Determinants of cholinesterase inhibition in pesticide handlers

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Overview

- Biology of cholinesterases (ChE)
- ChE monitoring in Washington State
- Study population and methods
- Preliminary results
- Discussion of findings and study limitations

Biology of cholinesterase

- Acetylcholine (ACh)
  - A chemical transmitter in the central and peripheral nervous systems
- Acetylcholinesterase (AChE)
  - Enzyme that hydrolyzes acetylcholine
  - The "off switch" at junctions between neurons and target cells
- ChE present in blood
  - AChE bound to RBCs
  - Butyrylcholinesterase (BuChE) in serum

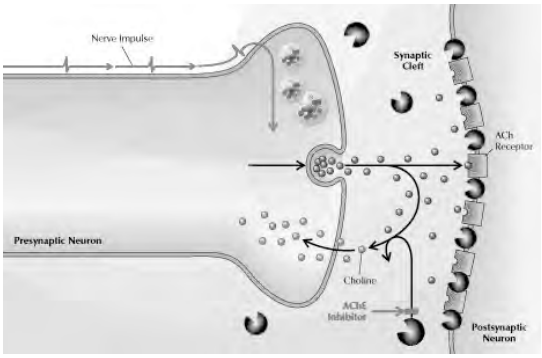


Diagram of cholinergic signaling (from Keifer, 2005)

ChE and pesticides

- ChE enzyme inhibited by certain classes of insecticides
  - Organophosphates (OPs)
  - *N*-methyl-carbamates (CBs)
- Various health endpoints associated with ChE inhibition
  - Dizziness, headaches, nausea, vomiting, anxiety, muscle twitching, fatigue, and blurred vision
  - Severe ChE inhibition can lead to seizures and respiratory failure
- AChE and BuChE measured in blood as markers of exposure and early biologic effects
  - High inter-individual variability in ChE activity
  - ChE levels recover naturally over time

ChE monitoring in Washington State

- Since 2004, ChE monitoring for agricultural workers who handle OP/CB insecticides
  - Employers contract with medical providers for monitoring services
  - ChE measurements performed by state laboratory from 2004-2006, and by private laboratory since 2007
- Handlers tested at baseline (i.e., pre-exposure) and during spray season to evaluate ChE inhibition from baseline
- Requirements for ChE depressions
  - > 20% AChE/BuChE inhibition → work practice investigation
  - ≥ 30% AChE inhibition → worker removal
  - ≥ 40% BuChE inhibition → worker removal
- Goals of the program:
  - Identify and correct unsafe work practices
  - Remove workers from exposure before development of pesticide-related illness

Study aims

1. Collect information about potential sources of pesticide exposure among participating handlers;
2. Characterize exposures and exposure-related behaviors; and
3. Determine which sources of exposure are associated with BuChE inhibition

Outcome of BuChE inhibition

- Focused on BuChE activity because:
  - High variability in AChE measurements in 2007
  - Little evidence of AChE inhibition among participants or in state program
  - BuChE more sensitive than AChE to chlorpyrifos
- Within-person change in BuChE activity from baseline (i.e., pre-exposure levels)
  - Etiologically relevant endpoint due to high inter-individual variability in ChE activity

Participation and inclusion criteria

- Completed surveys for 154 participants recruited during the 2006 and 2007 spray seasons
  - 50.7% participation rate
- Inclusion criteria:
  - Baseline and follow-up ChE test results available
  - Completed survey
- Handlers who participated in both years:
  - Selected record with greater degree of BuChE inhibition

Year of participation	N
2006	75
2007	57
2006 & 2007	22
Total:	154

Exposure history questionnaire

- Touch-screen tablet computer
- Uses pictures and audio
- English and Spanish versions
- Take survey in clinic or at worksite before ChE test results known
- Focuses on recent exposures (past 30 days)

OP/CB exposure score

- Based on algorithm for the Agricultural Health Study (Dosemeci *et al.*, 2002; Coble *et al.*, 2005; Hines *et al.*, 2008)

