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Original Contribution

Prenatal Organophosphate Metabolite and Organochlorine Levels and Performance on the Brazelton Neonatal Behavioral Assessment Scale in a Multiethnic Pregnancy Cohort

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Prenatal exposures to organophosphate pesticides and polychlorinated biphenyls have been associated with abnormal neonatal behavior and/or primitive reflexes. In 1998–2002, the Mount Sinai Children's Environmental Health Center (New York City) investigated the effects of indoor pesticide use and exposure to polychlorinated biphenyls on pregnancy outcome and child neurodevelopment in an inner-city multiethnic cohort. The Brazelton Neonatal Behavioral Assessment Scale was administered before hospital discharge ($n = 311$). Maternal urine samples were analyzed for six dialkylphosphate metabolites and malathion dicarboxylic acid. A random subset of maternal peripheral blood samples from the entire cohort ($n = 194$) was analyzed for polychlorinated biphenyls and 1,1′-dichloro-2,2′-bis(4-chlorophenyl)ethylene. Malathion dicarboxylic acid levels above the limit of detection were associated with a 2.24-fold increase in the number of abnormal reflexes (95% confidence interval: 1.55, 3.24). Likewise, higher levels of total diethylphosphates and total dialkylphosphates were associated with an increase in abnormal reflexes, as was total dimethylphosphates after paraoxonase expression was considered. No adverse associations were found with polychlorinated biphenyl or 1,1′-dichloro-2,2′-bis(4-chlorophenyl)ethylene levels and any behavior. The authors uncovered additional evidence that prenatal levels of organophosphate pesticide metabolites are associated with anomalies in primitive reflexes, which are a critical marker of neurologic integrity.

neonatal screening; pesticides; polychlorinated biphenyls; pregnancy; prenatal exposure delayed effects; reflex, abnormal

Abbreviations: BNBAS, Brazelton Neonatal Behavioral Assessment Scale; DDE, 1,1′-dichloro-2,2′-bis(4-chlorophenyl)ethylene; MDA, malathion dicarboxylic acid; PCB, polychlorinated biphenyl.

Prenatal exposures to organophosphate pesticides and polychlorinated biphenyls (PCBs) have been associated with abnormalities in neonatal behavior and/or primitive reflexes as measured by the Brazelton Neonatal Behavioral Assessment Scale (BNBAS) (1–5). Exposure levels and the specific behavioral or motor abnormalities reported have varied, as have sample sizes and ethnicities represented. Nevertheless, certain patterns have emerged.

To our knowledge, only one study has examined prenatal exposure to organophosphate pesticides and neonatal behavior. An increase in the number of abnormal primitive reflexes was associated with prenatal dialkylphosphate levels

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in maternal urine in an agricultural community (5). The majority of previous studies evaluating the impact of prenatal PCB exposure have reported motor abnormalities, including abnormalities in primitive reflexes (1–3) and in overall motor performance (including motor maturity and tone) (3). Also frequently reported were abnormalities in autonomic stability, which relates to signs of infant stress related to homeostatic adjustments, such as tremor, startle, and changes in skin color and vascularization (1, 2, 4). We know of no previous report of the effect of 1,1′-dichloro-2,2′-bis(4-chlorophenyl)ethylene (DDE) exposure on neonatal behavior.

Although pesticide use in the urban environment of New York City is widespread (6, 7), the extent and nature of individual exposure may vary greatly. The Mount Sinai Children's Environmental Health Center investigated the impact of pesticide and PCB exposure on pregnancy outcome (8–10) and child neurodevelopment in an inner-city multiethnic cohort of women recruited during pregnancy.

