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Appendix D:
Human Epidemiology: Effects in Children

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1.0. Introduction

Three major prospective epidemiologic cohort studies are looking at pre- and post-natal pesticide exposure in minority mothers and infants, birth outcomes, genetic susceptibility plus long-term childhood neurobehavioral and neurodevelopment outcomes. Funded by multiple Federal Agencies, including US EPA, the study sites are: (1) Columbia University, Mailman School of Public Health NYC, (2) Mt Sinai, School of Medicine, NYC, both with multi-ethnic urban poor women and infants, and (3) University of California at Berkeley (CHAMACOS, Center for Health Assessment of Mothers and Children of Salinas) with women and their children from low-income farm worker populations. The following sections provide a summary of the publications along with the strengths and weaknesses of these studies from the three epidemiologic research groups. There is a brief summary of results from Mt Sinai and Berkeley research teams who measured biomarkers of susceptibility e.g., paraoxonase (PON1) status in minority women and their infants to assess human polymorphisms in genetically determined organophosphate insecticide detoxification capacity. There is also a brief discussion of a recent related work (4) by Lu et al., (2008) who reported a rapid decline in child urinary 3,5,6-trichloro-2-pyridinol (TCP, chlorpyrifos metabolite) levels when switched to an organic diet of fruits and vegetables compared to a conventional diet. OPP has evaluated these studies with respect to their qualitative and quantitative relevance to the risk assessment of chlorpyrifos. This Appendix begins with a more extensive discussion of studies from Columbia University. The main document provides a weight of the evidence discussion where animal and human data on acetyl cholinesterase (AChE) inhibition, other modes/mechanisms of action, metabolism, genetic polymorphism, and epidemiology are discussed in an integrative manner.

The information described below is also summarized in Attachment A (Table A.1). Brief, descriptions of the five child neurodevelopment test scales used [Bayley, Brazelton, Child Behavior Checklist, Peabody Picture Vocabulary Test (PPTV) and, Wechsler Preschool and Primary Scale of Intelligence (WPPSI)] are included as Attachments B through F to this document.

2.0. Columbia University

The Columbia Center for Children's Environmental Health, in partnership with Centers for Disease Control and Prevention (CDC), began looking in 1997 at biomarkers of pesticide exposure, including chlorpyrifos and diazinon in maternal and cord blood from exposure from residential treatments and continue to follow this cohort to present day (Whyatt et al., 2001, 2003, 2004, 2007, 2008, Perera et al. 2005, Rauh et al. 2004, 2006, 2008). The Columbia cohort is composed of a total of 725 mother/newborn pairs of African American (65%) and Dominican/Latina (35%) descent. The cohort is comprised of non-smokers, non-

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illicit drug users who are without HIV, hypertension or diabetes. The subjects live in Northern Manhattan and the South Bronx. At the time of enrollment, approximately 25% of the mothers were married, 36% did not complete high school, and about 45% had incomes less than ten thousand dollars per year. Ninety one percent received Medicaid. Other characteristics of the population and standard covariates controlled for in the epidemiologic risk regression models are given in Table A-1, in Attachment A.

Whyatt et al. (2004) report chlorpyrifos and diazinon levels in umbilical cord blood plasma of 237 infants born before January 1, 2001. The Columbia research team also reports values for 77 infants born after the January 1, 2001 through about August 2003. Their later published studies report on additional post-cancellation cases. For the purposes of this review, the initial pre- and post-cancellation are of special interest, given the subsequent reports on long-term neurobehavioral and neurodevelopment deficits.

A number of data sources were utilized in the Columbia study including:

- **Maternal interviews (prenatal)** were conducted by trained bilingual interviewers to assess age, education, race/ethnicity, income, employment.

- **Biologic samples** of umbilical cord blood at delivery and maternal blood during pregnancy and delivery. Also meconium and urine samples were collected.

- **Environmental samples** of 48-hour personal air monitoring and stationary indoor air monitoring for two months during the third trimester of pregnancy.

- **Medical Records** were used to evaluate gestational age, gender, birth weight, length, head circumference, maternal height, pre-pregnancy weight and weight gain, medications, medical complications.

- **Observational measures of the home (2 years)** were made using a standardized instrument**,** called the HOME inventory.

- **Repeated child (1, 2, 3 years) developmental testing** was completed with the standardized Bayley Scales of Infant Development II (BSID-II). **Maternal testing** was done using non-verbal Maternal IQ (TONI-3). All tests were conducted by trained personnel with appropriate language skills.

- **Maternal Report (3 years).** These included the Child Behavior Checklist (CBCL), a standardized instrument with relevance for subsequent behavior problems and potential clinical diagnoses.

- **Wechsler Pre-School and Primary Scale of Intelligence-R (5 years)**

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- **Wechsler Intelligence Scale for Children IV (7 years)**

The study considered potential exposure to a number of compounds including 10 pesticides, air pollutants [polycyclic aromatic hydrocarbons (PAHs), particulate matter (PM) and environmental tobacco smoke (ETS)], allergens, metals (lead and mercury), and phthalate diesters. A total of 29 pesticides were measured in maternal and infant cord plasma blood. Some of the following pesticides were measured in both environmental and biologic samples: 4 OPs (chlorpyrifos, diazinon, malathion and methyl parathion), 4 carbamates (bendiocarb, carbaryl, carbofuran and propoxur) and *cis*-permethrin and *trans*-permethrin. The study also considered nutritional deficits (vitamins A, C, E) and social stressors (Rauh 2008, Presentation to EPA).

Type and amount of pesticide use in the home during pregnancy was measured via questionnaires. These data show that 85% of the women reported using some form of pest control during pregnancy, with 36% applied by exterminators and 49% applied by others (e.g., the women herself, other household member or apartment superintendent) (Whyatt et al. 2002, 2004, 2005, 2007 presentation to EPA). Of these women, 45% said their homes were sprayed more than once per month. In addition, the researchers were able to document that chlorpyrifos was heavily applied to these low-income residential settings between 1998 and 2001 because of a law in New York City that required pesticide control operators (PCOs) to report the amount of pesticides applied.

A unique aspect of this research is that the study was underway during the residential phase-out of chlorpyrifos, which was publically announced June 2000, and completed by December 2001. Chlorpyrifos levels dropped significantly in blood and air samples (Whyatt et al. 2005) following the 2000-2001 phase-out of most residential uses, as shown on Table 1. The study authors evaluated the impacts of this mitigation measure by comparing exposures and outcomes both before and after the phase-out. Surveys on pesticide sales in NYC stores confirm that the sale of chlorpyrifos products declined when the EPA announced the residential phase out in June 2000, and ceased when the cancellation took effect on January 2002.

The Columbia team included neurophysiologic (growth) measures (birth weight and length) and neurodevelopmental outcomes (on scales comparable to CHAMACOS and Mt Sinai) to the present time, as the birth cohort approaches school age. Specifically, the Columbia researchers have reported adverse impacts of prenatal chlorpyrifos on birth weight and length, and neurodevelopment in the first three years of life (Whyatt et al. 2004, Rauh et al. 2006). The study authors continue to collect and evaluate neurodevelopmental data for children five and seven years of age (Rauh 2008, Presentation to EPA).

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Preliminary analysis of these data suggests the neurodevelopmental effects persist in these children.

2.2. Chlorpyrifos Exposure Measurements in Biological and Environmental Media

Maternal Plasma, Umbilical Cord Plasma. Whyatt et al. (2003) report on levels of 29 pesticides or their metabolites in maternal and infant cord plasma between 1998 and 2001 from 199 women from this cohort. Chlorpyrifos was the most frequently detected OP pesticide and was present in 74% and 71% of samples from maternal and umbilical cord blood plasma, respectively. There were 148 and 150 samples, respectively above the Level of Detection (LOD) of 0.5-1 pg/g. The median values were 3.1 and 2.6 pg/g for moms and infants, respectively, while mean values were 4.8 and 4.7 pg/g, respectively. The range was from non-detectable (ND) to 35 pg/g for moms and ND to 63 pg/g for newborns.

Maternal plasma and infant cord blood plasma chlorpyrifos levels were highly correlated ($r=0.76$, $p < 0.001$). Maternal and cord plasma levels were also highly correlated for diazinon ($r=0.57$, $p<0.001$), the carbamates bendiocarb ($r=0.51$, $p<0.001$) and 2-isopropoxyphenol ($r=0.39$, $p<0.001$) (metabolite of propoxur) and the fungicides dicloran ($r=0.78$, $p<0.001$), and tetrahydrophthalimid (metabolite of captan and captafol) ($r=0.33$, $p<0.001$).

Chlorpyrifos in maternal personal air during pregnancy and maternal plasma and cord blood plasma samples at delivery are shown in Table 1. The geometric mean values dropped approximately 9 to12 fold for both maternal and cord blood between 1999 and 2001 (from 5.2 to 0.44 pg/g for maternal blood and from 3.74 to 0.42 pg/g for cord blood). The residential phase out was announced on June 2000, and completed by December 2001 when retail sales ceased. Air levels also dropped, but not as significantly. Geometric means are presented for comparison because the data tend to be log-normally distributed.

Table 1. Chlorpyrifos in Maternal Personal Air During Pregnancy and in Maternal and Cord Blood Plasma Samples at Delivery NYC Columbia University Study

Sample Media	G.M. (95% confidence Interval)						
	1999	2000	2001	2002	2003	2004	2005
48 Hour Personal air (ng/m3)	9.5 (7.87-11.49) (n=123)	6.63 (5.47-8.0) (n=126)	4.72 (3.66-6.07) (n=90)	3.13 (2.45-4.01) (n=59)	3.22 (2.54-4.07) (n=64)	1.49 (1.19-1.86) (n=63)	1.65 (1.29-2.09) (n=83)

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Sample Media	G.M. (95% confidence Interval)						
	1999	2000	2001	2002	2003	2004	2005
Maternal blood (pg/g)	5.2 (4.1-6.5) (n=71)	2.61 (2.1-3.23) (a) (n=120)	0.44 (0.37-0.54) (a,b,c) (n=86)	0.44 (0.35-0.55) (a,b,c) (n=59)	<LOD (n=58)	<LOD	NR
Cord blood (pg/g)	3.74 (2.92-4.8) (n=110)	2.05 (1.66-2.55) (a) (n=110)	0.42 (0.33-0.52) (a,b,c) (n=71)	0.47 (0.36-0.63) (a,b,c) (n=36)	<LOD (n=57)	<LOD	NR

Source: Whyatt et al. 2003, 2004, 2008
LOD= Limit of Detection for air is 2 ng/m3; for blood is 0.5 pg/g (96% samples) and 1 pg/g (4% samples); NR= not reported
Maximum Prior to Residential Phase Out: Personal air: 344.8 ng/m3; Maternal blood: 35 pg/g; Cord blood: 63 pg/g For levels of other pesticides see Whyatt (2003, 2004) publications.

Source: Whyatt et al. (2003, 2008 personal communication via email, 5/30/2008).
(a) P<0.05 test compared with 1999 levels based on Whyatt et al. 2003.
(b) P<0.05 test for linearity (ANOVA) based on Whyatt et al. 2003
(c) P<0.05 compared with 2000 levels (ANOVA) based on Whyatt et al. 2003.

Other pesticides were detected less frequently, including carbofuran in 45% of maternal and cord blood samples at mostly low levels, as the median is reported as non-detectable (<1 pg/g). Phthalimide, which is a metabolite of the fungicides folpet, and captan and the OP phosmet, was also detected with a frequency of 77% and 70% in maternal and cord blood, respectively, with reported medians of 25.2 and 24 pg/g (Whyatt et al. 2003).

African Americans had significantly higher exposures than did Dominicans to several of the insecticides, and they were significantly more likely to use can sprays than were Dominicans (Whyatt et al. 2003).

Air Monitoring. The Columbia University research team also measured eight pesticides, including chlorpyrifos via 48-hour personal air monitoring, and two week integrated stationary indoor air monitoring for two months among mothers during their third trimester of pregnancy between 1998 and 2001 (Whyatt et al. 2003). Four of the pesticides were found in 100% of the personal air samples, including chlorpyrifos, diazinon, propoxur and the fungicide *o*-phenylphenol. Chlorpyrifos had a median value of 7.1 ng/m^3, a mean concentration of 18.3 ng/m3 (± SD 36.8 ng/m^3) and a range of 0.7-345 ng/m^3. Air concentrations for diazinon, propoxur, and o-phenylphenol are even higher than chlorpyrifos with medians of 22.2 ng/m^3 (range 2.0- 6010 ng/m^3), 28 ng/m3 (range 3.1-1420 ng/m^3) and 29.4 ng/m^3 (range 7.8-743 ng/m^3), respectively (Whyatt et al. 2003). O-Phenylphenol is a widely used fungicide and antimicrobial

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agent for commercial and consumer purposes. Although present in air, o-phenylphenol was not detected in maternal or cord blood samples. Piperonyl butoxide was also detected in 45.5% of 2-week air samples with median and mean concentrations of <0.2 and 3.9 ng/m^3, respectively (range <0.2-608 ng/m^3) (Whyatt et al. 2007).

The air monitoring data indicates that multiple chemical exposure issues exist for this cohort and make for a more complex interpretation of health and exposure associations. In a subsequent publication, Whyatt et al. (2007) reported there was little within-home variability and no significant difference in air concentrations within homes. Between-home variability accounted for 88% of the variance in the indoor air levels of propoxur, 92% in chlorpyrifos and 94% in diazinon and 62% in piperonyl butoxide. Thus, pesticide values for air were more highly variable between treated homes, perhaps due to the episodic nature of indoor pesticide spraying (Whyatt et al. 2007).

A significant correlation was seen between personal air levels of chlorpyrifos, diazinon and propoxur and levels of these pesticides or their metabolites in maternal and/or cord blood ($p<0.05$). Chlorpyrifos levels in maternal personal air samples and cord blood were significantly higher in the summer compared with spring or fall ($p<0.05$) (Whyatt et al. 2003). Multiple linear regression models of chlorpyrifos in personal air were not linked to ethnicity or neighborhood. However, Whyatt et al. (2003) reported the following statistically significant associations between:

(a) chlorpyrifos in maternal plasma and ethnicity (p–value=0.008);
(b) chlorpyrifos and neighborhood for Harlem vs. Washington Heights (p–value=0.001) but not Harlem vs. South Bronx; and
(c) chlorpyrifos in umbilical cord plasma and ethnicity (p–value=0.03) and neighborhood (p–value=0.05) for Harlem vs. Washington Heights.

In sum, these results show that chlorpyrifos and other pesticide exposures vary among poor multi-ethnic populations and urban neighborhoods.

Meconium. Whyatt and Barr (2001) report on dialkyl phosphate (DAP) pesticide metabolites in meconium for a subset of infants from the Columbia University cohort (Table 2). Meconium is the fecal matter that accumulates in the intestines of the fetus *in utero* from the sixteenth week of gestation, but is not expelled until after birth. Therefore, it is an integrated measure of exposure over a longer time period during critical developmental stages of nervous system.

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Table 2. Selected organophosphate insecticides DAP metabolites in meconium

DAP OP Metabolites	Detects/ Samples	Detection Limits (µg/g)	Mean/ RSD (a)	Range (µg/g) (b)
DEP (includes chlorpyrifos) (c)	19/20	0.2	0.82/0.9	0.8-3.2
DETP (includes chlorpyrifos) (c)	20/20	0.09	2.6/ 1.4	2.0-5.6
DEDTP	1/20	0.05	NA	16
DMP	1/20	0.51	6.5/22	1.8
DMTP	ND	0.18	0	0
DMDTP	ND	0.08	0	0

DEP=diethyl phosphate DETP=diethylthiophosphate DEDTP=diethyldithiophosphate DMP= dimethyl phosphate DMTP= dimethylthiophosphate DMDTP=di methyldithiophosphate; ND = not detected, NA = not applicable; RSD = residual standard deviation
(a) From Whyatt, Barr (2001), Table 3, DAP concentration in meconium stored at room temperature
(b) = Specifications of the analytic methods.
(c) From metabolism of chlorpyrifos and/or possibly any of 8 crop use pesticides (Chlorethoxyphos, Chlorpyrifos, Coumafos, Diazinon, Disulfoton, Ethion, Parathion, Phorate, Sulfotepp, Terbufos). Except for residential uses of Chlorpyrifos and Diazinon, the others crop use insecticides are unlikely to be used near poor urban NYC mothers

Given the publication date, these meconium data likely represent values from residential pesticide exposures prior to the time of the phase out. The frequent presence of DEP and DETP concurrent with *in utero* development is of note.

Table 3 below presents the most common DAP organophosphate metabolites and identifies which pesticides may contribute to these metabolites in urine or other biological media. This table is provided for the reader as context.

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Table 3. Organophosphate Pesticide Metabolites Measured in Epidemiology Studies

Pesticide (CAS number)	Dimethyl-phosphate (813-79-5)	Dimethylthio-phosphate (1112-38-5)	Dimethyldithio-phosphate (756-80-9)	Diethyl-phosphate (598-02-7)	Diethylthio-phosphate (2465-65-8)	Diethyldithio-phosphate (298-06-6)
Azinphos methyl	•	•	•			
Chlorethoxyphos				•	•	
Chlorpyrifos				•	•	
Chlorpyrifos methyl	•	•				
Coumaphos				•	•	
Dichlorvos (DDVP)	•					
Diazinon				•	•	
Dicrotophos	•					
Dimethoate	•	•	•			
Disulfoton				•	•	•
Ethion				•	•	•
Fenitrothion	•	•				
Fenthion	•	•				
Isazaphos-methyl	•	•				
Malathion	•	•	•			
Methidathion	•	•	•			
Methyl parathion	•	•				
Naled	•					
Oxydemeton-methyl	•	•				
Parathion				•	•	
Phorate				•	•	•
Phosmet	•	•	•			
Pirimiphos-methyl	•	•				
Sulfotepp				•	•	
Temephos	•	•				
Terbufos				•	•	•
Tetrachlorvinphos	•					

From CDC (Centers for Disease Control and Prevention) 2005. Third National Report on Human Exposure to Environmental Chemicals. July. NCEH Pub. No. 05-057. Available at: http://www.cdc.gov/exposurereport/3rd/pdf/thirdreport.pdf

2.3. Prenatal Chlorpyrifos Exposure and Birth Weight and Length

Whyatt et al. (2004) report that chlorpyrifos umbilical cord blood plasma was inversely associated with birth weight and length. For each log unit increase in cord plasma chlorpyrifos level, birth weight decreased by 42.6 g ($p<0.03$) and birth length decreased by 0.24 cm ($p<0.04$). The Agency used the 'high' and 'low' chlorpyrifos designation by the study authors for convenience, and this does not necessarily reflect a judgment on the "cutoff" of chlorpyrifos exposure level. These data are depicted in Table 4.

The researchers evaluated the impact of the residential phase out for chlorpyrifos on birth weight and length. Children born pre-cancellation, before January 2001 (n =237), showed a statistically significant associations between chlorpyrifos for both birth weight (g) and birth length (cm) for chlorpyrifos (p–

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value=0.008 and 0.004, respectively) and the sum of chlorpyrifos and diazinon (p–value=0.007 and 0.004, respectively). For infants born prior to 01/01/01, birth weight decreased by 67.3 g and birth length decreased by 0.43 centimeters for each unit increase in log-transformed cord plasma chlorpyrifos levels. Both results are statistically significant at p=0.008 and 0.004, respectively (Whyatt et al.2004).

Evaluations for diazinon alone did not show significant correlations between maternal or cord blood levels and birth weight, birth length or head circumference. The propoxur metabolite 2-isopropoxyphenol in cord plasma was inversely associated with birth length (p=0.05) after controlling from chlorpyrifos and diazinon. In addition, after controlling for cord plasma 2-isopropoxyphenol (in addition to other potential confounders), the associations between birth weight and length and cord plasma (ln)chlorpyrifos as well as the sum of cord plasma (ln) chlorpyrifos and diazinon (in chlorpyrifos equivalents adjusted for relative potency) remained statistically significant (p≤ 0.02) and the effect size remained similar to that seen without 2-isopropoxyphenol in the model (Whyatt et al. 2004). There was no association between phthalimide (metabolite of folpet, captan and phosmet) and birth weight or length (personal communication with R. Whyatt, 8/8/08).

Table 4. Change in Birth Outcomes for Each Log Unit Increase in Chlorpyrifos Levels in Umbilical Cord Blood (pg/g)

Birth weight (grams)		Birth length (cm)		Head circumference (cm)	
-42.6 (-81.8 – -3.8)	P = 0.03	-0.24 (-0.47 – -0.01)	P = 0.04	-0.01 (-0.13 – 0.11)	P = 0.86

Model covariates were: gestational age of the newborn (in weeks), maternal pre-pregnancy weight and net weight gain during pregnancy (in kg), newborn gender (0 = male; 1 = female), parity (0 = nulliparous; 1 = at least one prior live birth), ethnicity (0 = Dominican; 1 = African American), ETS in the home (0 = no; 1 = yes) and season of delivery (dummy variable 1: 0 = summer; 1 = winter; dummy variable 2: 0 = summer; 1 = spring; dummy variable 3: 0 = summer; 1 = fall); models for head circumference included whether or not the delivery was by cesarean section (n=0, 1=yes).

The researchers have recently conducted additional analysis on birth weight based on discussions with EPA related to dose-response, imputing cord blood levels from maternal levels and evaluating additional potential confounders related to social economic status (SES), housing disrepair, material hardship, maternal satisfaction, prenatal alcohol consumption and prenatal exposure to PAHs and lead. Table 5 presents the results of these analyses comparing the results reported in Whyatt et al. (2004) with the updated analyses from Whyatt et al. (2008) (presentation to EPA, April). As shown in Table 5, the reduced birth

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weight and birth length for pre-cancellation chlorpyrifos levels in blood remain statistically significant at the highest exposure level, Group 4 (levels > 6.17 pg/g plasma), and no such relationship is seen post-cancellation for all additional analyses. Among infants born before the cancellation, birth weight for those with the highest chlorpyrifos exposures (group 4 levels >6.17 pg/g cord blood) averaged 192.2 (95% CI –358to -26.4) grams less than those with exposures below the limit of detection (group 1, <0.5-1 pg/g, p=0.02). After the cancellation, only one infant fell into the highest exposure group. These analyses controlled for the same potential confounders as in the previous analyses, including 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.

Table 5. Impact of Residential Cancellation on Association Between Chlorpyrifos Cord Blood and Birth Outcomes.

Change in birth weight/length for each log unit increase in chlorpyrifos (a)				
Time Period when Child Born	**Source/ Analysis Deviation**	**Sample Size**	**Mean and 95% CI**	
			Birth Weight (gm) (Mean and 95% CI)	**Birth Length (cm)**
Born prior to EPA regulations (1998-1/1/01)	Whyatt et al. 2004	222	B= - 67.3 (-116. to -17.8)** (p<0.008)	B= -0.43 (-0.73 to -.14)** (p<0.004)
	Whyatt et al. 2008 Updated Analysis	233	B= - 71.4 (-121.1 to –21.8)** (p<0.005)	NA
	Whyatt et al. 2008: Address imputation of cord levels from moms to cord blood (a1)	211	B= - 64.5 (-115.7 to –13.2)** (p<0.01)	
	Whyatt et al. 2008 Analyses to address additional confounding factors:			
	Marital status (b)	231	B= - 73.8 (-124 to –23.6)** (p<0.004)	NA
	Education ‘c)	226	B= -70.7 (-122.4 to –19)** (p<0.008)	
	Income (d)	222	B= - 68.7 (-120.3 to –17.2)** (p<0.009)	
	Housing disrepair (No versus one or more)	233	B= -70 (-119.7 to –20.3)** (p<0.006)	
	Number of disrepairs (e)	233	B= -71.5 (-121.3 to –21.7)** (p<0.005)	

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Change in birth weight/length for each log unit increase in chlorpyrifos (a)				
Time Period when Child Born	**Source/ Analysis Deviation**	**Sample Size**	**Mean and 95% CI**	
			Birth Weight (gm) (Mean and 95% CI)	**Birth Length (cm)**
	Lacked basic necessities (f)	227	B= -67.5 (-118.5 to –16.67)** (p<0.01)	
	Satisfied (yes vs no) (g)	231	B= -78.1 (-128.3 to –27.8)** (p<0.002)	
	Controlling for prenatal alcohol consumption: No vs yes	224	B= -78.3 (-128.7 to –27.9)** (p<0.002)	
	Controlling for prenatal alcohol consumption: No, some, frequent(h)	224	B= -77.7 (-128.2 to –27.2)** (p<0.003)	
	Prenatal PAH (i)	222	B= -68.7 (-120.6 to –16.7)** (p<0.01)	
	Prenatal lead (j)	156	B= -82 (-140.9 to –23.1)** (p<0.007)	
Born after EPA regulations (after 1/1/01)	Whyatt et al. 2004	77	B= 30.7 (-108.6 to 169.9) (p<0.66)	
	Whyatt et al. 2008 Updated Analysis	193	B= 32.7 (-59.3 to 124.7) (p<0.48)	

Source: Whyatt 2008, presentation to EPA, April 2008. Used with permission. Updated analysis from Whyatt et al. 2004 publication to address EPA comments.

