Occupational determinants of serum cholinesterase inhibition among organophosphate-exposed agricultural pesticide handlers in Washington State

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ABSTRACT

Objective To identify potential risk factors for serum cholinesterase (BuChE) inhibition among agricultural pesticide handlers exposed to organophosphate (OP) and N-methyl-carbamate (CB) insecticides.

Methods We conducted a longitudinal study among 154 agricultural pesticide handlers who participated in the Washington State cholinesterase monitoring program in 2006 and 2007. BuChE inhibition was analysed in relation to reported exposures before and after adjustment for potential confounders using linear regression. ORs estimating the risk of BuChE depression (>20% from baseline) were also calculated for selected exposures based on unconditional logistic regression analyses.

Results An overall decrease in mean BuChE activity was observed among study participants at the time of follow-up testing during the OP/CB spray season relative to pre-season baseline levels (mean decrease of 5.6%, $p<0.001$). Score for estimated cumulative exposure to OP/CB insecticides in the past 30 days was a significant predictor of BuChE inhibition ($\beta=-1.74$, $p<0.001$). Several specific work practices and workplace conditions were associated with greater BuChE inhibition, including mixing/loading pesticides and cleaning spray equipment. Factors that were protective against BuChE inhibition included full-face respirator use, wearing chemical-resistant boots and storing personal protective equipment in a locker at work.

Conclusions Despite existing regulations, agricultural pesticide handlers continue to be exposed to OP/CB insecticides at levels resulting in BuChE inhibition. These findings suggest that modifying certain work practices could potentially reduce BuChE inhibition. Replication from other studies will be valuable.

What this paper adds

- Agricultural pesticide handlers who are exposed to organophosphate and N-methyl-carbamate insecticides may experience inhibition of serum cholinesterase enzyme activity, a short-term marker of exposure and early biological effects.
- In this study, handlers who mixed/loaded pesticides or cleaned spray equipment had significantly greater serum cholinesterase inhibition than handlers who did not perform these activities.
- Several work practices appeared to protect against serum cholinesterase inhibition, including wearing a full-face respirator (rather than a half-face respirator), wearing chemical-resistant footwear and storing personal protective equipment in a locker at work.
- Results of this study suggest that models used to characterise occupational pesticide exposure for regulatory risk assessments may underestimate the degree of exposure attributable to specific work activities and practices.

BACKGROUND

Organophosphate (OP) and N-methyl-carbamate (CB) insecticides are widely used in agriculture. In Washington State, approximately 589 000 lb (267 166 kg) of azinphos-methyl, chlorpyrifos and carbaryl (three common OP/CB insecticides) were applied in apple orchards in 2007.[1] Other crops grown in Washington State are also frequently treated with OP/CBs including pears, cherries, grapes and potatoes.[1]

Acute effects of OP/CB exposure have been well documented; inhibition of neuronal acetylcholinesterase (AChE) enzyme activity is the main mechanism of OP/CB toxicity.[2] AChE hydrolyses the neurotransmitter acetylcholine, and thereby plays a critical role in regulating nerve transmissions in the central and peripheral nervous systems.[2] Cholinesterases (ChE) are found in blood in two different forms; AChE is associated with red blood cell membranes, and butyrylcholinesterase (BuChE) is present in serum.[3] Both AChE and BuChE inhibition are considered to be markers of early biological effects related to OP/CB exposure.[4] Generally, AChE inhibition is considered to be a better marker of toxicity, whereas BuChE inhibition is a more sensitive marker of exposure because it is inhibited more effectively than AChE by most OP/CBs including chlorpyrifos, diazinon and malathion.[5] BuChE measurements have been used successfully as endpoints in several previous studies of OP-exposed individuals.[6–8]

Among agricultural workers in the USA, OP/CBs continue to be responsible for a high proportion of pesticide poisonings,[9] likely due to their high acute toxicity and widespread use in agriculture. In an analysis of acute pesticide poisonings among US agricultural workers from 1998 to 2005, Calvert *et al* found that OP/CBs were implicated more frequently than any other class of pesticides.[9] There is also growing concern about a variety of health endpoints that may be associated with chronic exposure to OP/CB insecticides, including chronic neurological effects[10 11] and various cancers.[12]

Agricultural pesticide handlers are workers who are involved in the pesticide application process, which includes applying pesticides and related activities, such as mixing and loading pesticides into spray tanks and repairing application equipment. Handlers are generally considered to have higher levels of pesticide exposure than agricultural workers engaged in other tasks. However, relatively few studies have evaluated specific pesticide handling practices and conditions in relation to biological markers of exposure. Agricultural pesticide handlers may be exposed to OP/CBs as a result of dermal contact with pesticides or spray equipment,[13] inhalation,[14] accidental spills or spray equipment malfunction,[15] inadequate use of personal protective equipment (PPE),[16 17] and lack of decontamination facilities.[18]

In 2004, the Washington State Department of Labor and Industries initiated a monitoring program among agricultural workers who handle OP/CB insecticides. Workers who participate in this monitoring program are tested for AChE and BuChE activity at an annual baseline (ie, before the OP/CB spray season), and follow-up tests are conducted throughout the spray season to evaluate ChE inhibition relative to baseline levels. Follow-up tests are only required when workers have handled OP/CBs for 30+ h in a 30-day period. Generally, most handlers who return for follow-up testing have only one follow-up test each year, although some have multiple follow-up tests during the same spray season.[19] If a worker experiences >20% AChE or BuChE inhibition at follow-up relative to annual baseline levels, the employer must conduct a work practice investigation to determine possible sources of exposure. For ≥30% AChE inhibition or ≥40% BuChE inhibition, the worker is removed from handling activities (with wage protection) until his or her ChE activity returns to within 20% of baseline.

We recruited participants from the statewide ChE monitoring program for a study to identify workplace and behavioural factors associated with BuChE inhibition. This study addresses the need for further epidemiological research characterising relationships between pesticide use practices and biological markers of exposure, as suggested by Acquavella *et al*[20] and Quandt *et al.*[21] Relatively few studies have evaluated pesticide-related effects among agricultural pesticide handlers due to logistic challenges in accessing and following farmworker populations over time.[10 22] By recruiting participants from the statewide ChE monitoring program, we were able to investigate potential exposures and their relationship with BuChE inhibition among agricultural workers who handle OP/CB insecticides.

METHODS

We conducted a longitudinal study among agricultural pesticide handlers in Washington State during the OP/CB spray season (April–July) in 2006 and 2007. To recruit participants, we collaborated with two clinics that conducted ChE monitoring in eastern Washington State. Participants were recruited at the clinic or the worksite at the time of follow-up ChE testing. We used a computer-based survey instrument to collect information from participants. The survey was administered on tablet computers in either Spanish or English. All questions were displayed on the screen and audio-recorded, and icons or photos were used to represent possible responses for most questions.

