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Prenatal exposure to per- and polyfluoroalkyl substances and childhood autism-related outcomes

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Abstract

Background: Epidemiological evidence linking prenatal exposure to per- and polyfluoroalkyl substances (PFAS) with altered neurodevelopment is inconclusive, and few large studies have focused on autism-related outcomes. We investigated whether blood concentrations of PFAS in pregnancy are associated with child autism-related outcomes.

Methods: Ten cohorts from the National Institutes of Health (NIH)-funded Environmental influences on Child Health Outcomes (ECHO) Program were included (n=1429). Fourteen PFAS analytes were measured in maternal blood collected during pregnancy and 8 met detection criteria for analysis. We assessed quantitative autism-related traits in children via parent report on the Social Responsiveness Scale (SRS). In multivariable linear models, we examined relationships of each PFAS (natural log-transformed) with SRS scores. We further modeled PFAS as a complex mixture using Bayesian methods and examined modification of these relationships by child sex.

Results: Most PFAS in maternal blood were not associated with child SRS T-scores.

Perfluorononanoic acid (PFNA) showed the strongest and most consistent association: each 1-unit increase in ln-transformed PFNA was associated with greater autism-related traits (adj-β [95% CI]=1.5 [-0.1, 3.0]). The summed mixture, which included 6 PFAS detected in >70% of participants, was not associated with SRS T-scores (adj-β [95% Highest Posterior Density Interval]=0.7 [-1.4, 3.0]). We did not observe consistent evidence of sex differences.

Discussion: Prenatal blood concentrations of PFNA may be associated with modest increases in child autism-related traits. Future work should continue to examine the relationship between exposures to both legacy and emerging PFAS and additional dimensional, quantitative measures of childhood autism-related outcomes.

Keywords

Pregnancy; fluorocarbon; prenatal exposure; autism; PFAS; mixtures

INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals widely used for decades in industrial and consumer products as surfactants and repellent coatings. The structural stability and other chemical properties that have made PFAS desirable in industrial applications have also made them persistent and bio-accumulative in the environment. Human populations are chronically exposed to PFAS, mostly via diet, indoor dust, food packaging, and drinking water.^{1–6} Once ingested or inhaled, most PFAS can bind to serum proteins, resist degradation, and remain in the human body for years.⁷ Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), the most commonly studied PFAS,

have been linked to adverse health outcomes, including metabolic, immune, renal, and reproductive effects, in humans and animals.⁸ While exposure to these two compounds declined after their voluntary phase out in the United States (U.S.) in the early 2000s,⁹ these chemicals and other PFAS are still widely detectable in the U.S. population,^{10,11} including pregnant individuals,¹² fueling concerns about their developmental toxicity.

PFAS cross the placenta with differing efficiency¹³ and demonstrate potential to interfere with thyroid and steroid hormone signaling critical to neurodevelopment.¹⁴ Evidence from animal studies indicates that prenatal exposure to PFAS can alter neuronal cell development and cell differentiation, inducing neurobehavioral deficits.^{15–17} While prenatal PFAS exposure has also been associated with poorer neurodevelopmental outcomes in humans, this epidemiological literature is mixed and limited by mostly small sample sizes, low geographic coverage, and a restricted number of PFAS species.¹⁴

To date, few studies have examined PFAS in relation to endpoints pertinent to autism spectrum disorder (ASD), which affects 2% (~1.5 million) of U.S. children¹⁸ and encompasses disabilities in social communication, behavior, and sensory processing. ASD is more commonly diagnosed in males than in females, which implicates potential endocrine-disrupting mechanisms in the prenatal period.¹⁹ Studies of PFAS and ASD in U.S. and European-based samples have been equivocal; some suggest that higher levels of PFAS in mid-pregnancy blood increase the probability of ASD,^{20,21} whereas others report either null findings or inconsistent patterns of relationships, especially by sex.^{22–24} Studies that have examined quantitative measures of neurobehavior suggest moderate increases in autism-related traits, but associations have been inconsistent across PFAS and study populations, warranting clarification in larger samples.^{25–27}

Mixture methods, which can be used to model either co-adjusted or joint effects of multiple exposures, offer a novel approach to addressing the current uncertainty about the relationship between prenatal PFAS exposure and autism-related outcomes. The present study examined associations of prenatal exposure to PFAS and autism-related outcomes in a large, geographically diverse sample of U.S. children. We additionally analyzed PFAS as a mixture using Bayesian methods and effect measure modification by child sex.