**= statistically significant; NA= not applicable; additional analysis only conducted on birth weight.

(a) By multiple linear regression, independent variable: (ln)chlorpyrifos 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. For all models except a1, when umbilical cord blood chlorpyrifos levels were missing, values were inputted from maternal blood levels. In model a1, only (ln) umbilical cord blood levels (without any imputations from maternal blood for missing value) were included in the model as the independent variable. Models b-j control for the same covariates as above but with the addition of one additional covariate to each of the 11 models as indicated.

(b) Never married versus ever married.

(c) Less than high school versus high school or greater

(d) Annual household income less than $10,000 versus greater than $10,000.

(e) On a scale of 0-5 indices of housing disrepair are: holes in ceiling or walls, peeling paint, leaking pipes, water damage, or mold.

(f) Lacked shelter, food, clothing, heat, and/or medicine during pregnancy

(g) With overall living conditions

(h) Dummy variables: no versus some (< 1/day in any trimester); no vs frequent (≥1/day in any trimester)

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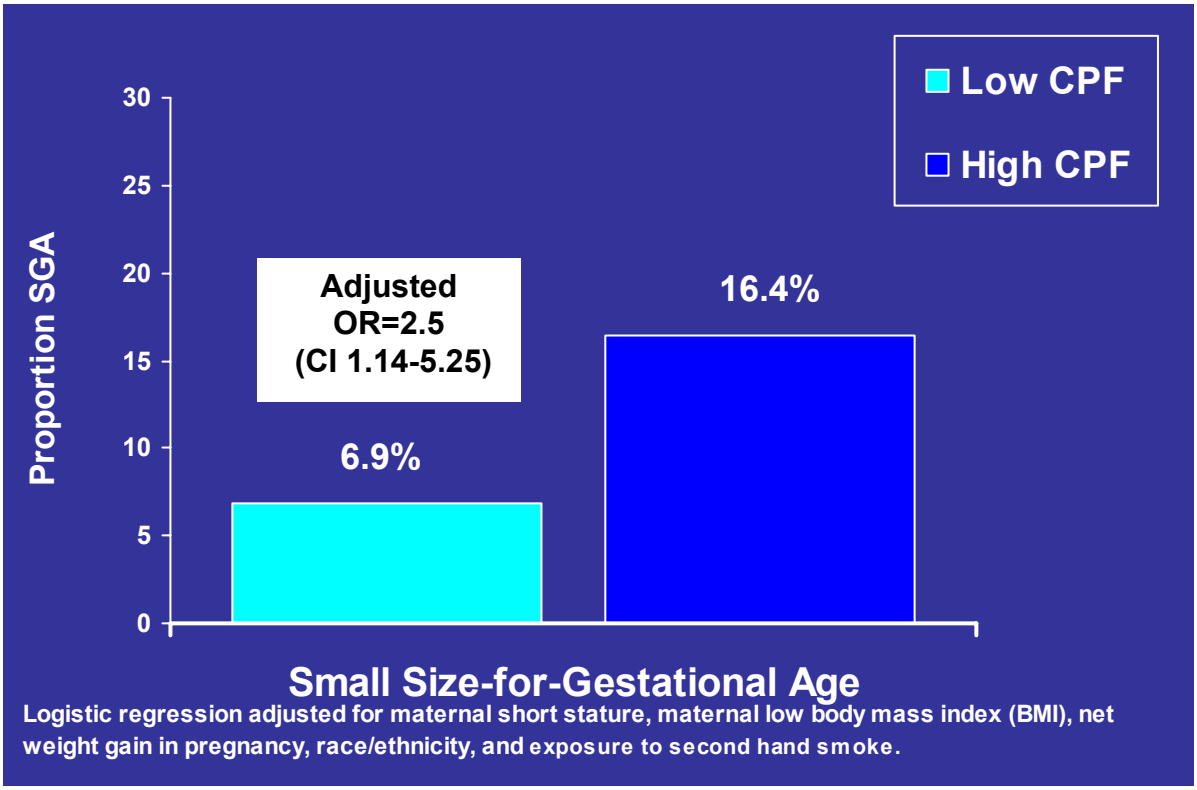
(i) Sum of PAHs measured in maternal 48 hour personal air samples during the3rd trimester of pregnancy.
(j) Cord lead all unclotted.

Figure 1 shows the increased proportion of infants that are small for gestational age (SGA) among those with high chlorpyrifos exposure based on cord blood levels > 6.17 pg/g (Rauh et al. 2007, Whyatt et al. 2007 presentation to EPA). The adjusted odds ratio for this comparison is OR=2.5 (CI 1.14, 5.53). This logistic regression analysis was adjusted for maternal short stature, maternal low body mass index (BMI), net weight gain in pregnancy, race/ethnicity, and exposure to second hand smoke.

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

An analysis of the personal air monitoring results during the third trimester of pregnancy was not associated with any of the three birth outcomes evaluated (birth weight, birth length or head circumference).

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In summary, for children born before chlorpyrifos residential cancellation, high chlorpyrifos exposure in cord plasma was significantly associated with decreased birth weight and length. In contrast, this relationship was no longer significant for newborns born after the cancellation because the blood levels dropped and only one child was in the high group (i.e., >6.17 pg/g). The chlorpyrifos air levels also went down. Therefore, multiple biologic and environmental measures in this study show an important pattern of pre-cancellation exposure and birth outcome changes.

2.4. Prenatal Chlorpyrifos Exposure and Neurodevelopmental Outcomes

As noted previously, this study evaluated children using the standardized Bayley Scales of Infant Development II (BSID-II) at 1, 2 and 3 years of age. The BSID-II is a widely used, normative value-referenced, developmental test for young children that is used frequently to diagnose developmental delay and is known to be sensitive to the effects of toxic exposures such as low-level lead exposure. This test assesses cognitive, motor, and behavioral development. Results in this study are reported as the Bayley Mental Development Index (MDI) and the Bayley Psychomotor Development Index (PDI). Each scale provides a developmental quotient (raw score/chronological age) which generates a continuous MDI and a corresponding PDI. Children's status can be classified as normal or delayed (scores of ≤ 85) on the basis of a standardized cutoff point of one standard deviation (SD). Behavior problems were measured through the Child Behavior Checklist (CBCL) for ages 1.5 to 5 years, which collects information on child behaviors occurring in the past 2 months. The CBCL reports results as total number of problems across a range of internalizing and externalizing behavior domains, and identifies children with high number of problems in each domain. All neurodevelopmental tests have adequate validity and reliability. See Attachments B and D for more information on these tests.

Measurable deficits in Mental and Psychomotor Development for children were seen when comparing children with umbilical cord blood levels in the highest quartile of chlorpyrifos exposure with those in the first 3 quartiles exposure to chlorpyrifos. Rauh et al., (2006) examined neurodevelopment outcomes for 254 children in the first three years of life and reported that high prenatal chlorpyrifos exposure (chlorpyrifos levels > 6.17 pg/g plasma in cord blood) was associated with statistically significant mental and psychomotor deficits at 3 years. Children with high chlorpyrifos prenatal exposure (chlorpyrifos > 6.17 pg/g) scored an average of 6.5 points lower on the Bayley PDI and 3.3 points lower on the Bayley MDI at three years of age, compared with the lower exposed group of children (chlorpyrifos < 6.17 pg/g). The high chlorpyrifos cut point of >6.17 pg/g was based on the previous report of reduced birth weight in this same cohort (Whyatt et al. 2004). Deficits in more highly exposed children

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included attention problems, ADHD problems and PDD problems at age three. More details about the motor and cognitive analyses are provided below.

Additional analyses were performed to evaluate the effects of high diazinon exposure on 3-year development (Rauh 8/12/08, personal communication with D. Smegal via email). With respect to the effect of chlorpyrifos on motor development or PDI, there is no significant effect of high diazinon exposure or any increase in the significance of the effect when chlorpyrifos and diazinon are combined. Regarding mental development or MDI, there is no diazinon effect, but the magnitude of the deficit is slightly increased when the combined chlorpyrifos and diazinon measure is used. These findings are very similar to effect of chlorpyrifos and diazinon on birth outcomes. In conclusion, these analyses do not reduce the chlorpyrifos effect for any of the 3-year outcomes.

Rauh (2008) [presentation to EPA in April] reported that follow-up of the Columbia University cohort children, including age appropriate IQ testing now extends to 60 and 84 months (five and seven years of age). Preliminary analyses of these data suggest that significant neurodevelopmental deficits observed at age 3 persist in older children.

In an earlier report (Rauh et al. 2004), the effects of environmental tobacco smoke (ETS) and maternal hardship on development were examined. At twenty-four months of age, there was a significant association between prenatal ETS exposure and lower 24-month Bayley MDI when the regression model was adjusted for race/ ethnicity, gender, gestational age at delivery, age at testing, marital status, maternal age, and level of PAH exposure. When tested for interaction effects only race/ ethnicity and gender were statistically significant. A second analysis of the neurotoxic effects of prenatal ETS and postnatal material hardship showed a significant interaction such that children with exposure to both ETS and maternal hardship had the greatest cognitive deficit (7.1 points). In a separate paper, PAH air pollutants were examined by Perera et al. (2005). The molecular dosimeter used by the Columbia team for PAHs was benzo(a)pyrene (B[a]P)-DNA adducts. Perera et al. (2005) reported a significant interaction between ETS and adducts, where combined exposure had a significant multiplicative effect on birth weight ($p = 0.04$) and head circumference ($p = 0.01$) after adjusting for confounders. It should be noted, however, that the chlorpyrifos findings previously discussed were still significant after controlling or both ETS exposure and maternal hardship.

Using logistic regression models, cognitive delay at 3 years is significantly associated with high prenatal chlorpyrifos exposure with an odds ratio (OR) of 2.383 or 138% excess in cognitive delay [confidence interval (CI) = 1.12, 5.08] as shown on Table 6. These cognitive development differences for high and low chlorpyrifos exposure are shown graphically in Figure 2 using General Linear Modeling (GLM), and in Figure 4 using logistic regression modeling. In addition,

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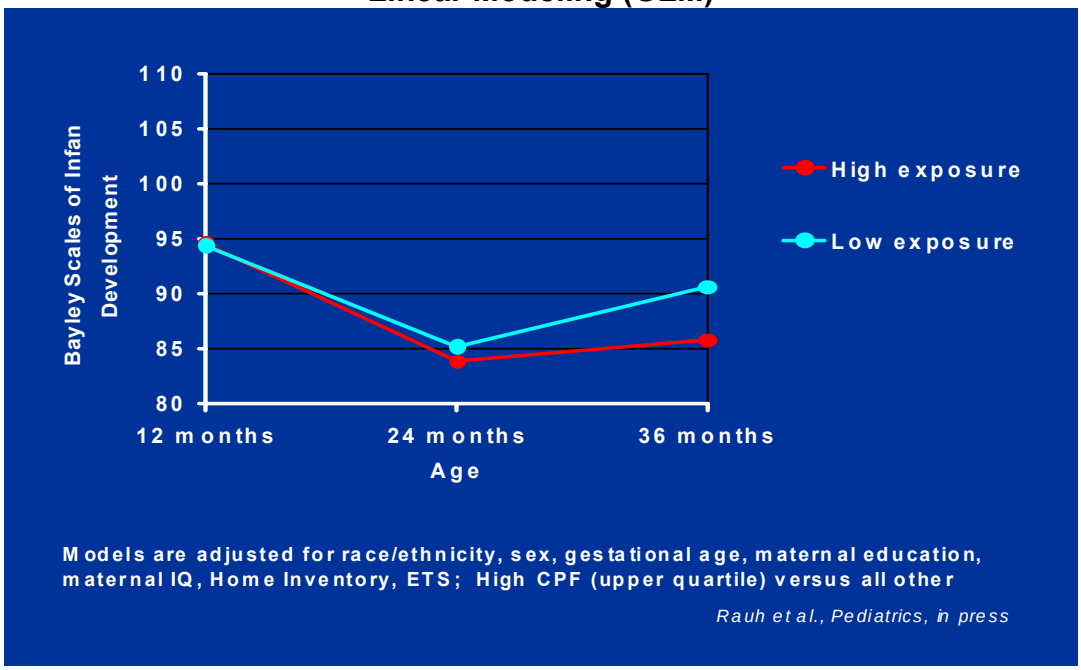
the regression model for Bayley psychomotor delay (PDI <85) is significant with OR = 4.938 or 393% excess in motor delay (CI = 1.78, 13.72) for 3 year olds. The psychomotor delay results are presented in Table 6, and depicted in Figures 3 and 5 using the GLM and logistic regression modeling, respectively.

Table 6. Logistic Regression Models Testing the Effects of Chlorpyrifos in Cord Blood on Adjusted Odds for Neurodevelopment Outcomes in Children (a)

Variable	12 Months		24 Months		36 Months	
	OR	95% CI	OR	95% CI	OR	95% CI
Significant Mental Delay (MDI<85) (Dependent Variable)						
ETS	0.582	0.25. 1.33	1.258	0.7, 2.26	1.232	0.65, 2.32
Chlorpyrifos	1.219	0.49, 3.06	1.754	0.86, 3.6	2.383	1.12, 5.08
Psychomotor Delay (PDI<85) (Dependent Variable)						
ETS	0.945	0.42, 2.15	0.771	0.33, 1.8	1.689	0.65, 4.41
Chlorpyrifos	1.883	0.78, 4.53	1.01	0.37, 2.76	4.934	1.78, 13.72

Source: Rauh et al. 2006, Pediatrics.
(a) N=228 children. Adjusted for race, gender, gestational age, maternal education, maternal IQ, ETS and home environment

Figure 2. Estimated Effects of Prenatal Chlorpyrifos Exposure on Cognitive Development in Children 12 through 36 months of Age, using General Linear Modeling (GLM)



Source: Rauh et al. 2006, Pediatrics.

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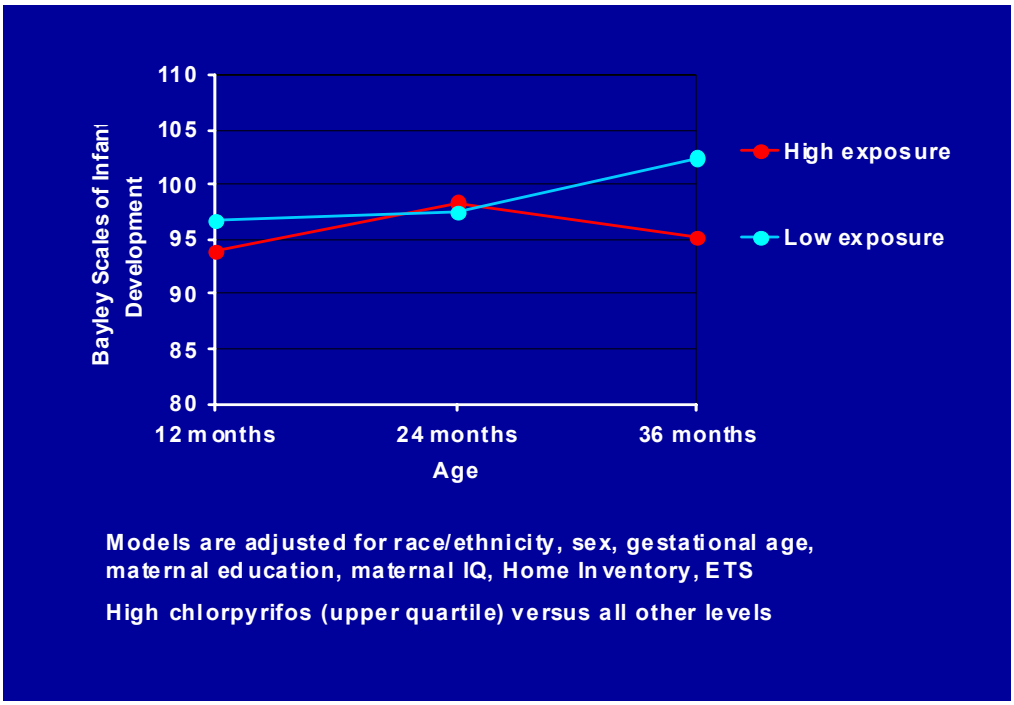
Low = umbilical cord blood < 6.17 pg/g plasma of chlorpyrifos
High = umbilical cord blood > 6.17 pg/g plasma of chlorpyrifos

Figure 3. Estimated Effects of Prenatal Chlorpyrifos Exposure on Motor Development in Children 12 through 36 months of Age, using General Linear Modeling (GLM)



Source: Rauh et al. 2006, Pediatrics.
Low = umbilical cord blood < 6.17 pg/g plasma of chlorpyrifos
High = umbilical cord blood > 6.17 pg/g plasma of chlorpyrifos

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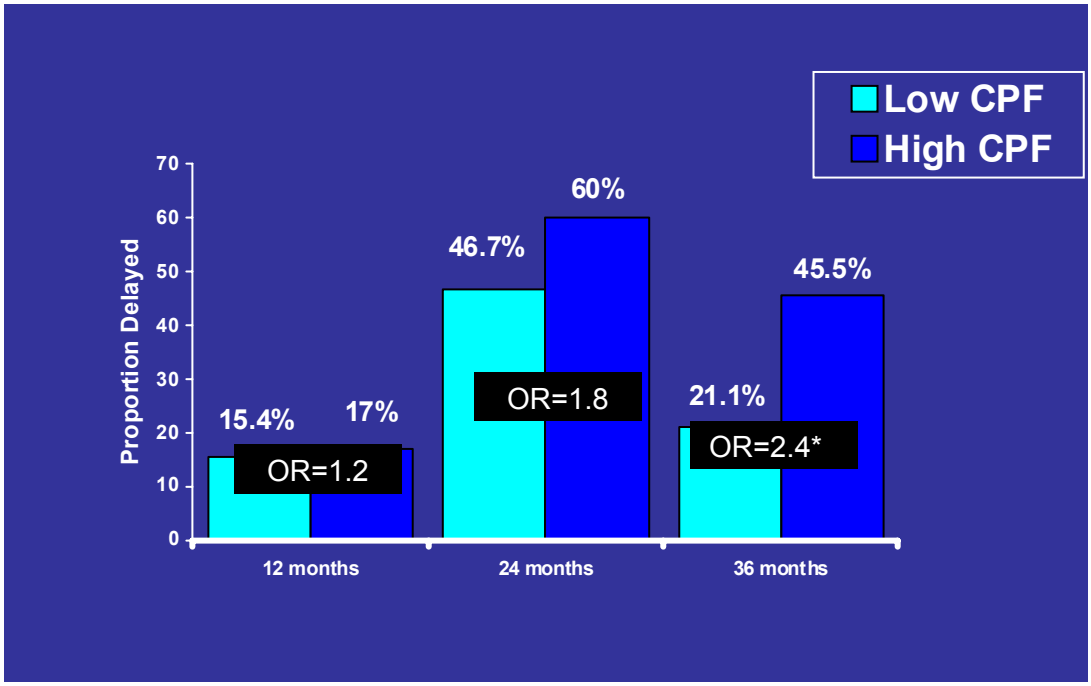
Figure 4. Cognitive Delay (< 85) at 12, 24 & 36 months on the Bayley, by level of chlorpyrifos exposure



N=228; Logistic regression adjusted for race/ethnicity, sex, gestational age, ETS, maternal IQ, maternal education, HOME Inventory
*p<0.01
Source: Rauh et al. 2006, Pediatrics

As shown in Figures 4 and 5, a significantly higher proportion of 3 year old children have cognitive and motor delays, which are likely to be educationally meaningful, associated with prenatal chlorpyrifos exposure. For children with low chlorpyrifos exposure there are 21.1% and 7.1% children with cognitive and motor delays, respectively compared to 45.5% and 31.1% of children with high chlorpyrifos exposure.

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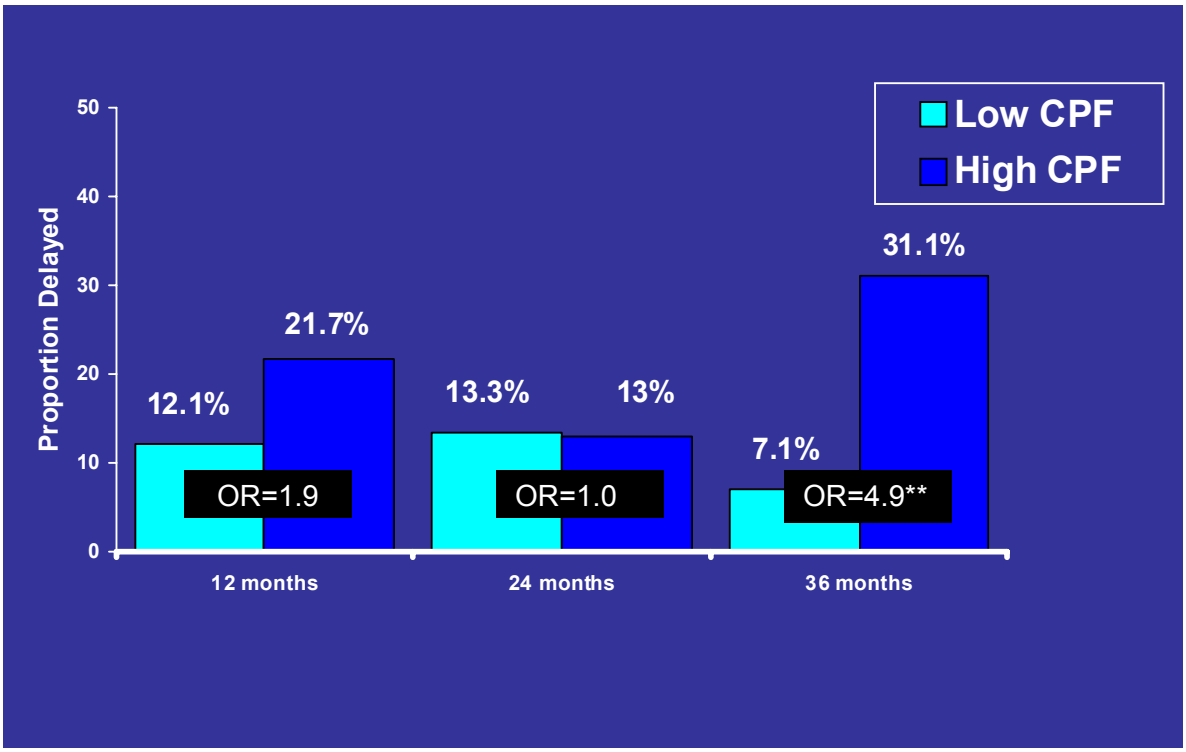
Figure 5. Motor Delay (< 85) at 12, 24 & 36 months on the Bayley, by level of chlorpyrifos exposure



Logistic regression adjusted for race/ethnicity, sex, gestational age, ETS, maternal IQ, maternal education, HOME Inventory

**p<0.001
Source: Rauh et al. 2006, Pediatrics

ADHD problems, attentional problems, and pervasive developmental problems (as measured by the CBCL) are also associated with prenatal chlorpyrifos exposure using logistic regression models, adjusting for race, sex, gestational age, maternal education, IQ, ETS and HOME environment. For attention problems at 36 months of age, the chlorpyrifos odds ratio (OR) is 11.63, a 1063 % excess (Rauh et al. 2006). For ADHD at 36 months of age, the chlorpyrifos OR is 6.30, or 530% excess. ETS is also significant at a comparable range of values. PDD problems are also associated with chlorpyrifos exposure in this NYC child cohort, with OR = 5.64 (a 464% excess). ETS is not significantly associated with attentional or PDD problems. These results are presented in Table 7.