MATERIALS AND 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 who delivered their infants at Mount Sinai Hospital between May 1998 and July 2001 (8, 9). Mother-infant pairs were recruited during pregnancy. Of 1,450 eligible women who were approached, 33 percent consented to participate ($n = 479$) (9). Of these women, 75 were excluded because of medical complications ($n = 3$), infant or fetal demise ($n = 2$), very premature birth (delivery before 32 completed weeks or at less than 1,500 g) ($n = 5$), miscarriage ($n = 1$), delivery of an infant with genetic abnormalities or malformations ($n = 5$), inability to collect biologic specimens before birth ($n = 12$), change of hospital or residence outside New York City ($n = 28$), or loss to follow-up or refusal to continue to participate ($n = 19$), leaving 404 for whom birth data were available.

We administered a questionnaire to participants during their third trimester of pregnancy to obtain information on environmental exposures, sociodemographic characteristics, medical history, and lifestyle factors. Maternal blood was obtained during routine venipuncture at a mean gestational age of 31.2 (standard deviation, 3.7) weeks, and a urine sample was collected at the same time. Delivery characteristics and birth outcomes were obtained from a perinatal database within the Mount Sinai Department of Obstetrics, Gynecology and Reproductive Science. The BNBAS was administered before hospital discharge ($n = 311$) by one of four examiners. Examiners were either trained and certified by the Brazelton Institute or trained by a certified examiner. Examinations took place in a quiet, semidarkened, warm room adjacent to the neonatal nursery, or in the mother's private room. The BNBAS was not administered if the infant was admitted to the Neonatal Intensive Care Unit ($n = 21$); if the infant was delivered and discharged over a weekend ($n = 43$); if the parent refused ($n = 5$); if the infant was not testable ($n = 2$); or if study personnel were unavailable ($n = 22$). 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 (Atlanta, Georgia) for six dialkylphosphate metabolites and malathion dicarboxylic acid (MDA). Laboratory and quality control methods (11–13), and between-batch coefficients of variation (12, 14, 15), have been reported previously. Metabolite values within a group (diethyl or dimethyl) were correlated (16); therefore, we imputed for samples with one or more missing values by using the method described by Eskenazi et al. (16). We extended this method to also include imputation of metabolite values for women who were missing two metabolites in a class. In that instance, six regression models were run for subjects with a complete set of metabolites. In each model, one metabolite was predicted from another single metabolite. Then, each missing metabolite was imputed from these models. Diethyl- and dimethylphosphate metabolites were then summed on a molar basis (as nm/liter) to obtain total diethylphosphates and total dimethylphosphates, respectively, and together to obtain total dialkylphosphates levels. Samples of urine that contained less than 20 mg/dl of creatinine ($n = 26$) were excluded from organophosphate metabolite analyses. A random subset ($n = 194$) distributed equally by maternal race/ethnicity of maternal peripheral blood samples from the entire cohort was analyzed for PCBs and DDE. PCBs were defined as the sum of congeners 118, 153, 138, and 180 (13). Total lipids (g/liter) were calculated by using cholesterol and triglycerides (17) determined on 174 plasma samples with sufficient volume. Of the infants who were administered the BNBAS, 151 had PCB and DDE levels measured prenatally.

The BNBAS includes 28 behavioral items and 18 primitive reflexes. The seven-cluster scoring method developed by Lester et al. (18) is typically used to reduce the dimensionality of the data; it divides infant behavior into seven domains: 1) habituation ($n = 183$), ability to respond to and inhibit discrete stimuli while asleep; 2) orientation ($n = 282$), attention to visual and auditory stimuli and quality of overall alertness; 3) motor ($n = 311$), motor performance and quality of movement and tone; 4) range of state ($n = 310$), a measure of infant arousal and state lability; 5) regulation of state ($n = 309$), ability to regulate state in the face of increasing levels of stimulation; 6) autonomic stability ($n = 310$), signs of stress related to homeostatic adjustments of the central nervous system; and 7) number and type of abnormal primitive reflexes ($n = 311$). Details of the Lester scoring method have been described previously (5, 18, 19).

