



Epidemiologic Studies of the Effects of Toxic Exposures on Brain and Behavior: Neuropsychological Assessment

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Why worry about the effects of pesticides on human health?

- 5000 new chemicals/year
- EPA estimates that at least 25% are neurotoxic
- High vulnerability of the developing brain
- Experimental animal evidence shows adverse effects of many chemicals on growth and development
- Some pesticides have been used as nerve gas in warfare because they were specifically designed to attack the mammalian central nervous system

**In many parts of the world,
this scene is commonplace**



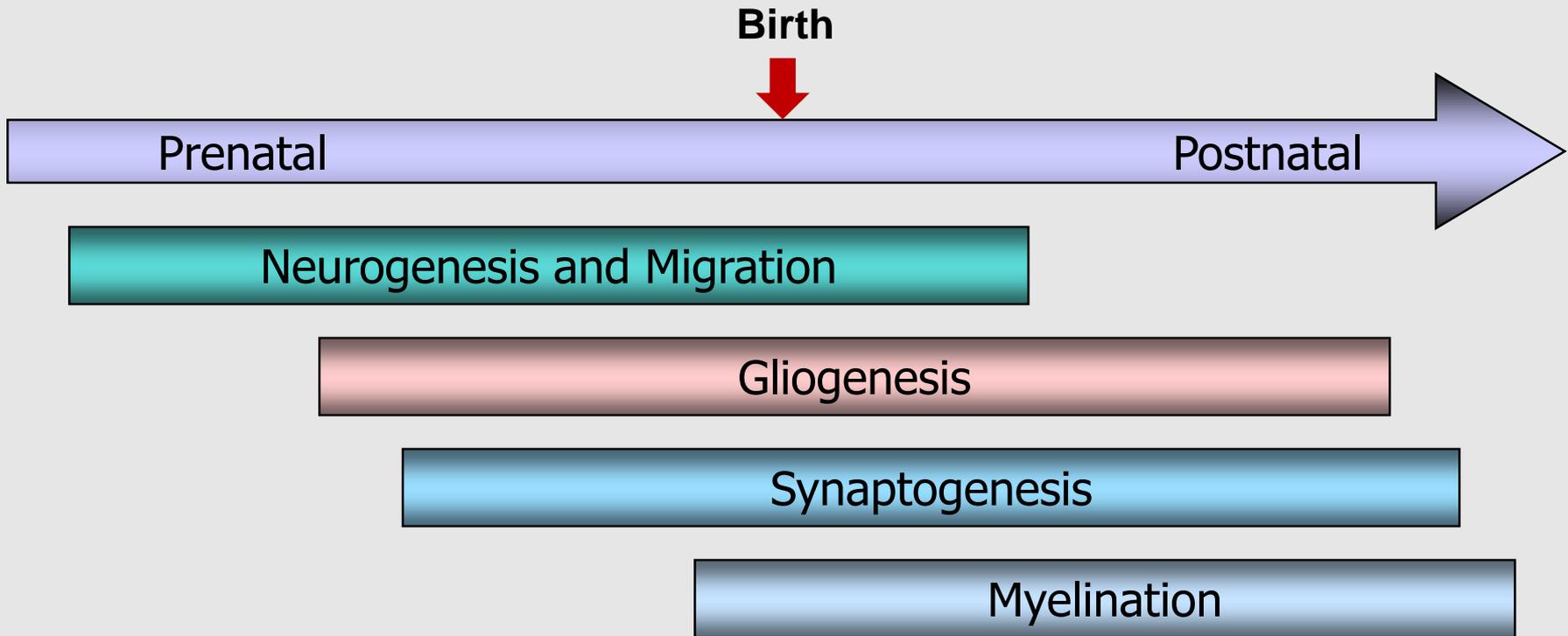
Overview

1. Identification of exposure (a widely-used OP pesticide) and population (inner-city minority pregnant women and their children)
2. Formulation of hypotheses (neuropsychological tests of cognition and behavior)
3. Choice of study design (prospective birth cohort)
4. Neuropsychological assessment: selection of tests (animal studies and current mechanistic understanding) and verification of neuropsychological domains in this population (confirmatory factor analysis)
5. Empirical demonstration of distinct neuropsychological profiles in this population (hierarchical cluster analysis)
6. Associations between exposure and profile (emergence of a phenotype or signature neuropsychological profile)

