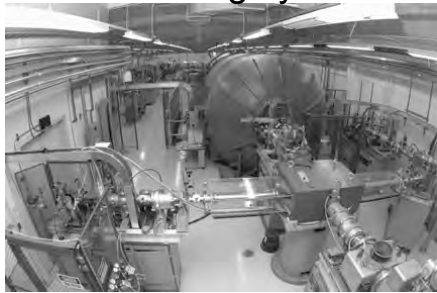


Novel Technologies to Measure Urinary Biomarkers of Pesticide Exposure

Shirley J. Gee and Bruce D. Hammock
Department of Entomology, University of California
Davis

Bruce A. Buchholz
Lawrence Livermore National Laboratories

Accelerator Mass Spectrometry Highly sensitive detection of ^{14}C



<https://www.llnl.gov/str/March06/Brown.html>



<https://www.llnl.gov/str/Knezovich.html>



Chest x ray:
50 microsieverts



1-hour plane flight at 9,000 meters:
5 microsieverts



Typical AMS nutrient study:
1 microsievert



Typical AMS drug
or toxin study:
1 nanosievert

A typical study exposure would result in a radiation exposure similar to a short airplane flight.

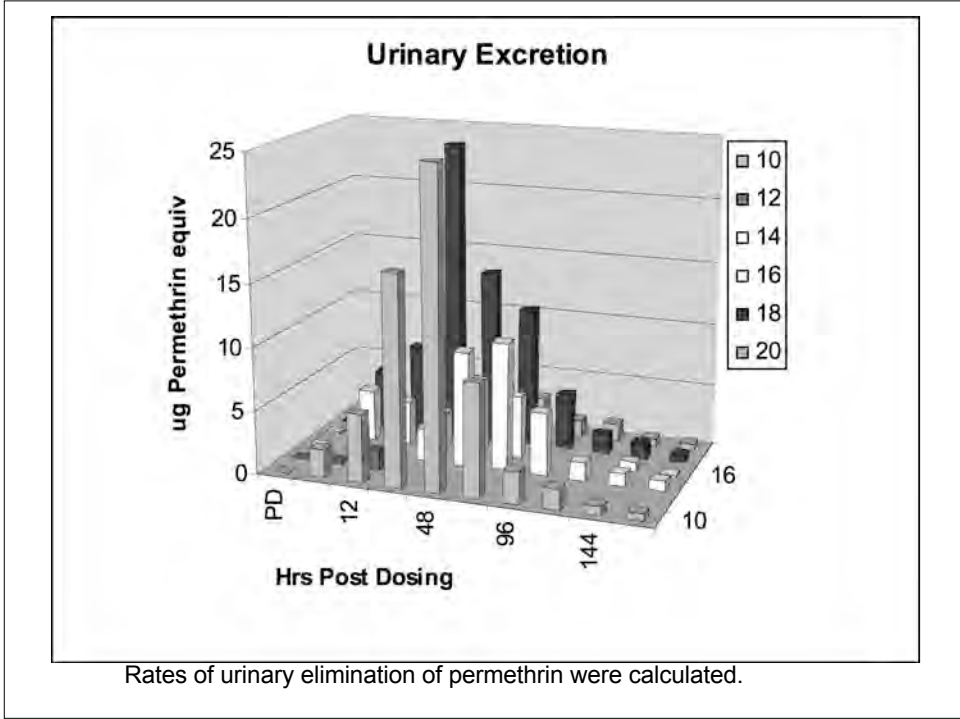
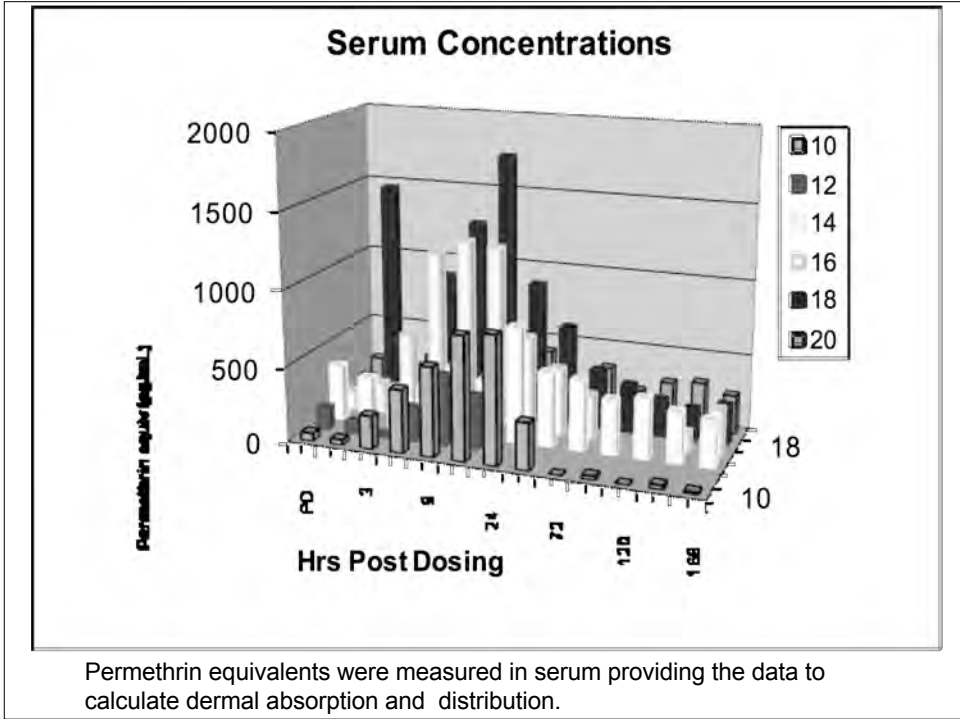
Permethrin exposure study