Work activity score

- Toxicity * (Handle + Apply + Clean) * PPE
- Characterizes exposure intensity for past 30 days

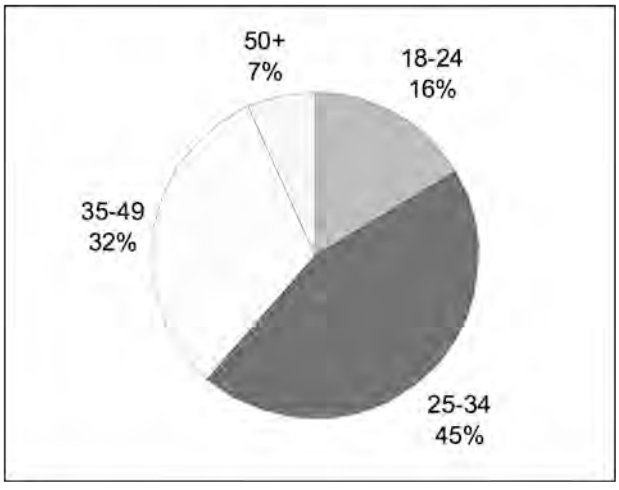
Statistical analyses

- Frequencies for demographics and exposure variables
- Multiple linear regression
  - BuChE inhibition
  - BuChE follow-up activity
  - Adjusted for: year, days since baseline ChE test, age, toxicity score, work activity score, PPE score

Participant characteristics (N=154)

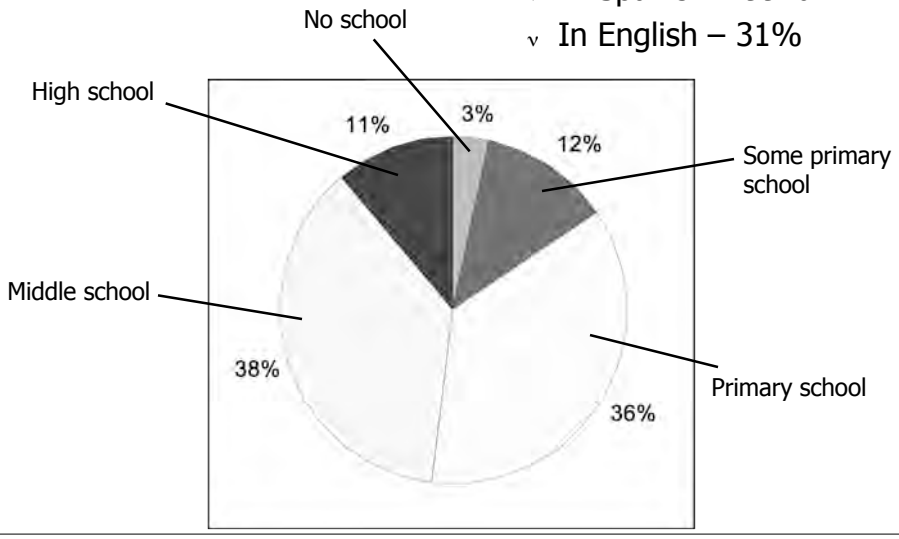
- All males
- 99% Latino
- 61% under 35 years old

Age of study participants



Education

- > 90% educated in Mexico
- Able to read:
  - In Spanish – 99%
  - In English – 31%

