Myopia and Exposure to Organophosphate and Pyrethroid Pesticides in the General United States Population

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Purpose. Previous research suggests that exposure to pesticides might be associated with human myopia, although data were obtained only from highly exposed individuals. The present study aimed to assess whether exposure to organophosphates and pyrethroids in the United States general population was associated with the prevalence of myopia.

Methods. Data were obtained from the National Health and Nutrition Examination Survey (NHANES, years 1999–2008). One-spot urine samples were used to estimate the concentration of several pesticide metabolites. Exposure data and equivalent spherical refraction errors were available for 5147 and 2911 individuals for organophosphates and pyrethroids, respectively. Multiple logistic regression models were used to assess the relation between log10-transformed urinary levels of pesticide metabolites and the risk of moderate (≤ -1 and > -5 diopters [D]) and high myopia (≤ -5 D) in adolescents (12- to 19-years old) and young adults (20- to 40-years old). Models were adjusted for sex, age, ethnicity, diabetes, creatinine, cadmium and lead concentrations, and income in both age groups, but also for education level and cigarette and alcohol consumption in the adult group.

Results. No association between organophosphates or pyrethroid metabolites and myopia was observed. However, after adjusting for education level and cigarette and alcohol consumption, a statistically significant decreased risk of high myopia in those with a 10-fold increase of dialkyl phosphate metabolites was found in adults but only in men ($P < 0.05$).

Conclusions. Our results suggest that exposure to organophosphates or pyrethroids do not increase the risk of myopia in the United States general population.

Keywords: myopia, organophosphates, pyrethroids, pesticide, NHANES

The prevalence of myopia (near-sightedness) is increasing drastically worldwide. In Asia, up to 90% of teenagers and young adults are myopic.[1] This dramatic increase has also been observed in Europe and the United States, where the prevalence of myopia has doubled in the last half century, and is now affecting approximately half of young adults.[2] As a result, one-third of the world population will likely be affected by myopia by 2020[1] and half by 2050.[2] These statistics demonstrate the need to investigate the causes of this phenomenon. It is known that genetics influence myopia; more than 100 regions of the genome have been associated with this condition.[1] However, this cannot fully explain the rapid increase of myopia observed worldwide and so it is likely that environmental factors are involved. Some interesting and intriguing data suggest that children who spent less time outside were at greater risk of developing myopia.[3,4] Other studies revealed a positive correlation between education level and myopia, suggesting that close work is involved in the development of this condition.[5,6] Another factor that has been associated with myopia is exposure to pesticides. For instance, in a rural community of Japan, high exposure to organophosphates was associated with a condition called the Saku disease, in which myopia was one of its major symptoms.[7]

Organophosphates are one of the most used classes of pesticides worldwide. They are used for crop protection, control of vector-borne disease, and residential pest control.[8] The primary source of exposure in humans is from pesticide residues on food. Studies on the impact of organophosphate exposure on children's health have mostly focused on the development of cognitive and motor functions. Prenatal exposure has been associated with mental development delays and lower intelligence quotient, attention deficit/hyperactivity disorder (ADHD)-like problems, and symptoms consistent with pervasive developmental disorder.[9–12] Postnatal exposure consequences include parent-reported problems with motor skills and behavior, poorer short-term memory and attention,[13] slower motor speed,[14] longer reaction time,[15] and ADHD in children.[16] Regarding ocular function, studies on a highly exposed population suggested that organophosphates may cause myopia.[7,17] Because these pesticides are known to increase levels of acetylcholine (ACh), it was suggested that this effect results of an over-stimulation of the muscarinic and nicotinic pathways. In support with this hypothesis, experi-

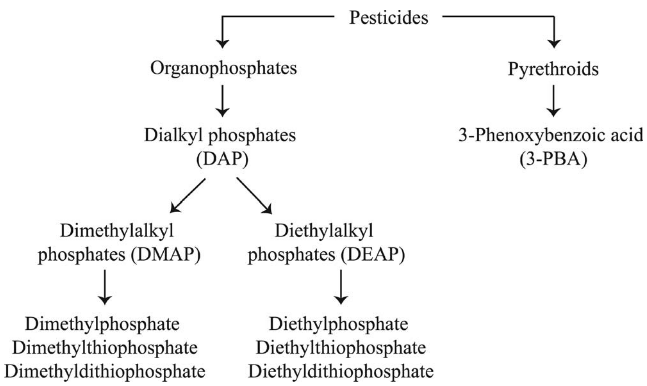

Figure. Graphical summary of the urinary metabolites used to estimate exposure to organophosphates and pyrethroids.

mental studies show that muscarinic agonists may increase myopia by increasing eye elongation,[18] whereas muscarinic and nicotinic antagonists clearly prevent myopia.[19–22] In humans, many studies reported that the muscarinic antagonist atropine is effective at preventing myopia in children.[23–26]

As mentioned above, the most known toxic mechanism of organophosphates is inhibition of acetyl cholinesterase (AChE), causing an accumulation of acetylcholine (ACh) in the synapses. Increasing concerns regarding organophosphates toxicity has driven the gradual replacement of these chemicals by pyrethroids, which now represent more than 30% of the amount of pesticides used in the recent years.[27] Residues on food and household pest control products are important sources of pyrethroid exposure.[28] The toxicologic effects of pyrethroids result from the alteration of the permeability of the sodium mediated ion channel in excited nerve cells, causing repetitive nerve impulses.[29] The symptoms of an accidental pyrethroid poisoning are well documented, and include blurry vision.[30,31] However, there seem to have been no studies assessing the role of common pyrethroid exposure on vision impairment. The purpose of the present study was to determine if exposure to organophosphates and pyrethroids is associated with myopia in the men and women of the general United States population.

