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Current-use flame retardants: Maternal exposure and neurodevelopment in children of the CHAMACOS cohort

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HIGHLIGHTS

- We measured organophosphate flame retardant (PFR) metabolites in urine from pregnant women.
- Metabolites of chlorinated tris and triphenyl phosphate (TPHP) were frequently detected.
- Cognition and behavior were assessed in 310 children at 7 years.
- Higher TPHP and total PFR exposure were associated with decreased IQ scores.

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ABSTRACT

Flame retardants are commonly used in consumer products found in U.S. households. Restrictions on the use of polybrominated diphenyl ether flame retardants have resulted in increased use of replacement chemicals, including Firemaster 550® (FM 550®) and organophosphate flame retardants (PFRs): tris(1,3-dichloro-2-propyl) phosphate (TDCIPP); tris(chloropropyl) phosphate (TCIPP); tris(2-chloroethyl) phosphate (TCEP); and triphenyl phosphate (TPHP). Animal research suggests that PFRs may affect neurodevelopment through noncholinergic mechanisms similar to some organophosphate (OP) pesticides. Despite the widespread presence of these compounds in home environments, and their structural similarity to neurotoxic OP pesticides, understanding of human exposure and health effects of PFRs is limited. We measured four urinary PFR metabolites from pregnant women in the CHAMACOS birth cohort study (n = 310) and assessed neurodevelopment of their children at age 7. Metabolites of TDCIPP (BDCIPP: bis(1,3-dichloro-2-propyl) phosphate) and TPHP (DPHP: diphenyl phosphate) were detected in >75% of urine samples, and isopropylphenyl phenyl phosphate (ip-PPP), a metabolite of one component of FM 550®, was detected in 72% of urine samples. We observed decreases of 2.9 points (95% Confidence Interval (CI): −6.3, 0.5) and 3.9 points (95% CI: −7.3,-0.5) in Full-Scale intelligence quotient and Working Memory, respectively, for each ten-fold increase in DPHP in adjusted regression models (n = 248). Decreases in Full-Scale IQ and Working Memory were greater in models of the molar sum of the PFR metabolites compared to the DPHP models. This is the first study to examine PFR and FM 550® exposures and potential neurodevelopmental outcomes in pregnant women and children. Additional research is warranted.

1. Introduction

Organophosphate flame retardants (PFRs) are persistent organic chemicals that are used in common consumer products found in U.S. households (ATSDR, 2012; Boethling and Cooper, 1985; Muir, 1984). PFRs and polybrominated diphenyl ethers (PBDEs) have been applied to polyurethane foams used in consumer products such as furniture found in the indoor environment in order to comply with California Technical Bulletin 117 (TB 117), in effect between 1977 and 2013 (Castorina et al., 2017; CDCA, 2000; Fang et al., 2013; Hoffman et al., 2015; Quirós-Alcalá et al., 2011;

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Abbreviations

ADHD	attention-deficit/hyperactivity disorder
BASC-2	Behavioral Assessment System for Children 2
CADS	Conners' ADHD/DSM-IV Scales
CHAMACOS	Center for the Health Assessment of Mothers and Children of Salinas
CI	confidence interval
DAP	dialkyl phosphate
HOME	Home Observation for Measurement of the Environment
IQ	intelligence quotient
OP	organophosphate
PBDE	polybrominated diphenyl ether
PFR	Organophosphate flame retardant
PPVT	Peabody Picture Vocabulary Test
WISC-IV	Wechsler Intelligence Scale for Children, 4th edition

Stapleton et al., 2009; Zota et al., 2008). All U.S. states are affected by the California standards because the furniture industry has applied them to products sold nationwide (Stapleton et al., 2012; US EPA, 2005). Due to concerns about the health risks of PBDEs, manufacturers began to phase out the use of PBDE flame retardants in consumer products in the early 2000s. Restrictions on the use of PBDE flame retardants have resulted in increased use of replacement flame retardants, including Firemaster 550® (FM 550®) and the OP triesters: tris(1,3- dichloro-2-propyl) phosphate (TDCIPP); tris(chloropropyl) phosphate (TCIPP); tris(2-chloroethyl) phosphate (TCEP); and triphenyl phosphate (TPHP) (Dodson et al., 2012; Stapleton et al., 2011).