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Table 7. Logistic Regression Models Testing the Effects of Chlorpyrifos and ETS on the Odds of Behavior Problems at 36 Months (a)

Prenatal Exposures	Attention Problems		ADHD Problems		Pervasive Developmental Disorder Problems	
	OR	95% CI	OR	95% CI	OR	95% CI
ETS	2.59	0.41. 6.52	7.88	1.17, 53.19	0.72	0.16, 3.29
Chlorpyrifos	**11.63**	1.82, 74.22	**6.3**	1.03, 38.42	**5.64**	1.23,25.72

Source: Rauh et al. 2006.
(a) N=228 children. Adjusted for race, gender, gestational age, maternal education, maternal IQ, ETS and home environment

In conclusion, the following neurodevelopment and neurobehavioral effects were reported for children highly exposed to chlorpyrifos prenatally in the Columbia cohort: (1) pre-natal exposure was associated with a 3.3 and 6.5 point adjusted mean decrement in 36-month development scores (Bailey MDI and PDI, respectively) in low income minority children; (2) the mean decrement resulted in a 2.4-fold risk of developmental delay (<85) on Bailey MDI and a 5-fold risk of delay on the PDI; and (3) prenatal chlorpyrifos exposure was associated with a significantly increased risk of behavior problems as measured by ADHD, attentional and PDD symptoms at age 3 years. As noted previously, the Agency used the 'high' and 'low' chlorpyrifos designation by the study authors for convenience, and this does not necessarily reflect a judgment on the "cutoff" of chlorpyrifos exposure level.

2.5. Strengths and Limitations

The Columbia cohort study has a number of strengths. First, it has a large sample size, and conducted a comprehensive evaluation of multiple chemicals, including chlorpyrifos, in both environmental and biological samples. Second, the study is prospective, with measures of exposure preceding the developmental evaluation. Another strength is that the Columbia study evaluated the population before and after the residential phase out of chlorpyrifos. Newborns born after January 2002 had substantially lower exposure and no association of fetal growth with chlorpyrifos exposure was apparent. Chlorpyrifos exposures were measured in the integrated media of blood, rather than as a metabolite in the urine, so it is definitive that these women and their fetus were exposed to chlorpyrifos. In addition, the chlorpyrifos maternal and umbilical cord blood measures are well correlated. The study also collected environmental measures of personal and room air monitoring to document exposure to chlorpyrifos. Other studies published by these authors correlated stationary air levels with the 48 hour personal air monitors. These

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data together show that the pregnant women had exposure to chlorpyrifos throughout pregnancy (Whyatt et al. 2007).

The Columbia University cohort study has limitations that are common to all human epidemiology studies, including multiple chemical exposures, such as diazinon and propoxur that are cholinesterase inhibitors, and o-phenylphenol, a disinfectant/fungicide, all of which were measured in 100% of air samples at higher median concentrations than chlorpyrifos. However, the mean umbilical cord levels were less than chlorpyrifos (1.1, 3.1 and 4 pg/g for diazinon, 2-isopropoxyphenol and chlorpyrifos, respectively). The study authors report that after controlling for both diazinon and 2-isopropxyphenol (metabolite of propoxur) exposure in cord plasma, the associations between birth weight and length and cord plasma (ln)chlorpyrifos remained statistically significant ($p \leq 0.02$) and the effect size remained similar to that seen without 2-isopropoxyphenol in the model (Whyatt et al. 2004). Some additional analyses were conducted on the neurodevelopmental effects to consider diazinon (Rauh 8/12/08, personal communication with D. Smegal vial email). These analyses show that the adverse impact of chlorpyrifos on birth weight and cognitive development is not due to diazinon exposure, and these analyses do not reduce the chlorpyrifos effect for any of the 3-year outcomes for MDI or PDI.

The Columbia population, similar to the Mt. Sinai and CHAMACOS cohorts, consisted of mostly children from low-income families, which were potentially at risk for poorer neurodevelopment. Infant neurodevelopment deficits may be multifactor in origins. It is not always possible to identify the sources of physiological and neurobehavioral deficits for each and every case.

There are not yet established National norms for chlorpyrifos in cord blood, although there are national population measures of the chlorpyrifos metabolite TCP reported in urine samples from the National Health and Nutrition Examination Survey (NHANES). Although cord blood lead levels were evaluated and determined not to be a confounder for the chlorpyrifos results on adverse outcomes in children, the postnatal blood lead data for the impacted children are not available for analysis. The researchers are analyzing the blood lead for these children at age seven.

Although the logistic regression analysis for small for gestational age (SGA) were adjusted for a number of factors, including maternal short stature, there does not appear to be an adjustment for father's short stature.

Among other things, the exposure groupings selected in this research were criticized as arbitrary by Cicchetti (2007) and defended as data derived by Rauh et al. (2007) in letters exchanged in *Pediatrics,* following publication of the Rauh et al. 2006 *Pediatrics* article on the impact of prenatal chlorpyrifos on neurodevelopment in the first 3 years of life. Some of the issues raised by Cicchetti (1997) were that the mean MDI scores were clinically meaningless,

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there were no standards defining “high” and “low” chlorpyrifos exposure, that Rauh et al. (2004) masked, but did not eliminate educational bias, the correlation of 0.76 for maternal and umbilical cord blood only explains 58% of variability in maternal plasma, the “high” and “low” exposure groups differed in their race/ethnicity characteristics which confounds race/ethnicity with exposure, and the finding that more high exposure children had ADHD is meaningless. Rauh et al. (2007) disagrees with Dr. Cicchetti claim that the significant chlorpyrifos effect on MDI was “clinically meaningless” and indicates that a Bayley developmental score < 85 prompts referral to early intervention services, and exposures that produce small shifts in the mean often result in more children who meet the diagnostic criteria. Dr. Rauh indicated the “high” and “low” groups were clearly defined based on the previous report of reduced birth weight among children with exposure levels above 6.17 pg/g (Whyatt et al. 2004). High school degree was used to adjust for maternal education because the sample was uniformly low income, and thus education was the preferred covariate for social class. Maternal intelligence, although controlled was not significant in their analysis. Because chlorpyrifos in maternal blood and/or adipose tissue are in steady state, cord blood provides a reasonable dosimeter for the amount of chlorpyrifos transferred to the fetus. Rauh et al. (2006) controlled for race/ethnicity in all models and also used a stratified analysis showing a significant chlorpyrifos effect within each ethnic group, independent of race. They used Achenbach’s Child Behavior Checklist (CBCL) to assess behavior problems rather than make a diagnosis because ADHD is hard to diagnose in preschool-aged children.

3.0. Mount Sinai Children’s Environmental Center

The Mount Sinai Children’s Environmental Center studied the relationship among prenatal pesticide exposure, maternal paraoxonase (PON1) activity, and infant growth and neurodevelopment in a prospective, multiethnic cohort study.

Cohort Characteristics: The Mount Sinai School of Medicine study is a prospective cohort of 404 births between May 1998 and May 2002 to pregnant NYC multiethnic women (Berkowitz 2003, Berkowitz et al. 2004). These birth years also span the period of the residential chlorpyrifos phase out. Like the Columbia University cohort, the women and their infants are the exposed to indoor residential pesticides, as well as air pollutants, and other chemicals including polychlorinated biphenyls (PCBs). Like the CHAMACOS cohort, they have collected PON1 information and PON1 levels. The study determined maternal paraoxonase (PON1), butyrylcholinesterase (BuChE) activity and PON1Q192R gene variant (Wolf et al. 2007). These women were relatively young, with 35% under age 20. The largest racial/ethnic group was predominantly Puerto Rican Hispanics (nearly 50%), followed by African Americans (nearly 28%) and whites (21%). At the time of enrollment, most of the women were single (47%), and had not completed high school (nearly 30%), although 50% were college graduates or had some college education. Forty six

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percent of household members used pesticides, while 71.5% reported indoor pesticide use in the home (Berkowitz et al. 2004).

Pesticide Exposure Measures: Berkowitz et al. (2003) evaluated pesticide exposure based on a questionnaire on pesticide use and urinary metabolite analysis for TCP (chlorpyrifos), phenoxybenzoic acid (PBA, some pyrethroid pesticides) and pentachlorophenol (PCP) for samples collected between May 1998 and July 2001. Spot urine measurements were collected during the third trimester (mean of 31.2 weeks) of pregnancy, and these data are shown in Table 8 below. The median TCP level was 7.5 µg/L, which is estimated to be equivalent to a median of 11.3 µg/g creatinine. The 90th percentile values were 61.2 µg/L and 70.2 µg/g creatinine, respectively (Berkowitz et al. 2003). Creatinine (a protein excreted at a constant rate per healthy individual) correction is helpful to account for the dilution effect where two people have the same dose but one consumes extra water and has a lower concentration value.

Almost 48% of the women reported having an insect problem, and approximately 46% said they or another household member applied pesticides in the home during pregnancy. When pesticide exposure by exterminators or building employees was considered, the estimate of indoor pesticide exposure increased to about 72%.

The TCP urine levels were significantly higher for women who had completed at least a high school education. However, the levels did not differ among other sociodemographic characteristics evaluated. There were no reported seasonal variations for TCP or PCP, nor did the urinary levels change during the sampling period (1998-2001). In addition, there was no reported association between the questionnaire data and pesticide metabolite data. The authors attribute this to the short half-lives of the pesticides measured, which reflect only recent exposure.

Table 8. Percentiles of Pesticide Metabolites for Maternal Spot Urine Samples Mt. Sinai Children's Environmental Health Study 1998-2001

Analyte	Sample Size	Percent Detect	Percentile				
			10th	25th	50th	75th	90th
Metabolite Concentration in Urine (ug/L)							
TCP	365	42%	0.3	1.8	7.5	25.7	61.2
PBA	307	55%	0.4	2.4	18.3	40.6	126.9
PCP	361	18%	1.0	2.0	7.0	18.0	52.0
Creatinine	373	NA	24.7	46.9	86.5	137.4	202.9
Metabolite Concentration as ug/g creatinine							
TCP	365	42%	0.4	1.8	11.3	31.7	70.2
PBA	307	55%	0.4	4.8	19.3	57.2	184.1
PCP	361	18%	1.1	2.4	7.3	28.4	67.0

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Source: Berkowitz et al. 2003; NA= not applicable
TCP= metabolite of chlorpyrifos
PBA= metabolite of pyrethroid pesticides including sumithrin, permethrin and cypermethrin
PCP= metabolite of pentachlorophenol, a wood preservative, and lindane and hexachlorobenzene.
Detection limits: TCP=11/12 ug/L; PBA=16 ug/L and PCP= 23 ug/L.

In a subsequent publication (Wolf et al. 2007), urinary concentrations of DAP, DMP, and DEPs were presented, along with BuChE activity and PON1 activity. PON1 tertiles in maternal plasma were designated as: (1) low = <96 mg/m * mL; (2) medium = 97-116.6 mg/m * mL; and (3) high=116.7-200 mg/m*mL. BuChE tertiles in maternal plasma were defined as: (1) < 1.9; (2) 1.9-2.43 and (3) 2.44-13.8. These data are shown on Table 9 below. In addition, they report urine data for malathion dicarboxylic acid (MDA, malathion metabolite) and blood levels for lead, and the organochlorine compounds DDE and PCBs. Urinary DMP metabolites were about three-fold higher than DEP metabolites (Wolf et al. 2007). MDA was detected in about 20% of maternal urinary samples.

Table 9. Urinary Concentrations in Maternal Spot Urine Samples Mt. Sinai Children's Environmental Health Study 1998-2002

Characteristics	Sample Size	Sum of DEPs (nm/L) Median (n)	Sum of DMPs (nm/L) Median (n)	Sum of DAPs (nm/L) Median (n)
PON tertile (Maternal Plasma) (mg/m* mL)				
1 (<96) (Low)	130	13.5 (119)	33.8 (120)	67.3 (119)
2 (97-116.6) (Medium)	123	16.4 (108)	40.1 (111)	69.3 (108)
3 (116.7-200) (High)	130	19.9 (111)	51.3 (117)	83.5 (111)
After January 1, 2001	34	22.8 (10)	26.7 (10)	60.0 (10)
BuChe Tertile (Maternal Plasma)				
1 (<1.9)	125	12.2 (101)	35.4 (104)	63.1 (101)
2 (1.9-2.43)	124	24.6 (108)	67.2 (112)	101.1 (108)
3 (2.44-13.8)	124	20.9 (113)	34.1 (116)	58.1 (113)
Maternal PON1Q192 genotype				
3: RR	95	22.6 (81)	55.6 (83)	90.4 (81)
2: RQ	174	14.0 (158)	34.4 (163)	64.3 (158)
1: QQ	120	25.6 (100)	47.8 (103)	82.6 (100)

Source: Wolf et al. 2007

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Birth Outcomes. Berkowitz et al. (2004) reported on *in utero* pesticide exposure as measured by urinary TCP in pregnant women, maternal PON1 activity and infant growth parameters (birth weight, head circumference and gestational age). PON1 activity was categorized into low, medium and high based on its tertile distribution (i.e., distribution divided into three subgroups).

Statistically significant reductions in head circumference at birth were associated with low maternal PON1 activity and detectable maternal TCP urine levels (>11 ug/L), even after adjusting for race/ethnicity, infant sex and gestation age. Table 10 presents these results. There was no association of PON1 activity or TCP and birth weight or birth length. Other potential covariates such as active and passive smoking, pre-pregnancy body mass index, maternal weight gain, blood lead levels, and cesarean section delivery were not included in the final models because they did not affect the results and only increased the variance. Marital status and educational levels were too closely correlated with race/ethnicity to be included in the analysis (Berkowitz et al. 2004). Smaller head size has been found to be predictive of subsequent lags in cognitive development, and thus the researchers suggest that chlorpyrifos may have a detrimental effect on fetal neurodevelopment among mothers with low PON1 activity.

Maternal PON1 levels alone, but not maternal PON1 genetic polymorphisms were also associated with smaller head size. Neither questionnaire data nor pesticide metabolite levels were associated with fetal growth indices (birth weight and length or fetal head circumference) or gestational age in this publication (Berkowitz et al. 2004).

Table 10. Head Circumference by Tertiles of Maternal PON1 and TCP urine levels

	Head Circumference (cm) (a)	
	Mean±SD	**Sample Size**
TCP<LOD		
Low PON	33.6±1.8	76
Medium PON	33.7±1.7	62
High PON	34.1±1.7	70
TCP>LOD		
Low PON	33.3±1.5**	47
Medium PON	34.0±1.5	57
High PON	34.1±1.6	55

Source: Berkowitz et al. (2004)
(a) Adjusted for race/ethnicity, infant sex and gestational age.
** P<0.014
LOD=11 ug/L

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Table 11. Standard CDC (2000) Infant Head Circumference Measurements at Birth

Percentile	Centimeters (cm)	
	White Male	White Female
5th	31.8	32.2
50th	35.8	34.7
95th	38.7	37.9

As shown in Table 11, an infant head circumference of 33.3 cm that was reported for the low PON group with TCP > 11 ug/L represents between the 5th and 50% percentile of the standard values for head circumference reported by the CDC for white children (Guo et al. 1988, 1997, Roche et al. 1987 as cited in CDC 2000). Race and ethnicity differences in head circumference have been debated in the pediatric literature, and one conclusion is that between race and ethnic groups are not as significant as within groups so the standard CDC tables apply [Pediatrics. 1985 Feb;75(2):318-20].

Wolf et al. (2007) report that head circumference was inversely associated with maternal PON1 activity (P=0.004). Head circumference was 0.62 ±0.18 cm smaller in the first activity (slow tertile PON1) compared with the third tertile of PON1 (adjusted n=382, p=0.0009). With slow activity PON1 of PON192, urinary DEPs were associated with decreased birth weight and DMPs were associated with shorter birth length. There were no associations between birth outcomes and BuChE.

In summary, Mt Sinai investigators reported changes in infant head circumference with measurable elevations in maternal urinary TCP while taking low maternal PON1 into account. Genetic susceptibility via polymorphic detoxification capacity may put some individuals at an excess risk and this is being evaluated in another section of this report.

Neurodevelopmental Outcomes: In a subsequent publication, Engel et al. (2007) evaluated child neurodevelopment using the Brazelton Neonatal Behavioral Assessment Scale (BNBAS) on a subset of 311 infants before hospital discharge. The BNBAS includes 28 behavioral items and 18 primitive reflexes.

In this study, maternal urine was measured for six dialkylphosphate metabolites and malathion dicarboxylic acid (MDA, malathion metabolite). In addition, blood samples from 194 women were collected and analyzed for PCBs and DDE. TCP urine levels were not discussed in this publication.

Overall, higher maternal urinary levels of DAP (n = 285, median 82.0 nm/liter, interquartile range 35.2-194.7 nm/liter, 96.5% detectable) and DEP (n = 285, median 24.7 nm/liter, interquartile range 8.9-52.8 nm/liter, 88.8%

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detectable) pesticide metabolites were associated with an increase in abnormal reflexes in infants, as was total DMP, when PON1 activity was considered. MDA above the level of detection (LOD) was also significantly associated (2.24 fold increase) with adverse reflexes. Abnormal primitive reflexes are a critical mark of neurologic integrity. No adverse associations were found for PCBs or DDT in maternal blood and any behavior in infants.

Table 12 shows the interaction between paraoxonase (PON1) expression levels by tertiles, and DAPs in maternal urine on the risk of abnormal neonate reflexes. Women in the lowest tertile of PON1 (i.e., slow organophosphate pesticide metabolizers) had a significantly increased risk of abnormal reflexes in their infants with increasing OP exposure for DMPs and total DAPs. No trend was observed for DEPs (chlorpyrifos is a DEP). For women in the highest tertile of PON1 expression, no increased risk of abnormal reflexes with increasing OP metabolite exposure was found.

Table 12. Interactions between PON1 Expression Levels, Urinary DAPs (nm/L) and Risk of Abnormal Reflexes in Exposed Neonates

Paraoxonase Expression Level	Total DEP (a)		Total DMP (a)		Total DAP (a)	
	Relative Risk	95% CI	Relative Risk	95% CI	Relative Risk	95% CI
Low: Tertile 1	1.78	1.01, 3.14	1.96*	1.27, 3.03*	2.38*	1.37, 4.15**
Medium: Tertile 2	1.42	0.85, 2.35	1.66*	1.03, 2.65*	1.75	0.96, 3.17
High: Tertile 3	1.56	1.01, 2.39	0.73	0.56, 0.96	0.76	0.48, 1.20

Source: Engel et al. (2007)
Interaction p≤ 0.01; ** interaction p<0.05.
CI=confidence interval
(a) Adjusted for examiner, anesthesia during delivery, paraoxonase 1 enzyme tertiles, and urinary creatinine by using Poisson regression. All models except total DEP were additionally adjusted for over dispersion.

Strengths and Limitations. This is a prospective study with a large sample size that evaluated multiple chemicals via urinary concentrations. The study also examined the influence of PON status on health outcomes. The study controlled for a number of covariates, including maternal age, race/ethnicity, BMI, pregnancy weight gain, country of birth, education, martial status, family income, preferred language, parity, and smoking in pregnancy. In addition, the study conducted comprehensive neurological infant behavior and physiology.

The results of this study are complicated because of exposure to multiple chemicals, including OPs such as malathion (MDA metabolite measured),

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pyrethroid pesticides (measured as PBA), and other compounds like PCP. In addition, this study collected spot urine samples which may not represent average exposure over time because these pesticides have short half lives. In addition, DAP, DMP and DEP are non-specific metabolites that result from several OP pesticides, so it is difficult to determine which compound may be contributing most to the adverse findings.

The Mt. Sinai population, similar to the Columbia and CHAMACOS cohorts, consisted of mostly children from low-income families, which were potentially at risk for poorer neurodevelopment.

4.0. CHAMACOS (Center for Health Assessment of Mothers and Children of Salinas), University of California at Berkeley

The CHAMACOS cohort includes 601 primarily Latina women from predominately farmworker populations in the Salinas Valley, California. There are several publications by this group that evaluate the exposure pathways (Eskenazi, 1999; Bradman et al, 2007), urinary metabolites in moms and children (Bradman et al, 2005, Eskenazi et al. 2007), birth outcomes (Eskenazi et al. 2004; Young and Eskenazi, 2005) and neurodevelopmental outcomes (Eskenazi et al. 2007) for children up to 24 months. Specifically, these studies have investigated the effects of organophosphate pesticide exposure during pregnancy on fetal growth and gestational duration (Eskenazi et al. 2004), and the relationship between prenatal and child organophosphate (OP) urinary metabolite levels with children's neurodevelopment (Eskenazi et al. 2007).

Cohort Characteristics. The majority of the women in this cohort were married (82%), did not have a high school degree (81%), and had low incomes. Approximately 28% of women worked in fields during pregnancy, another 14% had worked at other jobs in agriculture, including packing shed, nursery and green house work. About 85% of women had agricultural workers living in their homes during pregnancy. Very few of the women reported smoking (6%), drug use (2%) or alcohol consumption (1%) during pregnancy (Eskenazi et al. 2004). Half had symptoms of depression 1 year postpartum. The average maternal Peabody Picture Vocabulary Test (PPVT) score was in the low normal range, averaging 86±21.

PON1 status of these farm worker mothers and children and differential sensitivity to detoxify chemicals are discussed in other publications (Furlong et al. 2005, 2006, Holland et al. 2006).

Pesticide Exposure Measures. The mothers in this cohort are involved in agricultural activities or have husbands involved in agriculture, and thus have potential exposure to several different pesticides. Table A.1 below lists the multiple pesticides used in the area where the CHAMACOS population live and

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work. The published exposure studies from CHAMACOS cohort have focused on the six generic organophosphate dialkylphosphate (DAP) metabolites in maternal and child urine samples, as well as specific metabolites to malathion (malathion dicarboxylic acid, MDA), chlorpyrifos (TCP), and parathion (4-nitrophenol) metabolites in maternal urine sample, plus a total of 27 commonly used agricultural pesticides in house dust and urine of farmworker children (Bradman et al., 2003, 2005, Bradman 2007, Eskenazi et al, 2004, 2007). In addition, blood AChE and butyryl cholinesterase (BuChE) measurements were collected, and breast milk was evaluated for organochlorine and organophosphate pesticides. Breast milk data are not yet available for this cohort (personal communication with A. Bradman to D. Smegal, 8/12/08). Since the focus of this analysis is only chlorpyrifos as such, the interpretation of the outcome measures is complicated by multiple OP exposures. Even though the residential uses indoors are mostly gone, agricultural use in this area has not changed substantially. Mothers may have been exposed while at work in the field via the dermal or inhalation route, in the diet, or from take home exposures from their spouses (Bradman et al. 2007). Similarly, children were potentially exposed in utero, and from potential take home exposures from their parents working in agriculture.

Pesticide metabolites were measured in the spot urine samples of CHAMACOS mothers and in their infants at 6, 12 and 24 months of age. Pesticides were detected twice during pregnancy at 14 and 26 weeks, and once post delivery at 7 days postpartum, indicating that pesticide exposure was recurring and of long-term duration (Eskenazi et al. 2004, 2007). TCP levels were reported in two publications. Eskenazi et al. (2004) reported a median level of 3.3 ug/L, and a frequency of detection of 77% for TCP (n=482). In a subsequent publication, TCP was detected in 91% of women (average value from 445 women) at a median level of 3.54 ug/L. The diethyl phosphate (DEP, a metabolite of chlorpyrifos) was higher in the mothers (geometric mean of 18.1 nmol/L) compared to the children at 24 months of age (GM 10.5 nmol/L). In addition, total DAP, and dimethyl phosphate (DMP) urinary levels increased in children with age (Eskenazi et al. 2007). Maternal DAPs were uncorrelated with child DAPs in this study. The urinary TCP, DMP, DEP and DAP results for maternal urine are shown on Table 13.