Methods

Study Design

The National Health and Nutrition Examination Survey (NHANES) is a cross-sectional, population-based health survey of noninstitutionalized United States residents conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention.[32] This survey assesses the health of the United States general population by measuring several hundred variables on roughly 5000 subjects each year. NHANES uses a complex multistage probability sampling design, with oversampling of certain subgroups. Participants completed household surveys that included questions about demographics and health history. A certain proportion of participants underwent additional physical examinations, including visual function in a mobile center. Those participants also provided blood and urine samples for laboratory analyses, including measurement of environmental chemicals. NHANES was approved by the National Center for Health Statistics institutional review board, and all participants provided written informed consent. This study adhered to the tenets of the Declaration of Helsinki.

Study Population

The 1999 to 2008 NHANES cycles were used. Note that the measurements of the metabolite associated with pyrethroids exposure (3-phenoxybenzoic acid) were not available in the 2003 to 2004 and 2005 to 2006 cycles due to unacceptable measurement variance at or near the limit of detection. The present study was conducted on individuals aged 12 to 40 years. The upper limit (40-years old) was chosen to avoid age-related ocular confounders that could influence refraction such as the development of cataracts.[33] Data for organophosphates were available from 6517 subjects. However, the sample used for the analysis included 5147 subjects (79%). Subject exclusion is explained by: missing measurement of organophosphate exposure ($n = 264$), missing data for autorefractor ($n = 296$), eye surgery for near-sightedness or cataract ($n = 41$), missing data for one or several covariables ($n = 723$), or presenting moderate-to-high hyperopia ($n = 46$). Data for pyrethroids were available from 3799 subjects. The sample used for the analysis included 2911 subjects (77%). Subject exclusion is explained by: missing measurement of pyrethroid exposure ($n = 190$), missing data for autorefractor ($n = 154$), eye surgery for near-sightedness or cataract ($n = 25$), missing data for one or several covariables ($n = 495$), or presenting moderate-to-high hyperopia ($n = 24$).

Measurement of Objective Refraction

A NIDEK ARK-760 autorefractor (Nidek Co. Ltd., Tokyo, Japan) was used to measure the refractive error of each eye after removing corrective lenses. Because the NHANES protocol did not include cycloplegic refraction (see NHANES website[34] for the complete procedure of vision examination), we were limited in the present study to using noncycloplegic autorefraction data. As a result, despite the auto fogging used by the autorefractor to minimize accommodation, the measurements of refractive error may have been influenced by accommodation, particularly in younger participants. Three separate measurements of sphere, cylinder, and axis were acquired. Refractive errors were recorded in plus cylinder notation, and spherical equivalent (SphEq) was computed as the sphere measurement + half the cylinder measurement. Participants were categorized based on the refractive error measurement from the eye with the larger absolute value of SphEq. If one eye had surgery or was missing the refractive error measure, data from the other eye were used. Myopia was defined as SphEq less than or equal to −1.00 diopter (D). Because the nature and the consequences of myopia may vary as a function of its severity, the dependent variable for the present study was divided into two categories, that is moderate myopia (SphEQ ≤ -1.00 and > -5.00 D), and high myopia (SphEQ ≤ -5.00 D).[35] The reference group consisted of the subjects presenting emmetropia/low hyperopia, which was defined as a SphEq greater than −1.00 and less than 3.00. Therefore, subjects with moderate-to-high hyperopia (SphEq > 3.00 D) were excluded. The rational for excluding those subjects was to have a reference group in the normal range vision (i.e., that do not present a "disease") as it is commonly done in epidemiology.

Measurements of Urinary Pesticides

During the NHANES physical examination, one-spot urine samples were collected, aliquoted, and stored cold (2°–4°C) or frozen until they were shipped on dry ice to the Centers for Disease Control and Prevention for analysis. Metabolite concentrations resulting from the degradation of organophosphates and pyrethroids were determined. These chemicals reflect recent exposure to the parent compounds because they

Table 1. Comparison of Subsample Characteristics Between the Whole, Analytical, and Excluded Samples (NHANES 1999–2008, Individuals 12- to 40-Years of Age)

	Organophosphate Samples			Pyrethroid Samples		
	Whole ($N = 6517$)	Analytical ($N = 5147$)	Excluded ($N = 1370$)	Whole ($N = 3799$)	Analytical ($N = 2911$)	Excluded ($N = 888$)
Age, y						
Mean (SD)	22.2 (8.5)	22. 2 (8.5)	22.2 (8.6)	22.4 (8.6)	22.3 (8.6)	22.5 (8.7)
Interquartile range	14	14	14	14	14	15
Sex, %						
Male	47.2	47.4	46.4	47.0	46.8	48.3
Female	52.8	52.6	53.6	53.0	53.2	51.7
Ethnicity, %						
Mexican American	29.4	29.0	30.8	29.8	30.1	28.7
Non-Mexican Hispanic	5.7	5.7	5.7	7.3	7.2	7.5
Non-Hispanic White	34.0	35.2	29.5	33.9	35.5	28.8
Non-Hispanic Black	26.1	25.4	29.1	24.3	22.6	30.0
Other*	4.7	4.7	4.9	4.7	4.6	5.0
PIR, %						
<1.20	33.8	33.6	34.2	34.6	33.9	38.4
1.20–2.34	25.5	25.5	26.1	25.7	26.0	23.8
2.35–4.28	23.8	24.0	23.1	23.0	23.4	20.8
4.29–5.00	16.8	16.9	16.6	16.8	16.7	17.0
Diabetes, %						
Yes	1.2	1.2	1.1	1.1	1.0	1.2
No	98.8	98.8	98.9	98.9	99.0	98.8
Refraction, D						
Mean (SD)	−0.98 (2.2)	−1.06 (2.1)	−0.60 (2.2)	−0.95 (2.1)	−1.02 (1.8)	−0.60 (2.2)

