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## **Prenatal Exposure to Organophosphates, Paraoxonase 1, and Cognitive Development in Childhood**

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**Abbreviations:**

BSID-II	Bayley Scales of Infant Development, version II
CI	Confidence Interval
DDE	1,1'-dichloro-2,2'-bis(4-chlorophenyl)ethylene
$\Sigma$ DAP	sum of dialkylphosphate metabolites
$\Sigma$ DEP	sum of diethylphosphate metabolites
$\Sigma$ DMP	sum of dimethylphosphate metabolites
FSIQ	full-scale IQ
HOME	Home Observation for Measurement of the Environment
MDI	Mental Development Index
OP	organophosphate
PCBs	polychlorinated biphenyls
PDI	Psychomotor Development Index
PON1	paraoxonase 1
SD	Standard deviation
WISC-IV	Wechsler Intelligence Scale for Children - Fourth Edition
WPPSI-III	Wechsler Preschool and Primary Scale of Intelligence - Third Edition

## ABSTRACT

**Background:** Prenatal exposure to organophosphate pesticides has been shown to negatively impact child neurobehavioral development. Paraoxonase 1 (PON1) is a key enzyme in the metabolism of organophosphates.

**Objective:** To examine the relationship between biomarkers of organophosphate exposure, PON1, and cognitive development at ages 12 and 24 months, and 6 to 9 years.

**Methods:** The Mount Sinai Children's Environmental Health Study enrolled a multiethnic prenatal population in New York City between 1998 and 2002 (n= 404). Third trimester maternal urines were collected and analyzed for organophosphate metabolites (n = 360). Prenatal maternal blood was analyzed for *PON1* activity and genotype. Children returned for neurodevelopment assessments at ages 12 months (n = 200), 24 months (n = 276), 6 to 9 (n = 169) years.

**Results:** Prenatal total dialkylphosphate metabolite level was associated with a decrement in mental development at 12 months among blacks and Hispanics. These associations appeared to be enhanced among children of mothers who carried the *PON1* Q192R QQ genotype, which imparts slow catalytic activity for chlorpyrifos oxon. In later childhood, increasing prenatal total dialkyl- and dimethylphosphate metabolites were associated with decrements in perceptual reasoning in the maternal *PON1* Q192R QQ genotype, with a monotonic trend consistent with greater decrements with increasing prenatal exposure.

**Conclusion:** Our findings suggest that prenatal exposure to organophosphates negatively impacts cognitive development, particularly perceptual reasoning, with evidence of effects beginning at 12 months and continuing through early childhood. PON1 may be an important susceptibility factor for these deleterious effects.

## Background

Prior to 2001, residential exposure to organophosphate pesticides, including chlorpyrifos and diazinon, was common, even in an urban setting. Insecticides are used in multi-unit, inner city dwellings to control insect and rodent infestations within apartments, in common spaces, and around building exteriors. Despite the voluntary cancellation of residential use registrations of chlorpyrifos and diazinon in 2001 and 2004 respectively, between June 2005 and March 2006, 78% of randomly selected nationally representative US homes had measurable levels of chlorpyrifos, and 35% had measurable levels of diazinon, suggesting ongoing residential exposure (Stout et al. 2009). Additionally, exposure to organophosphate pesticides or their residues may occur via consumption of conventionally grown fruits and vegetables (Lu et al. 2008).

Neurodevelopmental consequences of human exposure to organophosphate pesticides have been demonstrated in urban (Bouchard et al. 2010; Engel et al. 2007; Rauh et al. 2006), rural (Eskenazi et al. 2010; Eskenazi et al. 2007; Young et al. 2005), and occupational settings (Handal et al. 2008; Roldan-Tapia et al. 2006), some of which specifically involve prenatal exposure (Engel et al. 2007; Eskenazi et al. 2010; Eskenazi et al. 2007; Marks et al. 2010; Rauh et al. 2006; Young et al. 2005). We have previously reported that prenatal pesticide exposure was associated with smaller head circumference (Berkowitz et al. 2004), and more abnormal primitive reflexes (Engel et al. 2007), particularly among children of mothers with low paraoxonase 1 (PON1) activity. PON1 is a key enzyme in the metabolism of organophosphate pesticides (Costa et al. 1999), and has been shown to be a biomarker of susceptibility to the toxic effects of organophosphate pesticides, both in animals (Costa et al. 2003), and in humans (Engel et al. 2007; Eskenazi et al. 2010; Lee et al. 2003; Nielsen et al. 2010).

## Objective

We undertook an investigation of the impact of prenatal organophosphate metabolite biomarker levels in relation to cognitive development at multiple times in childhood, while considering the modifying influence of maternal and child *PON1* genotype and enzymatic activity.

## Methods

The Mount Sinai Children's Environmental Health Cohort study is a prospective multiethnic cohort that enrolled primiparous women who presented for prenatal care with singleton pregnancies at the Mount Sinai prenatal clinic and two private practices, and were subsequently delivered at Mount Sinai Hospital between May 1998 and July 2001. The target population was healthy, first born infants with no underlying health conditions that might independently result in serious neurodevelopmental impairment. Therefore, women were considered eligible if they were primiparous with singleton pregnancies, had no underlying health conditions that might predispose them to have serious complications of pregnancy that might result in an at-risk infant, and ultimately delivered infants that were neither extremely preterm nor very low birth weight. (Berkowitz et al. 2003; Berkowitz et al. 2004) Mother-infant pairs were recruited early in pregnancy (n = 479). In brief, subsequent to delivery, 75 women were excluded because of medical complications, very premature births (delivery before 32 completed weeks or less than 1500 grams), delivery of an infant with a birth defect, inability to collect biologic specimens before birth, change of hospital or residence outside New York City, or refusal to continue to participate, leaving 404 women-infant pairs in the final cohort (Berkowitz et al. 2004).

