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Childhood exposure to per- and polyfluoroalkyl substances (PFAS) and neurobehavioral domains in children at age 8 years

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ABSTRACT

Background: Toxicological studies have raised concerns regarding the neurotoxic effects of *per-* and polyfluoroalkyl substances (PFAS). However, observational evidence from human studies investigating the association between childhood PFAS and neurobehavior is limited and remains unclear.
Objectives: To examine whether childhood PFAS concentrations are associated with neurobehavior in children at age 8 years and whether child sex modifies this relationship.
Methods: We used data from 208 mother-child dyads in the Health Outcomes and Measures of the Environment (HOME) Study, a prospective pregnancy and birth cohort (Cincinnati, OH, USA). We quantified PFAS in child serum at 3 and 8 years. We assessed neurobehavioral domains using the Behavior Assessment System for Children-2 at 8 years. We used multiple informant models to estimate score changes per ln-increase in repeated PFAS concentrations.
Results: Childhood PFAS were not associated with Externalizing or Internalizing Problems at 8 years. However, we noted effect measure modification by sex, with higher scores in Externalizing Problems among males per ln-unit increase in perfluorononanoate (PFNA) at 3 years ($\beta = 4.3$ points, 95% CI: 1.0, 7.7) while females had lower scores ($\beta = -2.8$ points, 95% CI: −4.7, −1.0). More Internalizing Problems were observed among males per ln-unit increase in concurrent PFNA concentrations ($\beta = 3.7$ points, 95% CI: 0.7, 6.8), but not in females ($\beta = -1.7$ points, 95% CI: −4.6, 1.2). Childhood PFNA concentrations were associated with lower scores for attention problems and activity of daily living.
Conclusion: While findings do not consistently support an association between childhood PFAS serum concentrations and neurobehavior, child sex may play a role in this relationship.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of environmentally persistent man-made chemicals that have both hydro- and lipophilic properties, making them highly desirable for commercial and industrial uses as surfactants, emulsifiers, and performance chemicals, such as hydraulic fluid and fuel additives. PFAS, which are chemically stable due to the strong C—F bond, are highly resistant to biological, chemical, and thermal degradation. PFAS are ubiquitous pollutants that have been detected in environmental media, wildlife species, and

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human tissues (Lau et al., 2007). Data from the National Health and Nutrition Examination Survey (NHANES) show that median concentrations of serum perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), and perfluorononanoate (PFNA) have declined among the US population (Dong et al., 2019). However, PFAS remain a public health concern because of mounting evidence linking them with cardiovascular disease, immunosuppression, and neurodevelopment (Bach et al., 2016; DeWitt et al., 2018; Liew et al., 2018; Rappazzo et al., 2017; Sunderland et al., 2019).

PFAS can disrupt thyroid hormone levels, alter neural cell differentiation, disturb neurochemical signaling and homeostasis, alter the susceptibility of the cholinergic system, stimulate neuronal cell apoptosis, and induce the formation of reactive oxidative stress (Berntsen et al., 2017; Eggers Pedersen et al., 2015; Johansson et al., 2009; Johansson et al., 2008; Lee and Viberg, 2013; Lee et al., 2016; Liu et al., 2013; Liu et al., 2015; Long et al., 2013; Reistad et al., 2013; Slotkin et al., 2008; Yu et al., 2016). Experimental studies in mice have reported disturbances in cognition and behavior from PFAS exposures occurring prenatally and neonatally (Fuentes et al., 2007; Johansson et al., 2009; Johansson et al., 2008; Viberg et al., 2013). In epidemiological studies, prenatal concentrations of PFAS have been associated with behavioral problems in children in the INUENDO (Biopersistent organochlorines in diet and human fertility) cohort (Hoyer et al., 2015), the Danish National Birth Cohort (DNBC) (Luo et al., 2020), the Health Outcomes and Measures of the Environment (HOME) Study (Vuong et al., 2021), the Upstate KIDS Study (Ghassabian et al., 2018), and in a Faroese cohort (Oulhote et al., 2019). Comparatively, the number of epidemiological studies investigating the potential role of childhood PFAS in neurobehavior are limited and results have been inconsistent. Some epidemiological studies have reported childhood PFAS were associated with behavioral problems in children (Gump et al., 2011; Oulhote et al., 2019; Oulhote et al., 2016), while others have reported protective or null associations (Stein et al., 2013, 2014). Further, findings regarding the relationship between childhood PFAS with internalizing behaviors and adaptive skills remains unclear (Oulhote et al., 2019; Oulhote et al., 2016; Stein et al., 2014).

Our study objectives were to: 1) examine the relationship of repeated measures of serum PFAS concentrations during childhood with neurobehavioral domains assessed at age 8 years, identifying possible windows of susceptibility; 2) identify potential sexual dimorphism in these associations; and 3) examine the persistence of our previously reported adverse associations between prenatal PFAS and neurobehavior taking into account childhood PFAS concentrations (Vuong et al., 2021).

2. Materials and methods

2.1. Study design and cohort

We used the HOME Study, a prospective pregnancy and birth cohort, for the present study. Between March 2003 and February 2006, we recruited pregnant women from the greater Cincinnati area that fulfilled the following inclusion criteria: 1) ≥18 years of age; 2) 16 ± 3 weeks of gestation; 3) residing in housing constructed before 1978; 4) receiving and planning to continue prenatal care and deliver at one of the collaborating obstetric practices; 5) HIV- status; and 6) not taking any medications related to seizures, thyroid disorders, or chemotherapy/radiation. A detailed description of the methodology for the HOME Study is described by Braun et al. (2020, 2017). Of the 468 enrolled women, 390 remained to deliver live singletons. We included in the present study 208 participants for whom information was available for at least one PFAS measurement during childhood and a neurobehavioral assessment at age 8 years. The study protocol was approved by the Institutional Review Board (IRB) at the Cincinnati Children's Hospital Medical Center (CCHMC). The Centers for Disease Control and Prevention (CDC) and collaborating institutions deferred to CCHMC IRB as the IRB of record.

