Chlorpyrifos

Human Data on Developmental and Reproductive Effects

Office of Environmental Health Hazard Assessment

Developmental Studies in Humans

Prospective cohort studies:

- **Columbia** *(Perera et al., '03, Whyatt et al., '04, '05, Rauh et al., '06)*
- **Mt. Sinai Hospital** *(Berkowitz et al., '04, Engel et al., '07, Wolff et al., '07)*
- **CHAMACOS** *(Eskenazi et al., '04, '07, Young et al., '05)*

Cohort study: *(Samarawickrema et al., '08)*

Case-control study of birth defects: *(Rull et al., '06)*

Case reports: (*Sherman '96, Sebe et al., '05)*

Related study of brain tumors: (*Searles Nielsen et al., 2005)*

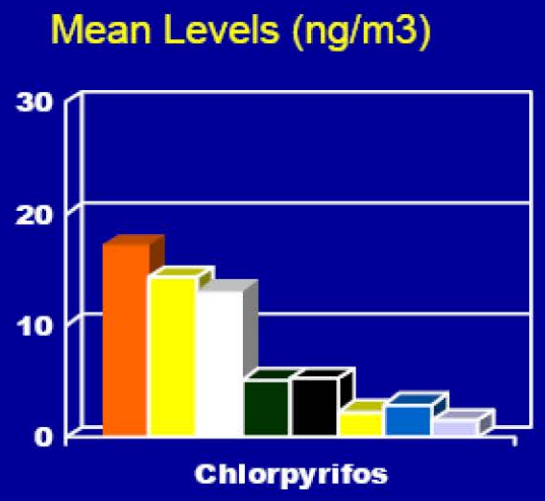
Columbia Cohort

Whyatt et al., 2004, 2005, Rauh et al., 2006

- African American and Dominican women in New York City
- Enrolled by 20th week of gestation
- 314 mother-newborn pairs
- Exposure measures - maternal and cord blood CPF, and personal air monitoring
- Multiple birth outcomes and neurodevelopmental outcomes
- Unique feature – pre/post cancellation of CPF for residential use

Updated analyses by Whyatt (Unpublished; prepared for US EPA)

Chlorpyrifos in 48 hour personal air samples during pregnancy by year of monitoring (N=621)

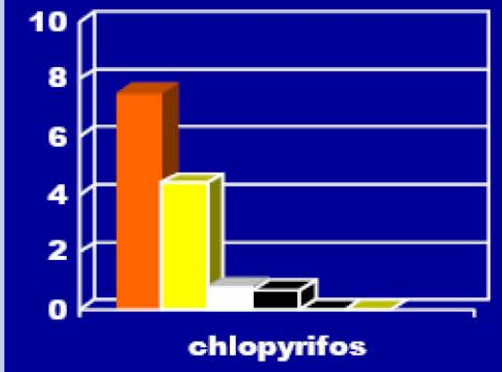


Year	N > LOD (%)
1999	123/123 (100%)
2000	126/126 (100%)
2001	89/90 (99%)
2002	59/59 (100%)
2003	64/64 (100%)
2004	62/63 (98%)
2005	81/83 (98%)
2006	12/13 (92%)

Updated analyses by Whyatt (Unpublished; prepared for US EPA)

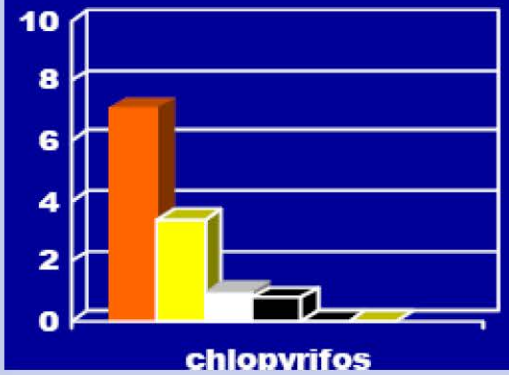
Chlorpyrifos levels in maternal and umbilical cord blood samples (pg/gram) at delivery by year of birth

Maternal blood (N=424)



Year	N > LOD (%)
1999	68/71 (96%)
2000	101/120 (84%)
2001	26/86 (30%)
2002	18/59 (31%)
2003	0/58 (0%)
2004	0/30 (0%)

Cord blood (N=395)



Year	N > LOD (%)
1999	93/110 (85%)
2000	88/110 (80%)
2001	18/71 (25%)
2002	14/36 (39%)
2003	0/43 (0%)
2004	0/25 (0%)

