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Prenatal Exposure to Perfluoroalkyl Substances and Child Behavior at Age 12: A PELAGIE Mother–Child Cohort Study

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BACKGROUND: Per- and polyfluoroalkyl substances (PFAS) are chemical substances spread throughout the environment worldwide. Exposure during pregnancy represents a specific window of vulnerability for child health.

OBJECTIVE: Our objective was to assess the impact of prenatal exposure to multiple PFAS on emotional and behavioral functions in 12-y-old children.

METHOD: In the PELAGIE mother–child cohort (France), prenatal exposure to nine PFAS was measured from concentrations in cord serum samples. Behavior was assessed at age 12 y using the parent-reported Strengths and Difficulties Questionnaire (SDQ) and the self-reported Dominic Interactive for Adolescents (DIA) for 444 children. Associations were estimated using negative binomial models for each PFAS. Bayesian kernel machine regression (BKMR) models were performed to assess the exposure mixture effect on children’s behavior.

RESULTS: In our study population, 73% of mothers had spent more than 12 y in education. Higher scores on SDQ externalizing subscale were observed with increasing cord-serum concentration of perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA) [adjusted mean ratio (aMR) = 1.18, 95% confidence interval (CI): 1.03, 1.34, and aMR = 1.14 (95% CI: 1.00, 1.29) for every doubling of concentration, respectively]. Results for the hyperactivity score were similar [aMR = 1.20 (95% CI: 1.04, 1.40) and aMR = 1.18 (95% CI: 1.02, 1.36), respectively]. With regard to major depressive disorder and internalizing subscales, perfluorodecanoic acid (PFDA) was associated with higher self-reported DIA scores [aMR = 1.14 (95% CI: 1.01, 1.27) and aMR = 1.11 (95% CI: 1.02, 1.21), respectively]. In terms of the anxiety subscale, PFDA and PFNA were associated with higher scores [aMR = 1.11 (95% CI: 1.02, 1.21) and aMR = 1.10 (95% CI: 1.01, 1.19), respectively]. Concurrent increases in the PFAS concentrations included in the BKMR models showed no change in the SDQ externalizing and DIA internalizing subscales scores.

CONCLUSION: Prenatal exposure to PFNA and PFOA were associated with increasing scores for measures of externalizing behaviors, specifically hyperactivity. We also identified associations between PFNA and PFDA prenatal exposure levels and increasing scores related to internalizing behaviors (general anxiety and major depressive disorder), which adds to the as yet sparse literature examining the links between prenatal exposure to PFAS and internalizing disorders. <https://doi.org/10.1289/EHP12540>

Introduction

Per- and polyfluoroalkyl substances (PFAS) form a group of more than 9,000 synthetic chemicals that have been used worldwide for more than 70 y. Their surfactant properties have ensured their entry into the composition of everyday consumer products—including protectants for paper and cardboard packaging, carpets, leather, textiles, food packaging, nonstick cookware, and firefighting foam.¹ As a result of their long half-lives (estimated between 2 and 30 y in humans), PFAS are both widespread and persistent in the environment.¹ For the general population, the major exposure routes are via food and water ingestion, though exposure can also occur through inhalation and dust ingestion.¹ The main PFAS dietary sources are fish, seafood and food items contaminated by coated food packaging.² Many studies have highlighted the ubiquity of contamination among humans,³ including among pregnant women.⁴ PFAS have been also detected in breast milk and cord serum samples,^{5,6}

and these findings highlight the ability of PFAS to cross the placental barrier,⁷ resulting in fetal exposure.^{8,9} Results from both animal and *in vitro* studies suggest that some PFAS are developmental neurotoxicants affecting several neurochemical targets in the developing brain.^{8,9} Mechanisms include changes in calcium homeostasis, protein kinase C, synaptic plasticity, cellular differentiation, and alterations to the thyroid hormones.^{10,11}

Epidemiological studies have examined the links between prenatal exposure to PFAS and neurodevelopment end points, in particular with regard to behavioral disorders. A 2018 review by Liew et al.¹² stated that these links were fairly inconclusive. For PFOA specifically, two other reviews drew the same conclusions.^{13,14} The majority of the 12 prospective cohort studies reviewed by Liew et al.¹² had examined the associations between prenatal exposure to some PFAS and attention-deficit/hyperactivity disorder (ADHD) (3 studies), behavior disorders (4 studies) or autistic traits (4 studies) among children 5–12 y of age. Just one study noted that prenatal exposure to perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) was associated with hyperactivity in children age 5–9 y old.¹⁵ Recent studies have since reported the adverse impact of prenatal exposure to PFAS on child behavior, including increased ADHD,^{6,16} attention deficits,² or externalizing behavioral difficulties^{17–23}—including, moreover, conduct disorders and oppositional defiant disorder. However, other recent studies reported reverse^{24,25} or no associations,^{26–30} leaving conclusions still unclear. Several studies observed females showing more externalizing disorders following prenatal^{2,6,19} or postnatal exposure³⁰ to several PFAS, in comparison with males. However, this literature remains both scarce and inconsistent.

Internalizing behavior disorders include emotional troubles, major depressive disorder, generalized anxiety disorder, and specific

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phobia.³¹ Children and adolescents showing internalizing disorders may also present with externalizing behavior problems, and some studies suggest that internalizing and externalizing disorders may involve common brain structures and circuits.^{32–34} Few human studies on the neurodevelopmental toxicity of PFAS have thus far considered internalizing behaviors,^{20,28,30} and even these have still provided only inconclusive results: Higher internalizing difficulties among children were reported in association with prenatal exposure to perfluorohexane sulfonic acid (PFHxS)²⁰ as well as to perfluorodecanoic acid (PFDA), perfluorononanoic acid (PFNA) and perfluoroheptane sulfonic acid (PFHpS),¹⁹ whereas in two other studies, no association was observed.^{28,30} Liew et al.¹¹ concluded that more studies are needed on internalizing difficulties such as depression and that (in the studies they reviewed) there was a lack of consideration of the PFAS mixture. Such effects of this mixture have been investigated in a few recent studies, with mixed results.^{16,18,19,30}

The aim of the present study was to assess the association between prenatal exposure to several PFAS and externalizing and internalizing behavior among 12-y-olds. PFAS were studied both individually and as a mixture.

Methods

Study Population

The Perturbateurs endocriniens: Etude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance (PELAGIE) mother-child cohort included 3,421 pregnant women from Brittany, France and covered the period 2002–2006. Women were recruited at their first prenatal visit by their gynecologist, obstetrician, or ultrasonography practitioner. The inclusion criteria required that women completed the inclusion questionnaire before 19 wk of pregnancy and were still pregnant at that time. At the time of delivery, cord-blood samples were collected from 2,138 women (62%). By the time the children reached the age of 12 y, 887 (41.5%) families were lost to follow-up, and for 60 families (2.8%) no cord blood sample remained; 1,191 were thus eligible for the clinical exam. A total of 933 (78.4%) of these families were reached by telephone, and 559 (59.9%) children agreed to undergo a clinical examination in a hospital setting (see Figure S1).

At inclusion and when their children were 2, 6, and 12 y of age, mothers completed a self-administered questionnaire at home on their family, social and demographic characteristics, diet and lifestyle, as well as on the child's health. At birth, hospitals provided information about the pregnancy, pregnancy outcome, and the child's health. Cord blood samples were collected, and persistent organic pollutants (POPs) were measured.³⁵

The adults participating in this study provided written informed consent and the 12-y-old children provided written assent. Written informed consent was obtained from parents/legal guardians for all participants under 18 y of age. The study was approved by the Advisory Committee on Information Processing in Health Research (CCTIRS; 2015; no. 15.326bis), the Committee for the Protection of Persons (CPP; 2015; no. 15/23-985), and the French National Commission for Information Technology and Civil Liberties (CNIL; 2002, 2015; no. 915420/2015-456).