The final survey consisted of 64 items. We collected information about: (1) OP/CB insecticides used and crops treated; (2) pesticide handling activities performed and spray equipment used; (3) duration and frequency of handling activities; (4) use, condition and storage of PPE; (5) decontamination practices; (6) acute exposure events; and (7) pesticide safety training. We also collected information about symptoms that may be related to OP/CB exposure, non-occupational risk factors for BuChE inhibition and demographic characteristics. Questions about potential sources of exposure and pesticide-related symptoms focused on the 30-day period prior to the interview and follow-up ChE test. We considered this 30-day period to be the most aetiologically relevant in terms of risk of BuChE inhibition because BuChE activity levels recover naturally over time.[5] Moreover, focusing on relatively recent exposures likely facilitated recall among study participants.

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

Exposure algorithm scores

Several algorithms were used to calculate scores for OP/CB toxicity, work activities and PPE use. The toxicity score was estimated by assigning values to specific OP/CBs based on the relative potency factors used in the United States Environmental Protection Agency (USEPA) cumulative risk assessments for OP and CB insecticides.[23 24] Relative potency factors were determined by the USEPA based on the degree of brain AChE inhibition in rat studies. As in the USEPA cumulative risk assessments, we assumed additive effects of exposures to multiple OP/CBs. It should be noted that one participant had an implausibly high toxicity score that was inconsistent with: (1) OP/CB insecticides registered for use on reported crops treated; and (2) OP/CB use by other participants from the same orchard. This record was therefore excluded from all analyses involving OP/CB toxicity score. Scores for work activities and PPE use were based on algorithms developed in the Agricultural Health Study[25]; these algorithms have been validated in several studies in different US regions with various chemicals and crops.[26 27] Work activity scoring was modified slightly to include tower sprayers and cleaning activities, and PPE scoring was modified to reflect greater use of PPE among participants in this study relative to Agricultural Health Study participants. PPE score was expressed in terms of the estimated likelihood of exposure: handlers wearing full PPE received a score of zero (lowest possible score), and handlers wearing no PPE received a score of 14 (highest possible score).

For all analyses, the exposure score variables were transformed into z scores (ie, standardised based on the mean and standard deviation) to allow for meaningful comparisons between these variables. We also calculated a score for cumulative OP/CB exposure in the last 30 days by adding the z score values for each of the individual exposure score variables:

$$Z_{total} = Z_{toxicity} + Z_{work\ activity} + Z_{PPE}$$

A detailed description of the scoring system used to estimate values for OP/CB toxicity, work activities, and PPE use is provided in Appendix A.

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 for laboratory testing. BuChE assays were performed by the Washington State Public Health Laboratories in 2006 and by Pathology Associates Medical Laboratories in 2007. Both laboratories measured BuChE activity using the Ellman method[28] with the ChE reagent kit from Roche Diagnostics. The Public Health Laboratories measured BuChE activity using an automated Dade Dimension AR system, and Pathology

Associates Medical Laboratories used an Olympus AU5421/AU2700 system. Both laboratories had high precision for BuChE measurements; the coefficients of variation were 2.5% in 2006 and 2.6% in 2007.[29] The main outcome in our study was BuChE inhibition, which was defined as the per cent change in BuChE activity comparing levels at follow-up during the OP/CB spray season against pre-season baseline levels for each handler.

We did not evaluate AChE inhibition because assays performed in 2007 had low precision (16.7% coefficient of variation),[29] and analyses of state monitoring program data found little overall evidence of AChE inhibition. In 2006, mean AChE inhibition among handlers with at least one follow-up test was 1.8%, and only two of the 472 handlers with follow-up tests had >20% AChE inhibition.[19] AChE inhibition may be a more relevant outcome for handlers in developing countries where higher levels of OP/CB exposure are generally observed.[30 31]

Sample selection

Records for 154 participants with complete surveys and both baseline and follow-up BuChE test results were included in this analysis. This represents 50.7% of the 304 pesticide handlers who were invited to participate in this study during the 2006 and 2007 spray seasons. Study participants were similar to all handlers in the statewide monitoring program in terms of age, ethnicity and gender. Mean age was 33.6 years for study participants and 32.9 years for all handlers in the state program, and almost all handlers (>99%) in both this study and the state program were Latino males.[19]

For handlers who participated in this study in either 2006 or 2007, we selected the first completed survey and corresponding BuChE data for this analysis. Some handlers participated in this study in both 2006 and 2007 (N=22). For those subjects, we chose only one record per subject to ensure independent results. The record with the larger value of BuChE inhibition was included to enhance the range of values for statistical testing. This would not introduce bias in the results because this choice was made without consideration of determinants of exposure.

Analysis

We evaluated BuChE inhibition in relation to overall OP/CB exposure during the past 30 days based on the algorithms described above. Cumulative OP/CB exposure score was modelled as a continuous predictor, and per cent change in BuChE activity from baseline level was modelled as a continuous outcome (ie, degree of BuChE inhibition per 1-unit increase in OP/CB exposure score). In another model, we evaluated OP/CB toxicity score, work activity score and PPE score as separate predictors of BuChE inhibition. Both models included year of participation, days since baseline ChE test and age in years as covariates. Linear regression with robust standard error estimates was used for each of these analyses.

Specific exposure variables were selected for multivariate analysis based on a priori hypotheses and preliminary bivariate analyses. Several potential confounding factors were included in the statistical models, including year of participation, days since baseline ChE test, age in years, toxicity score, work activity score and PPE score. Per cent change in BuChE activity from baseline levels (ie, BuChE inhibition) was used as the main endpoint in these analyses. Due to wide inter-individual variability in BuChE activity,[32] the relative change from baseline levels may be considered to be more biologically meaningful than the absolute level. Additionally, analyses were performed evaluating BuChE activity at follow-up (with baseline BuChE activity included as a covariate) and with log-transformed BuChE values. We also evaluated the risk of BuChE depression (>20% inhibition from baseline levels) in relation to specific exposure variables using multiple logistic regression adjusting for year of participation, days since baseline ChE test and age.

Differences were considered to be statistically significant if p values were <0.05. Analyses were performed using Intercooled Stata 9.2.