2.2. Childhood serum PFAS concentrations

Our analysis focused on PFOA, PFOS, PFHxS, and PFNA. These PFAS compounds were quantified in child sera collected at ages 3 ($n = 146$) and 8 years ($n = 193$) using on-line solid-phase extraction coupled to high-performance liquid chromatography-isotope dilution mass spectrometry with established quality control procedures (Kato et al., 2011). The limit of detection (LOD) was 0.1 ng/mL for all PFAS except PFOS, which was 0.2 ng/mL. We detected the four PFAS in all samples analyzed from HOME Study children aged 3 and 8 years.

2.3. Neurobehavioral assessment

We assessed neurobehavior in 8-year-old children by parent-report using the Behavioral Assessment System for Children-2 (BASC-2) (Reynolds and Kamphaus, 2004). The BASC-2 yields four composite scales: 1) Externalizing Problems, including hyperactivity, aggression, and conduct problem subscales; 2) Internalizing Problems, including anxiety, depression, and somatization subscales; and 3) Adaptive Skills, including adaptability, social skills, activity of daily living, and functional communication subscales; and 4) Behavioral Symptoms Index, including Externalizing Problems, Internalizing Problems, and attention problems, atypicality, and withdrawal subscales. All scales have a mean of 50 ± 10, with higher scores indicating poorer functioning or more of those behaviors. In contrast, for Adaptive Skills and its subscales, higher scores indicate better functioning.

2.4. Statistical methods

We analyzed data using descriptive statistics; we used the Student *t*-test for continuous variables and X^2 for comparison of categorical variables. To test the hypothesis that childhood PFAS concentrations are associated with poorer neurobehavioral outcomes in children, we used multiple informant models to estimate βs and 95% confidence intervals (CIs) between repeated measures of ln-transformed PFAS at ages 3 and 8 years and neurobehavior at age 8 years (Sanchez et al., 2011). This method utilizes a non-standardized version of generalized estimating equations for parameter estimation with the incorporation of separate linear regression models that are embedded into each set of estimating equations for each time period of interest. Thus, this model is able to incorporate repeated measures of PFAS concentrations during childhood to determine whether associations between PFAS measurements and BASC-2 scores differ by timing of measurement during childhood. To determine whether there are susceptible periods of vulnerability, we incorporated the interaction term between PFAS and age at measurement, with statistical significance considered present if $p < 0.10$. Because some interaction terms had a $p < 0.10$, we presented age-at-exposure-measurement-specific βs. Final models included covariates based on findings from Kingsley et al. (2018). Maternal sociodemographic and behavior factors included age, race/ethnicity, marijuana use, depression at enrollment, use of vitamin supplements during pregnancy, IQ, marital status, and whether the child was breastfed. We also adjusted for maternal blood lead level (16 ± 3 weeks gestation), maternal serum cotinine (16 ± 3 weeks gestation), household income, child sex, and the Home Observation for Measurement of the Environment (HOME) score at age 1 year, which is an assessment of how nurturing the home environment is by measuring the quality and quantity of encouragement and support for child development (Caldwell and Bradley, 1984). We additionally adjusted for prenatal concentrations of BDE-47 in a sensitivity analysis to determine if our conclusions remained the same.

Using generalized additive models, we tested for non-linear relationships between childhood PFAS concentrations and neurobehavior. We estimated sex-specific associations for males and females using the 3-way interaction term between PFAS (continuous), child sex (categorical), and visit at PFAS measurement (categorical), as well as all possible

2-way interactions (PFAS×child sex, PFAS×visit, visit×child sex), with a $p < 0.10$ indicating that statistically significant effect measure modification by child sex was present. Finally, we adjusted for prenatal PFAS concentrations to test whether associations differed between childhood PFAS and BASC-2 scores and to examine whether previous associations reported between prenatal PFAS and neurobehavior remained after taking into account childhood PFAS concentrations (Vuong et al., 2021).

For the secondary analyses, we re-examined the relationship between childhood PFAS and BASC-2 outcomes using body burden as the measure of exposure to account for the dynamic and complex uptake and elimination of PFAS during childhood that are influenced by collinear temporal changes, such as dilution due to physical growth. PFAS body burdens at ages 3 and 8 years were calculated using the formula: body burden (μg) = PFAS serum concentration (μg/mL) × volume of distribution (mL/kg bw) × body weight (kg) (Thompson et al., 2010). We used PFAS serum concentrations measured at children's ages 3 and 8 years. For volume of distribution, we used values provided by Thompson et al. (2010) for PFOA (170 mL/kg bw) and PFOS (230 mL/kg bw). For PFNA and PFHxS, volume distributions for PFOA and PFOS were used, respectively. This substitution, which was based on rodent studies, showed that the volume distributions of differing chain length perfluoroalkyl carboxylic acids are within the same range (Koponen et al., 2018). Childhood body weight (kg) was measured using a scale at ages 3 and 8 years.

3. Results

3.1. Study participants

The majority of women in the HOME Study were non-Hispanic white (59.4%), 25–34 years of age (57.5%), educated beyond a high school degree (72%), married or living with a partner (73%), nonsmokers (82.1%), and did not consume alcohol during pregnancy (55.1%) (Table 1). We found that PFOA concentrations at ages 3 and 8 years were significantly higher among children of mothers who were older, non-Hispanic white, had minimal or mild depressive symptoms, were married or living with a partner, and who breastfed their child. PFOA concentrations were additionally significantly higher among children from households with annual incomes ≥$80,000 and HOME scores ≥40. We also found significantly higher PFOS and PFHxS concentrations at ages 3 and 8 years among children from households with higher HOME scores and with two parents. Externalizing Problems scores at age 8 years were significantly higher among children of mothers who used marijuana during pregnancy and who were moderately/severely depressed.