METHODS

Overview

The Environmental influences on Child Health Outcomes (ECHO) Program is a consortium of 69 pediatric, longitudinal birth cohorts, cumulatively enrolling over 50,000 children, launched by the National Institutes of Health (NIH) to investigate the influence of early life environmental exposures on child health and development.²⁸ All ECHO cohorts were invited to participate in the present study based on participant consent for data sharing of the mother-child pairs within the ECHO consortium²⁹ and the availability of relevant exposure and outcome data. Eligible participants had a) PFAS concentrations measured in at least one serum or plasma biospecimen collected during pregnancy and b) data on a child's Social Responsiveness Scale (SRS) or clinical diagnosis of ASD. The study population was restricted to singleton births. Based on these criteria, our study population included 1429

mother-child pairs from 10 eligible cohorts (Table 1). With the exception of MARBLES, which has published on PFAS and ASD in a larger subset of their cohort,²¹ none of the remaining cohorts have examined the relationship between PFAS and these autism-related outcomes. The relationship between PFAS and ASD had been examined in a larger sample of the MARBLES cohort. At the time of analysis, child enrollment was ongoing for several cohorts and not all children had completed the follow-up visit (eTable 1). Cohorts submitted data to the ECHO Data Analysis Center (DAC) for analysis.

The study protocol was approved by the local (or central ECHO) institutional review board. Written informed consent or parent's/guardian's permission was obtained along with child assent as appropriate for participation in the ECHO-wide Cohort Data Collection Protocol and specific cohorts.

Serum/Plasma PFAS Measurements

Laboratory methods for PFAS measurements varied across the sample (Table 1) as cohorts analyzed PFAS in different years. A total of 14 PFAS were originally measured (ng/mL) in serum or plasma at three labs: the California Department of Toxic Substances Control,³⁰ the Centers for Disease Control and Prevention (CDC),^{21,31,32} and the Wadsworth Human Health Exposure Analysis Resource (HHEAR) lab.³³ Previous studies indicate that PFAS measurements in serum and plasma are comparable.^{34,35}

PFAS were included in the analysis if at least 50% of values were above the method limit of detection (LOD) or limit of quantification and if the analyte was measured in at least five cohorts. This reduced the number of PFAS to eight: PFOA, PFOS, perfluorononanoic acid (PFNA), perfluorohexanesulfonic acid (PFHxS), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFuNDA), N-methyl perfluorooctane sulfonamido acetic acid (NMFOSAA), and 2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid (EtFOSAA). We replaced observations below the LOD with the value of the LOD/ 2.³⁶ For 30% of mothers who had two measures in pregnancy (repeat specimens were collected in the same trimester 92% of the time), we applied the following rules to either select one of the measures or average repeat measures: in the case where an analyte was above the LOD in one measurement and below the LOD for another, we selected the measurement above the LOD. In the case where both measurements were either above or below the LOD, the measures were averaged. Thirty-nine percent, 29%, and 32% of PFAS measurements came from samples collected in the 1st, 2nd, and 3rd trimesters, respectively. PFAS values were non-normally distributed and were natural log-transformed for all primary analyses.

Assessment of Autism-Related Outcomes

Children's social and communication skills were assessed as a quantitative trait with the SRS.³⁷ Cohorts used either the pre-school (ages 2.5–4.5 years) or school-age (ages 4+) version of the SRS depending on the ages of their participants. Parents completed the SRS's 65 Likert response items about their child's social use of language, reciprocal social behavior, and restricted interests or repetitive behaviors. Higher composite scores on the SRS correspond to higher levels of autism-related traits in the child. SRS raw scores were converted to clinically informative T-scores, which are standardized by sex and

SRS version using an independent normative sample (mean 50 with a standard deviation of 10).³⁷ We further calculated T-scores on subscales of Social Communication and Interaction and Restricted Interests and Repetitive Behaviors. Previous studies demonstrate the SRS's psychometric reliability and validity in accurately identifying clinically significant social impairments in both general and clinical population samples.^{38–40} SRS scores also demonstrate low within-individual variability over time, especially over the age range of the school-age version of the SRS.^{41–43} We also obtained information on a clinical diagnosis of ASD from either parent report or abstraction of medical records.