I. Identification of the exposure: Chlorpyrifos (CPF)

- Since the 1960s, CPF (a broadband organophosphate insecticide) has been widely used for residential pest control and agricultural purposes
- In June 2000-2001 U.S. EPA banned indoor residential use
- Despite these restrictions, CPF remains one of the most heavily used insecticides world-wide
- Used on grain, cotton, corn, fruits, nuts and other vegetable crops; lawns, golf courses and road medians; to control, cockroaches, termites, lice; registered for direct use on sheep, turkeys, in dog kennels, and farm buildings
- Both air monitoring and blood-based biomarkers were available to measure this compound

Multiple Possible Mechanisms: Shifting Targets



Prenatal exposure to CPF inhibits acetylcholinesterase, which acts as a neurotropic factor during brain development; toxicity results from inhibition of cholinesterase and the consequent cholinergic hyperstimulation. **CPF also alters brain development through noncholinergic mechanisms, and at lower doses that cause only minimal acetylcholinesterase inhibition, yet result in developmental neurotoxicity.**¹

¹Slotkin, *Toxicol Appl Pharmacol*. 2004

2. Epidemiologic Hypotheses

Based on experimental animal evidence and the current mechanistic understanding, we hypothesized that:

Prenatal exposure to CPF would be associated with less optimal performance on neuropsychological tests in the areas of:

- **Attentional capacity**
- **Impulse control**
- **Memory**
- **Sensorimotor functioning**

3. Identification of the population and study design:

Columbia Cohort: 725 urban African American & Dominican mothers

Enrolled during pregnancy **1998-2006**; excluded active smokers, illicit drug users, women with HIV, hypertension or diabetes; current follow-up to age 14 years

Environment

- Questionnaire on pesticide use;



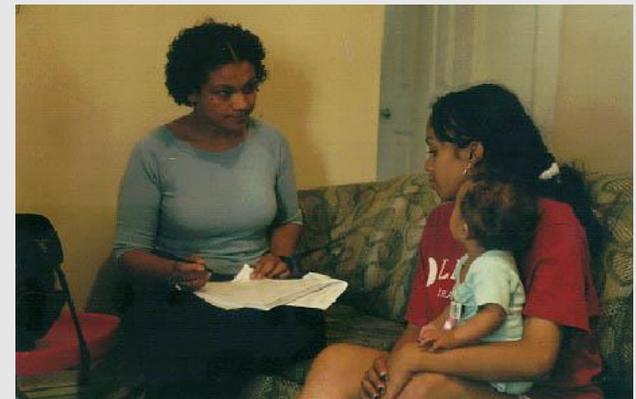
Biomarkers

- Pesticide compound in maternal and cord blood



Outcomes

- Cognition/Behavior
- Neuropsychological Function
- Brain Structure/Function (MRI/fMRI)





Why study this population?

Exposure was high in NYC communities and in this cohort among children who were born prior to the ban

Chlorpyrifos was detected in 99% of air and 70% of maternal and umbilical cord blood samples

- In 1997, the amount of insecticide applied by licensed applicators in NYC exceeded the amount applied in any other NY county, including farming regions
- 86% of cohort women reported using pest control methods (sprays and bombs) during pregnancy
- Maternal and newborn blood levels were highly correlated showing that these insecticides readily crossed the placenta
- Exposure levels in NYC cohort were comparable to levels in California farming communities



Over the course of the next 12-14 years, the cohort was assessed annually, using a range of physical and neurodevelopmental techniques.

Retention rates were high, due to continuity of staff and community input.