RESULTS

All of the participants in this study were male, and all with reported ethnicity, except for one participant, were Hispanic/Latino (table 1). Almost all participants completed the survey in Spanish (97%). Most participants were younger than 35 years of age (61%), and approximately half had a primary school education or less. Many participants had limited experience handling pesticides; approximately half had been employed as handlers for 3 years or less. Over three-fourths of our sample had baseline ChE tests within 60 days prior to their follow-up ChE

Table 1 Demographic characteristics of study participants (N=154)*

Characteristic	N	%
Sex		
Male	154	100.0%
Race/ethnicity		
Hispanic/Latino	152	99.3%
White, non-Hispanic	1	0.7%
Age in years		
18–24	25	16.3%
25–34	69	45.1%
35–49	49	32.0%
≥50	10	6.5%
Level of education		
Did not attend school	5	3.2%
Did not complete primary school	19	12.3%
Primary school	56	36.4%
Middle school	57	37.0%
High school	17	11.0%
Able to read		
In Spanish	152	98.7%
In English	48	31.4%
Years employed as a pesticide handler		
1 year or less	22	18.3%
2–3 years	37	30.8%
4–5 years	26	21.7%
6–10 years	22	18.3%
>10 years	13	10.8%
Location of home		
In town	76	50.0%
Rural area, away from orchards	23	15.1%
Rural area, near orchards	20	13.2%
In/next to orchards	28	18.4%
Other	6	3.9%
Survey language		
Spanish	150	97.4%
English	4	2.6%
Year of participation		
2006	82	53.3%
2007	72	46.8%
Days since baseline ChE test		
≤30 days	9	5.9%
31–60 days	109	71.2%
61–90 days	16	10.5%
>90 days	19	12.4%

*Missing values were excluded from percentages.
ChE, cholinesterase.

Table 2 Change in serum cholinesterase (BuChE) activity during the OP/CB spray season relative to baseline levels

Year	N	Per cent change in BuChE activity				BuChE depression*	
		Mean (SD)	p Value†	Median	p Value‡	N	%
Combined	154	−5.64% (11.65)	<0.001	−3.58%	<0.001	18	11.7%
2006	82	−4.82% (13.77)	0.0016	−2.23%	0.0076	13	15.9%
2007	72	−6.58% (8.64)	<0.001	−5.21%	<0.001	5	6.9%

*>20% BuChE inhibition from baseline activity level.
†Paired t test comparing mean baseline and follow-up BuChE activity.
‡Wilcoxon signed-rank test comparing baseline and follow-up BuChE activity.
BuChE, butyrylcholinesterase (serum cholinesterase); OP/CB, organophosphate/N-methyl-carbamate pesticide.

test; longer time since baseline testing was associated with greater BuChE inhibition (β=−0.145; p<0.001).

Overall, mean BuChE activity at follow-up was significantly lower than BuChE activity at baseline (p<0.001) (table 2). Mean BuChE inhibition was somewhat greater among handlers who participated in 2007 relative to participants in 2006; however, this difference was not statistically significant (mean (SD) of −4.8% (13.8) and −6.6% (8.6) for 2006 and 2007, respectively; p=0.34). Approximately 12% of the study sample had >20% BuChE depression, which was consistent with the frequency of BuChE depression in the statewide ChE monitoring program in 2006 and 2007.[29] More cases of BuChE depression were observed in 2006 than in 2007 (p=0.086, χ^2 test).

We observed a significant trend toward greater BuChE inhibition with increasing cumulative OP/CB exposure score (table 3). When analysed as separate predictors, OP/CB toxicity score and PPE score were significantly associated with BuChE inhibition, and there was a borderline significant association between work activity score and BuChE inhibition. Results were similar after several records with high outlying values for toxicity score or PPE score were excluded (data not shown). There was little evidence of correlation between OP/CB toxicity score, work activity score and PPE score in this analysis (correlation coefficients ranged from −0.06 to 0.12, p≥0.16).

Risk factors for BuChE inhibition

Several particular work activities were associated with greater BuChE inhibition (table 4) and risk of >20% BuChE depression (table 5). Unadjusted results are reported in appendices B and C. On average, handlers who reported mixing/loading pesticides had 5.25% greater BuChE inhibition than handlers who did not mix/load pesticides after adjusting for covariates (p=0.007). In the adjusted logistic regression analysis, we found that mixer/loaders were approximately twice as likely to experience BuChE depression as other handlers. Handlers who reported cleaning spray equipment had an average of 4.4% greater BuChE inhibition than handlers who did not clean spray equipment (p=0.033), and we observed a nine-fold increased risk of BuChE depression among handlers who cleaned spray equipment. Some other work activities and exposures were moderately, although not significantly associated with BuChE inhibition, including repairing spray equipment, cleaning out pesticide containers, cleaning up after pesticide spills, and reported use of azinphos-methyl, carbaryl or multiple OP/CBs in the last 30 days. There were no consistent associations of BuChE inhibition and methods of pesticide application, air blast or tower spraying.

Table 3 BuChE inhibition in relation to OP/CB exposure score in the last 30 days*

Exposure score variable	β Coefficient†	95% CI	p Value
Cumulative exposure score	−1.74	−2.61 to −0.86	<0.001
Toxicity score	−1.50	−2.93 to −0.06	0.041
Work activity score	−1.67	−3.39 to 0.05	0.057
PPE score	−2.03	−3.50 to −0.57	0.007

*Multiple linear regression with robust standard error estimates. Adjusted for year of participation, days since baseline ChE test, and age in years. Toxicity score, work activity score, and PPE score were all included in a single model when they were analysed as separate predictors. Analyses were restricted to participants with non-missing values for all covariates (N=118).
†Difference in per cent change in BuChE activity from baseline per 1-unit increase in score.
BuChE, butyrylcholinesterase (serum cholinesterase); ChE, cholinesterase; OP/CB, organophosphate/N-methyl-carbamate pesticide; PPE, personal protective equipment.

Recency of exposure did not appear to be associated with the degree of BuChE inhibition. There was some suggestion of an association between length of spray sessions and BuChE inhibition, with handlers who reported three to four spray sessions of 8 h or more having on average 6.9% greater BuChE inhibition than participants who reported no 8 h spray sessions in the last 30 days. However, this association was not statistically significant, and we did not see a consistent trend in the relationship between number of 8 h spray sessions and BuChE inhibition.

Greater BuChE inhibition was observed with increasing age after adjustment for covariates (p=0.048). Self-reported health status was also associated with BuChE inhibition, with participants who reported ‘poor’ or ‘fair’ health having 6.4% greater BuChE inhibition on average relative to participants who reported ‘excellent’ health status (p=0.02).

When analyses were repeated using log-transformed BuChE values, similar associations were observed for each of these exposures (results not shown).