Data were analyzed by using SAS software, version 9.1 (SAS Institute, Inc., Cary, North Carolina). Generalized linear models were used to analyze associations between biomarker levels and each domain except abnormal reflexes. Poisson regression was used to analyze the relation between biomarker levels and the number of abnormal reflexes because of the count nature of the data. The distribution of abnormal reflexes was skewed to the right, with a large number of zero values. When necessary, we corrected for overdispersion as indicated in the tables. Overdispersion may cause underestimation of the standard errors of regression

estimates. We adjusted for overdispersion by introducing a scale parameter estimated by the deviance divided by degrees of freedom.

Backward elimination was used to arrive at the final adjusted models. Covariates were eliminated if their exclusion caused less than a 20 percent change in the beta coefficient of the full model. The following were considered as potential confounders or effect modifiers: maternal age (continuous), race (White or non-White), marital status (single, married, living with the infant's father), education (<high school, high school, some college, ≥college degree), cesarean delivery (yes/no), delivery anesthesia (yes/no), infant age at examination (continuous), infant gender (male/female), infant jaundice (yes/no), and smoking (yes/no), alcohol consumption (yes/no), caffeine consumption (yes/no), or illicit drug use (yes/no) during pregnancy. Similar results were obtained when race was specified as a three-level class variable. Gestational age at delivery and birth weight were not evaluated for confounding because they are potentially causal intermediates. All models were adjusted for examiner. All biomarkers except MDA were included as $\log_{10}$ linear terms in the initial model selection stage. MDA was dichotomized at the limit of detection because only 21.6 percent of the population had detectable values. PCB models were additionally adjusted for laboratory batch. PCB and DDE models were run both with and without adjustment for serum lipids. Dialkylphosphate models were additionally adjusted for paraoxonase 1 enzyme level tertiles (20) and were run both with and without adjustment for urine creatinine. We also analyzed the possible interaction among paraoxonase 1, dialkylphosphate metabolite level, and abnormal reflexes. Paraoxonase 1 activity was determined as previously reported in plasma (μmol/minute per ml) by using phenylacetate as substrate (20).

A finding of two or more abnormal reflexes in a neonate often warrants further clinical investigation and possible intervention (19). Therefore, we conducted exploratory analyses of the association between multiple abnormal reflexes and prenatal pesticide levels, dichotomizing the number of abnormal reflexes at two or more in multivariable logistic regression models.

TABLE 1. Characteristics of the population in a multiethnic pregnancy cohort ($n = 311$), Mount Sinai Hospital, New York City, 1998–2002

Characteristic	No.	%
Maternal age (years)		
<20	111	35.7
20–24	101	32.5
25–29	38	12.2
30–34	47	15.1
≥35	14	4.5
Race		
White	63	20.3
Black	85	27.3
Latina	159	51.1
Other	4	1.3
Education		
<High school	98	31.5
High school graduate	59	19
Some college	80	25.7
≥Bachelor's degree	74	23.8
Marital status		
Married	89	28.6
Living with the infant's father	74	23.8
Single	148	47.6
Infant age at Brazelton Neonatal Behavioral Assessment Scale administration (days)*		
1	157	50.8
2	109	35.3
≥3	43	13.9
	Mean	SD
Gestational age at delivery (weeks)	39.3	1.5
Birth weight (g)	3,290	429

* Total does not add to 311 because, for two subjects, date of examination was not recorded when the examination was performed.

RESULTS

Participants in this study were predominantly young, Black and Latina women with low educational attainment (table 1). The majority were unmarried at enrollment. Severely preterm births were excluded by design; therefore, most women delivered term infants (92.9 percent) of normal birth weight (97.8 percent). All BNBAS examinations were completed within 5 days of delivery, the majority (86.1 percent) within 2 days of delivery.