Summary of results through 7 years of age:

Birth Weight: Cord blood CPF exposure (categorical) inversely associated with weight (deficit of 150.1 grams) (*Whyatt et al., EHP, 2004*)

Behavior and Development at 3 years: Cord blood CPF exposure (categorical) inversely associated with Bayley developmental score (deficit of ~ 6 points [motor] and ~ 5 points [mental]), and positively associated with increased behavior problems (attention, ADHD, and **pervasive developmental disorder problems**) (*Rauh et al., Pediatrics, 2006*)

Cognition at 7 years: Cord blood CPF (continuous) inversely associated with WISC-IV working memory (deficit of ~ 3.8 pts) and full-scale IQ (deficit of ~ 2 pts). Association is approximately linear, with no evidence of a threshold (*Rauh et al., EHP, 2011*)

Prenatal Exposure to Organophosphate Pesticides and IQ in 7-Year-Old Children

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Prenatal Exposure to Organophosphate Pesticides and Child Development in Childhood

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Seven-Year Neurodevelopmental Scores and Prenatal Exposure to Chlorpyrifos, a Common Agricultural Pesticide

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Convergence of observational evidence:

- All 3 papers reported significant 7 year cognitive deficits on the WISC, associated with prenatal organophosphate insecticide exposure
- The studies used three different populations: two urban, one rural/agricultural
- The studies used different biomarkers of exposure: one used measure of the compound in blood; two used urinary metabolites

4. Neuropsychological domains and factor analysis

At age 12 years, the NEPSY, Conners CPT, Children's Memory Scales, and Purdue Pegboard were selected based on hypothesized CPF toxicity. Test items were used in a factor analysis for data reduction and confirmation that items would load on expected conceptual domains, reflecting a range of cognitive abilities in this cohort and assuring generalizability to other populations. From all individual test items, eight latent factors emerged:

- **Verbal Memory**
- **Visual Memory**
- **Auditory Attention (Sustained)**
- **Auditory Impulse Control**
- **Auditory Impulse Control > Auditory Sustained (contrast)**
- **Visual Attention (Sustained)**
- **Visual Impulse Control**
- **Sensorimotor Function/Finger dexterity**

5. Profile construction (hierarchical cluster analysis)

Using these 8 factors, hierarchical clustering was conducted with the first 237 children who had reached 12 years of age and completed all measures. Each child was initially assigned to his/her own cluster. The algorithm then proceeds iteratively, at each stage joining the two most similar clusters, until there is a single cluster.

This method revealed neuropsychological heterogeneity in the community sample and yielded 3 distinct subgroups.

