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Determinants of Butyrylcholinesterase Inhibition Among Agricultural Pesticide Handlers in Washington State: An Update

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ABSTRACT

Objectives: Organophosphate (OP) and *N*-methyl-carbamate (CB) insecticides are used widely in agriculture to manage insect pests of economic importance. Agricultural workers are more likely to suffer exposure because of the widespread use of OP/CBs in agriculture, and pesticide-related illnesses among handlers may be more severe when compared to other farm workers. The goal of this study was to identify occupational and personal characteristics associated with butyrylcholinesterase (BuChE) inhibition in participants recruited from the Washington State Cholinesterase Monitoring Program from 2006 to 2011.

Methods: We conducted a longitudinal study among agricultural pesticide handlers in Washington State during the OP/CB spray season (March–July) over a 6-year period (2006–2011). Linear mixed effects regression models were used to evaluate BuChE inhibition in relation to self-reported occupational and personal characteristics.

Results: Relative to pre-season baseline levels, the mean decrease in BuChE activity during the OP/CB spray season over all years of the study period was 3.77% ($P < 0.001$). Greater BuChE inhibition was observed among handlers who reported using multiple OP/CBs ($\beta = -2.70$, $P = 0.045$), mixed or loaded OP/CBs ($\beta = -3.97$, $P = 0.002$), did not store personal protective equipment (PPE) in a locker at work ($\beta = -3.4$, $P = 0.014$), or did not wear chemical-resistant boots ($\beta = -16.6$, $P < 0.001$).

Discussion and Conclusions: The Washington State Cholinesterase Monitoring Program has provided a valuable opportunity to evaluate potential sources of OP/CB exposure among agricultural pesticide handlers. Several previously reported associations were confirmed in the current analysis, which included a larger number of pesticide handlers enrolled over a longer time period. The use of multiple OP/CBs and mixing/loading activities were significant risk factors, and the use of chemical-resistant boots and lockers for PPE storage were protective factors. Our findings point toward logical interventions to reduce exposure such as the implementation of engineering controls for mixing/loading activities, requirements for appropriate footwear, and the regular use of lockers for PPE storage.

KEYWORDS: agriculture; biological monitoring; cholinesterase; determinants of exposure; occupational exposure; organophosphorus compounds; pesticide; pesticide handler

INTRODUCTION

Organophosphate (OP) and *N*-methyl-carbamate (CB) insecticides are used widely in agriculture to manage insect pests of economic importance. OP/CB insecticides inhibit acetylcholinesterase (AChE), an enzyme critical in regulating nerve transmissions. The active site of the enzyme is bound by the OP/CB, rendering it unable to break down acetylcholine, a neurotransmitter. Acetylcholine then continues to stimulate the initiation of depolarization throughout the central and peripheral nervous system, causing overstimulation. In addition to acute neurotoxicity, there is concern that chronic exposures from low doses of OP/CBs over time can lead to negative health outcomes, such as cancers (Alavanja *et al.*, 2004; Cocco *et al.*, 2013), neurobehavioral deficits in children from *in utero* exposure (Marks *et al.*, 2010; Engel *et al.*, 2011; Rauh *et al.*, 2011) and in adults from occupational exposure (Ross *et al.*, 2013), and chronic bronchitis (Chakraborty *et al.*, 2009).

Agricultural workers are more likely to suffer exposure because of the widespread use of OP/CBs in agriculture (Calvert *et al.*, 2008). In Washington State, approximately half of the pesticide-related illness cases reported by agricultural workers between 2003 and 2008 were pesticide handlers (Morrissey, 2009). Pesticide-related illnesses among handlers were more likely to have moderate to severe outcomes when compared to other farm workers. Agricultural pesticide handlers are defined as workers who mix concentrated pesticides and load them into tanks or other application equipment, applicators, workers who decontaminate application equipment and personal protective equipment (PPE) that contain pesticide residues, mechanics who repair application equipment, and those that supervise handling activities. Agricultural pesticide handlers may be exposed to OP/CBs as a result of dermal contact with application equipment or residues on other surfaces, inhalation, spills and splashes, and inadequate or incorrect use of PPE.

Both AChE and another serine esterase, butyrylcholinesterase (BuChE), collectively called cholinesterase (ChE) in this article, are found in the blood and often used as biological markers of exposure to OP/CB insecticides. Although AChE is the enzyme associated with symptoms of acute exposure, BuChE has been more commonly used as a biological marker to test for low levels of exposure to OP/CB insecticides (Stefanidou *et al.*, 2009). Despite concerns about the sensitivity and specificity of BuChE as a marker, several studies have shown that BuChE is effectively inhibited (more than AChE) by certain OP/CB insecticides, including chlorpyrifos, and BuChE inhibition is considered a sensitive biological marker of exposure to these chemicals (Rodriguez *et al.*, 1997; Amitai *et al.*, 1998).