Table 1
Childhood serum concentrations of PFAS (ng/mL) and BASC-II scores [Mean(SD)] at 8 years in the HOME Study by various characteristics.

	n	3 years (GM)				8 years (GM)				Externalizing Problems
		PFOA	PFOS	PFHxS	PFNA	PFOA	PFOS	PFHxS	PFNA	
Overall		5.4	6.6	1.9	1.4	2.4	3.9	1.4	0.8	50(10)
Age, years										
<25	58	4.1*	5.2	1.3	1.1	2.0*	3.4	1.1*	0.6*	49(11)
25–34	119	5.7	7.0	2.1	1.5	2.5	4.0	1.5	0.8	50(9)
≥35	30	6.1	6.9	1.9	1.4	3.0	4.8	1.3	0.8	50(8)
Race/ethnicity										
Non-Hispanic White	123	6.5*	8.1*	2.5*	1.6*	2.8*	1.5*	1.6*	0.8	50(9)
Non-Hispanic Black and Others	84	3.9	4.5	1.1	1.1	2.0	3.2	1.1	0.7	50(11)
Household income										
<$40,000	88	4.1*	5.0*	1.2*	1.2	2.1*	3.6*	1.2	0.7	51(11)
$40,000–$79,999	67	5.9	6.8	2.1	1.5	2.5	3.8	1.4	0.8	49(9)
≥$80,000	52	6.8	9.1	2.9	1.5	3.0	4.6	1.6	0.9	49(8)
Maternal marijuana use										
No	192	5.5*	6.7	2.0*	1.4	2.5	4.0*	1.4	0.8	49(9)*
Yes	15	3.7	4.6	0.8	1.6	2.0	3.0	1.1	0.8	57(12)
Maternal depression										
Minimal or mild	185	5.6*	6.6	1.9	1.4	2.5*	4.0	1.4	0.8	49(9)*
Moderate or severe	20	4.2	6.2	1.9	1.4	2.0	3.1	1.1	0.7	56(11)
Maternal vitamin use										
Daily	159	5.5*	6.8	2.0	1.4	2.5	4.0	1.4	0.8	49(10)
<Daily	35	5.8	6.3	1.8	1.3	2.4	3.5	1.5	0.8	51(10)
Never	13	3.2	4.1	1.2	1.6	2.2	3.5	0.9	0.8	54(7)
Marital status										
Married or living with partner	151	5.9*	7.1*	2.1*	1.5	2.6*	4.2*	1.5*	0.8	49(9)
Not married or living alone	56	3.8	4.9	1.3	1.2	2.0	3.2	1.1	0.7	51(12)
HOME Score										
≥40	119	6.4*	8.0*	2.4*	1.6*	2.8*	4.3*	1.6*	0.8*	49(9)
35–39	40	3.9	4.4	1.4	1.1	1.9	3.3	1.2	0.6	52(12)
<35	34	3.8	4.7	1.0	1.3	2.2	3.7	1.1	0.7	50(8)
Ever breastfed current child										
No	40	3.8*	5.2	1.6	1.0*	2.0*	3.3*	1.1	0.7	50(10)
Yes	166	5.8	6.9	1.9	1.5	2.5	4.0	1.4	0.8	50(10)
Child Sex										
Male	93	5.1	6.1	1.7	1.3	2.3	3.8	1.3	0.7	51(10)
Female	115	5.6	7.0	2.0	1.5	2.5	4.0	1.4	0.8	48(9)

Abbreviations: GM - geometric mean.
* $p < 0.05$.

3.2. Longitudinal trends of PFAS in childhood

The median serum concentrations of PFOS in HOME Study children decreased from age 3 (6.2 ng/mL) to 8 years (3.6 ng/mL) (Supplemental Fig. S1). In contrast, the median body burden of PFOS increased from age 3 (21.5 μg) to 8 years (24.3 μg) (Supplemental Fig. S2). The serum concentrations of PFHxS also increased (6.5 μg to 8.1 μg), while PFNA concentrations were relatively stable (3.2 μg to 3.4 μg). However, serum PFOA concentrations and body burdens both decreased from age 3 to 8 years (5.4 ng/mL to 2.4 ng/mL for serum concentrations; 13.3 μg to 11.6 μg for body burdens).

3.3. Childhood serum PFAS and neurobehavior

Childhood serum PFAS concentrations were not statistically associated with Externalizing or Internalizing Problems or their corresponding subscales at age 8 years (Tables 2–3). While no relationship was observed between PFAS and Behavior Symptoms Index, we did observe a significant inverse association between PFNA concentrations at age 3 years and attention problems (Table 4). Associations between childhood PFAS and Adaptive Skills were not statistically significant, with point estimates close to the null (Table 5). We did, however, find one statistically significant inverse association between PFNA at age 8 years and scores for activity of daily living (β = −2.4 points, 95% CI: −4.4, −0.4), indicating poorer abilities. Additional adjustment for prenatal BDE-47 resulted in similar conclusions (results not shown). No evidence of a non-linear relationship between childhood PFAS and BASC-2 composites were observed. All non-linearity *p*-values in generalized additive models were ≥ 0.10 (results not shown).

3.4. Effect measure modification by child sex

Overall, our results did not suggest that child sex modified the relationships between childhood PFAS concentrations and Adaptive Skills (Supplemental Table S1). However, for Externalizing Problems, we noted significant modification by child sex with higher concentrations of PFNA at age 3 years (interaction term: PFNA × $visit_{3\ years}$ × child sex = 0.074) (Fig. 1). Significantly more externalizing behaviors were observed among males (β = 4.3 points, 95% CI: 1.0, 7.7), while less externalizing behaviors were noted among females (β = −2.8 points, 95% CI: −4.7, −1.0) with higher serum PFNA concentrations at age 3

Table 3
Estimated score differences (95% CIs) in BASC-2 Internalizing Problems scale and subscales at age 8 years by a ln-unit increase in childhood serum PFAS (ng/mL), HOME Study.[a]

