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Full Length Article

Prenatal exposure to organophosphate esters and behavioral development in young children in the Pregnancy, Infection, and Nutrition Study

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ABSTRACT

Organophosphate esters (OPEs) are commonly used as plasticizers and flame retardants in consumer products, and exposure is relatively ubiquitous in most populations studied. This may be of concern as some OPEs may be neurotoxic, endocrine-disrupting, and interfere with behavioral development; however, observational evidence is limited. We used data from the Pregnancy, Infection, and Nutrition Study, a prospective birth cohort study, to investigate associations between maternal OPE metabolite concentrations during pregnancy and behavioral development in offspring. Women provided a urine sample during pregnancy that was analyzed for concentrations of OPE metabolites, including diphenyl phosphate (DPHP), bis(1,3-dichloro-2-propyl phosphate) (BDCIPP), isopropyl-phenyl phenyl phosphate (ip-PPP), and 1-hydroxyl-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP). Offspring's behavioral development was assessed by the Behavioral Assessment System for Children (2nd Edition) (BASC-2) at approximately 36 months. Linear regression was used to estimate associations between tertiles in specific gravity-corrected OPE metabolite concentrations and children's scores on the BASC-2, adjusted for maternal age, maternal BMI, maternal race, maternal education, familial income, maternal depression, quality of the home environment, and sex. Higher BDCIPP concentrations were associated with higher scores on the Behavioral Symptoms Index (1st vs. 3rd tertile: $\beta = 3.03$; 95% CI = 0.40, 5.67) and Externalizing Problems (1st vs. 3rd tertile: $\beta = 2.49$; 95% CI: −0.12, 5.10) composites. Among BASC-2 scales, BDCIPP was most strongly associated with Withdrawal, Attention Problems, Depression, Hyperactivity, and Aggression. DPHP concentrations were also associated with higher scores on the Externalizing Problems and Behavioral Symptoms Index composites, but not as strongly as BDCIPP. Conversely, higher concentrations of ip-PPP were associated with fewer adverse behavioral symptoms, including an inverse association with the Internalizing Problems composite (1st vs. 3rd tertile: $\beta = -3.74$; 95% CI = −6.75, −0.74) and constituent scales. BCIPHIPP was not strongly associated with any measured behavioral outcomes. Our results suggest that greater maternal exposure to tris(1,3-dichloro-2-propyl phosphate) (TDCIPP, parent compound of BDCIPP) and, to a lesser degree, triphenyl phosphate (TPHP, parent compound of DPHP) during pregnancy is associated with adverse behavioral development in children. Our study contributes to the growing body of evidence pertaining to adverse developmental effects of prenatal OPE exposure and highlights the need for further research to characterize risks associated with this ubiquitous family of chemicals.

Abbreviations: ADHD, Attention Deficit Hyperactivity Disorder; BASC-2, Behavior Assessment System for Children 2nd Edition; BCIPHIPP, 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate; BCIPP, bis(1-chloro-2-propyl) phosphate; BDCIPP, bis13-dichloro-2-propyl) phosphate; CI, confidence interval; DPHP, diphenyl phosphate; ip-PPP, isopropyl-phenyl phenyl phosphate; IQR, interquartile range; MDL, method limit of detection; NHANES, National Health and Nutrition Examination Survey; OPE, Organophosphate Ester; PBDEs, polybrominated diphenyl ethers; PIN, Pregnancy Infection and Nutrition; SD, standard deviation; SG, specific gravity; tb-PPP, tert-butyl phenyl phenyl phosphate; TCEP, tris(2-chloroethyl) phosphate; TCIPP, tris(1-chloro-2-propyl) phosphate; TPHP, triphenyl phosphate; TDCIPP, tris13-dichloro-2-propyl) phosphate

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1. Introduction

Organophosphate esters (OPEs) are used in the production of a variety of consumer products. These compounds primarily function as flame retardants but are also used as plasticizers in other applications (ATSDR, 2012; van der Veen and de Boer, 2012; Wei et al., 2015). Utilization of OPEs has increased in recent years because they can be used to meet flammability standards for polyurethane foam in place of polybrominated diphenyl ethers (PBDEs), a phased-out class of flame retardant compounds (Wei et al., 2015; Stapleton et al., 2012; Cooper et al., 2016; Stapleton et al., 2009). In addition to polyurethane foam, other products also contain OPEs, including construction materials (van der Veen and de Boer, 2012; Marklund et al., 2003), electronics (van der Veen and de Boer, 2012; Zheng et al., 2017), children's products (Hoffman et al., 2015; Stapleton et al., 2011), nail polish (Mendelsohn et al., 2016), and recreational equipment (Keller et al., 2014; Gomes et al., 2016; Carignan et al., 2016). OPEs are applied as additive compounds that are not chemically bound to products during production, thus they subsequently volatilize and leach into surrounding environments and media (ATSDR, 2012; van der Veen and de Boer, 2012; Wei et al., 2015).

Because of their application to a wide variety of common consumer products and propensity to volatilize and leach, OPEs are present in many human environments including residential housing, office buildings, shopping centers, schools, child care facilities, and automobiles (Wei et al., 2015; Bergh et al., 2011; He et al., 2016; Reemtsma et al., 2008; Wu et al., 2016; Zhou et al., 2017). Inhalation and ingestion of suspended particles in indoor environments are well-documented sources of exposure (Wei et al., 2015; Meeker et al., 2013; Hou et al., 2016; Cequier et al., 2015; Dodson et al., 2014), though dermal exposure (Mendelsohn et al., 2016; Liu et al., 2017; Makinen et al., 2009) and other pathways (*e.g.*, dietary intake, drinking water) may also contribute to exposure (Wei et al., 2015; Hou et al., 2016; Li et al., 2014). Inside the body, OPEs are quickly metabolized, primarily to their respective diesters and monoesters, which are excreted in urine (ATSDR, 2012; Hou et al., 2016; Cooper et al., 2011; Van den Eede et al., 2013a). Although they have half-lives on the order of hours (Lynn et al., 1981; Nomeir et al., 1981; Minegishi et al., 1988; Sasaki et al., 1981), urinary OPE metabolite concentrations appear to be relatively consistent over periods from weeks to months. For example, validation studies among pregnant women indicate that singular spot assessments can reflect exposure across periods of months with fair to good reliability (Hoffman et al., 2014; Romano et al., 2017). As such, urinary concentrations of OPE metabolites are useful OPE biomarkers for biomonitoring surveys (Ospina et al., 2018) and epidemiologic studies (Cequier et al., 2015; Hoffman et al., 2017a; Castorina et al., 2017a; Butt et al., 2016; Hoffman et al., 2017b; Van den Eede et al., 2015), where they are detected with high frequency. The 2013–2014 National Health and Nutrition Examination Survey (NHANES) detected urinary biomarkers of triphenyl phosphate (TPHP) and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), two of the most well-studied OPEs, in greater than 90% of participants and with median concentrations of approximately 0.8 ng/ml (Ospina et al., 2018). Exposure is also pervasive among pregnant women and women of reproductive age (Cequier et al., 2015; Romano et al., 2017; Hoffman et al., 2017a; Castorina et al., 2017a; Butt et al., 2016; Feng et al., 2016; Carignan et al., 2017). Further, investigators have detected OPEs at the maternal-fetal interface (*e.g.*, placental tissue, chorionic villi and deciduae), indicating potential maternal-fetal transfer of exposure (Ding et al., 2016; Zhao et al., 2017).