Although BuChE is an established biological marker of exposure to OP/CB insecticides and has been used in many occupational exposure studies (Otto *et al.*, 1990; London *et al.*, 1997; Farahat *et al.*, 2003; Roldán-Tapia *et al.*, 2005; Abdel Rasoul *et al.*, 2008), relationships between ChE inhibition and some health outcomes, such as neurobehavioral performance, are not well established. Occupational exposure to OPs has been shown to adversely affect neurobehavioral performance in multiple studies (Rohlman *et al.*, 2011) though biological marker measures are rarely correlated with neurobehavioral performance. Biological markers including BuChE may not be sensitive enough for measuring repeated low levels of exposure, or OP/CBs may be targeting proteins other than ChEs resulting in inflammation and subsequent neurological effects (Banks and Lein, 2012). In addition, other factors, such as gender, age, physical activity, or weight, may impact BuChE levels (Lepage *et al.*, 1985; Zimmer *et al.*, 2012).

In 2004, the Washington State Department of Labor and Industries initiated a cholinesterase monitoring program for agricultural workers who handle OP/CB insecticides. The characteristics of this monitoring program have been described previously

(Hofmann *et al.*, 2010b; Keifer *et al.*, 2010). Briefly, handlers are tested for baseline ChE levels prior to OP/CB exposure, and follow-up tests are conducted throughout the growing season when OP/CBs are applied (March–August). Follow-up testing occurs if the handler uses OP/CBs for 30 or more hours in any consecutive 30-day period. An individual's ChE levels at follow-up are compared to his or her baseline levels to assess the degree of ChE inhibition relative to baseline.

Hofmann *et al.* (2010b) reported on occupational determinants of BuChE inhibition among agricultural pesticide handlers in Washington State, analyzing data collected in 2006 and 2007. The goal of this study was to identify occupational and personal characteristics associated with BuChE inhibition in participants recruited from the statewide monitoring program from 2006 to 2011. We sought to augment the 2006–2007 findings and confirm significant occupational determinants that persisted in the larger 2006–2011 data set.

METHODS

We conducted a longitudinal study among pesticide handlers in Washington State during the OP/CB spray season (March–July) over a 6-year period (2006–2011). To recruit participants, we collaborated with three clinics that conducted ChE monitoring in central and eastern Washington. Most of the participants were recruited at the clinics when they came for follow-up testing, but some participants were recruited at their workplaces. We used a computer-based survey instrument to collect information from participants at the time of follow-up testing (Hofmann *et al.*, 2010a). Most participants (75%) completed surveys within 2 weeks of the most recent use of OP/CBs, and the survey questions referred to the 30 days prior to follow-up testing. This is appropriate for short-term biomarkers such as BuChE, which has a half-life in plasma of 11 days (Mason, 2000). Field observations on a sample of 31 pesticide handlers from one workplace in 2006 who also participated in this study were used to test the validity of the survey instrument. In this subset, we observed high levels of agreement (≥89%) between survey data and field observations for PPE (respirator, boot, suit, and glove type), use of lockers for PPE storage, and type of OP/CB used. Of those who indicated they applied pesticides in the survey, 93% reported using the same type of application equipment that was observed in the field. Job titles were assigned to pesticide handlers during the field observation, and work activities selected on the survey matched the job title of the handler for all participants in the validation subset (e.g. all those with the 'mixer/loader' job title selected mixing and loading as a work activity). A previous evaluation of the survey instrument by Hofmann *et al.* (2010a) found high test–retest reliability for most of the selected variables (percent agreement: 71–98%, weighted kappa coefficients: 0.36–0.98).

All study procedures were approved by the Institutional Review Board at the University of Washington.

Serum cholinesterase (BuChE) measurements

We obtained participants' BuChE test results from the participating clinics in the statewide monitoring program. Clinic staff collected and processed serum samples, which were shipped cold overnight or transported in vehicles on cold packs for laboratory testing. BuChE assays were performed by the Washington State Public Health Laboratories in 2006 and by Pathology Associates Medical Laboratories (PAML) from 2007 to 2011. Both laboratories measured BuChE activity using the Ellman method (Ellman *et al.*, 1961) with the ChE reagent kit from Roche Diagnostics. The Public Health Laboratories measured BuChE activity using an automated Dade Dimension AR system, and PAML used an Olympus AU5421/AU2700 system. In 2008, PAML upgraded to a Janice Autopipetter. As part of the statewide monitoring program, external quality control replicate samples were collected from unexposed individuals at regular intervals during the spray season. Analyses of these quality control samples demonstrated high reproducibility for measurements of BuChE activity, with coefficients of variation ≤3.8% reported for both laboratories (Furman, 2008).

Sample selection

From 2006 to 2011, 596 invitations were extended to pesticide handlers to participate in the study. A total of 395 invitations were accepted (66%), and there were 201 refusals (34%). Each invitation does not necessarily represent a unique individual; one handler may have been invited to participate multiple times and refused in some instances. Since we did not collect identifying information from handlers who refused to participate, we were not able to compute a participation rate by individual.

Records were included for analysis if participants had completed surveys and both baseline and follow-up BuChE test results. We excluded follow-up visits if they were from handlers who came in for testing because a previous follow-up test exceeded the threshold of ChE inhibition (>20%) requiring a workplace investigation (i.e. depressionary follow-up visits). The purpose of depressionary follow-up visits is to monitor the recovery of BuChE levels, and these individuals may have been removed from handling and other work exposures to OP/CBs (though this is only a requirement when BuChE levels fall 40% or more from baseline) prior to the depressionary follow-up test. Because the purpose of depressionary follow-up visits is different than initial visits and BuChE levels are expected to be closer to baseline, they were not included in the analysis.