* Persons reporting their race/ethnicity as Chinese, Japanese, Korean, Asian Indian, Southeast Asian, Native American, Pacific Islander, or multiracial.

are quickly metabolized and excreted, with half-lives of 3 hours to a few days.[36,37] The Figure shows the associated metabolites for each pesticide type (organophosphates and pyrethroids). The estimation of organophosphate exposure was determined from six dialkyl phosphate (DAP) metabolites, which are associated with exposure to 28 or more organophosphates. DAP included three dimethyl alkylphosphate (DMAP) molecules (dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate) and three diethyl alkylphosphate (DEAP) molecules (diethylphosphate, diethylthiophosphate, diethyldithiophosphate). The estimation of pyrethroids exposure, on the other hand, was determined from 3-phenoxybenzoic acid (3-PBA). This metabolite represents exposure to one or more of the following pesticides: permethrin, cypermethrin, deltamethrin, allethrin, resmethrin, fenvalerate, cyhalothrin, fenpropathrin, and tralomethrin.[28] DAP concentrations were measured by lyophilization and chemical derivatization, followed by analysis with isotope-dilution gas chromatography/tandem mass spectrometry.[38] Concentrations of 3-PBA were measured through HPLC/tandem mass spectrometry using validated laboratory methods previously described.[39] Urinary creatinine concentration was also measured, and used to account for urine dilution. This value was obtained using an automated colorimetric method based on a modified Jaffe reaction on a Beckman Synchron AS/ASTRA clinical analyzer (Beckman Instruments, Inc., Brea, CA, USA) at the Fairview University Medical Center (Minneapolis, MN, USA).[27]

Covariates

Models were adjusted for potential sources of confounders based on previous literature and associations with ocular function. Sex, age, and creatinine concentration (indicator of urine dilution[40]) were systematically entered in the models. Several other covariates were also considered and included in the models when correlated with the SphEQ at P less than 0.2: (1) ethnicity, categorized as Mexican American, Non-Mexican Hispanic, Non-Hispanic White, Non-Hispanic Black, Other/multiracial; (2) cigarette smoking status, categorized as never, former, and current; (3) weekly alcohol consumption, categorized as zero, more than zero to less than one, and one or more drinks (note that cigarette and alcohol consumptions were not available for participants below 20-years old because of the confidentiality of these data); (4) type-2 diabetes, defined as 'present' or 'not present' as self-reported physician diagnosis; (5) the poverty income ratio (PIR), which is an continuous index representing the ratio of the household income to the poverty threshold after accounting for family size and ranging from zero to five (lower values indicating lower socioeconomic status); (6) education level, defined as the highest degree completed (<9th grade, 9th–11th grade, high school graduate, associate degree, college degree, or above); (7) blood concentrations of cadmium and lead exposures; and (8) household smoking, defined as the total number of cigarettes smoked in home per day.

Data Analysis

The Complex Samples module of SPSS 24.0 (IBM, Armonk, NY, USA) was used to conduct analyses, accounting for the multistage probability sampling design of NHANES. Strata, primary sampling units, and sample weights were used to obtain robust linearized standard errors and unbiased point estimates. Weights were recalculated according to NHANES

Table 2. Concentrations of Urinary Metabolites Associated With Exposure to Organophosphate and Pyrethroid Pesticides (NHANES 1999–2008, individuals 12- to 40-years of age; organophosphate: $N = 5147$, pyrethroid: $N = 2911$)

Metabolites	Detection Limit	*n* Below Detection Limit (%)	*n* Above Detection Limit (%)	Geometric Mean	Interquartile Range	Maximum
Organophosphate, nM						
Dimethylphosphate	3.7	2852 (55.4)	2295 (44.6)	6.4	32.0	2975
Dimethylthiophosphate	3.9	1753 (34.1)	3394 (65.9)	11.3	38.2	23476
Dimethyldithiophosphate	3.2	3720 (72.3)	1427 (27.7)	2.3	2.8	6997
Diethylphosphate	2.4	2887 (56.1)	2260 (43.9)	3.9	20.1	12753
Diethylthiophosphate	3.3	3003 (58.3)	2144 (41.7)	2.7	4.7	646
Diethyldithiophosphate	2.1	4742 (92.1)	405 (7.9)	0.9	1.1	183
∑Dimethyl phosphate	N/A	N/A	N/A	33.8	89.1	23480
∑Diethyl phosphate	N/A	N/A	N/A	12.0	25.2	12754
∑Dialkyl phosphate	N/A	N/A	N/A	57.2	121.0	23487
Pyrethroid (μg/L)						
3-phenoxybenzoic acid	0.1	740 (25.4)	2171 (74.6)	0.30	0.70	160

N/A, not applicable.

guidelines regarding the fusion of multiple NHANES cycles. The threshold for statistical significance was set at P less than 0.05. All statistical tests were two-sided.