Mounting evidence from both experimental and observational settings indicates that certain OPEs are biologically active and can affect behavioral development through both endocrine- and neurologically-mediated pathways, including thyroid-activity disruption (Meeker and Stapleton, 2010; Preston et al., 2017; Wang et al., 2015a; Wang et al., 2013), sex steroid-activity disruption (Meeker and Stapleton, 2010; Wang et al., 2015b; Liu et al., 2012; Liu et al., 2013a), and direct neurotoxic effects (Dishaw et al., 2011; Li et al., 2017; Wang et al., 2015c; Yuan et al., 2016). To date, only two observational studies of the behavioral effects of early life exposures to OPEs have been published (Lipscomb et al., 2017; Castorina et al., 2017b). Both studies found that exposure to these compounds was associated with behavioral problems in children, particularly externalizing behaviors. Together, the available mechanistic and observational evidence suggests that exposure to at least some OPE compounds in early life may be associated with adverse behavioral outcomes in children, particularly externalizing-like behaviors, such as hyperactivity and attention problems. However, currently available data from humans is limited and further investigation of behavioral effects of early life exposure to OPEs is warranted.

We used data from a prospective birth cohort study of children born to women living in North Carolina between 2004 and 2006 to investigate relationships between biomarkers of OPE exposures during pregnancy and behavioral outcomes among offspring at approximately three years of age.

2. Materials and methods

2.1. Study sample

The PIN Kids study is ancillary to the Pregnancy Infection and Nutrition Study - phase 3 (PIN3) and the PIN Postpartum Study (PIN — Pregnancy, Infection, and Nutrition Study 2018). PIN3 enrolled pregnant women before 20 weeks gestation from the University of North Carolina Hospital in Chapel Hill, NC and followed them to delivery to investigate risk factors for preterm birth; enrollment spanned 2001–2005. Women were recruited from prenatal care clinics before 20 weeks gestation if they were English-speaking, older than 16 years of age, carrying a singleton pregnancy, and intended to deliver at University of North Carolina hospitals. In 2003, the PIN Postpartum study began to enroll PIN3 participants who delivered live infants without major birth defects to study maternal weight retention and mental health for the first year postpartum (n = 689). Women were released from PIN Postpartum participation if they became pregnant again during follow-up or moved out of the area. Participants provided information about their health, nutrition, and lifestyle through interviews and questionnaires; they also provided second trimester urine and blood samples. In 2004, PIN Kids began to follow the growth and development of the children of PIN Postpartum participants age three years, by collecting data through maternal interview and child developmental assessment in the home (n = 577). Eligibility for this analysis required stored maternal prenatal urine to assay OPE metabolites and completion of the BASC2 at 3 years.

PIN protocols had been approved by the Institutional Review Board of the University of North Carolina at Chapel Hill and all participating mothers had given written informed consent and parental permission for their child's involvement.

2.2. Measurement of OPE metabolite concentrations

OPEs were measured in urine samples collected by UNC's General Clinical Research Center at approximately 24–29 weeks gestation (Median: 27; IQR: 27–28) (Hoffman et al., 2017a). Time of urine collection was not standardized, though > 95% of samples were collected between 0700 and 1200 h. Samples were aliquoted into polyethylene storage tubes and frozen at −80 °C until analysis (between 10 and 13 years after collection). Urine samples were analyzed for concentrations of six OPE metabolites, including diphenyl phosphate (DPHP), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), isopropyl-phenyl phenyl phosphate (ip-PPP), 1-hydroxyl-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP), bis(1-chloro-2-propyl) phosphate (BCIPP), and tert-butyl-phenyl phenyl phosphate (tb-PPP) (Fig. 1).