Exposure algorithm scores

As described by Hofmann *et al.* (2010b), we developed algorithm-based scores for work activities and PPE use, which were included in our statistical analyses to control for potential confounding by these factors. These algorithms were based on those developed for the Agricultural Health Study (Dosemeci, 2002). Although the algorithms in the Agricultural Health Study were recently updated based on new data (Coble *et al.*, 2011), these changes were unrelated to pesticide handling practices reported among participants in our study, so our exposure metrics were unchanged.

Comparison of 2006–2007 and 2006–2011 models

There were several key differences between the analysis of the 2006–2007 data set published by Hofmann *et al.* (2010b) and analysis of the 2006–2011 data set (Table 1). In the 2006–2007 data set, one record per person was included in the analysis. In the 2006–2011 data set, all follow-up visits were included except for the depressionary follow-up visits (tests done for handlers with a prior follow-up test showing >20% ChE inhibition). Individuals with multiple follow-up visits were accounted for by adding an individual random effect to our model. In addition, we added a workplace random effect because we expected that participants from the same workplace received the same training, used similar PPE, and shared facilities. By using this mixed effects model, we were able to maximize the statistical power for our analyses by including nearly all records while accounting for multiple observations per person.

The analysis of the 2006–2007 data set used a pesticide toxicity score, derived from relative pesticide potency factors for AChE inhibition in rat studies. Since OP/CBs inhibit AChE and BuChE differentially depending on the chemical, we calculated relative potency factors for BuChE inhibition based on the methodology used by the United

Table 1. Differences between the 2006–2007 and 2006–2011 analyses, including potential confounding variables included in baseline models

	2006–2007	2006–2011
Number of records/participant	One record	All records, excluding depressionary follow-ups
Random effects	Not applicable	Individual
		Workplace
Fixed effects	Age in years (continuous)	Age in years (categorical)
	Year of participation	Year of participation
	Days since baseline BuChE test	Season
Exposure algorithm scores	Pesticide toxicity	
	Work activity	Work activity
	PPE	PPE

States Environmental Protection Agency (EPA) for AChE potency factors for the 2006–2011 analysis (C. Simpson, unpublished data). However, these new pesticide potency scores based on BuChE data were homogenous across the OP/CB pesticides used by participants in this study. Therefore, in the current analysis of the 2006–2011 data set, a pesticide toxicity score was not included.

A variable that captured number of days since baseline BuChE testing was included in the base model in the 2006–2007 analysis but not in the 2006–2011 analysis. In general, participants from the same workplace went to the clinic as a group for their baseline and follow-up tests, so the 'days since baseline BuChE test' variable was captured by the 'workplace' random effect in our revised model.

Analysis

A linear mixed effects regression model was used because of dependence within the data set (Verbeke and Molenberghs, 2000). Individual and workplace were included as random effects to account for dependence since 64 individuals participated in the study on multiple occasions, and our study included participants from 37 separate workplaces, with a median (range) of 4 (1–44) participants per workplace. Several potential confounding factors were included as categorical variables in the statistical model as fixed effects. Age was included as a fixed effect because cholinesterase levels may differ by age group (Lepage *et al.*, 1985). Year was included as a fixed effect because of changes in lab procedures over the years and to account for seasonal variability and timing of pesticide applications each year. We also separated each year into 'early' and 'late' season because of the differences in pesticides used in the pre- and post-bloom stages of the crops, and we were concerned that PPE use might change because the warm temperatures in the 'late' season might lead to different behaviors in PPE use. We used May 15 as the date to transition from 'early' to 'late' because the switch from pre-bloom to post-bloom pesticide use practices generally occurs around that time (K. Lewis, personal communication).

All analyses were performed using R (Pinheiro *et al.*, 2011). The percent change in BuChE activity from baseline level was modeled as the continuous outcome variable. Percent change was used instead of absolute value of change because it accounted for differences in absolute measurements between years and wide inter-individual variability in baseline BuChE levels (Cocker *et al.*, 2002). Negative values of percent change indicate BuChE inhibition relative to baseline levels. The mean percent change in BuChE levels from baseline to follow-up was computed for each year of the study as well as the entire study, and baseline and follow-up BuChE levels were compared using one-sample *t*-tests.

The base model included individual and workplace as random effects, standardized work activity and PPE scores as continuous fixed effects, and age, year, and 'early'/'late' season as categorical fixed effects. We used this base model to test self-reported occupational characteristics, including self-reported pesticide use, work activities, and PPE use. Since self-reported work activities and PPE use were used to compute work activity and PPE scores, we did not include the standardized work activity score when investigating work activities and did not include standardized PPE scores when investigating PPE use.

We used the same base model to test personal characteristics, including pesticide handling experience and training, education and literacy, health awareness, smoking habits, alcohol consumption, and home location. The regression coefficients and standard errors were estimated with separate linear mixed effects models for each set of occupational and personal characteristics.