Table 13. Pesticide Metabolites for Maternal Spot Urine Samples

Analyte	Sample Size	Percent Detect	Concentration in Urine (ug/L), unless noted	
			Median, unless noted	Range, unless noted
Eskenazi et al. 2004 Results (2000-2001)				
TCP	482	76.3%	3.3	0.2-56.2
DMP	486	99.8%	101 nmol/L	5-6587 nmol/L

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Analyte	Sample Size	Percent Detect	Concentration in Urine (ug/L), unless noted	
			Median, unless noted	Range, unless noted
DEP	485	99.8%	22 nmol/L	2-680 nmol/L
Total DAP	485	99.8%	136 nmol/L	10-6854 nmol/L
Eskenazi et al. 2007 Results (2000-2003) (a)				
TCP	445	91%	3.54	Not provided
Total DMPs		99.8%	81.5 nmol/L (GM)	74.5-89.5 nmol/L (95% CI)
Total DEP		99.8%	18.1 nmol/L (GM)	16.7-19.7 nmol/L(95% CI)
Total DAP		99.8%	114.9 nmol/L(GM)	105.7-125 nmol/L(95% CI)

GM= geometric mean
(a) Values reported for average of 1st and 2nd pregnancy.

Cholinesterase measurements. Maternal DAPs and ChE levels collected concurrently at the 2nd pregnancy interview were not correlated, and a small positive, rather than an expected negative correlation was seen between the average DAP level during pregnancy and pre-delivery maternal blood and umbilical cord ChE levels.

Birth outcomes: Decreased length of gestation was associated with increased average urinary dimethylphosphate (DMA) metabolites of OPs measured at two points in pregnancy (p=0.02) and umbilical cord cholinesterase (p=0.001) (Eskenazi et al. 2004, 2005). Shortened gestational duration was most clearly related to increasing exposure levels based on maternal urinary measures in the latter part of pregnancy. They did not observe an adverse relationship with fetal growth measures (e.g., length, weight) and maternal urinary or blood measures of OP pesticide exposure. However, surprisingly the study found increases in body length and head circumference associated with some pesticide exposures. For example, increased maternal urinary DEP concentrations were significantly associated with increased head circumference, while increasing total maternal DAP and DMP urine concentrations were associated with both increased infant body length and increased head circumference (Eskenazi et al. 2004). All models of birth weight, length, head circumference and ponderal index were adjusted for gestational age and gestation age squared. The models include continuous variables for maternal age, pregnancy weight gain, week of prenatal care, and categorical variable for parity, infant sex, mother's county of birth, body mass index (BMI) and family income.

Lower levels of ChE in umbilical cord blood, but not BuChE cord blood or maternal blood ChE and BuChE, were associated with significantly shorter gestation, averaging 0.34 weeks (p=0.001) for each unit decrease in ChE (in micromoles per minute per milliter; range of ChE in cord blood is 4.4 units)

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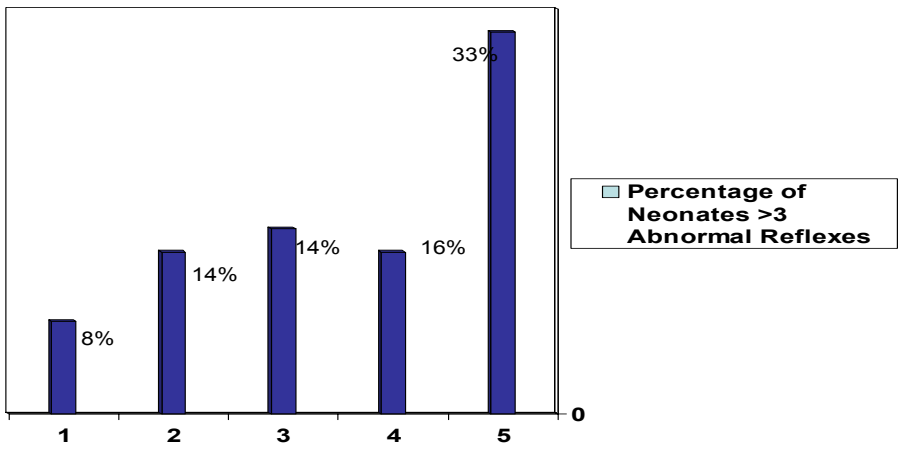
(Eskenazi et al. 2004), Decreasing levels of ChE in umbilical cord blood were also associated with increased risk of pre-term delivery (adjusted OF=2.3; 95% CI 1.1-4.8; p=0.02) and low birth weight (adjusted OR=4.3; 95% CI 1.1-17.5; p=0.04). However, in this study, 6 of the 11 low birth weight babies were also pre-term.

Neurodevelopmental Effects. Abnormal reflexes in neonates were also associated with urinary total DAP metabolites during pregnancy (Young et al. 2005). These reflexes are measured with the Brazelton Neonatal Behavioral Assessment Scales (BNBAS) that includes habituation, orientation, motor, range of state, regulation of state, autonomic and reflexes that are sensitive physiological indicators of overall neurologic function. Among the >3 day old infants, increasing average prenatal urinary metabolite levels were associated with both an increase in number of abnormal reflexes (total DAP: adjusted B=0.53, 95% CI=0.23, 0.82; DMA: adjusted B=0.41, 95% CI=0.12, 0.69, DEPs: adjusted B=0.37, 95% CI=0.09, 0.64) and the proportion of infants with more than three abnormal reflexes (total DAP: adjusted OR=4.9, 95% CI=1.5, 16.1; DMA: adjusted OR=3.2, 95% CI=1.1, 9.8, DEPs: adjusted OR=3.4, 95% CI=1.2, 9.9). No detrimental associations were found between postnatal urinary metabolites and any of the BNBAS clusters for infants ≤3 or > 3 days old at assessment. Figure 6 below depicts the increase in abnormal neonatal reflexes with increasing DAP maternal urinary levels (Young et al. 2005).

Figure 6. Newborns of Mothers with Higher OP Exposures Show More



CHAMACOS Quintile of Prenatal DAP metabolites (After Young et al., 2005)

In another publication of this same cohort, adverse mental development and pervasive developmental problems at 24 months of age were associated with prenatal urinary DAPs (Eskenazi et al. 2007). This study used Bayley Scale of Infant Development (BSID) and the Child Behavior Checklist (CBCL) to

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evaluate neurodevelopmental effects. The BSID consists of both the Mental Development (MDI) and the Psychomotor Development (PDI) Indices

Almost 30% of children of this cohort (105/355) had borderline PDD (> 93rd percentile) and 14% (51/355) had clinical manifestation of PDD. In addition, PDD was significantly associated with elevated prenatal (maternal) and child urinary DAP and DMP urinary levels, and child DEP levels at 24 months of age as shown in the Table 14 below. No significant associations were observed between maternal TCP urine levels and any Bayley or CBCL outcome. The only covariate that was found to be significantly related to risk of attention problems and ADHD was maternal depression. Mothers who had reported symptoms of depression 12 months postpartum had nearly 3-fold odds of reporting that their 2-year old had attention problems or ADHD (OR=3.1).

Table 14. Neurodevelopmental Outcomes Associated with Urinary Metabolites of OP Pesticides at 24 months (a) (n=355)

Urine Metabolites	Adjusted odds Ratios (OR) + 95% CI for CBCL Measures (b)		
	Attention (BL)	ADHD (BL)	PDD (CL)
Total Dialkyl phosphate (DAPs)			
Prenatal	0.77 (0.27–2.24	1.34 (0.50–3.59)	2.25 (0.99–5.16)**
Child	1.41 (0.75–2.64	1.11 (0.61–2.03	1.71 (1.02–2.87)**
Dimethyl phosphate (DMP)			
Prenatal	0.78 (0.31–1.96)	1.27 (0.53–3.04)	2.19 (1.05–4.58)**
Child	1.54 (0.85–2.76)	1.10 (0.63–1.94)	1.52 (0.94–2.45)*
Diethyl phosphate (DEP), includes chlorpyrifos			
Prenatal	0.78 (0.26–2.31)	0.59 (0.21–1.68)	0.88 (0.37–2.07)
Child	1.02 (0.61–1.71)	1.18 (0.72–1.94)	1.72 (1.12–2.64)**

*p ≤ 0.10; **p ≤ 0.05.
ADHD= Attention Deficit Hyperactivity Disorder; PDD= Pervasive Developmental Delay; CL= Clinical; BL= Borderline Clinical; CI= confidence intervals
Source: Eskenazi et al. 2007

(a) Models adjusted for sex, exact age at assessment, breast-feeding duration, HOME score, household income above poverty threshold, parity, maternal PPVT, and maternal depression. (PPVT = Peabody Picture Vocabulary Test, a measures of verbal ability).

(b) Adjusted ORs (95% CIs) for Syndrome Scores in the Clinical (CL) or Borderline Clinical (BL) Range on the Child Behavior Check List (CBCL) at 24 months of age for DAPs urinary metabolites.

At 24 months, children had increased mental delay (low MDI score < 85) that was significantly associated with both prenatal and child urinary DAP and DMP levels. Nearly half (184/369) or 50% of children in this cohort had MDI scores < 85 at 2 years. Children 12 months of age had significant MDI delays

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associated with DEP child urinary levels. These models were adjusted for psychometrician, location, exact age at assessment, sex, breast-feeding duration, HOME score, household income above poverty threshold, parity and maternal PPVT.

In summary, mothers and infants have measureable exposure to OP pesticides as indicated by the urinary metabolites DAP, DMP and DEP. Those same infants with OP exposure when followed over time have deficits on key neurodevelopmental parameters such as mental development and pervasive developmental problems at 24 months of age.

Strengths and Limitations. Some of the strengths of this study include that it is a prospective study with a large sample size that evaluated multiple chemicals via urinary concentrations in both moms and children. In addition, ChE in whole blood and butyrl ChE measurements were collected in both maternal and cord blood. The study also evaluated PON status in this population.

The results of this study are complicated because of exposure to multiple OPs, and other classes of pesticides in this study population. In this study, there was no statistically significant association between urinary TCP levels in mothers and adverse outcomes in their children at 6, 12 and 24 months for mental and psychomotor delays (MDI and PDI). However, chlorpyrifos use represented only about 10% of total OP pesticides in 2001 for the Salinas Valley (i.e., 55,000 lbs out of a total of 520,000 lbs (Eskenazi 2008, presentation to EPA). In addition, this study collected spot urine samples, which may not represent average exposure over time because these pesticides have short half-lives. In addition, DAP, DMP and DEP metabolites are non-specific metabolites that result from several OP pesticides, so it is difficult to determine which compound may be contributing most to the adverse findings. Total exposure patterns that are associated with pervasive developmental disorders may not be adequately captured. The lack of creatinine correction to account for hydration differences and better estimate physiological dose may introduce additional uncertainty for urinary pesticide values of both the mothers and children (Mage et al. 2004).

As noted by the study authors, about half of the women had postpartum depression, and this was a covariate that was found to be significantly related to risk of attention problems and ADHD. Mothers who had reported symptoms of depression 12 months postpartum had nearly 3-fold odds of reporting that their 2-year old had attention problems or ADHD (OR=3.1).

The CHAMACOS population, similar to the Columbia and Mt. Sinai cohorts, consisted of mostly children from low-income families, which were potentially at risk for poorer neurodevelopment. In addition, the study authors indicate that the mothers of this cohort had high levels of serum levels of certain organochlorine pesticides, such as DDT and DDE, because many women had originated from Mexico where these pesticides were used more recently.

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5.0 Longitudinal Dietary Exposure to Organophosphorus Pesticides in Children

In 2008, Dr. Chensheng Lu of Harvard University published a study evaluating dietary exposure of OPs in children. This study includes 23 children enrolled from age 3-11 in the Seattle, Washington and Atlanta, Georgia metropolitan areas. The study has two basic purposes of the study: 1) compare urinary metabolite concentrations on organic & conventional diets and 2) evaluate potential seasonal differences in exposure. Evaluation of longitudinal exposures (i.e., multiple consecutive days) in food is also a unique aspect of this study design.

The 2008 study is a larger version of a similar study published in 2006 by the same group (Lu et al, 2006) and a biological monitoring study of residential pesticide use among 110 children aged 2-5 years from 96 families in the Seattle metropolitan area (Lu et al 2001).

In the Lu et al. (2001) study, six DAP compounds, the common metabolites of OP pesticides were analyzed in spot urine samples of children in the spring and fall of 1998. In addition, the study authors conducted parental interviews on residential use of pesticides. OP metabolites were detected in the urine of nearly all children, as 99% children had at least one DAP metabolite, while DMTP and DETP were found in 70-75% of children. There were no reported differences in DAP levels related to season, community, sex, age, family income or housing type. DMP concentrations were higher than DEP levels for all age groups, as median concentrations were 0.11 and 0.04 umol/L, respectively. Pesticide use in home gardens was associated with a statistically significant increase in DAP (both DEP and DMP) metabolites in children, while indoor, lawn and pet use were not significantly associated with an increase in DAP (DMP or DEP) levels compared to children whose parents did not use pesticides.

In the Lu et al. (2006) study, conventional diets were substituted with organic food items for five consecutive days. Spot urine samples were collected twice (first morning void and before bedtime) for 15 days. The urine was analyzed for metabolites of several OPs, including chlorpyrifos (i.e., TCP). The median urinary concentration of specific metabolites for chlorpyrifos (TCP) and malathion (MDA) decreased to non-detectable levels immediately after the introduction of organic diets and remained non-detectable until conventional diets were introduced. The median urine concentration for other OP pesticides was also lower on an organic diet.

An expansion of this study was published in 2008, where 23 children (ages 3-11 years) were followed for an entire year (four seasons) between 2003 and 2004 (Lu et al. 2008). Children were switched to organic diets for five

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consecutive days in the summer and fall. Urinary metabolites were measured for chlorpyrifos (TCP) and malathion (MDA) in addition to other OP pesticides. Similar to the 2006 results, urinary metabolite concentrations were reduced to non-detectable levels or nearly non-detectable levels during the organic diet. A seasonal effect was also noted with the highest concentrations in the summer related to the consumption of fresh produce. TCP had the highest detection rate of 91% among the five OP pesticide metabolites tested. The mean urinary TCP concentration was 5.1 ug/L, with a 95th percentile of 14.7 ug/L, and a range of <0.2 to 32 ug/L. As shown on Figure 7, when organic diets were substituted for conventional diet, approximately two days were required for metabolite levels to approach zero. When the children were returned to conventional diets, it took approximately 2 days for TCP levels to return back to the previous conventional levels. The authors conclude that children are exposed exclusively to OP pesticides in their diets, and that an organic diet provides a “dramatic and immediate protective effect’ from exposure to organophosphate pesticides commonly used in agricultural production.

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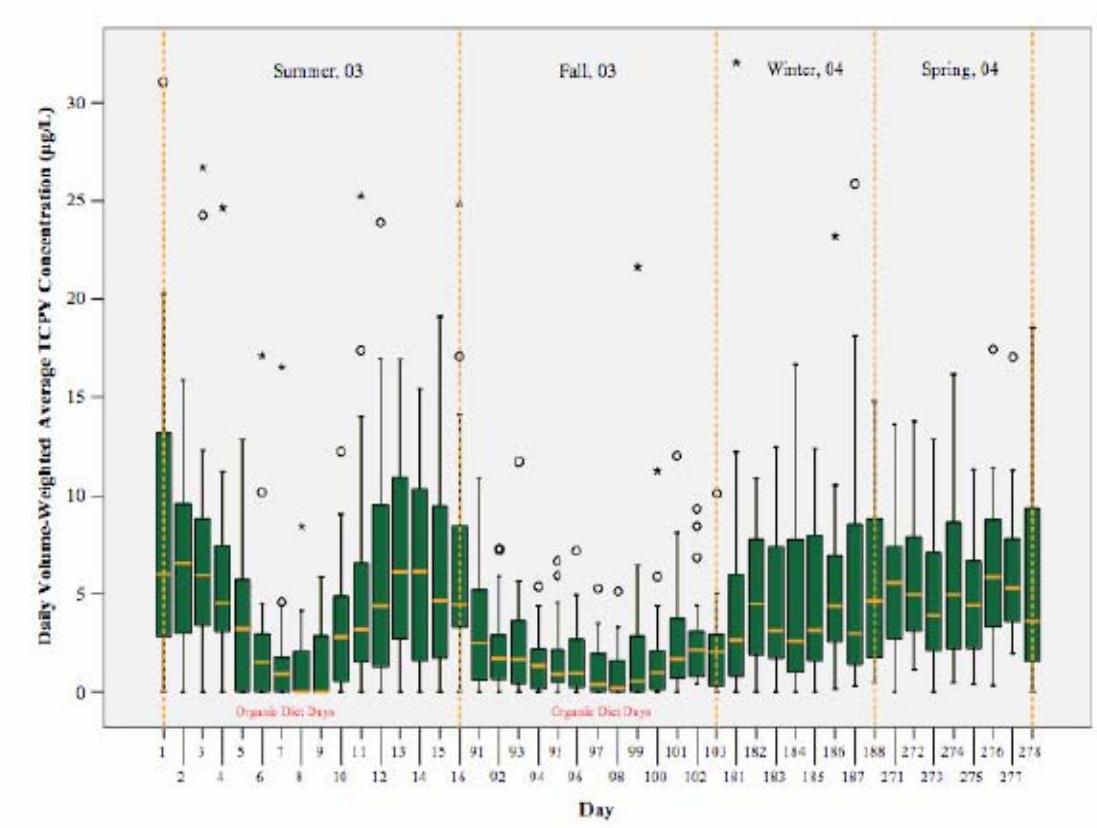
Figure 7. Plot of urinary metabolite data from Lu et al (2008) showing pattern of TCP in urine for children on an alternating conventional-organic-conventional diet



Source: Lu et al. 2008.

In 2000, the Agency identified residues of chlorpyrifos on tomatoes, grapes and apples as major sources of dietary exposure to young children, and as a result the food tolerances for these commodities were to be reduced substantially as part of the risk mitigation agreement with the registrants. In 2007, the Agency published a Federal Register notice intending to lower these tolerances (http://www.epa.gov/EPA-PEST/2007/August/Day-08/). The Agency expects to adopt the new tolerances in 2008. This action is expected to reduce chlorpyrifos exposure to young children.

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6.0 Discussion of Chlorpyrifos Blood and TCP Urine Levels in Context of Other Studies

7.0

The following text compares chlorpyrifos blood and TCP urine levels in various studies with levels measured in studies where administered dose is known and the cholinesterase inhibition was measured.

6.1. Chlorpyrifos Blood Levels and Cholinesterase Inhibition.

In this analysis, the Agency considered two human toxicity studies that measured both chlorpyrifos blood levels and corresponding plasma and/or RBC cholinesterase inhibition following a single dose exposure to adults (Nolan et al. 1982, Kisciki et al. 1999). Although there are differences in chlorpyrifos exposure (i.e, dermal/inhalation versus oral), and populations studied (pregnant women versus adult males and non–pregnant females), EPA believes a comparison provides useful information to assist in regulatory decision making.

As shown on Table 15 below, the chlorpyrifos blood levels measured in the epidemiology study (Whyatt et al. 2003, 2008) are orders of magnitude less than those detected in toxicity and pharmacokinetic studies with human subjects. The maximum detected levels in the epidemiology study are 0.063 ng/g in cord blood, and 0.035 ng/g in maternal plasma (Whyatt et a. 2003) before the residential phase-out of January 2002. In comparison, the highest detected concentration in the Nolan et al. (1982) study is 30 ng/g (476 fold higher than 0.063 ng/g), which was associated with approximately 88% plasma, but no RBC ChE inhibition following a single oral dose to males. However, in the Kisicki et al. (1999) study, no RBC ChE inhibition was observed at chlorpyrifos blood levels of up to 5.6 ng/g (i.e., 89-160 times higher than the maximum cord and maternal blood levels, respectively) following a single oral dose. In this same study, chlorpyrifos blood levels of 18 ng/g were associated with 28% RBC ChE inhibition in one female, which is approximately 285-514 fold higher than the maximum detected values in Whyatt et al. (2003). There are some uncertainties in this comparison. It is very likely that following repeated oral exposures in people, ChE inhibition would occur at lower chlorpyrifos blood levels than those repeated in the Kiscki (1999) and Nolan et al. (1982) studies. Based on a comparison of the single dose and repeated animal studies, repeated exposures may produce blood ChEI at approximately 3 to 17 fold lower doses than a single exposure (i.e., single dose versus repeated NOAELs of 0.5 mg/kg/day and 0.03 mg/kg/day, respectively and LOAELs of 1-1.5 mg/kg/day and 0.2-0.3 mg/kg/day, respectively from the 2000 chlorpyrifos RED). Thus, the single versus repeated exposure ChEI differences are likely to account for only a small fraction (i.e., 17 fold) of the human study responses for blood ChEI.

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Table 15. Comparison of Chlorpyrifos Blood Levels and Cholinesterase Inhibition

Study	Exposure Duration/Route/ Sample Size	Chlorpyrifos Blood Levels Mean±SD and/or Range (ng/g)	Percent Cholinesterase Inhibition		Administered Dose (mg/kg/day)
			Plasma	RBC	
Human Data					
Whyatt et al. (2003) (1998-2001) Mostly before Residential Phase Out	Long term/ inhalation and dermal (possibly incidental oral) (N=199 maternal blood; 211 cord blood)	**Maternal:** 0.0048±0.0055 (ND-0.035) (a)	Not measured		Not relevant (epidemiology study)
		Cord: 0.0047±0.0065 (ND-0.063) (a)	Not measured		
Whyatt et al. 2008 (presentation to EPA) (1998-2006) Pre and Post Phase out	Long term/ inhalation and dermal (possibly incidental oral) (N=425 maternal blood; 423 cord blood)	**Maternal:** 0.0028±0.0044 (ND-0.035) (a)	Not measured		
		Cord: 0.003.0±0.0053 (ND-0.063) (a)	Not measured		
Nolan et al. (1982) (n=6 males) (b)	Single oral dose	<5-30	12-89%	None-37%	0.5
	Single dermal dose	<5-10	None-26%	2-11%	5
Kiscki et al. (1999) ©	Single oral dose 3 M/2 F (QQ) 3 M/4 F (QR)	<1	Not measured	None	0.5
	1 M /2F (QQ) 2 M/2 F (QR) 1 M (RR)	<1		None	1
	2 M /1 F (QQ) 1 F (QR)	1.0-5.6			
	1 M/1 F (QQ) 4 M/1 F (QR) 1 F (RR)	<1		None	2
	1 M/2 F (QQ)	1.3-4.1			
	1 F (QQ)	2.5-18		28%	
Rat Data					
Mattsson et al. (1998) (d)	2 week gavage during gestation	<0.7	52% dam None fetus	39% dam None fetus	0.3
		2.55 dam 0.99-1.19 fetus	77% dam None fetus	87% dam None fetus	1
		109 dam 39-52 fetus	94% dam 86% fetus	99% dam 92% fetus	5

(a) limit of detection is 0.5 to 1 pg/g, or 0.0005-0.001 ng/g.

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(b) See Table A-2 for a detailed summary of these results.
(c) See Table A-3 for a detailed summary of these results.
(d) See Table A-4 for a detailed summary of these results.

It is also useful to compare the chlorpyrifos blood levels detected in pregnant rats and their fetuses with cholinesterase inhibition and administered dose. As shown on the Table 10 (and in Appendices A and B), at non-detectable chlorpyrifos blood levels of <0.7 ng/g, the dams had 52% and 39% plasma and RBC ChE inhibition, respectively which was associated with an administered dose of 0.3 mg/kg/day following a repeated 2 week daily gavage exposure. However, the fetuses had no cholinesesterase inhibition on gestational day 20. At higher doses of 1 mg/kg, detectable chlorpyrifos blood levels of 2.5 and 1.19 ng/g in the dam and fetus, respectively were associated with 77% and 87% plasma and RBC ChE inhibition in the dams, respectively (4 hours post dosing), but no ChE inhibition in the fetuses.