We administered a questionnaire to participants during their third trimester of pregnancy to obtain information on environmental exposures, sociodemographic characteristics, obstetrical and medical history, and lifestyle factors. Women self-identified as white, white Hispanic, black, black Hispanic, or other. For the purpose of this analysis, white Hispanic and black Hispanics were jointly considered Hispanic. Maternal blood and urine samples were also obtained during a routine clinical visit, generally between 26 and 28 weeks of gestation. Delivery characteristics and birth outcomes, including birth weight, length, head circumference, gestational age and infant gender, were obtained from a computerized perinatal database within the Department of Obstetrics, Gynecology and Reproductive Science at Mount Sinai Hospital.

The Bayley Scales of Infant Development (version II, BSID-II) was administered at the Mount Sinai Hospital at approximately 12 (n=200) and 24 months (n=276). The BSID-II provides age-standardized

norms of mental (MDI) and psychomotor (PDI) development. The MDI rates the child's cognitive ability in a number of areas, including memory, habituation, problem solving, early number concepts, generalization, classification, vocalizations, language and social skills. The PDI rates the child's fine and gross motor coordination. Scales are age-standardized to mean = 100, SD = 15 (Bayley 1993).

Interviews and examinations were conducted in English or Spanish as required. Children were invited to return for Wechsler psychometric intelligence tests between the ages of 6 and 9 years. Children that returned before 7 years of age were administered the Wechsler Preschool and Primary Scale of Intelligence - Third Edition (WPPSI-III). The WPPSI-III was administered in English or Spanish by one of 4 examiners. Block Design, Information, Matrix Reasoning, Vocabulary, Picture Concepts, Symbol Search, Word Reasoning, and Coding subtests were completed. Composite Verbal, Performance, Processing Speed, and Full-Scale IQ scores were derived using age-standardized WPPSI-III norms. Children who returned between the ages of 7 and 9 years were administered the Wechsler Intelligence Scale for Children - Fourth Edition (WISC-IV) by one of 4 examiners. Children provided witnessed assent prior to the start of the assessment. Block Design, Similarities, Digit Span, Picture Concepts, Coding, Vocabulary, Letter-Number Sequence, Matrix Reasoning, Comprehension and Symbol Search subtests were completed. Composite Verbal, Perceptual Reasoning, Working Memory, Processing Speed, and Full-Scale IQ scores were derived using age-standardized WISC-IV norms. Both the WPPSI-III and the WISC-IV were administered in a private room without the parent present. Mothers provided informed consent and children aged 7 and older provided verbal and witnessed assent. This study was approved by the Institutional Review Board of Mount Sinai School of Medicine.

Maternal urine samples were analyzed by the Centers for Disease Control and Prevention for six dialkylphosphate metabolites in two batches. Laboratory and quality control methods have been reported previously (Barr et al. 2005; Bravo et al. 2004; Wolff et al. 2005). In some cases, individual dialkylphosphate metabolite levels were missing due to analytic interference. In these cases, missing dialkylphosphate metabolite levels were imputed using regression analysis to predict the missing metabolite on the basis of the other non-missing metabolites measured for that woman within the group of correlated metabolites, as has been previously described (Engel et al. 2007). Samples below the LOD were defined as  $LOD/\sqrt{2}$ . Diethyl- and dimethyl-phosphate metabolites were summed (as  $\mu\text{m/L}$ ) to obtain

diethylphosphate ( $\Sigma$ DEP) and dimethylphosphate ( $\Sigma$ DMP) metabolites, respectively, and total dialkylphosphate ( $\Sigma$ DAP) levels. Very dilute samples of urine with  $< 20 \mu\text{g/dL}$  creatinine ( $n = 26$ ) were excluded from organophosphate metabolite analyses, in accordance with methods that have previously been applied for spot urine biomarker measures (Carrieri et al. 2001; Engel et al. 2007; Eskenazi et al. 2004; Wolff et al. 2007). Overall, approximately 97%, 89%, and 90% of the cohort had detectable levels of  $\Sigma$ DAP,  $\Sigma$ DEP, and  $\Sigma$ DMP metabolites respectively (Engel et al. 2007). A random subset of maternal peripheral blood samples from the entire cohort ( $n = 194$ ), distributed roughly equally by maternal race/ethnicity (66 blacks, 64 Hispanics, 64 whites, the number being dictated by budgetary considerations), was analyzed for polychlorinated biphenyls (PCBs) and 1,1'-dichloro-2,2'-bis(4-chlorophenyl)ethylene (DDE). PCBs were defined as the sum of congeners 118, 153, 138, and 180 (Wolff et al. 2005). Total lipids (g/liter) were calculated by using cholesterol and triglycerides (Phillips et al. 1989) determined on the 174 plasma samples with sufficient volume. Distributions of biomarker levels have been previously reported (Engel et al. 2007; Wolff et al. 2007).