Covariates

Using a directed acyclic graph, we *a priori* identified covariates that were either known or potential confounders or associated with child outcomes in previous literature (eFigure 1).^{44,45} The following covariates were included in the primary analysis: maternal age at delivery (continuous years), maternal educational attainment (high school degree or equivalent; some college, no degree; bachelor's degree), maternal race/ethnicity (Hispanic of any race, non-Hispanic Asian/Pacific Islander, non-Hispanic Black, non-Hispanic White, and non-Hispanic Other), parity (nulliparous, multiparous), and child sex. Race/ethnicity was included as a social construct that has been associated with both PFAS exposure and disparities in SRS sensitivity and ASD diagnosis.^{46,47} ECHO Cohort was used as a proxy for year of birth (ranged from 1991–2018 across cohorts) and geographic location.

Statistical Analysis

In the primary analyses, we used linear regression to estimate the change (with 95% confidence intervals [CI]) in overall SRS raw scores and T-scores per unit increase in ln-transformed PFAS blood concentration. All models were adjusted for cohort. Fully adjusted models additionally included the mother's age, race/ethnicity, educational attainment, and SRS version (preschool, school-age). Models for the SRS T-score did not adjust for child sex because the T-score is normed by sex. Participants with missing data were excluded from the adjusted models.

We performed several sensitivity analyses to assess the robustness of our results. To account for potential differences in the sensitivity of the SRS at younger ages, we stratified analyses by SRS version completed (preschool vs. school-age). We also conducted leave-one-out analyses to assess the stability of associations when each cohort was excluded from the adjusted model. Lastly, for PFAS with >20% of values imputed below the LOD, we re-ran models restricted to values >LOD to assess potential bias introduced by variability in LODs across laboratories and the imputation method.⁴⁸

We used two Bayesian approaches to examine PFAS as a complex mixture. These models considered only those PFAS that were most highly detected (quantified in >70% of participants), to maximize the sample size. First, we used Bayesian Weighted Sums, an approach that provides an overall estimate of the effect of a 1-unit increase in all exposures simultaneously and the percent contribution of each PFAS to that association.⁴⁹ Briefly, this approach imposes a Dirichlet prior distribution on the PFAS set to ensure that their values

(i.e., the percent of the summed effect) are constrained to a range of 0 to 1 and must sum to 1. A so-called ‘non-informative’ prior is applied to all covariates of interest.

Secondly, we estimated independent PFAS associations adjusted for co-pollutant exposures using a semi-Bayes shared mean prior on the PFAS compounds. This approach imposes the prior assumption that $\beta_i \sim N(\mu, \sigma)$ where the estimated coefficients for each PFAS analyte, β_i , are allowed to arise from a similar distribution; the variance, σ , is specified as weak to ensure that the estimates for each PFAS can diverge from the shared mean if the model and data support different values for them.^{50–52}

All Bayesian models were run for three chains of Markov Chain Monte Carlo (MCMC) sampling. Model convergence was determined using a Gelman Rubin Diagnostic (Rhat) value of 1.0 and a minimum effective sample size of 100.⁵³ We quantified statistical uncertainty with 95% Highest Posterior Density (HPD) intervals.

In the secondary analyses, we examined associations between PFAS and (1) T-scores on the two subscales of the SRS (Social Communication and Interaction and Restricted Interests and Repetitive Behaviors) and (2) a clinical ASD diagnosis. Our analysis of ASD diagnosis, which used logistic regression, was exploratory given the small number of cases in our study sample. We also examined sex differences in single-pollutant and mixture models of SRS scores stratified by child sex and in interaction models with a cross-product term between child sex and PFAS analyte.