Analytic methods of OPE urinalysis are described in detail

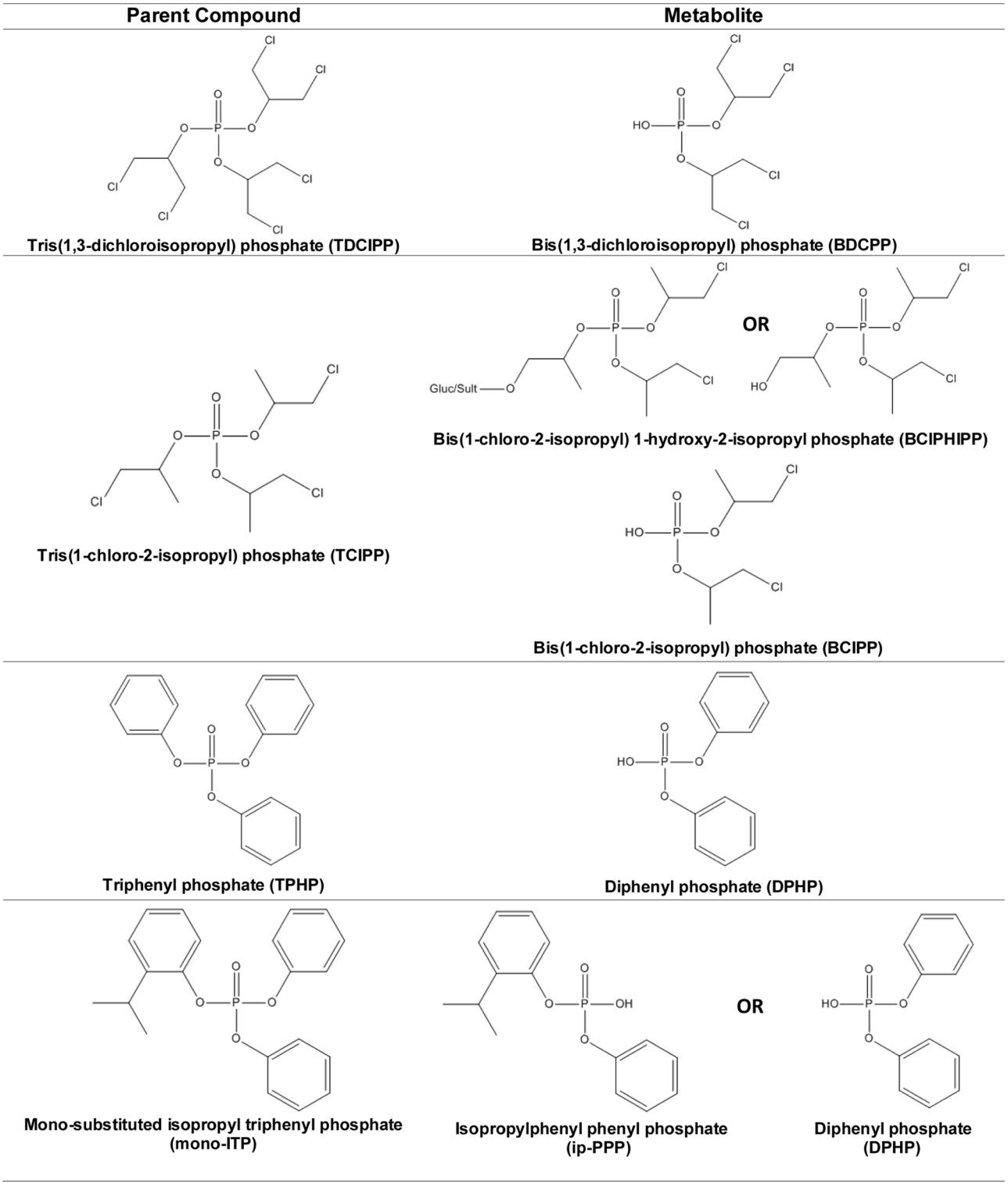

Fig. 1. OPE parent compounds and metabolites of interest to the Pregnancy, Infection, and Nutrition Study.

elsewhere (Hoffman et al., 2017a; Butt et al., 2016; Van den Eede et al., 2013b). Briefly, samples were extracted using previously described enzyme deconjugation and solid phase extraction techniques (Van den Eede et al., 2013b) adapted for 5 ml of urine (Butt et al., 2016). Samples were analyzed using electrospray ionization liquid chromatography tandem mass spectrometry. Standard reference materials were included to assess assay performance, and average of batch-specific coefficients of variation ranged from 11% to 16%. Method detection limits (MDLs) were calculated to be three times the standard deviation of the laboratory blanks, normalized to the average urine volume (3 ml). Samples were analyzed in three batches and MDLs were calculated separately for each batch; MDLs ranged from 127 to 243 pg/ml for DPHP, 60 to 197 pg/ml for BDCIPP, 37 to 177 pg/ml for ip-PPP, 3 to 33 pg/ml for BCIPHIPP, 136 to 333 pg/ml for BCIPP, and 213–846 pg/ml for tb-PPP.

Specific gravity (SG) was measured in each urine sample using a digital handheld refractometer (Atago). OPE metabolite concentrations were standardized for SG using the method proposed by Boeniger et al.

to account for urinary dilution (Boeniger et al., 1993).

2.3. Assessment of child's behavior

Children's behavior was assessed using the Behavioral Assessment System for Children, 2nd Edition (BASC-2) parent-rating scale for preschool children (PRS-P) (Reynolds and Kamphaus, 2004). The BASC-2 PRS-P is a parent-completed questionnaire of the parent's perceptions of their child's behavior, including both negative and positive behavioral qualities, which is valid for children ages 24–60 months (Reynolds and Kamphaus, 2004). Mothers completed the BASC-2 PRS-P questionnaire during the 36-month follow-up visit. Exposure status was unknown to mothers at time of completion of behavioral assessments.

To complete the BASC-2, mothers rated the frequency of their child's behaviors on 134 questions using a four-point scale (Never, Sometimes, Often, Almost Always) and responses were used to score four composites and sixteen scales (Supplemental Table S1). The four BASC-2 composites include Externalizing Problems, Internalizing Problems, Behavioral Symptoms Index, and Adaptive Skills. Higher scores on the Externalizing Problems, Internalizing Problems, and Behavioral Symptoms Index composites (and constituent subscales) indicate more behavioral problems, while higher scores on the Adaptive Skills composite (and constituent subscales) indicate more favorable behavioral abilities. The BASC-2 includes indices of internal validity to identify assessments that may have been inappropriately completed; these include the "F-Index" that assesses the possibility that a child's behavior was rated in an inordinately negative fashion, the "Response Pattern Index" that identifies inattentive reporting of behaviors (*e.g.*, all "Never" or all "Almost Always"), and the "Consistency Index" that identifies inconsistent responses to questions that are usually answered similarly. We omitted assessments with internal consistency indices indicating "Caution" or "Extreme Caution" (n = 13 (6.1%)). Raw scores on BASC-2 composites and scales were converted to age-specific T-scores of the BASC-2 standardized population (distributed with mean = 50, SD = 10) using the tables published in the manual.

2.4. Covariates

Throughout the PIN3, PIN Postpartum, and PIN Kids studies, women completed multiple interviews and questionnaires to provide information that characterized their health, nutrition, and life style (PIN — Pregnancy, Infection, and Nutrition Study 2018). PIN3 participants completed the Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977) to indicate the presence of depressive symptoms during pregnancy. PIN Kids staff administered a modification of the Home Observation for Measurement of the Environment (HOME) assessment to characterize physical and social influences on the child during the home visit at 3 years of age. While the HOME traditionally uses interview and observation to rate the several constructs that can impact the environment (Bradley and Caldwell, 1979), we scored only the three HOME subscales that comprised mostly interview items (Learning, Language Stimulation, and Academic Stimulation) to create a modified HOME score for these constructs. Thus, other constructs of the HOME, such as modeling behaviors or parent responsivity, are not reflected.

We identified covariates to include in our analyses using a Directed Acyclic Graph and a review of the literature (Supplemental Fig. 1). The Directed Acyclic Graph was used to model hypothesized unidirectional causal relations between variables and to identify variables to be included as covariates in order to block potential biasing pathways (*e.g.*, confounding pathways) (Greenland et al., 2019). Our covariate set included the following variables, identified as potential confounders or predictors of the outcomes that would reduce residual variance without introducing bias: maternal age in years (quadratic), maternal pre-pregnancy Body Mass Index (quadratic), maternal education in years (linear), income as a percentage of the 2001 poverty level (linear), maternal race (non-Hispanic White/all other races), maternal CES-D score ($< 17/\geq 17$), modified HOME Score (linear), and child's sex (male/female).