Plasma was separated from prenatal maternal peripheral blood and cord blood at delivery, and was used to measure PON1 activity by phenylacetate hydrolysis (Chen et al. 2005). Using maternal and child DNA, *PON1* polymorphisms were also measured using clamp-dependent and linking emulsion allele-specific PCR (Chen et al. 2005). We examined interactions between prenatal organophosphate exposure (dichotomized as above and below the median exposure) and tertiles of PON1 activity in maternal prenatal peripheral blood and child cord blood. We also examined interactions between prenatal organophosphate exposure and the maternal or child *PON1* Q192R, -108C>T, and L55M polymorphisms. We closely examined interactions if the type 3 F-test for the product-term in Proc GLM (either the class variable representing biomarker tertiles \* dichotomized genotypes or race/ethnicity, or the linear term for log<sub>10</sub> organophosphate metabolites \* dichotomized genotypes or race/ethnicity) was  $p < 0.20$ . The *PON1* Q192R polymorphism in particular has been shown to have a strong functional consequence on the relative rate of hydrolysis of certain organophosphate substrates (Li et al. 2000), and has been shown to affect the catalytic efficiency for chlorpyrifos but not diazinon (Richter et al. 2009).



Data were analyzed using the SAS system version 9.1 (Cary, NC). Generalized linear models were used to analyze the relationship between biomarker levels and the Mental and Psychomotor Development Indices (MDI and PDI respectively). In total, 200 children were administered the BSID-II at approximately 12-months of age (mean  $13.1 \pm SD=1.6$  months). Children were excluded from analyses if their refusal to do a large proportion of the items on the exam influenced their overall score (12-month MDI  $n = 1$ ). Two children were excluded from the 12-month analysis because their parent reported a diagnosis of pervasive developmental disorder. We also excluded observations when urine creatinine was  $< 20 \mu\text{g/dL}$  in maternal urine sample ( $n = 20$ ). Of the remaining 177 eligible children who completed 12-month exams, 174 had organophosphate metabolites measured in prenatal urine.

At the 24-month BSID-II (mean  $27.4 \pm SD=4.5$  months), 276 children completed the exam. Two were excluded from the 24-month analysis because the parent reported a diagnosis of pervasive developmental disorder. Samples of urine  $< 20 \mu\text{g/dL}$  creatinine ( $n = 23$ ) were also excluded. Of the remaining 251 eligible observations, 247 had organophosphate metabolites measured in prenatal urine. We could not compute the MDI scores for ten additional children because their refusals were too extensive to accurately calculate scores; however, they were included in the PDI analyses because their scores were valid..

In order to maximize the sample size of our models, we conducted analyses that combined the full-scale IQ (FSIQ), perceptual reasoning, verbal comprehension, and processing speed composite scores from children who came for at least one of the Wechsler psychometric intelligence exams—in the text & tables we refer to as the “combined populations” ( $n = 169$ ). For these analyses, we preferentially selected the WISC-IV composite scores, and substituted the WPPSI-III composite scores if the child did not return for the later exam. We included an indicator variable to account for whether the scores derived from a WISC-IV or WPPSI-III exam. The convergent validity between the WISC-IV and WPPSI-III has been previously reported. Specifically, in psychometric analyses, the correlation between WPPSI-III and WISC-IV FSIQ scores was 0.89, WPPSI-III Verbal IQ versus WISC-IV Verbal Comprehension = 0.83, WPPSI-III Performance IQ versus WISC-IV Perceptual Reasoning = 0.79 (Flanagan and Kaufman 2004). In our

study, among children who returned for both exams ( $n = 103$ ), the correlations between the composite scores on the WPPSI-III and WISC-IV were likewise very strong: FSIQ = 0.83, Verbal IQ/Verbal Comprehension = 0.84, and Performance IQ/Perceptual Reasoning = 0.78. For all models, covariates were retained if their exclusion caused more than a 10% change in the beta coefficient of the full model, or if they improved the precision of the main effect estimate. The following covariates were considered as potential confounders or effect modifiers: maternal age, race/ethnicity, marital status, education, breastfeeding, child sex, alcohol, smoking, or drug use during pregnancy, maternal IQ (measured by the Peabody Picture Vocabulary test), Home Observation for Measurement of the Environment (HOME) score, season of urine collection, maternal *PON1* activity or genotype, language spoken in the home, exact age at testing. Gestational age at delivery and birthweight were not evaluated for confounding because they are potentially causal intermediates (Moreno-Banda et al. 2009; Whyatt et al. 2004; Wolff et al. 2007). All models were adjusted for examiner and urinary creatinine.

## Results

The Mount Sinai Children's Environmental Health Center enrolled a multiethnic inner city cohort, the majority of whom were black or Hispanic women (approximately 80%) (Table 1). Most of the cohort was less than 25 years of age at enrollment. However, in some follow-up years, those who returned for follow-up assessments tended to be older women, with a disproportionately low fraction of women in the youngest category (less than 20 years at enrollment) being unreachable by any of our contact methods. Similar trends were found for education. Those returning for follow up did not differ substantially from the originally enrolled cohort with respect to racial/ethnic composition. Importantly, there were also no meaningful differences with respect to breastfeeding behaviors or alcohol use during pregnancy. In general, mothers who returned for follow-up assessments tended to have been older at enrollment and to have achieved a higher level of education. Allele frequencies for *PON1* polymorphisms vary by race, as has been previously reported for our population (Chen et al. 2003). In the population of subjects included in this analysis, the frequency of the A allele (resulting in Q amino acid) is 74% among whites, 36% among blacks, and 55% among Hispanics. The frequency of the -108T allele, and 55L alleles are also

differential by race. A detailed description of allele frequencies and metabolite concentrations according to race can be found in Supplemental Material, Table 1.