Factors protecting against BuChE inhibition

Wearing a full-face respirator appeared to protect against BuChE inhibition. Relative to full-face respirator users, handlers who wore half-face respirators had approximately 7.0% greater BuChE inhibition on average (p=0.034). Half-face respirator users were almost seven times as likely as full-face respirator users to experience BuChE depression. Wearing chemical-resistant footwear was also protective against BuChE inhibition. Handlers who did not wear chemical-resistant footwear had an average of 11.4% greater BuChE inhibition (p=0.041), and an estimated 7.6-fold increased risk of BuChE depression. Relative to handlers who wore nitrile gloves alone, those who wore nitrile gloves with cloth gloves underneath had somewhat less BuChE inhibition, although this difference was not statistically significant (p=0.087). In terms of PPE storage, handlers who reported storing PPE in a locker at work had less BuChE inhibition than handlers who did not use lockers. On average, handlers who did not use lockers for PPE storage had 7.6% greater BuChE inhibition, and were 5.8 times as likely to experience BuChE depression as handlers who did use lockers.

Contrary to expectations, handlers who reported wearing chemical-resistant aprons had somewhat greater BuChE inhibition than handlers who did not wear chemical-resistant aprons (p=0.119). Also, we did not observe any association between hand washing practices before breaks during pesticide applications and BuChE inhibition.

Table 4 Differences in BuChE inhibition in relation to selected exposures after covariate adjustment*

Exposure(s)	N	β Coefficient	95% CI	p Value
OP/CB compounds used[1]				
Chlorpyrifos	119			
No	49	Ref		
Yes	70	1.69	−3.52 to 6.89	0.522
Carbaryl	119			
No	80	Ref		
Yes	39	−2.05	−7.54 to 3.44	0.461
Azinphos-methyl	119			
No	99	Ref		
Yes	20	−3.68	−14.35 to 7.00	0.496
Multiple OP/CBs	119			
No	88	Ref		
Yes	31	−2.50	−8.35 to 3.35	0.399
Crops treated[2]				
Number of crops treated	114			0.651†
1 crop	83	Ref		
2 crops	22	−1.31	−6.30 to 3.68	0.604
3+ crops	9	−0.93	−8.29 to 6.42	0.802
Application methods[3]				
Air blast sprayer	116			
No	22	Ref		
Yes	94	0.58	−4.04 to 5.20	0.804
Tower sprayer	116			
No	100	−2.47	−7.81 to 2.86	0.360
Yes	16	Ref		
Handling activities[3]				
Mixing/loading	120			
No	39	Ref		
Yes	81	−5.25	−9.06 to −1.43	0.007
Entering pesticide storage area	120			
No	86	Ref		
Yes	34	0.71	−4.24 to 5.66	0.777
Early re-entry in treated area	120			
No	98	Ref		
Yes	22	0.07	−4.90 to 5.04	0.979
Repairing spray equipment	120			
No	106	Ref		
Yes	14	−3.16	−8.38 to 2.05	0.232
Cleaning activities[3]				
Cleaning PPE	120			
No	38	Ref		
Yes	82	−1.60	−6.60 to 3.40	0.526
Cleaning spray equipment	120			
No	53	Ref		
Yes	67	−4.39	−8.44 to −0.35	0.033
Cleaning pesticide containers	120			
No	89	Ref		
Yes	31	−2.73	−6.58 to 1.13	0.164
Cleaning pesticide storage space	120			
No	107	Ref		
Yes	13	0.60	−4.49 to 5.69	0.816
Cleaning pesticide spill	120			
No	114	Ref		
Yes	6	−4.12	−13.22 to 4.98	0.372
Exposure time[2]				
Days since last exposure	100			0.735†
Today	7	1.46	−11.11 to 14.04	0.818

Continued

Table 4 Continued

Exposure(s)	N	β Coefficient	95% CI	p Value
Yesterday	9	−1.34	−14.06 to 11.38	0.835
2−7 days ago	49	−3.05	−13.04 to 6.95	0.546
8−14 days ago	13	−0.97	−10.56 to 8.62	0.842
15−30 days ago	16	−0.16	−10.54 to 10.23	0.976
>30 days ago	6	Ref		
No. 8+ hour spray sessions	118			0.512†
None	8	Ref		
1−2 times	49	−3.48	−14.40 to 7.44	0.529
3−4 times	39	−6.86	−18.08 to 4.37	0.229
5+ times	22	−2.71	−13.85 to 8.44	0.631
Full-face respirator	118			
No (half-face)	85	−6.95	−13.36 to −0.55	0.034
Yes	33	Ref		
Powered air purifying respirator	90			
No (half-face)	85	−0.14	−6.68 to 6.41	0.966
Yes	5	Ref		
Disposable gloves under nitrile	108			
No	85	−0.19	−4.78 to 4.39	0.934
Yes	23	Ref		
Cloth gloves under nitrile	102			
No	85	−4.75	−10.21 to 0.71	0.087
Yes	17	Ref		
Chemical-resistant footwear	130			
No	5	−11.40	−22.35 to −0.45	0.041
Yes	125	Ref		
Rain suit	131			
No	16	−2.65	−8.50 to 3.20	0.372
Yes	115	Ref		
Chemical-resistant apron	131			
No	111	3.93	−1.03 to 8.88	0.119
Yes	20	Ref		
Locker for PPE[2]	116			
No	55	−7.58	−12.36 to −2.81	0.002
Yes	61	Ref		
No. activities without decontamination‡	118			0.750†
None	60	Ref		
One	27	−0.03	−5.08 to 5.02	0.990
Two	20	−3.85	−8.88 to 1.18	0.132
Three or more	11	5.34	−1.63 to 12.31	0.132
Demographics[2]				
Age category	118			0.048†
18−24	21	Ref		
25−34	52	1.87	−3.95 to 7.68	0.526
35−49	40	−1.03	−6.38 to 4.31	0.702
50+	5	−8.01	−19.87 to 3.85	0.184
Health status	118			0.032†
Excellent	17	Ref		
Good	67	−4.40	−9.69 to 0.89	0.102
Poor/fair	34	−6.40	−11.75 to −1.04	0.020

*Based on multiple linear regression with robust standard error estimates. All adjusted models included year of participation, days since baseline ChE test, and age in years. Additionally, the following covariates were included in specific analyses: [1]work activity score, PPE score; [2] toxicity score, work activity score, PPE score; [3] toxicity score, PPE score; [4] toxicity score, work activity score.
†Test for trend (continuous or ordered categorical exposure variable).
‡Includes not washing hands before drinking, eating, smoking, using a cellular phone, using a two-way radio, urinating in the orchard or field, or using a portable toilet.
BuChE, butyrylcholinesterase (serum cholinesterase); OP/CB, organophosphate/N-methyl-carbamate pesticide; PPE, personal protective equipment.