6.2 TCP Urine Levels

As discussed in Appendix A, TCP is the primary urinary metabolite of chlorpyrifos. The human studies show that following oral exposures, that chlorpyrifos absorption is fairly complete and the ½ life of elimination as TCP ranged from 15.5 to almost 36 hours. For dermal exposures, approximately 1-3% of the dermal dose was absorbed and the ½ life of elimination was approximately 30-40 hours (Nolan et al. 1982, Kiscki et al. 1999, Meuling et al. 2005).

In this analysis, the Agency considered the Kisciki et al. (1999) study that measured TCP urinary levels and corresponding RBC ChE inhibition following a single dose exposure to adults (Kisciki et al. 1999). The Nolan et al. (1982) study also made this comparison, but the TCP urine levels were reported as ug/hour, and the urine volume was not provided so the Agency was unable to determine the TCP concentration in ug/L for a useful comparison. Although there are differences in chlorpyrifos exposure (acute versus repeated), and populations studied (i.e., adult males/females versus pregnant females), EPA believes a comparison provides useful information to assist in regulatory decision-making. For example, the Berkowitz et al. (2004) epidemiology study evaluated pregnant females following crack and crevice treatments via primarily inhalation and dermal exposures over several weeks or months. The available human toxicity study involved single oral doses to adult males and females.

As shown on Table 16, some individuals had 12 hour TCP urine concentrations up to 15,323 ng/ml, without any RBC ChE inhibition. One female in this study, however, with low PON activity (QQ) exhibited 28% RBC ChE inhibition and excreted a maximum 12 hour TCP level of 8,270 ng/ml. These data seem to indicate a lot of human variability in TCP excretion levels

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associated with the same dose (2 mg/kg), and ChE inhibition response. In addition, the study authors conclude there is large variation in gastrointestinal absorption for chlorpyrifos and that this factor was more important than PON status with regard to observed inhibition of RBC AChE.

The Agency also considered other submitted registrant studies that measured both TCP and ChE activity, and these are shown on Table 16. In the Vaccaro et al. (1996) study, the maximum 12 hour TCP urinary concentrations for individuals that contacted freshly treated chlorpyrifos turf for 4 hours ranged from 10.5 to 41.6 ng/g (equivalent to ng/mL) (Vaccaro et al. 1996 (MRID 44167101; DP233282, HED DER Review 11/18/1998 D.Smegal to M. Hartman). The maximum inhibition reported in one individual for plasma ChE was 12%, while some subjects also had ChE activities above their baseline levels (i.e., +109%). No RBC ChE activity was measured in this study.

In the Honeycutt et al. 1993 (MRID 4306701) study, 3 workers had RBC ChE inhibition between 13 and 18%, which was associated with maximum 12 hour TCP urinary levels of between 93 and 117 ng/g. However, some other workers in this study excreted up to 93 ng/g and had no RBC ChE inhibition. In another study by Honeycutt et al. 1994 (MRID 43138102) of mixer/loader and applicator workers, the maximum 12 hour TCP levels were much higher and ranged from 23.7-593.2 ng/g. In this study the ChE measurements were unreliable, and the Agency was unable to estimate whether any of these workers had ChE inhibition. In addition, the Agency notes that some of the workers in this study had fairly high TCP baseline levels of up to 150 ng/g. However, these data confirm that workers tend to have higher urinary TCP levels than the general population. These data are presented in Appendix A, Tables A-7 and A-8.

In general, the TCP urine levels in the Berkowitz et al. (2004) epidemiology study are consistent with the urine TCP levels for residents following post-application of a crack and crevice treatment. As shown of Table 16 below, the spot maternal urine samples from the epidemiology study that were reported to be associated with adverse impacts on head circumference in infants were > 11 ug/L, with a 90^{th} percentile of 61.2 ug/L (average of 17.4 ug/L) (maximum value not reported). The median value for this population was 7.5 ug/L, and the frequency of detection was 42%. In comparison the maximum 12 hour urinary TCP levels from the Registrant study ranged from 6.8-31ug/L (equivalent to ng/mL) following a crack and crevice treatment of the kitchen and bathroom of three houses (Byrne et al. 1998, MRID 44458201, and reviewed in memorandum from D. Smegal, 12/3/98,EPA D242444, D240119, D239034).

The CHAMACOS study also reported TCP spot urinary concentrations for pregnant women from an agricultural community in the Salinas Valley, California (Eskenazi et al. 2004, 2007). As shown on the table below, their levels were lower than those reported in the NYC study, with a median value of between 3.3

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and 3.54 ug/L, and a range of 0.2-56.1 ug/L. However, the frequency of TCP detection was higher in this cohort, of between 76-91%. As discussed previously, chlorpyrifos represented approximately 10% of the total OP usage in the Salinas Valley, which may explain why the median values are lower in this cohort compared to those who had their homes treated with chlorpyrifos in NYC.

In addition, these maternal TCP levels can be compared to the levels in the U.S. population as measured by the National Health and Nutrition Examination Survey (NHANES). The 1999-2002 median (50th percentile) for all age groups evaluated in NHANES is 1.9 ug/L, with an 95th percentile value of about 11 ug/L for adults evaluated during the years 2001-2002. Thus, the level of greater than 11 ug/L reported in the Berkowitz et al. (2004) study represents about the 95th percentile for adults in the U.S. Figure 10 depicts these1999-2002 NHANES TCP spot urine concentrations graphically. It should be noted that the NHANES only contained data on 85 and 114 pregnant women for the 1999-2000 and 2001-2002 study years, respectively. The urinary TCP concentrations in pregnant women and concentrations in the U.S. general population may not be directly comparable because the chlorpyrifos toxicokinetics may differ during pregnancy (i.e., rates of absorption, distribution, metabolism and excretion).

Table 16. Comparison of TCP Urine Levels and Cholinesterase Inhibition for Humans

Study	Exposure Duration/ Route/ Sample Size	TCP Urine Levels (ng/ml or ug/L, unless reported)	Percent Cholinesterase Inhibition		Administered Dose (mg/kg/day)
			Plasma	RBC	
Berkowitz et al. 2003/2004 (NYC inner city cohort)	Long term/ inhalation and dermal (possibly incidental oral) n=365	**Maternal Spot Urine** (approx 32 weeks of pregnancy): Median= 7.5 ug/L; 90th percentile: 61.2 ug/L or 70 ug/g creatinine >11 ug/L associated with adverse birth outcomes in infants	Not measured		Not relevant (epidemiology study)
Eskenazi et al. 2004,2007 Results (2000-2003) (Agricultural cohort)	Long term/ inhalation, dermal and possibly incidental oral n=445-485	**Maternal Spot Urine**: Median 3.3-3.54 ug/L Range: 0.2-56.1 ug/L	Not measured		Not relevant (epidemiology study)

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Study	Exposure Duration/ Route/ Sample Size	TCP Urine Levels (ng/ml or ug/L, unless reported)	Percent Cholinesterase Inhibition		Administered Dose (mg/kg/day)
			Plasma	RBC	
Lu et al. 2008	Diet N=23 children ages 3-11 years	mean spot urine is 5.1 ug/L; 95th percentile of 14.7 ug/L (range of <0.2 to 32 ug/L	Not measured		Not relevant
Byrne et al. 1998 (MRID 44458201; 44331901; D242444, D240119, D239034 HED DER Review 12/03/1998 D.Smegal to M. Hartman	Single crack and crevice treatment with urine collection for 10 days post-treatment N=2M; 4F	Maximum TCP: 6.8-31.4 ng/mL; A.M.=17.4 ng/mL (baseline 2.7-22.6 ng/mL: A.M: 13 ng/mL) (12 hrs)	Not measured		Not Relevant; passive dosimetry/ biomonitoring study following crack and crevice treatment of kitchen and bathroom
NHANES 1999-2002	Adults 18-59 years	Median (50th Percentile) Concentrations= 1.9 ug/L Adults 95th percentile: 11 ug/L for 2001-2002	Not measured		Not relevant
Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) (2005)	Residential and diet N=128 Children 20 months-5.5 years (median 3.9 yrs) July 2000-March 2001 (pre-cancellation)	Spot Urine: Median: 5.3 Mean: 7.3 95th: 15.5 Range ND-104	Not measured		Not relevant
Kiscki et al. (1999)	Single oral dose (a) N=6/sex (QR=4F/3M; QQ=2F/3M)	509-4,292 (12 hrs)	Not measured	None	0.5
	N=6/sex (RR=1M; QR=3F/2M; QQ=3F/3M)	935-4,758 (12 hrs)		None	1
	N=1 F (RR)	5,275 (12 hrs)		None	2
	N=4M/1F (QR)	2,818-6,064 (12 hrs)			
	N=2M/3F (QQ)	2,224- 15,323 (12 hrs)			

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Study	Exposure Duration/ Route/ Sample Size	TCP Urine Levels (ng/ml or ug/L, unless reported)	Percent Cholinesterase Inhibition		Administered Dose (mg/kg/day)
			Plasma	RBC	
	N=1 F (QQ)	8,148 (12 hrs)		28%	
Vaccaro et al. 1996 (MRID 44167101; DP233282, HED DER Review 11/18/1998 D.Smegal to M. Hartman	Single lawn treatment with 4 hour exposure to treated turf N=5M; 4F	Maximum TCP: 10.5-41.6 ng/g (12 hrs)	Maximum 12% inhibition up to 109% of baseline	Not measured	Not Relevant; passive dosimetry/ biomonitoring study following granular lawn treatment
Honeycutt et al. 1993 (MRID 4306701) (b)	Pruners N=2	Maximum TCP: 117.7 ng/g (12 hours)	None	13-14%	Not Relevant; passive dosimetry/ biomonitoring study for pruners and re-entry workers
	Pruners: N=1	Maximum TCP: 93.1 ng/g (12 hours)	None	14-18%	
	Pruners N=6	Maximum TCP: 27.5-93.8 ng/g (12 hours) (baseline: <3-14.3 ng/g)	None		
	ReEntry workers N=5	Maximum TCP: 6.7-10.6 ng/g (12 hours) (baseline: 3.5-7.8 ng/g)	None		
Honeycutt et al. 1994(MRID 43138102) b)	Mixer/Loader (n=15)	Maximum TCP: 23-272.7 ng/g (12 hours) (baseline: 0.94-102.4 ng/g)	Cholinesterase data are not reliable (c)		Not Relevant; passive dosimetry/ biomonitoring study for mixer/loader/ applicators
	Applicator (n=15)	Maximum TCP: 23.7-593.2 ng/g (12 hours) (baseline: <3-150.2 ng/g)			

(a) See Tables A-5 and A-6 for a detailed summary of these results.
(b) See Tables A-7 and A-8 for a detailed summary of these results.
(c) Cholinesterase data are not reliable because the baseline values could not be duplicated or verified for a large number of subjects, and this affects the percent inhibition calculations

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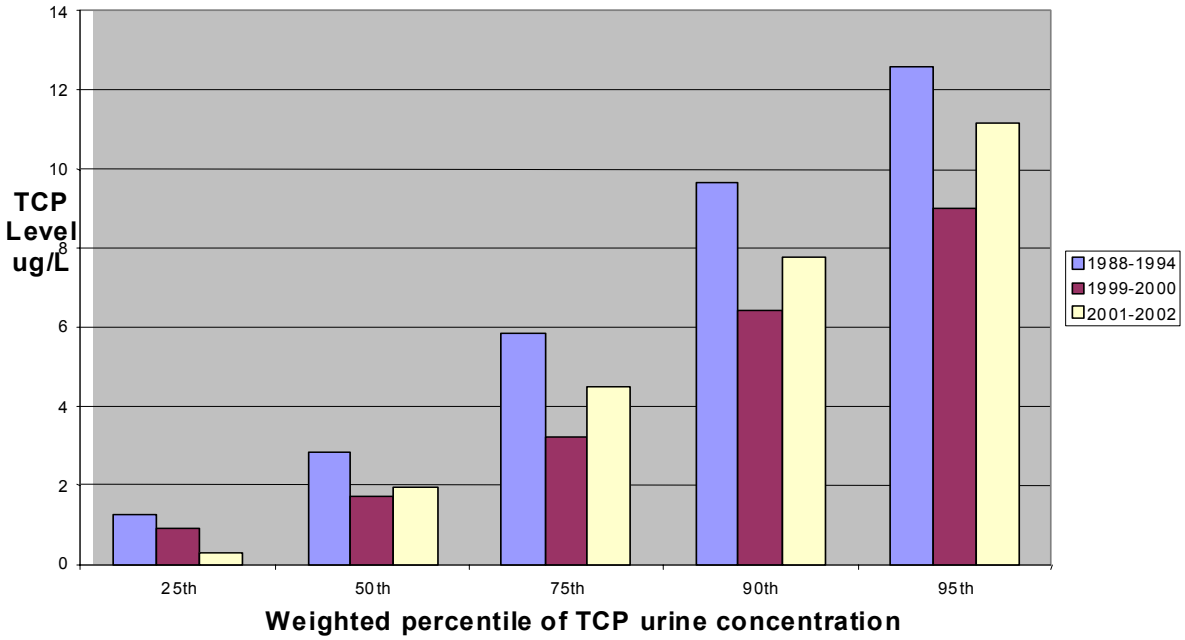
Figure 8. TCP urine concentration (ug/l) in adults (18 - 59 years)



Lu et al. (2008) also report TCP levels in the urine of children before they were switched to an organic diet. As shown on Table 16, the mean spot urine is 5.1 ug/L; with a 95th percentile of 14.7 ug/L, and a range of <0.2 to 32 ug/L. These data suggest that children have higher chlorpyrifos exposures than adults, based on a comparison of the TCP urinary levels in NHANES. As shown in Figure 11, below, the TCP spot urine 50th and 95th percentiles for children ages 6-11 years are about 3 ug/Land 15 ug/L for 2001-2002, respectively (CDC 2005). Thus, the 95th percentile for the Lu et al. (2008) data is similar to the 95th percentile NHANES result for children. In the Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) study (Morgan et al. 2005), the median TCP level was 5.3 ng/ml for 128 children ages 20 months to 5.5 years that had both residential and dietary exposure to chlorpyrifos between July 2000 and March 2001 (pre-cancellation of residential uses). The 95th percentile and maximum detected TCP concentrations were 15.5 and 104 ng/ml, respectively. The CTEPP TCP spot urine concentrations are higher than those reported in both Lu et al. (2008) and NHANES (CDC 2005), but this is not unexpected because the focus of the CTEPP study was to evaluate chlorpyrifos exposures, and other compounds, in the residential and pre-school environment whereas Lu et al. (2008) likely represents food only exposure.

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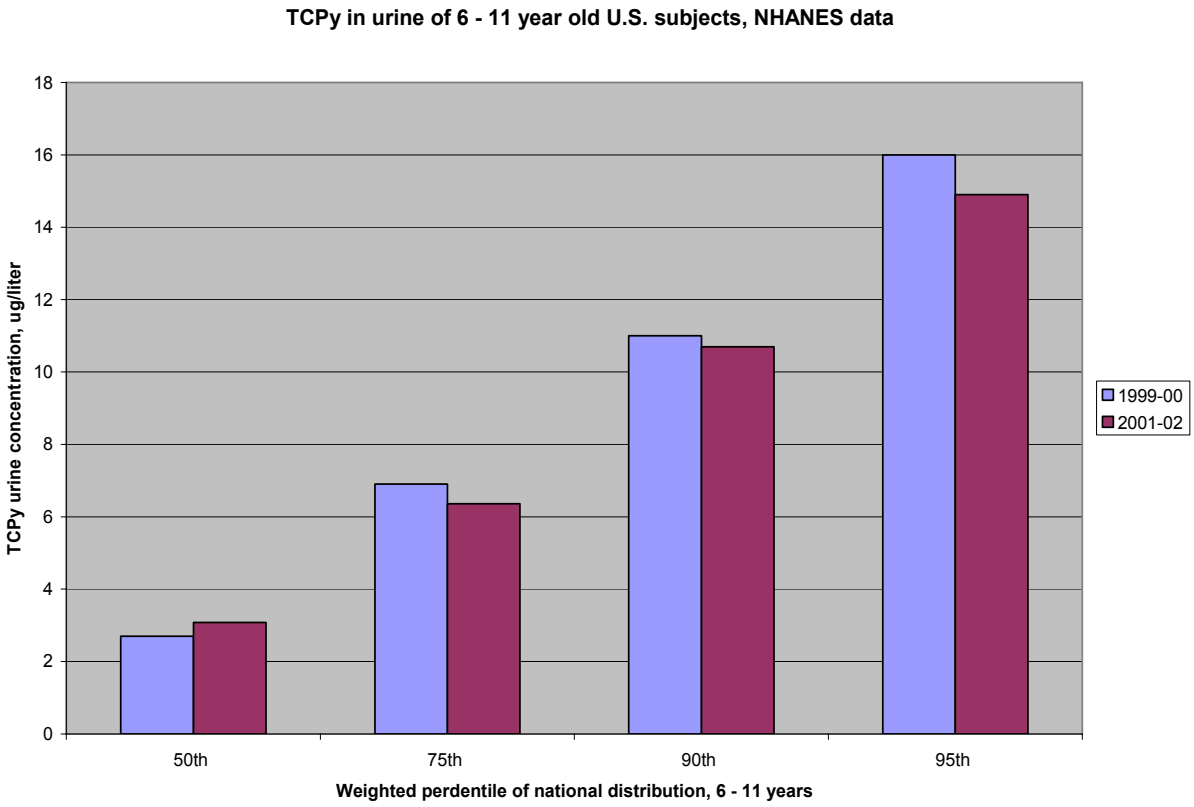
Figure 9. TCP in Urine of 6-11 year old children, NHANES Data



In conclusion, based on the TCP and ChE activity data available:

(1) There appears to be a large variability in response, which may be due to the route of exposure (i.e., oral in Kiscki et al. 1999 versus dermal and inhalation in the worker and residential studies by Honeycutt et al. 1993 and Vaccaro et al. 1996).

(2) The lowest TCP level associated with 13-18% RBC is 93 ng/ml (ng/g), while one individual excreted up to 15,000 ng/ml, without any RBC ChE inhibition.

(3) Based on factors 1 and 2, it is possible, but unlikely that some of the pregnant women in the Berkowitz et al. (2004) had low levels of ChE inhibition

(4) Another complication in this comparison is that the Berkowitz et al. (2004) results are for a spot sample, while the registrant TCP results represent a 12 hour urine sample. It should be noted that the registrant studies (worker, residential and Kisciki et al. 1999, Nolan et al. 1982) are

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all single day exposures while the epidemiology studies (Berkowitz et al. 2004) most likely represents repeated exposures throughout pregnancy.

As discussed previously, the ChEI response differs in animal studies by a factor of between 3 and 17 fold for acute and repeated exposures (based on a comparison of NOAELs and LOAELs from the 2000 RED).

7.0 Comparison of Chlorpyrifos Exposure Data for Crack and Crevice Treatments with the Columbia Epidemiology Study Results

It is useful to compare the chlorpyrifos air measurements from the registrant-submitted crack and crevice study to the levels detected in the Whyatt et al. (2003) epidemiology study from Columbia University.

The 2000 EPA risk assessment relied heavily on a registrant-submitted study to estimate exposure and risks from a crack and crevice treatment. In this study (MRID 44458201), three houses received a crack, crevice and spot treatment of a 0.5% chlorpyrifos spray to the kitchen and bathroom. The houses were then monitored up to 10 days post application, where air monitoring was collected at two heights in the kitchen (site of application) and family room (adjacent room). In addition, deposition measurements and dislodgeable residues were collected in the family room and a bedroom of each of the treated houses. Dislodgeable residues were measured on hard toys, and also on carpets to determine the amount of chlorpyrifos available for absorption. Details of the EPA review of this study can be found in memo from D. Smegal to M. Hartman (DP242444), 12/3/98.

In comparison, the 48-hour personal air monitoring data for chlorpyrifos in the epidemiology study (Whyatt et al. 2003) reported much lower average concentrations of 0.018 ug/m^3 for women in the study between September 1998 and May 2001. This value is approximately 32 times less than the 10-day TWA from the registrant study. The maximum detected concentration in the epidemiology study was 0.345 ug/m^3. It is not surprising that the epidemiology air concentrations are lower than the registrant crack and crevice study because the air levels were not correlated to timing of pesticide application in the epidemiology study. Nevertheless, they may reflect longer-term average chlorpyrifos air concentrations in the residential settings.

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Table 17. Crack and Crevice Treatment Comparison of Chlorpyrifos 2000 Risk Assessment and Epidemiology Results

Study	Mean Air Concentrations (ug/m3)
USEPA 2000 Risk Assessment based on Dow Crack and Crevice Study (44458201) (a)	Short-term 10 day TWA=0.58
	1 day Maximum=1.56
Whyatt et al. (2003) September 1998-May 2001 (b)	48-Hr personal air samples: 0.018 ±0.036 (0.0007-0.345)

TWA= Time-weighted average air concentration.
NE= not evaluated.
(a) USEPA Data Evaluation Record (DER) DP242444, Memo from D. Smegal to M. Hartman, December 3, 1998.
(b) Personal air samples collected for 48 hours during the third trimester of pregnancy, mostly before the residential phase-out on January 1, 2001.

8.0 Discussion of Epidemiology Studies and findings

It is unusual for the Agency to have data from three large, prospective cohorts for consideration in human health risk assessment. Each provides unique and somewhat complementary information:

- The Columbia University NYC cohort includes predominately African American and Dominican women and children. This team has reported indoor air, maternal and cord blood measures of parent chlorpyrifos, and multiple birth and neurodevelopmental outcomes. This cohort was exposed during pregnancy to chlorpyrifos and other pesticides indoors and in food. One focus of the publications from this group involves comparisons between pre- and post cancellation of indoor uses of chlorpyrifos.

- Mount Sinai NYC cohort includes women and children who are Puerto Rican Hispanic, African American and Caucasian. This team has associated urinary metabolites (TCP and/or DAPs) with some birth and neurodevelopmental outcomes. This group has placed an emphasis on relating outcome information with PON1 genetic status and/or activity level. The enrollment of the Mt. Sinai cohort overlapped with the cancellation of residential uses of chlorpyrifos. However, the researchers have not evaluated the impacts of the residential phase out on the health outcomes measured in their publications.

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- The CHAMACOS cohort includes mothers and children from farm families who live in the Salinas Valley, California and who are predominately of Mexican descent. This cohort is exposed to many pesticides, including multiple OPs (Table A-1), from multiple pathways such as occupational exposures and take-home exposures. This team has collected information on PON1 status but has not yet published findings associating PON1 status with health outcomes. This team has, however, associated urinary DAP metabolites with some birth and neurodevelopmental outcomes.

These studies have been performed by experienced investigators using prospective epidemiology methods where exposure measures come before measures of neurodevelopment deficits, including chemical measures on pesticide exposure in pregnant women and their infants. Chemical measures of blood and urine pesticide analytes are done by, or with, testing methods of the Centers Disease Control and Prevention (CDC) in ways that can be compared to reference ranges in the National Health and Nutrition Examination Survey (NHANES) for DAPs and/or TCP in urine and chlorpyrifos in blood (Columbia only).

All three studies used well developed and validated neurodevelopmental measures, which provide comparability. Both the Columbia and CHAMACOS cohorts used the BSID-II, a widely used, normative value-referenced, developmental test for young children that is used frequently to diagnose developmental delay and is known to be highly sensitive to low level intrauterine exposures. All three also used the CBCL to assess behavior problems such as attention problems, ADHD; and PDD disorders. The Mt. Sinai results for PDI and MDI are still in preparation (Engel et al., in prep).

