ORIGINAL ARTICLE

Blood acetylcholinesterase and butyrylcholinesterase as biomarkers of cholinesterase depression among pesticide handlers

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▶ Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/oemed-2014-102315).

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Received 8 May 2014
Revised 12 August 2014
Accepted 20 August 2014
Published Online First 4 September 2014

ABSTRACT

Objective Agricultural pesticide handlers are at an elevated risk for overexposure to organophosphate (OP) pesticides, but symptoms can be difficult to recognise, making biomarkers invaluable for diagnosis. Occupational monitoring programmes for cholinesterase depression generally rely on measuring activity of either of the two common blood cholinesterases which serve as proxy measurements for nervous-system acetylcholinesterase activity: red blood cell acetylcholinesterase (AChE) and plasma butyrylcholinesterase (BChE). These biomarkers, however, may be affected differentially by some OPs and the relationship between them has not been well characterised. We aim to determine the association between blood AChE and BChE activity levels and assess whether they produce comparable classifications of clinical cholinesterase depression among OP pesticide handlers.

Methods Using blood samples from 215 participants of the Washington State Cholinesterase Monitoring Program, we quantified changes in AChE and BChE activity from before and after exposure to OP pesticides and calculated Pearson correlation statistics for correlation of AChE and BChE changes in activity, as well as weighted κ statistics for agreement of classification of clinical cholinesterase depression based on AChE versus BChE measurements.

Results AChE and BChE activity measurements are weakly negatively correlated in our study population. Reaching a clinical threshold for diagnosis of cholinesterase depression based on the AChE marker did not correlate with reaching clinical depression based on the BChE marker.

Conclusions Both AChE and BChE should be measured in monitoring programmes because they may both give potentially important but disparate classifications of clinical cholinesterase depression.

What this paper adds

- Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activity in blood are commonly measured for assessing cholinesterase depression.
- Changes in AChE and BChE activity are only weakly correlated.
- Cholinesterase monitoring programmes should measure both AChE and BChE, as the two biomarkers can give disparate but potentially important classifications of clinical cholinesterase depression.

INTRODUCTION

Organophosphates (OPs) are among the most widely used pesticides in the USA. OPs act by inhibiting acetylcholinesterase (AChE), an enzyme that is essential for the proper functioning of the nervous system[1] as it hydrolyses the neurotransmitter acetylcholine in the nerve synapse.[2] On irreversible inhibition by an OP, AChE can no longer bind acetylcholine to terminate synaptic transmission. Inhibition of AChE in humans can cause many acute symptoms including dizziness, nausea, difficulty breathing and even death. The presence and severity of these symptoms depend in part on the degree of AChE depression, although symptoms are not always present in AChE-depressed individuals.

Occupational OP pesticide handlers are at an elevated risk for OP intoxication due to the potential for high exposure to these chemicals through skin contact, inhalation or accidental ingestion. Mixing or applying these pesticides can result in potentially harmful levels of exposure through one or more high exposure events or through chronic lower level exposure.[3] Owing to this risk, California and Washington have instituted longitudinal cholinesterase monitoring programmes for OP handlers in order to identify and treat instances of OP intoxication early on.[4 5]

Outward symptoms of OP intoxication can be difficult to recognise, so it is important to have a way of quantifying an individual's cholinesterase depression through objective measurement. In order to determine whether a case of OP intoxication has occurred, a measurement of relative change in cholinesterase activity is obtained by comparing a baseline, pre-exposure cholinesterase activity measurement with another measurement taken after the individual has been OP-exposed. A baseline measurement is necessary for comparison with the exposed measurement due to the substantial variability in baseline cholinesterase levels in the general population. From this relative measure it is determined if an individual has crossed a threshold into clinical cholinesterase depression, often defined as a 20% decrease in activity from baseline, requiring behavioural or medical intervention. Recently developed methods allow assessment of an individual's degree of cholinesterase inhibition without baseline biomarker measurement,[6]

To cite: Strelitz J, Engel LS, Keifer MC. *Occup Environ Med* 2014;**71**:842–847.

but use of a baseline measurement remains much more common.