At the 12-month BSID-II exam, the estimated effect of organophosphate metabolites on the MDI was strongly heterogeneous by race/ethnicity for the  $\Sigma$ DAP and  $\Sigma$ DMP metabolites (Table 2). Among nonwhites, increasing  $\Sigma$ DAP and  $\Sigma$ DMP tertiles of exposure were associated with a decrease in the MDI (log<sub>10</sub>  $\Sigma$ DAP Beta = -3.29, 95% CI -5.88, -0.70). However, among whites, the reverse pattern emerged, with higher exposure associating with better MDI scores (log<sub>10</sub>  $\Sigma$ DAP Beta = 4.77, 95% CI 0.69, 8.86). Similar trends in the effect estimates were found when we stratified by housing type (public versus private) rather than race/ethnicity. There was no heterogeneity in  $\Sigma$ DEP effect estimates according to race/ethnicity; and overall,  $\Sigma$ DEP metabolites were not associated with the 12-month BSID-II MDI. There was no relationship between organophosphate metabolites and the PDI at 12-months overall, and no interaction with race/ethnicity for any of the metabolite groups (Table 2). At the 24-month BSID-II, effect estimates were not heterogeneous by race/ethnicity (data not shown). Consistent with the 12-month assessment, prenatal maternal  $\Sigma$ DAP metabolite level was inversely associated with the 24-month MDI (B = -2.08, 95% CI -4.60, 0.44) in multivariate adjusted models, although the effect estimates were attenuated relative to the 12-month estimates and measured with comparable precision (see Supplemental Material, Table 2). The metabolites were not associated with 24-month PDI.

Because of the strong interaction between race/ethnicity and OP metabolites on the BSID-II MDI at 12-months, we examined the interaction between the *PON1* polymorphisms and  $\Sigma$ DAP and  $\Sigma$ DMP metabolites within strata of race/ethnicity; however, the white population was too small to further subdivide by genotype. Therefore we restricted this analysis to only the black or Hispanic population. Among blacks and Hispanics, the effects of  $\Sigma$ DAP,  $\Sigma$ DEP and  $\Sigma$ DMP were strongly differential according to *PON*Q192R genotype at 12-months, although not 24-months. At 12-months, children of mothers with the slow catalytic activity, *PON1* 192QQ genotype experienced an approximate 4 point decline on the MDI with each log<sub>10</sub> unit increase in  $\Sigma$ DAP and  $\Sigma$ DMP biomarker level, with essentially no effect among children of mothers with the QR/RR genotype. Although in some instances the point estimate among the

QR/RR group were quite elevated in a positive direction, they were always estimated with greater imprecision, despite the fact that this was the larger strata (Table 3). A similar pattern was found for the  $\Sigma$ DEP-*PON1* Q192R interaction, although the differences between genotypes were less pronounced. Results were consistent when we stratified by black and Hispanic ancestry (see Supplemental Material, Table 3), although the sample size was quite small. There was no interaction between the *PON1* Q192R polymorphism and OP metabolite level on the MDI at 24-months. There were also no interactions ( $p \geq 0.20$ ) between organophosphate metabolites and the L55M or -108C>T polymorphisms, nor with enzyme activity, on neurodevelopment at any age. Results were similar when we estimated associations according to the child's *PON1* Q192R genotype instead of the mother's genotype, but child genotype was only available for 57% of the population because cord blood was only collected on a subset. Therefore, we report only the maternal gene-OP interactions.

Increasing total DEP metabolite levels were associated with slight decrements in full scale IQ (log10 Beta = -2.89, 95% CI -6.15, 0.36) and perceptual reasoning (log10 Beta = -3.51, 95% CI -7.31, 0.30) on the combined psychometric exams, and also with Working Memory (log10 Beta = -3.48, 95% CI -7.29, 0.34) on the 7-9 year WISC-IV examination (Table 4), although the estimated effects were relatively modest and imprecise. These associations were not heterogeneous by race/ethnicity. However, for the combined population, there was again effect heterogeneity according to the *PON1* Q192R polymorphism for the associations between  $\Sigma$ DAP and  $\Sigma$ DMP biomarkers and perceptual reasoning domain., Children of mothers with the slow catalytic activity genotype (QQ) experienced a substantial decrement in the overall perceptual reasoning score with each log10 increment increase in exposure, while. there was no effect of  $\Sigma$ DAP and  $\Sigma$ DMP biomarkers on the perceptual reasoning score of children with mothers who carried the QR/RR genotypes (Table 5). Comparing biomarker tertiles according to genotype, increasing tertiles of  $\Sigma$ DAP,  $\Sigma$ DEP and  $\Sigma$ DMP among the children of mothers with the *PON1* 192QQ genotype were generally associated with monotonically declining LSMEANS in FSIQ and perceptual reasoning, while there was no consistent pattern in the QR/RR group (Figure 1). For perceptual reasoning, the first versus third tertile contrasts for  $\Sigma$ DAP and  $\Sigma$ DMP were statistically significant ( $p < 0.05$ ).

Maternal third trimester blood PCB concentration was inversely associated with mental development at 12 months ( $\log_e$  beta = -7.18, 95% CI -14.47, 0.11) but not at 24 months ( $\log_e$  beta = 1.60, 95% CI -6.57, 9.77), and was not associated with IQ (see Supplemental Material, Table 4). However, although the effect estimates for PCBs were sometimes quite large, they were also measured with extreme imprecision, as indicated by the very large confidence intervals. Maternal third trimester blood DDE was not associated with any of the outcome measures.