DAP metabolite concentrations were divided by their respective molar weight to calculate the sum (i.e., sigma or ∑) of DAP, DMAP, and DEAP concentrations in nanomoles per liter. These sums are then referred to ∑DAP, ∑DMAP, and ∑DEAP. Because metabolite concentrations were skewed toward lower values (positive skew), log-transformations (base-10) were applied to normalize the distributions. Concentration values that were below detection limit (see Table 2) were imputed using a multiple imputation procedure,[41] which assumes that values between 0 and the detection limit were normally distributed so that the frequency of imputation of a certain value corresponded to the frequency that would be observed in a normal distribution. The same multiple imputation technique was used for missing values of the DAP metabolites, which ranged from 0.1% to 1.8% depending on the metabolite.

Logistic regression analysis was used to estimate odds ratios (ORs) and 95% confidence intervals (CI) of having moderate or high myopia following a 10-fold increase in ∑DAP, ∑DMAP, ∑DEAP, and 3-PBA. Sensitivity analyses were also conducted using a 2-, 3- and 5-fold increase of these same metabolites. In all logistic regression analyses, the reference group (emmetropia/low hyperopia) was defined as SphEq greater than −1.00 and less than 3.00. Analyses were conducted separately in young (12- to 19-years old) and adult participants (20- to 40-years old) for two reasons. First, some important confounding variables (education, cigarettes, and alcohol) could not be included in the regression models for participants less than 20 years. Second, it is likely that this younger generation is influenced differently by environmental factors including those thought to play a role in the climbing of myopia prevalence, such as the increase of close work and the decrease of the time spent outside.[2]

Results

As shown in Table 1, the whole samples ($n = 6517$ and 3799 for organophosphates and pyrethroids, respectively) and the analytical samples ($n = 5147$ and 2911 for organophosphates and pyrethroids, respectively) had very similar characteristics. No significant difference between these two samples (P values > 0.1) was observed for age, sex, ethnicity, PIR, diabetes, or objective refraction.

Descriptive Statistics on the Concentrations of Urinary Metabolites

Depending on the DAP metabolite, the proportion of values below the detection limit varied from 34.1% to 92.1% (Table 2). For 89% of the subjects, at least one DAP metabolite had a detectable concentration. The proportion of values below the detection limit for 3-PBA was 25.4%. In bivariate analysis, ∑DAP, ∑DMAP, ∑DEAP, and 3-PBA concentrations were correlated with creatinine concentration (r values between 0.06 and 0.12; $P \leq 0.001$). The mean concentration of ΣDMAP (geometric mean: 33.8 nM) was considerably higher than the ∑DEAP (geometric mean: 12.0 nM). The geometric mean of 3-PBA concentration was 0.30 μg/L.

Prevalence of Myopia and Levels of Urinary Pesticide Metabolites With Respect to Different Sample Characteristics

Tables 3 and 4 present the prevalence of myopia and pesticide metabolite concentrations according to the different subsample characteristics. The prevalence of moderate myopia ranged between 22.2% and 36.7% for the organophosphates sample (Table 3) and 20.1% and 37.9% for the pyrethroid sample (Table 4), while high myopia ranged between 1.2% and 14.7% and 0.0% and 14.9%, respectively. Females were more affected than males by moderate myopia (organophosphate: $\chi^2_{(1,5147)} = 4.36$, $P = 0.038$; pyrethroid: $\chi^2_{(1,2911)} = 4.94$, $P = 0.026$) and high myopia (organophosphate: $\chi^2_{(1,5147)} = 7.02$, $P = 0.008$; pyrethroid: $\chi^2_{(1,2911)} = 4.93$, $P = 0.026$). This effect was not due to pesticide exposure since both sexes had similar metabolite levels, either for DAP (males: mean [M] = 162.0, SD = 685.0; females: M = 175.0, SD = 633.5) ($t_{(5145)} = 1.777$, $P = 0.076$) or 3-PBA (males: M = 1.14, SD = 3.79; females: M = 1.24, SD = 5.49) ($t_{(2909)} = 0.500$, $P = 0.617$). The prevalence of high myopia differed according to ethnicity (organophosphate: $\chi^2_{(4,5146)} = 23.8$, $P < 0.001$; pyrethroid: $\chi^2_{(4,2910)} = 11.8$, $P = 0.019$) with the highest rate in non-Hispanic white and multiracial people. This was not explained by variations in pesticide exposure because the different levels of DAP ($F_{(4,5146)} = 5.5$, $P < 0.001$) and 3-PBA ($F_{(4,2910)} = 12.7$, $P < 0.001$) between ethnicities were not following myopia