Environmental exposure distributions are shown in table 2. Regarding prenatal urinary organophosphate metabolites, there were strong and consistent associations between increased exposure and abnormal primitive reflexes (table 3). MDA levels above the limit of detection were associated with a 2.24-fold increase in the number of abnormal reflexes (95 percent confidence interval: 1.55, 3.24) in a Poisson regression model adjusted for examiner, anesthesia, paraoxonase 1 enzyme level, and creatinine. Likewise, relative to the first quartile, quartiles 2–4 of total diethylphosphates, total dimethylphosphates, and total dialkylphosphates were associated with an increased proportion of abnormal reflexes, although the associations did not increase monotonically and varied in their strength and precision (Web table 1) (This information is described in the first of four supplementary tables; each is referred to as "Web table" in the text and is posted on the *Journal*'s website (http://aje.oupjournals.org/).), suggesting a possible threshold effect. After we adjusted for examiner and age at examination, we found that subjects with prenatal MDA levels above the limit of detection delivered infants who were 3.6 times more likely to have at least two abnormal reflexes (95 percent confidence interval: 1.5, 8.8). Likewise, subjects with prenatal total diethylphosphates levels above the median delivered infants

TABLE 2. Organophosphate and organochlorine prenatal biomarker levels in a multiethnic pregnancy cohort, Mount Sinai Hospital, New York City, 1998–2002

Biomarker	No.	Median	% Detectable	Interquartile range
ΣDEP (nm/liter)*	285	24.7	88.8	8.9–52.8
Diethyldithiophosphate	273		8.8	
Diethylphosphate	257		45.9	
Diethylthiophosphate	283		83.7	
ΣDMP (nm/liter)*	297	47.8	90.2	15.6–149.2
Dimethyldithiophosphate	284		28.5	
Dimethylphosphate	268		57.5	
Dimethylthiophosphate	284		88.7	
ΣDAP (nm/liter)† (ΣDEP + ΣDMP)	285	82.0	96.5	35.2–194.7
Malathion dicarboxylic acid (μg/liter)‡	283	<LOD	21.6	
Polychlorinated biphenyls (sum of congeners 118, 153, 138, 180) (μg/liter)	151	0.8	95.4	0.6–1.3
1,1′-dichloro-2,2′-bis(4-chlorophenyl)ethylene (μg/liter)	151	0.6	98.0	0.4–1.3

* Limits of detection (LOD) for total diethylphosphates (ΣDEP) and total dimethylphosphates (ΣDMP) were based on an individual analyte value ≥LOD for any of the three metabolites in each class (1–4 nm/liter, 0.2–0.5 μg/liter).

† LOD for total dialkylphosphates (ΣDAPs) were based on an individual analyte value ≥LOD for any of the six metabolites when neither ΣDEP nor ΣDMP were missing.

‡ The LOD for malathion dicarboxylic acid was 0.3 μg/liter.

TABLE 3. Relation between third-trimester urinary organophosphate metabolite biomarkers (nm/liter) and neonatal behavior in a multiethnic pregnancy cohort, Mount Sinai Hospital, New York City, 1998–2002

	ΣDEP*			ΣDMP*			ΣDAP*			Malathion dicarboxylic acid		
	No.	β†	95% CI*	No.	β†	95% CI	No.	β†	95% CI	No.	β‡	95% CI
Habituation§	144	0.168	−0.230, 0.566	153	−0.024	−0.335, 0.288	144	0.08	−0.300, 0.460	148	0.440	−0.145, 1.025
Orientation¶	233	−0.106	−0.414, 0.201	244	0.018	−0.249, 0.285	233	−0.028	−0.336, 0.279	240	−0.100	−0.597, 0.405
Motor#	249	0.049	−0.077, 0.174	260	0.039	−0.068, 0.146	249	0.048	−0.078, 0.174	257	−0.050	−0.233, 0.156
Range of state**	253	0.035	−0.120, 0.189	264	0.035	−0.096, 0.167	253	0.015	−0.140, 0.169	256	−0.040	−0.281, 0.199
Regulation of state††	253	−0.047	−0.300, 0.207	264	−0.072	−0.283, 0.138	253	−0.026	−0.279, 0.227	256	−0.090	−0.480, 0.303
Autonomic stability‡‡	253	−0.154	−0.382, 0.075	264	0.000	−0.192, 0.193	253	−0.106	−0.334, 0.122	256	0.090	−0.274, 0.463
		RR*	95% CI		RR	95% CI		RR	95% CI		RR	95% CI
No. of abnormal reflexes§§	239	1.49	1.12, 1.98	250	1.13	0.90, 1.41	239	1.32	0.99, 1.77	242	2.24	1.55, 3.24