In a previous study, we demonstrated widespread exposure to TCEP, TPHP and TDCIPP and FM 550® in 310 pregnant women participating in the CHAMACOS birth cohort study in 1999–2000, with detection frequencies (DFs) > 70% (Castorina et al., 2017). Another large study (N = 349) in North Carolina also demonstrated ubiquitous exposure to these compounds among pregnant women (Hoffman et al., 2017b). Recent evidence suggests that BDCIPP and DPHP metabolite levels measured in spot urine samples collected from women during the second trimester of pregnancy are reliable over the entire pregnancy (Hoffman et al., 2014). The intraclass correlation coefficients (ICC) for BDCIPP and DPHP from multiple samples collected during the second trimester and at birth from this study were 0.5 (95% CI: 0.3, 0.7) and 0.6 (95% CI: 0.4, 0.7), respectively, indicating moderate to strong correlations.

Although PFR exposure has been associated with adverse neurologic, reproductive, and other outcomes (e.g., altered thyroid and liver weights) in laboratory animals (ATSDR, 2012; Babich, 2006; NRC, 2000; US EPA, 2005), to date no studies have examined the effects of PFRs on human neurodevelopment. PFRs are structurally similar to organophosphate (OP) pesticides, known human neurotoxicants, that can disrupt neural development through non-cholinergic mechanisms (Levin et al., 2002; Slotkin et al., 2007; Slotkin and Seidler, 2007; Umezu et al., 1998). *In-vitro* studies using PC12 cells (a widely used model that reproduces key mechanisms of developmental neurotoxicity resulting from OP pesticides) suggest that several PFR compounds are neurotoxic (Dishaw et al., 2011). In a direct comparison of the neurotoxicity of TDCIPP to the OP pesticide chlorpyrifos (Jameson et al., 2006; Qiao et al., 2001, 2005), researchers found that TDCIPP resulted in dose-dependent neurotoxicity, including inhibited DNA synthesis, decreased cell number, and altered neurodifferentiation, often with effects equivalent to or greater than equimolar concentrations (50 μM) of chlorpyrifos. The authors reported adverse effects from all PFRs tested in both the undifferentiated state and during neurodifferentiation, implying that the developing nervous system is likely to be vulnerable to disruption by PFRs beginning in the earliest stages of neural cell division and extending through the formation of neural circuits (Dishaw et al., 2011). In rat studies, TCEP has been shown to induce brain lesions in the hippocampus following acute- and intermediate-duration exposure and in the cerebral cortex and brain stem following chronic-duration exposure (NTP, 1991; Tilson et al., 1990). Studies in animal models suggest that neural systems in the hippocampus and frontal cortex may determine distinctive features of attention-deficit/hyperactivity disorder (ADHD) (di Michele et al., 2005).

There is also growing concern about the potential developmental health effects of exposure to TPHP, a widely used PFR and plasticizer (Mendelsohn et al., 2016). Several recent toxicological studies have linked TPHP exposure with reproductive and developmental toxicity, neurotoxicity, metabolic disruption, endocrine effects, and genotoxicity (Du et al., 2016; Mendelsohn et al., 2016; Zhang et al., 2016). TPHP has also been found to induce significant estrogenic activity *in-vitro* (Krivoshiev et al., 2016; Zhang et al., 2014).

In this study, we examine associations between *in utero* PFR exposure and cognitive and/or behavioral performance (attention) in 310 school-age children participating in the CHAMACOS birth cohort study. This is the first study to examine these exposures and potential neurodevelopmental outcomes in a cohort of pregnant women and children.

2. Materials and methods

Study Population: The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) is a longitudinal birth cohort study of predominantly Mexican-American families residing in California's Salinas Valley. Detailed information about recruitment and study methods are described elsewhere (Eskenazi et al., 2004, 2006). Briefly, 601 eligible pregnant women (≥18 years old, < 20 weeks gestation, Spanish- or English-speaking, qualifying for low-income health insurance, and planning to deliver at the public hospital) were recruited between October 1999 and October 2000 from community clinics resulting in 537 live births. Women were interviewed twice during pregnancy (at ~ 13 and 26 weeks gestation) and the children were followed through age 7. We analyzed OPFRs in 310 maternal urine samples (Castorina et al., 2017).