## Discussion

We report an association between prenatal increasing  $\Sigma$ DAP and  $\Sigma$ DMP urinary metabolite concentrations and poorer scores on the BSID-II MDI at 12-months among blacks and Hispanics. These associations were modified by maternal *PON1* Q192R genotype, such that negative effects appeared to be limited to children of mothers with the QQ genotype. Heterogeneity according to maternal race/ethnicity may indicate differences in exposure sources rather than any underlying susceptibility. As previously described, overall, 46.4% of our mothers reported that pesticides were applied in their home either by themselves or by a family member during their pregnancy (Berkowitz et al. 2003). However, there was a profound racial disparity in this behavior (Berkowitz et al. 2003). Among the women who returned for the 12-month BSID-II exam, 70.5% of the blacks and Hispanics reported buying pesticides, applying pesticides, or fumigating their house during their pregnancy, compared with only 31.6% of the whites, and yet we did not detect significant differences in their exposure distributions (Supplemental Material, Table 1). In our population, race/ethnicity was strongly associated with whether a subject lived in public or private housing. And indeed, similar trends in the effect estimates were found when we stratified by housing type (public versus private) rather than race/ethnicity, although the magnitude of the interaction was not as strong. This indicates that whites, or people living in private or owner-occupied housing, may have experienced a different source of exposure to pesticides or their metabolites that contributed substantially to their urinary concentrations; one possibility is that pesticide residues from fresh fruit and vegetable consumption account for a large fraction of the urinary metabolite levels among whites. Unfortunately this presents serious complications for exposure reconstruction using urinary metabolites. A recent examination of dialkylphosphate residues on fruits and vegetables found that over

half of the samples tested contained more pre-formed DAP residues than parent organophosphate pesticides (Zhang et al. 2008). These DAP residues were produced by abiotic hydrolysis, photolysis or plant metabolism (Zhang et al. 2008). Direct intake of the metabolite (i.e. pesticide residue) without the active oxon, rather than the parent pesticide, does not inhibit cholinesterase activity. Thus, for subjects for whom the primary source of pesticide exposure is fresh fruit and vegetable consumption, use of urinary metabolite concentrations as an indication of parent compound exposure may result in significant misclassification of exposure.

Between the ages of 6 and 9 years, prenatal  $\Sigma$ DEP urinary metabolite concentrations were associated with slight decrements in FSIQ, Perceptual Reasoning, and Working Memory. Furthermore, among children of QQ mothers,  $\Sigma$ DAP and  $\Sigma$ DMP urinary metabolite concentrations were associated with poorer scores on Perceptual Reasoning and FSIQ in a monotonically decreasing manner. In both cases, we observed stronger Q192R-interactions for  $\Sigma$ DAP and  $\Sigma$ DMP urinary metabolites, rather than  $\Sigma$ DEP metabolites. The reasons for this are unclear. *PON1* Q192R exhibits substrate specificity (Richter et al. 2009), but our strongest interactions were for dimethylphosphates, not diethylphosphates (into which chlorpyrifos and diazinon both metabolize). An alternative explanation is that our dimethylphosphate metabolite concentrations were simply higher (Engel et al. 2007), indicating more available substrate. Unfortunately the relevant parent compounds cannot be deduced on the basis of non-specific urinary DAPs; however, one well publicized source of DMP exposure during the period of enrollment was malathion spraying for West Nile carrying mosquitos (Nash et al. 2001; O'Sullivan et al. 2005). Interestingly, *PON1* status may indirectly influence methyl organophosphate metabolism when multiple organophosphate exposures are involved (Jansen et al. 2009).

The estimated effects we report for the BSID-II MDI are in line with what has recently been reported in the CHAMACOS study, a similarly designed prospective birth cohort that was enrolled in an agricultural community in the Salinas Valley of California (Eskenazi et al. 2010). Eskenazi et al. report evidence of negative effects of  $\Sigma$ DAPs on the MDI, but not the PDI, and particularly among children with the QQ genotype, although there did not appear to be strong effect heterogeneity in the DAP-MDI relationship

according to *PON* genotype.. Although we report interactions with maternal *PON1* Q192R genotype, very similar interactions and effect estimates were found for child genotype in our cohort (data not shown). In no cases was effect heterogeneity detected according to w *PON1* enzyme activity. This may be because genotype is a more stable, long-term predictor of metabolism potential. Reconciling estimated effects across studies can be complicated when only non-specific urinary metabolites are measured because these metabolites can derive from multiple parent compounds (2005) that may vary in the degree to which they interact with the Q192R genotype and influence neurodevelopment.

Ours was a multi-ethnic study population recruited at an inner city tertiary care hospital that serves a lower income minority population. As such, attrition resulting from the challenges of maintaining contact with this population may have impacted our study findings. Misclassification of parent compound exposure according to exposure route is another significant limitation of our study and other studies relying on urinary DAP biomarkers, and may explain the heterogeneity in effects observed according to race/ethnicity in our population. It may further complicate the comparison of exposure effects across studies where there exists heterogeneity in exposure sources. Additionally, we measured DAP biomarkers at one time during pregnancy, approximately the early third trimester. Although we accounted for seasonal variation in exposure levels in our model, other time-related variability may result in additional misclassification of exposure.

Finally, residual confounding by unmeasured covariates, including postnatal exposure, should be considered when evaluating our results. It should be noted that our study enrollment and evaluation periods overlapped with important regulatory changes in residential use of chlorpyrifos and diazinon, which occurred approximately midway through our study. Therefore, childhood exposure to these compounds may vary substantially pre versus post-ban, although we did not observe any significant interaction with study year (data not shown). However, these changes may explain the inconsistency between our 12 and 24-month BSID-II results. The 0-12-month exposure window may include less hand-to-mouth childhood exposure (from crawling exposure to house dust, and/or fresh fruit and vegetable consumption) than in the 12-24 month window; thus, more childhood exposure in the 12-24 month

window may modify the effect of prenatal exposure. Nonetheless, it is likely, overall, that childhood exposure to chlorpyrifos and diazinon was lower post-ban (Williams et al. 2008).

