Neurological Effects of Organophosphates on Farm Workers

Matthew C. Keifer MD MPH

Occupational Medicine Program, Departments of Medicine and Environmental Health,
University of Washington
Seattle, Washington

Principal Investigator: Matthew C. Keifer MD MPH
Co-investigators: Linda Rosenstock MD MPH
Harvey Checkoway PhD.
Richard Fenske PhD
Lawrence Robinson MD
Kent Anger PhD
Keith Claypoole (substituting for Sarah Raskind)
Larry Engle MS (graduate student)

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Significant findings:

This study demonstrated that no significant differences in neurobehavioral performance or neurological function attributable organophosphate pesticide (OPs) exposure were discernible among farm workers with exposure to OPs during thinning activities in sprayed fruit orchards in Washington State. An objective measure of exposure to OPs (acetylcholinesterase) validated the use of hours of thinning as an estimate of exposure. This estimate was derived from work questionnaires applied to workers. The absence of apparent effect was true whether the analysis was done including an estimate of past exposure or not. Measures of mood and neurological symptoms also did not differ significantly between exposed and unexposed workers.

Another significant finding of this study was the observation that acetylcholinesterase demonstrated a significant inverse relationship with hours worked in thinning orchard work. This represents evidence of biochemical effect from OP exposure in the task of fruit thinning in organophosphate treated orchards.

The Peabody Picture Vocabulary test in Spanish quite consistently predicted the performance on the neurobehavioral tests applied in this study. The Peabody also correlated quite highly ($r^2= 0.97$, $p<0.01$) with educational achievement as reported in years of education. This finding has implications for future studies of neurobehavioral performance among Hispanic farm workers. The Peabody is probably an excellent test for identifying the effect of education on performance on neurobehavioral tests and controlling for the differences in education between different groups of workers.

Usefulness of findings:

Both the peripheral nervous system findings (NCV's) and the neurobehavioral findings represent good news for workers with exposure comparable to that which occurred in the workers studied. This information should be reassuring to workers with similar low level exposure to OPs. Nevertheless, a note of caution is appropriate in generalizing from this study. This study population, recruited largely from orchards whose owners had clear commitment to worker health and safety, may represent a near best case scenario regarding exposure and so the absence of effects on the nervous system should be interpreted with caution. As OPs are known neurotoxins, generalization of the results of this study to all orchard thinning work in sprayed fields would be dangerous. It should not be interpreted to mean that exposure to these chemicals is not dangerous, nor should it be used to reduce the emphasis on preventing overexposure. Nevertheless, the results of this study suggest that low-level, non-intoxicating exposure to OP pesticides does not have a significant impact on the function of the brain or the peripheral nervous system in the studied population as can be measured with the tools used in this study.

Despite the absence of detectable effects on the brain or peripheral nervous system, this study does provide strong evidence that a biochemical effect of OPs is present in the workers studied. The cholinesterase activity levels measured in participants inversely

correlated with the reported hours of thinning during the thinning season. Cholinesterase is the direct target of the action of OPs and its inhibition in insects is generally thought to be the way it exerts it toxic effect. High levels of inhibition of cholinesterase in humans can lead to serious acute illness and even death. The findings in this study suggest that sufficient OPs are entering the body of these workers to have a definite effect on the target blood enzyme. The level of decreased activity identified is small, with the mean change in exposed workers representing less than a 5% decrease from the mean of the unexposed workers. We examined whether living near sprayed fields or exposure to cholinesterase inhibiting pesticides used in the home had an effect on cholinesterase activity. We did not find this to be true. There is some possibility that this finding might be due to something we did not measure in the workers but this is not likely as the most probable source of exposure was working in the sprayed fields.

Because education is one of the most important confounding effects on performance on neurobehavioral tests, the finding that the Peabody test was very good at measuring the effect of education is very important for future research in neurobehavioral effects of toxins. The Peabody is easily and quickly applied and has been translated to and standardized in Spanish. This study has shown that it represents an excellent tool for estimating educational achievement in an objective way.