2.1. Urine sample collection and analysis

Urine collection occurred during the 2nd prenatal study visit (mean gestational age was 26.0 (2.4) weeks) (n = 310). Samples were kept at −80 °C in the CHAMACOS biorepository and then transported on dry ice for analysis at Duke University. We measured four urinary metabolites: bis(1,3-dichloro-2-propyl) phosphate (BDCIPP); diphenyl phosphate (DPHP), and two FM 550® urinary metabolites: (isopropylphenyl phenyl phosphate (ip-PPP); and tert-butylphenyl phenyl phosphate (tb-PPP)). Samples were also analyzed for bis(1-chloro-2-propyl) phosphate (BCIPP), the metabolite of TCIPP. Analyses were performed at Duke University using liquid chromatography-tandem mass spectrometry (LC/MS-MS) (Butt et al., 2014). Laboratory methods were previously described in Castorina et al. (2017). Levels below the detection limit were imputed as the method detection limit (MDL) divided by the square root of 2 (Hornung and Reed, 1990). Results were standardized for urinary dilution using specific gravity (ng/ml) assessed

using a hand-held refractometer (National Instrument Company, Baltimore, MD). Analyses were performed on previously frozen samples.

2.2. Cognitive and behavior assessments

We assessed cognitive abilities when the children were 7-years of age using the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV) (Wechsler, 2003). All assessments were completed by a single bilingual psychometrician, who was trained and supervised by a pediatric neuropsychologist. Scores for four domains were calculated based on the following subtests: Verbal Comprehension (composed of Vocabulary and Similarities subtests), Perceptual Reasoning (Block Design and Matrix Reasoning subtests), Working Memory (Digit Span and Letter-Number Sequencing subtests), and Processing Speed (Coding and Symbol Search subtests). We administered all subtests in the dominant language of the child, which was determined through administration of the oral vocabulary subtest of the Woodcock–Johnson/Woodcock–Munoz Tests of Cognitive Ability in both English and Spanish (Woodcock and Munoz-Sandoval, 1990) at the beginning of the assessment. The psychometrician was blinded to exposure status. We standardized WISC-IV scores against U.S. population–based norms for English- and Spanish-speaking children. We did not administer Letter-Number Sequencing or Symbol Search subtests for the first 3 months of assessments, therefore 27 participants lack scores for Processing Speed and Working Memory domains. We excluded children with medical conditions that could affect neurodevelopmental assessment (n = 4, one child each with Downs syndrome, autism, deafness, and hydrocephalus). A total of 330 children were followed until 7 years of age. A Full-Scale IQ was available for 268 children with maternal urinary PFR metabolite measurements.

Children's behavior was assessed by maternal and teacher report at age 7 using the Behavior Assessment System for Children 2 (BASC-2) (Reynolds and Kamphaus, 2004) and the Conners' ADHD/DSM-IV Scales (CADS) (Conners, 2001). The behavior assessments were interviewer-administered to the mother (due to low literacy rates) and self-administered by the child's teacher. Both instruments have been validated in English and Spanish. The BASC-2 Parent Rating Scale asks how often the child exhibits certain behaviors in the home setting (160 questions), while the Teacher Rating Scale asks about similar behaviors at school (139 questions). Scales of interest from the BASC-2 were hyperactivity and attention problems. Responses were summed into raw scores and standardized T-scores were computed using age-standardized national norms, with higher values indicating more frequent problem behaviors. The CADS Parent and Teacher Forms assess attention and hyperactivity using 26 questions that correspond to the DSM-IV criteria for Attention-Deficit/Hyperactivity Disorder (ADHD). Answers were summed into raw scores and compared to national norms to generate T-scores standardized for age and sex for three DSM-IV-oriented scales (inattention, hyperactivity, and ADHD DSM-IV scales). The number of children with complete behavioral assessments ranged from 308 (CADS by maternal report) to 249 (CADS by teacher report). See Supplementary Material (SM) for more information.

2.3. Maternal interviews and assessments

Bilingual interviewers conducted maternal interviews in Spanish or English twice during pregnancy (~13 and 26 weeks gestation), after delivery and when the children were 6 months and 1, 2, 3.5, 5 and 7-years of age. Interviews obtained demographic information including maternal age, education, country of birth, number of years lived in the United States, marital status, paternal education, and family income. We conducted home visits shortly after enrollment (~16 weeks gestation) and when the child was 6 months and 1, 2, 3.5 and 5-years of age.

Mothers were administered the Peabody Picture Vocabulary Test (PPVT) for English speakers or the Test de Vocabulario en Imagenes Peabody (TVIP) for Spanish speakers at the six-month visit to assess maternal verbal intelligence (Dunn and Dunn, 1981). A short version of the HOME (Home Observation for Measurement of the Environment) inventory was completed during the 7-year visit to assess the home learning environment (Caldwell and Bradley, 2003).

