Cognitive Effects from Organophosphate Exposures

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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

Table 2. Studies that have used variants of the digit span test to assess pesticide exposure.

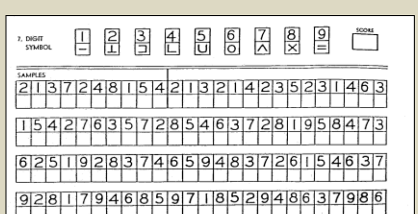
Study	Method	Outcome
Bazylewicz-Walczak et al. 1999	Polish NCTB	–0
Cole et al. 1997	NCTB	~
Farahat et al. 2003	Unknown	+
Fiedler et al. 1997	WAIS-R	–0
Kamel et al. 2003	BARS	+
London et al. 1997	NCTB	–0
Nishiwaki et al. 2001	NCTB	~
Reidy et al. 1992	WAIS-R	~
Rohlman et al. 2001b	BARS	+
Rosenstock et al. 1991	WAIS-R	+
Stephens et al. 1995	Unknown	–0
Stephens et al. 1996	NES	–0
Wesseling et al. 2002	NCTB	~
Yokoyama et al. 1998	Japanese WAIS	–0

Abbreviations and symbols: +, poorer performance in exposed group; ~, nonsignificant trend observed with poorer performance in exposed group; 0, no significant difference between control and exposed groups; NES, Neurobehavioral Evaluation System; WAIS-R, Weschler Adult Intelligence Scale–Revised.

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**

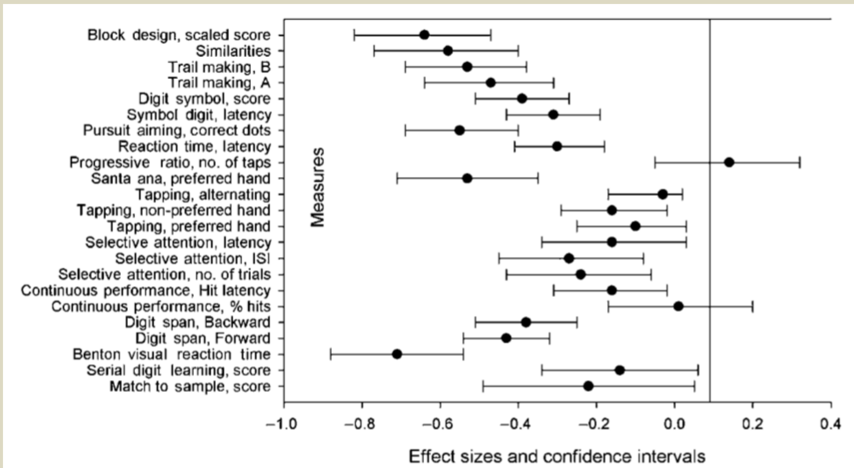
Complex Visual Motor/Executive Function (12 studies)
- **Digit Symbol, Symbol-Digit, Trailmaking**

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?



Ismail et al., 2012, *Occup Environ Med*

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

Potential reasons for the lack of correlation between biomarkers of OP exposure and neurobehavioral deficits

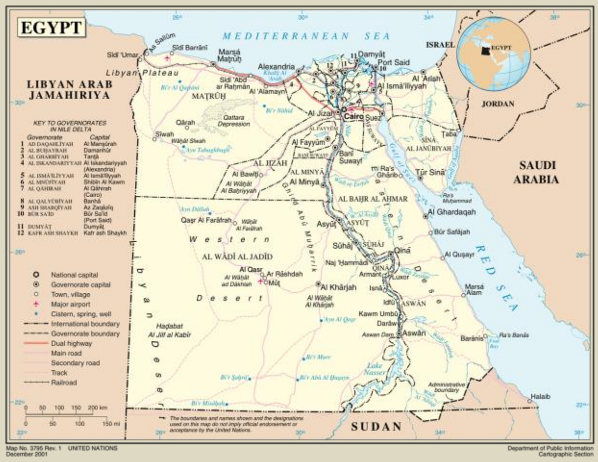
- **Exposure assessment**
  - Incomplete information on pesticide formulations
  - Lack of detailed data on workers' exposure history
- **Biological mechanisms**
  - Genetic differences in the expression and/or activity of enzymes that metabolize OPs or proteins that scavenge OPs differentially influence 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