There are two biomarkers that are commonly used to determine the extent of an individual's cholinesterase depression.[7] AChE activity from whole blood erythrocytes can be measured as a proxy for the cholinergic AChE that is found in the nervous system, as can BChE, which is measured in plasma. Both blood AChE and BChE inhibition are used as proxy measurements for cholinergic AChE inhibition.

In epidemiological research on cholinesterase depression, BChE is the more commonly used biomarker although this form of cholinesterase is not known to be directly involved in neuronal cholinergic processes. One reason for this may be that studies on the reliability of these biomarkers have found that assays for BChE tend to produce measurements that have greater correlation within and between laboratories, compared with measurement of AChE, meaning the BChE measurement has greater reproducibility.[8–10] For example, in the Washington State Cholinesterase Monitoring Program, within-laboratory coefficients of variation were around 2.5% for BChE measurements but were 16.7% for AChE measurements.[7] Since the goal of measuring cholinesterase in blood is to obtain a proxy measurement of cholinergic AChE activity in the central nervous system, which cannot be readily measured, questions remain as to whether red blood cell AChE or plasma BChE is the more appropriate marker and under what conditions.[11]

There is evidence supporting and against use of either biomarker. AChE measurement, although it has somewhat poorer reproducibility of measurements, is considered by some to be a more appropriate proxy for cholinergic AChE than is BChE due to the biological similarity of the enzymes.[11] Although BChE is more reliably measurable due to lower variability of the assay, the relevance of this enzyme as a proxy for cholinergic AChE is still debated[11]; BChE has been shown to be a significant predictor of symptoms of OP poisoning, but does not show a strong dose–response relationship[12] and is not inhibited by all OPs in the same way as AChE.[13] For these reasons, in the literature to date it is still unclear which biomarker is most appropriate for assessing OP intoxication.

In this study we have evaluated the relationship between AChE and BChE as biomarkers for cholinesterase depression from OP pesticides, and assessed whether these measures can be used interchangeably to diagnose cases of OP intoxication. The participants from whom the data were generated are OP pesticide handlers in Washington state participating in the statewide cholinesterase monitoring programme.

METHODS

Data collection

Enrolment

The Washington State Cholinesterase Monitoring Program was designed to actively monitor, record, manage and attempt to prevent occupational overexposures to cholinesterase inhibitors.[4] This programme is overseen and enforced by the Washington State Department of Labour and Industries and requires employers to offer cholinesterase activity level monitoring to agricultural pesticide applicators working in the state who meet certain temporal and chemical exposure criteria.[14] As part of this programme, OP handlers are invited to be seen at a clinic before and during the pesticide application season in order to undergo cholinesterase monitoring. Participants in the present study were recruited from the participants in the Washington State Cholinesterase Program, which is by law made available to employees who handle class I and II OP pesticides for at least 30 h within a 30-day period during the pesticide application season.[15] Participation in the cholinesterase monitoring programme is not mandatory—employees have the choice whether or not to participate in the programme. Therefore, in order to participate in the present study, the individual must have already chosen to participate in cholinesterase monitoring.

Participants for this study were recruited at three clinics of the cholinesterase monitoring programme between 2006 and 2011. Recruitment occurred at the time of a follow-up visit to the clinic during the pesticide spray season, and at that time consenting participants completed a self-administered computer-based survey in either English or Spanish to provide information on demographics, pesticide use, workplace practices including use of personal protective equipment and other details related to job duties and workplace pesticide exposure.[16] For two of the three clinics, enrolment occurred when the participant came for their blood draw. The third clinic travelled to worksites to provide cholinesterase monitoring on-site, and study personnel travelled with them to invite all participating pesticide handlers to join the study.