2.4. Data analysis

We log10-transformed continuous prenatal PFR urinary metabolite variables to reduce heteroscedasticity and the influence of outliers. Scores for cognition and behavior were approximately normally distributed and were modeled as continuous outcomes. Although we did not expect nonlinear relationships between neurodevelopment and exposure to these compounds, we used generalized additive models (GAMs) with a three-degrees-of-freedom cubic spline function to test for non-linearity. None of the digression from linearity tests were significant ($p < 0.05$). Therefore, we expressed urinary metabolite levels linearly (on the log10 scale) in separate multivariable linear regression models for each PFR metabolite. Regression coefficients thus represent mean change in cognition or behavior scores for each ten-fold increase in urinary metabolite level. In addition, we calculated the molar sum of the four urinary PFR metabolites (BDCIPP, DPHP, ip-PPP and tbutyl-PPP (ΣPFR)) for statistical analyses.

We selected model covariates *a priori* based on factors associated with child neurodevelopment in previous analyses (Bouchard et al., 2011; Gunier et al., 2017) [i.e., child's exact age at assessment, sex, maternal country of birth (Mexico vs. other) and HOME score at the 7-year visit (continuous)]. We considered the following variables as additional covariates in our models (Table 1): maternal age at delivery, maternal PPVT score (continuous), maternal education (≤6th grade vs. ≥ 7th grade), marital status at enrollment, and the Centers of Epidemiological Studies Depression Scale (CES-D) (Davila et al., 2009) for maternal depression (≥16 on CES-D) at the child's 7-year visit. In addition, we considered other covariates collected at the 7-year visit including housing density (number of persons per room), poverty (<federal poverty level vs. ≥ federal poverty level), presence of father in the home (yes/no), maternal work status, and location of assessment (field office or recreational vehicle). We also evaluated the average of organophosphate pesticide dialkyl phosphate (DAP) metabolites measured in maternal urine samples (Bradman et al., 2005) collected during pregnancy (at 13 weeks and 26 weeks gestation (n = 310)) in all models since these metabolite levels were related to Full-Scale IQ in a previous study of this cohort (Bouchard et al., 2011). We retained covariates that were significant ($p < 0.2$) in the final multivariate regression models. We included child age at assessment, sex, maternal country of birth, HOME score at the 7-year interview and prenatal DAPs in all models. We included maternal depression in all models except for those assessing CADS and BASC teacher reports. In all models of cognition and maternal reports of attention, we also included maternal education, maternal intelligence (PPVT) and household poverty level from the 7-year interview.

In separate sensitivity analyses, we controlled for exposure to other neurotoxicants, which we have found to be related to child IQ or attention-deficit/hyperactivity disorder in our cohort adjusting for the same covariates listed above (Eskenazi et al., 2013; Gaspar et al., 2015). Specifically, we considered log10-transformed lipid-

Table 1
CHAMACOS 7-year old child and maternal demographics (n = 310).

Cohort Characteristics	n (%)
Child intelligence (Full-Scale WISC score)	
≤74	9 (3.4)
75–99	100 (37.3)
≥100	159 (59.3)
Maternal PPVT score	
≤74	101 (33.6)
75–99	107 (35.6)
≥100	93 (30.9)
Maternal education	
≤6th grade	146 (47.1)
7-12th grade	99 (31.9)
≥ high school graduate	65 (21.0)
Maternal depression (CES-D>16)[a,b]	
No	205 (72.7)
Yes	77 (27.3)
Maternal age at delivery (years)	
18–24	132 (42.6)
25–29	103 (33.2)
30–34	51 (16.5)
35–45	24 (7.7)
Maternal country of birth	
Mexico	269 (86.8)
Other	41 (13.2)
Maternal years in US prior to birth	
≤1	68 (21.9)
2–5	80 (25.8)
6–10	83 (26.8)
≥11	49 (15.8)
Entire life	30 (9.7)
Family income at 7 years[c]	
At or below poverty threshold	223 (72.4)
Above poverty threshold	85 (27.6)
Parity prior to index child	
0	99 (31.9)
≥1	211 (68.1)
Breastfeeding duration of index child	
≤2 months	61 (19.7)
2-12 months	152 (49.0)
≥12 months	97 (31.3)
Sex of index child	
Female	140 (45.2)
Male	170 (54.8)