In conclusion, we found that prenatal maternal urinary dialkylphosphate metabolite concentrations were negatively associated with aspects of neurodevelopment at 12 and 24 months, and also at 6 to 9 years of age in an urban, inner city population. The evidence was strongest among the children of mothers with the *PON1* 192QQ genotype, which was present in approximately 30% of our population overall, although that varies according to racial ancestry. This important potential source of effect heterogeneity should be considered in future studies of organophosphate exposure.



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Table 1. Characteristics of the Mount Sinai Children's Environmental Health Study, Mount Sinai Medical Center 1998-2002

	Original Enrolled Cohort (N = 404)		12 month Follow-up (N = 200)		24 month Follow-up (N = 276)		6 - 9 year Follow-up (N = 169)	
	N	%	N	%	N	%	N	%
<b>Maternal Age at delivery</b>								
< 20 years	142	35.2	57	28.5	96	34.8	54	31.9
20-24 years	132	32.7	56	28.0	83	30.1	56	33.1
25-29 years	44	10.9	30	15.0	34	12.3	25	14.8
30-34 years	64	15.8	39	19.5	43	15.6	19	11.2
≥ 35 years	22	5.4	18	9.0	20	7.2	15	8.9
<b>Race/Ethnicity</b>								
White	86	21.29	57	28.5	63	22.8	31	18.3
Black	112	27.72	52	26.0	74	26.8	47	27.8
Hispanic	200	49.50	89	44.5	136	49.3	88	52.1
Other	6	1.49	2	1.0	3	1.1	3	1.8
<b>Marital Status</b>								
Married	117	29.0	71	35.5	81	29.4	38	22.5
Living with baby's father	98	24.3	41	20.5	62	22.5	40	23.7
Single	189	46.8	88	44.0	133	48.2	91	53.9
<b>Education</b>								
< High School	118	29.4	50	25.0	78	28.3	46	27.2
High School graduate	83	20.7	35	17.5	55	19.9	36	21.3
Some college	103	25.6	49	24.5	70	25.4	51	30.2
≥ Bachelor's degree	98	24.4	66	33.0	73	26.5	36	21.3
<b>Alcohol Use during Pregnancy</b>								
	59	14.0	31	16.0	36	13.4	28	16.9
<b>Duration of Breastfeeding</b>								
< 1 month	140	42.2	74	37.0	122	44.2	73	43.5
1-3 months	71	21.4	45	22.5	56	20.3	36	21.4
≥ 4 months	121	36.5	81	40.5	98	35.5	59	35.1
<b>Any organophosphate biomarker level</b>								
	383	94.8	190	95.0	267	96.7	165	99.0
<b>Maternal <i>PON1</i> Q192R</b>								
QQ	120	30.9	63	33.3	78	30.2	46	28.2
QR	174	44.7	83	43.9	119	46.1	78	47.9
RR	95	24.4	43	22.8	61	23.6	39	23.9
<b>Maternal paraoxonase enzyme activity (units/ml)</b>								
2964 – 9576 (Tertile 1)	130	33.9	69	37.5	91	35.7	57	34.8
9700 – 11660 (Tertile 2)	123	32.1	51	27.7	81	31.8	43	26.2
11665 – 20000 (Tertile 3)	130	33.9	64	34.8	83	32.6	64	39.0

Table 2. Prenatal Organophosphate Biomarker Levels and 12-month BSID-II Mental Development Index in the Mount Sinai Children's Environmental Health Study

