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Attention-Deficit/Hyperactivity Disorder and Urinary Metabolites of Organophosphate Pesticides



WHAT'S KNOWN ON THIS SUBJECT: Exposure to organophosphates has been associated with adverse effects on neurodevelopment, such as behavioral problems and lower cognitive function. Studies have focused, however, on populations with high levels of exposure, relative to the general population.



WHAT THIS STUDY ADDS: We conducted a study with 1139 children 8 to 15 years of age, representative of the US population. The findings showed that children with higher urinary levels of organophosphate metabolites were more likely to meet the diagnostic criteria for ADHD.

abstract

FREE

OBJECTIVE: The goal was to examine the association between urinary concentrations of dialkyl phosphate metabolites of organophosphates and attention-deficit/hyperactivity disorder (ADHD) in children 8 to 15 years of age.

METHODS: Cross-sectional data from the National Health and Nutrition Examination Survey (2000–2004) were available for 1139 children, who were representative of the general US population. A structured interview with a parent was used to ascertain ADHD diagnostic status, on the basis of slightly modified criteria from the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*.

RESULTS: One hundred nineteen children met the diagnostic criteria for ADHD. Children with higher urinary dialkyl phosphate concentrations, especially dimethyl alkylphosphate (DMAP) concentrations, were more likely to be diagnosed as having ADHD. A 10-fold increase in DMAP concentration was associated with an odds ratio of 1.55 (95% confidence interval: 1.14–2.10), with adjustment for gender, age, race/ethnicity, poverty/income ratio, fasting duration, and urinary creatinine concentration. For the most-commonly detected DMAP metabolite, dimethyl thiophosphate, children with levels higher than the median of detectable concentrations had twice the odds of ADHD (adjusted odds ratio: 1.93 [95% confidence interval: 1.23–3.02]), compared with children with undetectable levels.

CONCLUSIONS: These findings support the hypothesis that organophosphate exposure, at levels common among US children, may contribute to ADHD prevalence. Prospective studies are needed to establish whether this association is causal. *Pediatrics* 2010;125:e1270–e1277

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KEY WORDS

attention-deficit/hyperactivity disorder, pesticides, organophosphates, National Health and Nutrition Examination Survey

ABBREVIATIONS

DAP—dialkyl phosphate
DMAP—dimethyl alkylphosphate
DEAP—diethyl alkylphosphate
OR—odds ratio
CI—confidence interval
ADHD—attention-deficit/hyperactivity disorder
NHANES—National Health and Nutrition Examination Survey
DISC-IV—Diagnostic Interview Schedule for Children IV
PIR—poverty/income ratio
DSM-IV—*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*

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Approximately 40 organophosphate pesticides are registered with the US Environmental Protection Agency for use in the United States.¹ In 2001, 73 million pounds of organophosphates were used in both agricultural and residential settings. The Environmental Protection Agency considers food, drinking water, and residential pesticide use important sources of exposure.² Residential pesticide use is common, but the major source of exposure to pesticides for infants and children would be the diet, according to the National Academy of Sciences.³ The US Pesticide Data Program's 2008 Report indicates that detectable concentrations of the organophosphate malathion were found in 28% of frozen blueberry samples, 25% of strawberry samples, and 19% of celery samples.⁴ Children are generally considered to be at greatest risk from organophosphate toxicity, because the developing brain is more susceptible to neurotoxins⁵ and the dose of pesticides per body weight is likely to be larger for children. Children 6 to 11 years of age have the highest urinary concentrations of dialkyl phosphate (DAP) metabolites (markers of organophosphate exposure), compared with other age groups in the US population.⁶ Children have reduced expression of detoxifying enzymes, which contributes to their vulnerability.^{7,8} Epidemiological studies linking exposure to organophosphates and neurodevelopment have focused on populations with high levels of exposure, relative to the general population.^{9,10} Prenatal organophosphate exposure was associated with increased risk of pervasive developmental disorders, as well as delays in mental development at 2 to 3 years of age.^{11,12} Postnatal organophosphate exposure has been associated with behavioral problems, poorer short-term memory and motor skills, and longer reaction times in children.^{13–15}

A few epidemiological studies suggested that organophosphate exposure was associated with adverse neurodevelopmental outcomes, but no studies have addressed possible risks among children with average levels of exposure. By using data for a representative sample of US children, we examined the cross-sectional association between urinary DAP metabolite concentrations and attention-deficit/hyperactivity disorder (ADHD) prevalence in children 8 to 15 years of age.