* ΣDEP, total diethylphosphates; ΣDMP, total dimethylphosphates; ΣDAP, total dialkylphosphates; CI, confidence interval; RR, relative risk.

† β represents a $\log_{10}$ linear term for ΣDEP, ΣDMP, and ΣDAP.

‡ β reflects the contrast of above and below the limit of detection.

§ Adjusted for drug use during pregnancy, examiner, paraoxonase 1 (PON1) enzyme tertiles, and urinary creatinine by using a generalized linear model.

¶ Adjusted for prepregnancy body mass index, examiner, neonatal jaundice, PON1 enzyme tertiles, and urinary creatinine by using a generalized linear model.

Adjusted for infant age at examination, caffeine consumption during pregnancy, drug use during pregnancy, examiner, PON1 enzyme tertiles, and urinary creatinine by using a generalized linear model.

** Adjusted for infant age at examination, examiner, PON1 enzyme tertiles, and urinary creatinine by using a generalized linear model.

†† Adjusted for maternal education, examiner, PON1 enzyme tertiles, and urinary creatinine by using a generalized linear model.

‡‡ Adjusted for infant age at examination, examiner, smoking during pregnancy, PON1 enzyme tertiles, and urinary creatinine by using a generalized linear model.

§§ Adjusted for examiner, anesthesia during delivery, PON1 enzyme tertiles, and urinary creatinine by using Poisson regression. All models except for ΣDEP were additionally adjusted for overdispersion.

TABLE 4. Interactions among paraoxonase expression level, dialkylphosphate metabolites (nm/liter), and risk of abnormal reflexes in a multiethnic pregnancy cohort, Mount Sinai Hospital, New York City, 1998–2002

Paraoxonase expression level	ΣDEP†,‡		ΣDMP†,‡		ΣDAP†,‡	
	RR†	95% CI†	RR	95% CI	RR	95% CI
Tertile 1	1.78	1.01, 3.14	1.96*	1.27, 3.03*	2.38**	1.37, 4.15**
Tertile 2	1.42	0.85, 2.35	1.66*	1.03, 2.65*	1.75	0.96, 3.17
Tertile 3	1.56	1.01, 2.39	0.73	0.56, 0.96	0.76	0.48, 1.20

* Interaction $p \leq 0.01$; **interaction $p < 0.05$.

† ΣDEP, total diethylphosphates; ΣDMP, total dimethylphosphates; ΣDAP, total dialkylphosphates; RR, relative risk; CI, confidence interval.

‡ Adjusted for examiner, anesthesia during delivery, paraoxonase 1 enzyme tertiles, and urinary creatinine by using Poisson regression. All models except for ΣDEP were additionally adjusted for overdispersion.

who were 2.3 times more likely to have at least two abnormal reflexes (95 percent confidence interval: 1.1, 5.0). There was a strong interaction between paraoxonase 1 expression levels and total dimethylphosphates on risk of abnormal reflexes, such that infants born to women in the first (interaction $p = 0.002$) and second (interaction $p = 0.01$) tertiles (slower metabolizers) had a greater risk of abnormal reflexes than infants of those in the highest tertile (fast metabolizers). For women in the highest tertile of paraoxonase 1 expression, no increased risk of abnormal reflexes with increasing exposure was found. There was no interaction between total diethylphosphates and paraoxonase 1 (table 4).