In an effort to characterize the exposures and outcomes in the epidemiology studies, the Agency developed a series of detailed tables which compare blood levels of chlorpyrifos and urine levels of TCP in humans and animals across studies and compared these levels with AChE inhibition data where possible. Due to differences in study design between epidemiology and laboratory studies, the interpretation of these tables is challenging and problematic. For example, the chlorpyrifos blood levels measured in Whyatt et al. (2003, 2008) before the residential phase-out of January 2002 are three orders of magnitude less than those detected in toxicity and pharmacokinetic studies with human subjects where ChE inhibition was observed (Nolan et al, 1982; Kisicki et al, 1999). This finding suggests that the at least some of the outcomes reported in the epidemiology studies may have occurred at doses lower than those causing ChE inhibition. However, the magnitude of exposure to the mothers in the epidemiology studies is unknown and the level of chlorpyrifos in the blood is likely an underestimate of total exposure.

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With regard to the timing of exposure and the timing of measurements, in animal or human laboratory studies, time course studies provide valuable information such as the time of maximum effect and/or maximal blood or tissue levels and time to recovery. In contrast, in epidemiology studies, the timing of exposure is unknown and thus the timing of measurements in relation to when exposure occurred is also unknown. Specifically, the urine and blood measures in these studies are not timed with applications, so it is difficult to correlate these results with known exposures in the home or agricultural field. The epidemiology studies have taken spot samples of urine (as opposed to 24 hour samples). There is uncertainty associated with spot samples as they may not capture all the pesticide exposures because of the short-half life of the OP pesticides in the body. As noted previously, the TCP urine levels in the Mt. Sinai cohort above 11 ug/L represent about the 95th percentile of the NHANES TCP levels for adults in 2001-2002. These data are comparable because both NHANES and the Mt. Sinai data are spot urine samples.

Compared with epidemiology studies, laboratory studies, whether animal or human, are highly controlled situations where the amount, timing, and route of exposure are known. In laboratory experiments, important endpoints such as AChE inhibition and behavioral measures or clinical signs can be observed with care. In addition, the magnitude of exposure can be controlled for. Specifically, exposure pathways like food or residential exposures could be controlled for during a laboratory study compared with epidemiology studies where the magnitude of exposure may vary significantly among individuals and among different days. Similarly, the route of exposure is known for in the laboratory study but is unknown in the epidemiology studies. For example, in the human deliberate dosing studies, the subjects were exposed via the oral route. With regard to the epidemiology studies, mothers were likely exposed through the diet (oral) and from residential uses (dermal, inhalation).

While epidemiology results of this scope are new and complex to understand in a regulatory context, qualitatively, there are similar findings for neurodevelopment outcomes in all three studies, but some differences with respect to birth outcomes. Two studies show increases in abnormal reflexes in neonates, and all show adverse changes neurodevelopmental using validated test scales for Bayley and Brazelton and other neurologic metrics.

Birth Outcomes. There are some differences reported for associations between OP exposure and birth outcomes. For example, one group (Mt. Sinai) reported decreased head circumference (albeit small) when maternal urinary TCP levels were above the limit of detection (>11 ug/L) and PON1 status was considered (Berkowitz et al. 2004). In a follow-up study for the Mt. Sinai cohort, Wolf et al. (2007) report that head circumference was inversely associated with maternal PON1 activity (P=0.004). Head circumference was 0.62 $\pm$0.18 cm smaller in the first activity (slow tertile PON1) compared with the third tertile of PON1 (adjusted n=382, p=0.0009). With slow activity PON1 of PON192, urinary

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DEPs were associated with decreased birth weight and DMPs were associated with shorter birth length.

In contrast, increases in head circumference associated with increasing maternal urinary DAPs in the CHAMACOS cohort (Eskenazi et al. 2004). The Columbia team reported no changes in head circumference (Whyatt et al. 2004). Head size interpretation is complicated by smoking (Perera et al. 2003) and air pollutants (Perera et al. 2005). In conclusion, the head circumference data provide inconsistent results and are thus less robust than other outcomes.

The Mt. Sinai researchers reported that maternal PON1 levels alone, but not maternal PON1 genetic polymorphisms were associated with smaller head size. In addition, the study authors report that there was no interaction between PON1 and TCP urinary levels. These findings suggest that the decreased head circumference may not be solely attributed to TCP urinary levels.

With regard to changes in birth weight and birth length, the Columbia study reported decreased birth weight and length with increasing chlorpyrifos cord blood levels. However, Mt. Sinai cohort did not report any effects on birth weight and birth length associated with maternal urinary TCP levels. The CHAMCOS researchers also did not find an association between maternal DAP, DMP or DEP urinary levels and birth weight and length. Therefore, these three epidemiology studies report different findings, but they are also using different pesticide exposure measures (i.e., chlorpyrifos in blood, versus TCP or DAPs in urine) which could partially explain these differences. It is possible that cord blood measures of parent compound are more reliable than maternal urinary metabolites.

Neurodevelopmental Outcomes. Unlike the birth outcomes which show variable results across the cohorts, delays in mental development were reported in all three cohorts (Columbia, Mt. Sinai and CHAMACOS). Although these studies show qualitatively similar results (i.e., associations between OP exposure and neurodevelopmental outcomes), the degree to which chlorpyrifos is implicated these outcomes varies.

Both Mt. Sinai and CHAMACOS cohorts report abnormal reflexes in neonates associated with urinary maternal DAP levels. For each log10 unit increase in total DAPs, these authors report a 32 percent (Engel et al. 2007) and 26 percent (Young et al. 2005) increased risk of abnormal reflexes.

Increases in PDD disorder were reported in both the Columbia and CHAMACOS cohorts. In the Columbia study (Rauh et al. 2006) these effects in 3 year old children were associated with high (>6.17 pg/g) chlorpyrifos blood umbilical cord levels, while the CHAMACOS cohort reported these effects in 2

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year old children to be associated with increases in total urinary maternal and child DAPs and DMPs, and child DEPs for 12 month old children (Eskenazi et al. 2007).

All three cohorts have reported statistically significant delays in mental development in children 2 and 3 year of age associated with prenatal OP exposure as shown in the Figure 10 below. The Columbia results are associated with high chlorpyrifos cord blood levels, while the CHAMACOS and Mt. Sinai teams correlated urinary DAPs with mental delays in 2 year old children (Eskenzai et al. 2007, Engel et al. in preparation). The Mt. Sinai team has not yet published findings for the 2 year old children but these results are currently under preparation for publication. Based on preliminary information shared with the Agency, these results are expected to show increasing urinary DAPs are associated with lower MDI (Engel et al. in prep).

Thus, prenatal OP exposure has been reported to be associated with delays in mental development in 2 and 3 year old children, increased odds of abnormal reflexes in neonates, and increased odds of PDD disorder in children 2 and 3 years of age.

In the CHAMACOS study, however, TCP in maternal urine was not associated with any Bailey or CBCL adverse outcomes in children, and there were no reported associations between PDI or attentional deficits and urinary OP concentrations. It should be recognized that the Columbia study reported effects for 3 yrs olds, while the CHAMACOS study has only published data for 2 yr old children thus far. In addition, the urinary levels of TCP in the CHAMACOS study were much lower (median 3.5 ug/L compared to 7.5 ug/L for the Mt. Sinai cohort) than the Mt. Sinai cohort and this could partially explain the differences in the study results.

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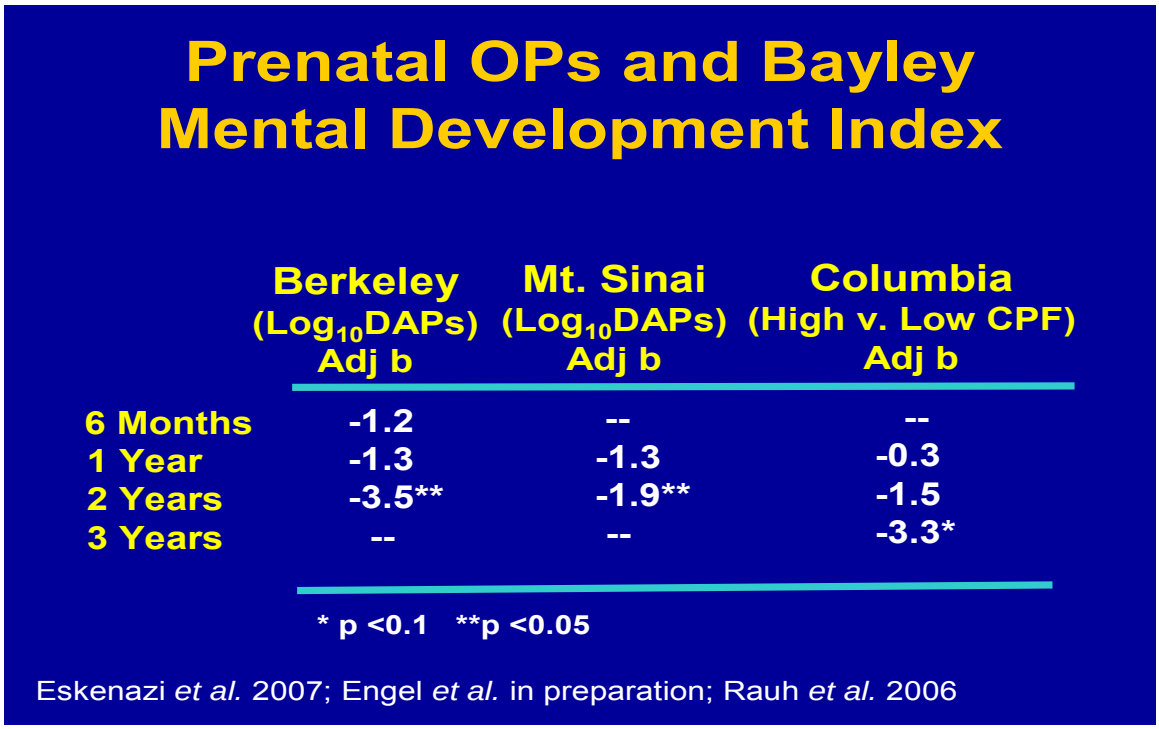
Figure 10. Prenatal Ops and Bayley Mental Developmental Index



Source: Rauh 2008, presentation to EPA, April. Used with permission.
CPF= chlorpyrifos

The Agency believes that the Columbia University studies provide the most relevant information for evaluating the human health effects of chlorpyrifos. These studies specifically evaluated chlorpyrifos in maternal and umbilical cord blood levels rather than the TCP and/or DAP urinary metabolites in maternal urine reported in the Mount Sinai and CHAMACOS studies. TCP is a common metabolite of chlorpyrifos, chlorpyrifos-methyl and trichlorpyr. It is also the primary environmental degradate of chlorpyrifos and is found on food treated with chlorpyrifos. As such, environmental and/or dietary exposures to TCP can also contribute to urinary TCP levels. As shown previously on Table 3, many of the 27 OPs can contribute to total urinary DAP concentrations, which complicates interpretation of the DAP data. The exposure of the CHAMACOS to multiple OPs reduces its usefulness in the chlorpyrifos risk assessment since one can not distinguish chlorpyrifos exposure (and thus outcomes) from other DEP- or DMP-forming OPs, some which are used in higher amounts in the Salinas Valley (Table A-1). Results reported by the Mount Sinai group are informative, particularly which regard to evaluating the relevance of PON1 status in health outcomes, but are less robust than the Columbia University studies.

In the Columbia University cohort, recruitment of study participants overlapped with residential use cancellation and there was a sharp decline in use. Chlorpyrifos levels dropped substantially in maternal personal air and plasma and cord blood plasma samples after cancellation. For children born

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before cancellation, high chlorpyrifos exposure in cord plasma was significantly associated with decreased birth weight and length. In contrast, this relationship was no longer significant for newborns born after the cancellation because the blood levels dropped and only one child was in the high group. Likewise, there was no association with chlorpyrifos and neurodevelopmental outcomes after the cancellation, again because all but one of the children had cord blood levels less than 6.17 pg/g. The low dose chlorpyrifos group represents children that may be exposed to chlorpyrifos from the diet (food and drinking water) and residual chlorpyrifos in the home from past applications that will go away with time. These children are closer to the average family in the U.S.

There were multiple chemical exposures in the Columbia University cohort study, including diazinon and propoxur that are potent cholinesterase inhibitors, and o-phenylphenol, a disinfectant/fungicide, all of which were measured in 100% of air samples at higher median concentrations than chlorpyrifos. However, the mean umbilical cord levels were less than chlorpyrifos (1.1, 3.1 and 4 pg/g for diazinon, 2-isopropoxyphenol and chlorpyrifos, respectively). Diazinon residential uses were also phased out, with retail sales for indoor uses ceasing by December 2002, a year after chlorpyrifos. It is also likely that the diazinon and propoxur metabolites in blood were probably underestimated, similar to chlorpyrifos, because of the relatively short half lives, and the lack of information to correlate the time of sample collection with pesticide application. The study authors report that after controlling for both diazinon and 2-isopropxyphenol (metabolite of propoxur) exposure in cord plasma, the associations between birth weight and length and cord plasma (ln)chlorpyrifos remained statistically significant ($p \leq 0.02$) and the effect size remained similar to that seen without 2-isopropoxyphenol in the model (Whyatt et al. 2004). Some additional analyses were conducted on the neurodevelopmental effects to consider diazinon (Rauh 8/12/08, personal communication with D. Smegal vial email). These analyses show that the adverse impact of chlorpyrifos on birth weight and cognitive development is not due to diazinon exposure, and these analyses do not reduce the chlorpyrifos effect for any of the 3-year outcomes for MDI or PDI. Given, that measured levels of chlorpyrifos have been statistically associated with multiple birth and neurodevelopmental outcomes, chlorpyrifos likely played a role in these outcomes.

While neurodevelopment deficits may be multifactor in origins, it is not always possible to identify the sources for each case, and there are not yet established national norms for chlorpyrifos in cord blood. As discussed previously, these children are from poor multi-ethnic populations and urban neighborhoods and may experience other health disparities that compound pesticide exposure. Such disparities are linked to health care access, low income and low education, as well as exposure to urban air pollutants.

Cicchetti (2007) published a critique of neurodevelopmental outcomes reported by Rauh et al. (2006). Specifically, Cichetti (2007) commented on the

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significance of differences in neurodevelopmental measures and suggested the mean MDI scores were clinically meaningless, and indicated that there were no standards defining “high” and “low” chlorpyrifos exposure. Dr. Cichetti also commented on the study population socio-demographics like maternal education- and said that Rauh et al. (2006) masked, but did not eliminate educational bias by dichotomizing maternal education into high school graduate or non-graduate. Furthermore, that the “high” and “low” exposure groups differed in their race/ethnicity characteristic which confounds race/ethnicity with exposure, and the finding that more high exposure children had ADHD is meaningless. Rauh et al. (2007) disagrees with Dr. Cicchetti claim that the significant chlorpyrifos effect on MDI was “clinically meaningless” and indicates that a Bayley developmental score < 85 prompts referral to early intervention services, and exposures that produce small shifts in the mean often result in more children who meet the diagnostic criteria. Dr. Rauh indicated the “high” and “low” groups were clearly defined based on the previous report of reduced birth weight among children with exposure levels above 6.17 pg/g (Whyatt et al. 2004). High school degree was used to adjust for maternal education because the sample was uniformly low income, and thus education was the preferred covariate for social class. Maternal intelligence, although controlled was not significant in their analysis. Rauh et al. (2006) controlled for race/ethnicity in all models and also used a stratified analysis showing a significant chlorpyrifos effect within each ethnic group, independent of race. They used Achenbach’s Child Behavior Checklist (CBCL) to assess behavior problems rather than make a diagnosis because ADHD is hard to diagnose in preschool-aged children.

In conclusion, the birth outcomes reported by the three groups do not provide a consistent pattern of effects. There is, however, a consistent pattern of health affects observed for the neurodevelopmental outcomes. Although there are some differences reported, the weight of the evidence supports the conclusion that exposure to OPs at sufficiently high levels during gestation, particularly in susceptible populations, may result in neurodevelopmental outcomes. Specifically, prenatal OP exposure at high levels may potentially be associated with increased odds of abnormal reflexes in neonates, delays in mental development in 2 and 3 year old children, and increased odds of pervasive developmental disorder in children 2 and 3 years of age. At lower exposure levels, particularly those found in food (and after the cancellation of indoor uses of chlorpyrifos), such effects have not been observed.

The Agency can not rule out the potential for multiple AChE-inhibiting pesticides impacting the health outcomes reported in the children. Individual chemical risk assessments for chlorpyrifos, diazinon and propoxur provided risk estimates above the Agency’s level of concern. As a result, the indoor uses for these three OP pesticides have been voluntary cancelled by the registrants. This conclusion regarding multi-chemical exposure does not preclude the potential contribution of chlorpyrifos in the reported health outcomes. Given, that

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measured levels of chlorpyrifos have been statistically associated with multiple birth and neurodevelopmental outcomes and these blood levels have been correlated in time with the chlorpyrifos phase-out, the Agency has preliminarily concluded that chlorpyrifos likely played a role in these outcomes.

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Attachment A. Data Tables

Table A.1.
Comparison of Key Epidemiological Studies of Mothers and Children with Pesticide Exposure

A. Study Place	Berkeley/CHAMACOS	Columbia University	Mt Sinai University	University of Washington
1.A. Location of Investigators	Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS), Center for Children's Environmental Health Research, University of California at Berkeley	Columbia Center for Children's Environmental Health, Mailman School of Public Health, Columbia University	Department of Community and Preventive Medicine, Mt. Sinai School of Medicine	University of Washington, Emory University
1.B. Place of Study	Salinas Valley, California	New York City, NY (Northern Manhattan and South Bronx)	New York City, NY	Surburban Seattle, WA
1C. Study Populations	Low income Latina pregnant women & their kids from agricultural community 82% married 81%<HS	NYC African American (35%) and Dominican (65%) pregnant women & their kids 25% married 35%<HS	NYC African American, White, Mexican & Puerto Rican preg. Women & kids 29% married 32%<HS 50% Hispanic; 28% Black and 21% White	White Suburban kids from Seattle

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A. Study Place	Berkeley/CHAMACOS	Columbia University	Mt Sinai University	University of Washington
2. Epidemiology Study Design (Type, Size, time frame)	Prospective cohort (n=601) 1990's to present. Primarily Latino women and their predominantly farmworker families in Salinas Valley, CA, where infants had pre and post natal exposure to a wider variety of agricultural pesticides, as measured by organophosphate metabolites (DAPs) associated with excess risk of pervasive developmental disorders and known PON1. N=355 infants (< 2 months) evaluated for neurodevelopmental effects (Eskenazi et al. 2007)	Prospective cohort & sub-studies (n= 725) (1998-present). Pregnant inner city minority women and their infants at high risk of adverse birth outcomes and more likely exposed to residential pesticides, environmental tobacco smoke (ETS), air polluants, polycyclic aromatic hydrocarbons (PAHs), and other pollutants. N=254 children evaluated at age 3 years for neurodevelopmental effects (Rauh et al. 2006)	Prospective cohort (n=404) (1990's to present) pregnant NYC multiethnic women and their infants exposed to indoor residential pesticides, polychlorinated biphenyls (PCBs), and with known PON1 genetic status for chemical detoxification capacity. Conducted questionnaire on pesticide use and urine and blood measurements. N=404 births evaluated for birth outcomes (Berkowitz 2004) N=311 infants (1-2 days old) evaluated for neurodevelopmental effects (Engel et al. 2007)	Exposure study in kids n= 23 kids 3-11yrs (2003-2004) Normal and organic diets & repeated measures of pesticide analytes in urine with diet switching for 1 year (2003-2004) over summer and fall sampling seasons with urine samples 2x/day for 7,12, or 15 consecutive days over 4 seasons
3. Methods (summary) (neurobehavioral & neurodevelopmental scores and pesticide exposure pre and/or postnatal	1.2+ Brazelton Bayley Scores DAPS & TCP in urine ChE in whole blood Butyryl ChE in maternal and umbilical cord blood	Bayley Scores in early yrs (pre and post residential cancellation of chlorpyrifos in an urban minority cohort Extensive maternal & infant characteristics.	~1.3+ Brazelton & DAPS negative 6.5** high to low Prenatal OPs	Switching organic & regular diet of fresh fruits and vegetables
3A. Exposure Metrics	Pesticide residues in urine twice during pregnancy via spot sample.	Pesticide residues in maternal blood at third trimester and delivery and in cord blood at delivery (pico g chlorpyrifos/g)	Pesticide residues in urine via spot sample during third trimester (31.2	Pesticide residues in urine via spot samples (malathion, chlorpyrifos &

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A. Study Place	Berkeley/CHAMACOS	Columbia University	Mt Sinai University	University of Washington
		Compared high >6.17 pg/g chlorpyrifos (CPY) in cord blood plasma to low <6.17 pg/g levels.	weeks) of pregnancy	
3B. Chemicals	TCP, dialkylphosphates (DAPs), DEPs, Dimethyl phosphates DMP, malathion (MDA). Median TCP=3.54 nmol/L. geometric mean for DEPs is 18.1 nmol/L in moms and 10.5 nmol/L in children at age 24 months. ~115 nmol/L Total DAPS (~ 80+dimethyl &~18 Diethyl)	29 pesticides measured in maternal and cord blood, including chlorpyrifos, diazinon and propoxur. Chlorpyrifos, diazinon, propoxur and o-phenylphenol in room air and personal monitor samples.	Maternal urine analyzed for TCP, phenoxybenzoic acid (PBA, pyrethroid pesticides), pentachlorophenol (PCP), 6 DAPS, and malathion dicarboxylic acid (MDA). Blood measured for PCBs and DDE (Berkowitz et al. 2003, Engel et al. 2007)	OP metabolite conc.
3C.Neurodevelopmental Outcomes Measured	Neurodevelopment Test Scores Brazelton Neonatal Development Bayley at 6m,1y,2y WPPSI at 3.5y, 5y Child Behavior Checklist at 2y,3.5y PPTV at 5y	Neurodevelopment Test Scores Bayley at 1y,2y,3y Child Behavior checklist at 3y WPPSI at 5 y	Neurodevelopment Test Scores Brazelton Neonatal Development. Bayley at 1y,2y WPPSI at 5 y	NA
4A. Risk Model Covariates	Maternal age, Race/ethnicity BMI, Pregnancy wt. gain Country of birth Education Martial status Family income Preferred language	Maternal age Race/Ethnicity Gender of newborn, Gestational age Maternal Education Maternal IQ, Maternal pre-pregnancy weight, and weight gain,	Maternal age Race/ethnicity Infant sex, gestational age Education Marital status Pesticide use by household	NA

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Table A.1. Comparison of Key Epidemiological Studies of Mothers and Children with Pesticide Exposure				
A. Study Place	**Berkeley/CHAMACOS**	**Columbia University**	**Mt Sinai University**	**University of Washington**
	Parity, Smoking in pregnancy Risk of pre-term or low birth weight Work status in pregnancy (ag) (Eskenazi 04)	Environmental tobacco smoke (ETS) Season of delivery, Home inventory Marital status Maternal body mass index (BMI) [wt (kg)/ ht (m^2)] Dietary PAH (fried, broiled or BBQ food in last 2 wks) Yr of delivery (pre/post cancellation)(Whyatt 04) Caesarian section (for head circumference) Maternal alcohol use (Perera 05)		
4B. Characteristics of Study Population (co-exposures)	Dimethyl phosphates: malathion, oxydemeton-methyl, dimethoate naled, methidathion Diethyl phosphates: diazinon, chlorpyrifos, disulfoton	Pesticides including diazinon, propoxur and o-phenyphenol Air and diet PAH Cord blood lead Postnatal ETS (in home 1st yrs) Alcohol consumption (Rauh 04)	pesticide use Berkowitz 04 TCP (chlorpyrifos metabolite) above and below LOD in urine; PCP, PBA, PCBs, DDE	TCPy Malathion Other OPs
4C. Neurophysiologic Results	Shortened gestational duration significantly associated with DMPs in urine, and decreased ChE activity in cord blood. Increased DMPs and DAPs in urine associated with increased head circumference and body length, and increased DEP maternal urine levels associated with increased	Decline in chlorpyrifos maternal and cord blood and air with phase out of residential use. Pre-cancellation high chlorpyrifos cord blood levels (>6.17 pg/g) associated with significant decrements in birth weight and birth length and an increase in small for gestational age (SGA) infants at birth. After phase out, only 1 child in high exposure group, and chlorpyrifos association with these adverse	Small and statistically significant decrease in infant head size at birth in mothers with low PON and TCP above limit of detection (Berkowitz 2004)	