Cholinesterase measurements

Blood samples for this analysis were collected as part of the WA Cholinesterase Monitoring Program. One baseline blood sample was collected from each participant during their preseason visit to the clinic, with BChE and AChE measurements made from the same blood sample. This captures each participant's 'normal' BChE and AChE activity levels, that is, at a time when they report not having been recently exposed to cholinesterase inhibiting pesticides. An additional sample was collected during the pesticide application season (April–July) at one or more follow-up visits.[17] This sample was intended to capture BChE and AChE activity at a time when the participant was likely to have been exposed, and possibly overexposed, to OPs.

Laboratory analysis of blood samples for BChE and AChE activity were conducted by the Washington State Public Health Laboratories (2006) and Pathology Associates Medical Laboratories from 2007 to 2011. Both laboratories used the Ellman colorimetric enzymatic assay to measure BChE and AChE activity.[18]

Subject and sample selection

Our analysis includes a total of 215 individuals who completed one baseline visit to the clinic and at least one follow-up visit during the pesticide spray season in any of the data collection years. One measurement per person of AChE activity proportion change from baseline and BChE activity proportion change from baseline are included in the following analyses.

Some participants had multiple follow-up visits during the study period, but for the purposes of the present analysis, we included only one visit per person, selecting the visit with the maximum BChE depression for that individual. We included the observation for each individual that reflected the greatest decrease in BChE activity level in order to capture the maximum depression observed for each participant over the study period. We used BChE measurement for determination of maximum cholinesterase depression because measured BChE activity is generally more sensitive to OP inhibition than is AChE activity.[19] A total of 61% of participants reported handling pesticides within 1 week prior to their clinic visit, including 19% who reported handling OPs within the past day.

Statistical analyses

The goal of the statistical analyses was to examine the nature of the relationship between AChE and BChE measurements, with a

particular focus on standard cut-offs used to indicate cholinesterase depression. Correlation coefficients and p values were obtained using PROC CORR in SAS. Fisher's transformation was used to calculate CIs for the correlation coefficients. Sensitivity analyses were conducted to test whether our method of sample selection (ie, sample showing maximum depression vs a random sample) affected the results. All analyses were performed using SAS V.9.3.

Quantifying cholinesterase inhibition

Our outcomes were calculated as proportion decreases in cholinesterase enzyme activity between measurements taken at baseline and follow-up visits. It is necessary to measure cholinesterase depression as a relative change in enzyme activity between baseline and follow-up because of the substantial population variability in baseline BChE and AChE activity levels.

We used least squares regression to examine change in AChE activity level as a predictor of change in BChE activity level across the strata of OP pesticide exposure and other factors. To estimate the effects of individual pesticides on the relationship between AChE and BChE proportion change while controlling for individual pesticide use, we constructed linear regression models adjusted for each pesticide used, representing the rarer pesticides (ie, those used by fewer than 20 participants: phosmet, diazinon, malathion, methidathion, methamidophos) with a single indicator variable for whether a participant reported use of any of the rare pesticides.

Although the degree of neurological effect of ChE depression appears to vary between individuals, 15% depression is considered to reflect OP overexposure,[20] while 40–50% depression is associated with mild neurotoxicity and more serious effects are anticipated after depression reaches 80%.[21] In the literature, cut-offs in per cent depression that are used for diagnosing cholinesterase depression vary, but are typically between 20% and 30% decreases in activity for AChE and BChE.[2 17 20 22] We used these cut-offs in the analysis to assess agreement of diagnosis of cholinesterase depression when using either biomarker.

Assessing agreement between biomarker measurements

In order to characterise the agreement of ChE proportion change measurements, distributions of AChE proportion change and BChE proportion change were categorised into tertiles which were examined for concordance using the weighted κ statistic.

