



# **Brain and Behavioral Effects of Prenatal Exposure to a Widely Used Pesticide**

Virginia A. Rauh, ScD.

COLUMBIA CENTER FOR CHILDREN'S ENVIRONMENTAL HEALTH  
COLUMBIA UNIVERSITY

"Neurodevelopment and Children's Environmental Health"  
Webinar hosted by NIEHS program:  
Partnerships for Environmental Public Health (PEPH)

No conflicts of interest to report

# 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

# This was then . . . .



and now . . . . .



In many parts of the world,  
this scene is commonplace





# **What is Chlorpyrifos?**

## **A broadband Organophosphate Insecticide**

- Since the 1960s, CPF has been widely used for residential pest control and agricultural purposes
- 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



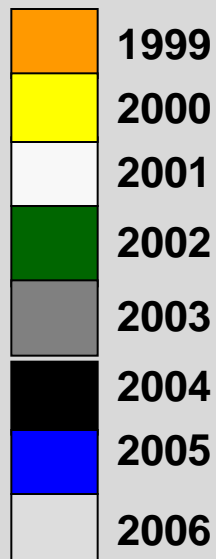
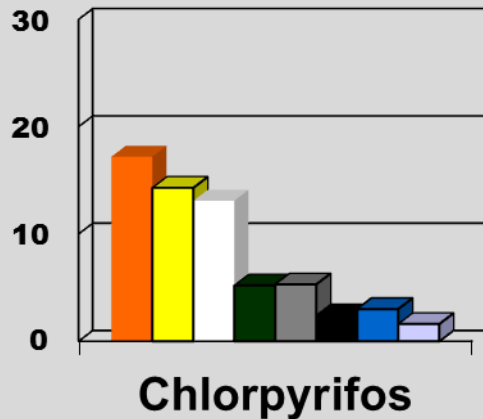
# Why study an urban population?

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

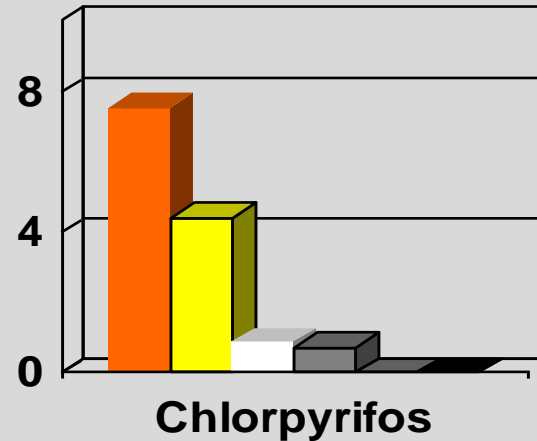
- In 1997, the amount of insecticide applied by licensed applicators in NYC exceeded the amount applied in any other NY county, including farming regions
- 86% of cohort women reported using pest control methods (sprays and bombs) during pregnancy
- Maternal and newborn blood levels were highly correlated showing that these insecticides readily crossed the placenta
- Exposure levels in NYC cohort were comparable to levels in California farming communities
- **Chlorpyrifos was detected in 99% of air and 70% of maternal and umbilical cord blood samples**

# Chlorpyrifos levels in personal air and blood declined immediately after EPA ban

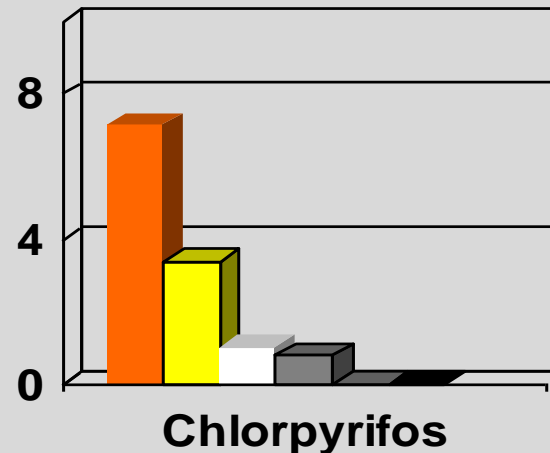
Personal air (ng/m<sup>3</sup>, n=621)