2.5. Statistical analysis

In our primary analyses, we used linear regression to estimate the covariate-adjusted change in children's scores on the BASC-2 composites and scales per tertile of SG-corrected OPE metabolite concentration measured in maternal urine during pregnancy. Because some participants' OPE metabolite concentrations were below the method detection limit (MDL; ranging from 0 to 16% of samples, depending on metabolite), we used multiple imputation to impute OPE metabolite values for these participants, as well as missing covariate data. Our multiple imputation procedure used Monte Carlo methods to generate 50 imputations conditional on covariates and OPE metabolite concentrations; OPE metabolite concentrations were imputed using a truncated log-normal model conditional on covariates and other OPE metabolite concentrations (Lubin et al., 2004). Analyses were performed on each of the imputed datasets and summary estimates were derived using Rubin's rules for multiple imputation (Rubin, 2019).

We performed multiple supplementary analyses. First, sex-specific effects are often observed for endocrine disrupting compounds (Frye et al., 2012; Gore et al., 2015; Venerosi et al., 2012), and some studies have observed sex-specific effects of OPE exposures (Preston et al., 2017; Wang et al., 2015a; Wang et al., 2015b; Liu et al., 2012; Liu et al., 2013a; Liu et al., 2016); therefore, we explored sex-specific effects by repeating our primary analyses in sex-stratified samples. Second, we repeated our primary analyses with OPE metabolite concentrations modeled as linear variables; specifically, we estimated the covariate-adjusted change in BASC-2 composites and scales per interquartile range (IQR) increase in SG-corrected log-10-transformed OPE metabolite concentrations. Third, we repeated our primary analyses with all OPE metabolites included in a single model. Fourth, we repeated our primary analyses with the omission of potentially influential variables, identified as variables with Cook's distance > 0.05.

3. Results

3.1. Study sample

In total, our study sample included 199 mother-child pairs who had both valid BASC-2 assessments and OPE metabolite measurements (Table 1). The median age of mothers in our study sample was 30 years (IQR: 27–33), and mothers were primarily White (82%) and highly educated (median years of education: 16; IQR: 15–18). Relative to the PIN Kids eligible cohort (n = 577), mothers in our analysis sample were slightly older, more likely to be White, had more years of education, and were of higher income.

3.2. OPE metabolite concentrations

We detected DPHP, BDCIPP, ip-PPP, and BCIPHIPP in > 80% of study participants (Table 2); tb-PPP and BCIPP were detected less frequently (2% and 51%, respectively), thus were omitted from bivariate analyses. Median concentrations were highest for ip-PPP (7.04 ng/ml), followed by BDCIPP (2.01 ng/ml), DPHP (1.38 ng/ml), and BCIPHIPP (0.45 ng/ml). The median concentrations specific gravity-uncorrected BDCIPP and DPHP in our study sample (1.15 ng/ml and 0.81 ng/ml, respectively) were similar to those more recently measured among females in the NHANES 2013–2014 cycle (0.89 ng/ml and 0.82 ng/ml, respectively) (Ospina et al., 2018). Correlations between the compounds ranged from -0.01 to 0.29. Descriptive statistics of SG-uncorrected OPE metabolite concentrations are located in Supplemental Table S2. Characteristics regarding OPE metabolite concentrations in PIN3 are detailed elsewhere (Hoffman et al., 2017a).

Table 1
Characteristics of the PIN Kids eligible population and analysis sample.

		PIN Kids Eligible n = 577	Analysis Sample[a] n = 199
Maternal Age at Child's Birth (years)	Median (IQR)	30 (26-33)	30 (27-33)
	Missing	0	0
Maternal Race	White	441 (77)	164 (82)
	Black	86 (15)	21 (11)
	American Indian	2 (0)	1 (1)
	Asian	20 (3)	2 (1)
	Other	27 (5)	11 (6)
	Missing	1	0
Maternal Education (years)	Median (IQR)	16 (14–18)	16 (15–18)
	Missing	0	0
Income as Percentage of 2001 Poverty Index	Median (IQR)	464 (232–596)	473 (263-596)
	Missing	18	3
Body Mass Index (kg/m^2)	Median (IQR)	23 (21–27)	23 (21–27)
	Missing	2	0
Modified HOME Score	Median (IQR)	19 (17–20)	19 (18–20)
	Missing	168	0
CES-D Score	0-16	352 (72)	138 (75)
	≥17	137 (28)	46 (25)
	Missing	88	15
Sex of the Child	Male	308 (53)	112 (56)
	Female	268 (47)	87 (44)
	Missing	1	0
Child's Age at BASC-2 (Months)	Mean (SD)	36 (36–38)	36 (36–38)
	Missing	245	0

Abbreviations: BASC-2, Behavioral Assessment System for Children 2nd Edition; CES-D, Center for Epidemiologic Studies Depression Scale; PIN, Pregnancy, Infection, and Nutrition Study.
[a] Participants with OPE metabolite measurements and valid BASC-2 assessments.

3.3. Assessment of child's behavior

In general, the distributions of the age-standardized BASC-2 composite and scale scores were similar to BASC-2 scores among the BASC-2 standardization population (Reynolds and Kamphaus, 2004); *i.e.*, the means of the age-standardized scores were approximately 50 and the standard deviations of these scores were approximately 10 (Table 3). However, the mean values of the BASC-2 Externalizing Problems, Internalizing Problems, and Behavioral Symptoms index were slightly lower than 50, whereas the mean value of the BASC-2 Adaptive Skills composite was slightly higher than 50, indicating slightly fewer behavioral problems and slightly better behavioral competencies in our study sample relative to the BASC-2 standardization population.

Table 3
Age-standardized, sex-combined BASC-2 T-scores in the analysis sample (n = 199).

		Mean	SD
Composites	Externalizing Problems	47	8.3
	Internalizing Problems	48	8.6
	Behavioral Symptoms Index	48	8.1
	Adaptive Skills	52	8.0
Scales	Aggression	47	8.7
	Hyperactivity	48	8.7
	Anxiety	49	9.0
	Depression	49	8.0
	Somatization	46	8.5
	Attention Problems	50	8.9
	Atypicality	48	8.9
	Withdrawal	48	8.9
	Activities of Daily Living	50	9.2
	Adaptability	54	8.9
	Functional Communication	52	8.4
	Social Skills	55	8.0

Abbreviations: BASC-2, Behavioral Assessment System for Children 2nd Edition; SD, standard deviation.