Child's Behavior Assessment

Parents assessed their children's behavior the year the child turned 12 y old, using the French version of the Strengths and Difficulties Questionnaire (SDQ).^{36,37} A total of 420 of 444 questionnaires (94.6%) were completed by the mother alone, 14 (3.2%) by the father alone, and 10 (2.2%) by both parents. The SDQ asks questions about 25 attributes divided between 5 scales of 5 items each. Respondents use a 3-point Likert scale scored from 0 (not true), 1

(somewhat true), to 2 (certainly true) to indicate the extent to which each attribute applies to the child in question. Scores ranging from 0 to 10 (sum of item responses) are then generated for emotional symptoms, conduct problems, hyperactivity-inattention, peer problems, and prosocial behavior.³⁷

Emotional symptoms and peer problems scores can be summed into an internalizing subscale, and hyperactivity/inattention and conduct problems scores can be summed into an externalizing subscale. As recommended by Goodman et al.,³⁸ these broader internalizing and externalizing composite scales (ranging from 0 to 20) can be fruitfully deployed in analyses of low-risk samples from the general population. The parent-reported French SDQ versions have been shown to accurately measure psychopathological symptoms in young people (especially in cases of externalizing troubles).³⁹

As children mature, the use of self-reported questionnaires should be considered, especially for measurement of internalizing troubles.³¹ The Dominic Interactive for Adolescents (DIA) was thus chosen for this study; it is a highly structured, multimedia self-report screen that uses visual and auditory stimuli to assess the current symptoms defining seven of the mental disorders most frequently found in adolescents 12–15 y of age. The DIA comprises 91 items (pictures and questions) and assesses four internalizing symptoms: major depressive disorder (MDD; 19 items), generalized anxiety disorder (GAD; 16 items), specific phobia (SPH; 8 items) and substance abuse disorder (SAD; 6 items), as well as three externalizing disorders: ADHD (18 items), oppositional defiant disorder (ODD; 9 items), and conduct disorder (CD; 15 items).⁴⁰ The internalizing composite scale is the sum of major depressive disorder, generalized anxiety disorder, specific phobia, and substance abuse disorder subscales (48 items), and the externalizing composite scale is the sum of ADHD, ODD, and CD subscales (42 items). The health personnel conducting the clinical exam, who had no access to the results at the end of the test, offered the children the option of completing it alone in a room to minimize possible inhibition or control in children's answers. The psychometric properties of the DIA were evaluated,^{40,41} and they showed good internal consistency; the range of coefficients remained fairly constant across age, sex, language, and type of subsample (school, clinical).⁴¹

In the main analysis, the parent-reported SDQ was used to assess children's hyperactivity and externalizing disorders to allow comparisons, because evaluation of children's behavioral disturbances in the literature is typically based on parents' (or teachers') reports. As mentioned above, the self-reported DIA test was preferred for the assessment of internalizing disorders, general anxiety, and major depressive disorder, because these are self-reported by children and meet the classification of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM).^{40–42}

Given that both informants (parent and child) might provide unique information about child's behavior,⁴³ complementary analyses were conducted on the externalizing and ADHD scales derived from the self-reported DIA and on internalizing behaviors derived from the parent-reported SDQ.

Where one or two items were missing from questionnaire data, the corresponding subscale score was extrapolated from the remaining available items (done for $n = 16$, including five participants with two missing values for hyperactivity scale and $n = 14$, including one participant with two missing values for the conduct troubles scale). Eleven children were excluded either because questionnaire data were missing ($n = 1$) or because they had three (or more) missing SDQ items within a scale ($n = 4$), and some because they were receiving regular or prolonged treatment in the form of tranquilizers or antidepressant or psychostimulant neuroleptic drugs ($n = 6$).

Exposure Assessment

Prenatal exposure to nine PFAS [perfluorobutanoic acid (PFBA), perfluorohexanoic acid (PFHxA), PFOA, PFNA, PFDA, perfluoroundecanoic acid (PFUnDA), perfluorobutane sulfonic acid (PFBS), PFHxS, and PFOS] was assessed by measuring biomarkers in cord blood serum samples by the Centre de Toxicologie du Québec (CTQ) at the Institut National de Santé Publique du Québec (INSPQ).

The PFAS were extracted from the cord serum samples (100 μ L) using the solid phase extraction (SPE) technique with a weak anion exchange resin. The extracts were evaporated to dryness and taken up in a 5 mM ammonium acetate in 40% methanol solution. Samples were analyzed using ultra performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS); the method has been described in more detail elsewhere.⁴⁴ The limit of detection (LOD) varied from 0.05 to 0.2 μ g/L, depending on the analytes. The LOD was calculated using 3 times the standard deviation (SD) on 30 replicates, analyzed in different analytical runs, of a serum sample at a concentration between 5 and 7 times the estimated concentration, yielding a signal to noise of 3:1. Intraday precision varied between 2.6% and 8.1%, and interday precision varied between 5.4% and 7.4%, depending on the analytes.

Of the 548 children who had undergone the clinical examination at 12 y of age and did not meet the exclusion criteria (i.e., missing behavior score or treated on a regular or prolonged basis with tranquilizers or antidepressant or psychostimulant neuroleptic drugs, $n=11$), 444 had their cord blood samples analyzed for PFAS measurements. Concentrations were reported as wet weight (units of micrograms per liter). Missing data, because of analytical interferences in some samples for the analytes PFNA ($n=11$), PFDA ($n=38$), and PFUnDA ($n=22$), confirmed by qualifying ion ratio that did not meet the expected criterion of $\pm 20\%$, were then excluded.

Several POPs had already been measured, as reported previously,³⁵ and these measurements were used in this study for sensitivity analysis on the influence of coexposures. Analyses were performed in cord-blood serum samples by the CTQ at the INSPQ: 14 polychlorinated biphenyls (PCBs), (PCBs 28, 52, 74, 99, 101, 118, 138, 153, 170, 180, 183, 187, 194, 203), 8 organochlorine pesticides [OCPs: β -hexachlorocyclohexane (β -HCH), γ -hexachlorocyclohexane (γ -HCH), hexachlorobenzene (HCB), dieldrin, dichlorodiphenyltrichloroethane (p,p' -DDT), dichlorodiphenyldichloroethylene (p,p' -DDE), heptachlor epoxide, α -Endosulfan], and 3 polybrominated diphenyl ethers (PBDEs), 47, 99, 209. Total lipid level data (g/L) were also available.

Covariates and Potential Confounding

Potential covariates were identified *a priori*, based on existing literature on *in utero* risk factors for mental health and behavior [maternal stress and anxiety,^{45–47} smoking, drug or alcohol consumption during pregnancy,^{31,45–49} maternal prepregnancy body mass index (BMI),⁴⁸ hypertensive disorders during pregnancy, preeclampsia and other pregnancy complications,^{46,48,49} exposure to environmental chemicals,^{31,45,47} and low birth weight and prematurity^{45–47,49}]. Refined selection was made using a directed acyclic graph (DAG) approach (see Figure S2).⁵⁰ Covariates were obtained from the PELAGIE questionnaires completed at inclusion (early in pregnancy), at birth, and up to the child's 12th birthday. We considered child sex, birth weight, and small for gestational age (SGA; $<10\%$); maternal age at inclusion, number of years in full-time education for mother, marital status at birth (single or not), parity, prepregnancy BMI, smoking status and alcohol intake during pregnancy, usual fish intake before pregnancy (because fish and seafood may accumulate persistent organic contaminants and

heavy metals), and pregnancy complications (hypertension, infection *in utero*). The final adjustment set included: maternal age (in years), maternal prepregnancy BMI (in three categories: <18.5 kg/m², 18.5–25 kg/m², ≥ 25 kg/m²), number of years in full-time education for mother (in three groups: <12 y, 12 y, >12 y), parity (nulliparous vs. multiparous), maternal prepregnancy fish intake (<1 once a month, at least once a month, at least twice a week), smoking status at inclusion (smoker vs. nonsmoker), and child sex. Selected covariates had very few missing data ($<1\%$) and were then simply imputed with the mode.