Abstract:

This study was designed to determine if exposure to OPs in the process of apple thinning had a detectable effect on neurobehavioral performance and neurological function and whether years of historical exposure to orchard thinning showed an effect on the same functions after recovery from any acute effects. A battery consisting of the World Health Organization Core Test Battery, several tests of memory, mood and neurological symptoms, acetylcholinesterase, vibration threshold, and paraoxonase, as well as nerve conduction tests were applied cross- sectionally to 137 farm workers and a group of age, gender and education matched controls. Tests were first performed at the end of the thinning season, when recent exposure would be at its cumulative peak and again after a 6-9 month exposure respite. 90 workers were tested a second time.

Results: No consistent significant effect from exposure as estimated by reported hours of thinning since the beginning of the year nor cumulative years of past thinning could be found on neurobehavioral performance, mood, neurological symptoms nor nerve conduction in either round of testing. The exposure estimate of reported hours of thinning was validated by a significant and strong inverse correlation with acetylcholinesterase activity. The Peabody Picture Vocabulary test showed a consistent correlation with neurobehavioral performance on most tests and showed a very high correlation with reported years of education ($r^2= 0.97$, $p<0.01$) demonstrating its validity as an objective estimate of educational achievement.

Conclusion: The recent and life long exposure to OPs experienced by this cohort of workers did not evidently adversely effect their performance on a broad battery of neurobehavioral tests, tests of mood, sensation and neurological symptoms. Acetylcholinesterase activity, an objective measure of physiological effect of exposure inversely correlated with reported recent exposure. The Peabody Picture Vocabulary test proved to correlate highly with reported years of education and frequently correlated with neurobehavioral performance.

Background:

OPs are used heavily in the northwest for the control of the codling moth, the major pest in apples. The predominant OP employed for the past 30 years for this purpose has been azinphos-methyl, a class 1 toxicity compound. Most OP applications in apples in the Northwest occur during the early summer and are contemporaneous with the fruit thinning processes. Fruit thinning involves reducing the number of apples on a tree to enhance the growth of the remaining fruit. It is done exclusively by hand and is dependent on large number of farm laborers and in most of the west involves principally migrant Hispanic laborers.

OPs have been clearly identified in many studies as a cause of both central and peripheral chronic neurological effects in persons who have sustained a heavy exposure (Keifer 1997, Rosenstock 1991, Steenland 1994, Savage 1988, McConnell 1994, Lotti 1986). It is important to note that nearly all cases of chronic neurological effects attributed to OPs resulted from overexposure which caused acute severe clinical illness. The few well controlled studies of subjects with less severe acute exposures or only chronic low level exposure have not observed these chronic neurological outcomes (Ames 1995, Fiedler 1997, Engel 1998). To date only a single well controlled study has been published which examined the effect of chronic low level exposure to OPs on central nervous system function (Fiedler 1997). This study found no significant difference between life long orchardists and a well matched control group on a broad group of neurobehavioral tests.

The present study examines the effect of exposure to OPs during the task of apple orchard thinning on neurobehavioral and neurophysiological parameters. The study compares post exposure performance of a group of orchard thinners to baseline performance on the same battery of tests. Changes in performance due to learning or changes in testing techniques are controlled for by recruiting and simultaneously testing a matched control for each exposed worker.

The specific aims of this study as stated in the original proposal are:

1. Are there abnormalities in neurological function in female farm workers with chronic low-level OP exposure immediately following an exposure season as compared to a reference group with little or no current and little or no historical OP exposure?

2. Do identified abnormalities show a relationship with estimates of recent past exposure?

3. Do identified abnormalities persist after six months on non exposure and do they show a relationship with estimates of historical exposure?

4. Does paraoxonase and chlorpyrifos oxonase activity levels show a protective effect and thus help predict performance on the neurological battery when compared with historical exposure.

5. Is muscarinic receptor density affected by chronic exposure to op pesticides as estimated by work time in fumigated orchards.