Maternal plasma (pg/gm, n=424)

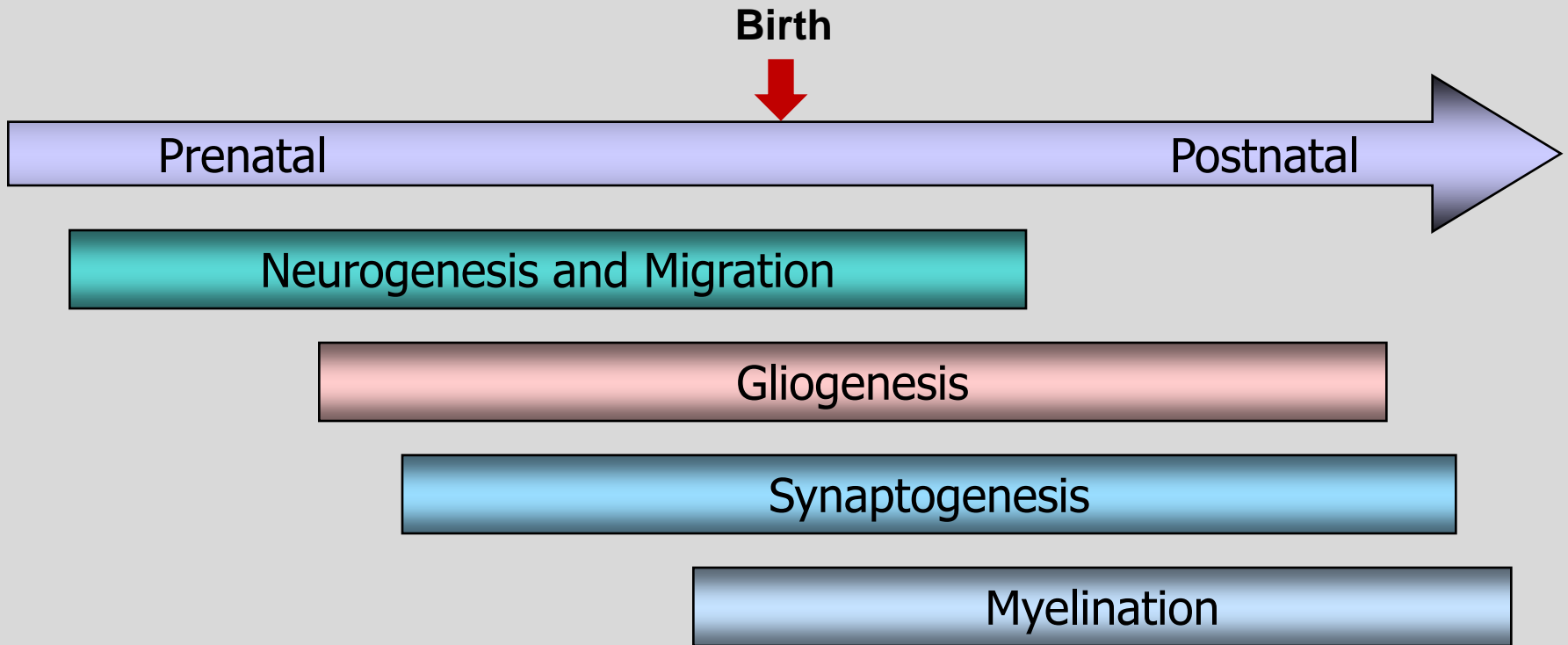


Cord plasma (pg/gm, n=395)





# 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.<sup>1</sup>**

<sup>1</sup>Slotkin, *Toxicol Appl Pharmacol.* 2004

# 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;
- 48-hour personal air sampling



## Biomarkers

- Pesticide compound in maternal and cord blood



## Outcomes

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



# 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 of attentional capacity, impulse control, memory, and sensorimotor functioning;
- Reduced overall brain size, and this overall reduction will be driven by disproportionate reductions in the size of sub-regions of the brain in the heteromodal association cortices (prefrontal, parietal, and lateral temporal) and in the hippocampus--regions that subserve higher cognitive functions.