Table 3. Urinary Dialkyl Phosphates Concentrations (nM) and Prevalence of Moderate Myopia (≤–1.00 D, >–5.00 D) or High Myopia (≤–5.00 D) (NHANES 1999–2008, Individuals 12- to 40-Years of Age; $N = 5147$)

	n (%)	Geometric mean (95%CI)*	Moderate Myopia (%)	High Myopia (%)
Sex				
Male	2439 (47.4)	55.3 (52.5–58.2)	653 (26.8)	123 (5.0)
Female	2708 (52.6)	59.0 (56.1–62.1)	796 (29.4)	184 (6.8)
P value		0.076	0.037	0.008
Ethnicity				
Mexican American	1495 (29.0)	58.1 (54.3–62.2)	417 (27.9)	63 (4.2)
Non-Mexican Hispanic	294 (5.7)	58.8 (50.7–68.2)	82 (27.9)	20 (6.8)
Non-Hispanic White	1811 (35.2)	52.8 (49.7–56.1)	524 (28.9)	129 (7.1)
Non-Hispanic Black	1305 (25.4)	64.5 (60.0–69.3)	355 (27.2)	69 (5.3)
Other/multiracial	242 (4.7)	48.0 (40.8–56.5)	71 (29.3)	26 (10.7)
P value		<0.001	0.852	< 0.001
PIR				
<1.20	1731 (33.6)	60.7 (57.0–64.6)	458 (26.5)	68 (3.9)
1.20–2.34	1310 (25.5)	55.4 (51.6–59.5)	329 (25.1)	84 (6.4)
2.35–4.28	1235 (24.0)	52.7 (48.9–56.8)	382 (30.9)	72 (5.8)
4.29–5.00	871 (16.9)	60.1 (55.2–65.5)	280 (32.1)	83 (9.5)
P value		0.017	<0.001	<0.001
Diabetes				
Yes	60 (1.2)	52.0 (35.7–75.7)	22 (36.7)	7 (11.7)
No	5087 (98.8)	57.3 (55.2–59.4)	1427 (28.1)	300 (5.9)
P value		0.573	0.140	0.061
Education Level†				
<9th grade	161 (6.7)	53.1 (43.5–64.9)	40 (24.8)	2 (1.2)
9th–11th grade	450 (18.7)	48.7 (43.2–55.0)	100 (22.2)	11 (2.4)
High school graduate	551 (22.9)	47.1 (42.4–52.4)	149 (27.0)	28 (5.1)
Associate degree	770 (32.0)	51.0 (46.6–55.9)	226 (29.4)	62 (8.1)
College degree	475 (19.7)	57.6 (51.1–65.0)	158 (33.3)	70 (14.7)
P value		0.135	0.003	<0.001

* Geometric mean of dialkyl phosphate concentration is presented with 95%CI in parentheses.
† Data for education level exclude subjects between 12- and 19-years old.

occurrence (Tables 3, 4). Furthermore, the prevalence of moderate myopia (organophosphate: $\chi^2_{(4,5147)} = 20.0$, $P < 0.001$; pyrethroid: $\chi^2_{(4,2911)} = 11.4$, $P = 0.010$) and high myopia (organophosphate: $\chi^2_{(4,5147)} = 33.0$, $P < 0.001$; pyrethroid: $\chi^2_{(4,2911)} = 16.7$, $P = 0.001$) differed depending on the socioeconomic status. Again, this was not explained by variations in pesticide exposure because the different levels of DAP ($F_{(4,5146)} = 3.4$, $P = 0.017$ and 3-PBA ($F_{(4,2910)} = 7.3$, $P = < 0.001$) between socioeconomic classes were not following myopia occurrence. In accordance with the literature, it is of interest to mention that myopia differed as a function of education level in the organophosphate (moderate: $\chi^2_{(4,5147)} = 15.7$, $P = 0.003$; high: $\chi^2_{(4,5147)} = 68.8$, $P < 0.001$) and pyrethroid samples (moderate: $\chi^2_{(4,2911)} = 12.7$, $P = 0.013$; high: $\chi^2_{(4,2911)} = 45.7$, $P < 0.001$).

Odds of Myopia With Respect to Pesticide Metabolite Concentrations

Table 5 presents ORs of having moderate or high myopia following a 10-fold increase of ∑DAP, ∑DMAP, ∑DEAP, or 3-PBA concentration for people between 12- and 19-years old. Regression models were adjusted for sex, age, ethnicity, diabetes, creatinine, cadmium and lead concentrations, as well as PIR (crude ORs are available in Supplementary Table S1). Either for crude or adjusted analyses, there was no significant association between any pesticide exposure and myopia, regardless of sex (male versus female).

Table 6 presents ORs of having moderate or high myopia following a 10-fold increase of ∑DAP, ∑DMAP, ∑DEAP, or 3-PBA concentrations for people between 20- and 40-years old. Regression models were adjusted for the same variables that were used for the 12- to 19-year age group: sex, age, ethnicity, diabetes, creatinine, cadmium and lead concentrations, as well as PIR (crude ORs are available in Supplementary Table S2). Again, there was no significant association between any pesticide exposure and myopia for both crude and adjusted data when the whole sample was considered. However, analyses stratified by sex revealed fewer cases of high myopia in adult males that approached the level of significance for ∑DAP and ∑DMAP (Table 6). To further investigate these associations, a sensitivity analysis was conducted by re-running the regression models with additional important confounders that is education level as well as alcohol and cigarette consumption (Table 7). Indeed, it is well known that those factors are implied in the causal pathway of many ocular disorders not only of refraction error, but also of other eye diseases such as cataracts and AMD.[42–44] Results showed, in men only, that high myopia significantly decreased following a 10-fold increase of ∑DAP (OR: 0.52; 95%CI: 0.29–0.96); this association was actually due to the contribution of ∑DMAP (OR: 0.52; 95%CI: 0.29–0.96).