[a] Assessed at 7-year visit.
[b] Yes = CES-D score>16.
[c] Poverty levels were calculated using the U.S. Department of Health and Human Services' thresholds for the year 2007. A family of four with an annual income of $ 20,650 or less was considered to be at or below the poverty level.

adjusted concentrations (ng/g-lipid) of p, p'-dichlorodiphenyltrichloroethylene (DDT), p, p'-dichlorodiphenyldichloroethylene (DDE) (n = 219) (Bradman et al., 2007) and polybrominated diphenyl ether flame retardants (PBDEs) (n = 221) (Castorina et al., 2011) measured in prenatal maternal blood samples. We used the sum of the four major congeners (BDE-47, -99, −100, and −153) to estimate penta-PBDE exposure (Eskenazi et al., 2013). In other sensitivity analyses, we excluded outliers identified with studentized residuals greater than three. To control for potential selection bias due to loss to follow-up, we ran regression models with weights determined as the inverse probability of inclusion in our analyses (Hogan et al., 2004). We determined probability of inclusion using multiple logistic regression models with baseline covariates as potential predictors. All analyses were conducted in STATA 13 (StataCorp, College Station, TX).

3. Results and discussion

3.1. Demographic characteristics

Eighty-five percent of CHAMACOS study pregnant women were born in Mexico, with 48% having spent <5 years in the United States. Their mean age was 26 years, and nearly all lived within 200% of the federal poverty level. Table 1 presents CHAMACOS 7-year old child and maternal characteristics (n = 310). Additional demographic information on this population has been published previously (Eskenazi et al., 2004).

3.2. Prenatal urinary metabolite levels

Urinary flame retardant metabolite concentrations for pregnant women from the CHAMACOS cohort have been reported previously (Castorina et al., 2017). Briefly, metabolites of TDCIPP (BDCIPP) and TPHP (DPHP) were detected in 78% and 79% of 310 prenatal urine samples (see SM Table S1). Specific gravity corrected median (max) PFR metabolite levels in the CHAMACOS cohort were 0.4 (53.1) ng/ml for BDCIPP and 0.9 (54.1) ng/ml for DPHP. The presumed urinary metabolite (ip-PPP) of isopropylphenyl diphenyl phosphate (ip-PDPP) was detected in 72% of samples with a median (max) of 0.3 (5.5 ng/ml) (Castorina et al., 2017). The compound ip-PDPP is a component of FM® 550, although it can also be used as a plasticizer in other applications. The metabolite tb-PDPP was detected in 15% of samples. BCIPP was not detected in any samples. Statistical analyses for this paper focused on three metabolites with detection frequencies >70%: BDCIPP, DPHP and ip-PPP. These three metabolites significantly correlated with each other, albeit weakly (Pearson r's = 0.14–0.30, p-values<0.05) (See SM Table S4). Compared to urinary BDCIPP, DPHP and ip-PPP levels measured in 349 pregnant women from North Carolina in the early 2000s (Hoffman et al., 2017b), the median (max) levels in the CHAMACOS cohort (BDCIPP: 0.4 (53.1) ng/ml, DPHP: 0.9 (54.1) ng/ml, and ip-PPP: 0.3 (5.5) ng/ml) were lower than the North Carolina study (BDCIPP: 1.9 (140) ng/ml, DPHP: 1.3 (112) ng/ml, and ip-PPP: 7.1 (69) ng/ml).

Table 2
Change in cognitive scores in children tested at 7 years for each 10-fold increase in prenatal urinary metabolite concentration (ng/ml).[a]

Cognitive test	n	BDCIPP β (95% CI)[b]	DPHP β (95% CI)[b]	ip-PPP β (95% CI)[b]	∑PFR metabolites β (95% CI)[d]
WISC-IV scale					
Full-Scale IQ	248	0.2 (−1.9,2.4)	−2.9 (−6.3,0.5)†[c]	−0.0 (−4.3,4.2)	−3.8 (−8.2,0.5)†
Working memory	249	−0.2 (−2.3,2.0)	−3.9 (−7.3,-0.5)**[c]	1.1 (−3.1,5.3)	−4.6 (−8.9,-0.3)**
Perceptual reasoning	275	0.7 (−1.8,3.2)	−1.0 (−4.9,2.8)	−0.6 (−5.4,4.2)	−1.6 (−6.5,3.3)
Verbal comprehension	275	−0.1 (−2.2,2.0)	−1.8 (−5.0,1.4)	−0.4 (−4.3,3.6)	−2.9 (−6.9,1.2)
Processing speed	249	−0.4 (−2.6,1.7)	−2.6 (−6.1,0.7)	1.0 (−3.1,5.2)	−3.1 (−7.3,1.2)