METHODS

Study Design and Population

The National Health and Nutrition Examination Survey (NHANES) is a population-based, health survey of noninstitutionalized US residents conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention. The NHANES uses a complex, multistage, probability sampling design, with oversampling of certain subgroups. Participants completed household surveys, which included questions about demographic features and health history, and blood and urine samples were collected during physical examinations at mobile centers.¹⁶ We used data from 2000–2004, years for which ADHD was assessed in children 8 to 15 years of age; diagnoses were available for 3998 children. Urinary DAP metabolite levels were measured for a random subsample of the NHANES participants. From 2000 to 2002, the sampling rate was 50% for ages 6 to 11 years and 33% for ages 12 to 15 years. From 2003 to 2004, the sampling rate was 33% for all ages. Measurements of urinary DAP levels were available for 1481 children among those with ADHD diagnoses. The NHANES was approved by the National Center for Health Statistics institutional review board, and all participants provided written informed consent.

Children who received newborn care in an ICU or premature nursery ($n = 167$) and those with birth weights of <2500 g ($n = 126$) were excluded because these are important risk factors for developmental disorders.¹⁷ We excluded 24 children with extremely dilute urine (creatinine levels of <20 mg/dL) and 1 outlier with respect to urinary DAP concentrations. Children with missing data were excluded (poverty/income ratio [PIR], $n = 43$; fasting duration, $n = 38$; urinary creatinine level, $n = 1$).

ADHD Assessment

The Diagnostic Interview Schedule for Children IV (DISC-IV), a structured diagnostic interview designed for use in epidemiological studies,¹⁸ was used to assess the presence of ADHD on the basis of slightly modified criteria from the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV)*.¹⁹ The interview was conducted with the mother or another caretaker over the telephone by trained interviewers, 2 to 3 weeks after the physical examination. The interview was conducted by bilingual interviewers in English or Spanish. The DISC-IV has evidence of substantial validity,¹⁸ reliability for both its English¹⁸ and Spanish^{20,21} versions, and successful use via telephone in DSM-IV field trials.²² The use of DISC-IV data is restricted for confidentiality reasons; therefore, we accessed the data through the National Center for Health Statistics Research Data Center.

The DISC-IV scoring algorithms determine ADHD diagnostic status for the previous year, as well as ADHD subtype, that is, predominately inattentive subtype, predominately hyperactive/impulsive subtype, or combined subtype. The diagnosis is based on the presence, during the previous 12 months, of symptoms related to inattention, hyperactivity, and impulsivity,

with significant impairment in ≥ 2 settings (eg, at school and home).²³ The DSM-IV criterion that symptoms must not occur in conjunction with another neuropsychiatric disorder is not assessed by the DISC-IV. We chose not to use the DSM-IV criterion that symptoms must have been present before 7 years of age, because our hypothesis was that urinary DAP concentrations would be associated with increased odds of concurrent ADHD.

The DISC-IV identified 119 cases of ADHD, but 30 children did not meet the diagnostic criteria although the parent reported use of doctor-prescribed ADHD medication during the previous year. Because the diagnostic interview addresses the presence of symptoms, children whose symptoms were well controlled with medication would not meet the diagnostic criteria. Therefore, we also conducted analyses in which ADHD cases were defined as either meeting the DISC-IV diagnostic criteria or regularly taking ADHD medication during the previous year.

Measurement of Urinary Metabolites of Organophosphate Pesticides

During the physical examination, "spot" urine specimens were collected from participants, divided into aliquots, and stored cold (2–4°C) or frozen. Samples collected for DAP measurements were shipped on dry ice to the Centers for Disease Control and Prevention's National Center for Environmental Health. Six urinary DAP metabolites, resulting from the degradation of ≥ 28 different organophosphates, were measured in urine to provide an indicator of the body burden of common organophosphates.³ The urinary DAP metabolites measured were 3 dimethyl alkylphosphate (DMAP) molecules (dimethyl phosphate, dimethyl thiophosphate, and dimethyl dithiophosphate) and 3

diethyl alkylphosphate (DEAP) molecules (diethyl phosphate, diethyl thiophosphate, and diethyl dithiophosphate). DMAP metabolites are derived from *O,O*-dimethyl-substituted organophosphate pesticides such as malathion; DEAP metabolites result from the degradation of *O,O*-diethyl-substituted organophosphates such as chlorpyrifos. The measurements were performed through lyophilization and chemical derivatization, followed by analysis through isotope-dilution gas chromatography-tandem mass spectrometry.²⁴ Stable-isotope analogues were used as internal standards for each of the metabolites, which resulted in a high degree of accuracy and precision, with low analytical limits of detection. Concentrations below the detection limit were imputed a value corresponding to the value of the detection limit divided by $\sqrt{2}$.²⁵ Urinary creatinine concentrations were determined using an automated colorimetric method based on a modified Jaffe reaction on a Beckman Synchron AS/ASTRA clinical analyzer (Beckman Instruments, Inc., Brea, CA) at the Fairview University Medical Center, Minneapolis, Minnesota.²⁶