All analyses were performed using the R statistical software package. Bayesian models utilized the Just Another Gibbs Sampler (JAGS) software.

RESULTS

Project Viva and the New Hampshire Birth Cohort Studies accounted for the majority of study participants, contributing 467 and 318 out of 1429 participants, respectively (Table 1). Compared with other cohorts, Project Viva also had the earliest birth years (eTable 2). In the pooled sample, most participating mothers were non-Hispanic White (64%) and had at least a bachelor’s degree (64%) (Table 2). Most mothers gave birth between ages 30–34 (40%), and 61% of children were born in 2011–2018. The mean T-score (SD) for participating children was 47.4 (7.3). The prevalence of ASD among participants with ASD diagnostic information was 3.9% (n=38).

Median blood concentrations of PFHxS, PFOA, and PFOS were higher than other PFAS in the pooled sample (eFigure 2) and within cohorts (eFigure 3). Furthermore, these three compounds, along with PFNA, were detectable in >98% of participants (eTable 3). EtFOSAA and PFuNDA were not measured in some cohorts and were also detected above the LOD least frequently (eFigure 3). Compared with other cohorts, Project Viva had markedly higher median concentrations of EtFOSAA, NMFOSAA, PFOA, and PFOS. PFOA, PFOS, and NMFOSAA were more highly correlated with one another (Spearman correlation, $\rho > 0.7$) than with other PFAS, although several were moderately correlated with one another ($\rho > 0.4$) (eFigure 4).

In both unadjusted and adjusted models, most PFAS were not associated with SRS T-scores, though the direction of effect was positive for all PFAS except PFHxS and PFUNDA (Table 3). PFNA showed the strongest association with greater autism-related traits: a 1-unit increase in blood levels of PFNA correlated with a 1.5 point increase in SRS T-score (95% CI= -0.1, 3.0). Models of the SRS raw scores provided similar inference to models of T-scores (eTable 4).

While PFNA was associated with higher SRS scores, regardless of SRS version used, point estimates for other PFAS tended to be larger among participants who used the school-age version relative to the preschool version (eTable 5). Leave-one-out analyses suggested that most estimates, including for PFNA, were generally robust to exclusion of specific cohorts (eFigure 5). However, excluding Project Viva attenuated the point estimates for several PFAS and reversed the direction of association for NMFOSAA and PFOS. Among analytes with >20% non-detected values, results were generally similar when re-running models restricted to observations quantified above the LOD (eTable 6).

Mixture analyses examined a subset of the six most highly detected PFAS: PFOA, PFOS, PFNA, PFHxS, NMFOSAA, and PFDA. The summed PFAS mixture obtained with Bayesian Weighted Sums was not associated with SRS scores (β [95% HPD]=0.7 [-1.4, 3.0]) (Table 4). In the semi-Bayes shared mean model, which examined individual PFAS associations mutually-adjusted for other PFAS, the estimated associations of each PFAS with SRS scores were generally attenuated or similar to the results in Table 3 (Table 4). One exception was PFOS, which had a larger point estimate in these models (adj- β [95% HPD]=1.1 [-0.6, 3.5]).

Estimates of the associations between PFAS and SRS subscales were generally null, with the exceptions of PFDA and PFNA, which were associated with higher T-scores on the Social Communication and Interaction subscale (adj- β [95% CI] PFDA =1.1 [-0.3, 2.4]; PFNA= 1.8 [0.2, 3.4] (eTable 7).

In logistic models examining PFAS and odds of ASD, we did not observe a clear relationship and estimates were imprecise (eTable 8). The summed PFAS mixture was not associated with ASD, though the direction of effect was positive (adj-OR=1.4, 95% HPD: 0.2, 10.2) (eTable 9). All PFAS contributed similarly to the summed association. Co-pollutant-adjusted PFAS models also did not indicate clear associations between specific PFAS and ASD risk and HPD intervals were imprecise.