Table 5 Adjusted odds ratios for BuChE depression in relation to selected exposures based on unconditional logistic regression*

Exposure(s)	Cases (%)†	OR	95% CI
OP/CB compounds used			
Chlorpyrifos			
No	11 (21%)	Ref	
Yes	6 (7%)	0.43	0.11 to 1.63
Carbaryl			
No	6 (6%)	Ref	
Yes	11 (27%)	3.38	0.95 to 11.99
Azinphos-methyl			
No	11 (10%)	Ref	
Yes	6 (25%)	1.23	0.20 to 7.48
Multiple OP/CBs			
No	10 (10%)	Ref	
Yes	7 (19%)	1.05	0.26 to 4.26
Crops treated			
Number of crops treated			
1 crop	11 (10%)	Ref	
2 crops	4 (13%)	0.93	0.22 to 3.92
3+ crops	2 (22%)	0.98	0.13 to 7.41
Application methods			
Air blast sprayer			
No	2 (7%)	Ref	
Yes	15 (13%)	1.66	0.30 to 8.99
Tower sprayer			
No	17 (12%)	Undefined‡	
Yes	0 (0%)		
Handling activities			
Mixing/loading			
No	2 (4%)	Ref	
Yes	15 (15%)	2.23	0.42 to 11.68
Entering pesticide storage area			
No	9 (8%)	Ref	
Yes	8 (18%)	2.08	0.60 to 7.18
Early re-entry in treated area			
No	14 (12%)	Ref	
Yes	3 (9%)	0.57	0.12 to 2.79
Repairing spray equipment			
No	15 (12%)	Ref	
Yes	2 (9%)	0.64	0.11 to 3.73
Cleaning activities			
Cleaning PPE			
No	5 (11%)	Ref	
Yes	12 (11%)	0.98	0.28 to 3.45
Cleaning spray equipment			
No	2 (3%)	Ref	
Yes	15 (18%)	9.15	1.66 to 50.30
Cleaning pesticide containers			
No	11 (10%)	Ref	
Yes	6 (15%)	1.29	0.36 to 4.66
Cleaning pesticide storage space			
No	15 (11%)	Ref	
Yes	2 (11%)	1.25	0.22 to 7.08
Cleaning pesticide spill			
No	16 (11%)	Ref	
Yes	1 (10%)	0.35	0.03 to 3.58
Exposure time			
Time of last exposure			
Within the last week	10 (12%)	1.41	0.37 to 5.33
>1 week ago	4 (8%)	Ref	
No. 8+ hour spray sessions			
None	2 (14%)	Ref	
1–2 times	6 (9%)	0.34	0.05 to 2.33
3–4 times	9 (19%)	0.77	0.12 to 4.89
5+ times	1 (3%)	0.15	0.01 to 2.09
Personal protective equipment			
Full-face respirator			
No (half-face)	14 (13%)	6.77	1.05 to 43.69
Yes	2 (6%)	Ref	
Powered air purifying respirator			
No (half-face)	14 (13%)	2.88	0.25 to 33.62
Yes	1 (20%)	Ref	
Disposable gloves under nitrile gloves			
No	11 (11%)	1.02	0.22 to 4.81
Yes	3 (11%)	Ref	
Cloth gloves under nitrile gloves			
No	11 (11%)	0.88	0.14 to 5.66
Yes	2 (10%)	Ref	
Chemical-resistant footwear			
No	4 (67%)	7.64	1.03 to 56.61
Yes	14 (10%)	Ref	
Rain suit			
No	3 (16%)	2.30	0.48 to 11.02
Yes	15 (11%)	Ref	
Chemical-resistant apron			
No	15 (11%)	0.81	0.17 to 3.90
Yes	3 (15%)	Ref	
Locker for PPE			
No	11 (17%)	5.83	1.52 to 22.40
Yes	5 (6%)	Ref	
Decontamination practices			
No. activities without decontamination§			
None	9 (11%)	Ref	
One	4 (12%)	1.67	0.40 to 6.96
Two	4 (16%)	2.37	0.52 to 10.85
Three or more	1 (7%)	0.79	0.08 to 8.03
Demographics			
Age in years			
18–24	1 (4%)	Ref	
25–34	7 (10%)	2.05	0.21 to 19.73
35–49	8 (16%)	3.89	0.41 to 37.13
50+	2 (20%)	8.19	0.54 to 124.1
Health status			
Excellent	1 (4%)	Ref	
Good	11 (13%)	5.19	0.53 to 50.84
Poor/fair	6 (13%)	4.25	0.40 to 44.70

*Adjusted for year of participation (2006, 2007), days since baseline ChE test (≤60 days, 61–90 days, >90 days), and age category (18–24, 25–34, 35–49, 50+ years). Two records with missing data for days since baseline ChE test or age were excluded from the adjusted analyses.
†Cases of BuChE depression were defined as >20% decrease from baseline BuChE activity. Percentages refer to the proportion of cases of BuChE depression within each exposure category.
‡No cases of BuChE depression were observed among handlers who used tower sprayers (N=20).
§Includes not washing hands before drinking, eating, smoking, using a cellular phone, using a two-way radio, urinating in the orchard or field, or using a portable toilet.
BuChE, butyrylcholinesterase (serum cholinesterase); ChE, cholinesterase; OP/CB, organophosphate/N-methyl-carbamate pesticide; PPE, personal protective equipment.

Results were essentially unchanged when these analyses were repeated using log-transformed BuChE values, except that the association between chemical-resistant footwear use and less BuChE inhibition was only borderline significant (p=0.053).

DISCUSSION

This study identified several work activities that were associated with BuChE inhibition, and some PPE use practices that appeared to prevent BuChE inhibition. Results were generally consistent with the findings of other studies.[21 33] Handlers who mix/load pesticides are generally considered to have relatively high exposures,[25] and this activity may be particularly hazardous due to potential exposure to OP/CBs in their concentrated forms (ie, before being diluted for application). We also found that handlers who cleaned spray equipment had significantly greater BuChE inhibition. Similarly, Arbuckle *et al* found that washing spray equipment was associated with elevated urinary levels of the herbicide 2,4-D.[34] Although 2,4-D is not a ChE-inhibiting pesticide, the exposure pathway is likely to be similar. There was also some suggestion in our study that handlers may have been exposed while cleaning out pesticide containers or cleaning up after pesticide spills, although these factors were not significantly associated with BuChE inhibition after adjustment.

Use of PPE has been shown to minimise pesticide exposures effectively.[16 21 34 35] In our study, handlers who wore full-face respirators and chemical-resistant footwear had significantly lower levels of BuChE inhibition. Storing PPE in a locker was also protective against BuChE inhibition. Handlers who change into chemical-resistant boots for applications and store PPE in a locker at work may have less 'take home' exposure. Although such PPE use and storage practices may afford greater protection, it is also possible that these variables could be surrogates for safer handling practices in general.