PFAS	Internalizing Problems	Anxiety	Depression	Somatization
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
PFOA				
3 years	0.4 (−2.1, 2.9)	−0.4 (−3.0, 2.2)	0.2 (−2.3, 2.7)	1.4 (−1.2, 4.0)
8 years	−0.7 (−3.5, 2.0)	−1.1 (−4.1, 1.9)	−0.2 (−2.9, 2.6)	−0.3 (−3.2, 2.6)
PFOS				
3 years	0.6 (−1.5, 2.6)	1.4 (−0.9, 3.8)	−0.2 (−2.1, 1.6)	0.1 (−2.2, 2.4)
8 years	0.2 (−2.3, 2.7)	−0.2 (−3.2, 2.8)	0.7 (−1.5, 3.0)	−0.1 (−2.8, 2.7)
PFHxS				
3 years	0.6 (−0.6, 1.7)	0.8 (−0.6, 2.1)	0.8 (−0.4, 1.9)	−0.2 (−1.7, 1.3)
8 years	0.2 (−1.5, 1.9)	0.3 (−1.7, 2.3)	0.4 (−1.2, 1.9)	−0.3 (−2.2, 1.6)
PFNA				
3 years	0.1 (−2.3, 2.5)	0.5 (−1.8, 2.9)	−0.5 (−2.4, 1.4)	0.3 (−2.2, 2.8)
8 years	0.7 (−1.6, 3.0)	0.1 (−2.6, 2.7)	1.0 (−1.2, 3.2)	0.7 (−1.6, 3.0)

PFAS were ln-transformed.
[a] Adjusted by maternal age, race/ethnicity, household income, maternal marijuana use, maternal blood lead, maternal serum cotinine, maternal depression, vitamin use during pregnancy, maternal IQ, marital status, Home Observation for Measurement of the Environment Score, whether the child was breastfed, and child sex.

Table 2
Estimated score differences (95% CIs) in BASC-2 Externalizing Problems scale and subscales at age 8 years by a ln-unit increase in childhood serum PFAS (ng/mL), HOME Study.[a]

PFAS	Externalizing Problems	Hyperactivity	Aggression	Conduct problems
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
PFOA				
3 years	0.1 (−2.7, 2.8)	−0.4 (−3.3, 2.6)	−0.2 (−2.8, 2.4)	0.7 (−2.0, 3.5)
8 years	0.2 (−2.8, 3.1)	−0.3 (−3.4, 2.8)	−0.2 (−3.1, 2.7)	1.0 (−2.4, 4.4)
PFOS				
3 years	−1.3 (−3.5, 1.0)	−1.2 (−3.4, 1.1)	−1.0 (−3.4, 1.5)	−1.2 (−3.4, 1.1)
8 years	−1.4 (−4.1, 1.3)	−1.8 (−4.7, 1.0)	−1.6 (−4.2, 0.9)	−0.2 (−3.1, 2.7)
PFHxS				
3 years	0.02 (−1.6, 1.6)	−0.3 (−1.9, 1.2)	0.1 (−1.5, 1.7)	0.4 (−1.3, 2.1)
8 years	−1.1 (−2.8, 0.6)	−1.9 (−3.9, 0.04)	−1.2 (−2.9, 0.5)	0.1 (−1.9, 2.1)
PFNA				
3 years	−0.7 (−2.5, 1.1)	−0.8 (−2.5, 0.9)	−0.7 (−2.6, 1.2)	−0.4 (−2.2, 1.4)
8 years	−0.03 (−1.9, 1.9)	0.8 (−1.4, 3.0)	−0.9 (−3.0, 1.1)	−0.003 (−2.2, 2.1)

PFAS were ln-transformed.
[a] Adjusted by maternal age, race/ethnicity, household income, maternal marijuana use, maternal blood lead, maternal serum cotinine, maternal depression, vitamin use during pregnancy, maternal IQ, marital status, Home Observation for Measurement of the Environment Score, whether the child was breastfed, and child sex.

Table 4
Estimated score differences (95% CIs) in BASC-2 Behavior Symptoms Index and subscales at age 8 years by a ln-unit increase in childhood serum PFAS (ng/mL), HOME Study.[a]

PFAS	Behavior Symptoms Index	Atypicality	Withdrawal	Attention problems
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
PFOA				
3 years	0.3 (−2.4, 3.0)	0.3 (−2.4, 3.1)	1.9 (−0.8, 4.6)	−0.2 (−3.2, 2.9)
8 years	0.4 (−2.5, 3.2)	0.04 (−3.2, 3.3)	2.1 (−1.1, 5.3)	0.7 (−2.8, 4.2)
PFOS				
3 years	−0.7 (−2.7, 1.3)	−0.1 (−2.0, 1.7)	1.1 (−1.2, 3.3)	−1.9 (−4.1, 0.3)
8 years	−0.3 (−2.8, 2.2)	0.2 (−2.1, 2.4)	1.0 (−1.7, 3.7)	0.2 (−2.9, 3.3)
PFHxS				
3 years	0.2 (−1.0, 1.5)	−0.3 (−1.4, 0.7)	0.9 (−0.6, 2.4)	−0.1 (−1.6, 1.4)
8 years	−0.8 (−2.5, 0.8)	−1.3 (−2.8, 0.2)	0.6 (−1.5, 2.7)	−0.5 (−2.5, 1.4)
PFNA				
3 years	−1.1 (−2.9, 0.7)	−0.5 (−2.1, 1.1)	−0.5 (−2.8, 1.9)	−1.8 (−3.4, −0.2)
8 years	0.5 (−1.6, 2.6)	−0.1 (−2.1, 2.0)	0.5 (−2.2, 3.3)	1.4 (−0.8, 3.6)

PFAS were ln-transformed.
[a] Adjusted by maternal age, race/ethnicity, household income, maternal marijuana use, maternal blood lead, maternal serum cotinine, maternal depression, vitamin use during pregnancy, maternal IQ, marital status, Home Observation for Measurement of the Environment Score, whether the child was breastfed, and child sex.