With some exceptions, most relationships between PFAS and SRS scores were similar across sexes (eTable 10). PFOS was associated with higher SRS T-scores among girls (adj- β [95% CI]=1.3 [-0.6, 3.2]) but not among boys (adj- β [95% CI]=-0.8 [-3.5, 1.8]; p-int=0.02). Additionally, though HPD intervals were wide, the summed mixture estimate was associated with greater autism-related traits in girls (T-score adj- β [95% HPD]=1.5 [-1.2, 4.3]) but not in boys (T-score adj- β [95% CI]=-0.6 [-4.1, 2.8]) (eTable 11). After adjusting for co-pollutants in semi-Bayes shared mean models, the sex difference in the association between PFOS and SRS scores was diminished (eTable 11).

DISCUSSION

In this large, pooled sample of over 1000 children from NIH ECHO birth cohorts across the U.S., most PFAS levels in maternal blood were associated with small and statistically imprecise increases in autism-related trait scores. PFNA demonstrated the largest and most consistent association with autism-related traits. This association was consistent in both preschool and school-age children and after exclusion of individual cohorts. The association was also more pronounced on the subscale of social communication and interaction scores relative to restricted interests and repetitive behaviors. When we examined the 6 most commonly detected PFAS as a mixture, their Bayesian weighted sum was not associated with autism-related traits in children. We did not observe an association between PFAS and ASD, although we had few ASD cases and therefore limited statistical power. While PFOS was more strongly associated with higher SRS T-scores in girls relative to boys, this sex difference was not robust in Bayesian models.

Previous epidemiological studies have presented a mixed empirical picture of the neurotoxicity of PFAS, especially with respect to autism-related outcomes.¹⁴ The Ohio-based HOME study is the only other study of prenatal PFAS exposures and the SRS. In contrast to our results, they reported no association with PFNA and an inverse relationship between PFOA and lower T-scores (i.e., fewer autism-related traits) in 4- to 5-year-old children, although the sample was relatively small (n=175) and the estimates were statistically imprecise in the chemical mixture model using semi-Bayesian hierarchical regression.²⁵ Findings have been equivocal in other studies that have examined prenatal PFAS in relation to other neurobehavioral outcomes that typically show overlap with autism-related traits. For example, a follow-up study in HOME observed associations between prenatal PFOS, PFHxS, and PFNA and externalizing and internalizing behavior problems, hyperactivity, and somatization at ages 5–8 years (n=240).²⁷ Meanwhile, a large study in the Faroe Islands reported no association between prenatal PFAS exposure and behavioral problems in 7-year-old children (n=539).⁵⁴

Case-control studies focused specifically on ASD diagnosis have also been inconclusive with regards to whether autism-related endpoints are sensitive to early-life exposure to PFAS. A study in California reported inverse associations of PFOA and PFOS with ASD,²² although these associations were attenuated when adjusted for co-pollutants in a Bayesian model.⁵¹ Two additional California studies have reported conflicting evidence, with one study finding PFHxS and PFOS to be associated with higher odds of ASD,²⁰ whereas the other study, in an ASD sibling cohort, found PFOA and PFNA to be associated with higher odds of ASD and PFHxS with lower odds.²¹ Large studies in Scandinavian registries have suggested further nuances in this relationship. A Norwegian study observed a U-shaped association between prenatal PFOA and ASD and inverse associations with PFDA and PFUnDA.²⁴ However, a Danish study observed mostly null associations, although they noted a potential U-shaped association between PFHxS and ASD.²³

While results stratified by sex suggested more pronounced relationships between some PFAS and autism-related traits in girls, we did not observe strong evidence of sex differences. Sex-specific patterns in the literature have been similarly inconclusive. Several

studies reported relationships between prenatal PFOS and PFOA exposure and greater autism-related outcomes among boys (SRS²⁸ and ASD^{20,25,27}) while others have linked PFOS and PFHxS to ASD among girls,^{20,24} or found no evidence of sex differences.^{22,23} However, because ASD is more prevalent among boys, the sample size of girls in research to date has typically been too small to reliably detect differences. However, studies of other neurodevelopmental outcomes support the possibility that PFAS may have stronger adverse influences on social development in girls. Two large studies have found that prenatal exposure to PFAS, specifically PFOS, PFOA, and PFNA, was associated with poorer verbal and communication skills⁵⁵ and delays in personal social skill development among girls.²⁶