Other sensitivity analyses were conducted (data not shown). First, all regressions were conducted considering 2-, 3- and 5-fold increase of pesticide metabolites. In all cases, results were the same as in the main analyses. For example, as observed for the 10-fold increase ∑DMAP, a 5-fold (OR: 0.64;

Table 4. Urinary 3-Phenoxybenzoic Acid Concentrations (ug/L) and Prevalence of Moderate Myopia (≤−1.00 D, >−5.00 D) or High Myopia (≤−5.00 D) (NHANES 1999–2008, Individuals 12–40 Years of Age; $N = 2911$)

	n (%)	Geometric Mean (95% CI)*	Moderate Myopia (%)	High Myopia (%)
Sex				
Male	1363 (46.8)	0.302 (0.278–0.329)	352 (25.8)	66 (4.8)
Female	1548 (53.2)	0.293 (0.271–0.319)	457 (29.5)	105 (6.8)
P value		0.617	0.026	0.026
Ethnicity				
Mexican American	876 (30.1)	0.249 (0.226–0.275)	236 (26.9)	36 (4.1)
Non-Mexican Hispanic	211 (7.2)	0.230 (0.186–0.284)	65 (30.8)	12 (5.7)
Non-Hispanic White	1033 (35.5)	0.291 (0.260–0.324)	294 (28.5)	74 (7.2)
Non-Hispanic Black	657 (22.6)	0.430 (0.384–0.480)	187 (28.)	36 (5.5)
Other/multiracial	134 (4.6)	0.283 (0.210–0.381)	27 (20.1)	13 (9.7)
P value		<0.001	0.235	0.019
PIR				
<1.20	986 (33.9)	0.342 (0.311–0.377)	249 (25.3)	39 (4.0)
1.20–2.34	757 (26.0)	0.322 (0.289–0.358)	196 (25.9)	44 (5.8)
2.35–4.28	680 (23.4)	0.257 (0.224–0.294)	216 (31.8)	43 (6.3)
4.29–5.00	488 (16.8)	0.245 (0.211–0.284)	148 (30.3)	45 (9.2)
P value		<0.001	0.010	0.001
Diabetes				
Yes	29 (1.0)	0.416 (0.246–0.701)	11 (37.9)	2 (6.9)
No	2882 (99.0)	0.297 (0.280–0.315)	798 (27.7)	169 (5.9)
P value		0.264	0.221	0.814
Education Level†				
<9th grade	93 (6.7)	0.284 (0.205–0.394)	22 (23.7)	0 (0.0)
9th–11th grade	274 (19.7)	0.315 (0.264–0.376)	61 (22.3)	5 (1.8)
High school graduate	325 (23.4)	0.314 (0.264–0.374)	80 (24.6)	17 (5.2)
Associate degree	441 (15.1)	0.269 (0.230–0.316)	132 (29.9)	36 (8.2)
College degree	255 (8.8)	0.284 (0.227–0.355)	87 (34.1)	38 (14.9)
P value		0.667	0.013	< 0.001

* Geometric mean of 3-phenoxybenzoic acid concentration is presented with 95%CI in parentheses.
† Data for education level exclude subjects between 12- and 19-years old.

95%CI: 0.42–0.97), 3-fold (OR: 0.74; 95%CI: 0.55–0.98), and even 2-fold (OR: 0.82; 95%CI: 0.69–0.99) increase $\sum$DMAP were still significantly associated with a decreased prevalence of high myopia. Second, analyses were conducted accounting for passive smoking, which was done by adding household smoking in the regression models. The addition of this covariate did not change the results. Third, we tested whether the period of the year (November to April versus May to October) during which the urinary sample was taken could affect the results. Again, the addition of this covariate in the models did not affect the results. Finally, the results remained the same when subjects presenting moderate-to-high hyperopia were included in the reference group.

Discussion

The present study showed that a 10-fold increase of urinary metabolites resulting from organophosphate or pyrethroid exposure was not significantly associated with myopia in the adolescent or young adult general population. Both for the whole sample and the sex-stratified samples, similar regression results were found, before and after adjustments for control variables (age, ethnicity, diabetes, creatinine, cadmium and lead concentrations, PIR). Analyses in the adult group that included further adjustment for education, cigarette, and alcohol consumption revealed a significant decreased risk of high myopia in men, such that a 10-fold increase of $\sum$DAP and $\sum$DMAP both resulted in a 48% lower odds of presenting high myopia (Table 7). It was impossible to apply the same approach to the 12- to 19-year-old group because the education level was too strongly correlated with age, and cigarette and alcohol consumption data were confidential, and therefore not publicly available. However, considering the modest changes of the odds ratios in the adult group (i.e., from 0.57/0.58 [Table 6] to 0.52/0.52 after adjustment [Table 7]), it is very unlikely that the small odds ratios observed in the adolescents (Table 5) would have reached the level of statistical significance. Among all the sensitivity analyses, none showed changes in the results, including the one that included moderate-to-high hyperopia subjects in the reference group, which is not surprising considering the very small number of those subjects (organophosphate sample: $n = 46$, pyrethroid sample: $n = 24$).