The κ coefficient (K) tests agreement of paired measures. The value of the κ statistic takes into account the agreement between the variables that could be due to chance by subtracting out the proportion of concordant results that would be expected to occur by chance alone.[23] A κ of 1 indicates perfect agreement between the measures, while a negative κ indicates that there is less agreement than would be expected by chance alone. Generally a κ value of ≥0.80 is considered to indicate good concordance between the measures, and $0.67<K<0.80$ indicates moderate concordance.[23]

On the basis of common guidelines for diagnosing ChE depression using these biomarkers, the cohort was also categorised into those who had at least 20% BChE depression between baseline and follow-up and those who did not, and separately stratified into those who had 20% AChE depression between baseline and follow-up and those who did not. We then performed κ tests to assess agreement of classification into category of 'ChE Depressed' or 'Not ChE Depressed' based on measurement by either biomarker.

Pesticide exposure score algorithm

To quantify each participant's cumulative recent OP pesticide exposure, we employed an exposure algorithm developed for the Agricultural Health Study.[19] Based on this method, we created a z-score that quantified relative total exposure to cholinesterase-inhibiting pesticides in the 30 days prior to the participant's clinic visit. The algorithm used the exposure information from the participant survey, incorporating self-reported use of individual pesticide, total hours of pesticide use, whether the participant mixed and/or applied pesticides, consistency of use and types of personal protective equipment (eg, gloves), methods of application (eg, backpack sprayer, tractor, as well as use of an enclosed cab), personal hygiene (time between pesticide mixing/application and washing, usual time until changing clothes after a spill) and other factors influencing exposure. Each factor was weighted based on published industrial hygiene literature and on pilot study data from the Agricultural Health Study.

Sensitivity analysis

To test whether selection of the follow-up visit with the most extreme decrease in BChE activity per participant affected the observed correlations between AChE and BChE, we performed a sensitivity analysis. For this analysis we repeated all tests for correlation after calculating AChE and BChE proportion changes using the first follow-up observation for each participant rather than the follow-up observation with the most extreme percent decrease in BChE activity.

RESULTS

Descriptive statistics

Two hundred and fifteen individuals with one baseline and at least one follow-up observation each were included in the analyses. Study participants were almost entirely Latino men younger than age 50, with more than half of the study population under age 35 (table 1). The majority had completed education through only middle school and most were not able to read in English. Most participants completed the self-administered computer survey in Spanish (data not shown).

At their follow-up visit, participants reported the type of pesticides they handled or applied in the prior 30 days (see online supplementary table S1). The OP chlorpyrifos was overall the most commonly reported pesticide in this cohort, reportedly used by over half of all participants. The carbamate pesticide carbaryl was the second most commonly reported pesticide, reported by over one-quarter of participants. Other pesticides were used by only a minority of the cohort, with no other pesticide reported by more than 13.5% of participants.

Although some participants reported using multiple pesticides in the 30 days prior to their visit, the majority of participants reported only one (see online supplementary table S2), chlorpyrifos being the most common among single pesticide users (data not shown). Few participants reported using more than 2 pesticides during the 30 days prior to their follow-up clinic visit. Among those who reported using multiple pesticides, the most common combination was carbaryl with chlorpyrifos (n=17), followed by carbaryl with azinphos methyl (n=7) or malathion with azinphos methyl (n=7). No more than 6 participants used any other given combinations of pesticides. We did not perform separate analyses for participants who used multiple pesticides because of these small numbers.

Measurements of BChE proportion change were approximately normally distributed with a median value of −0.05, a mean value of −0.06, with a SD of 0.10 and a range of −0.39–

Table 1 Characteristics of participants at enrolment

Characteristic	n=215	Per cent
Gender		
Male	213	99.07
Missing	2	0.93
Age category (years)		
18–24	36	16.74
25–34	95	44.19
35–49	68	31.63
50+	15	6.98
Missing	1	0.47
Ethnicity		
Black	1	0.47
White	1	0.47
Latino	210	97.67
Missing	3	1.40
Education completed		
None	8	3.72
Partial primary school	33	15.35
Primary school	74	34.42
Middle school	70	32.56
High school	29	13.49
Missing	1	0.47
Able to read English		
No	137	63.72
Yes	76	35.35
Missing	2	0.93

0.19. Measurements of AChE proportion change were also normally distributed but were more varied, with a median value of −0.01, a mean value of 0.00 and a SD of 0.14, and a range of −0.50–0.76.