Table 5
Estimated score differences (95% CIs) in BASC-2 Adaptive Skills and its subscales at age 8 years by a ln-unit increase in childhood serum PFAS (ng/mL), HOME Study.[a]

PFAS	Adaptive Skills	Adaptability	Social skills	Activity of daily living	Functional communication
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
PFOA					
3 years	−0.8 (−3.7, 2.1)	−1.0 (−4.3, 2.3)	−2.3 (−5.4, 0.8)	−1.2 (−4.1, 1.8)	2.1 (−1.0, 5.2)
8 years	−1.2 (−4.7, 2.2)	−0.3 (−3.9, 3.2)	−1.3 (−5.1, 2.4)	−2.1 (−5.3, 1.2)	0.3 (−3.2, 3.7)
PFOS					
3 years	0.7 (−1.5, 2.9)	1.1 (−1.2, 3.4)	−0.02 (−2.5, 2.5)	1.1 (−1.3, 3.4)	0.8 (−1.3, 2.9)
8 years	−2.2 (−5.1, 0.6)	−0.1 (−3.1, 2.9)	−2.5 (−5.7, 0.6)	−1.4 (−4.2, 1.4)	−1.8 (−4.4, 0.8)
PFHxS					
3 years	0.4 (−1.1, 1.8)	0.5 (−1.0, 2.0)	0.9 (−0.7, 2.5)	0.2 (−1.4, 1.7)	0.7 (−0.7, 2.1)
8 years	0.1 (−1.7, 1.9)	0.8 (−1.0, 2.5)	0.9 (−1.3, 3.1)	0.1 (−1.8, 2.1)	0.2 (−1.5, 1.9)
PFNA					
3 years	0.7 (−1.3, 2.6)	−0.02 (−1.9, 1.9)	0.4 (−1.6, 2.5)	0.9 (−1.3, 3.1)	0.9 (−0.9, 2.7)
8 years	−2.1 (−4.3, 0.1)	−1.6 (−3.9, 0.7)	−1.8 (−4.1, 0.6)	−2.4 (−4.4, −0.4)	−1.7 (−4.0, 0.6)

PFAS were ln-transformed.
[a] Adjusted by maternal age, race/ethnicity, household income, maternal marijuana use, maternal blood lead, maternal serum cotinine, maternal depression, vitamin use during pregnancy, maternal IQ, marital status, Home Observation for Measurement of the Environment Score, whether the child was breastfed, and child sex.

years. In addition, concurrent PFOA was associated with higher aggression scores in males (β = 2.7 points, 95% CI: −0.7, 6.2), but lower scores in females (β = −3.4 points, 95% CI: −7.5, 0.7) (interaction term: PFOA × $visit_{8\ years}$ × child sex = 0.051), albeit sex-stratified associations were not statistically significant. We also observed effect modification by sex with concurrent PFNA concentrations, with significantly higher internalizing behaviors and depression scores among males (Internalizing Problems: β = 3.7 points, 95% CI: 0.7, 6.8; Depression: β = 5.1 points, 95% CI: 2.5, 7.7), while there were null associations among females (Internalizing Problems: β = −1.7 points, 95% CI: −4.6, 1.2; Depression: β = −2.1 points, 95% CI: −4.8, 0.6). Similar findings were also noted between PFNA and PFOA at age 8 years and Behavior Symptoms Index (Supplemental Fig. S3).