Other studies have shown that glove use is associated with lower levels of exposure while mixing or applying pesticides.[34 35] We did not see any strong associations between glove use and BuChE inhibition in this study, but it should be noted that only one participant did not wear chemical-resistant gloves. As such, we could only evaluate differences in BuChE inhibition between handlers who wore chemical-resistant gloves alone (67%) and handlers who wore chemical-resistant gloves in combination with disposable gloves (18%) or cloth gloves (13%).

We found that handlers who wore chemical-resistant aprons had somewhat greater BuChE inhibition than other handlers; this association was not in the anticipated direction. Chemical-resistant aprons are generally worn by handlers while mixing/loading pesticides, which is an activity with an inherently higher risk of exposure. Although we attempted to control for handling activities in this analysis, there may have been residual confounding due to generally higher exposures among handlers who wore aprons relative to other handlers.

In terms of decontamination practices, Curwin *et al* found that hand washing significantly reduced the concentration of acephate residues on the hands of tobacco harvesters.[36] We did not see an association between hand washing practices and BuChE inhibition, which may be due to exposure misclassification. We collected self-reported information about 'usual' hand washing practices, but were unable to ascertain the frequency and consistency of such practices after each application. Such misclassification might also explain the lack of associations between decontamination before breaks (ie, potential contamination from using a cellular phone, eating or urinating) and BuChE inhibition. More detailed observations of decontamination practices as potential sources of exposure are warranted.

In addition to potential sources of exposure, we evaluated BuChE inhibition in relation to exposure to specific OP/CBs. In the unadjusted analysis, chlorpyrifos users had less BuChE inhibition relative to handlers who were exposed to other OP/CBs, including compounds that were more acutely toxic (eg, azinphos-methyl). However, the association between BuChE inhibition and chlorpyrifos use was not significant after adjustment for covariates, suggesting that confounding may have been present. In particular, days since baseline test may have been an important confounder in this analysis because chlorpyrifos is typically applied early in the spray season, whereas other OP/CBs (including azinphos-methyl) are usually applied later in the season when there is greater potential for cumulative BuChE inhibition over time. The association between use of multiple OP/CBs and BuChE inhibition is more plausible; recent studies suggest that mixed exposures can potentiate the toxic effects of specific OPs.[37]

We did not observe any association between recency of exposure and degree of BuChE inhibition. However, it should be noted that approximately two-thirds of our study population had handled pesticides within the week preceding their follow-up ChE test, and only 8% of the sample was exposed >30 days previously. This pattern suggests that there may not have been enough heterogeneity in our sample to determine the association between recency of exposure and BuChE inhibition.

Relative to handlers who reported 'excellent' health status, handlers who reported 'poor' or 'fair' health status had significantly greater BuChE inhibition. It is possible that handlers with poorer health were susceptible to BuChE inhibition, or their BuChE activity may have recovered more slowly following OP/CB exposure. However, it is also possible that handlers with greater BuChE inhibition may have experienced symptoms of pesticide-related illness, and reported poorer health *as a consequence of* OP/CB exposure. Because self-reported health status was determined cross-sectionally at the time of follow-up ChE testing, we were unable to characterise the temporal relationship between health status and BuChE inhibition. Future studies with prospective data collection may provide additional information about this association.

Study strengths and limitations

The implementation of a ChE monitoring program in Washington State provided a valuable opportunity to evaluate potential sources of exposure to OP/CB insecticides among agricultural pesticide handlers. Because participants were unaware of the results of their follow-up ChE tests at the time of the interview, and acute pesticide-related symptoms are relatively uncommon in this population, reporting or healthy worker survivor effect biases on risk estimates in this study were probably minimal. Results were not materially changed after excluding 15 participants with self-reported symptoms of pesticide-related illness (data not shown). We also repeated the analyses after excluding eight participants with a previous BuChE depression (ie, >20% BuChE inhibition on the prior follow-up visit). Again, results were essentially unchanged for most exposures, with the following exceptions: (1) cleaning pesticide containers was statistically significantly associated with greater BuChE inhibition (p=0.033); (2) the association between cleaning spray equipment and greater BuChE inhibition was only borderline significant (p=0.053); and (3) the association between wearing a full-face respirator and less BuChE inhibition was no longer significant (p=0.13).

Previous studies have noted the potential for exposure misclassification in self-reported data.[38] In the present study, reliance on self-reported exposure information may have resulted in missing data for some variables (12% for OP/CB insecticides used) and misclassification of other exposures. In particular, exposure misclassification may have been a concern for worker behaviours, which are somewhat more subjective. However, since participants were unaware of their ChE results at the time of data collection, we would expect any misclassification to be non-differential, resulting in under-estimated associations. Future studies may be able to validate self-reported exposures against direct workplace observations.

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. It is likely that this limited our ability to characterise the risk of BuChE inhibition associated with individual OP/CBs.

Due to an administrative change in the statewide monitoring program, ChE assays were performed by different laboratories, with differing measurement methods, in 2006 and 2007. This was unlikely to have been an important bias, however. Absolute BuChE levels did differ by year, yet the per cent of BuChE inhibition from baseline levels was not substantially different in 2006 and 2007 (means were 4.8% and 6.6%, respectively). Furthermore, year of participation was included as a covariate in all adjusted analyses. Moreover, when we evaluated follow-up BuChE activity as the outcome variable, results were generally consistent with the findings based on BuChE inhibition reported above (results not shown).

Finally, statistical power was limited in this study, particularly for evaluating associations with relatively uncommon exposures (eg, not wearing chemical-resistant boots). Thus, our findings should be replicated in other populations with greater heterogeneity of PPE use and other exposure-related factors. Although risk estimates from logistic regression analyses were based on a small number of cases of BuChE depression (N=18), the associations were generally in the same direction as those observed in the linear regression analyses, and several strong associations were observed.

Implications for policy and practice

Findings from this study suggest that continued efforts are needed to promote and enforce safe pesticide handling practices among agricultural pesticide handlers. We investigated modifiable worker behaviours and workplace conditions, as well as specific high-risk handling activities. These findings may ultimately inform future targeted interventions to reduce pesticide exposures.

Furthermore, evidence of an association between OP/CB exposure and BuChE inhibition in this study suggests that current regulatory exposure assessment models may underestimate exposure.[39] Estimates of occupational exposure in pesticide risk assessments could be refined based on associations between BuChE inhibition and specific work activities and practices observed in this study. It should be noted that greater use of PPE was reported among participants in this study relative to pesticide handlers in other regions of the USA and in developing countries.[25 30 31 40] Nonetheless, the general pattern of consistency of our findings with those from previous studies of pesticide exposure determinants offers some reassurance that our results have relatively broad generaliseability.