As shown in table 2, SDs tended to be slightly larger for the AChE measurements. This table also shows mean proportion change for both AChE and BChE among participants with ≥10%, ≥15% or ≥20% decrease from baseline in either one. Participants who had at least 10%, 15% or 20% decrease in activity of one biomarker did not tend to show a similar amount of decrease in activity of the other biomarker. For those with at least 20% decrease in BChE activity, their AChE activity slightly increased from baseline, on average, by 5%. Among participants in the top tertile for pesticide exposure score, there was a negative average change for BChE but a positive average change for AChE. We only had five participants with at least 30% BChE depression, and just four participants with at least 30% AChE depression.

Table 2 Mean BChE and AChE activity proportion change from before to after pesticide exposure (N=215)

	N	Mean BChE	SD	Mean AChE	SD
Entire cohort	215	−0.06	0.10	0.00	0.14
≥10% BChE depression	70	−0.18	0.07	0.02	0.15
≥10% AChE depression	34	−0.06	0.09	−0.18	0.09
≥15% BChE depression	39	−0.22	0.06	0.02	0.13
≥15% AChE depression	17	−0.04	0.09	−0.24	0.10
≥20% BChE depression	22	−0.26	0.05	0.05	0.13
≥20% AChE depression	7	−0.03	0.05	−0.32	0.11
Handled only chlorpyrifos*	92	−0.04	0.10	−0.02	0.08
Top tertile of exposure score	56	−0.10	0.11	0.04	0.16

*Refers to participants who reported handling only chlorpyrifos in the 30 days prior to the date of their follow-up blood draw.
AChE, acetylcholinesterase; BChE, butyrylcholinesterase.

Cholinesterase depression in our study sample is similar to that of the full population of the Washington State Cholinesterase Monitoring Program participants (see online supplementary table S3). Overall the percentages of individuals with at least 20% cholinesterase depression in each year were similar between participants in our study and the state cholinesterase monitoring programme.[24] Although the percentage of workers with at least 20% depression is somewhat higher among participants in the present study in some years of data collection, the numbers of participants in these subgroups tend to be small.

Correlation between proportion changes of AChE and BChE

AChE and BChE proportion change are weakly negatively correlated, with correlation coefficient −0.14 (95% CI −0.27 to −0.01; figure 1). When restricting the analysis to individuals who had 10% or 15% AChE or BChE activity depression, the two biomarkers remained weakly negatively correlated (table 3). The same trend persisted when restricting to participants who had applied OP pesticides within the past week or who had used only chlorpyrifos within the past 30 days. We were unable to restrict to sole users of other pesticides due to small numbers of participants using other pesticides. Correlation coefficients are not shown for AChE or BChE depression of ≥20% due to small numbers.

κ statistic

We observed little concordance between AChE and BChE activity when categorised either by tertile of enzyme activity change or as above or below the clinical threshold for cholinesterase depression (table 4). The κ coefficients indicated a weak negative association between measured AChE and BChE activity levels.

Sensitivity analysis

For a sensitivity analysis, z-transformed correlation coefficients and corresponding 95% CIs based on AChE and BChE proportion change measurements from the first follow-up visit of each participant were calculated (see online supplementary table S4). This is compared with data in table 3, which shows