We evaluated the possibility that infant age may modify these associations by using a $\log_{10}$ linear term for total diethylphosphates, total dimethylphosphates, and total dialkylphosphates, a dichotomized term for MDA (at the limit of detection), and a dichotomized term for age at BNBAS administration (1 day vs. $\geq$2 days of age). With the exception of MDA, there appeared to be stronger associations between metabolite levels and abnormal reflexes for examinations performed after the first day of life, although only the total dimethylphosphates interaction p value was $\leq$0.10. In contrast, an interaction ($p = 0.10$) with MDA showed the reverse; that is, for infants examined before day 2, the relation between MDA and abnormal reflexes was stronger (relative risk = 2.51, 95 percent confidence interval: 1.61, 3.90) compared with that for infants examined later (relative risk = 1.34, 95 percent confidence interval: 0.72, 2.49) (Web table 2).

Of the 308 infants for whom we were able to evaluate all 18 primitive reflexes, 198 (64 percent) had all reflexes within the normal range, 55 (18 percent) had one abnormal reflex, 25 (8 percent) had two abnormal reflexes, and 30 (10 percent) had three or more abnormal reflexes. The majority of abnormal reflexes were due to hypotonicity (which includes a reflex not elicited after stimulation), although a small number of hypertonic ($n = 5$) and asymmetric ($n = 6$) reflexes were observed. In exploratory analyses, we examined whether specific abnormal reflexes might be related to elevated organophosphate pesticide metabolite levels. In chi-square analyses, MDA levels above the limit of detection were associated with both abnormal ''crawling'' and ''resist arms'' reflexes ($p < 0.01$). Likewise, a higher total diethylphosphates level was associated with an abnormal ''crawling'' reflex when the Wilcoxon signed-rank test was used ($p < 0.05$).

Prenatal PCB level was associated with range of state, but in an unexpected direction. Increasing PCB level was positively associated with an improved range of state, in a dose-dependent manner (table 5 and Web table 3). A similar trend, but less strong, was seen for regulation of state. There were no notable associations between prenatal DDE levels and infant behavior or reflexes (table 5 and Web table 3).

DISCUSSION

A high prevalence of pesticide use in two New York City pregnancy cohorts has been reported (8, 21). All of the women who participated in personal air monitoring of their home had detectable levels of diazinon, chlorpyrifos, carbamate propoxur, and *o*-phenylphenol (21). In our cohort, 46 percent reported that pesticides had been applied in their home during their pregnancy (8). Prenatal exposure to organophosphate pesticides was associated with reduced infant head circumference after considering paraoxonase 1 in our cohort (9) and reduced infant birth weight and length (22) in another inner-city minority cohort. Additionally, organophosphate pesticide metabolites were associated with decreased length of gestation in a rural, agricultural population (16).

We found an association between both generic and pesticide-specific biomarkers of prenatal organophosphate pesticides and an increased number of abnormal primitive reflexes in neonates, consistent with a previously reported association in a more highly exposed cohort (5). Although there did not appear to be a substantially increased risk of abnormal reflexes due to dimethylphosphates overall, once we accounted for paraoxonase 1 expression level, women in lower tertiles of paraoxonase 1 expression (i.e., slow organophosphate pesticide metabolizers) had a significantly increased risk of abnormal reflexes. In addition, subjects with detectable MDA, or greater than the median total diethylphosphates levels, had an elevated risk of delivering infants with two or more abnormal reflexes, a clinically significant

TABLE 5. Relation between third-trimester serum organochlorine biomarkers (μg/liter) and neonatal behavior in a multiethnic pregnancy cohort, Mount Sinai Hospital, New York City, 1998–2002