6. Emergence of Community-Based Clusters/Profiles

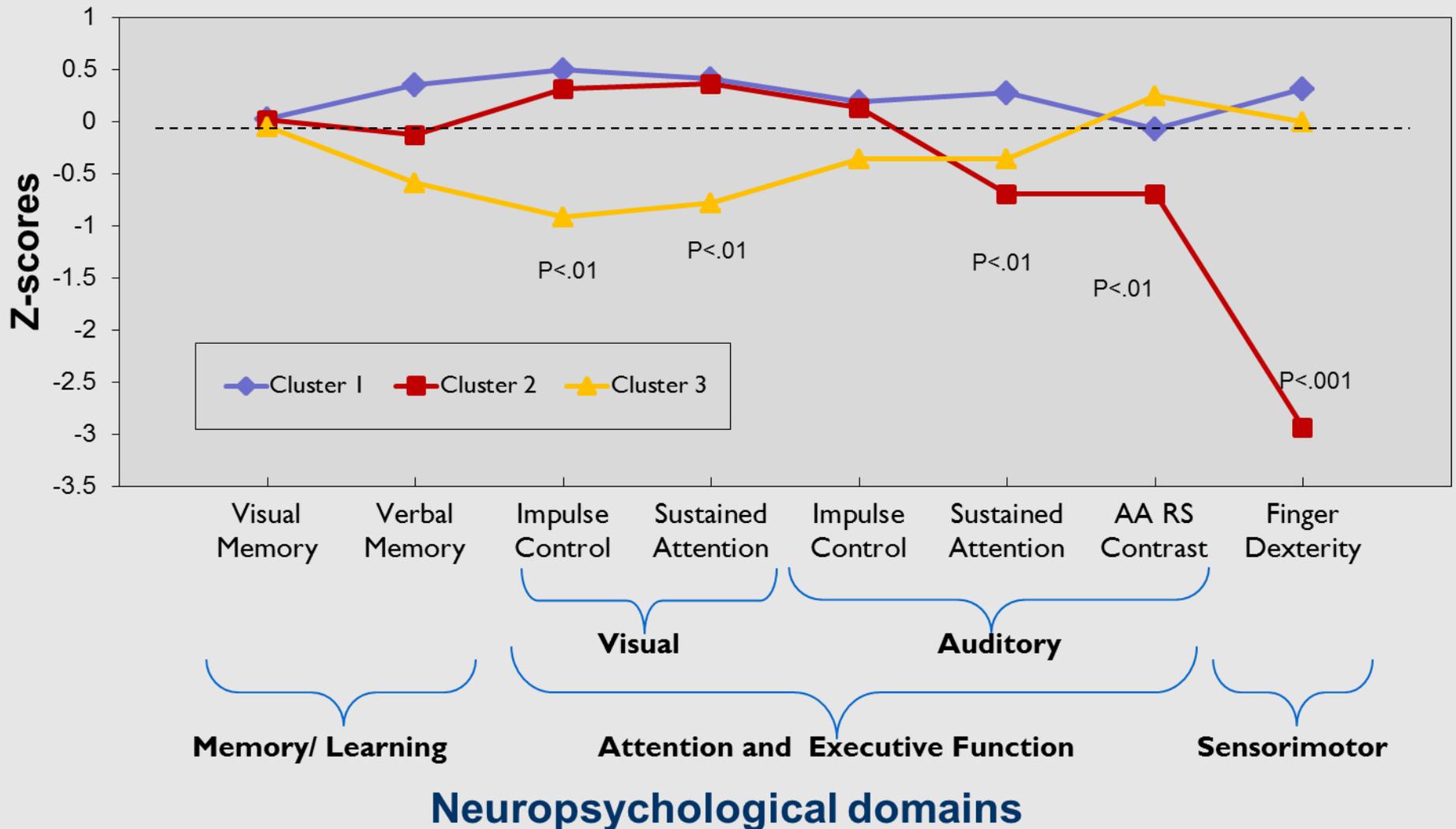
At age 12 years, extensive neuropsychological testing was conducted. Hierarchical clustering of factor-analyzed test items yielded 3 distinct subgroups of children:

Cluster 1 ('normal'): This group scored at or above the population mean on all domains and average full-scale IQ (9.9% high CPF exposure).

Cluster 2 ('atypical inattentive'): This group is disproportionately made up of highly exposed children (65.4% high CPF), with average visual and verbal memory skills, good inhibitory control (low impulsivity, few commission errors), but low full-scale IQ (93 points), poor auditory attention to simple tasks (e.g., numbers forward), and weak fine motor skills/finger dexterity.

Cluster 3 ('impulsive'): This group scored poorly on impulse control, memory and attention, and had low full-scale IQ (94 points). This group is disproportionately black (49.1%) and male (62.6 %). Only 12.5% of this group had high CPF exposure.

Comparison of 3 distinct profiles on 8 neuropsychological domains, adjusted for exact age at testing, sex, race, prenatal environmental tobacco smoke, general ability index, and maternal ADHD



Conclusions

1. Children with high pesticide exposure cluster together to form a distinct **behavioral phenotype** characterized by poor fine motor dexterity, auditory attention deficits, good impulse control and no visual memory problems.
2. This phenotype puts children at risk for potential clinical emotional or learning impairments **(IQ deficits and learning problems have important implications for school performance and developmental trajectories)**.
3. Cognitive and behavioral deficits associated with this phenotype may be mapped to **alterations in brain regions and function**.
4. Dr. Peterson will discuss the use of MRI modalities and findings specific to this population.



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