We analyzed most of the same variables in the 2006–2011 data set as the 2006–2007 data set, including a re-evaluation of all of the factors that were significantly associated with BuChE inhibition in the 2006–2007 analysis. Due to the increased sample size, it was possible to evaluate additional occupational and personal characteristics in the current analysis. We re-evaluated all occupational determinants if they were significant in the 2006–2007 analysis and included additional variables because the mixed model allowed us to include all of the available survey records.

Because different models were used when analyzing the 2006–2007 and 2006–2011 data sets, in sensitivity analyses, we also evaluated whether differences in the models changed our results. We applied the current mixed model approach to the data set used in the 2006–2007 analysis, but we removed the individual random effect since there was only one record per individual in the earlier data set. We also restricted the

2006–2011 data set to 2006–2007 and analyzed those data using the mixed model approach; this data set included more records than the original 2006–2007 analysis because there was more than one record per person.

Differences were considered to be statistically significant if P values were <0.05.

RESULTS

There were 375 surveys completed by 215 unique individuals at follow-up visits. Of those individuals, 151 completed a single survey, while 64 completed more than one survey. The time between baseline and follow-up testing ranged from 19 to 162 days. All participants were male pesticide handlers who primarily worked in the tree fruit industry. Almost all participants self-identified as Latino and completed the survey in Spanish (98%). Over half of the participants were under 35 years of age (57%) (Table 2).
In a subset of the data consisting of one randomly chosen follow-up visit from each of the 215 participating handlers, the average percent change in BuChE levels from baseline to follow-up for the entire study period was a decrease of 3.77% ($P < 0.001$; Table 3). Among the 215 participating handlers, 70 (33%) experienced >10% BuChE inhibition relative to pre-exposure baseline levels, and 22 (10%) had a >20% depression in BuChE activity (Table 3).

Determinants of BuChE inhibition

Results for determinants of BuChE inhibition are shown in Table 4.

Handlers who reported using multiple pesticides had on average a 2.70% greater decrease in BuChE activity levels when compared to handlers who used just one pesticide ($P = 0.045$). There were no significant associations between specific pesticides and BuChE inhibition.

Handlers who mixed or loaded OP/CBs had on average a 3.97% greater decrease in BuChE activity than those who did not mix or load these chemicals ($P = 0.002$). There were no significant associations between cleaning or repairing spray equipment, cleaning PPE, or cleaning pesticide containers and BuChE inhibition.

Handlers who did not wear chemical-resistant boots had on average a 16.63% greater decrease in BuChE activity ($P < 0.001$). Mixers and loaders who wore aprons had on average a 4.09% greater decrease in BuChE activity than those who do not wear aprons ($P = 0.014$). Those who reported they did not have

Table 2. Demographic characteristics of study participants from 2006 to 2011

Characteristic	*N*	%
Sex[a]		
Male	215	100.0
Race/ethnicity[a]		
Hispanic/Latino	214	99.5
White, non-Hispanic	1	0.5
Survey language[a]		
Spanish	211	98.1
English	4	1.9
Number of total visits by each participant[a]		
1	151	70.2
2	24	11.2
3	14	6.5
4	10	4.7
5+	16	7.4
Age in years[b]		
18–24	50	13.4
25–34	161	43.3
35–49	131	35.2
50+	30	8.1
Number of visits each year[b]		
2006	91	24.3
2007	93	24.8
2008	65	17.3
2009	53	14.1
2010	45	12.0
2011	28	7.5
Season[b]		
Early (before or on May 15)	231	61.6
Late (after May 15)	144	38.4

[a]$N = 215$, based on number of unique individuals.
[b]$N = 375$, based on number of total visits; missing values were excluded from percentages.

Table 3. Change in serum cholinesterase (BuChE) activity during the OP/CB spray season relative to baseline levels[a]

Year[a]		Percent change in BuChE activity		Frequency of BuChE inhibition[b]	
				>10% decrease	>20% decrease
	n	Mean (SD)	*P* value[c]	*n* (%)	*n* (%)
2006	85	−2.74 (11.01)	0.024	18 (21)	10 (12)
2007	79	−6.44 (8.55)	<0.001	25 (32)	6 (08)
2008	46	−7.65 (8.71)	<0.001	17 (40)	5 (11)
2009	51	−6.94 (11.48)	<0.001	18 (35)	5 (10)
2010	43	−3.23 (7.32)	0.005	11 (26)	0 (0)
2011	28	2.66 (12.4)	0.266	5 (18)	1 (04)
2006–2011	215	−3.77 (9.95)	<0.001	70 (33)	22 (10)

[a]For participants with multiple follow-up visits in the same year, the first visit was selected. For the 2006–2011 summary, one visit from each participant was chosen at random.
[b]Defined as having decreased BuChE activity at the specified level at any follow-up visit relative to pre-exposure baseline level, by year and for 2006–2011 combined.
[c]Two-sided *P* values for one-sample *t*-tests.

lockers for PPE storage had on average a 3.40% greater decrease in BuChE activity ($P = 0.004$). There were no significant associations between respirators, glove type, or taking breaks to smoke or use cellular phones and BuChE inhibition.