BNBAS* domain	PCBs*			DDE*		
	No.	β	95% CI*	No.	β	95% CI
Habituation†	78	1.07	−0.49, 2.63	78	−0.248	−1.09, 0.59
Orientation‡	127	−0.333	−1.54, 0.88	127	0.308	−0.35, 0.96
Motor§	136	−0.035	−0.45, 0.39	136	0.098	−0.14, 0.33
Range of state¶	134	0.555	−0.07, 1.00	134	0.028	−0.27, 0.32
Regulation of state#	135	0.335	−0.46, 1.13	135	0.279	−0.15, 0.71
Autonomic stability**	134	0.342	−0.54, 1.23	134	−0.309	−0.79, 0.18
		RR*	95% CI		RR	95% CI
No. of abnormal reflexes††	130	0.56	0.18, 1.67	127	0.95	0.49, 1.84

* BNBAS, Brazelton Neonatal Behavioral Assessment Scale; PCBs, polychlorinated biphenyls; DDE, 1,1′-dichloro-2,2′-bis(4-chlorophenyl)ethylene; CI, confidence interval; RR, relative risk.

† PCB model was adjusted for prepregnancy body mass index, examiner, neonatal jaundice, total lipids, and laboratory batch. DDE model was adjusted for examiner and total lipids.

‡ PCB model was adjusted for race, prepregnancy body mass index, examiner, total lipids, and laboratory batch. DDE model was adjusted for race, neonatal jaundice, examiner, and total lipids.

§ PCB model was adjusted for examiner, total lipids, and laboratory batch. DDE model was adjusted for caffeine consumption during pregnancy, examiner, and total lipids.

¶ PCB model was adjusted for race, infant age at examination, examiner, total lipids, and laboratory batch. DDE model was adjusted for race, infant age at examination, caffeine consumption during pregnancy, examiner, and total lipids.

PCB model was adjusted for maternal education, examiner, total lipids, and laboratory batch. DDE model was adjusted for maternal education, examiner, and total lipids.

** PCB model was adjusted for infant age at examination, maternal age, prepregnancy body mass index, examiner, total lipids, and laboratory batch. DDE model was adjusted for infant gender and age at examination, maternal age, prepregnancy body mass index, smoking during pregnancy, examiner, total lipids, and neonatal jaundice.

†† Poisson models were adjusted for overdispersion. PCB model was additionally adjusted for examiner, race, anesthesia during delivery, total lipids and laboratory batch. DDE model was additionally adjusted for examiner, race, anesthesia during delivery, and total lipids.

threshold (19). Our findings are remarkably consistent with those of Young et al. (5); for each $\log_{10}$ unit increase in total dialkylphosphates, these authors reported a 26 percent increased risk of abnormal reflexes, and we found a 32 percent increased risk. The magnitudes of association were also similar for total diethylphosphates (relative $\text{risk}_{\text{Young}} = 1.25$, relative $\text{risk}_{\text{Engel (current study)}} = 1.49$) and total dimethylphosphates (relative $\text{risk}_{\text{Young}} = 1.20$, relative $\text{risk}_{\text{Engel (current study)}} = 1.13$). Both studies found stronger associations between dialkylphosphate metabolites and abnormal reflexes for older children, although, in our study, the statistical interaction with day of administration was not significant.

The relation between MDA, a malathion-specific metabolite, and abnormal reflexes in our cohort appeared to be age dependent, such that the most pronounced effect was observed in infants evaluated within the first day of life. This finding conflicts with the suggestion of later effects for dialkylphosphate metabolites, including dimethylphosphates, which encompasses nonspecific metabolites of malathion. Because the class of dimethylphosphates includes breakdown products of numerous organophosphate pesticides, it is unclear what pesticides may be driving this disparity.