3.4. Associations between OPE metabolite concentrations and child's behavior

Higher concentrations of BDCIPP were associated with higher scores on the Behavioral Symptoms Index and Externalizing Problems composites (Table 4), which includes direct associations with scores on several specific scales, including Withdrawal, Attention Problems, Depression, Hyperactivity, and Aggression. These associations were generally monotonic, though the associations between BDCIPP and the Withdrawal and Attention Problems scales indicated a stronger association in the second tertile than the third tertile. Similar associations were observed for DPHP, though they were not as strong as those for BDCIPP. Higher concentrations of ip-PPP were associated with lower scores on the Internalizing Problems and Behavioral Symptoms Index composites, which were primarily driven by inverse associations between ip-PPP and the Anxiety, Atypicality, Somatization, and Depression scales. BCIPHIPP concentrations were not strongly associated with any of the BASC-2 composites or scales.

3.5. Supplementary analyses

Our results were robust to several supplementary analyses. First, sex-specific associations between OPE metabolite concentrations and BASC-2 scores were generally consistent with sex-combined associations (Supplemental Table S3, Supplemental Fig. 2). As a general trend, adverse associations appeared stronger among females than males, particularly for BDCIPP and DPHP. However, the results of these sex-specific analyses were highly imprecise and must be interpreted cautiously. Second, specifying OPE metabolite concentrations as linear terms produced results similar to our primary analyses specifying exposure in tertiles, although associations with DPHP were notably

Table 2
Specific gravity-corrected OPE metabolite concentrations (ng/ml) measured in prenatal urine samples provided by the analysis sample (n = 199).

		Percentiles					Spearman Correlation Coefficients			
Metabolite	% < MDL	5	25	50	75	95	DPHP	BDCIPP	ip-PPP	BCIPHIPP
DPHP	16	< MDL	0.80	1.38	2.37	9.55	1	0.29	0.19	0.19
BDCIPP	5	< MDL	0.93	2.01	3.75	11.92		1	−0.01	0.23
ip-PPP	0	1.93	4.35	7.04	10.63	22.34			1	0.11
BCIPHIPP	3	0.11	0.25	0.45	0.86	6.57				1

Abbreviations: BCIPHIPP, 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate; BDCIPP, bis(1,3-dichloro-2-propyl) phosphate; DPHP, diphenyl phosphate; ip-PPP, isopropyl-phenyl phenyl phosphate; MDL, method detection limit; OPE, organophosphate ester.

Table 4
Estimated change in age-standardized, sex-combined BASC-2 T-scores per tertile of specific gravity-corrected OPE metabolite concentration, adjusted for covariates.

		DPHP				BDCIPP				ip-PPP				BCIPHIPP			
		2nd Tertile (0.99–1.89 ng/ml)		3rd Tertile (1.93–111.56 ng/ml)		2nd Tertile (1.19–3.09 ng/ml)		3rd Tertile (3.10–139.60 ng/ml)		2nd Tertile (5.01–8.99 ng/ml)		3rd Tertile (8.99–69.00 ng/ml)		2nd Tertile (0.31–0.68 ng/ml)		3rd Tertile (0.68–97.96 ng/ml)	
		Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
Composites	Externalizing Problems	0.26	[−2.40, 2.92]	1.44	[−1.22, 4.09]	0.34	[−2.23, 2.91]	2.49	[−0.12, 5.10]	−0.99	[−3.64, 1.66]	−1.75	[−4.51, 1.00]	−0.85	[−3.58, 1.88]	−0.64	[−3.28, 2.00]
	Internalizing Problems	−0.28	[−3.23, 2.66]	0.21	[−2.73, 3.15]	1.03	[−1.84, 3.89]	1.33	[−1.58, 4.24]	−0.99	[−3.89, 1.90]	−3.74	[−6.75, -0.74]	0.97	[−2.02, 3.96]	−1.48	[−4.38, 1.42]
	Behavioral Symptoms Index	1.07	[−1.61, 3.75]	1.98	[−0.70, 4.66]	1.87	[−0.72, 4.47]	3.03	[0.40, 5.67]	−1.12	[−3.80, 1.56]	−2.06	[−4.84, 0.72]	−0.66	[−3.42, 2.09]	−1.30	[−3.97, 1.36]
	Adaptive Skills	−1.11	−3.77, 1.54]	−0.53	[−3.18, 2.12]	−0.32	[−2.90, 2.27]	−1.42	[−4.05, 1.21]	0.91	[−1.75, 3.56]	−0.06	[−2.82, 2.69]	0.24	[−2.48, 2.97]	0.40	[−2.24, 3.03]
Scales	Aggression	0.14	[−2.73, 3.01]	1.40	[−1.46, 4.26]	−1.08	[v3.85, 1.68]	2.09	[v0.72, 4.90]	−0.56	[−3.42, 2.31]	−1.59	[−4.57, 1.38]	−2.03	[−4.96, 0.90]	−1.43	[−4.26, 1.41]
	Hyperactivity	0.18	[−2.67, 3.04]	1.14	[−1.71, 3.98]	1.60	[−1.16, 4.37]	2.32	[−0.49, 5.12]	−1.33	[−4.18, 1.51]	−1.66	[−4.61, 1.29]	0.42	[−2.50, 3.35]	0.14	−2.69, 2.97]
	Anxiety	0.22	[−2.82, 3.25]	0.57	[−2.47, 3.60]	1.05	[−1.90, 4.00]	−0.72	[−3.72, 2.29]	−1.33	[−4.33, 1.67]	−3.60	[−6.71, -0.48]	0.36	[−2.74, 3.45]	−1.74	[−4.74, 1.26]
	Depression	−0.63	[−3.40, 2.15]	−1.20	[−3.98, 1.57]	1.66	[−1.03, 4.35]	2.43	[−0.30, 5.17]	−1.53	[−4.30, 1.23]	−2.27	[−5.13, 0.60]	0.65	[−2.18, 3.49]	−1.08	[−3.83, 1.67]
	Somatization	−0.35	[−3.22, 2.52]	1.16	[−1.70, 4.03]	−0.65	[−3.44, 2.14]	1.15	[−1.69, 3.99]	0.49	[−2.35, 3.32]	−2.64	[−5.58, 0.31]	1.26	[−1.67, 4.19]	−0.44	[−3.28, 2.40]
	Attention Problems	0.31	[−2.52, 3.14]	2.39	[−0.44, 5.22]	2.86	[0.11, 5.61]	2.36	[−0.43, 5.16]	−1.08	[−3.93, 1.77]	−1.12	[−4.08, 1.83]	−1.10	[−4.02, 1.81]	−1.70	[−4.52, 1.12]
	Atypicality	2.72	[−0.19, 5.62]	1.96	[−0.94, 4.86]	0.42	[−2.43, 3.27]	1.44	[−1.45, 4.34]	−2.07	[−4.96, 0.82]	−3.45	[−6.45, -0.45]	−1.36	[−4.35, 1.63]	−1.29	[−4.19, 1.61]
	Withdrawal	1.95	[−1.08, 4.98]	2.99	[−0.03, 6.02]	3.09	[0.15, 6.04]	2.92	[−0.08, 5.91]	1.89	[−1.16, 4.93]	0.87	[−2.30, 4.03]	0.12	[−3.02, 3.25]	−0.42	[−3.45, 2.61]
	Activities of Daily Living	−0.77	[−3.80, 2.25]	0.53	[−2.49, 3.55]	0.82	[−2.11, 3.75]	−1.74	[−4.72, 1.24]	2.19	[−0.82, 5.20]	0.17	[−2.96, 3.29]	0.62	[−2.48, 3.73]	0.67	[−2.33, 3.67]
	Adaptability	−0.94	[−3.89, 2.00]	−0.76	[−3.70, 2.19]	−1.65	[−4.51, 1.21]	0.31	[−2.59, 3.22]	−0.54	[−3.48, 2.40]	0.27	[−2.79, 3.33]	−0.90	[−3.89, 2.10]	1.69	[−1.21, 4.59]
	Functional Communication	−1.19	[−3.91, 1.53]	−1.10	[−3.82, 1.62]	0.06	[−2.58, 2.71]	−1.38	[−4.07, 1.31]	0.29	[−2.42, 3.00]	−1.50	[−4.32, 1.31]	0.71	[−2.07, 3.49]	−1.16	[−3.85, 1.52]
	Social Skills	−0.49	[−3.13, 2.15]	−0.10	[−2.74, 2.54]	−0.51	[−3.07, 2.05]	−1.79	[−4.40, 0.81]	0.98	[−1.66, 3.61]	1.00	[−1.73, 3.74]	0.31	[−2.40, 3.01]	−0.03	[−2.65, 2.58]