Licensed handlers had on average a 3.06% greater decrease in BuChE activity than unlicensed handlers with training ($P = 0.014$) and 1.89% greater decrease than those with no license or training. Those who reported they were not concerned with the health effects of pesticides had on average a 12.12% greater decrease in BuChE activity than those handlers who reported that they were very concerned ($P = 0.021$). The majority of handlers (81%) reported being very concerned about pesticides affecting their health.

Self-reported health status and symptoms, years handling pesticides, level of education completed, English literacy, smoking habits, alcohol consumption, acetaminophen use, and home location were not associated with BuChE inhibition.

Several findings from the 2006–2007 analysis by Hofmann *et al.* (2010b) were confirmed in the current analysis, while others failed to replicate (Table 5). All factors that were significant in the Hofmann *et al.* (2010b) analysis were significant or borderline significant in the 2006–2007 mixed models (both with and without the individual random effect) except for the use of full-face respirators. Several risk factors, such as wearing PPE aprons and applying multiple OP/CBs, were significant in the current study but not in the Hofmann *et al.* (2010b) analysis. However, when the mixed model analysis was applied to the 2006–2007 data set, these factors were found to be significant or borderline significant. Cleaning spray equipment was a significant risk factor in all 2006–2007 analyses but was not significant in the 2006–2011 analysis.

DISCUSSION

In this investigation, we sought to replicate and expand on findings from a previous analysis of determinants of BuChE inhibition among OP/CB-exposed agricultural pesticide handlers in Washington State. While findings in the updated analysis are not statistically independent since 154 of the 215 participants (72%) were included in both analyses, the current study included more than twice the number of new records (221 of 375 records were new; 59% of the total number of records), which provided better power to detect associations and gives us more confidence in the updated analysis. Several factors that were previously associated with greater BuChE inhibition (e.g. mixing and loading pesticides) or that were protective against

Table 4. Differences in BuChE inhibition in relation to selected exposures after adjusting for random and fixed effects. β coefficients represent the estimated difference in percent change in BuChE activity compared to the reference group for each factor. The reference group was selected *a priori* as the group that was thought to be the least exposed/most protected.

Exposure(s)	N[a]	β coefficient	SE	*P* value[b]
Pesticide[c]				
Chlorpyrifos	311			
No	118	Ref	—	—
Yes	193	−2.10	1.57	0.179
Azinphos-methyl	311			
No	249	Ref	—	—
Yes	62	1.45	1.65	0.379
Carbaryl	311			
No	223	Ref	—	—
Yes	88	−2.49	1.39	0.073
Multiple OP/CBs	311			
No	229	Ref	—	—
Yes	82	−2.70	1.35	0.045
Work activity[d]				
Mixing/loading	346			
No	82	Ref	—	—
Yes	264	−3.97	1.28	0.002
Repairing spray equipment	346			
No	266	Ref	—	—
Yes	80	−0.18	1.25	0.883
Cleaning PPE	350			
No	86	Ref	—	—
Yes	264	0.03	1.18	0.978
Cleaning spray equipment	350			
No	142	Ref	—	—
Yes	208	−1.47	1.02	0.149
Cleaning pesticide containers	350			
No	219	Ref	—	—
Yes	131	−1.23	1.09	0.257

Table 4. *Continued*

Exposure(s)	N[a]	β coefficient	SE	P value[b]
PPE[e]				
Respirator	347			
Full-face	38	Ref	—	—
Half-face	285	−2.65	2.28	0.244
Powered air purifying respirator	24	−3.16	3.11	0.310
Gloves	347			
Disposable gloves under nitrile gloves	71	Ref	—	—
Nitrile gloves alone	221	−0.29	1.37	0.834
Cloth gloves under nitrile gloves	55	2.12	1.78	0.235
Chemical-resistant boots	359			
Yes	353	Ref	—	—
No	6	−16.63	3.82	<0.001
Apron[f]	274			
Yes	43	Ref	—	—
No	231	4.09	1.67	0.014
Storage of PPE in locker	354			
In a locker at work	209	Ref	—	—
In another location	145	−3.40	1.19	0.004
Breaks and hand-washing				
Smoke break	341			
Never stopped spraying	319	Ref	—	—
Always washed before smoking	19	0.64	2.36	0.787
Sometimes/never washed before smoking	3	−2.78	5.37	0.605
Cell phone break	341			
Never stopped spraying	236	Ref	—	—
Always washed before using phone	29	−1.48	2.08	0.476
Sometimes/never washed before using phone	76	−0.57	1.37	0.676
Work experience and training				
Applicator license and/or training from licensed pesticide applicator in last 12 months	326			
Yes, applicator license	112	Ref	—	—
Yes, training	181	3.06	1.25	0.014
No, no training and no license	33	1.89	1.94	0.330