Statistical Analyses

Comparisons between individuals included in this study and the original cohort were performed using chi-square or Fisher tests for categorical variables or Student's *t*-tests for mean comparison after having checked applicability criteria. For behavioral scores, internal consistency was checked using polychoric and tetrachoric Cronbach's alpha calculation. Because some items in the DIA are used in multiple subscales, Cronbach's alpha for composite internalizing and externalizing subscales was calculated using items only once. Score distributions between males and females were compared using Wilcoxon tests. Spearman rank correlations were used to estimate agreement between parent-reported SDQ and self-reported DIA internalizing and externalizing behavior scores.

Compounds detected in fewer than 5% of cord-serum samples were excluded from the analyses; this was the case for PFBA, PFHxA and PFBS. The other six PFAS cord-serum concentrations were detected in $>70\%$ of the samples and were then \log_2 -transformed after graphical check for distribution log-normality.

Because all machine concentration readings below the LOD were available for PFAS, these values (rather than data imputation) were used for the analyses. Spearman rank correlation coefficients were calculated between values of cord-serum PFAS concentrations.

Overall, 444 individuals with prenatal exposure to at least one PFAS compound measured in cord-serum sample and behavior scores were included in the analyses.

Single-pollutant approach. For each PFAS, we used negative binomial regression models to explore associations between \log_2 -transformed cord-serum concentrations and each of the scores (after checking for data overdispersion). Results are presented as adjusted mean ratio (aMR) of behavioral score with 95% confidence intervals (CIs), reflecting the relative change in the mean score with every doubling of the PFAS cord-serum concentration. All models were adjusted for the potential confounders listed above.

Linearity was investigated using generalized additive models (GAM) including restricted cubic splines. Nonlinearity was quantified using the effective degree of freedom (edf), which is a summary statistic of GAM. An edf equal to 1 reflects a linear relationship, $1 < \text{edf} < 2$ corresponds to a weakly nonlinear relationship, and $\text{edf} \geq 2$ can be considered a nonlinear relationship.^{51,52} Models with exposure categorized in quartiles were retained when the edf was >1.5 . To evaluate the possible sex-specific effect of PFAS on behavioral scores, we introduced to the models a cross-product term between child sex and PFAS exposure variable and retained statistical significance at $p < 0.10$. Stratified analyses on child sex were then performed to determine whether results were qualitatively similar between groups.

Multiple-pollutant approach: Bayesian kernel machine regression. We used Bayesian kernel machine regression (BKMR), a non-parametric high-dimensional exposure–response function resulting from component-wise variable selection (50,000 iterations) to

investigate nonlinear associations, interactions, and joint effects of the mixture of PFAS.⁵³ Each compound within the model is assigned a posterior inclusion probability (PIP), indicating the relative importance of each exposure. We used a threshold for posterior inclusion probability of 0.5 (i.e., considered an important compound in a mixture).⁵⁴

Only the six PFAS exposures accounting for more than 70% of detected data were included as continuous log₂-transformed data and were centered on the median and interquartile range (IQR) scaled. Although our outcomes were score counts, we used Gaussian regression in the BKMR models. Potential confounders identified earlier were included in the models.

Sensitivity analysis. Although breastfeeding has been associated with children's mental health, it was not considered in our *a priori* set of potential confounders because prenatal PFAS concentrations have previously been associated with decreased breastfeeding duration.^{55,56} To control for possible confounding, single-pollutant models were additionally adjusted for breastfeeding duration (none, ≤3 months, >3 months) (excluding missing data *n* = 76).

Both maternal and paternal mental health have been associated with children's internalizing and externalizing behavior.^{57,58} Furthermore, parental mental health can influence the way they evaluate their own child's behavior.⁵⁹ At age 12 y, the respondent parent's mental health was measured using the Short-Form 36 (SF36) questionnaire's emotional well-being subscale.⁶⁰ The parent completing the questionnaire (overwhelmingly the mother, as mentioned above) was answering both the SDQ for the child and the SF36 part for his or her own mental health. When both parents declared having answered the questionnaire (*n* = 10), we assumed that the SF36 questionnaire was completed by the mother. We then performed a second sensitivity analysis, adding this covariate into the single-pollutant models for each behavioral score.

Because prenatal exposure to other pollutants [including POPs such as PCBs and PBDEs or organochlorine (OC) pesticides] has already been identified as linked to child behavior,^{61,62} we performed a third sensitivity analysis to verify the potential influence of coexposures. PCBs, PBDEs, and OC pesticides were available for a reduced sample size, ranging from 401 to 415, depending on the compound considered. Values below the LOD were randomly imputed from a log-normal probability distribution, the parameters of which were estimated using a maximum-likelihood method.⁶³ Concentrations were log₂-transformed for compounds detected in >70% of the population and categorized for compounds detected in <70%. Compounds detected in fewer than 5% of samples were not included in the analyses. To identify potential coexposures linked to outcomes, we used separate negative binomial regression models adjusted for potential confounders (same set as for the main models). Given that PCBs, OC pesticides, and PBDEs are lipophilic compounds,⁶⁴ total lipid levels were included in these models as covariates. Spearman rank correlation coefficients were also calculated between cord serum PFAS and POP concentrations. For each outcome and each PFAS, models performed in the single-pollutant approach were then run adding all coexposure variables linked at *p* < 0.1 with the outcome. The multiple pollutant approach with BKMR models was also performed, adjusting models for potential confounders and coexposures.

Analyses were performed using R (version 4.0.2; R Development Core Team). Results were considered statistically significant when the *p*-value was <0.05.

Results

Mother-child pairs' characteristics are shown in Table 1. Mothers were a mean of 30.8 (SD = 3.9) years of age at inclusion, and 13% (*n* = 68) were overweight or obese prior to pregnancy. Twenty-

Table 1. Study population characteristics (*n* = 444), PELAGIE mother-child cohort, Brittany, France.

Characteristics	<i>n</i> (%) or mean ± SD
Mother	
Age at inclusion	
Mean ± SD y	30.80 (± 3.88)
Age group	
<20 y	0
20 to <25 y	23 (5.2)
25 to <30 y	172 (38.7)
30 to <35 y	178 (40.1)
35 to <40 y	67 (15.1)
40 y or more	4 (0.9)
Parity	
No child	169 (38.1)
At least one child	275 (61.9)
Prepregnancy BMI	
Mean ± SD kg/m ²	22.05 (± 3.73)
BMI group	
<18.5 kg/m ²	38 (8.6)
18.5 kg/m ² to <25 kg/m ²	345 (78.2)
25 kg/m ² to <30 kg/m ²	45 (10.2)
30 kg/m ² or more	13 (2.9)
Mother's education	
<12 y	48 (10.8)
12 y	71 (16)
>12 y	325 (73.2)
Tobacco status ^a	
No or former smoker	341 (77.3)
Smoker at the beginning of pregnancy	100 (22.7)
Food intake-fish consumption before pregnancy ^b	
<1 once/month	52 (11.7)
At least once a month	256 (57.8)
At least twice a week	135 (30.5)
Birth	
Gestational age	
Mean ± SD gestational weeks	39.58 (± 1.25)
Birth weight	
Mean ± SD g	3,467.26 (± 439.17)
Small for gestational age (<10%)	
No	431 (97.1)
Yes	13 (2.9)
Breastfeeding ^c	
None	106 (28.8)
≤3 months	103 (28)
>3 months ≤6 months	98 (26.6)
>6 months	61 (16.6)
Parental mental health (when child was age 12 y)	
Short-Form 36 – Emotional well-being ^{d,e}	
Mean ± SD	69.13 (± 13.57)
Child sex at birth	
Males	224 (50.5)
Females	220 (49.5)
Child age at examination	
Mean ± SD y	12.81 (± 0.13)

Note: BMI, body mass index; SD, standard deviation.