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APPENDIX A: EXPOSURE ALGORITHM SCORES FOR OP/CB TOXICITY, WORK ACTIVITIES AND PPE USE

Category	Score
Toxicity score	
Chlorpyrifos	1.0 (ref)
Azinphos-methyl	1.32
Carbaryl	0.95
Malathion	0.01
Dimethoate	4.29
Phosmet	0.36
Diazinon	0.16
Methidathion	6.25
Methamidophos	21.43
Other	1.0
Maximum score	36.8
Work activity score	
Application method	
Airblast sprayer	9
Boom sprayer	3
Tower sprayer	3
Backpack application	1
Other	3
Enclosed cab correction	×0.5
Handling activities	
Mix/load pesticides	9
Repair spray equipment	2
Enter pesticide storage room	2
Early re-entry into treated orchard	2
Cleaning activities	
PPE	1
Spray equipment	2
Pesticide containers	3
Pesticide storage space	3
Pesticide spill	3
Maximum score	46
PPE score	
Respirator/chemical-resistant headwear	
Powered air purifying respirator	7
Full-face respirator	5
Half-face respirator with:	
Goggles and/or face shield	4
Safety glasses	3
Other/no eye protection	2
Other/no respirator with:	
Eye protection	1
No eye protection	0
Hood/rain hat	+2
Chemical-resistant glove use	
Nitrile gloves, disposable gloves underneath	2
Nitrile gloves, with or without cloth gloves underneath	1
Leather gloves, other gloves, no gloves	0
Chemical-resistant footwear use	
Chemical resistant boots	2
Leather boots, other boots, no boots	0
Chemical protective clothing score	
Rain suit with apron	3
Rain suit, no apron	2
Tyvek suit or apron, no rain suit	1
Other/none	0
Maximum total	14

OP/CB, organophosphate/N-methyl-carbamate pesticide; PPE, personal protective equipment.

APPENDIX B: RESULTS OF UNADJUSTED LINEAR REGRESSION ANALYSES EVALUATING BUCHE INHIBITION IN RELATION TO SELECTED EXPOSURES*

Exposure(s)	N	Mean	β Coefficient	95% CI	p Value
OP/CB compounds used					
Chlorpyrifos	136				
No	52	−9.40%	Ref		
Yes	84	−3.46%	5.95	1.56 to 10.33	0.008
Carbaryl	136				
No	95	−3.98%	Ref		
Yes	41	−9.80%	−5.82	−10.09 to −1.56	0.008
Azinphos-methyl	136				
No	112	−4.40%	Ref		
Yes	24	−11.97%	−7.58	−14.41 to −0.74	0.030
Multiple OP/CBs	136				
No	100	−4.21%	Ref		
Yes	36	−9.95%	−5.74	−10.20 to −1.28	0.012
Crops treated					
Number of crops treated	146				0.006†
1 crop	106	−4.50%	Ref		
2 crops	31	−7.87%	−3.37	−7.63 to 0.88	0.119
3+ crops	9	−12.63%	−8.14	−13.79 to −2.48	0.005
Application methods					
Air blast sprayer	147				
No	28	−3.86%	Ref		
Yes	119	−6.20%	−2.34	−6.21 to 1.54	0.236
Tower sprayer	147				
No	127	−6.66%	−6.71	−10.32 to −3.11	<0.001
Yes	20	0.05%	Ref		

Continued

Continued

Exposure(s)	N	Mean	β Coefficient	95% CI	p Value
Handling activities					
Mixing/loading	151				
No	54	−0.96%	Ref		
Yes	97	−8.10%	−7.14	−10.59 to −3.69	<0.001
Entering pesticide storage area	151				
No	106	−5.65%	Ref		
Yes	45	−5.29%	0.36	−3.91 to 4.62	0.869
Early re-entry in treated area	151				
No	119	−5.70%	Ref		
Yes	32	−4.98%	0.72	−3.84 to 5.28	0.755
Repairing spray equipment	151				
No	128	−5.88%	Ref		
Yes	23	−3.70%	2.17	−2.41 to 6.76	0.350
Cleaning activities					
Cleaning PPE	152				
No	45	−6.07%	Ref		
Yes	107	−5.46%	0.61	−3.69 to 4.91	0.780
Cleaning spray equipment	152				
No	68	−3.50%	Ref		
Yes	84	−7.37%	−3.87	−7.34 to −0.40	0.029
Cleaning pesticide containers	152				
No	112	−4.40%	Ref		
Yes	40	−9.12%	−4.71	−8.63 to −0.80	0.019
Cleaning pesticide storage space	152				
No	134	−5.75%	Ref		
Yes	18	−4.85%	0.89	−4.16 to 5.95	0.728
Cleaning pesticide spill	152				
No	142	−5.47%	Ref		
Yes	10	−8.05%	−2.58	−9.28 to 4.11	0.447
Exposure time					
Days since last exposure	132				0.942†
Today	8	−6.46%	0.89	−8.72 to 10.50	0.855
Yesterday	12	−6.10%	1.26	−6.50 to 9.01	0.749
2–7 days ago	64	−4.57%	2.78	−4.51 to 10.08	0.451
8–14 days ago	19	−4.39%	2.96	−4.37 to 10.30	0.426
15–30 days ago	18	−4.52%	2.83	−5.52 to 11.18	0.504
>30 days ago	11	−7.35%	Ref		
No. 8+ hour spray sessions	154				0.306†
None	14	−2.63%	Ref		
1–2 times	64	−3.81%	−1.18	−8.01 to 5.66	0.734
3–4 times	47	−10.64%	−8.01	−15.36 to −0.66	0.033
5+ times	29	−3.06%	−0.43	−7.68 to 6.82	0.907
Personal protective equipment					
Full-face respirator	140				
No (half-face)	105	−7.15%	−6.56	−10.71 to −2.40	0.002
Yes	35	−0.59%	Ref		
Powered air purifying respirator	110				
No (half-face)	105	−7.15%	5.86	−0.27 to 11.99	0.061
Yes	5	−13.01%	Ref		
Disposable gloves under nitrile	128				
No	101	−5.81%	1.97	−1.92 to 5.85	0.318
Yes	27	−7.78%	Ref		
Cloth gloves under nitrile	121				
No	101	−5.81%	−3.14	−8.17 to 1.88	0.218
Yes	20	−2.67%	Ref		
Chemical-resistant footwear	153				
No	6	−20.01%	−14.90	−22.52 to −7.28	<0.001
Yes	147	−5.10%	Ref		
Rain suit	154				
No	19	−6.77%	−1.29	−5.94 to 3.36	0.584
Yes	135	−5.48%	Ref		
Chemical-resistant apron	154				
No	134	−4.93%	5.50	1.16 to 9.85	0.013
Yes	20	−10.43%	Ref		
Locker for PPE	150				
No	66	−9.54%	−7.15	−10.84 to −3.46	<0.001
Yes	84	−2.39%	Ref		
Decontamination practices					
No. activities without decontamination‡	154				0.806†
None	80	−5.00%	Ref		
One	34	−6.29%	−1.30	−5.85 to 3.25	0.573
Two	25	−8.69%	−3.69	−8.67 to 1.28	0.145
Three or more	15	−2.54%	2.46	−3.05 to 7.97	0.379
Demographics					
Age category	153				0.067†
18–24	25	−4.53%	Ref		
25–34	69	−4.75%	−0.22	−5.33 to 4.90	0.933
35–49	49	−6.92%	−2.39	−7.81 to 3.04	0.386
50+	10	−8.51%	−3.97	−12.16 to 4.21	0.339
Health status	154				0.022†
Excellent	23	−1.32%	Ref		
Good	85	−5.66%	−4.34	−9.47 to 0.79	0.097
Poor/fair	46	−7.77%	−6.45	−11.76 to −1.14	0.018