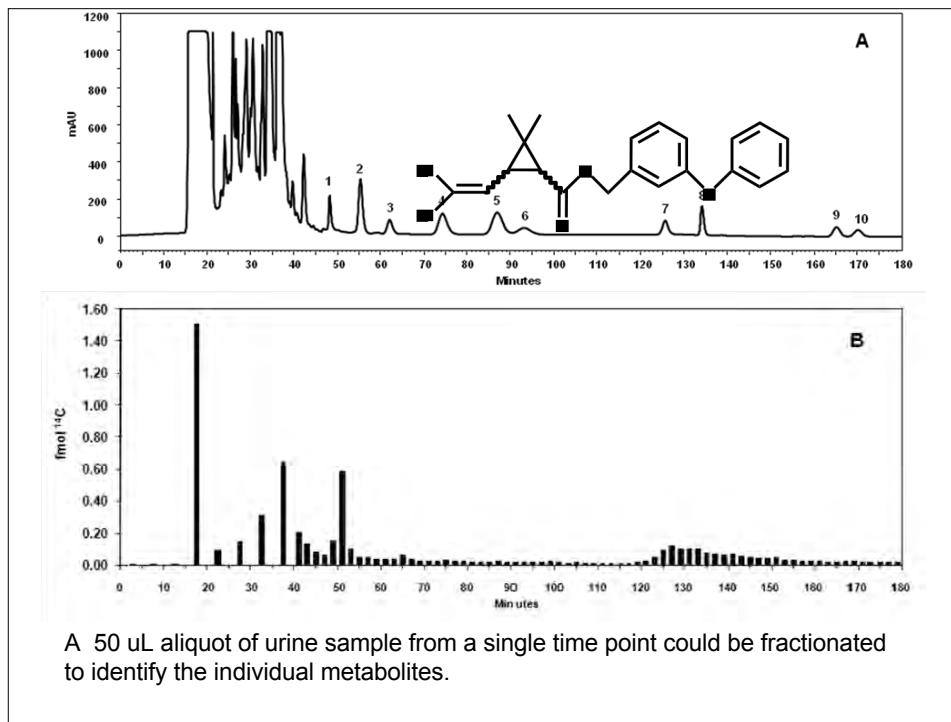
- 6 healthy adults
- No known exposure to pyrethroids in last 3 months
- 25 mg Permethrin in isopropyl alcohol + 1 μ Ci
- 8 hr Dermal exposure
- Saliva, blood, urine collected for 7 days
- Analyze by accelerator mass spectrometry