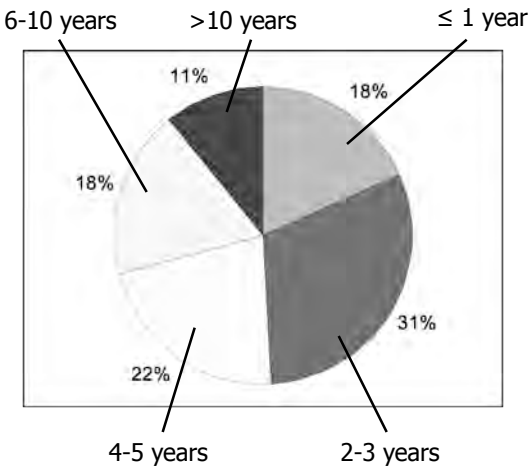


Work history

- 37% had applicator license

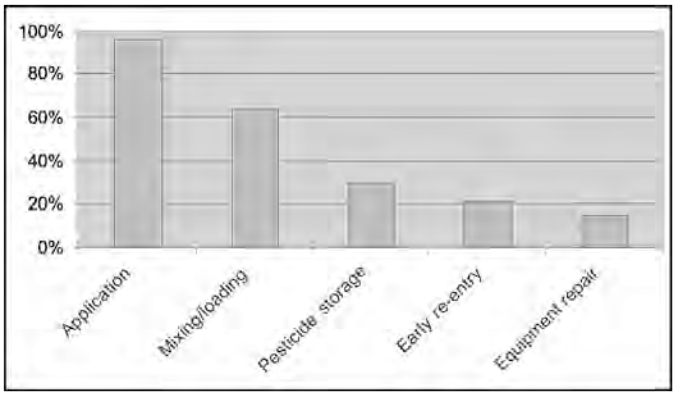
- 80% of non-licensed applicators trained in last 12 months
- ~50% employed as handlers for 3 years or less

Length of employment as a handler*



* Missing for 34 individuals

Handling activities, last 30 days



Reported OP/CB use, last 30 days

OP/CB insecticides	N	%*
Chlorpyrifos	84	61.8%
Carbaryl	41	30.2%
Azinphos-methyl	24	17.7%
Dimethoate	13	9.6%
Malathion	7	5.2%
Other OP/CB	19	14.0%

* Percentages exclude 18 records with missing/unknown data

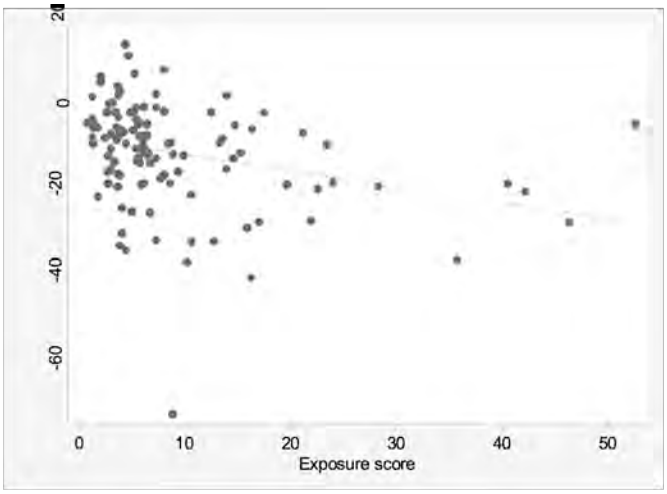
BuChE inhibition among study participants

		Percent BuChE inhibition		BuChE depression*	
Group	**N**	**Mean (SD)**	**P value†**	**N**	**%**
Combined	154	-5.64% (11.65)	< 0.001	18	11.7%
2006	82	-4.82% (13.77)	0.0016	13	15.9%
2007	72	-6.58% (8.64)	< 0.001	5	6.9%

* >20% BuChE inhibition from baseline activity level
† Paired t-test comparing mean baseline and follow-up BuChE activity

Exposure intensity score

(Toxicity * work activity score * PPE)



β = -0.37; P = 0.003

Exposure algorithm results

		Descriptive statistics				Linear regression*		
Score	N	Mean	(SD)	Mdn	Range	β†	(95% CI)	P value
Toxicity‡	135	1.64	(1.48)	1	0.16-10.54	-1.33	(-2.22, -0.45)	0.003
Work activity	149	18.1	(6.75)	19	0-32	-0.38	(-0.65, -0.11)	0.006
PPE	141	4.51	(1.68)	5	1-15	-1.28	(-2.15, -0.40)	0.005

* Robust linear regression with toxicity score, work activity score, and PPE score included in the same model; restricted to participants with non-missing values for each exposure score variable (N=118)

† Difference in percent change in BuChE activity per 1 unit increase in score

‡ One record with an implausibly high toxicity score value was excluded from this analysis

- Toxicity, work activity and PPE scores all predict BuChE inhibition

How are handlers getting exposed?