Table 4. *Continued*

Exposure(s)	*N*[a]	*β* coefficient	SE	*P* value[b]
Years handling pesticides	317			
More than 10 years	30	Ref	—	—
6–10 years	78	1.54	2.18	0.482
4–5 years	74	2.21	2.31	0.337
2–3 years	84	0.31	2.35	0.894
1 year or less	51	−0.64	2.62	0.807
Health awareness				
Concern about health affected by pesticide exposure	340			
Very concerned	275	Ref	—	—
A little bit concerned	48	−0.40	1.53	0.795
Not at all concerned	3	−12.12	5.27	0.021
I do not have an opinion	14	−0.89	2.61	0.734
Self-rated health status	341			
Excellent	42	Ref	—	—
Good	194	0.67	1.70	0.692
Poor or fair	105	−0.82	1.85	0.655
Experience symptoms related to pesticide exposure	301			
No symptoms or illnesses	247	Ref	—	—
Selected symptom(s) from list and/or other	30	−0.32	1.87	0.863
Level of education	341			
Completed high school	40	Ref	—	—
Completed middle school	97	−1.22	1.96	0.533
Completed primary school or part of primary school	197	−1.11	1.91	0.560
Did not attend school	7	−1.74	4.06	0.668
Read in English	340			
Fairly well or very well	55	Ref	—	—
Not at all or not very well	285	−0.75	1.44	0.602
Smoke cigarettes	340			
Not at all	249	Ref	—	—
Some days	62	−0.77	1.45	0.592
Every day	29	−2.35	1.94	0.227
Alcohol consumption	328			
Never	124	Ref	—	—

Table 4. ***Continued***

Exposure(s)	N[a]	β coefficient	SE	P value[b]
Rarely to every day	204	1.64	1.16	0.156
Acetaminophen use	258			
Never	140	Ref	—	—
Sometimes or often	118	−0.03	1.15	0.980
Home location	340			
In town	185	Ref	—	—
In country, away from orchards	37	−1.25	1.83	0.494
In country, near orchards	38	0.08	1.76	0.962
Within or next to orchard	71	−1.76	1.55	0.255
Other	9	−0.09	3.34	0.978

[a]Number of records included in regression.
[b]Two-sided *P* value.
[c]Work activity and PPE exposure algorithm scores were included as fixed effects covariates in the statistical models.
[d]PPE exposure algorithm score was included as a fixed effect covariate in the statistical models.
[e]Work activity exposure algorithm score was included as a fixed effect covariate in the statistical models.
[f]Restricted to those who selected mixing/loading as a work activity.

BuChE inhibition (e.g. using a locker for PPE storage, use of chemical-resistant footwear) were confirmed in this analysis, whereas other associations failed to replicate (e.g. full-face respirator use, cleaning spray equipment). Other factors that were not significantly associated with BuChE inhibition in the previous analysis, such as the use of multiple OP/CBs, emerged as significant in this analysis, which included a much larger sample size (N_{survey} = 375, $N_{subject}$ = 215 versus $N_{survey/subject}$ = 154), continued follow-up over a longer time period (2006–2011 versus 2006–2007), and a higher response rate (66% versus 51%).

One specific activity, mixing and loading pesticides, was associated with greater BuChE inhibition in both the 2006–2007 and 2006–2011 analyses. This is an important exposure route where interventions to improve safety could reduce workers' exposure. The state of California requires the use of closed loading systems for many operations, an engineering control that could be implemented in orchards in Washington State to reduce exposures when mixing/loading chemicals (Thongsinthusak and Ross, 1994). Using water-soluble packets has also been shown to reduce pesticide exposures when compared to the open pouring of liquids or dumping of wettable powders (Rutz and Krieger, 1992).

The use of lockers for storage of PPE was found to be protective in both the 2006–2007 and 2006–2011 data sets. One possible explanation for this finding is that the availability of lockers is a surrogate for workplaces with higher safety standards and more facilities for workers. While we found that reported usage of lockers for PPE storage was not independent of workplace ($P < 0.01$), when we examined the effect of locker usage within several of the larger orchards with heterogeneous locker usage, locker usage remained a protective factor. This suggests that the locker effect might not simply indicate safer practices by the entire workplace but may indicate a more direct effect on exposure reduction through the use of the lockers themselves.

Not wearing chemical-resistant boots was strongly associated with BuChE inhibition. This should be interpreted with caution since only six handlers reported not wearing chemical-resistant boots, five of which were records from the 2006–2007 analysis.

Use of multiple OP/CBs in the 30 days prior to follow-up ChE testing was associated with BuChE inhibition in the 2006–2011 analysis. While the association in the 2006–2007 analysis was of similar magnitude ($\beta = -2.5$ in 2006–2007; $\beta = -2.7$ in 2006–2011), it was not statistically significant in the original investigation with fewer subjects. This finding

Table 5. Sensitivity analysis comparing selection criteria and analysis methods used in 2006–2007 and 2006–2011