^aMissing data: *n* = 3.

^bMissing data: *n* = 1.

^cMissing data: *n* = 76.

^dMissing data: *n* = 15.

^eResponding parent: mother, *n* = 415/429 (96.7%).

three percent of mothers reported that they were smokers at the beginning of pregnancy. Seventy-three percent had spent more than 12 y in education. At the time of the clinical exam, the children were 12.8 y of age on average.

In comparison to the original cohort (*n* = 3,421), women included in this study (*n* = 444) were better educated [47% with ≥15 y vs. 32% for women not included (*n* = 2,977), *p* < 0.001], were older [mean age (SD) 30.80 y (3.88) vs. 29.99 y (4.35), *p* < 0.001], were less likely to be primipara (*p* = 0.003), had lower prepregnancy BMI (*p* = 0.03), smoked less at the beginning of pregnancy (77% were nonsmoker vs. 71%, *p* = 0.01), and ate fish

Table 2. Parent-reported SDQ. Scores at age 12 y, PELAGIE mother–child cohort, Brittany, France ($n = 444$). Values reported are the median (minimum–maximum) scores.

Sex	Externalizing	Hyperactivity	Internalizing
Total ($n = 444$)	3 (0–17)	2 (0–10)	3 (0–15)
Males ($n = 224$)	4 (0–17)	3 (0–10)	3 (0–14)
Females ($n = 220$)	2 (0–13)	1 (0–9)	4 (0–15)
p -Value ^a	8.2×10^{-8}	3×10^{-9}	0.009

Note: SDQ, Strengths and Difficulties Questionnaire.

^aWilcoxon test p -value comparing males vs. females. See Table S2 for results for every subscale.

more often before pregnancy ($p < 0.001$). Their babies were born with higher gestational age [mean \pm SD: 39.58 wk (1.25) vs. 39.10 wk (2.48)] than those not included in the study ($p < 0.001$) had higher birth weights [mean \pm SD: 3,467 g (439 g) vs. 3,334 g (570 g), $p < 0.001$], and were less likely to be small for gestational age ($p < 0.001$) than those not included. Details are displayed in Table S1.

Tables 2 and 3, respectively, present the results for parent-reported SDQ, as well as self-reported DIA scores for the entire sample population and for males and females separately. Detailed results of the SDQ and DIA subscales are available in Tables S2 and S3. Males had higher median scores than females on both the SDQ externalizing subscale [4 (range: 0–17) vs. 2 (range: 0–13), respectively; $p < 0.001$] and the hyperactivity subscale [3 (range: 0–10) vs. 1 (range: 0–9) for males and females, respectively; $p < 0.001$] as well as for externalizing [8 (range: 0–28) vs. 6 (range: 0–24); $p < 0.001$] and ADHD scores [6 (range: 0–18) vs. 4 (range: 0–16); $p < 0.001$] for males and females, respectively, on the DIA scale. Females had higher median scores than males on the DIA internalizing subscale [9 (range: 0–39) vs. 11 (range 0–31), respectively; $p = 0.002$].

Cronbach's alpha (polychoric) was 0.84 and 0.86 for the SDQ hyperactivity and externalizing subscales, respectively, and 0.81 for the SDQ internalizing behavior subscale. For the DIA anxiety, major depressive, and internalizing subscales, tetrachoric Cronbach's alpha was 0.80, 0.87, and 0.89, respectively, and 0.91 and 0.90 for ADHD and externalizing subscales (see Tables S4 and S5 for the coefficients for other subscales). Spearman rank correlations between SDQ and DIA were higher for externalizing behaviors ($\rho = 0.47$) than for internalizing ($\rho = 0.33$).

Table 4 shows the distribution of cord serum concentrations of PFAS in our sample. Concentrations above the LOD were measured in more than 70% of samples for PFHxS (100%), PFOS (100%), PFOA (100%), PFNA (99.8%), PFDA (88.4%), and PFUnDA (73.2%). PFOS and PFOA had the highest medians [4.6 μ g/L IQR = 3.5–5.9 and 2.2 μ g/L IQR = 1.7–2.8, respectively, and the strongest correlations were between PFNA and PFDA ($\rho = 0.78$) and PFNA and PFOA ($\rho = 0.73$); see Figure S3].

Associations between Cord-Blood PFAS Concentrations and Child Behavioral Scores

Single-pollutant approach: main analysis. Table 5 presents the main results for the associations between PFAS prenatal exposure

and SDQ externalizing and hyperactivity scores and DIA internalizing, general anxiety, and major depressive disorders scores. Linearity was not rejected for any PFAS (see Figure S4 for results of the splines regression models), and no heterogeneity was identified between males and females (results for interaction term significance and for stratified models are displayed in Figures S5 and S6 and Table S6). Higher scores on the SDQ externalizing subscale were observed with increasing cord serum concentration of PFOA and PFNA [aMR = 1.18 (95% CI: 1.03, 1.34) and aMR = 1.14 (95% CI: 1.00, 1.29) for every doubling of PFAS concentration, respectively]. Hyperactivity score results were similar [aMR = 1.20 (95% CI: 1.04, 1.40) and aMR = 1.18 (95% CI: 1.02, 1.36), respectively]. Concerning major depressive and internalizing subscales, higher PFDA concentrations were associated with higher scores [aMR = 1.14 (95% CI: 1.01, 1.27) and aMR = 1.11 (95% CI: 1.02, 1.21), respectively, for every doubling of concentration]. In terms of the anxiety subscale, both PFDA and PFNA concentrations were associated with higher scores [aMR = 1.11 (95% CI: 1.02, 1.21) and aMR = 1.10 (95% CI: 1.01, 1.19), respectively].

Single-pollutant approach: complementary analysis. When using externalizing and ADHD scores obtained from self-reported DIA, the results were similar on the whole: Associations were in the same direction (see Table 5), although they were diminished and no longer statistically significant for PFOA and PFNA and became statistically significant for PFDA. When using the parent-reported SDQ subscale for internalizing behavior, no association was identified with any PFAS.

BKMR models. Concurrent increases in concentrations of PFAS included in the models (reference: 25th percentile) showed no, or only a very slight, increase in both SDQ externalizing and DIA internalizing scores (Figure 1), CI for mixture–outcome associations crossed the null value.

For the externalizing score, PFOA was identified as the predominant component of the joint effect (PIP = 0.46; see Figure 1) followed by PFUnDA (PIP = 0.20). The single-exposure effect of PFOA suggested a weak association with a higher score on the SDQ externalizing subscale (see Table S14 and Figure S7A).

For the internalizing subscale, PFDA was the predominant component of the joint effect (PIP = 0.47; see Figure 1), followed by PFNA (PIP = 0.15). The single-exposure effect of PFDA suggested a weak association with higher score on the DIA internalizing subscale (see Table S14 and Figure S7B).

No evidence of either interaction or nonlinear association was identified, other than for PFDA prenatal exposure and DIA internalizing subscale, where the response curve seemed to reach a plateau as concentration increased (see Figures S8 and S9 and Table S15 for exposure levels).

Sensitivity Analyses

Sensitivity analyses performed for the single-pollutant approach on multivariable negative binomial regression models revealed results similar, on the whole, to those of the main analyses rerun on the same sample sizes (see Tables S7 and S8). The association

Table 3. Self-reported DIA. Scores at age 12 y, PELAGIE mother–child cohort, Brittany, France ($n = 444$). Values reported are the median (minimum–maximum) scores.