Organophosphate Biomarkers		Combined Race/Ethnicity <sup>a</sup> (N = 149)		Black/Hispanic Subjects <sup>b</sup> (N = 111)		White Subjects <sup>b</sup> (N = 38)		interaction p-value
		Adjusted Mean MDI	95% CI	Adjusted Mean MDI	95% CI	Adjusted Mean MDI	95% CI	
<b>ΣDAP</b>	<b>T3</b>	96.1	93.1, 99.0	91.5	88.3, 94.7	103.7	98.5, 108.8	< 0.001
	<b>T2</b>	95.8	92.5, 99.1	94.4	91.2, 97.5	95.9	90.6, 101.3	
	<b>T1</b>	97.0	93.7, 100.3	96.2	92.9, 99.4	92.0	85.4, 98.7	
	<b>log<sub>10</sub> Beta</b>	(-1.00)	(-3.28, 1.28)	(-3.29)	(-5.88, -0.70)	(4.77)	(0.69, 8.86)	
<b>ΣDEP</b>	<b>T3</b>	97.5	94.3, 100.6	95.2	91.9, 98.6	100.6	94.6, 106.5	0.82
	<b>T2</b>	95.4	92.3, 98.6	93.8	90.4, 97.1	96.8	90.8, 102.9	
	<b>T1</b>	95.9	92.9, 98.9	94.3	90.9, 97.6	97.3	91.8, 102.7	
	<b>log<sub>10</sub> Beta</b>	(0.03)	(-2.23, 2.29)	(-0.33)	(-3.00, 2.35)	(0.86)	(-3.16, 4.87)	
<b>ΣDMP</b>	<b>T3</b>	96.1	93.4, 99.0	92.1	89.0, 95.2	103.3	97.9, 108.7	< 0.01
	<b>T2</b>	96.1	92.9, 99.3	94.2	91.0, 97.4	97.2	91.1, 102.6	
	<b>T1</b>	96.8	93.5, 100.0	96.3	93.0, 99.5	92.2	85.6, 98.7	
	<b>log<sub>10</sub> Beta</b>	(-1.12)	(-3.14, 0.89)	(-3.35)	(-5.64, -1.06)	(4.45)	(0.82, 8.08)	
		Adjusted Mean PDI	95% CI	Adjusted Mean PDI	95% CI	Adjusted Mean PDI	95% CI	interaction p-value
<b>ΣDAP</b>	<b>T3</b>	92.5	88.5, 96.6	94.2	89.5, 98.9	90.8	83.3, 98.2	0.65
	<b>T2</b>	96.6	92.1, 101.1	97.5	93.0, 102.1	97.0	89.2, 104.7	
	<b>T1</b>	95.3	90.9, 99.8	97.7	93.1, 102.4	90.0	80.5, 99.6	
	<b>log<sub>10</sub> Beta</b>	(-0.52)	(-3.66, 2.62)	(-1.52)	(-5.21, 2.16)	(2.07)	(-3.83, 7.96)	
<b>ΣDEP</b>	<b>T3</b>	93.6	89.3, 98.0	95.6	91.0, 100.2	91.7	83.5, 99.9	0.25
	<b>T2</b>	94.5	90.1, 98.9	95.9	91.2, 100.6	94.4	86.0, 102.7	
	<b>T1</b>	95.3	91.2, 99.5	97.7	93.1, 102.4	92.1	84.6, 99.6	
	<b>log<sub>10</sub> Beta</b>	(-0.20)	(-3.28, 2.87)	(-0.48)	(-4.11, 3.16)	(0.46)	(-5.12, 6.03)	
<b>ΣDMP</b>	<b>T3</b>	94.5	90.6, 98.5	96.4	92.0, 100.8	92.5	84.9, 100.2	0.83
	<b>T2</b>	93.7	89.3, 98.0	94.5	90.1, 99.0	94.4	86.7, 102.1	
	<b>T1</b>	95.1	90.7, 99.5	97.8	93.2, 102.4	89.5	80.2, 98.8	
	<b>log<sub>10</sub> Beta</b>	(-0.92)	(-3.68, 1.85)	(-1.81)	(-5.07, 1.45)	(1.36)	(-3.83, 6.56)	

<sup>a</sup> General linear model adjusted for race/ethnicity, maternal age at enrollment, child sex, examiner, maternal PON1 enzyme activity, season of urine collection, laboratory batch, HOME Score, alcohol consumption during pregnancy, and urinary creatinine.

<sup>b</sup> General linear model adjusted for maternal age at enrollment, child sex, examiner, maternal PON1 enzyme activity, season of urine collection, laboratory batch, HOME Score, alcohol consumption during pregnancy, and urinary creatinine, and including a biomarker-race interaction term.

Table 3 *PON1* Q192R Interaction with Total Dialkyl and Dimethylphosphate Biomarker levels on the BISD-II Mental Development Index in the Mount Sinai Children's Environmental Health Study

	12-MONTH BSID-II Black/Hispanic Subjects N = 110 <sup>c</sup>					24-MONTH BSID-II Total Population N = 191				
	<i>PON1</i> 192 QR/RR (FAST) N = 82		<i>PON1</i> 192 QQ (SLOW) <sup>b</sup> N = 28		interaction p-value	<i>PON1</i> 192 QR/RR (FAST) N = 140		<i>PON1</i> 192 QQ (SLOW) N = 57		interaction p-value
	log <sub>10</sub> Beta	95% CI	log <sub>10</sub> Beta	95% CI		log <sub>10</sub> Beta	95% CI	log <sub>10</sub> Beta	95% CI	
$\Sigma$ DAP <sup>a</sup>	5.45	-0.79, 11.69	-4.71	-7.59, -1.83	<0.01	-1.04	-6.06, 3.99	-1.27	-4.40, 1.84	0.93
$\Sigma$ DEP <sup>a</sup>	3.55	-1.14, 8.23	-1.60	-4.99, 1.79	0.08	-0.55	-4.79, 3.70	-0.15	-3.51, 3.21	0.88
$\Sigma$ DMP <sup>a</sup>	2.52	-2.71, 7.74	-4.30	-6.89, -1.71	0.02	0.12	-4.17, 4.42	-0.48	-3.27, 2.30	0.81

<sup>a</sup> General linear model adjusted for maternal age at enrollment, child sex, examiner, HOME Score, alcohol consumption during pregnancy, laboratory batch, season of urine collection, urinary creatinine, and including a biomarker-*PON1* Q192R genotype interaction. The 24-month model was additionally adjusted for maternal race/ethnicity.

<sup>b</sup> Note that in this category, 7 are black and the remainder Hispanic.