Significant factors	2006–2007 (154 records)[a]			2006–2011 (375 records)[b]			2006–2007 (184 records)[c]			2006–2007 (154 records)[d]		
	n	β (SE)	*P* value	*n*	β (SE)	*P* value	*n*	β (SE)	*P* value	*n*	β (SE)	*P* value
Significant in all analyses												
Mixed/loaded chemicals	120	−5.25 (1.93)	0.007	346	−3.97 (1.28)	0.002	163	−5.17 (1.65)	0.001	120	−4.47 (1.98)	0.016
Used chemical-resistant boots	130	−11.40 (5.53)	0.041	359	−16.63 (3.83)	<0.001	176	−16.10 (3.72)	<0.001	130	−13.50 (4.82)	0.005
Used locker for PPE	116	−7.58 (2.41)	0.002	354	−3.40 (1.19)	0.004	171	−5.30 (1.65)	0.001	116	−7.63 (2.20)	0.001
Significant in 2006–2007[a]												
Cleaned spray equipment	120	−4.39 (2.04)	0.033	350	−1.47 (1.02)	0.149	165	−3.09 (1.43)	0.031	120	−4.35 (1.85)	0.019
Used full-face respirator (versus half-face respirator)	118	−6.95 (3.23)	0.034	323	−1.78 (2.44)	0.466	153	−0.48 (3.10)	0.878	118	−2.97 (3.68)	0.418
Self-rated health status as poor/fair (versus excellent)	118	−6.40 (2.70)	0.02	341	−0.82 (1.85)	0.655	160	−4.59 (2.36)	0.051	118	−5.22 (2.99)	0.081
Significant in 2006–2011[b]												
Wore an apron (restricted to mixers/loaders)[e]	88	−2.97 (2.93)	0.314	274	−4.09 (1.67)	0.014	122	−4.80 (2.27)	0.014	97	−4.45 (3.23)	0.168
Used multiple OP/CBs	119	−2.50 (2.95)	0.399	311	−2.70 (1.35)	0.045	141	−6.00 (1.89)	0.002	119	−4.20 (2.67)	0.116

[a]Results from the 2006–2007 analysis published by Hofmann *et al.* (2010b).
[b]Results from the 2006–2011 analysis described in this article.
[c]Results from restricting the 2006–2011 analysis to the years 2006–2007.
[d]Results from using the Hofmann *et al.* (2010b) data and analyzing using the mixed effects model described in this article. The individual random effect was removed because there was only one record per individual in this data set.
[e]Results reported by Hofmann *et al.* (2010b) did not restrict analysis to mixers/loaders, so *n* reported here is different than in the Hofmann *et al.* (2010b) publication.

may be indicative of greater cumulative exposure during the time period before follow-up ChE testing among those handlers reporting multiple OP/CB use. Alternately, mixed exposures may potentiate the toxic effects of specific OPs (Jansen *et al.*, 2009), thus resulting in greater BuChE inhibition.

The 2006–2007 analysis found that, contrary to expectations, handlers who wore chemical-resistant aprons had greater BuChE inhibition than other handlers; however, this association was not statistically significant. In the 2006–2011 analysis, we restricted our analysis to handlers who reported performing mixing and loading activities because they were the most likely to wear aprons as certain pesticide labels require them when mixing and loading. We found that mixer/loaders who wore aprons had greater BuChE inhibition ($P = 0.014$) than those who did not. One explanation is that aprons are required when handling more highly toxic chemicals, and the aprons were not fully protective against these chemicals. Another explanation is that PPE decontamination training might not emphasize the need to clean aprons, so aprons may not be decontaminated together with other PPE, resulting in exposure to OP/CB residues with each use (K. Galvin, personal communication).

Differences between the 2006–2007 and 2006–2011 analyses can be largely explained by the selection criteria and the additional subjects and records included in the 2006–2011 analysis. When the records used by Hofmann *et al.* (2010b) were analyzed using the mixed model approach (with the exclusion of the individual random effect because there was only one record per person), the results were similar, suggesting that differences between the models did not have a notable influence on the results. When the 2006–2011 data were restricted to 2006–2007 and analyzed using the mixed model approach, including the selection criteria that allowed more than one record per person, several factors that were not found significant by Hofmann *et al.* (2010b) were significant in this analysis.

Cleaning spray equipment was a significant risk factor in all three of the 2006–2007 analyses, but not in the 2006–2011 analysis, which may indicate that safer decontamination practices have been adopted over the years, leading to decreased exposures when cleaning equipment. The use of full-face respirators was found to be a significant protective factor in the Hofmann *et al.* (2010b) analysis, but not in any of the other analyses, which suggests that the original finding may have been due to a Type I error (i.e. detection of an erroneous significant effect).

Study strengths and limitations

The cholinesterase monitoring program in Washington State has provided a valuable opportunity for us to evaluate potential sources of exposure to OP/CB insecticides among agricultural pesticide handlers. By design, all subjects enrolled in this study had been handling OP or carbamate pesticides for a minimum of 30 h in the 30 days prior to ChE testing. It is highly likely that most, if not all, subjects received some exposure to these compounds even if a significant (i.e. >20%) depression in an individual's BuChE activity was not observed. BuChE activity is, unfortunately, a somewhat insensitive measure of an individual subject's exposure. Due to variations in assay performance and within-individual biological variation, even at the 20% depression threshold used in the Washington State Cholinesterase monitoring program, the false-positive rate for an individual subject is between 1 and 6% (Kalman *et al.*, 2006). However, when the BuChE data are aggregated across multiple samples and individuals, statistically significant changes in BuChE are observable at the group level well below the 20% threshold used for individual samples. For example, as we demonstrate in Table 3, a depression of ~3% in BuChE activity is statistically significant for aggregated data. Similarly, a prior analysis of Washington State's Cholinesterase monitoring data observed a statistically significant depression of 4.8% in BuChE activity comparing subjects' pre-season baseline sample to their first periodic follow-up sample (Kalman *et al.*, 2006). While our study subjects reported few symptoms of possible acute pesticide-related illness, previous studies have indicated that neurological effects were observed in Egyptian cotton farmers in association with pesticide exposure that resulted in BuChE inhibition of <20% (Rohlman *et al.*, 2011).