Sex	Externalizing	ADHD	Internalizing	Anxiety	Major depressive disorder
Total ($n = 444$)	7 (0–28)	5 (0–18)	10 (0–39)	5 (0–15)	3 (0–17)
Males ($n = 224$)	8 (0–28)	6 (0–18)	9 (0–39)	5 (0–15)	3 (0–17)
Females ($n = 220$)	6 (0–24)	4 (0–16)	11 (0–31)	5 (0–15)	3 (0–12)
p -Value ^a	0.0001	2.8×10^{-5}	0.002	0.002	0.4

Note: ADHD, attention-deficit/hyperactivity disorder; DIA Dominic Interactive for Adolescents.

^aWilcoxon test p -value comparing males vs. females. See Table S3 for results for every subscale.

Table 4. Distribution of cord-blood serum concentrations of PFAS, including machine concentration readings below the LOD available, PELAGIE mother–child cohort, Brittany, France (*n* = 444).

	LOD (µg/L)	<i>n</i>	<i>n</i> ND (%)	Min	5%	10%	25%	50%	75%	90%	95%	Max
PFOA	0.07	444	0 (0)	0.31	1.04	1.32	1.70	2.24	2.84	3.72	4.14	7.36
PFNA	0.09	433	1 (0.2)	0.07	0.16	0.20	0.26	0.35	0.46	0.58	0.67	1.55
PFDA	0.06	406	47 (11.6)	0.02	0.05	0.06	0.07	0.10	0.13	0.16	0.18	0.51
PFUnDA	0.05	422	113 (26.8)	0.00	0.00	0.03	0.05	0.07	0.09	0.12	0.14	0.30
PFHxS	0.06	444	0 (0)	0.06	0.26	0.31	0.42	0.56	0.70	0.95	1.09	3.17
PFOS	0.2	444	0 (0)	0.54	2.24	2.78	3.52	4.61	5.89	7.63	8.50	27.17

Note: PFBA, PFHxA, and PFBS were detected in <5% of samples (0.5%, 2.5% and 1.3% respectively). LOD, limit of detection; ND, nondetected; PFBA, perfluorobutanoic acid; PFBS, perfluorobutanesulfonic acid; PFDA, perfluorodecanoic acid; PFHxA, perfluorohexanoic acid; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFUnDA, perfluoroundecanoic acid.

between prenatal exposure to PFDA and the SDQ hyperactivity score became statistically significant (aMR = 1.18; 95% CI: 1.00, 1.38, *p* < 0.05) after controlling for breastfeeding (Table S7). When models were rerun on the sample using data for parental emotional well-being measured by SF36 (with and without adjusting for SF36-EWB, see Table S8), effect sizes for anxiety and internalizing behavior scores were slightly amplified, and the association between PFOA and anxiety score appeared statistically significant (aMR = 1.10; 95% CI: 1.00, 1.20).

Potential persistent organic pollutant coexposures considered in sensitivity analyses included PCB 118, 138, 153, 170, 180, 183, 187, 194, 203, 74, and 99; PBDE 209, 47, and 99; dieldrin; heptachlor epoxide; HCB; *p,p'*-DDE; γ -HCH; and β -HCH. The proportion of nondetected values and the distributions for each compound is displayed in Table S9. Correlations between coexposure (OC, PCB, and PBDE) and PFAS are displayed in Figure S3. Higher correlations were seen between PFUnDA and PCBs (0.27 for PCB118 to 0.34 for PCB153). Detailed results for models studying associations between prenatal exposure to POP and behavior scores are displayed in Table S10. Furthermore, γ -HCH, detected vs. nondetected, was associated (*p* < 0.1) with higher scores on the SDQ hyperactivity scale (aMR = 1.4; 95% CI: 1.0, 2.0) and with DIA internalizing, major depressive, and anxiety subscales (aMR = 1.3; 95% CI: 1.0, 1.6). Dieldrin (values greater than median in reference to nondetected values) was also negatively associated with major depressive subscale [aMR_{values > LOD and < median vs. nondetected} = 1.1 (95% CI: 0.9, 1.3) and aMR_{values > median vs. nondetected} = 0.8 (95% CI: 0.7, 1.0)]. When models were further adjusted for these coexposures, the conclusions were no different from the main analysis. The associations between PFUnDA and anxiety and internalizing subscales became statistically significant [aMR = 1.10 (95% CI: 1.01, 1.20) and aMR = 1.09 (95% CI:

1.00, 1.18), respectively; see Table S11]. In the BKMR models, results were also similar to those of the main analysis (see Table S12).

Discussion

Our objective was to assess the link between prenatal exposure to several PFAS and behavior among 12-y-olds. The results of the single-pollutant approach showed increased scores on externalizing and hyperactivity subscales associated with increasing cord-blood serum levels of PFOA and PFNA. The study also highlighted the fact that prenatal exposures to increasing levels of PFNA and PFDA were associated with increasing scores on depressive, anxiety, and internalizing subscales. Our results also suggested a weak association between prenatal PFUnDA and scores on anxiety and internalizing subscales. For both internalizing and externalizing subscales, the overall mixture effect from BKMR modeling suggested no, or very slight, deleterious effects.

In light of our findings, the discussion below focuses on studies also conducted in European areas and on the associations between prenatal exposure to PFOA and PFNA and externalizing behaviors, on the one hand, and on PFDA and PFNA and any other PFAS and internalizing behaviors on the other.

The cord serum samples used in our study were collected between 2002 and 2006. The median cord serum concentration of PFAS was generally within the range reported by other contemporaneous studies using cord blood measurements. For PFOA, we observed a median of 2.25 µg/L, whereas studies in European countries observed median values of 3.4 µg/L in Germany,⁶⁵ 3.7 µg/L from the Danish Birth Cohort study,⁶⁶ 0.88 µg/L in a Norwegian study⁶⁷ (*n* = 123), 1.5 µg/L in Belgium⁶⁸ (*n* = 346), and 1.27 µg/L [geometric mean (GM)] in a pooled analyses of

Table 5. Associations between prenatal exposure measured in cord serum and behavior scores at age 12 y. Results from the single-pollutant approach, PELAGIE mother–child cohort, Brittany, France (*n* = 444).

Outcome	Exposure					
	PFOA (log ₂ µg/L) <i>n</i> = 444	PFNA (log ₂ µg/L) <i>n</i> = 433	PFDA (log ₂ µg/L) <i>n</i> = 406	PFUnDA (log ₂ µg/L) <i>n</i> = 422	PFHxS (log ₂ µg/L) <i>n</i> = 444	PFOS (log ₂ µg/L) <i>n</i> = 444
	aMR (95% CI)	aMR (95% CI)	aMR (95% CI)	aMR (95% CI)	aMR (95% CI)	aMR (95% CI)
Main analysis						
SDQ externalizing	1.18 (1.03, 1.34)	1.14 (1.00, 1.29)	1.07 (0.94, 1.21)	1.00 (0.88, 1.13)	1.02 (0.90, 1.16)	1.06 (0.94, 1.21)
SDQ hyperactivity	1.20 (1.04, 1.40)	1.18 (1.02, 1.36)	1.11 (0.96, 1.30)	0.99 (0.85, 1.14)	1.07 (0.93, 1.23)	1.08 (0.94, 1.26)
DIA internalizing	1.05 (0.96, 1.14)	1.08 (1.00, 1.17)	1.11 (1.02, 1.21)	1.06 (0.97, 1.15)	1.02 (0.94, 1.10)	1.02 (0.94, 1.11)
DIA anxiety	1.06 (0.98, 1.16)	1.10 (1.01, 1.19)	1.11 (1.02, 1.21)	1.07 (0.98, 1.16)	1.02 (0.94, 1.11)	1.01 (0.93, 1.10)
DIA depression	1.08 (0.97, 1.21)	1.11 (1.00, 1.23)	1.14 (1.01, 1.27)	1.05 (0.95, 1.17)	1.02 (0.92, 1.13)	1.02 (0.92, 1.14)
Complementary analysis						
DIA externalizing	1.05 (0.94, 1.17)	1.03 (0.93, 1.14)	1.13 (1.01, 1.27)	1.04 (0.94, 1.16)	1.03 (0.93, 1.14)	1.00 (0.90, 1.11)
DIA ADHD	1.07 (0.96, 1.19)	1.06 (0.96, 1.18)	1.16 (1.04, 1.30)	1.05 (0.95, 1.16)	1.06 (0.96, 1.17)	1.03 (0.92, 1.14)
SDQ internalizing	1.06 (0.94, 1.20)	0.94 (0.84, 1.05)	0.97 (0.86, 1.09)	0.95 (0.85, 1.06)	1.04 (0.93, 1.17)	1.00 (0.88, 1.13)