Other Covariates

The following variables were considered as potential confounders: gender, age (in months), child race/ethnicity (non-Hispanic white, black, Mexican American, or other/multiracial), PIR (the ratio of self-reported family income to the family's appropriate poverty threshold value, on the basis of Census data, recoded into 4 categories), BMI (in quartiles), blood lead concentrations (logarithmically transformed), maternal age at birth, maternal smoking during pregnancy (yes or no), and time since last consuming food or drink, recorded at the time of blood and urine sampling (recorded on a scale of 1 [0–2 hours] to 7 [21–24 hours]).

Data Analyses

We used the complex samples module of SPSS 17.0 (SPSS Inc, Chicago, IL) to conduct all analyses, with accounting for the multistage probability sampling design of the NHANES. Strata, primary sampling units, and sample weights were used to obtain robust linearized SEs and unbiased point estimates. The threshold for statistical significance was set at $P < .05$. All statistical tests were 2-sided. The DAP metabolite concentrations were divided by the respective molecular weights before being summed to yield DEAP, DMAP, and total DAP concentrations. Because DMAP and DEAP metabolites might have different relationships to the outcomes,¹² they were examined separately. Because the distributions of total DAP, DMAP, and DEAP concentrations were skewed, logarithmic transformations (base 10) were applied. Correlates of urinary DAP metabolite concentrations were examined with a general linear model. Logistic regression analyses were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for ADHD (any subtype) and ADHD subtypes, per 10-fold increases in total DAP, DMAP, and DEAP metabolite concentrations (in nanomoles per liter). Results are presented for crude analyses and adjusted analyses. Gender- and age-related differences were examined in subpopulation analyses and, because there were no differences in effect estimates and no significant interaction, these variables were included as covariates in the models. In addition, race/ethnicity, PIR, fasting time, and logarithmically transformed urinary creatinine concentrations (as recommended by Barr et al,²⁶ to adjust for urine dilution) were included in the models because they are important potential confounders; other covariates also were examined in sensitivity analyses. Because individual urinary

TABLE 1 Concentrations of Urinary DAP Metabolites (*N* = 1139)

	<i>N</i>	Below Detection Limit, <i>n</i> (%)	Urinary Metabolite Level, nmol/L			
			Geometric Mean	Interquartile Range	Minimum	Maximum
			Diethyl phosphate	1133	534 (46.9)	4.7
Diethyl thiophosphate	1121	487 (42.8)	2.0	0.4–7.6	0.3	650
Diethyl dithiophosphate	1139	911 (80.0)	0.5	0.3–0.3	0.2	36
Dimethyl phosphate	1139	581 (51.0)	10.7	2.8–39.0	2.8	1324
Dimethyl thiophosphate	1139	407 (35.7)	13.7	1.9–58.8	0.9	9929
Dimethyl dithiophosphate	1133	664 (58.3)	1.7	0.4–7.3	0.3	7006
DEAPs	1139	253 (22.2)	11.0	2.1–35.0	0.8	5905
DMAPs	1139	209 (18.3)	41.3	10.1–130.7	4.5	10 068
Total DAPs	1139	71 (6.2)	68.3	24.4–186.0	6.0	10 195

DAP metabolite levels were below the analytical limit of detection for a large proportion of children (Table 1), which might bias effect estimates;²⁷ we conducted further analyses on the metabolite with the highest detection frequency, namely, dimethyl thiophosphate. Cases were categorized as below the limit of detection or lower or higher than the median of detectable concentrations adjusted for creatinine levels.

RESULTS

Descriptive Statistics

Our study sample included 1139 children 8 to 15 years of age, with charac-

teristics similar to those of children not included in the sample (Table 2). One hundred nineteen children met the diagnostic criteria for any ADHD subtype, which corresponds to a population prevalence of 12.1% (95% CI: 9.6%–15.1%). The prevalence estimates were 7.6% (95% CI: 5.5%–10.4%) for inattentive subtype, 1.5% (95% CI: 0.8%–2.7%) for hyperactive/impulsive subtype, and 3.0% (95% CI: 2.1%–4.3%) for combined subtype. When children taking ADHD medication were included as case subjects, there were 148 cases.