Notes: Adjusted for maternal age (quadratic), maternal BMI (quadratic), income as percentage of 2001 poverty index (linear), maternal education (linear), maternal race (White non-Hispanic/Other), maternal CES-D score (< 17/ ≥ 17), HOME Score (linear), and sex (male/female).

attenuated (Supplemental Table S4). Third, when all OPE metabolites were included in a single model, many of the strongest associations we observed in the primary analyses persisted and the directionality of most associations was unchanged relative to the primary analyses (Supplemental Table S5), though precision decreased, and some estimates were attenuated. Finally, when influential observations were removed from analyses, we similarly observed that most of the associations present in our primary analyses were unchanged, indicating that the primary analyses were not unduly influenced by a small number of influential observations (Supplemental Table S6).

4. Discussion

In this prospective birth cohort study, we observed that concentrations of certain OPE metabolites measured in maternal urine during pregnancy were associated with offspring's scores on the BASC-2, as reported by their mothers, at approximately three years of age. BDCIPP concentrations were positively associated with a variety of adverse behavioral symptoms, including more withdrawal, attention problems, depression, and hyperactivity. DPHP concentrations were also associated with higher behavioral symptom scores, including withdrawal, attention problems, and atypicality, though to a lesser degree than BDCIPP. Conversely, ip-PPP concentrations were generally associated with fewer behavioral symptoms, particularly internalizing behaviors such as anxiety, depression, and somatization, as well as atypicality. BCIPHIPP was consistently not associated with behavioral symptoms in this study.

Our results contribute to the growing body of epidemiologic evidence relating prenatal and early life OPE exposure to early-life behavioral development. Lipscomb et al. performed a cross-sectional study among preschool-aged children (ages 3–5 years, n = 72) and reported that greater environmental ΣOPE exposure (assessed by passive silicone samplers) was associated with fewer responsible behaviors and more externalizing behaviors, as assessed by teacher report using the Social Skills Improvement Rating Scale (Lipscomb et al., 2017). The investigators did not estimate associations with individual OPE compounds, but their summed exposure metric included parent compounds of several of the metabolites investigated in our study, including tris (1,3-dichloro-2-propyl) phosphate (TDCIPP, a parent compound of BDCIPP), triphenyl phosphate (TPHP, a parent compound of DPHP), tris(1-chloro-2-propyl) phosphate (TCIPP, a parent compound of BCIPHIPP), and tris(2-chloroethyl) phosphate (TCEP). More recently, Castorina et al. reported that higher concentrations of BDCIPP measured in maternal prenatal urine were associated with higher scores on the Attention Problems subscale of the BASC-2 (teacher report, n = 247), and also that higher concentrations of ip-PPP measured in maternal prenatal urine were associated with higher scores on the Hyperactivity subscale of the BASC-2 among children at 7 years (maternal report, n = 281) (Castorina et al., 2017b).

In our study, we observed similar associations between BDCIPP and increased externalizing behaviors and other behavioral symptoms, such as attention problems and hyperactivity; we did not, however, observe that ip-PPP concentrations were associated with more externalizing behavioral symptoms, and conversely observed that ip-PPP concentrations were associated with fewer internalizing behaviors. Thus, some, but not all, associations have been somewhat consistently observed across the small number of available studies. Some discrepancies in results among these studies may be attributable to differences in exposure levels among the study populations, methods of exposure assessment, and children's ages at behavioral assessments. First, while both our study and Castorina et al. (2017b) measured OPE metabolite concentrations in maternal urine sampled during pregnancy to assess prenatal exposure, the median concentration of ip-PPP in our study population was approximately twenty times that measured in Castorina et al.'s study population (7.04 ng/ml vs. 0.34 ng/ml), and median DPHP and BDCIPP concentrations were approximately two to three times the median concentrations of these metabolites in Castorina et al.'s study population, using the same analytic technique. Direct comparison of results to Lipscomb et al.'s study is more challenging because the investigators assessed postnatal exposure among children at three to five years of age and used passive silicone samplers to measure environmental concentrations of OPEs. Although measurements of OPEs in silicone wristbands correlate with urinary metabolites of these compounds (Hammel et al., 2016), such differences in methods of exposure assessment may have contributed to differences in observed associations. Another potential contributing factor to inconsistent study findings is differences in children's ages at behavioral assessments among the three studies; children in our study were assessed at approximately three years of age, whereas Lipscomb et al. assessed children at three to five years and Castorina et al. assessed children at approximately seven years. While each of these studies used age-appropriate behavioral assessments completed by persons familiar with the children's behavior (*e.g.*, parents or teachers), the norms around behavior do change with age and scores may also be impacted by perceptions and reporting. Additionally, some behavioral aspects and developmental abnormalities may only become apparent at later ages, which would not be picked up in our study of young children. Altogether, despite some discrepant findings, the available evidence across observational studies indicates that early life exposure to certain OPEs, particularly TDCIPP, are potentially associated with adverse behavioral effects, particularly externalizing behaviors.