Note: Negative binomial regression models adjusted for maternal age at the beginning of pregnancy (in years), maternal prepregnancy BMI (in three categories: <18.5 kg/m², 18.5–25 kg/m², ≥25 kg/m²), maternal education (in three groups: <12 y, 12 y, >12 y), parity (nulliparous vs. multiparous), maternal prepregnancy fish intake (<1 once/month, at least once a month, at least twice a week) smoking status at inclusion (smoker vs. nonsmoker) and child sex. ADHD, attention-deficit/hyperactivity disorder; aMR, adjusted mean ratio; BMI, body mass index; CI, confidence interval; DIA, Dominic Interactive for Adolescents; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFUnDA, perfluoroundecanoic acid; SDQ, Strengths and Difficulties Questionnaire.

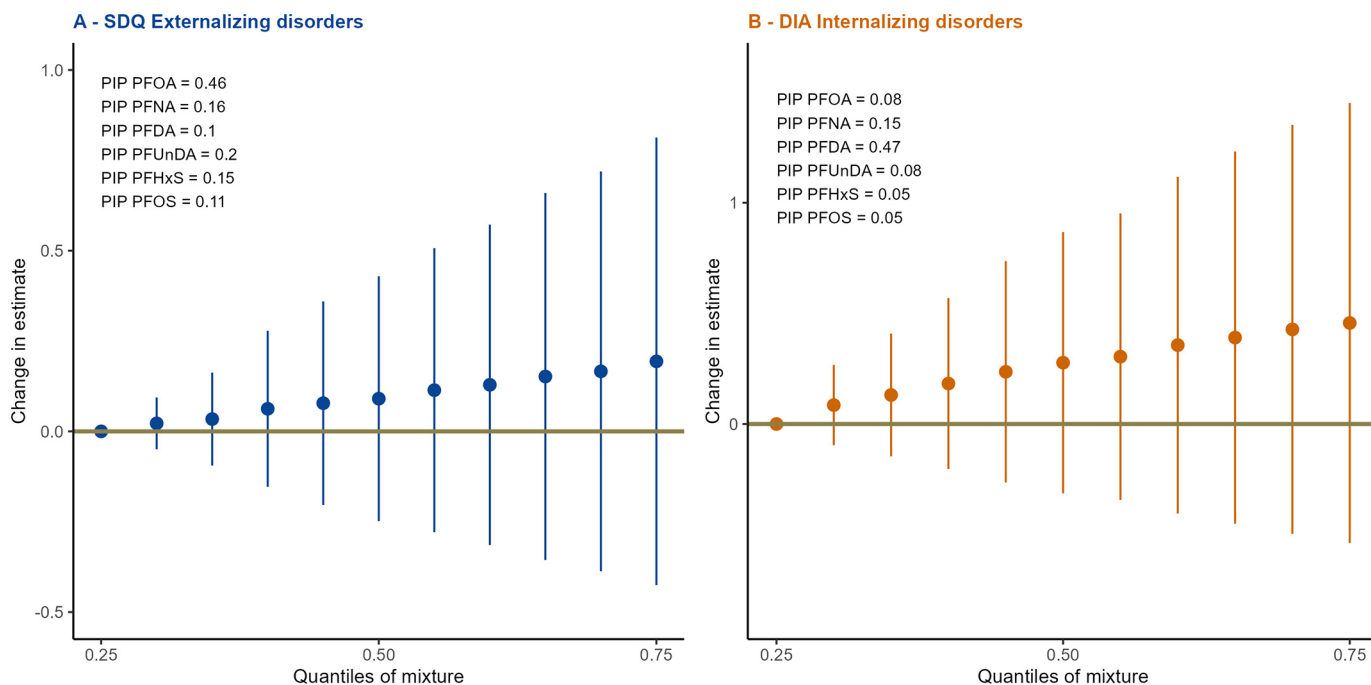


Figure 1. Overall PFAS mixture effect for SDQ Externalizing subscale (A) and DIA internalizing subscale (B) from BKMR models ($n = 378$), PELAGIE mother–child cohort, Brittany, France. Change in estimate are spotted as blue and orange dots and 95% CIs as blue and orange bars (for externalizing and internalizing subscales, respectively) for concurrent increase of exposures in comparison with the 25th percentile. PIP are provided on the figure. Mixture was composed of PFOA, PFNA, PFDA, PFUnDA, PFHxS, and PFOS. Models were adjusted for maternal age at the beginning of pregnancy (in years), maternal prepregnancy BMI (in three categories: $<18.5 \text{ kg/m}^2$, $18.5\text{--}25 \text{ kg/m}^2$, $\geq 25 \text{ kg/m}^2$), maternal education (in three groups: $<12 \text{ y}$, 12 y , $>12 \text{ y}$), parity (nulliparous vs. multiparous), maternal prepregnancy fish intake (<1 once/month, at least once a month, at least twice a week), smoking status at inclusion (smoker vs. nonsmoker) and child sex. Numeric data can be found in Table S13. Note: BKMR, Bayesian kernel machine regression; BMI, body mass index; CI, confidence interval; DIA, Dominic Interactive for Adolescents; PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFUnDA, perfluoroundecanoic acid; PIP, posterior inclusion probabilities; SDQ, Strengths and Difficulties Questionnaire.

four cohorts from Belgium and Slovakia.⁶⁹ The median cord concentration of PFNA of $0.35 \mu\text{g/L}$ in our study was slightly higher than in the pooled analysis from Belgium and Slovakia (GM of $0.21 \mu\text{g/L}$) or the Norwegian study (median $0.12 \mu\text{g/L}$). The median level of cord-blood PFDA in our study ($0.10 \mu\text{g/L}$) was higher than in the Norwegian study (median $0.04 \mu\text{g/L}$) and similar to that found in the Faroese Islands^{70,71} (GM of $0.07 \mu\text{g/L}$ and $0.11 \mu\text{g/L}$). For PFOS cord serum concentration, median concentration was $4.6 \mu\text{g/L}$ in our sample and was lower than that found in other studies (medians of $7.3 \mu\text{g/L}$ in Germany⁶⁵ and $11.0 \mu\text{g/L}$ in Denmark⁶⁶).