Whyatt et al., 2005

- Significant decreases in birth weight and birth length
- Birth weight averaged 215.1 g (95% CI -384.7, -45.5) less in those with highest exposure compared with those with lowest combined cord plasma CPF and diazinon (p = 0.01)

Birth weight – for each log unit increase in CPF

Born *before* 1/1/01

- 67.3 gm (95% CI, -116.6 to – 17.8) p = 0.0008

Born *after* 1/1/01

30.7 (95% CI, -108.6 to 169.9)

Birth Length – similar statistically significant decreases were seen in infants born before and after 1/1/01

Updated analyses (Unpublished; Prepared for EPA)

Change in birth weight for each log unit increase in chlorpyrifos levels in umbilical cord plasma among infants born before and after 1/1/01

	B (95% CI)	P-value
1. Total cohort (n=426)	-12.8 (-44.2, 18.6)	p=0.43
2. Before ban (n=233)	-71.4 (-121.1, -21.8)	p=0.005
3. After ban (n=193)	32.7 (-59.3, 124.7)	p=0.48

By multiple linear regression. Independent variable: (ln)pesticide controlling for active and passive smoking, ethnicity, parity, maternal pre-pregnancy weight and net weight gain during pregnancy, gender and gestational age of the newborn, and season of delivery

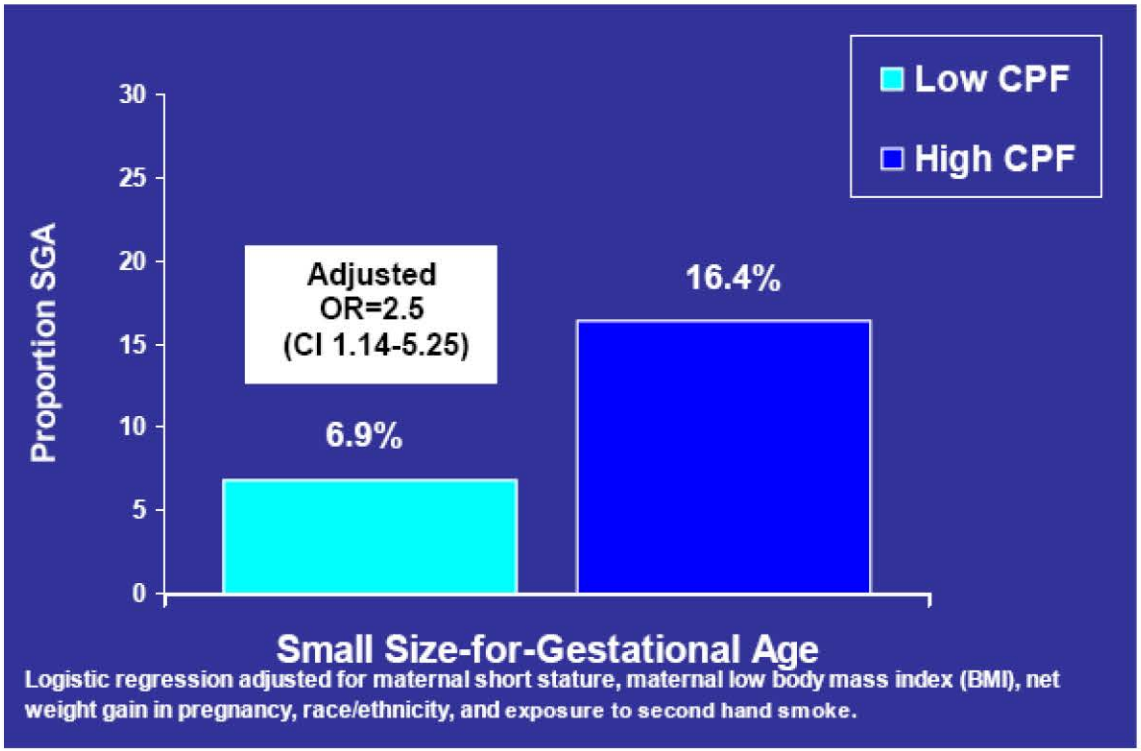
Additional Analyses by Whyatt Presented to US EPA (cont.)