The proportions of children with urinary DAP concentrations below the de-

tection limit were between 35.7% and 80.0%, depending on the metabolite (Table 1). Most children (93.8%) had ≥ 1 detectable metabolite, of the 6 DAPs measured. In a multivariate analysis, higher DAP concentrations were associated with higher creatinine concentrations ($P < .001$), younger age ($P = .03$), lower blood lead concentrations ($P = .06$), and higher PIR ($P = .10$). DAP concentrations were higher for children examined in 2003–2004 (adjusted mean: 18.15 nmol/L; SE: 1.15 nmol/L), compared with those examined in 2000 (mean: 12.62 nmol/L; SE: 1.24 nmol/L) and 2001–2002 (mean: 11.69 nmol/L; SE: 1.18 nmol/L), although not significantly ($P = .10$). Gender, race/ethnicity, and fasting duration were not significantly associated with DAP metabolite concentrations (all $P > .3$).

Any Subtype of ADHD

The odds of meeting the DISC-IV criteria for ADHD increased with the urinary concentrations of total DAP metabolites (Table 3). Adjustment for covariates attenuated the estimates (for a 10-fold increase in total DAP concentration, unadjusted OR: 1.31 [95% CI: 1.06–1.63]; adjusted OR: 1.21 [95% CI: 0.97–1.51]). This association was driven by DMAP metabolites, for which the association was statistically significant even after adjustment (OR: 1.55 [95% CI: 1.14–2.10]). When children taking ADHD medication were included as case subjects, slightly higher effect estimates were obtained for DMAPs (adjusted OR: 1.72 [95% CI: 1.31–2.28]). A 10-fold difference in DMAP concentrations corresponds approximately to the increase from the 25th to 75th percentile of children's concentrations (Table 1). DEAP metabolite levels were not significantly associated with the odds of ADHD, whether cases were defined strictly according to the DISC-IV criteria or included children taking ADHD medication (Table 3).

TABLE 2 Comparison of Characteristics of Children 8 to 15 Years of Age Not Included and Included in Study Sample

	Not Included	Included
Male, <i>n</i> (%) ^a	2247 (51.2) (<i>N</i> = 4578)	570 (53.2) (<i>N</i> = 1139)
Race/ethnicity, <i>n</i> (%) ^a		
Non-Hispanic white	1138 (59.6) (<i>N</i> = 4578)	325 (63.3) (<i>N</i> = 1139)
Black	1533 (16.0) (<i>N</i> = 4578)	342 (13.2) (<i>N</i> = 1139)
Mexican American	1555 (11.7) (<i>N</i> = 4578)	382 (11.6) (<i>N</i> = 1139)
Other/multiracial	352 (12.7) (<i>N</i> = 4578)	90 (12.0) (<i>N</i> = 1139)
PIR, <i>n</i> (%) ^a		
<1.0	1390 (23.4) (<i>N</i> = 4145)	363 (21.4) (<i>N</i> = 1139)
1.0–1.84	1009 (20.7) (<i>N</i> = 4145)	274 (21.3) (<i>N</i> = 1139)
1.85–3.0	730 (20.6) (<i>N</i> = 4145)	214 (21.6) (<i>N</i> = 1139)
>3.0	1016 (35.3) (<i>N</i> = 4145)	288 (35.8) (<i>N</i> = 1139)
Maternal smoking during pregnancy, <i>n</i> (%) ^a	697 (19.8) (<i>N</i> = 4509)	152 (17.7) (<i>N</i> = 1125)
ADHD, <i>n</i> (%) ^a		
Any subtype	295 (12.2) (<i>N</i> = 2859)	119 (12.1) (<i>N</i> = 1139)
Inattentive subtype	133 (5.9) (<i>N</i> = 2865)	69 (7.6) (<i>N</i> = 1139)
Hyperactive subtype	81 (3.2) (<i>N</i> = 2860)	21 (1.5) (<i>N</i> = 1139)
Combined subtype	81 (3.1) (<i>N</i> = 2865)	29 (3.0) (<i>N</i> = 1139)
Age, weighted mean \pm SE, mo	144 \pm 0.6 (<i>N</i> = 4578)	143 \pm 1.0 (<i>N</i> = 1139)
BMI, weighted mean \pm SE, kg/m ²	20.8 \pm 0.1 (<i>N</i> = 4502)	20.6 \pm 0.2 (<i>N</i> = 1139)
Blood lead level, weighted mean \pm SE, μ g/dL	1.4 \pm 0.04 (<i>N</i> = 4036)	1.4 \pm 0.04 (<i>N</i> = 1093)
Maternal age at birth, weighted mean \pm SE, y	26.3 \pm 0.2 (<i>N</i> = 4432)	26.4 \pm 0.3 (<i>N</i> = 1107)

^a Proportions were weighted.