Permethrin Urinary Excretion

Subject	Total ug Permethrin equiv excreted in 168 hr	% of dose administered
010	63.6	0.25
012	1.84	0.01 (only sampled for first 12 hr)
014	30.4	0.12
016	22.6	0.09 (missing samples)
018	68.7	0.27
020	16.2	0.06

Permethrin equivalents were readily measured in all samples, although no counts were detected by liquid scintillation counting.





Outlook

- Accelerator mass spectrometry is an available, viable method to determine the metabolism of a pesticide in humans.
- Well-characterized urinary pesticide metabolites can be used as biomarkers of exposure.
- Accelerator mass spectrometry can also be used to identify other types of biomarkers of exposure or effect.
- Such knowledge could then be used to develop clinically useful tests of exposure.

Cholinesterase Monitoring Washington State: Biomonitoring at a State Level

Matthew Keifer MD MPH
Pacific Northwest Agricultural Health
Center
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The Washington State Cholinesterase Monitoring System

- ✖Background and Brief History
- ✖Present ChE rule in Washington State
- ✖Results 2004-2006
- ✖Problems in 2007
- ✖Interpretation of Results
- ✖Lessons Learned

Protein adducts as biomarkers of pesticide exposure

Chris Simpson, PNASH

Session II

Panel: *Biomonitoring State of the Science – Where is New Technology Going?*

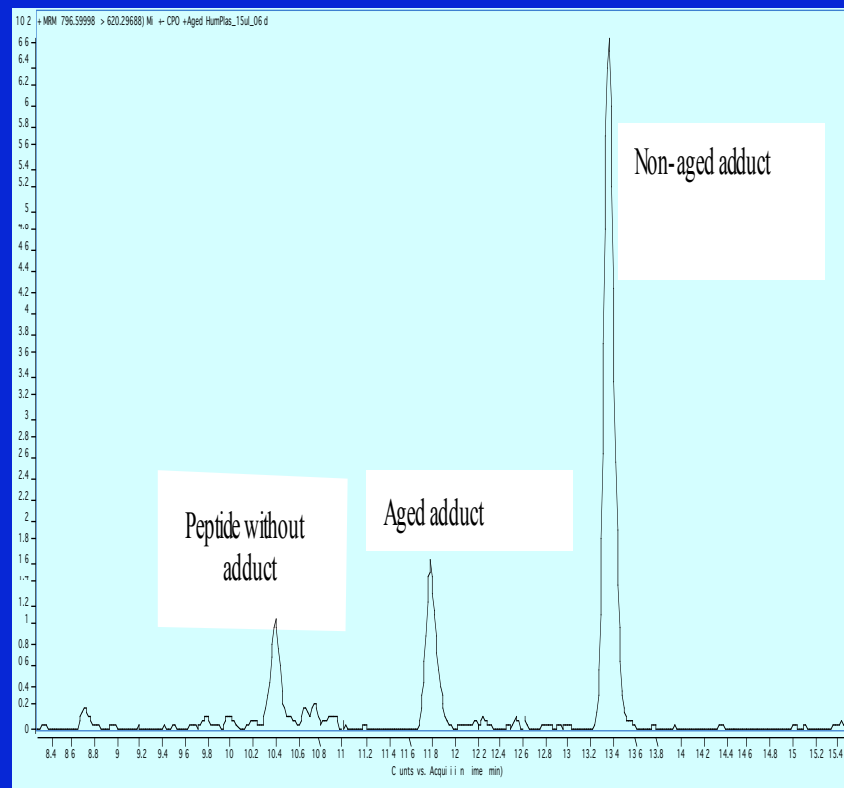
New Paths Conference, Nov. 11-13, 2008
Cle Elum, WA