Methods:

Farm workers of both sexes were recruited to participate from several orchards in and around Wenatchee, Washington. Several exposed and control workers were also recruited by word of mouth through previously recruited workers. For each worker recruited from an orchard, a worker was recruited from a non-agricultural workplace and was matched on age, education to within 3 years and gender. Non-agricultural workplaces included garment manufacturing, hotels and restaurants. All workers were recruited and tested during the thinning season in the Wenatchee area April-August and controls and exposed were tested simultaneously. Testing was performed by trained interviewers and testers with previous training in the tests applied. Testers were blinded to the exposure status of the workers. The testing was conducted in a temperature stable building throughout the mid-day and evening whenever workers could be scheduled. The order of the testing was randomly assigned and generally four subjects were tested at the same time.

The test battery included sensory and motor nerve conduction and repetitive stimulation electromyography and neurobehavioral tests including the World Health Organization Neurobehavioral Core Test Battery, the block design from the Weschler Adult Intelligence Revised test, the paired associates memory tests, the Peabody Picture Vocabulary test and the depression scale from the Brief Symptom Inventory, the Q16 neurological symptom questionnaire in Spanish and the Vibratron IIR testing device for vibration threshold (Sensortek Inc., Clifton, N,J). In pretesting of the battery several tests were eliminated due to technical problems with their applications or time constraints. These included the visual evoked potentials, the Lanthony 15 hue desaturated panel, and the Beck depression inventory.

Two tests included in the panel but not originally planned when the study was submitted included the R-R interval testing and the Brief Symptom Inventory depression scale. An additional biological assay, diazinon oxonase, was available by the time the study began so this was added to the blood enzyme activity testing.

A structured interview which asked about demographics, alcohol intake, home pesticide exposure, work history, health history and general social history was applied in the first round. For the second round of testing an icon based work-calendar questionnaire was developed to improve work history acquisition. In a separate but parallel study a comparison between the Icon-Calendar questionnaire and the structured interview based questionnaire was performed. A draft of the preliminary manuscript is attached.

Venous blood was drawn on all willing subjects during the first round of testing. Tests of biochemical effects of exposure included acetylcholinesterase. This testing was done using the EQM Testmate OP. Testing was also done for paraoxonase, chlorpyrifos oxonase and diazinon oxonase on a most participants. White cell buffy coat samples were extracted from phycol sedimented blood and transported to the laboratory in Seattle within 48 hours where they were preserved and frozen at -80 to later be tested for white cell muscarinic receptor density. Testing was done twice, once at the end of the exposure season and once before the beginning of the next.

Differences between the first and second round of testing included the following.
The icon-calendar questionnaire was applied to obtain work histories.
Only finger stick blood sampling was done for cholinesterase testing.
The Peabody Picture Vocabulary test was not applied a second time.
Testing was done before the season began.

In addition to minor modifications in the test battery, several more important changes were made from the original proposal. These included a change of venue for the study and a change in cohort gender profile. Testing was done in the Wenatchee area instead of the Yakima area. The agricultural practices and the workforce in the two areas are quite similar but this change allowed us to take advantage of free testing facilities and an overlapping recruitment process being carried out for another study which examined pesticide exposure in orchard farm workers. The cohort originally planned to be all female was opened to include males and females. This was done to assure sufficient numbers for the study. Changes in the law in Washington on the presence of children in the agricultural workplace which were put in place during the first year of the study reduced the number of females working in thinning and threatened the power of the study.

Due to the fact that most able bodied blue collar workers in the Wenatchee area participate in the cherry harvest, most workers did report some work exposure. As the cherry harvest is short (<2 weeks), and no OPs are used on cherries near the harvest, this work experience which was common even among our "unexposed" controls, was not considered to be significant in terms of pesticide exposure.

Questionnaires were coded where necessary by the investigator. Data were double entered by a professional data entry service. Analysis was carried out with SPSS or SAS statistical packages. Multiple linear regression were used for continuous outcome variables. Logistic regression was used for some binomial variables and non-parametric chi square was used to evaluate ordinal variables and the Fischer exact test was applied dichotomous variables. $P<0.05$ two tailed testing was employed for significance.