†p < 0.1; **p < 0.05; Abbreviations: BDCIPP = bis(1,3-dichloro-2-propyl) phosphate; DPHP = diphenyl phosphate; ip-PPP = isopropylphenyl phenyl phosphate.
[a] Concentrations were standardized for specific gravity to account for urine dilution. Models were adjusted for maternal education, PPVT scores, CES-D scores, country of birth and prenatal urinary DAP metabolite levels; HOME z-score; language of WISC testing; child sex and age at assessment; and household poverty.
[b] β = beta coefficient from regression models, CI = confidence interval.
[c] β's for Full-Scale IQ and working memory increase when the 2 outliers are removed (see text) (β = −4.3; 95% CI: −7.6, −0.9 and β = −4.9; 95% CI: −8.2, −1.6), respectively.
[d] ∑PFR metabolites represent the molar sum (nmol/L) of the four urinary PFR metabolites (BDCIPP, DPHP, ip-PPP and tb-PPP).

3.3. Prenatal PFR exposure and cognitive function

The mean (standard deviation) Full-Scale IQ score for the CHAMACOS cohort at the 7-year visit was 103.1 (14.3) (See SM Table S5). In general, IQ scores were lower across all domains with increasing DPHP and ΣPFR metabolite levels (See Table S6 and Table 2). We observed an estimated 3.9-point decrease (95% CI: −7.3, −0.5) in Working Memory for each ten-fold increase in urinary DPHP metabolites (n = 249) and a 2.9-point decrease (95% CI: −6.3, 0.5) in Full-Scale IQ (n = 248) (Table 2). In addition, we found lower Working Memory and Full-scale IQ scores with increasing ΣPFR metabolite levels. We observed an estimated 4.6-point decrease (95% CI: −8.9, −0.3) in Working Memory for each ten-fold increase in ΣPFR metabolites (n = 249) and a 3.8-point decrease (95% CI: −8.2, 0.5) in Full-Scale IQ (n = 248). Relationships between DPHP and ΣPFR metabolites and Full-Scale IQ, however, were not statistically significant (p = 0.09 and 0.07, respectively). Fig. 1 presents prenatal PFR molar sum quintiles and children's working memory. The effect of PRF exposure on IQ is most notable at the highest quintile. Similar patterns were seen for Full-Scale IQ (not shown). There were no significant relationships between urinary BDCIPP and ip-PPP levels and Full-Scale IQ or any of the WISC-IV subscales (Table 2). We did not observe significant interaction by sex for the association of prenatal metabolite concentrations with any of the subscales so boys and girls were analyzed together.

Previous studies of the CHAMACOS cohort have reported decrements in WISC Full-Scale IQ scores among 7-year-olds with increasing concentrations of maternal prenatal urinary DAPs (Bouchard et al., 2011) and serum PBDEs (Eskenazi et al., 2013). For each ten-fold increase in maternal DAP concentrations, a decrease of 5.6 (95% CI: −9.0, −2.2) was reported in children's Full-Scale IQ controlling for maternal education and intelligence, HOME scores at the 6-month study visit, and language of assessment (n = 297) (Bouchard et al., 2011). Due to the strength of the association found between maternal prenatal DAP metabolite levels and Full-Scale IQ in CHAMACOS children, we controlled for prenatal DAP metabolite levels as well as other important covariates in our models (see Table 2).