The action of PFAS on glucocorticoid and reproductive hormones could plausibly cause neurotoxic susceptibility to differ by sex.¹⁴ However, as demonstrated in experimental studies, PFAS have potential to disrupt a diversity of biological targets that are instrumental to neurodevelopment,¹⁴ including neuronal signaling and differentiation.⁵⁶ PFAS can bind, with varying potency, to the thyroid hormone transport protein transthyretin, blocking thyroxine binding^{57,58} and consequently limiting the supply of thyroid hormone reaching the fetus.⁵⁹ Another potential toxicological pathway is PFAS's capacity to differentially activate peroxisome proliferase-activated receptors (PPARs), key regulators of placental function.⁶⁰ Heterogeneity in toxicity across PFAS may also stem from differences in their ability, due to size and functional groups, to cross the placenta; for example, PFOA and PFHxS reach the fetus more readily than PFNA and PFOS.¹³ PFNA, specifically, has been linked to pathways of thyroid disruption,⁶¹ oxidative stress,⁶² and PPAR dysregulation⁶³ in toxicology studies.

This study is not without limitations. Children with PFAS measurements who had yet to complete the follow-up visit with SRS collection were not included in the analysis. However, we expect participation to-date has been non-differential with respect to child exposure and outcome. Since some PFAS levels may decline in maternal circulation across pregnancy,⁶⁴ the trimester of sample collection may have influenced PFAS concentrations. While the timing of specimen collection was not related to child outcome, it could have introduced non-differential exposure misclassification, thereby reducing statistical power and attenuating our findings toward the null. While we adjusted for several known strong confounders, we cannot rule out that unmeasured confounding by factors such as smoking, which is possibly correlated with higher PFAS⁴⁵ and also higher SRS scores⁶⁵ and could have produced our modest positive findings. We also noted high heterogeneity across cohorts, particularly with respect to birth year and PFAS exposure profiles, consistent with temporal PFAS exposure trends in the US.¹¹ Although leave-one-out analyses suggested that the pooled results were robust to exclusion of specific cohorts, the heterogeneity of the ECHO cohorts reduced the precision of our estimates and raises the possibility that our overall findings may be masking subgroups with differential sensitivity to PFAS exposure.⁶⁶ The diversity and growing size of ECHO presents an opportunity for future studies to better understand how factors, such as co-exposures, postnatal exposures, social determinants of health, and genetics, may modify susceptibility to PFAS.

The 6 PFAS we excluded due to low detection may warrant continued scrutiny with respect to autism-related outcomes. PFNA also had a narrower exposure range relative to other PFAS, which could have influenced estimates in our study.⁶⁷ We also lacked

sufficient data to examine effect measure modification by potential protective factors against neurotoxicants, such as duration of exclusive breastfeeding and vitamin supplementation during pregnancy. Lastly, because of the low U.S. prevalence of ASD (2%)¹⁸ combined with the mostly population-based cohort designs of the contributing ECHO sites, our sample ultimately had too few ASD diagnoses to rigorously examine PFAS associations with ASD.

Our study has several key strengths, including its large sample size for the SRS analyses and geographic and sociodemographic heterogeneity, capturing a diverse cross-section of the U.S. pediatric population. Additionally, we focused on the SRS, a quantitative measure of autism-related traits; this enhanced the study's power to detect more subtle relationships,⁶⁸ rather than relying on a clinical diagnosis of ASD, which is often subject to delays or under-detection due to inequitable access in screening and diagnostic assessments in healthcare settings.⁶⁹ Although clinical diagnosis of ASD is strongly correlated with high SRS T-scores, the psychometric domains of the instrument are also affected, though to a lesser degree, by other developmental conditions that may be linked to PFAS exposure such as ADHD.^{70,71}