Results:

137 workers were tested on round one, 90 workers were tested on round two and 87 had complete enough exams to be included in the analysis for year 2. All exposed workers and controls recruited were primarily Spanish speaking. No significant seasons of total

thinning, age, education as measured by the Peabody or percent female were evident between testing rounds. The percent of workers who had participated in some form of agricultural work in 94 did decrease as a percentage of workers tested in year 2. The total years of farm work by those who had worked in farm work in 1994 also increased slightly in year 2.

Table 2 presents the coefficients for effect in multilinear regression for the variables included in the equation. The tests listed in Table 2 represent those which a priori were thought unlikely to be influenced significantly by education. The base model therefore does not include the Peabody Picture Vocabulary score. This table demonstrates that the effect of the previous season of thinning, is significant in predicting performance in only one test of those applied. This test, the Santa Ana pegboard (dominant hand) is a measure of rapidity of motor skills. The direction of the effect was in the predicted direction with an inverse correlation between seasons reported and performance on the test. This isolated effect is difficult to interpret given the absence of an effect on several other tests of motor function. The effect of the reported number of hours of thinning in 1994, a variable with clear effects on acetylcholinesterase activity, never reached a level of statistical significance in any of the other tests examined.

Table 3 includes the coefficients for exposure variables and confounders on those tests which a priori were thought likely to be influenced by education. The Peabody is therefore included in the model testing these results. In this table the effect of recent thinning is predictive of performance on none of the tests. Past seasons of thinning was predictive on two tests, Trails A and Digit span backward. The absence of influence of these variables on the vast majority of tests performed is apparent. The strong predictive value of the Peabody Picture vocabulary is clear in the majority of tests presented. Notably the Peabody score does not show significant predictive influence on changes (year 2- year 1) in performance on all but one of the tests.

The influence of paraoxonase, chlorpyrifos oxonase and diazinon oxonase on performance of all the above tests was examined by adding these variables to the model separately and in groups. There was no significant change in the equation's predictive power when any or all of these variables were added. Notably, these levels specifically did not influence in any significant way the predictive power of the equation for acetylcholinesterase activity. These variables were thus dropped from the model for subsequent analysis.

Analysis of dichotomous and ordinal depression and neurological symptoms indicated that no significant effect of recent exposure in thinning was evident for subjects with thinning exposure greater than 2 weeks as compared to subjects with less than two weeks.

The effects of exposure on nerve conduction and repetitive stimulation electromyography are described in the attached article (Engel et al. 1997). The relationship between exposure and R-R interval testing is presently being evaluated as a thesis project by Dr David Suchard a fellow in the Occupational Medicine Program at the University of Washington. Repeat testing of the peripheral nervous system by nerve conduction

velocities and repetitive stimulation electromyography are presently being analyzed by Larry Engel MS a graduate student in the department of Epidemiology. The results of these evaluations are pending at the time of the writing of this report.

The samples collected for muscarinic receptor density were never analyzed. The Costa laboratory, which was planning on performing this analysis lost funding for such support and could not perform the analysis as planned. Some 100 samples remain frozen at -80 and a laboratory in the U.K. has been contacted who may be able to perform the procedure. Further options for analyzing these samples will be explored.

Discussion:

Due to the difficulty of obtaining sufficient female farm workers for the study we recruited both female and male farm workers for this study. The study examined the effect of a season of low -level exposure to OPs on neurobehavioral and neurophysiological performance among a controlled cohort of orchard thinners. We used the worker's hours of reported thinning since January '94 as our primary exposure variable. This measure of exposure was strongly predictive of an objective, well established biological marker for organophosphate pesticide exposure, AChE. We also measured educational level with a previously validated achievement test, the Peabody Picture Vocabulary to control for the effects of education. This test correlated very highly with reported years of education suggesting that it was a very valid estimate of education. The Peabody was, as expected, strongly predictive of most of the neurobehavioral tests which would be thought to be influenced by education.

