Cognitive Effects from Organophosphate Exposures

Diane S. Rohlman¹, Ph.D. and Pamela J. Lein², Ph.D.

¹Center for Research on Occupational and Environmental Toxicology
Oregon Health & Science University

²Department of Molecular Biosciences
School of Veterinary Medicine, UC Davis

Funding: NIH R21 ES017223 (Diane S Rohlman, PI)
NIH R01 ES016308 (W Kent Anger & Pamela J Lein, MPI)

Collaborators:
WK Anger, PJ Lein, FM Farahat, M Lattal, JR Olson, MR Bonner, RA Fenske, K Galvin,
TM Farahat, A Ismail, G Abdel Rasoul, O Hendy, M El Batanony,

Conflict of Interest: OHSU and Dr. Rohlman have a significant financial interest in Northwest Education Training and Assessment, LLC, a company that may have a commercial interest in the results of this research and technology. This potential conflict of interest was reviewed and a management plan approved by the OHSU Conflict of Interest in Research Committee was implemented.

Studies examining low-dose exposures

• Range of occupational groups in different countries (> 20 studies)
  – Pesticide workers, sheep dippers, greenhouse workers, tree-fruit farmers, farmworkers and residents on farms
  – US (migrant farmworkers), Ecuador, Egypt, South Africa, Spain, Brazil, UK, United Arab Emirates, Israel
  – Adults and adolescents occupationally exposed

• Majority of studies observed neurobehavioral differences in occupational groups
Studies examining low-dose exposures

Not all studies have found deficits associated with exposure (Maizlish 87, Rodnitzky 75, Daniell 92, Ames 95)

Results are not consistent across studies

Why are there variations in neurobehavioral performance?

Method – Procedure – Population

• Range of methods used (computer/paper, parameters)
• Cross sectional designs (may not provide information about previous exposures)
• Small sample size (N < 100)
• Populations with low education, limited writing/computer, language/culture
Why are there variations in neurobehavioral performance?

Exposure Classification

- **Pesticide Source Information**: pesticide use, home inventory, proximity to agricultural field, job classification
- **Environmental Monitoring**: indoor air, dust samples (vehicle/home), surface wipes
- **Biomarkers**: plasma ChE, urinary metabolites

Usually can’t establish the exposure history

---

Do repeated low-dose exposures cause neurotoxicity in humans?

Review of 24 studies indicate deficits in exposed vs. controls in several functional domains:

- **Motor Speed/Coordination** (10 studies)
  - Finger Tapping, Pegboard, Aiming
- **Information Processing Speed** (8 studies)
  - Simple Reaction Time, Syntactic Reasoning
- **Complex Visual Motor/Executive Function** (12 studies)
  - Digit Symbol, Symbol-Digit, Trailmaking
- **Attention/Short-term Memory** (9 studies)
  - Digit Span
- **Memory** (6 studies)
  - Benton Visual Retention
  - Match to Sample

Rohlman et al., 2011, *Neurotoxicology*
Do repeated low-dose exposures cause neurotoxicity in humans?

- **Weight of evidence**
  - (19 of 24 studies) suggests that occupational exposures to OPs are associated with neurobehavioral deficits

- **However,**
  - A relationship between OP dose and behavioral deficits has not been defined in humans
  - Only 2 of 24 studies have demonstrated a link between neurobehavioral performance and current biomarkers of OP exposure: blood cholinesterase (ChE) activity and urinary levels of OP metabolites

Ismail et al., 2012, *Occup Environ Med*
Potential reasons for the lack of correlation between biomarkers of OP exposure and neurobehavioral deficits

- **Exposure assessment**
  - Incomplete information on pesticide formulations
  - Lack of detailed data on workers’ exposure history

- **Biological mechanisms**
  - Genetic differences in the expression and/or activity of enzymes that metabolize OPs or proteins that scavenge OPs differentially influence peripheral versus central outcomes.
  - ChE inhibition may not be mechanistically related to chronic OP neurotoxicity

Hypotheses

- OP-induced neurobehavioral deficits are dose-related
- Biomarkers based on alternative, non-cholinergic mechanisms may be better predictors of OP neurotoxicity or improve prediction when used in conjunction with ChE inhibition
  - oxidative stress
  - inflammation
Setting of Human Studies