TABLE 3 ORs for Any ADHD Subtype for 10-Fold Increases in Urinary DAP Metabolite Levels (*N* = 1139)

	OR (95% CI)			
	Cases Identified With DISC-IV (<i>n</i> = 119)		Cases Identified With DISC-IV or ADHD Medication (<i>n</i> = 148)	
	Unadjusted	Adjusted ^a	Unadjusted	Adjusted ^a
DEAPs	1.02 (0.74–1.41)	0.94 (0.69–1.28)	0.88 (0.66–1.18)	0.80 (0.60–1.05)
DMAPs	1.66 (1.24–2.22)	1.55 (1.14–2.10)	1.87 (1.42–2.47)	1.72 (1.31–2.28)
Total DAPs	1.31 (1.06–1.63)	1.21 (0.97–1.51)	1.48 (1.20–1.82)	1.35 (1.10–1.67)

^a Adjusted for gender, age, race/ethnicity, PIR, fasting duration, and logarithmically transformed urinary creatinine concentration.

TABLE 4 ORs for Any ADHD Subtype According to Creatinine Level-Adjusted Urinary Dimethyl Thiophosphate Concentration (*N* = 1139)

Dimethyl Thiophosphate Concentration	OR (95% CI)			
	Cases Identified With DISC-IV (<i>n</i> = 119)		Cases Identified With DISC-IV or ADHD Medication (<i>n</i> = 148)	
	Unadjusted	Adjusted ^a	Unadjusted	Adjusted ^a
Below detection limit (<i>n</i> = 407)	1.0 (reference)			
Lower than median (<i>n</i> = 366) ^b	1.11 (0.63–1.97)	1.05 (0.57–1.95)	1.36 (0.76–2.44)	1.22 (0.65–2.27)
Higher than median (<i>n</i> = 366) ^c	1.83 (1.18–2.82)	1.93 (1.23–3.02)	2.04 (1.30–3.22)	2.12 (1.32–3.41)

^a Adjusted for gender, age, race/ethnicity, PIR, and fasting duration.

^b Range: 0.9 to 30.4 nmol × g of creatinine per L; median: 11.2 nmol × g of creatinine per L.

^c Range: 30.4 to 7932.0 nmol × g of creatinine per L; median: 97.6 nmol × g of creatinine per L.

The associations between DMAP concentrations and ADHD were similar for girls (with cases defined as meeting DISC-IV criteria or taking ADHD medication, adjusted OR: 2.09 [95% CI: 1.39–3.15]) and boys (adjusted OR: 1.60 [95% CI: 1.09–2.36]; *P* for interaction = .48). Similarly, we examined age differences in DMAP effect estimates for children 8 to 11 years and 12 to 15 years of age but found no difference (*P* for interaction = .55).

The metabolite dimethyl thiophosphate was the most commonly detected DMAP metabolite (64.3% of children) and accounted for 50% of total DMAP metabolites. Children with creatinine-adjusted dimethyl thiophos-

phate concentrations above the median of detectable values had twice the odds of ADHD, compared with children with concentrations below the detection limit (adjusted OR: 1.93 [95% CI: 1.23–3.02]) (Table 4). The OR was higher when children taking ADHD medication were included as case subjects (adjusted OR: 2.12 [95% CI: 1.32–3.43]).

Sensitivity Analyses

The urinary creatinine level was a potential confounder because higher concentrations were associated both with greater odds of ADHD and with higher DAP concentrations. We examined different analytical approaches to

account for creatinine concentrations in our analyses. We used creatinine-adjusted DAP, DMAP, and DEAP concentrations (metabolite/creatinine concentrations), and results were essentially the same as those obtained in the main analyses, in which adjustment for the creatinine level was achieved by including it as an independent variable along with the other covariates.

To evaluate possible cohort effects, we added a term for year of data collection to the models, but this term was not significant and did not change the effect estimates appreciably. The effect estimates were not affected by adjustments for blood lead concentrations, maternal age at birth, or maternal smoking during pregnancy. Given that ADHD medication use could change the metabolism of pesticides and influence their excretion in urine, we excluded the 40 children who were taking such medication, but the results were similar (adjusted OR for 10-fold increase in DMAP levels: 1.80 [95% CI: 1.18–2.76]).

Subtypes of ADHD

The odds of meeting the diagnostic criteria for hyperactive/impulsive ADHD subtype increased significantly with higher DEAP (adjusted OR for 10-fold increase in concentration: 2.15 [95% CI: 1.06–4.40]), DMAP (adjusted OR for 10-fold increase in concentration: 2.13 [1.08–4.20]), and total DAP (adjusted OR for 10-fold increase in concentration: 1.85 [1.04–3.27]) levels (Table 5).