Using Peabody Picture Vocabulary score, age, gender and hemoglobin as a control for nutritional status as control variables, tests of neurobehavioral and neurological performance were examined against previous and recent exposure. No evidence of a consistent influence of recent or distant past exposure could be found in performance on these tests. When change in performance was examined between baseline testing and peak exposure testing, no consistent significant influence was discernible. The addition of variables which would potentially be protective of the effects of pesticide exposure, paraoxonase, chlorpyrifos oxonase and diazinon oxonase did not change the predictive value of the equations.

The ordinal scores from the depression scale from the BSI and the dichotomous answers to the neurological symptoms from the Q16 were examined for their relationship to exposure and none of the symptom questions asked showed a significant relationship. Given the number of tests applied to this cohort, chance alone would predict a finding of significance on one or two of these questions but none was seen.

The predictive value of the Peabody for reported education and its clear predictive value for neurobehavioral tests helps validate it as an estimate of education. This triangulation of correlation between the Peabody test score, years of education and neurobehavioral performance also helps to support the validity of the results of the neurobehavioral tests

performed. Additionally, the Peabody score was not very predictive of changes in performance except on a single test. The too would support the validity of the Peabody and the testing. A persons past educational level would not be expected to have a significant influence on changes on a test taken twice over 6 months.

Some limitations of the quality of testing were undoubtedly introduced by the fact that subjects were often tested after working hours when fatigue might have been an important factor. The likelihood is that this would have most influenced the performance of the farm workers who tended to most often come in after work and who generally reported working 6-7 day weeks. This does not appear to have influenced the outcome of the study as no significant effect is apparent from thinning.

The loss to follow-up of 47 participants may have had a significant negative impact on the power of this study to detect a difference between years 1 and 2. There were definite changes in the number of workers who had performed recent farm work in 1994 between testing sessions. If this did have an influence the effect would be apparent in the changes in testing between round 1 and round 2. As the influence of thinning was not pronounced even in year 1, the loss to follow-up would not likely influence the final results of this study as they stand now.

We have no independent objective manner for validating the information we received from workers regarding their previous work experience. A study comparing the first round work history questionnaire to the second round work history questionnaire, the Icon-calendar questionnaire showed that the information provided on the first questionnaire was of limited precision and probably underestimated the total seasons of farm work (manuscript attached). The imprecision may have biased results toward the no effect direction.

The relative rarity of positive associations between recent and past thinning exposure and performance on neurobehavioral tests given the number of statistical tests that were applied to this data supports the conclusion that there was very little effect of exposure on the performance of this cohort. While it is possible that the tests were insensitive to the subtle effects of OP exposure on the central nervous system, the symptom reporting in this case probably would have detected at least some subjective influence.

Conclusion:

No consistent significant effect of a season of orchard thinning in organophosphate sprayed orchards on neurobehavioral or neurophysiological parameters was found. No effect of recent exposure as judged by orchard work for more than two weeks in the past year was detectable on mood or neurological symptoms. Past history of thinning in agriculture as estimated by seasons reported also did not show an effect on neurobehavioral or neurophysiological performance. Acetylcholinesterase activity was noted to be significantly inversely correlated with recent thinning activities suggesting a distinct biochemical effect of exposure to OPs.

An analysis of the peripheral nervous system data from the exposure round of testing has been completed and published (Engel et al 1998). No significant differences were noted between workers acutely exposed or those with a history of chronic exposure (as evidenced by a history of farm work) and unexposed, age, education and gender matched controls. Neither motor nerve conduction, sensory nerve conduction nor repetitive stimulation electromyography showed a significant difference.

The Peabody Picture vocabulary correlated very well with reported years of education and was a strong predictor of performance on many of the neurobehavioral tests applied.

Specific Aims addressed individually:

1. Are there abnormalities in neurological function in female farm workers with chronic low-level OP exposure immediately following an exposure season as compared to a reference group with little or no current and little or no historical OP exposure?

Because of the difficulty recruiting female farm workers for the study we recruited a balanced gender population to participate in the study. Only a single neurobehavioral test and one symptom demonstrated a statistically significant association with exposure as estimated by hours of thinning in the recent season. These association may have occurred by chance given the number of tests applied.