The epidemiologic evidence of behavioral effects of early life OPE exposures is supported by a growing body of observational and experimental evidence linking OPE exposure to physiological processes related to behavioral development. Human behavior and behavioral development are largely governed by the endocrine and neurological systems (Clark et al., 2019; Filley, 1995; Fink et al., 2011; Wilkinson and Brown, 2015), and these physiological systems develop rapidly in early life, particularly during the prenatal period, and are highly sensitive to perturbations caused by exogenous pollutants (Bondy and Campbell, 2005; Grandjean and Landrigan, 2014; Rice and Barone, 2000; Miodovnik, 2011). Available evidence indicates that OPEs may exert endocrine-disrupting and neurotoxic effects, which may affect behavioral development through both direct exposure and maternally-mediated effects that occur as a result of maternal exposure to OPEs during the prenatal period. For example, observational studies have reported associations between thyroid hormone concentrations and TDCIPP and TPHP concentrations in house dust (Meeker and Stapleton, 2010) and also DPHP concentrations in urine (Preston et al., 2017); experimental studies have similarly reported associations between OPE exposures and altered thyroid function (Wang et al., 2013; Farhat et al., 2013; Kim et al., 2015; Xu et al., 2015), including altered thyroid hormone concentrations and altered thyroid-related gene and protein expression. Maternal thyroid function during pregnancy and offspring's thyroid function during early life are critical to both the development of anatomical structures and the government of physiological processes related to behavior (Gore et al., 2015; Ghassabian et al., 2014; de Escobar et al., 2004; Haddow et al., 1999; Williams, 2008); further, mounting evidence suggests that maternal thyroid dysfunction during pregnancy is associated with ADHD-like behaviors in offspring (Modesto et al., 2015; Ghassabian et al., 2012; Andersen et al., 2014; Vermiglio et al., 2004; Pakkila et al., 2014). As such, maternal exposures to OPEs, including TDCIPP, during pregnancy may result in maternal thyroid dysregulation that leads to thyroid-mediated developmental effects on the fetus, which manifest as adverse behavioral symptoms among children that are consistent with ADHD (*e.g.*, externalizing-like behaviors). In a similar manner, sex steroid exposure during the prenatal period and early life is important to behavioral development (Gore et al., 2014), and available evidence indicates OPE exposures can influence sex steroid expression and function (Wang et al., 2015b; Liu et al., 2012; Liu et al., 2016; Liu et al., 2013b; Zhang et al., 2014; Krivoshiev et al., 2016), which can lead to sex steroid-

mediated behavioral effects of OPE exposure. Experimental studies have found associations between OPE exposure and sex steroid concentrations in exposed animals (Wang et al., 2015b; Liu et al., 2012; Liu et al., 2013a; Liu et al., 2016) and sex steroid mRNA and protein expression in in vitro settings (Wang et al., 2015b; Liu et al., 2013a; Liu et al., 2016). Further still, experimental evidence indicates OPE exposure may cause potential neurotoxic effects, such as cytotoxicity to neuronal cells (Li et al., 2017; Pei et al., 2016; Crump et al., 2012; Ta et al., 2014) and alteration of neurotransmitter levels (Wang et al., 2015a; Wang et al., 2015c). Of particular concern are neurotoxic effects that follow direct exposure to the fetus during the prenatal period as a result of maternal-fetal transmission of exposure; such maternal-fetal transmission of exposure is supported by observational studies that have detected OPEs at the maternal-fetal interface (*e.g.*, placental tissue, chronic villi and deciduae) (Ding et al., 2016; Zhao et al., 2017). In summary, available evidence suggests that early life exposure to certain OPEs, including TDCIPP and DPHP, may be associated with behavioral development, particularly externalizing behaviors, through both endocrine- and neurologically-mediated pathways.

As a contribution to the evidence of behavioral effects of early life OPE exposures, our study is noteworthy for its prospective design, assessment of exposures during the sensitive prenatal period, and investigation of a broad array of behavioral outcomes. Yet, certain study characteristics bear upon our results and interpretation. Assessment of exposure during the prenatal period is valuable, as the prenatal period is a uniquely sensitive period for development, including development of anatomical structures related to behavioral development (Bondy and Campbell, 2005; Grandjean and Landrigan, 2014; Rice and Barone, 2000; Miodovnik, 2011). We measured concentrations of OPE metabolites in a single spot urine sample collected from mothers during the 25th to 29th weeks of pregnancy, which would not capture potential variability in exposure throughout different sensitive windows of fetal development across pregnancy. Although this may result in some exposure misclassification, previous studies have characterized variability in OPE metabolite concentrations throughout pregnancy and observed moderate to good consistency (Hoffman et al., 2014; Romano et al., 2017). Still, future investigations will likely benefit from exposure assessments that occur at multiple points during pregnancy across the sensitive prenatal and early childhood periods.

Measured OPE metabolite concentrations in urine are imperfectly sensitive and specific markers of OPE parent compound exposure. For instance, multiple metabolites may result from a single parent compound, such that measurement of a single metabolite may not fully characterize an individual's exposure to that parent compound (Hou et al., 2016; Van den Eede et al., 2013a; Van den Eede et al., 2015); conversely, some metabolites, such as DPHP, may result from multiple compounds (Nishimaki-Mogami et al., 1988; Ballesteros-Gomez et al., 2015), and may even be used in products in their own right (Makiguchi et al., 2013; Makiguchi et al., 2011). Such limitations in sensitivity and specificity may be reduced by identifying and measuring additional OPE metabolites.

Detection of OPE metabolites has varied over time (Hoffman et al., 2017b). We detected four OPE metabolites with high frequency (> 80%) from urine samples collected between 2002–2005 from the PIN Study. The median concentrations of specific-gravity uncorrected BDCIPP and DPHP were 1.15 ng/ml and 0.81 ng/ml (respectively), which is similar to those more recently measured among females in the NHANES 2013–2014 cycle (0.89 ng/ml and 0.82 ng/ml, respectively) (Ospina et al., 2018). However, ip-PPP concentrations in our sample were higher than those reported by other observational studies (Butt et al., 2016; Carignan et al., 2017), which may increase our likelihood of detecting effects of exposures at higher levels, but also may limit the generalizability of our results. The sources of high ip-PPP exposure in our study sample are unclear. Data to characterize and compare TCIPP exposure in the population, and its most frequently detected metabolite BCIPHIPP, are limited (Van den Eede et al., 2015; Schindler et al., 2009). For the four metabolites reported here, non-detect rates were low, still we used multiple imputation to handle values below the limit of detection, which is superior to alternative approaches, such as single value replacement or single imputation (Lubin et al., 2004).