Study participants were unaware of their follow-up ChE test results when they took the surveys, and acute-pesticide-related symptoms were relatively uncommon in this population, so the impact of reporting or healthy worker survivor effect biases on our findings in this study was probably minimal.

Because we relied on self-reported exposure information, it is possible that there may have been some non-differential misclassification of exposure in our analyses, which would attenuate the observed associations. High levels of agreement between survey data and field observations demonstrate the validity of the survey instrument, and previous analyses of test–retest reliability showed substantial agreement for selected survey questions (Hofmann *et al.*, 2010a). Nonetheless, future studies would benefit from incorporating other strategies for obtaining information on pesticide use, work activities, and PPE use to reduce the potential for this misclassification.

We evaluated BuChE inhibition in relation to use of specific OP/CBs during the preceding 30 days. However, due to time constraints, we were unable to collect detailed information regarding the degree of exposure to specific OP/CBs, such as quantity and type of pesticide handled for each work activity. This limited our ability to characterize the risk of BuChE inhibition associated with specific OP/CBs. Despite the large sample size in this investigation, we were unable to thoroughly evaluate some relatively uncommon exposures, such as not using chemical-resistant gloves. Thomas *et al.* (2010) found a significant increase in 2,4-D urinary metabolites in applicators who did not wear rubber gloves. Because all participants in our study reported wearing nitrile gloves, we could not evaluate whether or not use of these gloves was associated with BuChE inhibition, and we did not see associations with specific combinations of gloves used.

BuChE levels have been shown to vary by biological factors such as gender, age, and degree of overweightness (Lepage *et al.*, 1985; Zimmer *et al.*, 2012). While gender and age were accounted for, weight data were not collected and could not be adjusted for as a potential confounding variable in this analysis. However, accounting for age may have also captured variability due to overweightness. Lepage *et al.* (1985) found that although BuChE activity increased with overweightness, this also correlated with increases by age category as well.

Although the number of workers providing baseline samples under the Washington State Cholinesterase monitoring rule stayed fairly constant at ~2000 workers per year since 2007, the number of workers who returned for follow-up testing after meeting the 30-h threshold declined from 386 workers in 2007 to 186 workers in 2011 (Furman, 2011). It is possible that workplaces are distributing jobs, so there are more workers participating in handling activities but fewer workers who participate in handling activities for more than 30 h in 30 days, after which follow-up testing is required. The number of workplace investigations resulting from the cholinesterase monitoring program steadily declined from 2004 to 2011. Of the 580 pesticide handlers who participated in the WA State monitoring program and came in for at least one follow-up test in 2004, 16.7% (n = 97) were above the 20% threshold level and 3.8% (n = 22) resulted in workplace removals. In 2011, 186 handlers came in for follow-up testing, and 3.2% (n = 6) were above the 20% threshold level and there were no workplace removals (Kalman *et al.*, 2006; Furman, 2011).

The trend in decreasing workplace investigations from 2007 to 2011 may also be attributed in part to the phase out of azinphos-methyl for use on apple crops in Washington State. In 2006, the EPA issued its final decision to phase out the remaining uses of azinphos-methyl by the end of the 2012 growing season. Starting in 2007, there have been mandatory decreasing application rates and more restrictions when using azinphos-methyl. Approximately 300 000 pounds of azinphos-methyl, chlorpyrifos, and carbaryl, three commonly used OP/CB insecticides, were applied in Washington State apple orchards in 2011 (National Agricultural Statistics Service, 2012), a 49% decrease from 2007 (National Agricultural Statistics Service, 2008). The use of azinphos-methyl decreased by 83% and the use of chlorpyrifos decreased by 30%, when comparing use in 2007–2011. The introduction of an integrated pest management program by Washington State University (WSU) extension also impacted pesticide use in the state. The Pest Management Transition Project was started by WSU in 2008 in response to the tree fruit industry's concern about available insect management tools after the phase out of azinphos-methyl. The program has expanded since its start in 2008 and promotes the adoption of non-OP insecticides along with other tools, such as biological control (Brunner *et al.*, 2010).

CONCLUSIONS

The findings of this study confirm that several factors previously reported to be associated with BuChE inhibition are important determinants of OP/CB exposure among agricultural pesticide handlers. We confirmed that mixing and loading chemicals were associated with greater BuChE inhibition and that the use of chemical-resistant boots and lockers for PPE storage were protective factors. Our findings point toward logical interventions to reduce exposure such as the implementation of engineering controls for mixing/loading activities, requirements for appropriate footwear, and the regular use of lockers for PPE storage. Studies are needed to assess whether aprons are being used correctly during mixing/loading and decontaminated adequately to determine whether aprons are effective at reducing exposure. This study built upon an existing state mandated biological monitoring program. When properly designed, biological monitoring programs can provide opportunities for identifying deficiencies in worker protection and emphasize the positive impact of safe pesticide handling practices.

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DISCLAIMER

The authors have not been involved in legal testimony or consultancy related to material in the paper.

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