TABLE 5 ORs for Subtypes of ADHD for 10-Fold Increases in Urinary DAP Metabolite Levels (*N* = 1139)

	OR (95% CI)					
	Hyperactive/Impulsive Subtype (<i>n</i> = 21)		Inattentive Subtype (<i>n</i> = 69)		Combined Subtype (<i>n</i> = 29)	
	Unadjusted	Adjusted ^a	Unadjusted	Adjusted ^a	Unadjusted	Adjusted ^a
DEAPs	2.29 (1.25–4.21)	2.15 (1.06–4.40)	0.77 (0.52–1.14)	0.70 (0.49–1.01)	1.29 (0.68–2.43)	1.22 (0.59–2.50)
DMAPs	2.26 (1.33–3.86)	2.13 (1.08–4.20)	1.61 (1.10–2.37)	1.47 (0.99–2.19)	1.30 (0.56–2.99)	1.30 (0.48–3.48)
Total DAPs	1.95 (1.18–3.22)	1.85 (1.04–3.27)	1.26 (0.91–1.75)	1.14 (0.81–1.61)	1.09 (0.59–2.01)	1.05 (0.51–2.16)

^a Adjusted for gender, age, race/ethnicity, PIR, fasting duration, and logarithmically transformed urinary creatinine concentration.

The odds of inattentive subtype increased with higher concentrations of DMAP metabolites (adjusted OR: 1.47 [95% CI: 0.99–2.19]), although this did not reach the level of significance. Concentrations of DAP metabolites were not significantly associated with the odds of combined subtype.

DISCUSSION

We report an association between urinary DMAP metabolite concentrations, which are indicators of exposure to dimethyl-containing organophosphate pesticides, and increased odds of ADHD for children 8 to 15 years of age. There was a 55% to 72% increase in the odds of ADHD for a 10-fold increase in DMAP concentration, depending on the criteria used for case identification. This association was not explained by gender, age, PIR, race/ethnicity, fasting duration, or creatinine concentration. Whether DAP metabolite concentrations are more strongly associated with a specific subtype of ADHD is unclear, because of the small numbers of cases, although the association was stronger for the predominantly hyperactive/impulsive subtype. This study should be generalizable to the US population because the NHANES sample is nationally representative, unlike previous studies of groups with higher exposure levels.^{11–15} With respect to the importance of these findings, organophosphates are among the most widely used pesticides, and the concentrations of DAP metabolites among children did not decrease from 2000 to 2003–2004.

The most important limitation of the present study is the assessment of organophosphate exposure through measurement of DAP metabolites in only 1 spot urine sample. Given that long-term exposure to organophosphates likely would be necessary to produce neurochemical changes causing ADHD-like behaviors, serial mea-

surements of urinary metabolites of organophosphates over a longer time period would provide a better assessment of average exposure, but the NHANES does not include longitudinal follow-up assessments. For organophosphates coming from the diet, the measurement of organophosphate metabolites in a single urine sample may reflect average exposure levels reasonably well, to the extent that diet is consistent. Given that organophosphates are eliminated from the body after 3 to 6 days,²⁸ the detection of DAPs in the urine of most children indicates continuing exposure. An additional consideration is that urinary DAP levels might reflect not only exposure to organophosphates but also direct exposure to DAPs present in the environment, resulting from degradation of organophosphates through hydrolysis or photolysis. Significant amounts of DAPs have been found on several types of fruits and vegetables.²⁹ In any case, misclassification of exposure on the basis of measurements of urinary DAP levels should be nondifferential and should bias effect estimates toward the null.

Given the cross-sectional nature of our analysis, we cannot rule out the possibility that children with ADHD engage in behaviors that expose them to higher levels of organophosphates. If this were the case, however, we would have expected to see higher levels of urinary DEAP metabolites as well, which was not the case. Another limitation is measurement error, in that the concentrations of individual DMAP metabolites were below the analytical limit of detection for large proportions of children. This problem, however, does not apply to the analysis showing that children with levels higher than the median of detectable dimethyl thiophosphate concentrations were twice as likely to be diagnosed as having

ADHD as were those with undetectable concentrations.

The present study uses a larger sample size than previous investigations on neurodevelopmental effects of organophosphate exposure, as well as DSM-IV–based diagnostic outcomes. Comparisons across studies are difficult because of differences in exposure levels, timing of exposure, outcomes assessed, and age at assessment. Higher blood chlorpyrifos concentrations during pregnancy were found to be associated with poorer mental and motor development at 3 years,¹¹ and greater postnatal exposure to organophosphates was associated with difficulties with memory, attention, motor tasks, behavior,¹⁴ and reaction time.¹³ Prenatal exposure to organophosphates also was associated with poorer mental development at 2 years of age and, as in our study, the association was with DMAP rather than DEAP metabolites.¹² The stronger association with DMAP metabolites could be explained by greater exposure to organophosphates metabolized into DMAP metabolites, or it might indicate greater toxicity of these organophosphates.