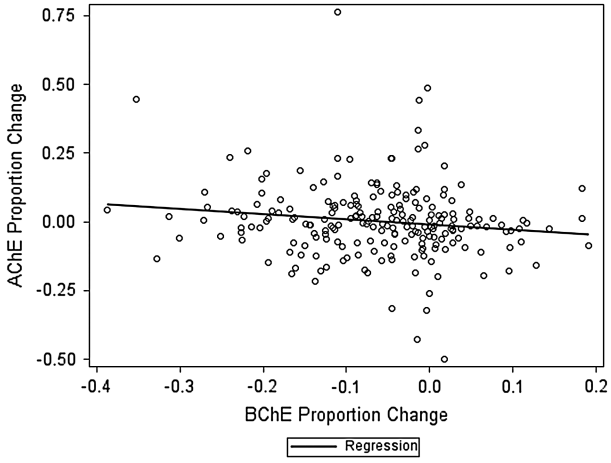

Figure 1 Correlation between acetylcholinesterase and butyrylcholinesterase proportion change (N=215).

Table 3 Correlation of AChE and BChE proportion change measurements

Subset of cohort	N	Correlation coefficients*	95% CI
Entire cohort	215	−0.14	−0.27 to −0.01
≥10% BChE depression	70	−0.11	−0.33 to 0.13
≥10% AChE depression	34	−0.24	−0.54 to 0.10
≥15% BChE depression	39	−0.28	−0.55 to 0.04
≥15% AChE depression	17	−0.19	−0.61 to 0.32
Handled only chlorpyrifos	92	−0.15	−0.34 to 0.06
Handled OP within past 7 days	132	−0.10	−0.27 to 0.07
Handled only chlorpyrifos within past 7 days	59	−0.16	−0.40 to 0.10
Top tertile of exposure score	56	−0.20	−0.44 to 0.07

*Presented Pearson correlation coefficients have been z-transformed, but are identical to the untransformed Pearson correlation coefficients to the tenths decimal place.
AChE, acetylcholinesterase; BChE, butyrylcholinesterase; OP, organophosphate.

z-transformed correlation coefficients for AChE and BChE proportion change when sampling the follow-up visit with the greatest decrease in BChE activity from baseline.

All correlation coefficients that are presented in table 3 are captured within the 95% confidence limits of the correlation coefficients obtained using the alternative sampling method, that is, using the first follow-up measurement rather than the one showing the greatest change in BChE from baseline. The correlation results obtained using either method are not statistically significantly different.

A κ statistic was also calculated to quantify concordance of tertile assignment using the alternative sampling method. The weighted κ was −0.07 (95% CI −0.18 to 0.03). This was similar to the value calculated using the participant samples with the greatest proportion change and is within the confidence limits of the κ value reported for tertile agreement in table 4, demonstrating that the sampling method does not significantly affect the estimated concordance.

DISCUSSION

The present study found a weak negative correlation between BChE and AChE measurements of cholinesterase depression among pesticide handlers. The amount of recent exposure to cholinesterase-inhibiting insecticides did not have an appreciable effect on the observed correlations. These data suggest that diagnosis of cholinesterase depression in this population is contingent on which biomarker, AChE or BChE, is used for diagnostic testing.

Blood BChE and AChE activity levels have both been used as surrogate biomarkers for cholinergic AChE depression in screening programmes as well as in epidemiological studies. However, we were unable to identify any published research on the comparability of these two biomarkers, particularly in regard to differences in their identification of clinically-relevant cholinesterase depression of 20% or greater.

Table 4 Concordance between AChE and BChE for different categorisations of cholinesterase depression (N=215)

Categories	κ	95% CI
≥20% Depression by BChE and AChE	−0.05	(−0.08 to −0.02)
Tertile of BChE and AChE Depression*	−0.11	(−0.21 to −0.01)

*Weighted κ coefficients are used to quantify tertile agreement.
AChE, acetylcholinesterase; BChE, butyrylcholinesterase.

Our results show a weak negative correlation between measurement of AChE and BChE proportion change. This relationship persisted after restricting to those with relatively high exposure to OP insecticides, those who used chlorpyrifos only and those who had been classified as cholinesterase depressed based on current guidelines.