# 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

Maryse F. Bouchard,<sup>1,2</sup> Jonathan Chevrier,<sup>1</sup> Kim G. Harley,<sup>1</sup> Katherine Kogut,<sup>1</sup> Michelle Vedar,<sup>1</sup> Norma Calderon,<sup>3</sup> Celina Trujillo,<sup>1</sup> Caroline Johnson,<sup>1</sup> Asa Bradman,<sup>1</sup> Dana Boyd Barr,<sup>4</sup> and Brenda Eskenazi<sup>1</sup>

<sup>1</sup>University of California–Berkeley, Center for Environmental Research and Children's Health, School of Public Health, Berkeley, California, USA; <sup>2</sup>CHU Sainte-Justine Research Center and Université de Montréal, Department of Environmental and Occupational Health, Montreal, Quebec, Canada; <sup>3</sup>Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS), Clinica de Salud del Valle de Salinas, Salinas, California, USA; <sup>4</sup>Emory University, Rollins School of Public Health, Atlanta, Georgia, USA

and Cognitive  
Canfield,<sup>5</sup>

## Prenatal Exposure to Organophosphate Pesticides and Child Development in Childhood

Stephanie M. Engel,<sup>1</sup> James Wetmur,<sup>2,3</sup> Jia Chen,<sup>1</sup> and Mary S. Wolff<sup>1</sup>

<sup>1</sup>Department of Preventive Medicine, <sup>2</sup>Department of Microbiology, and <sup>3</sup>Sinai School of Medicine, New York, New York, USA; <sup>4</sup>School of Public Health, Cornell University, Ithaca, New York, USA

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

Virginia Rauh,<sup>1</sup> Srikanth Arunajadai,<sup>2</sup> Megan Horton,<sup>3,4</sup> Frederica Perera,<sup>4</sup> Lori Hoepner,<sup>4</sup> Dana B. Barr,<sup>5</sup> and Robin Whyatt<sup>4</sup>

<sup>1</sup>Heilbrunn Center for Population and Family Health, Mailman School of Public Health, <sup>2</sup>Department of Biostatistics, Mailman School of Public Health, <sup>3</sup>Sergieievsky Center, and <sup>4</sup>Columbia Center for Children's Environmental Health, Mailman School of Public Health, Columbia University, New York, New York, USA; <sup>5</sup>Emory University, Atlanta, Georgia, USA

# Convergence of observational evidence:

- All 3 papers reported significant 7 year cognitive deficits on WISC subscales 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