<sup>c</sup> Note that 1 subject identified mixed ethnicity

Table 4 Prenatal Organophosphate Biomarker Levels and Psychometric Intelligence at 6-9 years in the Mount Sinai Children's Environmental Health Study

	N	log <sub>10</sub> ΣDAP		N	log <sub>10</sub> ΣDEP		N	log <sub>10</sub> ΣDMP	
		Beta	95% CI		Beta	95% CI		Beta	95% CI
<b>Combined Populations (6-9 years)<sup>a</sup></b>									
Full Scale IQ	140	-1.39	-4.54, 1.77	140	-2.89	-6.15, 0.36	142	-0.46	-3.17, 2.26
Perceptual Reasoning		-2.36	-6.04, 1.31		-3.51	-7.31, 0.30		-1.15	-4.31, 2.02
Verbal Comprehension		-0.42	-3.45, 2.62		-1.20	-4.35, 1.96		-0.05	-2.64, 2.54
<b>WISC-IV (7-9 years)<sup>a</sup></b>									
Full Scale IQ	114	-1.10	-5.01, 2.81	114	-3.15	-7.19, 0.89	115	-0.39	-3.64, 2.86
Perceptual Reasoning		-2.39	-6.97, 2.19		-4.37	-9.10, 0.36		-1.24	-5.05, 2.57
Verbal Comprehension		0.56	-3.11, 4.23		-0.08	-3.91, 3.76		0.39	-2.65, 3.42
Processing Speed		-1.05	-5.57, 3.46		-2.11	-6.81, 2.59		-0.79	-4.52, 2.94
Working Memory		-0.53	-4.24, 3.18		-3.48	-7.29, 0.34		0.29	-2.81, 3.38
<b>WPPSI-III (6 years)<sup>a</sup></b>									
Full Scale IQ	96	-1.14	-4.55, 2.28	96	-1.40	-5.27, 2.47	98	-0.56	-3.68, 2.56
Perceptual Reasoning		-2.07	-5.66, 1.52		-1.59	-5.68, 2.50		-1.46	-4.74, 1.83
Verbal Comprehension		-1.16	-4.59, 2.27		-2.27	-6.14, 1.60		-0.52	-3.67, 2.62
Processing Speed		-1.22	-5.12, 2.67		-1.85	-6.25, 2.56		-0.84	-4.35, 2.67

<sup>a</sup> Generalized linear models adjusted for sex, race/ethnicity, maternal education, language in the home, maternal PON1 enzymatic activity, alcohol use in pregnancy, batch season of urine collection, and urinary creatinine. Combined Population models additionally adjusted for whether the score came from the WISC-IV or WPPSI-III instrument.

Table 5 Joint Prenatal Organophosphate Biomarker and *PON1* Q192R Effect on Combined IQ Domains at 6-9 years in the Mount Sinai Children’s Environmental Health Study

	FSIQ <i>PON1</i> 192 QR/RR (Fast) N = 101		FSIQ <i>PON1</i> 192 QQ (Slow) N = 39		interaction p-value
	Beta	95% CI	Beta	95% CI	
$\log_{10} \Sigma\text{DAP}^a$	-0.66	-4.33, 3.00	-2.33	-8.40, 3.74	0.64
$\log_{10} \Sigma\text{DEP}^a$	-2.32	-6.49, 1.86	-3.13	-8.21, 1.96	0.80
$\log_{10} \Sigma\text{DMP}^a$	0.28	-2.89, 3.44	-1.79	-6.83, 3.25	0.49
	Perceptual Reasoning <i>PON1</i> 192 QR/RR (Fast)		Perceptual Reasoning <i>PON1</i> 192 QQ (Slow)		interaction p-value
	Beta	95% CI	Beta	95% CI	
$\log_{10} \Sigma\text{DAP}^a$	-0.56	-4.80, 3.68	-7.52	-14.53, -0.51	0.09
$\log_{10} \Sigma\text{DEP}^a$	-3.24	-8.11, 1.62	-4.80	-10.73, 1.13	0.68
$\log_{10} \Sigma\text{DMP}^a$	0.71	-2.96, 4.38	-6.15	-11.99, -0.31	0.05
	Verbal Comprehension <i>PON1</i> 192 QR/RR (Fast)		Verbal Comprehension <i>PON1</i> 192 QQ (Slow)		interaction p-value
	Beta	95% CI	Beta	95% CI	
$\log_{10} \Sigma\text{DAP}^a$	-0.33	-3.87, 3.20	-0.73	-5.12, 6.59	0.76
$\log_{10} \Sigma\text{DEP}^a$	-0.45	-4.51, 3.60	-1.20	-6.13, 3.74	0.81
$\log_{10} \Sigma\text{DMP}^a$	0.12	-2.93, 3.16	0.24	-4.60, 5.09	0.97

<sup>a</sup> Generalized linear models adjusted for sex, race/ethnicity, maternal education, language in the home, alcohol use in pregnancy, batch season of urine collection, urinary creatinine, and an indicator variable to designate the WISC-IV or WPPSI-III instrument.



**Figure 1 Legend**

Multivariate adjusted mean estimates and 95% confidence limits according to tertiles of exposure and *PON1* Q192R genotype are presented. Among the children of mothers with the *PON1* 192QQ genotype (black triangles), increasing tertile of  $\Sigma$ DAP,  $\Sigma$ DEP and  $\Sigma$ DMP exposure was generally associated with a monotonic decline in the combined WISC-IV/WPPSI-III FSIQ and Perceptual Reasoning domains, adjusted for sex, race/ethnicity, maternal education, language in the home, alcohol use in pregnancy, batch and season of urine collection, urinary creatinine, and an indicator variable to designate the WISC-IV or WPPSI-III instrument. There were no consistent patterns in the QR/RR genotype group (gray squares). There was considerable imprecision in all estimates. The 1<sup>st</sup> versus 3<sup>rd</sup> tertile contrasts for Perceptual Reasoning were significantly different at  $p < 0.05$  for  $\Sigma$ DAP and  $\Sigma$ DMP. Overall these results suggest a role for genetic susceptibility in the deleterious effects of prenatal pesticide exposure.

Figure 1. FSIQ and Perceptual Reasoning scores at 6-9 years according to summed dialkylphosphate metabolite tertiles and *PON1* Q192R genotype (n = 140)