Agricultural workers involved in OP (chlorpyrifos) application to cotton fields located in Menoufia, Egypt situated in the Nile River delta north of Cairo

[Map of Egypt]

Occupational Cohort

Egyptian Cotton Workers

- **Applicator** – applies CPF using a backpack sprayer
- **Technician** – walks with an applicator to direct the path of the applicator and point out heavy insect infestation
- **Engineer** – periodically walks the fields but more often directs application from the edge of the fields
Experimental Strategy

Biomarkers of Organophosphorus Pesticide-Induced Neurotoxicity

**EGYPTIAN WORKER COHORT**
- Quantify human exposures (blood and urine biomarkers)
- Assess metabolic polymorphisms
- Identify behavioral effects, determine persistence
- Reanalyse behavioral effect data with novel biomarker
- Identify predictive human biomarkers

**LONG EVANS RAT MODEL**
- Approximate human exposures in rat model
- Learning-deficit rat model: identify behavioral effects
- Test novel biomarkers in learning-deficit rat model: Select mechanistically-based biomarkers
- Set exposures based on CHE depression

**PBPK/PD Model:**
- Estimate biologically effective doses
- Estimate human dose-response relationships for chlorpyrifos-induced biomarker responses and behavioral deficits

Typical pesticide application schedules to cotton fields in Menoufia Egypt

**Human Exposure Pattern (in Menoufia, Egypt)**

<table>
<thead>
<tr>
<th></th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>Sep/Oct</th>
</tr>
</thead>
<tbody>
<tr>
<td>nB</td>
<td>nB</td>
<td>nB</td>
<td>nB</td>
<td>nB</td>
</tr>
<tr>
<td>BI</td>
<td>BI</td>
<td>BI</td>
<td>BI</td>
<td>BI</td>
</tr>
</tbody>
</table>

Teams consisted of Applicators, Technicians, & Engineers, who have two or three dosing patterns.
Neurobehavioral Studies in Occupational Cohort: Trailmaking test *

A – draw line from 1 to 2 to 3...
B – draw a line from 1 to A to 2 to B to 3 to C ...

Test of complex visual scanning with a motor component and is sensitive to many types of brain damage (esp. part B).

* Farahat et al. (2003) found deficits on this test (both A & B) in engineers + technicians vs. Ministry of Agriculture controls. Significant differences found in 5 of 5 studies of OP-exposed workers in which the Trailmaking test has been used.

Analysis of Neurobehavioral Data

• **Generalized Estimating Equations (GEE),** a regression analysis that tests for the effects of variables on non-independent repeated measures
  – gave people the same Trailmaking test 4-5 times (when learning was expected to improve performance) during (July, Aug) and after (October) chlorpyrifos applications.

• **Variables**
  – Age
  – Years of education
  – Cholinesterase inhibition (based on June ChE measure) on days of testing
  – TCPy on days of testing
  – Years working for the Ministry of Agriculture
  – Job title (Applicator, Tech, Engineer) < only significant factor
Long Evans Rat Model Based on Human Exposure Data

• CPF exposure in Egyptian cotton workers is primarily dermal, so administered CPF daily via subcutaneous injection

• Preliminary dose range finding studies identified doses that upon repeated daily s.c. injections produced levels of blood cholinesterase reduction in rats comparable to that found in the Egyptian workers at the end of chlorpyrifos application cycle
  – 3 and 10 mg/kg daily (s.c.)

Experimental design in rat studies
Ongoing biomarker analysis in rat models of occupational CPF exposure

- **Current biomarkers**
  - Plasma ChE, urinary TCPy

- **Oxidative stress***
  - F2-isoprostanes (brain and urine)
  - Prostaglandin E2 (brain)

- **Inflammation**
  - GFAP and Iba1 immunoreactivity (brain)
  - Inflammatory cytokines (brain, blood)
  - C-reactive protein (blood)

*Isoprostane and PGE2 analyses performed by Dejan Milatovic and Miki Aschner, Vanderbilt University*