CPF findings remained statistically significant after:

- Omitting imputed cord blood values
- Evaluating potential confounders – related to SES, housing disrepair, material hardship, maternal satisfaction, prenatal alcohol consumption, prenatal exposure to polycyclic aromatic hydrocarbons (PAHs) and lead
  - Previously controlled for diazinon and propoxur

Additional analyses by Whyatt and Rauh presented to US EPA

Figure 1. Logistic regression showing the effect of high chlorpyrifos exposure on the odds of SGA in a cohort of inner city children



Source: Rauh et al., 2007, presentation at the International Conference of Toxicology Montreal. CHLORPYRIFOS EXPOSURE, FETAL GROWTH RESTRICTION, AND POSTNATAL CATCH-UP GROWTH IN CHILDREN
SGA=size for gestation age. N=385.
Low = umbilical cord blood < 6.17 pg/g plasma of chlorpyrifos
High = umbilical cord blood > 6.17 pg/g plasma of chlorpyrifos

Columbia Study

Rauh et al., 2006

- Neurodevelopmental outcomes:
  - — Bayley Scales of Infant Development II to assess mental (cognitive) and psychomotor development at 12, 24, and 36 months of age
  - — Childhood Behavioral Checklist to measure behavior problems
  - — Home Observation for Measurement of the Environment (HOME) to measure the quality of the caretaking environment

Odds Ratio (95% Confidence Interval) of the Effects of High Exposure to CPF

Mental Delay (Months)			
	12	24	36
CPF	1.22 (0.48 – 3.06)	1.75 (0.86 – 3.60)	2.37 (1.08-5.19)
Psychomotor Delay (Months)			
	12	24	36
CPF	1.88 (0.78-4.53)	1.01 (0.37-2.76)	4.52 (1.31-12.70)
Behavioral Disorder (at 36 months)			
	Attention Problems	ADHD Problems	PDD Problems
CPF	11.26 (1.79-70.99)	6.50 (1.09-38.69)	5.39 (1.21-24.11)

Mt. Sinai Study

Berkowitz et al., 2004, Wolff et al., 2007 (related) Engel et al., 2007 (related)

- Puerto Rican Hispanic, African American and Caucasian women in New York City
- Enrolled by 20th week of gestation
- 404 mother-newborn pairs
- Measured urinary TCPY and dialkyl phosphate (DAP) metabolites
- Multiple birth outcomes and neurodevelopmental outcomes
- Unique feature – measured PON1 status and/or activity

Berkowitz et al., 2004

- Significant reductions in head circumference at birth were associated with detectable levels of TCPY (>11 ug/L) in maternal urine and low maternal PON1 activity ($p = 0.014$)

- No association between PON1 activity or TCPY and birth weight or birth length

Wolff et al., 2007 (related study)

- As in Berkowitz et al., 2004, there was an inverse association between maternal PON1 activity and head circumference ($p = 0.004$)
- A significant 163 g deficit in birth weight between extremes of the interaction - fast activity-PON1 genotype/low Σdiethyl phosphate (DEP) and slow activity-PON1/high ΣDEP ($p = 0.042$)
- A similar effect was seen between the extremes for PON192*DEP interaction (199 g deficit) ($p = 0.02$)

Engel et al., 2007 (related study)

- Infants whose mothers had total DEP levels above the median were 2.3 times more likely to have at least 2 abnormal reflexes (95% CI, 1.1 to 5.0)

CHAMACOS

Eskenazi et al., 2004, 2007, Young et al., 2005 (related study)

- Women living in the Salinas Valley in California
- Predominantly of Mexican descent
- Enrolled at 20 weeks gestation
- 488 mothers-child pairs
- Measured TCPY, specific and total DAPs in urine
- Multiple birth outcomes and neurodevelopmental outcomes

Eskenazi et al., 2004

- No association between TCPY and parameters of fetal growth or gestational age
- Decreases in gestational duration associated with increased urinary dimethyl phosphates (DMP) levels and decreased levels of cholinesterase in umbilical cord blood

Eskenazi et al., 2007

- No associations observed between TCPY levels and MDI or PDI scores or CBCL outcomes
- Elevated odds ratios seen between total DAPs and pervasive development disorder (PDD)

Young et al., 2005 (related study)

- Association between DEP and the number of abnormal reflexes (similar associations seen with total DAP and DMP)

Samarawickrema et al., 2008

- Compared pregnant women exposed during spray season and in between spray seasons in Sri Lanka
- CPF detected in one maternal and cord blood sample
- Lower mean cord blood BuChE activity seen during the spray season compared with the between-spray season
- Evidence of increased oxidative stress and high molecular weight fragmentation activity in fetal DNA during the spray season