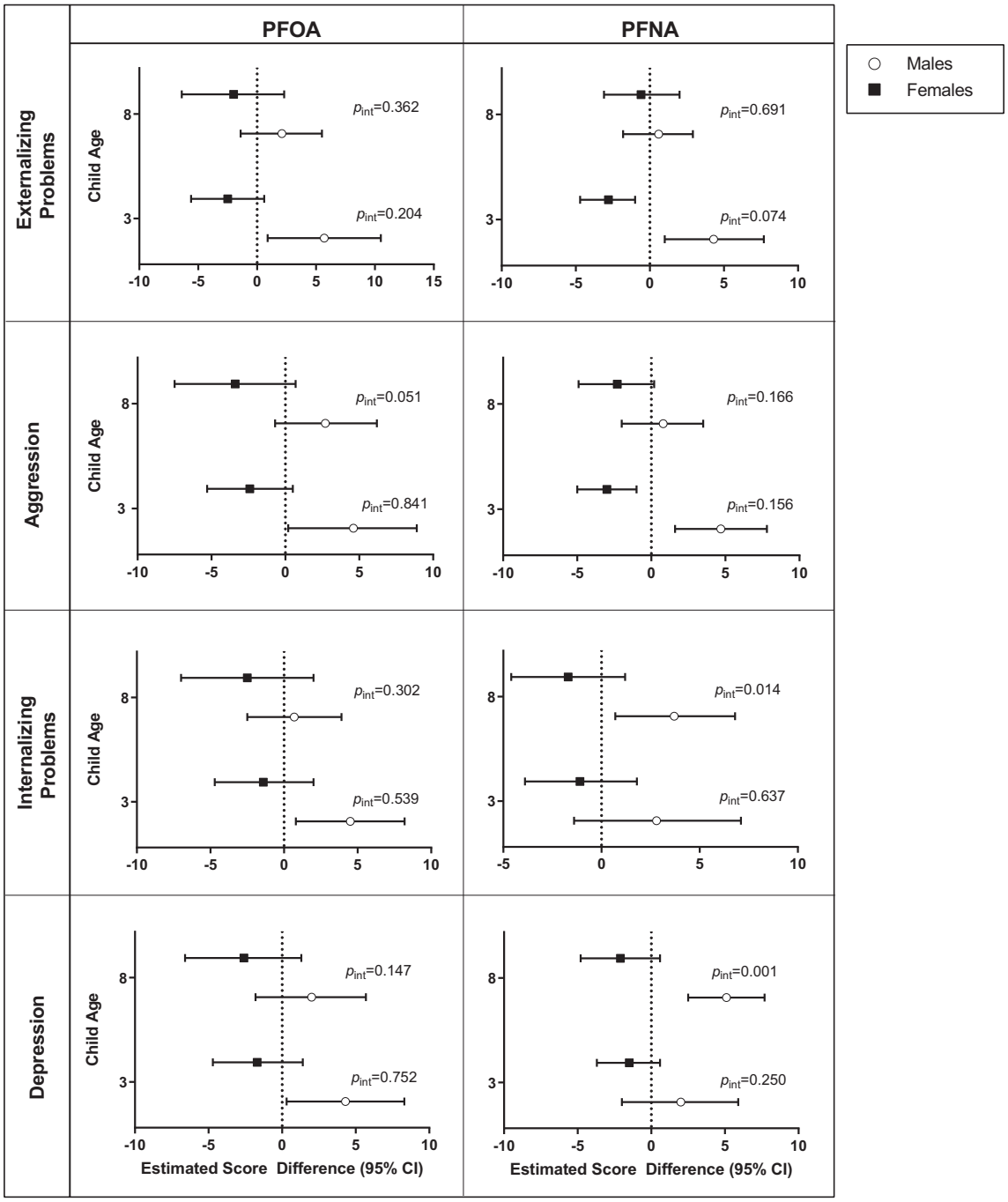

Fig. 1. Estimated score differences and 95% CIs in BASC-2 scores at age 8 years by a ln-unit increase in child serum PFAS concentrations (ng/mL) by child sex, HOME Study. Adjusted by maternal age, race/ethnicity, household income, maternal marijuana use, maternal blood lead, maternal serum cotinine, maternal depression, vitamin use during pregnancy, maternal IQ, marital status, Home Observation for Measurement of the Environment Score, and whether the child was breastfed.

3.5. Childhood body burden of PFAS and neurobehavior

Childhood body burdens of PFAS were not associated with Externalizing Problems (Supplemental Table S2). However, there was a statistically significant inverse relationship between body burdens of PFHxS at age 8 years and hyperactivity ($\beta = -2.1$ points, 95% CI: −3.9, −0.4) that was not present when we examined serum PFHxS. We observed comparable associations between body burden of PFAS and Internalizing Problems and Adaptive Skills as with PFAS serum concentrations (Supplemental Tables S3–4). However, there were two additional statistically significant inverse associations, specifically between the body burden of PFHxS at age 8 years and atypicality scores and between the body burden of PFOS at age 3 years and attention problems (Supplemental Table S5). We observed several significant sex interactions among associations between PFAS body burden and neurobehavior that were previously noted in our analyses of PFAS serum concentrations, including effect measure modification by sex in the associations between: 1) PFOA at age 8 years and aggression; 2) PFNA at age 8 years and Internalizing Problems and depression; and 3) PFOA at age 8 years and atypicality (Supplemental Table S6).

3.6. Prenatal and childhood PFAS and neurobehavior

We observed no relationship between prenatal PFAS concentrations

and Adaptive Skills in models adjusted for childhood PFAS concentrations (Supplemental Table S7). However, we continued to observe an inverse relationship between PFNA at 8 years and activity of daily living. There were several positive associations between prenatal PFAS and Externalizing Problems after adjusting for childhood concentrations (Fig. 2). Prenatal PFOS (β = 3.2 points, 95% CI: 1.0, 5.3), PFHxS (β = 2.1 points, 95% CI: 0.6, 3.7), and PFNA (β = 3.7 points, 95% CI: 1.1, 6.3) were significantly associated with higher scores on Externalizing Problems. These findings are concordant with those previously reported in the HOME Study that did not take into account childhood PFAS concentrations (Vuong et al., 2021). Statistically significant positive associations were also observed between prenatal PFOS, PFHxS, and PFNA with hyperactivity, aggression, and conduct problems. Adjusting for prenatal PFAS resulted in similar null associations between childhood PFAS and Externalizing Problems. Finally, mutual adjustment for prenatal and childhood PFAS resulted in significantly higher scores for Internalizing Problems, somatization, Behavior Symptoms Index, and attention problems in children with increased prenatal PFHxS (Supplemental Table S8–9). We also continued to observe null findings between childhood PFAS with Internalizing Problems and its subscales (Supplemental Table S8) as well as a significant inverse relationship between PFNA at 3 years with attention problems (Supplemental Table S9).

4. Discussion

In this prospective cohort study, we observed that childhood serum concentrations of PFAS at ages 3 and 8 years were not associated with externalizing or internalizing behaviors in children at age 8 years. However, there is suggestive evidence that child sex modifies the associations between childhood PFAS and neurobehavior, where higher serum concentrations of PFOA and PFNA are associated with more externalizing problems, internalizing problems, and behavior symptoms in males compared to females. We also noted significant associations between PFNA and lower scores for activity of daily living and attention problems, though findings do not suggest childhood serum concentrations of PFOA, PFOS, or PFHxS are associated with behavior symptoms or adaptive skills. Lastly, prenatal PFAS concentrations remain associated with more externalizing and internalizing problems in children even after taking into account childhood concentrations.

Our null findings between childhood PFAS and behavioral problems are not consistent with the findings from the Faroese cohort (Oulhote et al., 2019; Oulhote et al., 2016). PFNA concentrations at age 5 years were significantly associated with more externalizing problems in children at age 7 years (Oulhote et al., 2016). Oulhote et al. (2016) also reported more internalizing problems in children at 7 years with higher