It is possible that postentry exclusions in our cohort may have biased our findings if they were associated with both organophosphate pesticide levels and neonatal behavior. Most of our excluded women were those who moved out of the study area, were lost-to-follow up, or lacked prenatal biologic specimens, which largely reflect socioeconomic condition. We recently described that, compared with Blacks or Hispanics, Whites had slightly higher levels of dimethylphosphates and that a larger proportion of older women, relative to younger women, had MDA levels above the limit of detection (10). However, in our analysis, maternal age and race were not associated with number of abnormal reflexes. Additionally, the consistency of our findings with those of Young et al. (5) suggests that bias is unlikely to account for the entire effect. The BNBAS was not administered to a substantial fraction (23 percent) of the initial cohort for various reasons, including weekend infant delivery. Thus, any factors related to weekend delivery (e.g., fewer inductions) would be underrepresented among our tested subjects. However, this possibility alone does not impose a bias on our findings, even if those factors are related to exposure or disease status. That is, fewer induced deliveries with pesticide exposure, for example, would affect precision, but that alone would not affect the validity of the estimates. Only under the scenario in which a different exposure-disease relation existed between those tested and not tested would bias result.

The Mount Sinai Children's Environmental Health Center enrolled subjects between 1998 and 2002, which overlaps with the period when chlorpyrifos and diazinon were being phased out of residential use (23, 24). However, in 1997, chlorpyrifos was the most heavily used insecticide by pest control operators at the New York City Housing Authority (7). Chlorpyrifos, which is metabolized to diethylphosphates, is a known neurotoxicant (7). Systemic toxicity of organophosphates is achieved through the inhibition of cholinesterases. Accumulating evidence suggests that developmental toxicity may result from a disruption in the maturation of neuronal cells and the organization of synaptic networks (25). A comparative analysis of developmental and systemic toxicity related to chlorpyrifos and diazinon found them to be similar, although diazinon, which is also metabolized to diethylphosphates, appeared slightly less potent by some measures (25).

Behavioral sequelae of prenatal organophosphate pesticide exposure have been reported in animal studies. Pups prenatally exposed to low levels of chlorpyrifos during pregnancy exhibited a significant alteration in cliff avoidance and the righting reflex at postnatal days 1 and 3 (26). Some chlorpyrifos-associated behavioral anomalies have been linked to disruption of the serotonin systems in the perinatal period, leading to global upregulation of serotonin-related synaptic proteins (27). Additionally, some chlorpyrifos-associated behavioral anomalies appear to persist into adulthood (28). However, this study reflects only pesticide metabolites, and it is unclear how their levels relate to the parent organophosphate.

PCBs are among the most widely studied neurotoxicants. In previous studies that have used the BNBAS to evaluate their in utero effects on infant behavior, altered motor performance and reflexes (1–3), habituation (2, 4), range of state (1), and autonomic stability (1, 2, 4) have been reported. The differences in these findings are likely due to variability in PCB level and congener mixture and to the populations under study. In our population, PCB levels were quite low overall and were related to both country of origin and fish consumption (13). We report an unexpected association between increasing PCB levels and improved range of state that was dose dependent and monotonic. A similar trend was seen for regulation of state. A recent small study linked decosahexaenoic acid levels in breast milk to improved range of state in infants at 9 days of age (29). Decosahexaenoic acid is an omega-3-fatty acid present in fish, particularly cold-water fish. It is possible, therefore, that our results suggesting a positive effect for PCB exposure are actually attributable to their correlation with fish consumption and therefore omega-3-fatty acid levels during pregnancy.

Observing two or more abnormal reflexes in a neonate is considered clinically significant and often results in a more intensive neurologic examination and possible intervention (19). In our cohort, very few maternal, peripartum, or neonatal characteristics predicted abnormal reflexes except infant age, gestational age at delivery, and anesthesia use during delivery (Web table 4). However, the prognostic utility of a single measurement of infant behavior shortly after delivery is unclear. Likewise, organophosphate pesticide metabolites have short half-lives, and our exposure measurements rely on a single urine specimen taken during the third trimester. However, if sources and patterns of exposure (e.g., residential pesticide use or exposure from a food source) are unchanged, we can assume that a single measurement reflects a typical measurement at any time during pregnancy. Even so, misclassification of both exposure and outcome is probable in this study, although they are likely to be independent of one another. Additional research is under way to assess their impact on subsequent markers of neurodevelopment, and therefore the persistence of these associations over time.

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