- Overall decrease from baseline and highly significant relationship with cumulative exposure score indicate that handlers are being exposed at levels resulting in BuChE inhibition
- Which specific factors, if any, lead to over-exposure?
  - Want to identify potential sources of exposure for future prevention efforts

OP/CB compounds used

Exposure	N	β	95% CI	P value
Chlorpyrifos	119			
No	49	Ref	---	---
Yes	70	1.69	-3.52, 6.89	0.522
Carbaryl	119			
No	80	Ref	---	---
Yes	39	-2.05	-7.54, 3.44	0.461
Azinphos-methyl	119			
No	99	Ref	---	---
Yes	20	-3.68	-14.35, 7.00	0.496
Multiple OP/CBs	119			
No	88	Ref	---	---
Yes	31	-2.50	-8.35, 3.35	0.399

Crops treated and application methods

Exposure	N	β	95% CI	P value
No. crops treated	114			0.651*
1 crop	83	Ref	---	---
2 crops	22	-1.31	-6.30, 3.68	0.604
3+ crops	9	-0.93	-8.29, 6.42	0.802
Air blast sprayer	114			
No	21	Ref	---	---
Yes	93	0.46	-4.01, 4.94	0.838
Tower sprayer	114			
No	99	-1.65	-7.44, 4.14	0.574
Yes	15	Ref	---	---

* Test for trend

Handling activities

Exposure	N	β	95% CI	P value
Mixing/loading	118			
No	37	Ref	---	---
Yes	81	-3.68	-7.45, 0.08	0.055
Pesticide storage	118			
No	85	Ref	---	---
Yes	33	2.81	-2.22, 7.84	0.271
Early re-entry	118			
No	96	Ref	---	---
Yes	22	1.85	-2.72, 6.42	0.425
Repairing equipment	118			
No	104	Ref	---	---
Yes	14	-1.50	-6.77, 3.77	0.574

Mixing/loading pesticides

- Potential for higher levels of exposure
- Splashes, spills, contact with contaminated equipment
- Exposure to concentrated product

USDA, 2006

Cleaning activities

Cleaning	N	β	95% CI	P value
PPE	118			
No	38	Ref	---	---
Yes	80	-1.45	-6.54, 3.64	0.574
Spray equipment	118			
No	52	Ref	---	---
Yes	66	-4.93	-8.87, -0.99	0.015
Pest. containers	118			
No	87	Ref	---	---
Yes	31	-1.48	-5.44, 2.48	0.460
Pest. storage space	118			
No	105	Ref	---	---
Yes	13	2.26	-2.87, 7.39	0.384
Pest. spill	118			
No	112	Ref	---	---
Yes	6	-3.00	-10.93, 4.94	0.456

Cleaning spray equipment

- Associated with elevated urinary levels of 2,4-D in study by Arbuckle *et al.* (2002)
- Handlers may not wear PPE for cleaning activities

USDA, 2006

Time of exposure

Exposure	N	β	95% CI	P value
Last exposed	100			0.735*
Today	7	1.46	-11.11, 14.04	0.818
Yesterday	9	-1.34	-14.06, 11.38	0.835
2-7 days ago	49	-3.05	-13.04, 6.95	0.546
8-14 days ago	13	-0.97	-10.56, 8.62	0.842
15-30 days ago	16	-0.16	-10.54, 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, 7.44	0.529
3-4 times	39	-6.86	-18.08, 4.37	0.229
5+ times	22	-2.71	-13.85, 8.44	0.631