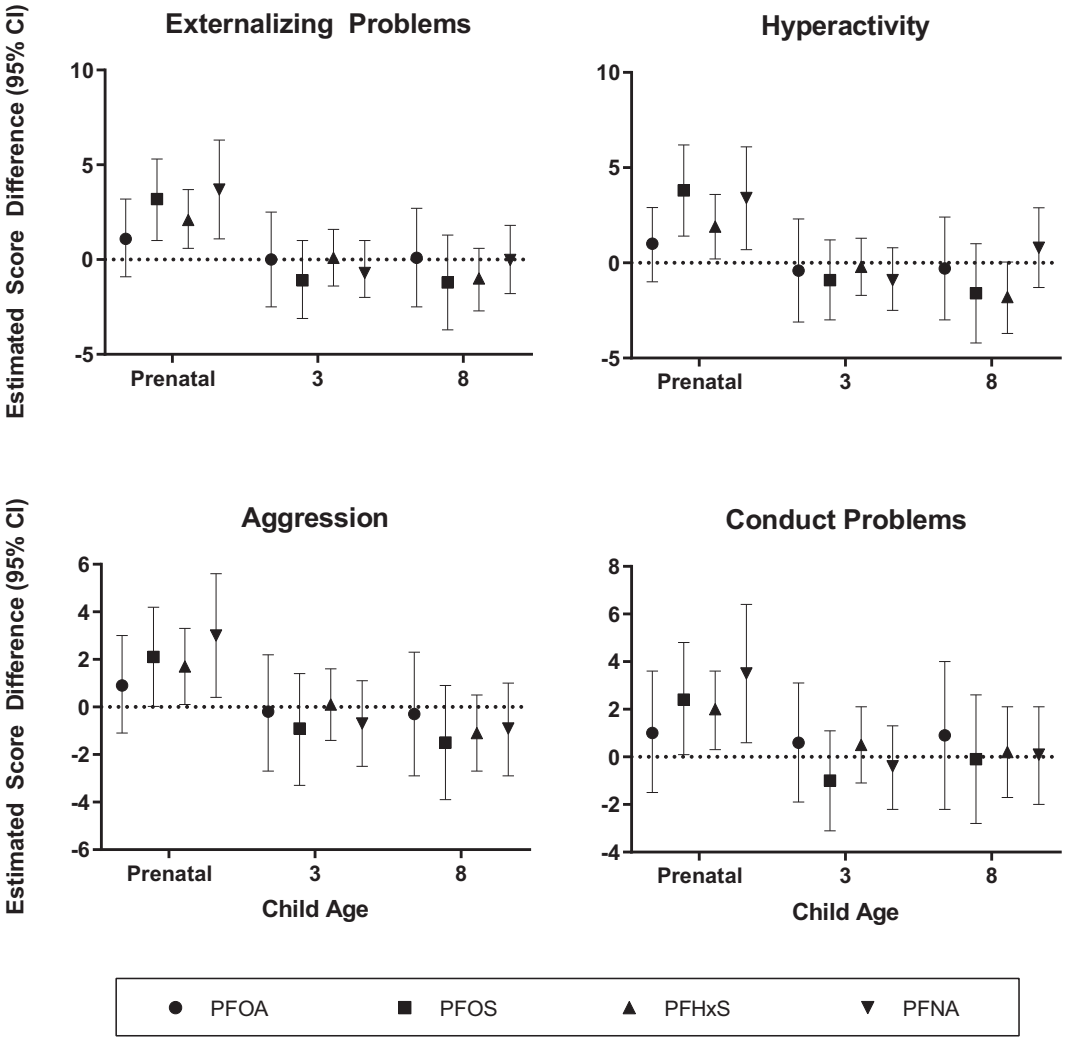

Fig. 2. Estimated score differences (95% CIs) in BASC-2 Externalizing Problems scale and subscales at age 8 years by a ln-unit increase in serum PFAS during gestation and childhood (ng/mL), HOME Study. Adjusted by maternal age, race/ethnicity, household income, maternal marijuana use, maternal blood lead, maternal serum cotinine, maternal depression, vitamin use during pregnancy, maternal IQ, marital status, Home Observation for Measurement of the Environment Score, whether the child was breastfed, and child sex.

PFOA serum concentrations at age 5 years. In the examination of early life exposures to chemical mixtures, PFNA concentrations at 5 years was significantly associated with higher total Strengths and Difficulties Questionnaire (SDQ) scores, indicating worse behavioral problems (Oulhote et al., 2019). However, no relationship was noted between concurrent PFAS concentrations and behavioral problems at 7 years (Oulhote et al., 2016). In a cross-sectional study examining serum PFAS at age 10 years, higher concentrations of several PFAS were associated with shorter inter-response times, indicating impulsivity (Gump et al., 2011). In contrast, the C8 Health Study reported mixed findings, with protective and null associations between childhood PFAS and behavioral problems, including inattention and impulsivity (Stein et al., 2013, 2014). The only adverse relationship observed in the HOME Study was between prenatal PFAS and externalizing and internalizing behaviors, which was previously reported by Vuong et al. (2021) and replicated in the current study after taking into account childhood concentrations. Our findings align with those from the INUENDO cohort examining prenatal PFAS concentrations (Hoyer et al., 2015), but they contrast with the protective associations reported in the Taiwanese and Danish cohorts (Fei and Olsen, 2011; Lien et al., 2016) and the null associations in the Dutch cohort (Quaak et al., 2016).

No other study has formally examined childhood PFAS and Adaptive Skills using BASC-2, but a few have investigated aspects of this behavioral domain, including peer-social behaviors and self-help. Those studies, which mainly focused on PFAS during gestational development, mirror the results of the present study (Chen et al., 2013; Fei et al., 2008; Goudarzi et al., 2016; Oulhote et al., 2016). In contrast, Lien et al. (2016) observed better prosocial behavior and fewer peer problems in children at 7 years with increased cord PFOA concentrations. Hoyer et al. (2015) reported worse scores on the SDQ in children 7–9 years of age with higher prenatal PFOA and PFOS concentrations. Our study similarly lacked convincing evidence of a relationship between childhood PFAS and Adaptive Skills. This finding is inconsistent with the Faroese cohort where higher PFOA concentrations at age 5 years were associated with problems with peer relationship, though further adjustment for prenatal PFAS resulted in null associations (Oulhote et al., 2016).