2. Do identified abnormalities show a relationship with estimates of recent past exposure?

No consistent relationship was identified between past seasons of thinning and neurobehavioral, neurophysiological function or measures of mood or neurological symptoms.

3. Do identified abnormalities persist after six months on non exposure and do they show a relationship with estimates of historical exposure?

Changes in performance between year one and year two were examined using new variable created by simply subtracting one year's score from another year's score. No effect was apparent on initial testing and no new effect of exposure was found across the six month respite from exposure. Previously tested exposure variables demonstrated no consistent relationship with changes in performance across the six month respite.

4. Do paraoxonase and chlorpyrifos oxonase activity levels show a protective effect and thus help predict performance on the neurological battery when compared with historical exposure.

These two enzymes and an added enzyme, diazinon oxonase, did not show a significant effect on neurobehavioral, or biochemical effects of exposure (AChE activity).

5. Is muscarinic receptor density affected by chronic exposure to OP pesticides as estimated by work time in fumigated orchards.

This test has not yet been performed. The receptor density has not yet been tested because the laboratory which offered to do the test at no cost lost funding and could not perform the analysis. The samples are still viable and a capable laboratory will be sought to do the assay.

Journal articles:

Neurophysiological Function of Farm Workers Exposed to Organophosphate Pesticides. L. Engel, M. Keifer, Checkoway H, L. Robinson, T Vaughan. Archives of Environmental Health 53(1):7-13, 1998.

Planned publications:

M. Keifer, M. Engel L. Fenske R, Checkoway H. Neurobehavioral effects of organophosphate pesticide exposure among apple thinners in Washington State.

Engel L, Keifer M, Vaughan T, Robinson L, Checkoway H. Neurological effects of Organophosphate Pesticide Exposure among Apple Thinners in Washington State: a six month follow-up.

Keifer M, Engel L, A Comparison of a traditional questionnaire with an Icon/calendar based questionnaire to assess occupational histories.

Keifer K, Engel L, Thompson M, Zahm S. A Test-Retest evaluation of an Icon-calendar work history questionnaire

Zahm S, Keifer M, Engel L. Primary - Surrogate work history comparison using an Icon-Calendar work history questionnaire.

Keifer M, Claypoole K, Engel L. The Peabody Picture Vocabulary accurately estimates educational level in Migrant and Seasonal Farm Workers.

Suchard D, Keifer M, Engel L, Pfeifer M. R-R interval testing as a tool for physiological effect of exposure to Organophosphate pesticides.

Keifer M. Muscarinic receptor density and Organophosphate exposure.

Thesis:

Engel L: Neurophysiological function among farm workers exposed to organophosphate pesticides. Thesis, University of Washington 1996.

Proceedings:

Keifer M. Icon Questionnaire vs. Traditional Questionnaire: A comparison of data obtained and work data entry methods. National Cancer Institute Workgroup on Farmworker Epidemiology, San Antonio, TX. February 24-25, 1998.

Engel L*, Keifer M, Checkoway H, Vaughn T. Neurological effects of chronic organophosphate exposure in orchard workers. (poster) American Public Health Association Meeting Nov 17-21, 1997. New York City.

M. Keifer, L. Engel, R. Fenske, Neurobehavioral effects of organophosphate pesticide exposure among apple thinners in Washington State. (poster) American Public Health Association Meeting Nov 17-21, 1998, Washington D.C.

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Savage E P. Keefe T J. Mounce L M. Heaton R K. Lewis J A. Burcar PJ. Chronic neurological sequelae of acute organophosphate pesticide poisoning. Arch-Environ-Health. 1988 Jan-Feb. 43(1). P 38-45.

Steenland K, Jenkins B, Ames R G, O'Malley M, Chrislip D and Russo J. Chronic neurological sequelae to organophosphate pesticide poisoning. Am J Public Health 1994;84(5):731-736.