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Table A.1. Comparison of Key Epidemiological Studies of Mothers and Children with Pesticide Exposure				
A. Study Place	**Berkeley/CHAMACOS**	**Columbia University**	**Mt Sinai University**	**University of Washington**
	head circumference (Eskenazi et a. 2004)	outcomes was not longer significant.		
4.D Neurodevelopmental Results	Statistically significant abnormal neonatal reflexes, adjusted for maternal age at delivery, smoking, minutes since fed at BNBAS BNBAS interviewer and mean diastolic and systolic blood pressure (Young, Eskenazi 05, Table 4) [Bazelton Neonatal Behavioral Assessment Scale (BNBAS) includes Habituation, orientation, Motor performance, range of state, regulation of state, autonomic stability and reflexes]	Pre-cancellation high chlorpyrifos cord blood levels (>6.17 pg/g) associated with mental and psychomotor delays and increased attention problems, ADHD and pervasive developmental disorders at 3 yrs. After phase out, only 1 child in high exposure group, and chlorpyrifos association with these adverse outcomes was not longer significant. Development lags seen even with children receiving services for neurodevelopmental & neurobehavioral lags that adversely impact school performance. Bailey Psychomotor Developmental Index (PDI) = 6.5 points lower Bailey Mental Developmental Index (MDI) = 3.3 points lower for high vs low chlorpryrifos exposed children. Proportion of high vs low kids is 5x greater for PDI, and 2.4x greater for MDI (Rauh et al. 2006)	Total DEPs and Total DAPs associated with excess abnormal reflexes, as well as total DMPS once PON1 status taken into account PCBs and DDE not associated with any abnormal reflexes findings at birth. Malathion dicarboxylic acid (MDA) above LOD assoc. with 2.24-fold increase in abnormal reflexes (95 %CI 1.55-3.24).	
5. Study Strengths	Prospective cohort design Large sample size Pesticide measure, pregnancy	Prospective cohort design Large sample size Pesticide measure during pregnancy	Prospective cohort design Large sample size Pesticide measure,	Repeated pesticide measures in the same children

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A. Study Place	Berkeley/CHAMACOS	Columbia University	Mt Sinai University	University of Washington
	and birth Good on covariate controlled PON1 known Comprehensive neurological infant behavior and physiology	and birth (mom and cord blood correlated well) Chlorpyrifos exposure confirmed by presence in blood, rather than metabolite (no other source or exposure) Air monitoring conducted, both personal and stationary in rooms Ability to evaluate exposure and outcomes for pre- and post- residential cancellation Controlled for a large number of covariates Comprehensive neurological infant behavior and physiology	pregnancy and birth Good on covariate controlled PON1 known Comprehensive neurological infant behavior and physiology	Unique study design
6. Study Limitations & Challenges	Multiple pesticide exposures. Urinary DAPs reflect actual real world exposures, but hard to determine which pesticide contributes most to adverse outcomes. Difficult to link adverse outcomes to cholinesterase inhibition.	Only blood measures of chlorpyrifos collected, and meconium for different DAP metabolites. These are appropriate for integrated exposure estimates. No cholinesterase measurements, so difficult to link to chlolinesterase inhibition. Chlorpyrifos air monitoring is variable. due to potential for different spraying pattern across neighboring apartments. Air monitoring does not correlate with blood measures for chlorpyrifos very well. Pesticide exposure via dietary exposures not considered. Only cord blood lead was considered. Blood lead for postnatal period not available for analysis.	Multiple chemical exposures. Study publications did not discuss impact of PBA or PCP results on TCP associations. No cholinesterase measurements so difficult to link to cholinesterase inhibition.	Smaller sample size

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Table A.1.
Comparison of Key Epidemiological Studies of Mothers and Children with Pesticide Exposure

A. Study Place	Berkeley/CHAMACOS	Columbia University	Mt Sinai University	University of Washington
Notes	*Chlorpyrifos metabolite TBA LOD TCPY=0.26ug/l median 3.3ug/l Maternal education, marital status, parity, country of birth, poverty, smoked during pregnancy, alcohol use during pregnancy, caffeine use during pregnancy, cesarean delivery, general anesthesia, Demographics, breast feeding initiated after delivery, infant sex (see Table1, Young, Eskenazi 2005)	Broad spectrum of developmental and behavioral delays seen in more highly exposed children, including attention problems, attention deficit/hyperactivity disorder problems, and pervasive developmental disorder problems that impact school performance and may require early intervention services.	PCBs and DDT done in random subset of maternal plasma	
Study citations	Bradman 03, 05, 07 Eskenazi 99, 04, 07 Furlong…Eskenazi 06 Young, Eskenazi 05	Perera 03, 05 Rauh 04, 06, 07 Whyatt 01, 03, 04, 07	Berkowitz et al. 2003, 2004 Chen et al. 2003 Engel et al. 2007	Lu 08

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Table A-2
Blood Chlorpyrifos Levels and Corresponding Plasma and RBC Cholinesterase Inhibition Following a Single Dose to Humans (a, b)
Nolan et al. (1982)

Hours Post-dosing	Male Subjects											
	A		B		C		D		E		F	
	Blood Level (ng/g) (c)	% Plasma /RBC ChEI	Blood Level (ng/g)	% Plasma/ RBC ChEI	Blood Level (ng/g)	% Plasma/ RBC ChEI	Blood Level (ng/g)	% Plasma/ RBC ChEI	Blood Level (ng/g)	% Plasma/ RBC ChEI	Blood Level (ng/g)	% Plasma ChEI
Single Oral Dose of 0.5 mg/kg												
0	ND	--	ND	--	ND	--	ND	--	ND	--	9	--
1	ND	--	ND	--	ND	--	6	--	5	--	9	--
2	ND	+/11%	ND	--	19	64%/2%	--	--	11	70%/**37%**	15	30%/6%
4	ND	--	ND	--	9	--	21	--	8	--	9	--
6	ND	57%/11%	12	12%/+	12	**88%**/+	21	72%/+	8	84%/+	18	78%/+
8	ND	--	7	--	30	--	ND	--	ND	--	ND	--
10	ND	--	8	--	6	--	ND	--	ND	--	ND	--
12	ND	70%/18%	28	64%/+	15	85%/2%	ND	**86%**/+	ND	**89%**/+	ND	**83%**/+
24	--	71%/16%	--	**84%**/+	--	86%/5%	NA	84%/+	NA	85%/+	NA	80%/+
Single Dermal Dose of 5 mg/kg												
0	NT	NT	7	--	ND	--	ND	--	10	--	ND	--
2	NT	NT	ND	13%/+	ND	+	ND	10%/+	8	12%/4%	ND	+/2%
6	NT	NT	ND	+/+	5	--/3%	5	12%/+	ND	14%/4%	ND	+/2%
10	NT	NT	ND	--	ND	--	ND	--	10	--	5	--
12	NT	NT	--	+/+	--	12%/11%	--	14%/+	--	22%/10%	--	+/+
24	NT	NT	ND	+/+	ND	15%/+	6	18%/+	7	19%/+	ND	19%/6%
48	NT	NT	ND	6%/+	ND	18%/+	--	18%/+	--	26%/+	-	+/4%

NT (not tested at this dose; dermal dose 0.5 mg/kg); ND not detected at detection limit of 0.005 µg/mL; NA=not available;
--= not analyzed; +=value greater than pre-dose level; #=Value same as predose.
Bolded values represent peak time of cholinesterase inhibition.

(a) Source: Nolan et al. 1982, MRID 00124144
(b) Percent inhibition following oral dose is relative to subject's pre-test mean value; following dermal dose is relative to subject's day 30 post oral dose value
(c) Values reported as ng/ml which is equivalent to ng/g.

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Table A-3
Chlorpyrifos Blood Levels and Corresponding RBC Cholinesterase Inhibition Following a Single Oral Dose to Humans (a)
Kiscki et al. (1999)

Administered Dose (mg/kg)	Subject Number/ Gender	PON Status	Hours Post Dosing							
			2 Hr		4 Hr		8 Hr		12 Hr	
			Blood Level (ng/g)	% RBC ChEI	Blood Level (ng/g)	% RBC ChEI	Blood Level (ng/g)	% RBC ChEI	Blood Level (ng/g)	% RBC ChEI
0.5	3 M/2 F 3 M/4 F	QQ QR	All subjects < 1 ng/kg and had no RBC ChE inhibition during this time frame							
1	1 M/2 F	QQ	8/12 subjects < 1 ng/kg and had no RBC ChE inhibition during this time frame							
	2 M/2 F	QR								
	1 M	RR								
	1 M (11)	QQ	1	None	<1	None	<1	None	<1	None
	1 M (14)	QQ	<1	None	2.7	None	1.5	None	<1	None
	1 F (21)	QQ	5.6	None	2.9	None	<1	None	<1	None
	1 F (30)	QR	<1	None	<1	None	1.1	None	<1	None
2	1 M/1 F	QQ	8/12 subjects < 1 ng/kg and had no RBC ChE inhibition during this time frame							
	4 M/1 F	QR								
	1 F	RR								
	1 M (47)	QQ	3.1	None	1.3	None	3.4	None	1.8	None
	1 F (49)	QQ	3.1	None	xx	None	1.7	None	<1	None
	1 F (56)	QQ	<1	None	<1	None	18	**28**	2.5	None
	1 F (59)	QQ	2.2	None	4.1	None	4.1	None	1.5	None

xx=not analyzed; M=Male; F=Female; (subject #)

(a) Source: Kisciki et al. 1999, MRID 44811002; Brzak 2000 MRID 45144101

Bolded value represents peak time of cholinesterase inhibition.

Table A-4
Comparison of Cholinesterase Inhibition with Chlorpyrifos Blood and Milk Levels in Rat Dams and Pups (Exposure GD 6-LD 11)
Mattsson et al. 1998; DNT Companion Study (a)

Dose (mg/kg/ day)	% ChE Inhibition at peak time		Mean Chlorpyrifos in Blood (ng/g)				Mean Chlorpyrifos oxon in Blood (ng/g)	Mean Chlorpyrifos in Milk (ng/g)		
	Dams (b)	Fetus/Pups c)	GD 20 (Dams/ Fetus)	LD 1	LD 5	LD11	GD 20 (Dams/ Fetus)	LD 1	LD 5	LD 11
5	LD 1: 89% forebrain, 80% hindbrain; 89% heart; 94% plasma; 99% RBC	GD 20: 60% Forebrain, 56% Hindbrain, 82% Heart; 92% RBC, 85% plasma	108.78/ 52.8-39.4 (dam is 0.131% of dose)	14	14	ND	ND/0.97-0.94	3022	1534	19.8
1	GD 20: 49% Heart; LD5 10% forebrain; 12% Hindbrain; LD 1: 77% plasma; 87% RBC	None	2.55/ 0.99-1.19 (dam is 0.015% of dose)	ND		ND	ND	139.5	81.8	ND
0.3	LD1: 10% Heart; 52% plasma; 39% RBC	None	ND	ND		ND	ND	20.6	13.5	ND

GD= gestation day
LD= lactation day
ChEI= cholinesterase inhibition
ND=< 0.7 ng/g for chlorpyrifos and chlorpyrifos-oxon.

(a) DNT Companion Study (44648101), Mattsson et al. 1998.
(b) For both dams and pups, the cholinesterase (ChE) activity was measured 4 hours post dosing on GD 20.
© Lactation day ChE measurements for pups was 2 hours post dosing to dams pups. ChE measurements of 2 hr postdosing dams not at ideal time, and probably missed the peak. On page 39 of MRID 44648102, it states that "most of the exposure from milk would likely have occurred between 3 and 6 hours post-maternal dosing (based on peak blood levels Fig.7) with a further delay of a few hours due to time necessary to digest the milk (based on Byczkowski et al., 1994)."

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Table A-5
TCP Urine Levels and Corresponding RBC Cholinesterase Inhibition
Following a Single Oral Dose to Humans (a)
Kisicki et al. (1999)

Dose (mg/kg)	Subject # (females)	PON Status	Hours Post Dosing									
			0-6 Hr		6-12 Hr		12-24 Hr		24-36 Hr		36-48 Hr	
			TCP Level (ng/mL)	% RBC ChEI	TCP Level (ng/mL)	% RBC ChEI	TCP Level (ng/mL)	% RBC ChEI	TCP Level (ng/mL)	% RBC ChEI	TCP Level (ng/mL)	% RBC ChEI
Controls	20	QQ	2.3		BLOQ		2.2		4.3		6.0	
	23	QQ	7.5		3.6		BLOQ		3.7		3.6	
	27	QQ	BLOQ		3.4		3.4		3.3		3.4	
	29	QR	9.9		BLOQ		4.4		4.5		3.2	
	32	QQ	12		10.6		BLOQ		8.7		BLOQ	
	34	QR	BLOQ		2.2		6.1		2.4		3.6	
0.5	19	QR	618		1292		**4292**		2065		974	
	22	QR	1389		719		**1578**		490		755	
	26	QQ	284		344		**2057**		716		805	
	28	QR	500		238		**509**		360		366	
	31	QQ	347		393		**730**		365		590	
	36	QR	622		468		608		671		**726**	
1	21	QQ	517		2859		2402		1240		**2593***	
	24	QQ	1126		1784		1302		1744		**2054**	
	25	QQ	808		1422		1472		**2341**		740	
	30	QR	722		3332		1914		2515		**2642**	
	33	QR	169		737		1278		852		**1289**	
	35	QR	390		466		**935**		451		806	
2	49	QQ	882		2183		**3310**		1453		1897	

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Table A-5
TCP Urine Levels and Corresponding RBC Cholinesterase Inhibition
Following a Single Oral Dose to Humans (a)
Kisicki et al. (1999)

Dose (mg/kg)	Subject # (females)	PON Status	Hours Post Dosing									
			0-6 Hr		6-12 Hr		12-24 Hr		24-36 Hr		36-48 Hr	
			TCP Level (ng/mL)	% RBC ChEI	TCP Level (ng/mL)	% RBC ChEI	TCP Level (ng/mL)	% RBC ChEI	TCP Level (ng/mL)	% RBC ChEI	TCP Level (ng/mL)	% RBC ChEI
	51	QR	1413		3688		**6064**		1786		3736	
	55	RR	676		1850		**5275**		1658		4537	
	56	QQ	1246	-	7966	25 (8) 30 (12)	**8148**	30 (12 hr) 28 (24 hr)	6270	28 (24 hr) 21 (36 hr)	7068	21 (36) 23 (48)
	58	QQ	1690		994		1868		2304		**2458**	
	59	QQ	1881		4682		5036		**6822**		3822	

M=Male; F=Female; values are % ChEI (hours)

(b) Source: Kisicki et al. 1999, MRID 44811002; Brzak 2000 MRID 45144101

Limit of quantitation 2.0 ng/mL; BLOQ: below limit of quantitation

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Table A-6
TCP Urine Levels and Corresponding RBC Cholinesterase Inhibition
Following a Single Oral Dose to Humans (a)
Kisicki et al. (1999)

Dose (mg/kg)	Subject # (males)	PON Status	Hours Post Dosing									
			0-6 Hr		6-12 Hr		12-24 Hr		24-36 Hr		36-48 Hr	
			TCP Level (ng/mL)	% RBC ChEI	TCP Level (ng/mL)	% RBC ChEI	TCP Level (ng/mL)	% RBC ChEI	TCP Level (ng/mL)	% RBC ChEI	TCP Level (ng/mL)	% RBC ChEI
Controls	2	RR	BLOQ		BLOQ		BLOQ		5.9		5.8	
	6	QR	4.1		3.2		3.8		4.1		8.6	
	8	QR	BLOQ		BLOQ		2.9		6.7		4.2	
	10	QR	3.5		4.4		9.4		2.3		5.7	
	13	QQ	5.4		4.8		7.6		3.4		2.5	
	17	QQ	3.6		2.6		4.2		5.2		5.3	
0.5	3	QR	98		593		964		636		**980***	
	5	QQ	520		724		907		**1440**		739	
	9	QQ	1396		1217		940		**1512**		1472	
	12	QQ	152		427		678		712		**1239***	
	15	QR	417		**1256**		**1256**		687		ns	
	18	QR	200		482		1388		836		**1688**	
1	1	QQ	498		1373		**3217**		2224		2896	
	4	RR	212		1779		1636		2034		**1334***	
	7	QR	1286		2499		2941		**3706**		1126	
	11	QQ	2128		2764		**4758**		1967		3109	
	14	QQ	4496		7952		3602		3382		**4526****	
	16	QR	1614		658		716		**3666**		2370	
2	38	QR	1308		2030		2794		**3263**		2822	
	40	QQ	189		578		**2224**		1196		1931	

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Table A-6
TCP Urine Levels and Corresponding RBC Cholinesterase Inhibition Following a Single Oral Dose to Humans (a)
Kisicki et al. (1999)

Dose (mg/kg)	Subject # (males)	PON Status	Hours Post Dosing									
			0-6 Hr		6-12 Hr		12-24 Hr		24-36 Hr		36-48 Hr	
			TCP Level (ng/mL)	% RBC ChEI	TCP Level (ng/mL)	% RBC ChEI	TCP Level (ng/mL)	% RBC ChEI	TCP Level (ng/mL)	% RBC ChEI	TCP Level (ng/mL)	% RBC ChEI
	42	QR	878		3282		6164		4532		**2818***	
	43	QR	850		1314		2672		5188		**5725**	
	47	QQ	1506		4704		**15323**	+ (12 hr) 4 (24 hr)	5549	+ (48 hr)	8610**	
	48	QR	949		784		**3360**		3262		2662	

M=Male; F=Female; * higher value next interval; **remained high; values are % ChEI (hour); ns no sample

(c) Source: Kisicki et al. 1999, MRID 44811002; Brzak 2000 MRID 45144101

Limit of quantitation 2.0 ng/mL; BLOQ: below limit of quantitation

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Table A-7 . Honeycutt et al. 1993 MRID 43062701 Worker Re-entry Study							
Subject/time	**Plasma ChE (% inhibition)**	**Baseline Plasma ChE**	**RBC ChE (% inhibition)**	**Baseline RBC ChE**	**Range**	**Post-dose Peak(s) (ng/g)**	**Time to Post-dose Peak (hrs)**
Pruner							
JG PR 1 3/25 3/28 3/31	 8751 9690 9210	 - √9221 9221	 8604 7038 **6721 (14)**	 - √7821±1107 7821	Pre-dose range: 5.3 – 8.8 Post-dose range: 10.5 – 117.7	117.7	12-24
JM PR 2 3/25 3/28 3/31	 6243 6243 6195	 - **?** 6201 6201	 9320 9320 7711 (14/**18**♪)	 - **?** 8984 8985	Pre-dose range: 4.2-5.9 Post-dose range: 4.5-93.1	93.1	12-24
JG PR 3 3/25 3/28 3/31	 9411 9969 9984	 - √ 9690 9690	 9576 8378 8162	 - √ 8977±847 8977±847	Pre-dose range: 3.3-3.7 Post-dose range: <3.0-66.8	66.8	12-24
RL PR 4 3/25 3/28 3/31	 6879 6840 6660	 - √ 6859 6860	 12161 11269 **10231 (13)**	 - √11715±630 11715	Pre-dose range: <3.0-3.7 Post-dose range: 4.8-117.7	117.7	36-48
IL PR 5 3/25 3/28 3/31	 4998 5409 4974	 - √ 5203 5204	 12689 11925 11872	 - √ 12307 12307	Pre-dose range: 7.5-10.1 Post-dose range: 10.8-44.5	44.5	12-24
JLP PR 6 3/27 3/30 4/4	 5384 7830 6492 (2)*	*6607±1730** - **?** 5938 5938	 10089 9955 12653	 - √ 10022 10022	Pre-dose range: <3.0-<3.0 Post-dose range: <3.0-93.8	93.8	24-36
RC PR 7 3/27 3/30 4/4	 2734 4275 3213	*3504*±1090 - **?** 2973 2973	 10757 11645 12838	 - √ 11201 11201	Pre-dose range: 5.3-7.6 Post-dose range: <3.0-56.3	56.3	12-24

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Table A-7 . Honeycutt et al. 1993 MRID 43062701 Worker Re-entry Study							
Subject/time	**Plasma ChE (% inhibition)**	**Baseline Plasma ChE**	**RBC ChE (% inhibition)**	**Baseline RBC ChE**	**Range**	**Post-dose Peak(s) (ng/g)**	**Time to Post-dose Peak (hrs)**
FD PR 8		*7003±1899*			Pre-dose range: 3.9-5.8	56.4	12-24
3/27	5660	-	10144	-			
3/31	8346	-	9178	√ 9661	Post-dose range:		
4/4	7029	**?** 7687	14453	9661	7.2-56.4		
ML PR 9		*6800±1962**			Pre-dose range: 9.6-14.3	37.7	84-96
3/27	5412	-	8890	-			
3/31	8187	**?** 5622	9110	√ 9000	Post-dose range:		
4/4	**5832 (14)***	5622	12702	9000	<3.0-37.7		
MM PR 10		*5992±720*		*10594±2368*	Pre-dose range: <3.0-3.0	27.5	12-24
3/27	5482	-	8920	-			
3/31	6501	**?** 6715	12269	**?** 12152	Post-dose range:		
4/4	6930	6715	12035	12152	<3.0-27.5		
Re-entry							
SG RE 1					Pre-dose range: <3.0-4.2	10.0	24-36
11/25	8496	√ 8433	10388	√ 10177			
12/2	8370	8433	9967	10177	Post-dose range:		
12/6	8415	8433	9710 (5)	10177	<3.0-10.0		
RG RE 2					Pre-dose range: <3.0-7.8	7.7	12-24
11/25	9177	√ 8715±653	10811	√ 10984			
12/2	8253	8715	11158	10984	Post-dose range:		
12/6	8481 (3)	8715	11307	10984	<3.0-7.7		
JC RE 3					Pre-dose range: 4.7-5.1	10.3	12-24
11/25	7236	√ 7011	10194	√ 10164			
12/2	6786	7011	10134	10164	Post-dose range:		
12/6	6783 (4)	7011	10835	10164	3.2-10.3		
JLG RE 4					Pre-dose range: <3.0-6.1	6.7	12-24
11/25	8481	√ 8230±354	11834	√ 11553			
12/2	7980	8230	11272	11553	Post-dose range:		
12/6	7644 (8)	8230	10967 (6)	11553	<3.0-6.7		
MB RE 5					Pre-dose range:	10.6	12-24

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Table A-7 . Honeycutt et al. 1993 MRID 43062701 Worker Re-entry Study							
Subject/time	**Plasma ChE (% inhibition)**	**Baseline Plasma ChE**	**RBC ChE (% inhibition)**	**Baseline RBC ChE**	**Range**	**Post-dose Peak(s) (ng/g)**	**Time to Post-dose Peak (hrs)**
					3.0-3.5		
11/25	6066	√ 6216	12593	√ 12652			
12/2	6366	6216	12712	12652	Post-dose range:		
12/6	6540	6216	12102	12652	<3.0-10.6		

√ baseline mean confirmed; **?** baseline mean not confirmed; (% inhibition); *mean ± s.d.* (my calculation); ♪ % inhibition using pre-exposure value

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Table A-8. Mixer, Loader, Clean-up Study with Chlorpyrifos Honeycutt et al. 1994 (MRID: 43138102) (a)						
ID	**Baseline TCP Range(ng/g)**	**Post-Dose TCP Range (ng/g)**	**Max TCP(ng/g)**	**Time to Peak (hours post dosing)**	**Max Range of TCP for all Mixer/ Loaders (ng/g)**	**ChEI**
Mixer/Loaders						
ML-FS-1	35.1 - 47.4	48.4 - 113.8	113.8	60 - 72	23.0 - 272.7	ChE data are unreliable. Unable to confirm baseline values for large number of subjects, and this affects the % ChE inhibition estimates.
ML-FS-2	0.94 - 1.07	24.4 - 77.1	77.1	24 - 36		
ML-FS-3	21.0 - 35.4	4.9 - 87.0	87.0	48 - 60		
ML-FS-4	22.6 - 29.8	18.9 - 132.5	132.5	72 - 84		
ML-FS-5	17.6 - 19.0	32.9 - 105.3	105.3	24 - 36		
ML-FS-6	102.4[a]	27.6 - 207.6	207.6	0 - 12		
ML-FS-7	5.4 - 6.3	<3.0 - 47.5	47.5	24 -36		
ML-FS-8	<3.0 - 3.5	<3.0 - 133.9	133.9	36 - 48		
ML-FS-9	3.1 - 4.3	<3.0 - 27.5	27.5	24 - 36		
ML-FS-10	33.9 - 52.8	23.7 - 272.7	272.7	12 - 24		
ML-FS-11	21.1 - 24.3	13.4 - 63.0	63.0	12 - 24		
ML-FS-12	9.6 - 20.9	13.7 - 44.0	44.0	72 - 84		
ML-FS-13	<3.0 - 12.4	5.5 - 23.0	23.0	60 - 72		
ML-FS-14	33.2 - 75.0	7.4 - 135.9	135.9	24 - 36		
ML-FS-15	<3.0 - 7.0	11.8 - 216.2	216.2	12-24 and at 60-72		
Applicators						
AP-FS-1	30.0 - 54.7	19.6 - 303.8	303.8	36 - 48	23.7 – 593.2	
AP-FS-2	48.4 - 57.4	38.6 - 67.3	67.3	24 - 36		
AP-FS-3	36.3 - 116.7	24.8 - 112.9	112.9	12 - 24		
AP-FS-4	8.0 - 15.7	18.8 - 40.7	40.7	72 - 84		
AP-FS-5	8.0 - 32.1	10.3 - 75.0	75.0	96 - 108		
AP-FS-6	58.1[a]	17.2 - 97.3	97.3	0 - 12		
AP-FS-7	26.4 - 84.8	25.1 - 114.8	114.8	60 - 72		
AP-FS-8	13.2 - 25.0	25.5 - 593.2	593.2	60 - 72		
AP-FS-9	14.4 - 17.1	16.8 - 69.9	69.9	12 - 24		
AP-FS-10	<3.0 - 9.5	4.7 - 131.9	131.9	60 - 72		
AP-FS-11	71.8 - 150.2	25.3 - 148.3	148.3	12 - 24		
AP-FS-12	8.4 - 11.9	13.0 - 44.0	44.0	24 - 36		

Table A-8. Mixer, Loader, Clean-up Study with Chlorpyrifos Honeycutt et al. 1994 (MRID: 43138102) (a)						
ID	**Baseline TCP Range(ng/g)**	**Post-Dose TCP Range (ng/g)**	**Max TCP(ng/g)**	**Time to Peak (hours post dosing)**	**Max Range of TCP for all Mixer/ Loaders (ng/g)**	**ChEI**
AP-FS-13	31.1 - 49.9	77.7 - 111.0	111.0	72 - 84		
AP-FS-14	11.0 - 17.5	<3.0 - 23.7	23.7	24 - 36		
AP-FS-15	3.4 - 8.7	28.8 - 156.9	156.9	0 -12		

A: only one pre-dose TCP measurement recorded in the raw data

(a) only values for mixer/loader and applicator recorded here.