In the INUENDO cohort (Greenland, Ukraine, and Poland), prenatal exposure to PFOA was associated with a small-to-moderate effect on behavior (especially hyperactivity) in 5- to 9-y-olds, as assessed using the parent-reported SDQ (0.5 point higher hyperactivity score for high exposure in comparison with low exposure).¹⁵ In the Faroese Islands birth cohort, prenatal PFOA exposure was reported to be associated with higher externalizing behaviors, as assessed using the SDQ at age 7 y.²¹ In contrast, in the Danish Birth Cohort study, increased prenatal exposure to PFOA (second and third quartiles) was statistically significantly associated with lower odds ratios (OR) of children scoring higher in terms of total difficulties, emotional symptoms, and hyperactivity assessed using the SDQ, in comparison with the lowest quartile of exposure.⁷²

Studies focusing on ADHD diagnosis or symptoms were also identified. In a nested case–control study in the Norwegian Mother, Father and Child Cohort study (MoBa), prenatal exposure to PFOA was associated with a higher risk of ADHD.¹⁶ In the Human Milk Study (HUMIS), exposure to PFOA measured in breast milk was positively associated with ADHD in single-pollutant analysis—though

this association disappeared in the multipollutant approach.⁶ In the Danish Birth Cohort study, even though no association was found between prenatal exposure to PFAS and risk of an ADHD diagnosis, when categorized in quartiles, the PFOA concentration was positively associated with ADHD [relative risk (RR)_{third vs. first quartile} = 1.46 (95% CI: $1.14, 1.88$) and RR_{fourth vs. first quartile} = 2.02 (95% CI: $1.49, 2.75$)],⁷³ where PFAS were entered into models simultaneously.

In the present study, we detected no difference between females and males in the association between prenatal PFOA exposure and scores on externalizing and hyperactivity subscales. Effect modification by sex has been reported previously,^{2,17–20,24,29,74,75} but few studies have found any such effect for the association between prenatal PFOA exposure and externalizing behaviors. In the Dutch mother–child cohort Linking Maternal Nutrition to Child Health (LINC), males having the highest prenatal exposure to PFOA showed lower scores on the Externalizing Problem Scale of the Child Behavior Checklist.⁷⁴ In Denmark, though no associations between maternal PFOA concentrations in serum and symptoms of ADHD at the age of 2.5 or 5 y were seen overall, a sex difference was suggested, with a higher risk among females (though the association was not statistically significant).⁷⁵ In a meta-analysis performed on nine European birth cohorts, Forns et al. identified no overall association between prenatal exposure to PFOA and ADHD, but they did suggest a sex-specific association between early-life exposure (including prenatal) to PFOA and ADHD, with females being more at risk than males, as seen in the two studies cited previously.¹⁴

Ten other European studies have also reported no association between prenatal exposure to PFOA and externalizing behaviors, making it difficult to draw any firm conclusions from these

studies. For sex-specific effects, it is even more complicated, although the studies identified do tend to suggest that females are at a higher risk.^{2,19,25,26,28,30,72,73,76,77}

In our study, prenatal PFNA exposure (cord-serum median concentration 0.35 ng/mL) was associated with higher externalizing and hyperactivity subscales scores measured by SDQ. This finding is in accordance with the literature. A study from Greenland, Ukraine, and Poland²² reported prenatal PFNA exposure (median maternal plasma concentration 0.64 ng/mL) to be associated with higher SDQ total difficulties and hyperactivity scores among children 5 to 9 y of age. In the Danish National Birth Cohort,¹⁹ an increasing prenatal PFNA exposure was associated with externalizing difficulties at 7 and 11 y of age, reported (via SDQ) by parents and children; no sex-specific differences were identified, although associations measured at age 7 y appeared stronger in females.

In Europe, in the other nine studies identified, no associations were seen in term of links between prenatal exposure to PFNA and externalizing behaviors.^{2,16,21,25,26,30,73–75}

Beyond Europe, three studies have suggested associations between prenatal exposure to PFNA and externalizing behavior: lower scores for inattention and oppositional defiant subscales in 7-y-olds,⁷⁸ increased hyperactivity and hyperactive-type ADHD symptoms and diagnostic criteria in children 5 and 8 y of age,²⁰ higher ADHD and hyperactivity-impulsivity scores among males.²⁴

Our study reported that prenatal exposure to PFNA was associated with higher scores on the anxiety subscale (as well as on internalizing subscale, though to a lesser extent) measured by the self-reported DIA. A similar association was also suggested with SDQ self-reported internalizing difficulties at 11 y of age in the Danish National Birth Cohort.¹⁹ Beyond Europe, a study has suggested an association between prenatal exposure to PFNA and less somatic complaints problems in 2- and 4-y-old males in China.¹⁸

Using the DIA, we also observed that prenatal PFDA exposure was associated with higher scores for internalizing, depressive, and anxiety subscales. Although there is a scarcity of literature regarding the effect of prenatal PFDA exposure on behavior, our result is in line with that of the study in the Danish National Birth Cohort, in which a suggestive association was also observed between prenatal PFDA exposure (median maternal plasma concentration 0.17 ng/mL) and higher SDQ self-reported internalizing difficulties scores at 11 y of age.¹⁹

In our study, a further association was suggested between PFUnDA and scores on anxiety and internalizing subscales. Only one study (performed in China) identified prenatal exposure to both PFUnDA and perfluorotridecanoic acid (PFTrDA) as associated with internalizing problems, measured using the Child Behavior Checklist (CBCL) in 2- and 4-y-old males.¹⁸ Few other studies have explored associations between prenatal exposure to PFAS and internalizing disorders. In Europe, a study of the association between prenatal exposure to PFOA and PFOS and depression diagnosis identified through the prescription of antidepressants reported no association,²⁸ whereas an inverse association was identified between PFOS and SDQ parent-reported internalizing subscale score in children 3 to 7 y of age.²⁵ In the United States, among 8-y-olds, prenatal PFHxS was associated with more severe internalizing problems, especially depression and somatization, measured using the Behavior Assessment System for Children, 2nd edition (BASC-2).²⁰

Because we are all exposed to a mixture of many intercorrelated PFAS, including before we are born, there is a growing interest in addressing this issue, including through the use of more complex statistical approaches.^{79–81} We therefore used a BKMR model, retaining six compounds in the mixture: PFOA, PFNA, PFDA, PFUnDA, PFHxS, and PFOS. We did not find any statistically significant association of the PFAS mixture observed in

our French population with externalizing or internalizing behavior scores of children. The fact that we contrasted all PFAS compounds in the mixture model may have somewhat diluted or concealed stronger compound-specific effect. However, in the BKMR models, when we introduced only the PFAS that were associated with outcomes in the single-pollutant approach, the mixture effect still did not reach statistical significance. This mixture approach did not raise any concern about interactions between PFAS, and suggested that it may be PFOA and PFDA that predominantly affect behavioral scores (externalizing and internalizing, respectively), although both PIP were <0.5, which is the threshold identified in the literature at which a compound is considered to be of importance.⁵⁴ In contrast with findings using the single-pollutant approach, PFNA was not identified as contributing much to the mixture effect on behavior scores. The associations seen with PFNA and externalizing behavior and internalizing behavior may be because of strong correlations between PFOA and PFNA and PFDA and PFNA (see Figure S2).

Using Weighted Quantile Sum (WQS) regression, Luo et al.¹⁹ found that the mixture of six PFAS (PFOA, PFNA, PFDA, PFOS, PFHxS, PFHpS) was associated with higher total SDQ difficulties in Danish children at age 7 y, though the effect sizes of the associations decreased by the age of 11 y. They also identified higher (though not statistically significant) externalizing troubles scores in parental and child reports at 7 and 11 y, associated with the prenatal PFAS mixture, with PFNA contributing most to the mixture effect. Using structural equation modeling with mutual adjustment for pre- and postnatal exposures, Oulhote et al. did not identify any effect of prenatal exposure to a PFAS mixture (PFOA, PFNA, PFDA, PFOS, PFHxS) on the SDQ in 7-y-olds from the Faroe Islands, though they did find that postnatal exposure to PFAS measured at 5 and 7 y of age was associated with the latent function of several SDQ subscales, i.e., total difficulties, externalizing subscale, conduct problems, and autism spectrum disorder scores.³⁰ Again, no association between PFAS mixture (PFOA, PFNA, PFDA, PFUnDA, PFOS, PFHxS, PFHpS) and risk of ADHD was observed using quantile G-computation, though there was a lower risk of autism spectrum disorder among prenatally exposed Norwegian children (PFNA, PFOA, and PFOS had the highest positive influence in the mixture, whereas PFDA, PFUnDA, PFHpS, and PFHxS had the highest negative influence).¹⁶ A few studies have included several families of POPs in a mixture model. Oulhote et al.²¹ identified an overall adverse effect of a mixture of mercury, PCBs, and PFAS measured prenatally on behavioral scores measured via the SDQ and using the G-formula combined with SuperLearner.