Our assessment of children's behavior also possessed strengths and limitations. The BASC-2 has demonstrated validity and correlates well with other behavioral assessments (Reynolds and Kamphaus, 2004). In our study, we used a well validated parent-rating scale, the BASC-2. Assessments that rely on parent report may be partial to a parent's perceptions about typical child behavior that may be influenced by a variety of factors, including their own behaviors and the behaviors of other children. In our analyses, we included mothers' scores on the Center for Epidemiologic Studies Depression Scale (CES-D) as a covariate, which may have reduced reporting biases related to maternal behavior. However, future studies should consider including direct neurobehavioral assessments. Additionally, the behavioral assessment scores produced by the BASC-2 may not be as immediately significant as a clinical diagnosis of a behavioral disorder, though among young children they can have predictive utility for future behavioral disorders and subclinical differences (Reynolds and Kamphaus, 2004). Even subclinical effects can greatly influence children's behavior over the course of their lifetimes and may be more relevant for studies of environmental toxicants, where effects on behavior are likely to be modest. Future investigations of behavioral effects of OPE exposure will benefit from continued use of these instruments and other similar instruments to assess subclinical differences, but clinically relevant assessments may also be valuable. A final limitation of our behavioral assessments is that we administered them at approximately three years of age, which may have limited our ability to accurately assess certain behavioral dimensions that become more pronounced with age. The body of evidence relating prenatal and early life OPE exposures to behavioral development would benefit from behavioral assessments administered at multiple ages.

The PIN studies provided an efficient setting to explore our research question, including OPE metabolite concentrations, developmental assessments, and considerable data on covariates important for assessment of OPEs and behavior. The PIN Kids protocol did not begin until the final years of the PIN3 and PIN Postpartum studies, which limited the sample size; but the characteristics of the mother-child pairs in our sample were generally similar to the baseline cohort. We adjusted for maternal age, race, and education in our analyses to reduce any potential bias resulting from selectivity by these factors. To investigate potential selection bias resulting from differences in OPE metabolite concentrations between the PIN3 participants with OPE metabolite concentrations and our analysis sample, we compared OPE metabolite concentrations between these samples (Supplemental Table S7) and observed only modest differences between the larger sample with OPE metabolite concentrations and our analysis sample. We similarly compared BASC-2 scores between the larger sample that had these scores and our analysis sample (Supplemental Table S8) and similarly only observed modest differences in BASC-2 scores between these two populations. These differences did not persist after covariate adjustment for variables included in our analyses (Supplemental Table S9). Therefore, although the mothers in our analysis sample differed somewhat from the larger PIN3 population, which potentially limits the generalizability of our results, it does not seem that the exposure or outcomes differed substantially between these samples in a manner that would have substantially biased our findings. Nonetheless, future studies would benefit from larger study populations to allow for greater generalizability and greater statistical power, particularly to assess associations among boys and girls separately.

In our study, we performed an exploratory analysis of sex-specific associations between prenatal OPE exposure and measures of behavioral development. Because our sex-stratified analysis samples were small, sex-specific associations were imprecise and we advise caution in interpreting these results. Still, we observed that prenatal BDCIPP

concentrations appeared to be more strongly associated with adverse behavioral development among females than males. In particular, we observed that BDCIPP was associated with lower scores on the Adaptive Skills Composite and constituent scales among females, but not among males (with the exception of a weak inverse association with the Social Skills scale). This finding is interesting because in a recent investigation of prenatal OPE exposure in relation to cognitive development in this same cohort Doherty et al. (2019) we observed stronger adverse associations between BDCIPP and performance on the Mullen Scales of Early Learning (a measure of early life cognitive development) among females than males. With consideration of the cautions noted above, further investigation of these findings suggesting potentially greater developmental toxicity of BDCIPP among females than males is warranted in future studies, especially given the endocrine disrupting compounds (Frye et al., 2012, Gore et al., 2015, Venerosi et al., 2012).

Our study contributes to a growing body of evidence that OPEs may adversely affect behavioral development. Pervasive exposure among women of reproductive age and young children to an environmental toxicant potentially capable of producing adverse behavioral effects is of immediate public health significance. Early life, particularly the prenatal period, is a uniquely sensitive period for human development, and even subtle perturbations in developmental trajectories can amount to significant effects throughout the lifecourse (Bondy and Campbell, 2005; Grandjean and Landrigan, 2014; Rice and Barone, 2000; Miodovnik, 2011). Behavioral disorders, such as ADHD, autism, and other behavioral conditions, incur steep costs to individuals, their families, and society, and even subclinical impairments to behavioral development can impact an individual's ability to thrive. As such, the identification of intervenable risk factors for suboptimal behavioral development is of chief importance to public health. OPEs are promoted as replacements for brominated flame retardants (*e.g.*, PBDEs), which were phased-out amid concerns of their toxicity, particularly their neurodevelopmental toxicity, and environmental persistence. The available evidence suggests that OPEs should be highly scrutinized as a suitable alternative. Further investigation of developmental effects of OPEs, including observational studies with greater statistical power and improved exposure assessment, will aid in the characterization of the risks involved with such pervasive exposure to these compounds, and will inform decisions regarding their continued usage.

5. Conclusions

In a prospective birth cohort study, we observed that higher concentrations of BDCIPP in prenatal maternal urine were associated with more adverse behaviors in offspring, including Withdrawal, Attention Problems, and others. We observed similar associations with DPHP concentrations, although associations were not as strong as those observed for BDCIPP. Conversely, ip-PPP was associated with fewer behavioral symptoms, particularly those associated with internalizing behaviors. BCIPHIPP was not apparently associated with behavioral symptoms in this study. Our study contributes to the growing body of evidence pertaining to the behavioral effects of early life exposure to OPEs that indicates that OPEs may adversely affect early childhood development. However, further research is needed to better characterize the toxicity of specific OPE compounds and identify developmental endpoints most sensitive to such toxicity. The results of this study can be used in combination with other data on these chemicals to inform decision making regarding the usage of OPEs.

Declaration of interest

None.

Submission declaration

All of the authors have read and approved the paper, and it has not been published previously nor is it currently being considered by any other peer-reviewed journal.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neuro.2019.03.007.

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