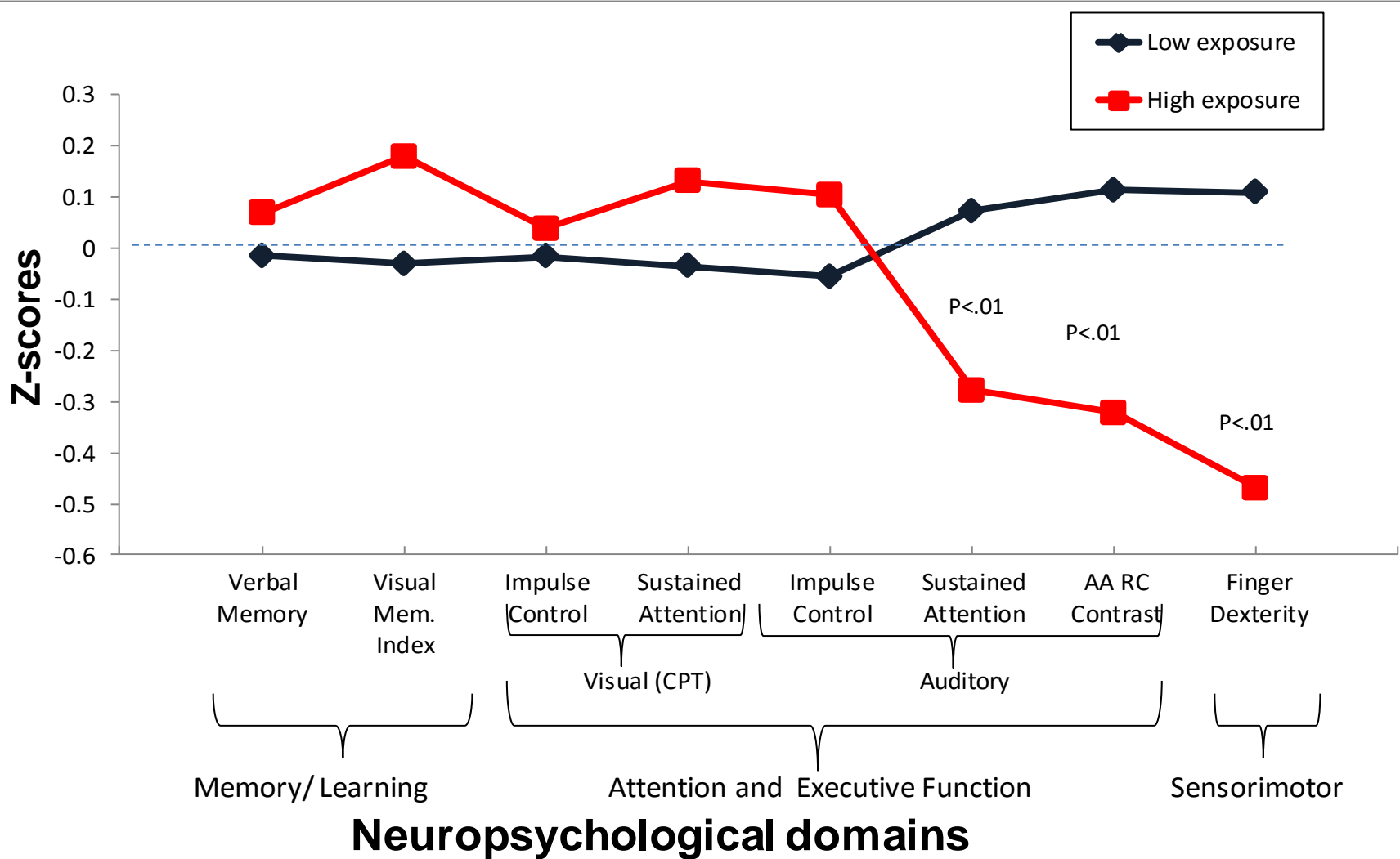


# Neuropsychological Outcomes at 12 Years

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**

# Comparison of neuropsychological profiles at 12 years by CPF exposure level (high/low), adjusted for age, sex, ethnicity and ETS



# Mapping to alterations in brain structure and function

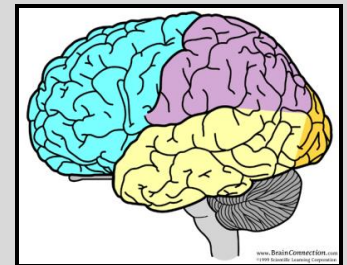
We conducted a pilot MRI study (N=40) in this cohort when the children were 6-11 years of age

## High exposure group (n=20)

- CPF level  $\geq 4.39$  pg/g, reflecting upper tertile
- No prenatal ETS exposure
- Low prenatal PAH exposure