* Test for trend

Personal protective equipment

Exposure	N	β	95% CI	P value
Full-face respirator	113			
No (half-face)	84	-7.71	-14.29, -1.13	0.022
Yes	29	Ref	---	---
PAPR	89			
No (half-face)	84	-2.01	-9.05, 5.04	0.572
Yes	5	Ref	---	---
Gloves	101			
Nitrile only	79	-0.06	-5.00, 4.89	0.982
Disposable + nitrile	22	Ref	---	---
Gloves	95			
Nitrile only	79	-3.74	-9.94, 2.46	0.234
Cloth + nitrile	16	Ref	---	---

Half-face respirators

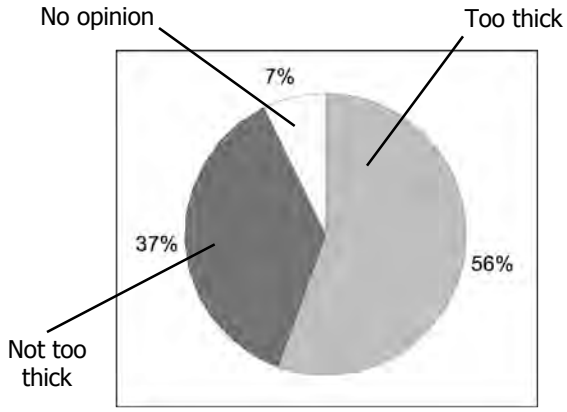
- Half-face respirator use reported in many investigations of ChE depression by L&I

- Potential for dermal contamination on upper portion of face

Glove use

- Other studies have shown that glove use is one of the most effective ways of reducing exposure (de Cock 1995, Arbuckle 2002)
- Why don't we see an association in our study?
  - Chemical-resistant glove use nearly universal
  - Consequently, we can only assess use of other gloves in combination with chemical-resistant gloves

Glove thickness*



* Perception of glove thickness for performing certain tasks such as unclogging spray nozzles, adjusting the pressure regulator, or repairing spray equipment

- Participants who thought that gloves were too thick were six times as likely to unclog spray nozzles with bare hands or disposable gloves (40% vs. 7%; P=0.001)

Personal protective equipment

Exposure	N	β	95% CI	P value
Chem-resistant footwear	118			
No	5	-9.24	-20.49, 2.02	0.107
Yes	113	Ref	---	---
Rain suit	118			
No	16	1.07	-5.11, 7.25	0.732
Yes	102	Ref	---	---
Chem-resistant apron	118			
No	102	6.05	0.85, 11.24	0.023
Yes	16	Ref	---	---
Locker for PPE	116			
No	55	-7.58	-12.36, -2.81	0.002
Yes	61	Ref	---	---

Footwear and lockers

- Take home exposure pathway
  - Wearing work boots home
  - Not using a locker

- May be surrogates for other sources of exposure

Unexpected findings

- Why was chemical-resistant apron use associated with *greater* BuChE inhibition?
- Generally used with more toxic compounds or for high exposure handling activities

Discussion

- Able to identify modifiable work practices and conditions as well as specific activities for targeted interventions

Risk factors for BuChE inhibition identified:

- Handling activities
  - Mixing/loading pesticides
  - Cleaning spray equipment
- PPE use
  - Half-face respirator (vs. full-face)
  - Not wearing chemical-resistant footwear
  - Not using a locker for PPE storage

Limitations

- ChE measurements performed by different labs in 2006 and 2007
- Lack information on extent of exposure to specific OP/CBs
- No information about knowledge and beliefs about pesticides

Next steps

- Continue to recruit and enroll pesticide handlers in this study through 2010 spray season
- Conduct further analyses with larger sample

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Roundtable Discussion

Risk factors for pesticide over-exposure among agricultural pesticide handlers

10:45 AM - 12:00 PM

Questions?

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