Several biological mechanisms might underlie an association between organophosphate pesticides and ADHD. A primary action of organophosphates, particularly with respect to acute poisoning, is inhibition of acetylcholinesterase,³⁰ and disruptions in cholinergic signaling are thought to occur in ADHD.³¹ At doses lower than those needed to inhibit acetylcholinesterase, certain organophosphates affect different neurochemical targets, including growth factors, several neurotransmitter systems, and second-messenger systems.^{32,33} Exposure to some of these organophosphate compounds was shown to cause hyperactivity and cognitive deficits in animal studies.^{34,35} Developmental exposure to

organophosphates might have persistent effects on multiple neural systems that may underlie ADHD behaviors, such as inattention and cognitive deficits, similar to the effects of developmental nicotine exposure.^{36,37}

CONCLUSIONS

The present study adds to the accumulating evidence linking higher levels of

pesticide exposure to adverse developmental outcomes. Our findings support the hypothesis that current levels of organophosphate pesticide exposure might contribute to the childhood burden of ADHD. Future studies should use a prospective design, with multiple urine samples collected over time for better assessment of chronic exposure and critical windows of exposure,

and should establish appropriate temporality.

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REFERENCES

1. US Environmental Protection Agency. Pesticide reregistration status for organophosphates. Available at: www.epa.gov/pesticides/reregistration/status_op.htm. Accessed January 15, 2010
2. Cohen Hubal EA, Sheldon LS, Burke JM, et al. Children's exposure assessment: a review of factors influencing Children's exposure, and the data available to characterize and assess that exposure. *Environ Health Perspect*. 2000;108(6):475–486
3. US National Research Council, Committee on Pesticides in the Diets of Infants and Children. *Pesticides in the Diets of Infants and Children*. Washington, DC: National Academy Press; 1993
4. US Department of Agriculture, Agricultural Marketing Service. *Pesticide Data Program: Annual Summary, Calendar Year 2008*. Washington, DC: US Department of Agriculture; 2009. Available at: www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5081750. Accessed January 15, 2010
5. Weiss B. Vulnerability of children and the developing brain to neurotoxic hazards. *Environ Health Perspect*. 2000;108(suppl 3):375–381
6. Barr DB, Bravo R, Weerasekera G, et al. Concentrations of dialkyl phosphate metabolites of organophosphorus pesticides in the US population. *Environ Health Perspect*. 2004;112(2):186–200
7. Furlong CE, Holland N, Richter RJ, Bradman A, Ho A, Eskenazi B. *PON1* status of farmworker mothers and children as a predictor of organophosphate sensitivity. *Pharmacogenet Genomics*. 2006;16(3):183–190
8. Holland N, Furlong C, Bastaki M, et al. Paraoxonase polymorphisms, haplotypes, and enzyme activity in Latino mothers and newborns. *Environ Health Perspect*. 2006;114(7):985–991
9. Rosas LG, Eskenazi B. Pesticides and child neurodevelopment. *Curr Opin Pediatr*. 2008;20(2):191–197
10. Jurewicz J, Hanke W. Prenatal and childhood exposure to pesticides and neurobehavioral development: review of epidemiological studies. *Int J Occup Med Environ Health*. 2008;21(2):121–132
11. Rauh VA, Garfinkel R, Perera FP, et al. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. *Pediatrics*. 2006;118(6). Available at: www.pediatrics.org/cgi/content/full/118/6/e1845
12. Eskenazi B, Marks AR, Bradman A, et al. Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. *Environ Health Perspect*. 2007;115(5):792–798
13. Grandjean P, Harari R, Barr DB, Debes F. Pesticide exposure and stunting as independent predictors of neurobehavioral deficits in Ecuadorian school children. *Pediatrics*. 2006;117(3). Available at: www.pediatrics.org/cgi/content/full/117/3/e546
14. Ruckart PZ, Kakolewski K, Bove FJ, Kaye WE. Long-term neurobehavioral health effects of methyl parathion exposure in children in Mississippi and Ohio. *Environ Health Perspect*. 2004;112(1):46–51
15. Rohlman DS, Arcury TA, Quandt SA, et al. Neurobehavioral performance in preschool children from agricultural and non-agricultural communities in Oregon and North Carolina. *Neurotoxicology*. 2005;26(4):589–598
16. Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey: questionnaires, data sets, and related documentation. Available at: www.cdc.gov/nchs/nhanes/about_nhanes.htm. Accessed April 6, 2010
17. Vohr BR, Wright LL, Dusick AM, et al. Neurodevelopmental and functional outcomes of extremely low birth weight infants in the National Institute of Child Health and Human Development Neonatal Research Network, 1993–1994. *Pediatrics*. 2000;105(6):1216–1226
18. Shaffer D, Fisher P, Lucas CP, Dulcan MK, Schwab-Stone ME. NIMH Diagnostic Interview Schedule for Children Version IV (NIMH DISC-IV): description, differences from previous versions, and reliability of some common diagnoses. *J Am Acad Child Adolesc Psychiatry*. 2000;39(1):28–38
19. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 4th ed. Washington, DC: American Psychiatric Association; 1994
20. Bravo M, Ribera J, Rubio-Stipec M, et al. Test-retest reliability of the Spanish version of the Diagnostic Interview Schedule for Children (DISC-IV). *J Abnorm Child Psychol*. 2001;29(5):433–444
21. Canino G, Shrout PE, Rubio-Stipec M, et al. The DSM-IV rates of child and adolescent disorders in Puerto Rico: prevalence, correlates, service use, and the effects of impairment. *Arch Gen Psychiatry*. 2004;61(1):85–93
22. Lahey BB, Applegate B, McBurnett K, et al. DSM-IV field trials for attention deficit hyperactivity disorder in children and adolescents. *Am J Psychiatry*. 1994;151(11):1673–1685
23. Shaffer D, Fisher P, Lucas C, Comer J. *Scoring Manual: Diagnostic Interview Schedule for Children (DISC-IV)*. New York, NY: New York State Psychiatric Institute, Columbia University; 2007
24. Bravo R, Caltabiano LM, Weerasekera G, et al. Measurement of dialkyl phosphate metabolites of organophosphorus pesticides in human urine using lyophilization with gas chromatography-tandem mass spectrometry and isotope dilution quantification. *J Expo Anal Environ Epidemiol*. 2004;14(3):249–259
25. Hornung RW, Reed DL. Estimation of average concentration in the presence of nondetectable values. *Appl Occup Environ Hyg*. 1990;5:46–51