Three Prospective Cohorts

Columbia African - American, Dominican	Maternal and cord blood CPF	↓ Birth wt and length ↑ SGA, ↑ Delays in mental and psychomotor develop., ↑ behavioral disorders
Mt. Sinai PR Hispanic, African-American Caucasian	TCPY, PON1 Median TCPY = 7.6 ug/L	↓ Head circumference
CHAMACOS Mexican	TCPY Median = 3.3 ug/L	No effects

Female Reproductive Studies of Chlorpyrifos in Humans

Wagner et al., 1990

CPF was detected in:

- 1/11 human milk samples (0.436 ug/kg)
- 3/11 cervical fluid samples (0.62ug/kg – 6.83 ug/kg)
- Was not detected in follicular fluid

Sanghi et al., 2003

- Breast milk samples from 12 women in Bhopal, India
- CPF was detected in all samples (mean 0.230 mg/L, range 0.085 -0.355 mg/L)
- Estimated intake in infants would be 0.041 mg/kg, 4.1 times greater than recommended by WHO
- Other pesticides detected included endosulfan, malathion, methyl-parathion

Male Reproductive Studies of Chlorpyrifos in Humans

Six studies:

- **One case control** *(Swan et al., 2003)*
- **Five cross-sectional** *(Meeker et al., 2004a, 2004b, 2006a, 2006b, 2008)*
- **Two related studies** **(***Perez-Herrera et al., '08, Salazar-Arredondo et al., '08)*
    - in-vitro
    - multiple pesticide study - interaction with PON1 data

Swan et al., 2003

- Case–control study conducted in men from a multi-centered study of semen quality in fertile men
- Men chosen from Missouri and Minnesota
- Cases – men having poor semen quality
- Odds Ratio for low semen quality
  - — Missouri OR = 6.4 (95% CI, 0.5 to 86.3)
  - — Minnesota OR = 0.5 (95% CI, 0.04 to 8.3)

Meeker et al., 2004a, 2004b, 2006a, 2006b, 2008

- Cross-sectional studies
- Males in sub-fertile couples from an andrology clinic
- TCPY in urine
- 360 men, mean age 36.1 years, mostly Caucasian
- Men excluded on the basis of specific gravity values being too concentrated (>1.030) or too dilute (<1.010)

Meeker et al.

- **Higher TCPY associated with two measures of sperm DNA integrity**

  Suggesting DNA damage in human sperm

- **Suggestive associations between higher TCPY and**

  ↓ sperm concentration, sperm motility, and straight-line velocity, no association with sperm morphology, or other motion parameters

- **Higher TCPY associated with**

  ↓ testosterone levels, no association with FSH, LH, inhibin B, SHBG, free androgen index

  ↓ decrease in T4 levels and ↑ in TSH, no association with total T3

  ↓ in estradiol levels, no association with prolactin

Two Related Male Reproductive Studies

Salazar-Arredondo et al., 2008

- In vitro exposure to CPF, methyl-parathion, and diazinon and their oxon
- Oxon metabolites and parent compounds have ability to damage mature sperm chromatin, particularly DNA

Perez-Herrera et al., 2008

- Cross-sectional study, agricultural workers in Mexico
- Sperm DNA integrity and semen quality parameters (viability, motility, and morphology) modified by PON1Q192R polymorphisms

Summary - Developmental

Animal Data

- No malformations or effects on viability & body weight

Neurodevelopment

- Oral exposures: indicative of effects on behavior, brain morphometry
- Subcutaneous exposure: cholinergic & cellular effects on hippocampus & cerebral cortex, biochemical changes affecting synaptic nerve terminals & synaptic activity

Human Data

- ↓ birth weight, ↓ birth length, ↓ head circumference

Neurodevelopment

- ↑ neurodevelopmental delays (MDI, PDI), ↑ behavioral disorders (attention, ADHD, PDD)

Summary - Female Reproductive

Animal Studies

- **Presence of CPF in milk**
- **↓ pup weight, ↑ pup mortality in F1 rats at 5 mg/kg-day**

Human Studies

- **Presence of CPF in breast milk**

Summary – Male Reproductive

Animal Studies

- Some DNA damage (genotoxicity studies)

Human Studies

- ↓ DNA integrity in sperm suggesting DNA damage
- ↓ sperm concentration, sperm motility, and straight-line velocity, no association with sperm morphology, or other motion parameters
- ↓ testosterone levels, no association with FSH, LH, inhibin, SHBG, free androgen index, ↓ T4 levels, ↑ TSH, no association with total T3
- ↓ estradiol levels, no association with prolactin