The fact that a significant difference in myopia prevalence following an increase of $\sum$DAP and $\sum$DMAP concentrations was only found in men is challenging to explain. This apparent sex discrepancy might be explained by differential mechanisms of action of organophosphates. For instance, we know from epidemiologic studies[10,45,46] and experimental studies[47,48] that exposure to organophosphates can affect the development of the brain in different ways as a function of sex. Although the causes of such sex differences remain unclear, organophosphate exposure has been shown to disrupt sex hormones.[49–52]

Another challenging finding to explain is that the only significant result was a decreased prevalence of high myopia in relation to organophosphate exposure. By contrast, seminal works in Japan in the 1960's describing the so-called Saku disease suggested that high exposure to organophosphates

Table 5. Adjusted Odds Ratios for Adolescents of Having Moderate Myopia (≤−1.00 D, >−5.00 D) or High Myopia (≤−5.00 D) Following a 10-Fold Increase in Urinary Concentration of Dialkyl Phosphate or 3-Phenoxybenzoic Acid (NHANES 1999–2008, Individuals 12–19 Years of Age; Dialkyl Phosphate: $N = 2740$, 3-Phenoxybenzoic Acid: $N = 1523$)

	Moderate Myopia		High Myopia	
	OR Adjusted* (95%CI)	*P*	OR Adjusted* (95%CI)	*P*
Dialkyl phosphate				
All	1.10 (0.85–1.43)	0.458	0.91 (0.62–1.35)	0.641
Male	1.05 (0.76–1.46)	0.764	0.89 (0.46–1.75)	0.739
Female	1.18 (0.84–1.65)	0.344	0.91 (0.49–1.69)	0.755
Dimethylalkyl phosphate				
All	1.09 (0.88–1.34)	0.446	0.88 (0.62–1.27)	0.496
Male	1.00 (0.78–1.30)	0.975	1.05 (0.61–1.80)	0.867
Female	1.20 (0.90–1.60)	0.219	0.77 (0.44–1.34)	0.345
Diethylalkyl phosphate				
All	0.99 (0.76–1.29)	0.919	1.08 (0.67–1.74)	0.739
Male	0.93 (0.67–1.29)	0.639	0.88 (0.40–1.93)	0.749
Female	1.07 (0.78–1.48)	0.661	1.27 (0.66–2.45)	0.463
3-phenoxybenzoic acid				
All	0.85 (0.65–1.11)	0.225	1.03 (0.49–2.15)	0.945
Male	0.74 (0.53–1.05)	0.094	0.81 (0.41–1.61)	0.540
Female	0.96 (0.66–1.38)	0.802	1.19 (0.41–3.39)	0.745

* Adjusted for sex, age, ethnicity, PIR, diabetes, and creatinine cadmium and lead concentrations.

Table 7. Adjusted Odds Ratios for Young Adults of Having Moderate Myopia (≤−1.00 D, >−5.00 D) or High Myopia (≤−5.00 D) Following a 10-Fold Increase in Urinary Concentration of Dialkyl Phosphate or 3-Phenoxybenzoic Acid (NHANES 1999–2008, Individuals 20–40 Years of Age; Dialkyl Phosphate: $N = 2407$, 3-Phenoxybenzoic Acid: $N = 1388$)

	Moderate Myopia		High Myopia	
	OR Adjusted* (95%CI)	*P*	OR Adjusted* (95%CI)	*P*
Dialkyl phosphate				
All	1.03 (0.84–1.25)	0.807	0.76 (0.50–1.15)	0.188
Male	1.07 (0.80–1.44)	0.631	0.52 (0.29–0.96)	0.035
Female	0.97 (0.76–1.25)	0.831	0.99 (0.61–1.60)	0.965
Dimethylalkyl phosphate				
All	1.01 (0.84–1.22)	0.926	0.77 (0.52–1.13)	0.181
Male	1.09 (0.83–1.43)	0.529	0.52 (0.29–0.96)	0.037
Female	0.92 (0.74–1.15)	0.465	0.98 (0.64–1.49)	0.911
Diethylalkyl phosphate				
All	0.94 (0.75–1.18)	0.582	1.00 (0.72–1.40)	0.994
Male	0.97 (0.71–1.32)	0.840	0.87 (0.53–1.44)	0.582
Female	0.92 (0.66–1.27)	0.598	1.17 (0.73–1.90)	0.508
3-phenoxybenzoic acid				
All	0.96 (0.76–1.22)	0.758	1.05 (0.72–1.53)	0.802
Male	1.02 (0.73–1.43)	0.885	1.35 (0.77–2.37)	0.285
Female	0.88 (0.65–1.20)	0.421	0.89 (0.55–1.45)	0.639

* Adjusted for sex, age, ethnicity, diabetes, poverty income ratio, creatinine cadmium and lead concentrations, cigarette and alcohol consumptions, and education level.

increased myopia.[7] Because these pesticides are known to increase levels of ACh, this effect is thought to be related to alterations of the muscarinic and nicotinic processes in the eye.[17] In support of this hypothesis, several experimental studies have clearly demonstrated the crucial role of the muscarinic and nicotinic pathways in the process of eye elongation and therefore myopia.[20–22] Moreover, it has been clearly demonstrated that the muscarinic antagonist atropine is effective at preventing myopia in children.[23–26] Despite being in contradiction with the notion that organophosphates may increase myopia, our results are nevertheless in agreement with some experimental research in chicks. In a study using chlorpyrifos, an organophosphate pesticide widely used in the United States, deprivation-induced myopic animals showed shorter vitreal chambers and were thus less myopic after exposure (sex was not specified in that study).[53] Decreased myopia was also found in a similar experimental model following exposure to diisopropylfluorophosphate, an indirect cholinomimetic.[54] The mechanisms underlying these atypical (reverse) results are unclear. First, it has been shown that the effect of some myopigenic compounds might differ depending on dose. For instance, intravitreal drug exposure to reserpine in chicks can make the eye more myopic at high doses (>1000 nmol) but less myopic at low doses (<100 nmol).[55] Second, the over-stimulation of the muscarinic pathway can lead to its desensitization because of a decrease in the number of muscarinic receptors.[56] Third, a possible mechanism to account for a decrease of high myopia as a function of exposure to organophosphates is through thyroid dysfunction. Indeed, it is known that these pesticides can alter thyroid function by decreasing the metabolic concentration of T3.[57–59] In parallel, some studies in patients with hyperthyreosis (excessive production of thyroid hormone) reported the presence of high myopia.[60–62]