Our main findings on childhood PFAS concentrations and neurobehavior were discordant with those of the Faroese cohort (Oulhote et al., 2019; Oulhote et al., 2016). This discrepancy may relate to a number of factors. In the Faroese study, PFAS serum concentrations were measured at ages 5 and 7 years, while in the HOME Study concentrations were measured at ages 3 and 8 years. In the Faroese study, significant adverse associations were only noted between PFAS at 5 years, while null associations were observed with PFAS at 7 years with behavioral outcomes. There were generally null findings in the present study, though PFNA at 3 and 8 years was inversely associated with attention problems and activity of daily living scores, respectively. Inconsistent conclusions between the studies regarding the timing of childhood PFAS exposure with neurobehavior does not indicate a specific time period during childhood in which PFAS are more detrimental to neurobehavior. Concentrations also differed between the cohorts, with PFOS and PFOA median concentrations higher in Faroese children at age 7 years (PFOS: 15.26 ng/mL; PFOA: 4.37 ng/mL) compared to HOME Study children at age 8 years (PFOS: 3.6 ng/mL; PFOA: 2.4 ng/mL). The administered neurobehavioral assessments also varied between studies. We used the BASC-2, while Oulhote et al. (2019; 2016) used the SDQ, which provides the scales of Externalizing Problems (consisting of subscales: hyperactivity/inattention and conduct problems) and Internalizing Problems (consisting of subscales: emotional symptoms, peer relationship problems, and prosocial behavior). They focused on subscales of the SDQ that pertained to adaptive skills, including peer relationship problems and prosocial behavior, which may have differed from our behavioral assessment of Adaptive Skills using BASC-2. Lastly, statistical approaches varied between studies. While we used multiple informant models that took into account repeated measures of PFAS, Oulhote et al. (2016) used negative binomial regression models. They did, however, used structural equation modeling (SEM) as well as G-formula combined with Superlearner to examine the joint associations between prenatal and childhood PFAS along with other potential environmental contaminants (Oulhote et al., 2019; Oulhote et al., 2016).

Interestingly, both the HOME Study and the Faroese cohort reported potential sexual dimorphism between childhood PFAS and behavioral domains. In the HOME Study, we noted more externalizing problems with higher concentrations of PFNA at age 3 years and higher aggressions scores with increasing PFOA concentrations at age 8 years in males, but not in females. In the Faroese cohort the opposite was found, with female children appearing more sensitive to PFAS (Oulhote et al., 2016). Higher PFOS, PFNA, and PFHXs concentrations at age 7 years were associated with more externalizing problems, hyperactivity/inattention, and conduct problems in females, but not in males. It is unclear as to why there is a discrepancy between the studies regarding effect modification by sex results for childhood concentrations. While it remains to be determined whether males or females may be more sensitive to PFAS' potential neurotoxic effects, there is evidence from both studies that the associations may differ by sex. The mechanisms that come into play have yet to be elucidated, though eliminations rates between sexes may contribute to sex-specific findings. Toxicological studies in mice and rodents have shown differences in the clearance of PFAS by sex, with PFOA, PFNA, and PFOS having longer half-lives in males because of slower elimination rates (Kennedy Jr. et al., 2004; Ohmori et al., 2003; Tatum-Gibbs et al., 2011). In humans, PFOS and PFHxS have also been observed to have longer half-lives in males than in females (Li et al., 2018).

The present study has several strengths, including prospective childhood PFAS exposure assessment and extensive covariate information, including maternal depression, caregiving quality/quantity, and measurements of other potential neurotoxicants. We had repeated measurements of PFAS during childhood, and examined associations taking into account prenatal PFAS concentrations. Third, we utilized multiple informant models to identify potential windows of susceptibility. Lastly, we relied on the BASC-2 to assess neurobehavior in children, which is a reliable and valid neurobehavioral battery.

We also recognize some study limitations. First, the sample size limits our power to examine effect measure modification by sex. Although some findings would suggest potential sexual dimorphism, our results should be interpreted cautiously given the sample size. Second, while we had two serum PFAS measurements during childhood, earlier measurements during infancy and around toddler age may be more detrimental to neurodevelopment. Third, we do not have information regarding water consumption sources or frequency within the HOME Study for children at 3 and 8 years. Quantification of PFAS concentrations in child serum occurred after Cincinnati implemented water treatment technology to remove PFAS. Given that the main route of human exposure to PFAS is via water consumption and that 60% of water volume consumed by US children 4–13 years is from tap water sources, it is likely that our findings may be biased toward the null (Drewnowski et al., 2013; Sunderland et al., 2019). Multiple comparisons remains a potential concern despite the use of multiple informant models to reduce the total number of models in the study because there is no reduction in type 1 error. However, given our generally null associations, type 1 error is not a serious concern. In addition, we did not examine mixtures of PFAS, which may have additive, synergistic, or negative effects. Lastly, findings may not be entirely generalizable as some PFAS concentrations in the HOME Study are higher than those reported in NHANES. While the geometric mean (GM) of PFOS concentrations in HOME Study mothers (12.8 ng/mL) was comparable to that of pregnant women in NHANES 2003–2004 (12.3 ng/mL), PFOA concentrations are higher in the HOME Study (5.3 ng/mL compared to 2.39 ng/mL) (Woodruff et al., 2011). Childhood concentrations of PFOA and PFOS were also higher in the HOME Study at 3 and 8 years

compared to concentrations quantified in serum of children at 3–5 and 6–11 years in NHANES 2013–2014 (Ye et al., 2018).

5. Conclusions

Findings from the HOME Study do not support a relationship between childhood serum concentrations of four PFAS compounds and neurobehavior. Nevertheless, there is some evidence to suggest that sex may modify the relationship between childhood PFAS and neurobehavior, with males being more sensitive to increasing concentrations of PFOA and PFNA. The null association observed between PFAS concentrations in all HOME Study children and BASC-2 outcomes may be due to the inverse associations observed among females that attenuated the positive associations in male children. However, because of our limited sample size, results should be interpreted cautiously and replicated in sufficiently powered birth cohorts. Last, we also confirmed previous findings from the HOME Study between higher prenatal PFAS concentrations and more externalizing and internalizing problems in children after taking into account childhood PFAS concentrations.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the CDC. Use of trade names is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the US Department of Health and Human Services. The authors declare no competing financial interest.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ntt.2021.107022.

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