*Based on linear regression with robust standard error estimates.
†Test for trend (continuous or ordered categorical exposure variable).
‡Includes not washing hands before drinking, eating, smoking, using a cellular phone, using a two-way radio, urinating in the orchard or field, or using a portable toilet.
BuChE, butyrylcholinesterase (serum cholinesterase); OP/CB, organophosphate/N-methyl-carbamate pesticide; PPE, personal protective equipment.

APPENDIX C: UNADJUSTED ODDS RATIOS FOR BUCHE DEPRESSION IN RELATION TO SELECTED EXPOSURES BASED ON UNCONDITIONAL LOGISTIC REGRESSION

Exposure(s)	Cases (%)*	OR	95% CI
OP/CB compounds used			
Chlorpyrifos			
No	11 (21%)	Ref	
Yes	6 (7%)	0.29	0.10 to 0.83
Carbaryl			
No	6 (6%)	Ref	
Yes	11 (27%)	5.44	1.85 to 15.97

Continued

Continued

Exposure(s)	Cases (%)*	OR	95% CI
Azinphos-methyl			
No	11 (10%)	Ref	
Yes	6 (25%)	3.06	1.00 to 9.32
Multiple OP/CBs			
No	10 (10%)	Ref	
Yes	7 (19%)	2.17	0.76 to 6.22
Crops treated			
Number of crops treated			
1 crop	11 (10%)	Ref	
2 crops	4 (13%)	1.28	0.38 to 4.34
3+ crops	2 (22%)	2.47	0.45 to 13.38
Application methods			
Air blast sprayer			
No	2 (7%)	Ref	
Yes	15 (13%)	1.88	0.40 to 8.72
Tower sprayer			
No	17 (12%)	Undefined†	
Yes	0 (0%)		
Handling activities			
Mixing/loading			
No	2 (4%)	Ref	
Yes	15 (15%)	4.76	1.04 to 21.65
Entering pesticide storage area			
No	9 (8%)	Ref	
Yes	8 (18%)	2.33	0.84 to 6.49
Early re-entry in treated area			
No	14 (12%)	Ref	
Yes	3 (9%)	0.78	0.21 to 2.88
Repairing spray equipment			
No	15 (12%)	Ref	
Yes	2 (9%)	0.72	0.15 to 3.37
Cleaning activities			
Cleaning PPE			
No	5 (11%)	Ref	
Yes	12 (11%)	1.01	0.33 to 3.06
Cleaning spray equipment			
No	2 (3%)	Ref	
Yes	15 (18%)	7.17	1.58 to 32.59
Cleaning pesticide containers			
No	11 (10%)	Ref	
Yes	6 (15%)	1.62	0.56 to 4.71
Cleaning pesticide storage space			
No	15 (11%)	Ref	
Yes	2 (11%)	0.99	0.21 to 4.74
Cleaning pesticide spill			
No	16 (11%)	Ref	
Yes	1 (10%)	0.88	0.10 to 7.37
Exposure time			
Time of last exposure			
Within the last week	10 (12%)	1.49	0.44 to 5.03
>1 week ago	4 (8%)	Ref	

Continued

Continued

Exposure(s)	Cases (%)*	OR	95% CI
No. 8+ hour spray sessions			
None	2 (14%)	Ref	
1–2 times	6 (9%)	0.62	0.11 to 3.46
3–4 times	9 (19%)	1.42	0.27 to 7.50
5+ times	1 (3%)	0.21	0.02 to 2.59
Personal protective equipment			
Full-face respirator			
No (half-face)	14 (13%)	2.54	0.55 to 11.77
Yes	2 (6%)	Ref	
Powered air purifying respirator			
No (half-face)	14 (13%)	0.62	0.06 to 5.91
Yes	1 (20%)	Ref	
Disposable gloves under nitrile gloves			
No	11 (11%)	0.98	0.25 to 3.79
Yes	3 (11%)	Ref	
Cloth gloves under nitrile gloves			
No	11 (11%)	1.10	0.22 to 5.39
Yes	2 (10%)	Ref	
Chemical-resistant footwear			
No	4 (67%)	19.0	3.19 to 113.2
Yes	14 (10%)	Ref	
Rain suit			
No	3 (16%)	1.50	0.39 to 5.76
Yes	15 (11%)	Ref	
Chemical-resistant apron			
No	15 (11%)	0.71	0.19 to 2.73
Yes	3 (15%)	Ref	
Locker for PPE			
No	11 (17%)	3.16	1.04 to 9.61
Yes	5 (6%)	Ref	
Decontamination practices			
No. activities without decontamination‡			
None	9 (11%)	Ref	
One	4 (12%)	1.05	0.30 to 3.68
Two	4 (16%)	1.50	0.42 to 5.37
Three or more	1 (7%)	0.56	0.07 to 4.81
Demographics			
Age in years			
18–24	1 (4%)	Ref	
25–34	7 (10%)	2.71	0.32 to 23.21
35–49	8 (16%)	4.68	0.55 to 39.76
50+	2 (20%)	6.0	0.48 to 75.34
Health status			
Excellent	1 (4%)	Ref	
Good	11 (13%)	3.27	0.40 to 26.75
Poor/fair	6 (13%)	3.30	0.37 to 29.19

*Cases of BuChE depression were defined as >20% decrease from baseline BuChE activity. Percentages refer to the proportion of cases of BuChE depression within each exposure category.
†No cases of BuChE depression were observed among handlers who used tower sprayers (N=20).
‡Includes not washing hands before drinking, eating, smoking, using a cellular phone, using a two-way radio, urinating in the orchard or field, or using a portable toilet.
BuChE, butyrylcholinesterase (serum cholinesterase); OP/CB, organophosphate/N-methyl-carbamate pesticide; PPE, personal protective equipment.