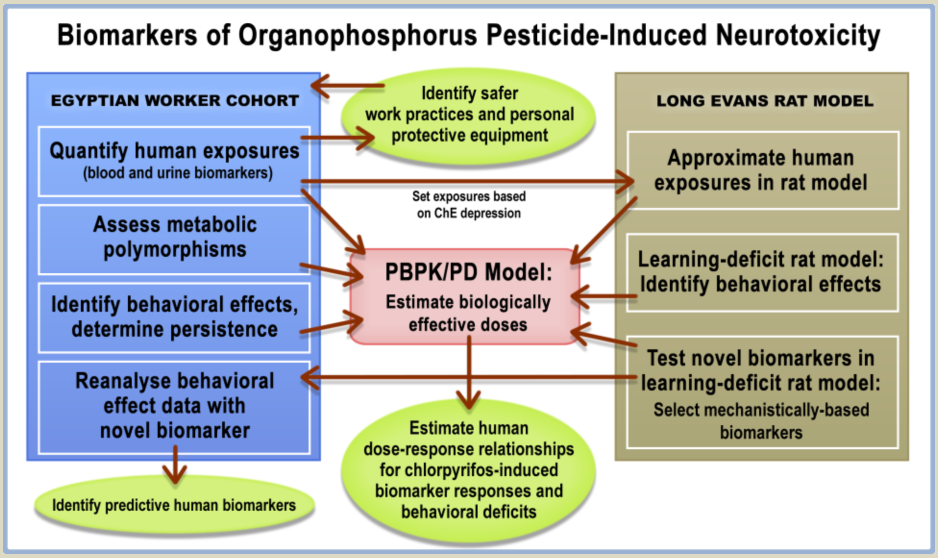


Occupational Cohort

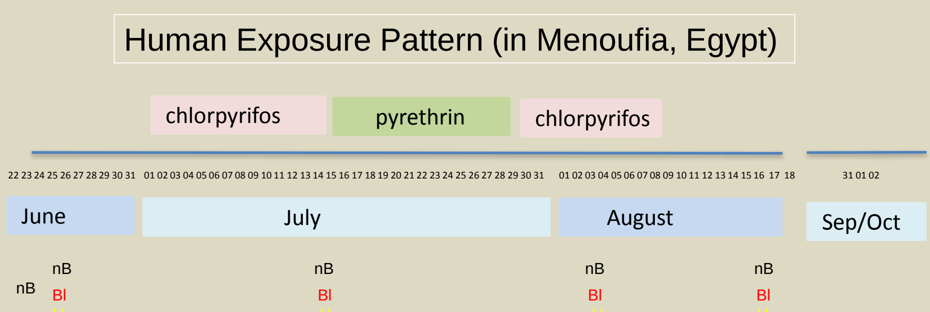
Egyptian Cotton Workers

- **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



Typical pesticide application schedules to cotton fields in Menoufia Egypt



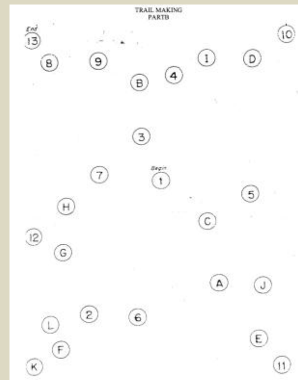
Teams consisted of Applicators, Technicians, & Engineers, who have two or three dosing patterns

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.

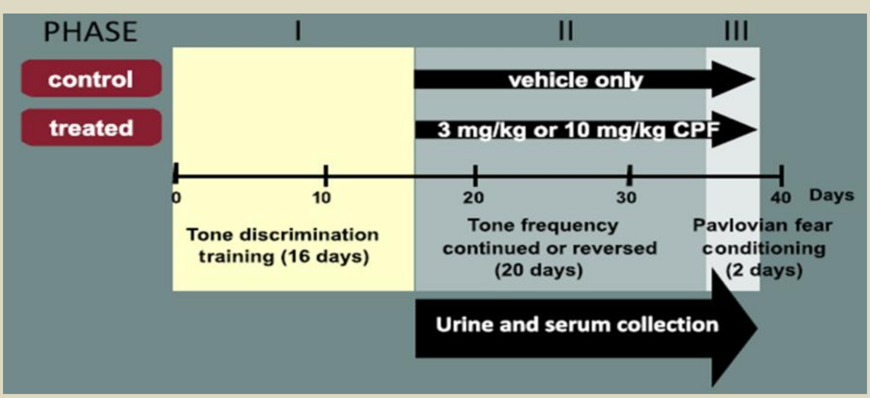
Analysis of Neurobehavioral Data

- **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***