In animal studies (mostly focused on PFOA and PFOS), the most consistent neurobehavioral deficit associated with developmental exposure has been impaired motor activity⁸² (increased or decreased, both of which were associated with disruption of the cholinergic system as reported earlier for PCBs and PBDEs⁸³). Starnes et al. argued in their review that the interpretation of tests relying on exploratory and motor behavior could be impaired due to concurrent physical and emotional (anxiety-like) reactions in the face of new environments.⁸² Impaired learning and memory were also identified, though less frequently, in the literature. A few studies have focused on other PFAS (mostly long-chained),⁸⁴ and data on PFNA and PFDA are sparse; Kawabata et al. did not identify PFDA exposure as being associated with alteration in cognitive function or anxiety in adult rat models,⁸⁵ and Johansson et al. identified no effect in rats exposed neonatally to PFDA.⁸³

The major mechanisms identified to explain PFAS neurotoxicity are modification in calcium homeostasis, alterations in neurotransmitters [glutamate, γ -aminobutyric acid (GABA), dopamine, acetylcholine, and serotonin levels] and neuroendocrine

disruption (including sex, stress, and thyroid hormones).⁸² PFAS neurotoxicity might increase with carbon chain length and fluorination level.⁸² It has also been suggested that the biological mechanisms of developmental neurotoxicity could include inhibition of DNA synthesis, deficits in cell numbers and growth, oxidative stress, and reduced cell viability, differing from one PFAS to another.⁸⁶ PFAS toxicity to the brain and behavior might also express itself through indirect pathways, such as alteration of immune functions, or dysregulation of peroxisome proliferator-activated receptors (PPARs), which intervene in regulating cellular differentiation, proliferation, and metabolic routes.⁸²

One strength of our study is that we used different tests to evaluate both externalizing and internalizing behaviors based on the parental reports and the child self-reports, respectively. The SDQ has been shown to accurately capture externalizing disorders in low-risk community samples³⁸ and has been very widely used in studies on associations between chemical prenatal exposure and behavior in childhood. This approach allowed us to compare our results to other published data. When considering externalizing behavior assessed with the DIA, associations with prenatal exposures to PFAS were on the whole similar, highlighting the consistency of these results. Given that, thus far, internalizing disorders have rarely been studied at ages at which children were capable of self-reporting, the DIA has not been used in such studies. Nevertheless it remains a very promising tool because it is easy to implement and allows the assessment of disorders meeting the DSM classification.^{40–42} The fact that our complementary analysis using the parent-reported SDQ internalizing composite scale did not show any association highlights the importance of using self-reported questionnaire to accurately assess internalizing behaviors in adolescents.

Prenatal exposure to PFAS was assessed in cord-blood samples collected at delivery in our study. We believe that this is likely to represent direct fetal exposure to PFAS; indeed, it has been shown that transplacental transfer of PFAS from maternal blood to fetal blood differs, depending on type of PFAS (sulfonate compounds such as PFOS and PFHxS show lower transplacental transfer rates than carboxylates) and carbon chain length.^{87,88} The relationship between transplacental transfer rate and carbon chain length seemed U-shaped: Compounds including 8 carbons (such as PFOA) showed a higher level of transplacental transfer rate than compounds with 9, 10, or 11 carbons (such as PFNA, PFDA, and PFUnDA, respectively), and the rate seemed to increase then with chain length^{87,88}; this observation might be explained by differences in binding affinity to serum proteins according to chain length. It has also been reported that the PFAS concentration in maternal blood decreases throughout the pregnancy.⁸⁹ Cord-blood samples might reflect exposure at the end of pregnancy and then underestimate it by assuming the PFAS transplacental transfer rate to be constant throughout pregnancy. Had they been available, maternal blood samples collected in early pregnancy would certainly have contributed other valuable information and potentially allowed the indirect effects of PFAS exposure on behavior in later life to be included via mediation related to alterations in maternal thyroid function⁹⁰ and, to a lesser extent, in newborns and infants⁹¹ as a result of exposure to PFAS. These alterations in maternal thyroid function may impair a child's neuropsychological development, including in terms of externalizing behaviors such as ADHD and internalizing behaviors like anxiety and depression.⁹²

As we stated earlier, postnatal exposure to PFAS has been shown to be associated with behavioral outcomes among children. In our study population, we did not measure postnatal exposure to PFAS during childhood. Breastfeeding is known to be a source of exposure to PFAS during infancy, and because of their long half-

lives, we can assume that postnatal exposure in infancy is driven by breastfeeding. For our study, we performed a sensitivity analysis adjusting our models for breastfeeding (as a way of somehow controlling for early postnatal exposure), and there was no effect on results, which supports our findings regarding associations between prenatal exposure to some PFAS and behavior at age 12 y.

Although maternal education level and age in our study sample were higher than those in the original cohort, all regression models were adjusted for these two covariates, which minimized the effect of a potential selection bias on the results. In this prospective longitudinal cohort, we were able to take account of many confounding factors, including prenatal coexposure to other persistent organic pollutants, such as PCBs,⁶¹ PBDEs,⁶² and OCPs. We cannot, however, exclude residual confounding from other unmeasured or unknown confounders. Because the socioeconomic status of our study population is both high and fairly homogenous, confounding linked to socioeconomic status is unlikely to occur. We measured parent emotional well-being using the SF36 questionnaire; it allowed us to partially take parent mental health into consideration in our study. However, it is worth noting that parent mental health assessment occurred at the same time as the child behavior assessment. Previous measure was not available in our study.

In recent years, exposure levels to PFOA and PFOS have decreased for the general population in Europe and the United States, as a result of the inclusion of PFOA and PFOS in the Stockholm convention list of banned or restricted POPs.^{93,94} Yet this trend is mixed; exposure levels to PFNA and PFDA and other PFAS (such as PFBA, PFHxA or PFBS that were detected in <5% in our study) are increasing.^{93–95} Both these compounds and the new generation of PFAS (such as GenX and C604) are now spreading worldwide and demand fresh investigation.

Conclusion

This study has highlighted prenatal PFOA and PFNA exposures as being associated with higher scores on externalizing and hyperactivity subscales among 12-y-olds, which is in accordance with the literature regarding younger children. We also found prenatal exposure to PFNA and PFDA to be associated with scores on internalizing subscale; this result adds some information to the as yet sparse literature examining the links between prenatal exposure to PFAS and internalizing disorders. The toxicological plausibility of the association between prenatal exposure to PFAS and internalizing behavior has yet to be studied, but the links and relationships between externalizing and internalizing behaviors (especially among adolescents) are known, and this field warrants further studies. Indeed, internalizing disorders such as depression and anxiety can impact future mental health.

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The PELAGIE cohort data comply with the European regulation on the protection of personal data (25 May 2018). This regulation is based on a logic of compliance and increased responsibility of the actors who access the data. In addition, the cohort study complies with the French “informatique et liberté” law (law no. 78-17, January 1978, 2018). Access to data is thus possible following the agreement of the cohort principal investigators (C.C., C.W.) and on condition that the actors demonstrate respect for these European and French principles of personal data protection to strengthen the rights of individuals. Further enquiries can be directed to the corresponding author.

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