Protein Adducts as biomarkers of pesticide exposure

- A “protein adduct” is the compound formed when a chemical binds (irreversibly) to a protein within our bodies.
- Potential advantages:
 - Retrospective
 - Sensitive
 - specific

OP adducts to cholinesterase

- OPs form stable adducts with cholinesterases
- Butyryl cholinesterase is isolated from human blood (plasma fraction)
- Isolated BuChE is digested to peptides
- Active site peptide separated, identified and quantified using HPLC/MS/MS.
- Adducts expected to persist after cholinesterase activity has returned to normal
- Specificity - you know what is bound to the BuChE.
- Sensitivity to ~ 5% inhibition



Future directions

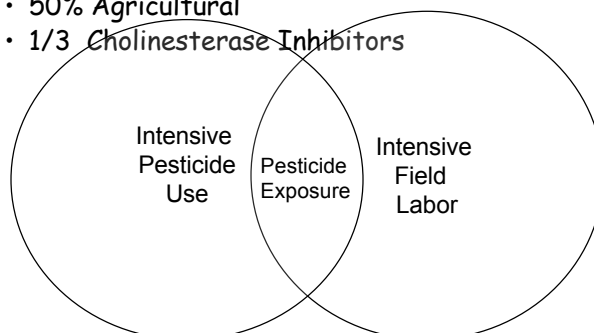
- Adducts to other more abundant proteins
 - e.g. Albumin
- Adduct profiling to assess exposures to mixtures of pesticides
- Adduct-omics
 - Which proteins are reacting with the pesticide(s)
 - Do these proteins link to potential biological responses or adverse health effects?

Cholinesterase Monitoring

- Biomarker for OP and carbamate exposure
- California has oldest state ChE requirement in US
 - Filmore and Lessenger (1993) - Found 24% of workers had ChE depression requiring removal in California program
 - Ames et al. (1985) found that 5% of 500+ samples on CA workers required removal.-cooperation on data call in was poor.
 - Das R. Could not get good cooperation from providers in California ChE program
 - Yearly 1981-1991 >200000 samples over ten years found 0.44% of structural applicators were removed due to depression.

Washington Agriculture, Workers and Pesticides

- Crops
 - Apples, Pears, Cherries, Hops, Potatoes, Milk
- Workers
 - 100,000+ Agricultural Workers at peak
- Pesticide Incident Reporting Panel
 - 250-400 Pesticide Poisonings per year
 - 50% Agricultural
 - 1/3 Cholinesterase Inhibitors



What Happened in Washington State?
Dep. Labor and Industries (State OSHA) a Key Player

- Farm workers Representatives & Legal Services request rulemaking on ChE or L&I 1986-1988
 - No action taken
- 1993, Centro Campesino again petitions for a rule
 - L&I adopts a "voluntary rule"
 - Organizes the Technical Advisory group TAG
- 1994, Technical Advisor Group finds cholinesterase monitoring effective in improving the health of pesticide exposed workers
- 1997 a small epidemic of organophosphate pesticide poisonings in Washington State
 - 2 farmworkers, Rios and Ferias file suit against L&I

