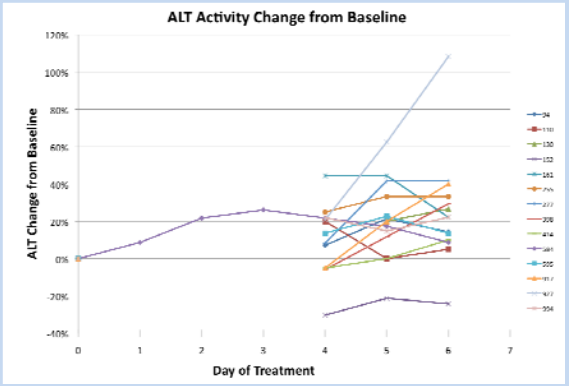

Figure 7: Combined data for change in ALT

Results and Discussion: The mean pseudocholinesterase activity increased from baseline for each day of treatment (Table 2, Figure 6). The majority of subjects displayed an increase in PChE activity. The large diversification in data produced extremely high standard deviations. The mean AST and ALT values showed an increase in activity between day 4 and day 6 (Figure 7).

Conclusions:

- **Taking 75% of the maximum daily dosage of acetaminophen does not decrease the serum pseudocholinesterase activity of healthy adults.**
- **Acetaminophen consumption at 75% the maximum dosage over 6 days does not affect organophosphate exposure testing results.**

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References: **[1]** Wilson, B.W. (2001) Handbook of pesticide toxicology: Agents **[2]** Kuntz, E. (1992) Hepatology: Principles and Practice 103-105. **[3]** Watkins, P.B. et al. (July 5,2006) JAMA. **[4]** Ellman, G.L. et al. (1967) J Biochemical Pharmacology 7:88-95.