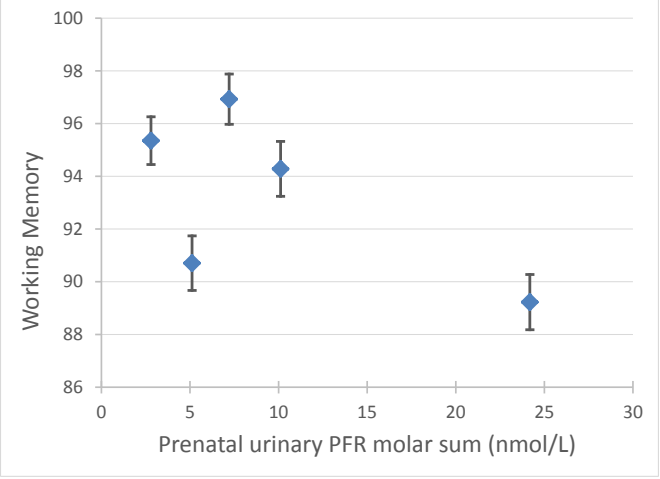

Fig. 1. Mean ± SE WISC-IV score per quintile of prenatal urinary PFR molar sum concentration and Working Memory. The medians (ranges) for ∑PFR quintiles (nmol/L) are as follows: first quintile, 2.8 (0.7–4.0); second, 5.1 (>4.0–6.1); third, 7.2 (>6.1–8.6); fourth, 10 (>8.6–14); and fifth, 24.2 (>14-220). Estimates were adjusted for maternal education, PPVT scores, CES-D scores, country of birth and prenatal urinary DAP metabolite levels; HOME z-score; language of WISC testing; child sex and age at assessment; and household poverty.

Eskenazi et al. (2013) studied the relationship between prenatal penta-PBDE congener (BDE-47, -99, −100 and −153) levels in serum and cognition in 7-year-old CHAMACOS children. That study found decrements of 4.7 (95% CI: −9.4, 0.1) in Full-Scale IQ with each ten-fold increase in ∑PBDEs (ng/g lipids) in multivariable regression models controlling for child's age, sex, home score at the 6-month study visit, language of assessment, and maternal years living the U.S. before giving birth (n = 231) (Eskenazi et al., 2013). The strength of the reported associations between prenatal PBDE levels and Full-Scale child IQ scores were higher than those we found for ΣPFR metabolites. Results from the sensitivity analyses of the PFR models with and without inclusion of PBDEs are described below.

3.3.1. Sensitivity analysis

Results were similar for PFR metabolite levels and WISC-IV cognition scales with and without the inclusion of DDT, DDE and PBDEs (not shown) even though the sample sizes were smaller for the models that included these covariates (e.g., n = 184 for Full-Scale IQ model including PBDEs). Excluding the relatively few outliers for BDCIPP (n = 1), DPHP (n = 2) and ip-PPP (n = 1) did not change our results other than to strengthen the association between urinary DPHP levels and Full-Scale IQ (β = −4.3; 95% CI: −7.6, −0.9) and working memory scores (β = −4.9; 95% CI: −8.2, −1.6). Associations between DPHP metabolite levels and Full-Scale IQ were similar (β = −2.6; 95% CI: −7.0, 0.8) to our final model when we used inverse probability weighting to adjust for potential selection bias.

3.4. Prenatal PFR exposure and children's behavior

The associations of prenatal TDCIPP, TPHP and ip-PDPP metabolite concentrations with child behavior as assessed by the BASC-2 and CADS are shown in Table 3. We did not observe significant interaction by sex for the association of prenatal metabolite concentrations with any of the subscales so boys and girls were analyzed together.

Higher maternal urinary ip-PPP concentrations during pregnancy were associated with increased hyperactivity in 281 boys and girls at age 7 years according to mothers' report from the BASC-2, but not for teachers' report. Each ten-fold increase in prenatal ip-PPP concentrations was associated with an increase in hyperactivity scores of 2.4 points (95% CI: 0.1, 4.7) by mothers' report (Table 3). Prenatal ip-PPP concentrations were not associated with inattention or hyperactivity in CHAMACOS children using the CADS at age 7.

No significant associations were observed with prenatal BDCIPP, DPHP or ∑PFR metabolite concentrations and attention or hyperactivity, although for most scales, the point estimates trended towards increased behavior problems.

3.5. Limitations

This study had several limitations. We measured urinary flame retardants once during pregnancy. Given that these metabolites have relatively short half-lives in the body, this measurement may not reflect chronic exposures (Meeker et al., 2013). There is evidence, however, to suggest that urinary BDCIPP and DPHP levels measured in samples collected during the second trimester are moderately to strongly reliable over the course of the entire pregnancy (Hoffman et al., 2014), likely due to constant sources of exposure in the home environment. Further, documented PFR exposure levels have increased over the last decade, and in some cases, urinary metabolite levels (e.g., BDCIPP) are dramatically higher today than they were in the early 2000's (Hoffman et al.,