26. Barr DB, Bravo R, Weerasekera G, et al. Concentrations of dialkyl phosphate metabolites of organophosphorus pesticides in the U.S. population. *Environ Health Perspect.* 2004;112:186–200
27. Cole SR, Chu H, Nie L, Schisterman EF. Estimating the odds ratio when exposure has a limit of detection. *Int J Epidemiol.* 2009;38(6):1674–1680
28. Bradway DE, Shafik TM, Lores EM. Comparison of cholinesterase activity, residue levels, and urinary metabolite excretion of rats exposed to organophosphorus pesticides. *J Agric Food Chem.* 1977;25(6):1353–1358
29. Zhang X, Driver JH, Li Y, Ross JH, Krieger RI. Dialkylphosphates (DAPs) in fruits and vegetables may confound biomonitoring in organophosphorus insecticide exposure and risk assessment. *J Agric Food Chem.* 2008;56(22):10638–10645
30. Sultatos LG. Mammalian toxicology of organophosphorus pesticides. *J Toxicol Environ Health.* 1994;43(3):271–289
31. Coccini T, Crevani A, Rossi G, et al. Reduced platelet monoamine oxidase type B activity and lymphocyte muscarinic receptor binding in unmedicated children with attention deficit hyperactivity disorder. *Biomarkers.* 2009;14(7):513–522
32. Verma SK, Kumar V, Gill KD. An acetylcholinesterase-independent mechanism for neurobehavioral impairments after chronic low level exposure to dichlorvos in rats. *Pharmacol Biochem Behav.* 2009;92(1):173–181
33. Slotkin TA, Seidler FJ. Comparative developmental neurotoxicity of organophosphates in vivo: transcriptional responses of pathways for brain cell development, cell signaling, cytotoxicity and neurotransmitter systems. *Brain Res Bull.* 2007;72(4–6):232–274
34. Timofeeva OA, Sanders D, Seemann K, et al. Persistent behavioral alterations in rats neonatally exposed to low doses of the organophosphate pesticide, parathion. *Brain Res Bull.* 2008;77(6):404–411
35. Timofeeva OA, Roegge CS, Seidler FJ, Slotkin TA, Levin ED. Persistent cognitive alterations in rats after early postnatal exposure to low doses of the organophosphate pesticide, diazinon. *Neurotoxicol Teratol.* 2008;30(1):38–45
36. Heath CJ, Picciotto MR. Nicotine-induced plasticity during development: modulation of the cholinergic system and long-term consequences for circuits involved in attention and sensory processing. *Neuropharmacology.* 2009;56(suppl 1):254–262
37. Slotkin TA. Cholinergic systems in brain development and disruption by neurotoxicants: nicotine, environmental tobacco smoke, organophosphates. *Toxicol Appl Pharmacol.* 2004;198(2):132–151

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