## Low exposure group (n=20)

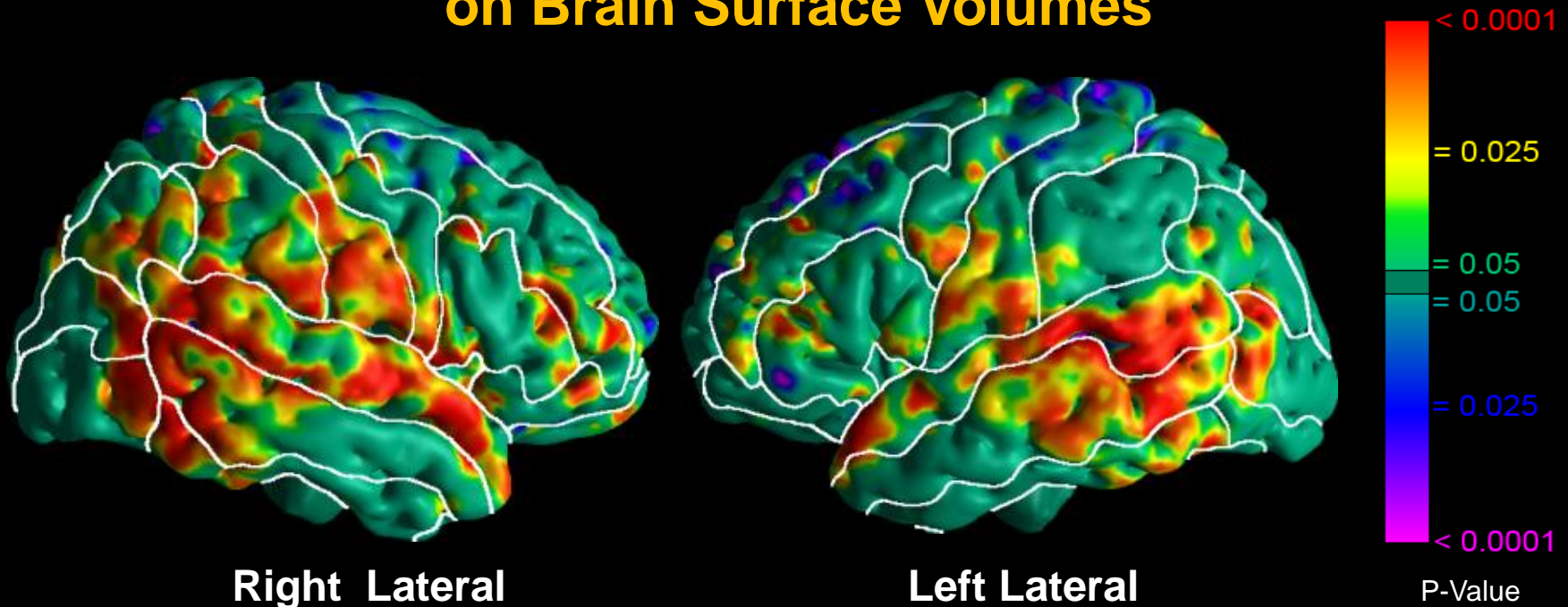
- CPF level  $< 4.39$  pg/g (lower tertiles)
- No prenatal ETS exposure
- Low prenatal PAH exposure



# Results #1: Morphology of the Cerebral Surface

- Overall brain size did not differ significantly across exposure groups, unadjusted or adjusted for age, sex, and height;
- There were volumetric differences and deformations in specific brain regions, with or without correction for overall brain size;
- Enlargements at the cerebral surface derived primarily from enlarged underlying white matter.

# Main Effects of Prenatal Chlorpyrifos Exposure on Brain Surface Volumes



- High chlorpyrifos exposure is associated with significant structural abnormalities (unilateral and bilateral enlargements; inward deformations). Warm colors indicate enlargement of underlying white matter and cool colors indicate indentation deformation.
- Regions with abnormalities sub-serve attention, receptive language, social cognition, reward, emotion, and inhibitory control.

*Rauh et al., PNAS, 2012*

# Cognitive and behavioral processes subserved by the affected cortical regions

- **Attention and receptive language** (posterior temporal regions)
- **Social cognition** (mesial superior frontal gyrus, cuneus, precuneus, and superior temporal gyrus)
- **Reward, emotion, and inhibitory control** (gyrus rectus and related orbitofrontal regions)
- **Executive function** (inward deformations in the dorsal and mesial surfaces of the left superior frontal gyrus)