Table 3
Change in scores for attention-related outcomes in CHAMACOS 7-year-old children, for each 10-fold increase in maternal prenatal urinary metabolite concentration (ng/ml; specific gravity standardized) using adjusted linear models.[a]

	n	BDCIPP β (95% CI)	DPHP β (95% CI)	ip-PPP β (95% CI)
Conner's Rating Scale (CADS)				
– Maternal Report (T-score)				
ADHD Index	282	0.5 (−0.7, 1.6)	−1.2 (−3.0, 0.6)	0.6 (−1.6, 2.8)
Inattention DSM-IV	282	0.4 (−0.7, 1.5)	−0.9 (−2.6, 0.8)	0.3 (−1.8, 2.4)
Hyperactive/Impulsive DSM-IV	282	0.8 (−0.4, 2.0)	−0.8 (−2.7, 1.1)	0.4 (−1.9, 2.7)
Total subscale DSM-IV	282	0.7 (−0.4, 1.8)	−0.9 (−2.7, 0.9)	0.3 (−1.9, 2.5)
BASC-2 – Maternal Report (T-score)				
Hyperactivity scale	281	0.9 (−0.3, 2.1)	−0.6 (−2.5, 1.3)	2.4 (0.1, 4.7)**
Attention problems scale	281	0.5 (−1.0, 2.1)	0.8 (−1.7, 3.2)	1.2 (−1.9, 4.2)
Conner's Rating Scale (CADS)				
– Teacher Report (T-score)				
ADHD Index	243	1.0 (−0.9, 2.8)	1.4 (−1.6, 4.4)	0.4 (−3.3, 4.2)
Inattention DSM-IV	246	0.5 (−0.9, 1.8)	1.0 (−1.3, 3.3)	−0.8 (−3.6, 2.0)
Hyperactive/Impulsive DSM-IV	246	0.9 (−0.8, 2.6)	1.0 (−1.8, 3.8)	1.1 (−2.3, 4.6)
Total subscale DSM-IV	242	0.8 (−0.8, 2.4)	1.1 (−1.6, 3.7)	0.1 (−2.3, 3.2)
BASC-2 – Teacher Report (T-score)				
Hyperactivity scale	247	0.5 (−1.1, 2.1)	1.4 (−1.2, 3.9)	1.1 (−2.1, 4.3)
Attention problems scale	247	1.1 (−0.1, 2.3)†	1.0 (−0.9, 3.0)	0.0 (−2.4, 2.5)

†$p < 0.1$; **$p \leq 0.05$; Abbreviations: ADHD = attention-deficit/hyperactivity disorder; CI = confidence interval; BDCIPP = bis(1,3-dichloro-2-propyl) phosphate; DPHP = diphenyl phosphate; ip-PPP = isopropylphenyl phenyl phosphate.

[a] Models adjust for sex, age at assessment, maternal country of birth, HOME score at 7-years, prenatal DAPs, and maternal depression and education (included in Maternal Report models only). We observed no significant differences in the results between analyses of the individual metabolites and ΣPFRs (data not shown).

2017a). Thus, research is needed to assess potential neurodevelopmental health effects related to current exposure levels. Future studies should also evaluate both prenatal and postnatal metabolite levels based on multiple measurements made during pregnancy and childhood and investigate exposure to other flame retardants that were not measured here.

4. Conclusions

This is the first study to examine PFR and FM 550® exposures during pregnancy and potential neurodevelopmental outcomes in children. We tested associations between cognitive and behavioral assessment results and four prenatal urinary PFR metabolites in a birth cohort of 7-year-old children. We observed decreases in Full-Scale intelligence quotient and Working Memory with increasing prenatal DPHP and total PFR metabolite levels in multivariable regression models adjusted for important confounders. However, no significant associations were found between neurodevelopment and metabolites of TDCIPP (BDCIPP) or ip-PDPP (ip-PPP). Given widespread exposure to these compounds among pregnant women (Castorina et al., 2017; Hoffman et al., 2017b) and children (Butt et al., 2014; Cequier et al., 2015) and growing concern about potential neurodevelopmental effects of these toxicants, more studies are needed with a wider range of biological measurements to investigate potential neurodevelopmental effects of PFR exposure in this population.

Statement of financial interest

Dr. Asa Bradman is a volunteer member of the Board for The Organic Center, a non-profit addressing scientific issues around organic food and agriculture. None of the other authors declares any actual or potential competing financial interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2017.09.037.

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