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Attachment B.
Bayley Scales of Infant Development

Definition

The Bayley Scales of Infant Development (BSID) measure the mental and motor development and test the behavior of infants from one to 42 months of age.

Purpose

The BSID are used to describe the current developmental functioning of infants and to assist in diagnosis and treatment planning for infants with developmental delays or disabilities. The test is intended to measure a child's level of development in three domains: cognitive, motor, and behavioral.

Cognitive Development

Cognition can be defined as a process by which knowledge is gained from perceptions or ideas. Cognitive development refers to how an infant perceives, thinks, and gains an understanding of the world. Within the history of developmental psychology, the work of Jean Piaget (1896–1980), the Swiss psychologist, has had the greatest impact on the study of cognitive development. Piaget's theory is focused on the processes of cognitive development and states that the child is born with an innate curiosity to interact with and understand his/her environment. It is through interaction with others that the child actively constructs his/her development.

Motor Development

During the first two years of life, infants grow and develop in many ways. Two types of motor development occur at this stage. Cephalocaudal development occurs in the following sequence: head before arms and trunk and arms and trunk before legs. Proximodistal development occurs as follows: head, trunk, arms before hands and fingers. Motor development has a powerful impact on the social relationships, thinking, and language of infants. Large motor development allows infants to have more control over actions that help them move around their environment, while small motor development gives them more control over movements that allow them to reach, grasp, and handle objects. The sequence of these developments is similar in most children; however, the rate of growth and development varies by individual.

Behavioral Development

Temperament is the set of genetically determined traits that organize the child's approach to the world. They are instrumental in the development of the child's distinct personality and behavior. This behavioral style appears very early in life—within the first two months after birth—and undergoes development, centered on features such as intensity, activity, persistence, or emotionality.

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Besides measuring normal cognitive, motor, and behavioral developmental levels, the BSID are also used in cases in which there are significant delays in acquiring certain skills or performing key activities in order to qualify a child for special interventions. Specifically, they are also used to do the following:

- identify children who are developmentally delayed
- chart a child's progress after the initiation of an intervention progrlim
- teach parents about their infant's development
- conduct research in developmental psychology

Description

The BSID were first published by Nancy Bayley in *The Bayley Scales of Infant Development* (1969) and in a second edition (1993). The scales have been used extensively worldwide to assess the development of infants. The test is given on an individual basis and takes 45–60 minutes to complete. It is administered by examiners who are experienced clinicians specifically trained in BSID test procedures. The examiner presents a series of test materials to the child and observes the child's responses and behaviors. The test contains items designed to identify young children at risk for developmental delay. BSID evaluates individuals along three scales:

- Mental scale: This part of the evaluation, which yields a score called the mental development index, evaluates several types of abilities: sensory/perceptual acuities, discriminations, and response; acquisition of object constancy; memory learning and problem solving; vocalization and beginning of verbal communication; basis of abstract thinking; habituation; mental mapping; complex language; and mathematical concept formation.
- Motor scale: This part of the BSID assesses the degree of body control, large muscle coordination, finer manipulatory skills of the hands and fingers, dynamic movement, postural imitation, and the ability to recognize objects by sense of touch (stereognosis).

- Behavior rating scale: This scale provides information that can be used to supplement information gained from the mental and motor scales. This 30-item scale rates the child's relevant behaviors and measures attention/arousal, orientation/engagement, emotional regulation, and motor quality.

The BSID are known to have high reliability and validity. The mental and motor scales have high correlation coefficients (.83 and .77 respectively) for test-retest reliability.

Precautions

BSID data reflect the U.S. population in terms of race, ethnicity, infant gender, education level of parents, and demographic location of the infant. The BSID was standardized on 1,700 infants, toddlers, and preschoolers between one and 42 months of age. Norms were

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established using samples that did not include disabled, premature, and other at-risk children. Corrected scores are sometimes used to evaluate these groups, but their use remains controversial.

The BSID has poor predictive value, unless the scores are very low. It is considered a good screening device for identifying children in need of early intervention.

Preparation

Before giving the BSID test to a child, the examiner explains to the parents what will happen during the test procedure. This is to allow the examiner to establish a focused rapport with the child once the procedure has started and avoid diverting attention from the child to the parents during the test. The parents are also asked not to talk to the child during the BSID test to avoid skewing results.

Risks

There are no risks associated with the BSID test.

Parental Concerns

As of 2004 it was recognized that parental involvement in the developmental assessment of their children is very important. First, because parents are more familiar with their child's behavior, their assessment may indeed be more indicative of the child's developmental status than an assessment that is based on limited observation in an unfamiliar clinical setting. The involvement of parents in their child's development testing also improves their knowledge of child development issues and their subsequent participation in required intervention programs, if any. In cases of developmental problems, parents should bear in mind that the scoring and interpretation of the test results is a highly technical matter that requires years of training and experience. Besides the BSID, parents should be aware that three other infant development scales are commonly used:

- Brazelton Neonatal Behavioral Assessment Scale: This scale tests an infant's neurological development, interactive behavior, and responsiveness to the examiner, and need for stimulation. This test is administered during the newborn period only.
- Gesell Developmental Schedules: These schedules test for fine and gross motor skills, language behavior, adaptive behavior including eye-hand coordination, imitation, object recovery, personal-social behavior such as reaction to persons, initiative, independence, and play response.
- Denver Developmental Screening Test: This test is used to identify problems or delays that should be more carefully evaluated. It measures four types of development: personal/social, fine-motor/adaptive, language, and gross motor skills.

See also Cognitive development; Personality development; Personality disorders.

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Resources

Books

Amiel-Tison, Claudine, et al. *Neurological Development from Birth to Six Years: Guide for Examination and Evaluation.* Baltimore, MD: Johns Hopkins University Press, 2001.

Sattker, Jerome M. *Assessment of Children: Behavioral and Clinical Applications*, 4th ed. Lutz, FL: Psychological Assessment Resources Inc., 2001.

——. *Assessment of Children: Cognitive Applications*, 4th ed. Lutz, FL: Psychological Assessment Resources Inc., 2001.

Periodicals

Glenn, S. M., et al. "Comparison of the 1969 and 1993 standardizations of the Bayley Mental Scales of Infant Development for infants with Down's syndrome." *Journal of Intellectual Disability Research* 45, no. 1 (February 2001): 55–62.

Provost, B., et al. "Concurrent validity of the Bayley Scales of Infant Development II Motor Scale and the Peabody Developmental Motor Scales in two-year-old children." *Physical and Occupational Therapy in Pediatrics* 20, no. 1 (2000): 5–18.

Voigt, R. G., et al. "Concurrent and predictive validity of the cognitive adaptive test/clinical linguistic and auditory milestone scale (CAT/CLAMS) and the Mental Developmental Index of the Bayley Scales of Infant Development." *Clinical Pediatrics (Philadelphia)* 42, no. 5 (June 2003): 427–32.

Organizations

American Academy of Child & Adolescent Psychiatry (AACAP). 3615 Wisconsin Ave., N.W., Washington, DC. 20016–3007. Web site: www.aacap.org.

American Academy of Pediatrics (AAP). 141 Northwest Point Boulevard, Elk Grove Village, IL 60007–1098. Web site: www.aap.org.

American Psychological Association (APA). 750 First Street, NE, Washington, DC 20002–4242. Web site: www.apa.org.

Child Development Institute (CDI). 3528 E. Ridgeway Road, Orange, CA 92867. Web site: www.childdevelopmentinfo.com.

Web Sites

"Assessments for Young Children." *LD Online*. Available online at www.ldonline.org/ld_indepth/early_identification/assessment_devareas.html (accessed November 23, 2004).

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[Article by: Monique Laberge, Ph.D.]

http://www.answers.com/topic/bayley-scales-of-infant-development?cat=health
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Attachment C

BHBAS: Understanding the Baby's Language

While babies may not speak their first word for a year, they are born ready to communicate with a rich vocabulary of body movements, cries and visual responses: all part of the complex language of infant behavior.

The Brazelton Neonatal Behavioral Assessment Scale
(BNBAS) was developed in 1973 by Dr. T. Berry Brazelton and his colleagues. The scale represents a guide that helps parents, health care providers and researchers understand the newborn's language. "The Scale gives us the chance to see what the baby's behavior will tell us," says Dr. Brazelton, professor emeritus, Harvard Medical School. "It gives us a window into what it will take to nurture the baby."

The Scale, looks at a wide range of behaviors and is suitable for examining newborns and infants up to two months old. By the end of the assessment, the examiner has a behavioral "portrait" of the infant, describing the baby's strengths, adaptive responses and possible vulnerabilities. The examiner shares this portrait with parents to develop appropriate caregiving strategies aimed at enhancing the earliest relationship between babies and parents.

Scale reveals infant's individuality
When the Scale was published in the early 1970s, people were just beginning to appreciate the infant's full breadth of capabilities, and the only tests available were designed to detect abnormalities. The Scale was designed to go beyond available assessments by revealing the infant's strengths and range of individuality, while still providing a health screen.

The BNBAS is based on several key assumptions. First, infants, even ones that seem vulnerable, are highly capable when they are born. "A newborn already has nine months of experience when she is born," Dr. Brazelton notes. "She is capable of controlling her behavior in order to respond to her new environment."

Second, babies "communicate" through their behavior, which, although it may not always seem like it, is a rational language. Not only do infants respond to cues around them, like their parents' faces, but they also take steps to control their environment, such as crying to get a response from their caregivers.

Third, infants are social organisms, individuals with their own unique qualities, ready to shape as well as be shaped by the caregiving environment.

Assessing the baby's capabilities
In an effort to reveal everything the infant has to say, the Scale was built to 28 behavioral and 18 reflex items. The exam does not yield a single score but instead assesses the baby's capabilities across different developmental areas and describes how the baby integrates these areas as she deals with her new environment.

When infants are born they face four developmental tasks vital to their growth. The Scale examines how well the infant manages these interrelated tasks and sees if the baby may need extra caregiving support in some areas.

The most basic challenge facing newborns is to regulate their breathing, their temperature and the rest of their autonomic system, which needs to be functioning properly before infants can concentrate on other developmental areas. High-risk infants may spend most of their energy trying to maintain their autonomic systems, so they cannot focus on other areas of growth. Sights and sounds may overtax them, so looking at their mother's face may disturb their breathing or noise may set off tremors, startles or color changes, signals that are assessed by the Scale.

Next, infants strive to control their motor system. Inhibiting random movements and controlling activity levels lets the newborn focus her energy on other developmental tasks vital to growth. If the baby is having difficulty in this area, caregivers can help her by providing as much tactile support as necessary to help her settle down, such as holding or swaddling her. The Scale assesses the quality of the baby's tone, activity level and reflexes.

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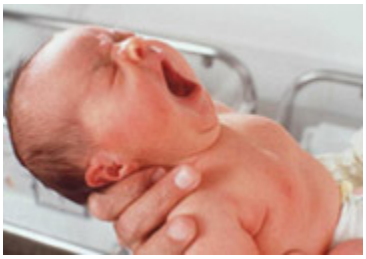
Once the baby can manage motor behavior, she will be ready to tackle the next sphere in her developmental agenda: "state" regulation. State is a key developmental concept that describes levels of consciousness, which range from quiet sleep to full cry. The infant's ability to control her states enables her to process and respond to information from her caregiving environment. The NBAS examiner looks at how an infant controls her states, and at the transition from one state to another.



For example, the exam reveals how an infant responds to light, sound and touch during the sleep state. The examiner briefly shines a light in a sleeping baby's eyes. Generally, the child blinks and squirms in irritation. When we repeat the process several times, the infant usually tunes out the stimulation and remains asleep. The baby's ability to ignore the stimulation allows her to conserve energy and to develop. If a baby has trouble blocking out stimulation during the exam, parents will know that they need to support their child, perhaps by being quiet or keeping her from bright light.

Finally, when an infant's autonomic, motor and state systems are in equilibrium, she is ready to interact socially, the ultimate developmental task. The Scale shows how babies are ready to be engaged in their new world from the first moments of life. In this portion of the assessment, the examiner looks to see how a baby follows a red ball, a face and a voice. It is a powerful experience for parents to see their new child respond to their voices or study their faces.

Best performance

NBAS examiners are trained to get the best performance from the child by doing everything possible to support the infant in "succeeding." For example, one part of the exam looks at an infant's ability to self-console when she is upset. Some infants console themselves easily, while others have a more difficult time. If the infant cannot console herself, the examiner takes measured steps to help her. Not only do we learn how much support the infant may need at home, but also how far along the child is at completing her developmental agenda.

By the end of the exam, the examiner has developed a vibrant portrait of the newborn, which can be used to tailor caregiving to the baby's specific physical needs and behavioral style. Does the baby like to be handled? Is the baby receptive to social interaction? Does the baby easily calm herself? "One of the important things about the Scale is that it parallels what parents are looking for," Dr. Brazelton says. "It puts health care providers on the same wavelength as parents."

Reference:

Brazelton, T.B., & Nugent, J.K. (1995). The Neonatal Behavioral Assessment Scale. Mac Keith Press, Cambridge.

The NBAS requires training to be able to administer it effectively and reliably. For more information, please see the Training Program section.

http://www.brazelton-institute.com/intro.html (downloaded 5 29 08)

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Attachment D
Child Behavior Checklist

Purpose: Designed to assess "social competence" and "behavior problems" in children.

Population: Ages 4-18.

Score: Five scale scores.

Time: Not reported.

Authors: Thomas M. Achenbach and Craig Edelbrock.

Publisher: Thomas M. Achenbach.

Description: The Child Behavior Checklist (CBCL) was designed to address the problem of defining child behavior problems empirically. It is based on a careful review of the literature and carefully conducted empirical studies. It is designed to assess in a standardized format the behavioral problems and social competencies of children as reported by parents.

Scoring: The CBCL can be self-administered or administered by an interviewer. It consists of 118 items related to behavior problems which are scored on a 3-point scale ranging from not true to often true of the child. There are also 20 social competency items used to obtain parents' reports of the amount and quality of their child's participation in sports, hobbies, games, activities, organizations, jobs and chores, friendships, how well the child gets along with others and plays and works by him/herself, and school functioning.

Reliability: Individual item intraclass correlations (ICC) of greater than .90 were obtained "between item scores obtained from mothers filling out the CBCL at 1-week intervals, mothers and fathers filling out the CBCL on their clinically-referred children, and three different interviewers obtaining CBCLs from parents of demographically matched triads of children." Stability of ICCs over a 3-month period were .84 for behavior problems and .97 for social competencies. Test-retest reliability of mothers' ratings were .89. Some differences were found between mothers' and fathers' individual ratings.

Validity: Several studies have supported the construct validity of the instrument. Tests of criterion-related validity using clinical status as the criterion (referred/non-referred) also support the validity of the instrument. Importantly, demographic variables such as race and SES accounted for a relatively small proportion of score variance.

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Norms: Normative data, obtained from parents of 1,300 children, were heterogeneous with respect to race and socioeconomic status and were proportionate to the composition of the general U.S. population.

Suggested Uses: It is suggested that the CBCL is a viable tool for assessing a child's behaviors, via parent report, in a clinical or research environment**.**

http://cps.nova.edu/~cpphelp/CBCL.html (downloaded 5 29 08)

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Attachment E
Peabody Picture Vocabulary Test

The Peabody Picture Vocabulary Test measures verbal ability or scholastic aptitude. It can be used with children aged two years and upwards, and reliably with seven years and up. Now in its fourth edition, PPTV takes less then 15 minutes to complete. It does not require the child to read, and scoring is rapid and objective. Scores can be converted to an IQ score.

http://www.preventionaction.org/reference/peabody-picture-vocabulary-test
(downloaded 5 28 08)

Peabody Picture Vocabulary Test

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The PPVT-III is an untimed, individual intelligence test, orally administered in 11 to 12 minutes or less. Extensively revised, this test measures an individual's receptive (hearing) vocabulary for Standard American English. In addition, it provides a quick estimate of verbal ability or scholastic aptitude. Specifically, the PPVT-III can also be used for assessing the English vocabulary of non-English-speaking individuals and assessing adult verbal ability. Two parallel forms (IIIA and IIIB) can be used for testing and retesting. No reading is required by the client, and scoring is rapid and objective. Item responses are made by pointing or multiple choice selection, dependant upon the subject's age. The total score can be converted to a percentile rank, mental age, or a standard deviation IQ score. No special training is required to administer, score, or interpret the PPVT-III.

The national norms of the PPVT-III have been extended to include ages 2-6 to 90+ years of age. This edition also was developed from adult norms obtained on 828 persons ages 19 to 40 selected to be nationally representative of geographical regions and major occupational groups. No people with handicaps were included in the norm population. A technical supplement gives detailed standardization data.

The PPVT-III provides an estimate of the client's verbal intelligence and has been administered to groups who had reading or speech problems, had mental retardation, or were emotionally withdrawn. Because the manner of the client's response to stimulus vocabulary is to point in any fashion to one of four pictures that best fits the stimulus work, these tests also apply to rehabilitation clients who have multiple physical impairments, but whose hearing and vision are intact. The test also has high interest value, and this can establish good rapport with the client. For its administration, the examiner

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presents a series of pictures to each client. There are four pictures to a page, and each is numbered. The examiner states a word describing one of the pictures and asks the client to point to or say the number of the picture that the word describes.

The test is not useful in its present form for blind and deaf people, but can be useful for people with mental retardation, for whom no modifications in instructions or format are needed. The only possible problem is that the illustrations for about the first 50 items often use children. These may not be acceptable to the adult with mental retardation.

http://en.wikipedia.org/wiki/Peabody_Picture_Vocabulary_Test (downloaded 5 29 08)

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Attachment F

Wechsler Preschool and Primary Scale of Intelligence

From Wikipedia, the free encyclopedia

• *Ten things you may not know about images on Wikipedia* •

The **Wechsler Preschool and Primary Scale of Intelligence (WPPSI)** is an intelligence test designed for children ages 2 years 6 months to 7 years 3 months developed by David Wechsler in 1967. It is a descendent of the earlier Wechsler Adult Intelligence Scale and the Wechsler Intelligence Scale for Children tests.

It has since been revised twice, in 1989 and 2002.

The current revision, WPPSI–III, is published by Harcourt Assessment. It provides subtest and composite scores that represent intellectual functioning in verbal and performance cognitive domains, as well as providing a composite score that represents a child's general intellectual ability (i.e., Full Scale IQ).

Subtests

The WPPSI–III is composed of 14 subtests.

Block Design - While viewing a constructed model or a picture in a Stimulus Book, the child uses one- or two-colour blocks to re-create the design within a specified time limit.

Information - For Picture Items, the child responds to a question by choosing a picture from four response options. For Verbal Items, the child answers questions that address a broad range of general knowledge topics.

Matrix Reasoning - The child looks at an incomplete matrix and selects the missing portion from 4 or 5 response options.

Vocabulary - For Picture Items, the child names pictures that are displayed in a Stimulus Book. For Verbal Items, the child gives definitions for words that the examiner reads aloud.

Picture Concepts - The child is presented with two or three rows of pictures and chooses one picture from each row to form a group with a common characteristic.

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Symbol Search - The child scans a search group and indicates whether a target symbol matches any of the symbols in the search group.

Word Reasoning The child is asked to identify the common concept being described in a series of increasingly specific clues.

Coding - The child copies symbols that are paired with simple geometric shapes. Using a key, the child draws each symbol in its corresponding shape.

Comprehension - The child answers questions based on his or her understanding of general principles and social situations.

Picture Completion - The child views a picture and then points to or names the important missing part.

Similarities - The child is read an incomplete sentence containing two concepts that share a common characteristic. The child is asked to complete the sentence by providing a response that reflects the shared characteristic.

Receptive Vocabulary - The child looks at a group of four pictures and points to the one the examiner names aloud.

Object Assembly - The child is presented with the pieces of a puzzle in a standard arrangement and fits the pieces together to form a meaningful whole within 90 seconds.

Picture Naming - The child names pictures that are displayed in a Stimulus Book.

Scoring

The WPPSI–III provides Verbal and Performance IQ scores as well as the Full Scale IQ. In addition, the Processing Speed Quotient (known as the Processing Speed Index on previous Wechsler scales) can be derived for children aged 4:0 - 7:3, and a General Language Composite can be determined for children in both age bands (2:6–3:11 & 4:0–7:3). Children in the 2:6-3:11 age band are administered only five of the subtests: Receptive Vocabulary, Block Design, Information, Object Assembly, and Picture Naming.

Quotient and Composite scores have a mean of 100 and a standard deviation of 15. Subtest scaled scores have a mean of 10 and a standard deviation of 3. For Quotient and Composite scores, below 70 is Extremely Low, 70-79 is

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Borderline, 80-89 is Low Average, 90-109 is Average, 110-119 is High Average, 120-129 is Superior, 130 and above is Very Superior. This is true for all Wechsler Scales.

http://en.wikipedia.org/wiki/Wechsler_Preschool_and_Primary_Scale_of_Intelligence (downloaded 5 29 08)

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