Table 6. Adjusted Odds Ratios for Young Adults of Having Moderate Myopia (≤−1.00 D, >−5.00 D) or High Myopia (≤−5.00 D) Following a 10-Fold Increase in Urinary Concentration of Dialkyl Phosphate or 3-Phenoxybenzoic Acid (NHANES 1999–2008, Individuals 20–40 Years of Age; Dialkyl Phosphate: $N = 2407$, 3-Phenoxybenzoic Acid: $N = 1388$)

	Moderate Myopia		High Myopia	
	OR Adjusted* (95%CI)	*P*	OR Adjusted* (95%CI)	*P*
Dialkyl phosphate				
All	1.07 (0.88–1.30)	0.512	0.84 (0.56–1.26)	0.396
Male	1.10 (0.84–1.45)	0.473	0.57 (0.32–1.04)	0.067
Female	1.02 (0.80–1.30)	0.865	1.07 (0.67–1.72)	0.765
Dimethylalkyl phosphate				
All	1.05 (0.87–1.26)	0.615	0.86 (0.59–1.24)	0.417
Male	1.12 (0.870–1.44)	0.378	0.58 (0.32–1.03)	0.065
Female	0.97 (0.78–1.21)	0.781	1.07 (0.70–1.61)	0.759
Diethylalkyl phosphate				
All	0.95 (0.76–1.19)	0.659	1.02 (0.74–1.41)	0.915
Male	0.97 (0.72–1.31)	0.845	0.86 (0.54–1.37)	0.531
Female	0.94 (0.68–1.28)	0.677	1.16 (0.74–1.83)	0.522
3-phenoxybenzoic acid				
All	0.95 (0.74–1.21)	0.655	1.00 (0.69–1.45)	0.997
Male	1.05 (0.75–1.46)	0.788	1.23 (0.67–2.27)	0.491
Female	0.87 (0.62–1.21)	0.390	0.87 (0.54–1.41)	0.569

* Adjusted for sex, age, ethnicity, poverty income ratio, diabetes, creatinine cadmium and lead concentrations.

Alternatively, one cannot exclude the possibility that the statistically significant associations discussed above are false positives, although their effect sizes were the largest compared with the others, and the same pattern of results was observed for crude and adjusted analyses. One explanation that could account for spurious results is the influence of intermediary factors. For instance, fruits and vegetables are known to be a source of DAP,[63] but they are also known to be a source of nutrients that may be beneficial to ocular function.[64] As a consequence, fruits and vegetables consumption, which was not considered in the present study, could confound our results by increasing DAP concentrations and possibly preventing high myopia, although at this time, there are no studies showing a protective effect of antioxidants on myopia. Another potential confounder that could have affected the results is passive smoking. Indeed, it has been reported that household smoking is associated with more hypermetropic objective refractions in children,[65,66] which could result from an alteration of the ocular nicotinic pathways. However, our sensitivity analyses showed that household smoking did not influence our results.

The first and main limitation of this study is associated with the NHANES databank, which consists of a cross-sectional population-based survey. In fact, the NHANES design only allows establishing associations between factors with no regard to the underlying mechanisms. Furthermore, additional concerns apply when using a one-spot urine sample to estimate pesticide exposure because the latter can change relatively quickly over time (e.g., summer versus winter). To minimize this limitation, one should control for the period of the year during which the urinary sample was obtained but this information was available only through two categories (i.e., either November to April or May to October). Of note, our sensitivity analyses using this variable showed no evidence of confounding effects. In the same vein, the measured metabolites have half-lives ranging from a few hours to a few days,[36,37] whereas myopia takes years to develop. The present study, as well as any NHANES study investigating other developmental disorders such as the attention deficit/hyperactivity disorder,[16,67,68] must thus rely on the assumption that diet, occupation, and environment are in general stable for most people, and that therefore a single urine sample can reasonably reflect the average exposure, at least for some years. Our results must be taken with caution because we do not know earlier environmental conditions in which myopia was developing during childhood (i.e., the nature of activities, and thus exposure may have changed since then). A second limitation is that a part of DAP exposure might be the result of direct exposure to these metabolites instead of the degradation of organophosphates. However, it was assumed that this effect should affect the whole sample equally, and therefore, should not significantly affect the global results. A third limitation is the proportion of values below the detection limit (see Table 1), which results in a considerable number of imputed values, although 89% of the subjects had at least one detectable DAP metabolite in their urine sample.

To our knowledge, the present research is the first to show that common exposure to organophosphate pesticides does not have a significant impact on the prevalence of myopia in the general United States population. We also showed that exposure to pyrethroids, a class of pesticide that often is used to replace organophosphates and is very extensively used worldwide, was not associated with myopia. However, the findings of the present study must be interpreted with caution because of the limitations raised above. Further epidemiologic and experimental studies in low-exposed individuals using serial measurements are necessary to confirm the potential contribution of pesticide exposure to the development of myopia, but also to other ocular diseases such as cataracts.

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