Table 1. Demographics and farm work of workers in round 1 and round 2 testing

Characteristic	round 1	round 2
mean age	28	28
% female	45	42
% Spanish speaking	99	100
% farm workers in 94	88	60
% ever did farm work	89	90
Peabody standard grade score	6.07	6.02
Yrs farm work (those who worked in '94)	2.26 (SD 5.40)	2.73 (SD 6.7)
number participating	137	90

Table 2. Coefficients for exposure and confounders for first year results and changes in results from first to second year (Δ values). Tests where previous education unlikely to affect outcome.

Test	Gender	Hgb	Age	Thinning hours '94	Pre 1994 thinning (seasons)	p-value for model
Vibratron hand	-0.08	0.13	0.22	-0.07	-0.06	0.58
Vibratron foot	0.04	-0.08	0.22	0.07	-0.05	0.53
Santa Ana dominant	0.04	0.07	-0.21	-0.21	-0.07	0.23
Santa Ana non-dominant	0.00	0.70	-0.10	-.025	-0.21	0.23
Δ Vibration hand	-0.06	0.08	0.09	0.21	0.05	0.67
Δ Vibration foot	-0.06	0.14	0.06	0.27	0.08	0.17
Δ Santa Ana dominant	0.14	0.11	0.03	-0.16	0.16	0.31
Δ Santa Ana non dominant	**0.48**	-0.19	-0.01	0.09	**-0.26**	**0.02**
Δ Reaction time	0.03	0.08	-0.03	-0.00	0.11	0.88
Acetylcholinesterase yr 1	**0.20**	**0.57**	NA	**-0.18**	NA	**0.00**

Model includes; gender, age, hemoglobin, thinning hours '94 and seasons of thinning.
Bold indicates significant $p<0.05$.
"Δ" calculated delta score: score year 2- score year 1.

Table 3. Coefficients for exposure variables and confounders for outcomes of first year results and changes in results from first to second year (Δ values). Tests where previous education probably affects outcome.

Test	Gender	Hgb	Age	Peabody Score	Thinning hours in 94	Pre 1994 thinning (seasons)	p-value for model
Trails A	0.05	-0.08	0.16	**-0.59**	0.11	**-0.26**	<0.00
Symbol digit	0.28	0.07	-0.11	**0.64**	-0.10	-0.18	<0.00
Reaction time	-0.32	0.08	-.09	**-0.32**	-0.18	-0.08	0.05
Pursuit aiming (good)	0.09	0.18	-0.53	**0.48**	-0.16	0.22	**0.00**
Paired associations tough	-0.32	**0.43**	0.19	0.18	0.08	-0.14	0.22
Paired association easy	-0.26	0.34	0.13	**0.28**	0.21	-0.21	0.08
Digit span forward	**-0.49**	**0.41**	0.11	0.18	-0.11	-0.13	**0.04**
Digit span backward	-0.20	-0.01	0.02	**0.57**	-0.07	0.24	**0.01**
Block design	-0.01	0.09	0.11	**0.54**	-0.01	-0.02	**0.01**
Benton	-0.29	0.31	0.01	**0.46**	-0.06	-0.11	**0.00**
Δ Trails	0.07	-0.09	-0.11	**0.42**	0.23	0.12	0.06
Δ Symbol digit forward	0.15	-0.82	**-0.44**	-0.00	-0.15	0.10	0.09
Δ Pursuit aiming (good)	0.24	-0.19	0.04	**-0.34**	0.04	0.27	0.19
Δ Paired associations tough	-0.13	-1.01	-0.05	-0.18	0.12	0.06	0.62
Δ Paired association easy	-0.00	-0.17	-0.12	-0.10	0.00	0.23	0.52
Δ Digit span forward	0.05	-0.04	-0.16	0.01	-0.06	0.18	0.93
Δ Digit span backward	-0.08	0.17	0.18	0.03	0.17	**-0.35**	0.38
Δ Block design	-0.01	-0.03	-0.03	-0.12	0.08	-0.01	0.97
Δ Benton	0.20	-0.22	-1.14	-0.15	0.01	0.10	0.76

Model includes; gender, age, hemoglobin, Peabody Picture Vocabulary score, thinning hours '94 and seasons of thinning.
Bold indicates significant $p<0.05$.
"Δ" calculated delta score: score year 2- score year 1.