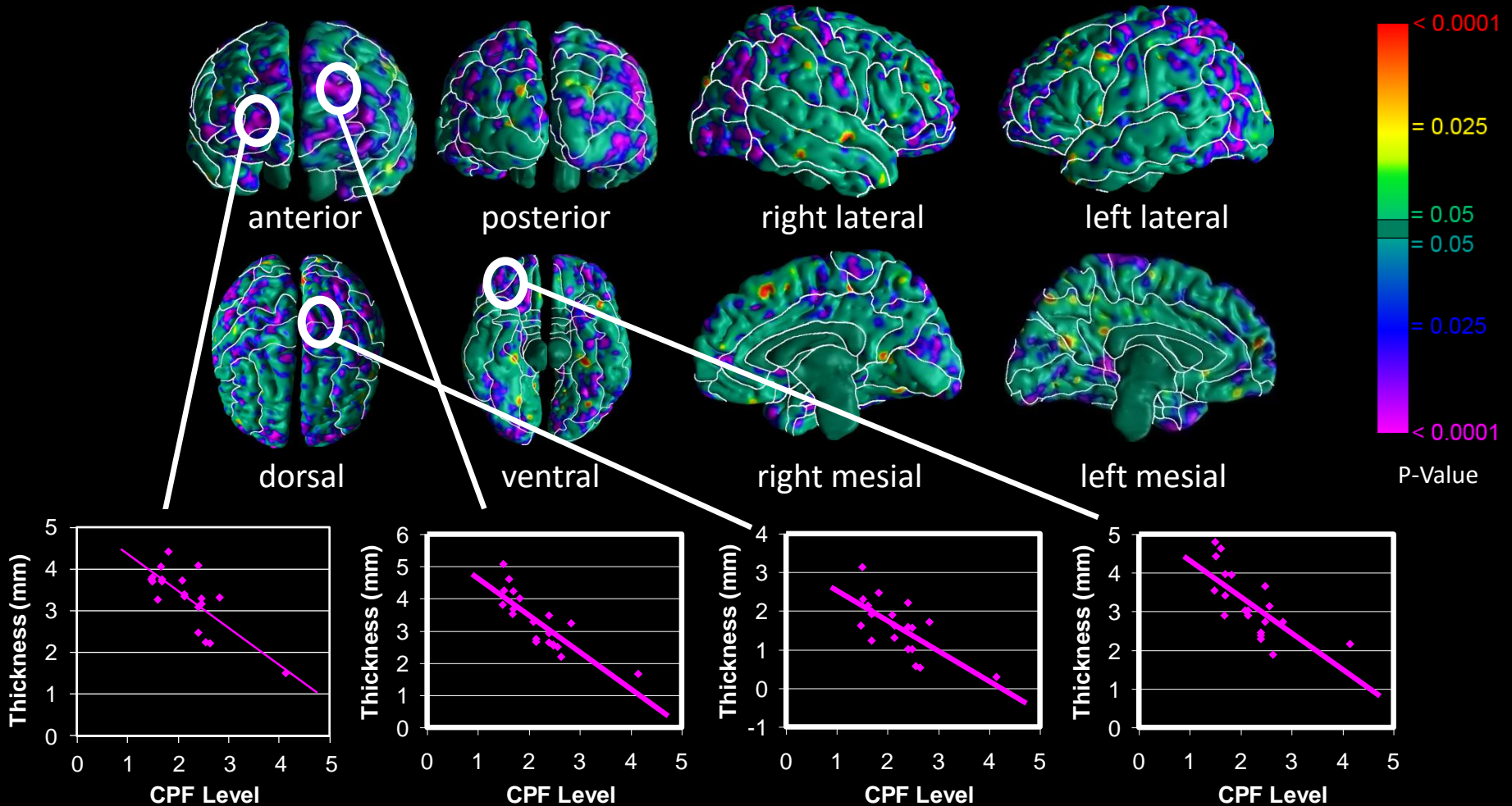


## **Results #2 :**

### **Association with Cortical Thickness**

- Reduced thickness of dorsal parietal and frontal cortices in the high exposure group
- Within the high exposure group, an inverse dose-response relationship of cortical thickness with CPF exposure levels