Rios V. Dept Labor and Industries
2002- WA Supreme Court



"L&I Do Your Duty" Monitor for
ChE Depression

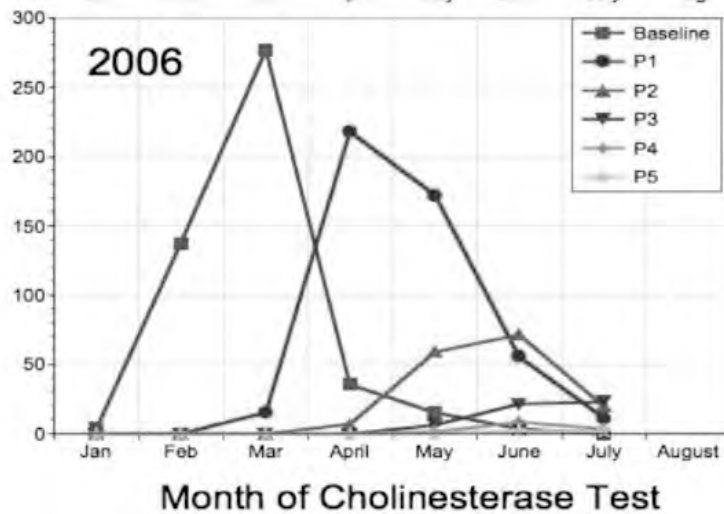
Washington Cholinesterase Rule

- **Workers Covered**
 - Handlers of Class I and II organophosphates (and/or) carbamates
 - 30 hours of exposure in 30 days
 - Voluntary participation (by worker)
- **Laboratory**
 - Both AChE and BChE on all workers
 - Baselines required
 - Single laboratory with central reporting
 - Handling hours reported on each worker
- **Response**
 - Removal at >30% AChE or >40% BuChE depression
 - Warning-workplace investigation at >20% depression of either enzyme
- **Scientific Advisory Committee-years 2004-2006**

ChE Tests Done

Year/test type	2004	2005	2006
Baselines tests	2655	2263	1889
Follow-up tests	580 (22%)	611 (27%)	471 (25%)
Working Baselines	?	164	48

Testing by Month

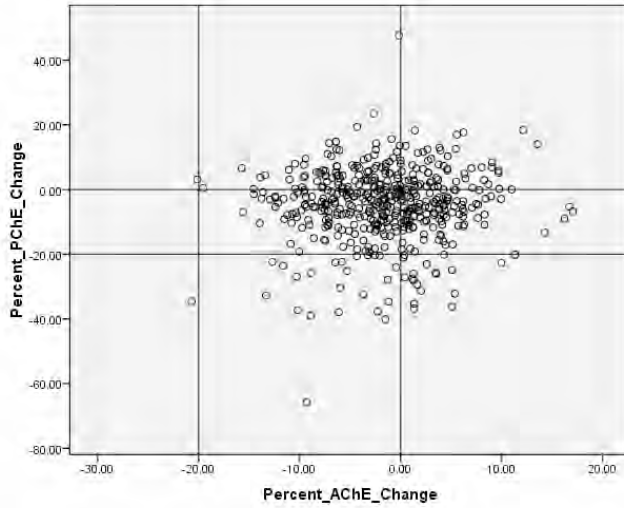


Cholinesterase Depressions

Year/test type	2004	2005	2006
Follow-up tests	580	611	471
Depressions > 20%	119 (21%)	59 (10%)	57 (12%)

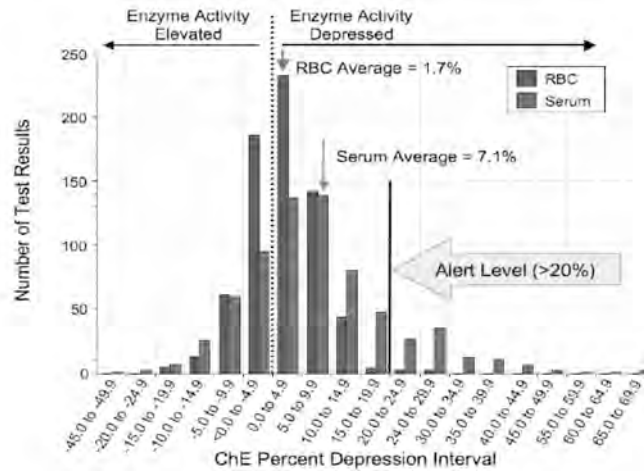
Across 3 yrs average BuChE depression = 6.8%