The weighted κ coefficients for agreement between categories of cholinesterase depression based on the two biomarkers also showed a weak negative association. Since individuals who reach at least 20% BChE depression are not likely to also have at least 20% AChE depression, and vice versa, these biomarkers cannot be used interchangeably for diagnostic purposes. Furthermore, sensitivity analyses showed that the observed results are robust to our sampling method.

Our correlation results were additionally robust to the method of selecting data samples. Sensitivity analyses indicated that selecting follow-up observations for each individual that reflected the greatest decrease in BChE activity for that individual produced generally similar correlation coefficients between AChE and BChE as selecting the first follow-up observation.

There are important factors to consider when interpreting the results of this study. Over the course of the WA cholinesterase monitoring programme, the number of events of cholinesterase depression among participants has decreased.[25] This may be a result of the effectiveness of the cholinesterase monitoring programme in reducing incidence of overexposure to OP insecticides, or may be due to the fact that OP insecticide use in Washington state decreased between 2006 and 2011.[26] In addition, these markers of cholinesterase depression may not be sufficiently sensitive at low exposure levels,[6] although this is less of a concern for this relatively high-exposure population. It is possible, however, that this issue of sensitivity could be contributing to the differences observed between the two biomarkers.

This study has a number of strengths. It is large for a study of its kind, and has both baseline and follow-up blood samples from participants. We were able to quantify both AChE and BChE from a single blood draw so as to more appropriately compare the measures. The generalisability of our results is improved by the fact that the study cohort is similar to the statewide cholinesterase monitoring programme participants with respect to cholinesterase depression as well as demographics.[17 27]

Certain limitations of this study must be considered. Owing to the self-reported nature of our exposure data, misclassification of exposure is possible. However, a validation study of the survey instrument shows that problems with recall in this study are likely minimal and do not introduce a significant amount of bias.[16] Since we do not have complete data on which pesticides were used by our participants and exactly how much of each was used, we are unable to fully account for the differential effects of various OPs (eg, chlorpyrifos) on BChE and AChE activity, although we accounted for this with the available data.

We are unaware of any other studies that have examined the relation between AChE and BChE activity measurements in a human population. Our analyses suggest little agreement between the two measures, at least at the exposure levels experienced by these farm workers, and that differences in patterns of these markers must be considered in interpreting results. More research is needed to determine the conditions under which AChE vs BChE is the more appropriate biomarker. Our study lacked the necessary data to address this question. Thus, for the present, we recommend that both be measured in cholinesterase

monitoring programmes or for determining cholinesterase depression in other occupational settings.

Acknowledgements The authors would like to acknowledge all participants in the current study for making this research possible. The authors would also like to acknowledge the Washington Department of Labor and Industries, who maintain the Washington State Cholinesterase Monitoring Program and who collected and assayed the biological specimens that were used in the current study. The authors would also like to acknowledge Jonathan Hofmann for providing information on the data collection procedures used for the parent study.

Contributors JS contributed to the planning, conduct and reporting of this research planned, executed the analysis and wrote the manuscript. LSE contributed to formation of the research hypothesis, helped to plan the analysis and offered comments on drafts of the manuscript. MCK provided the data for this project, contributed to the generation of the research questions and offered comments on drafts of the manuscript.

Funding Centers for Disease Control and Prevention/National Institute for Occupational Safety and Health (U50OH07544 and T42OH008433) and National Institute of Environmental Health Sciences (5T32ES007018-37, P30ES07033, T32ES07262, P42ES04696, and ES009883).

Competing interests None.

Patient consent Obtained.

Ethics approval The University of North Carolina Institutional Review Board deemed the current analysis exempt from IRB approval. The study which collected the data that were used in the current analysis was approved by the University of Washington Institutional Review Board.

Provenance and peer review Not commissioned; externally peer reviewed.

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Blood acetylcholinesterase and butyrylcholinesterase as biomarkers of cholinesterase depression among pesticide handlers

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Occup Environ Med 2014 71: 842-847 originally published online September 4, 2014
doi: 10.1136/oemed-2014-102315

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