# Correlation of Cortical Thickness with CPF Exposure Levels in Children with High CPF Exposure (N=20)



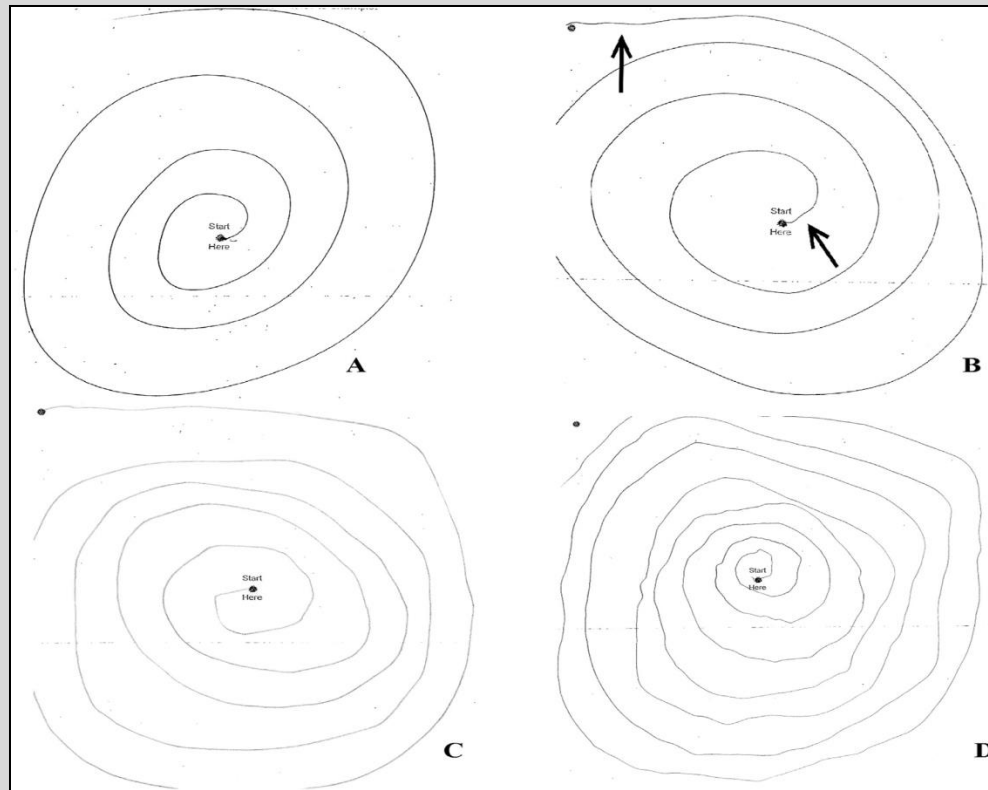


# Motor Findings: Tremor Measure

- N=271 children, 9-13 years of age
- Tremor assessed by the Archimedes Spiral, a free-hand motor test rated by a senior neurologist (prevalence of 9.1% measureable tremor in this age group<sup>\*</sup>).
- Ratings range from 0 (no tremor) to 2 (moderate amplitude oscillations present throughout the spiral)

*<sup>\*</sup>Louis et al., Pediatr Neurol, Feb, 2015*

# Examples of spiral ratings



(A) Rating=0 (no tremor)

(B) Rating=0.5 (subtle, low amplitude oscillations are present in a few spots [see arrows] but are not consistently present throughout the spiral)

(C) Rating=1.0 (low amplitude oscillations are present in multiple places)

(D) Rating=1.5 (low amplitude oscillations are present in multiple places and oscillations can at times reach moderate amplitude)

# CPF effects on Tremor

Children in the high CPF exposure group showed:

- Higher rates of clinically meaningful tremor in unadjusted analyses.
- Logistic regression (adjusted for sex, age at testing, ethnicity, medications) showed positive associations of high CPF group with tremor in the dominant hand ( $p=0.015$ ), tremor in either hand ( $p=0.028$ ), tremor in both hands ( $p=0.027$ ), and marginal association with tremor in the non-dominant hand ( $p=0.055$ ).
- Tremor is presumed to originate at the sub-cortical level.

## **To summarize the MRI findings:**

These brain anomalies suggest that prenatal CPF exposure, even at low levels consistent with standard usage, is significantly associated with structural changes in the developing brain that persist into middle childhood, and may be related to longer-term neuropsychological and motor problems.





# Acknowledgments

## Co-Investigators

F. Perera, V. Rauh, R. Whyatt, H. Andrews,  
R. Miller, B. Peterson, D. Camann, D. Barr,  
M. Horton, D. Tang, L. Hoepner, A.  
Rundle, R. Bansal, A. Margolis

## Research Workers

D. Diaz, M. Reyes, F. Arias, D. Holmes, J.  
Ramirez, G. Badia, A. Peralta, R. Martinez,  
Y. Nunez

## Funders

- NIH (NIEHS)
- EPA
- Private foundations and individuals

**Thanks to the mothers and  
children of NYC who have so  
generously given their time  
and effort to this work!**