Percent Change in ChE 2006 Baseline-1st Follow-up



Enzyme	%Change
BuChE	-4.6
AChE	-1.7

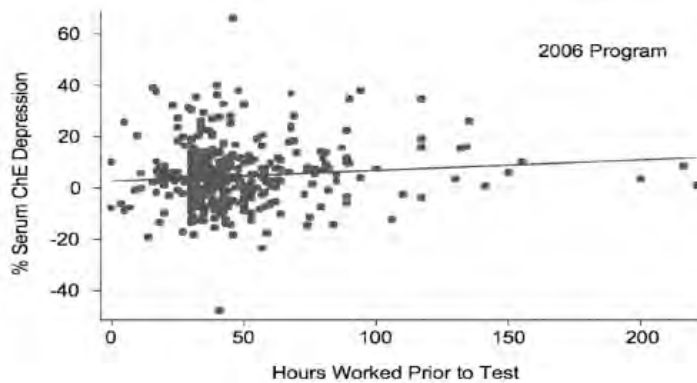
Cholinesterase Depression 2006 All Follow-up Results



Mean change in BChE and AChE significant at $p < 0.00$

Hours of Exposure and ChE Reported With Follow-up Testing

- A slight but significant relationship was found between hours of exposure and BChE depression
- Less dramatic for hours and AChE depression



Was it Pesticides? Working "Baselines"

- Workers with exposure before baseline testing
 - Had lower baselines than the non-exposed population
- Lower "baselines" for both AChE and BuChE

Year	AChE	BuChE
2005 (n=164)	-2.8%	-6.9% *
2006 (n=48)	-2.3%	-5.4%

* P < 0.05

New Laboratory in 2007

- Variability rose in AChE
- Many more AChE based investigations
- BuChE CV similar to past years.

Table 1: Precision for RBC and serum ChE results, 2004-2007

	RBC ChE				Serum ChE			
	2004	2005	2006	2007	2004	2005	2006	2007
I. Replicate Precision: Blinded Field QC Samples								
N pairs	53	186	*88	107	53	176	*89	107
% CV	6.5%	4.2%	3.8%	16.7%	1.6%	2.1%	2.5%	2.6%
dates/person	1 or 2	up to 7	*up to 5	up to 6	1 or 2	up to 7	*up to 5	up to 6
II. Estimated Within-Person % CV from Monitoring Results (baseline vs first periodic test)								
% CV	9%	5.5%	4.3%	15.1%	10%	6.6%	8.3%	7.1%

*through 7/31/2006 only

Investigations by L&I Depressions and Quitters

Depression Risk Factors?

- Airblast sprayer commonly reported among those with >20% depression
- Ball cap use common as well
- Poor PPE management
- Poor workplace hygiene practices

Quitters (30 interviewed)

- Owner applied
- Eliminated ChE inhibitors
 - Changed pesticides
 - Went organic
 - Increased IPM
- Increased employees
- Increased spray rate-decreased hours

ChE Monitoring in Washington State What We Learned

- No illnesses reported among workers with depressed ChE
- Workplace exposure is resulting in ChE depression in the worker population
- BuChE consistently more depressed
- Airblast sprayers most common method of application found (no reference)
- Growers reduced exposure to avoid testing

Good News-Bad News

- Very few depressions to the point of removal
 - A consistent trend across three years
- Broad population change in ChE activity,
 - Principally seen in BuChE activity but also seen in ACHE
- Several risk factors may be related to depression
 - No reference information to confirm the strength of these potential risk factors

Too Late to Close the Barn Door?



- An opportunity to study what is depressing ChE in pesticide handlers

Acknowledgements

- Other Scientific Advisory Committee Members
 - David Kalman Ph.D.
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