Despite the relatively small effect estimates, our findings suggest that some of the most widely detected PFAS could be associated with increases in autism-related traits, an association of potential public health relevance. Body burdens of PFOA and PFOS in the U.S. have dropped substantially since industry voluntarily phased out these chemicals in the early 2000s.^{11,72} However, other chemicals, such as PFNA, have increased and thousands of other PFAS, many of unknown toxicity, remain on the global market and are widely detectable in environmental and biological samples.⁷³ While our study focused on so-called legacy PFAS, which continue to pose risks to health because of their long biological half-lives,^{74,75} emerging replacement PFAS and their molecular byproducts also show signs of developmental neurotoxicity despite being engineered to bioaccumulate less readily.⁷⁶ Recognizing that toxicity data cannot feasibly keep pace with the rapid escalation of these structurally similar but understudied new PFAS, a class-based regulatory approach to PFAS has received growing support as a path to effectively manage both the known and unknown health risks of these chemicals of concern.⁷⁷

CONCLUSION

In summary, findings from our U.S. consortium suggest that prenatal exposure to PFNA may be associated with a modest increase in autism-related traits in children. Prenatal exposure to other legacy PFAS, including their summed mixture, was not strongly associated with autism-related traits, although several had weak, positive directions of effect. Based on the mixed literature to-date, closer scrutiny of PFAS remains warranted, especially with respect to sex differences and other potentially sensitive subgroups. Large studies with dimensional, quantitative measures of neurobehavior and gestational measurement of emerging and current-use PFAS will help clarify the relationship between this diverse class of chemicals and early brain development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Description of the Cohorts Included

Cohort name	N	Cohort features	Location	Years of birth	Trimester(s) of PFAS sample	Biological media of PFAS sample	SRS and/or ASD
Project Viva	467	Cohort is predominantly non-Hispanic White and college-educated.	Boston, Massachusetts	1999–2003	1 st	Plasma ^a	SRS-2 (School-age version), ASD
New Hampshire Birth Cohort Study (NHBCS)	318	Participants are mostly residents living in rural areas and primarily non-Hispanic White, reflective of the underlying population of the region.	Concord, New Hampshire Lebanon, New Hampshire	2009–2013	2 nd to 3 rd	Plasma ^a	SRS-2 (Preschool and School-age versions), ASD
Healthy Start	184	Cohort is racially and economically diverse	Colorado	2010–2013	2 nd , 3 rd	Serum ^a	SRS-2 (School-age version), ASD
University of California-Markers of Autism Risk in Babies (MARBLes)	31	ASD sibling cohort (ASD familial enriched risk)	Northern California	2009–2015	1 st , 2 nd , 3 rd	Serum ^a	SRS-2 (Preschool and School-age versions), ASD
Emory University Atlanta	144	Black/African American cohort from metropolitan Atlanta.	Atlanta, Georgia	2014–2018	1 st , 2 nd	Serum ^b	SRS-2 (Preschool and School-age versions), ASD
Pregnancy Environment and Lifestyle Study (PETALS)	78	Cohort is racially and economically diverse	California	2014–2017	1 st , 2 nd , 3 rd	Serum ^b	SRS-2 (School-age version), ASD
Rochester	102	Enriched for maternal sociodemographic diversity and psychosocial stress through recruitment at clinics serving disproportionately high-need patients.	Rochester, New York	2016–2019	2 nd	Serum ^b	SRS-2 (Preschool version)
Kaiser Permanente Research Bank (KPRB)	12	Cohort is racially and economically diverse	California	2014	1 st	Serum ^b	ASD
Chemicals in our Bodies (CIOB)	67	Cohort is urban, racially and economically diverse	San Francisco, California	2015–2018	2 nd , 3 rd	Serum ^c	SRS-2 (School-age version), ASD
Illinois Kids Development Study (IKIDS)	26	Cohort is from a mid-size mid-western college town, predominantly non-Hispanic White and college-educated.	Urbana - Champaign, Illinois	2015–2016	2 nd	Serum ^c	SRS-2 (Preschool and School-age versions)

ASD, autism spectrum disorder; PFAS, per- and polyfluoroalkyl substances; SRS, Social Responsiveness Scale.

^aPFAS in plasma and serum measured by Centers for Disease Control and Prevention (Kato et al. 2011; Sagiv et al. 2018; Oh et al. 2021; Honda, Robinson, and Kannan 2018)

^bPFAS in serum measured by Human Health Exposure Analysis Resource (HHEAR) (Honda, Robinson, and Kannan 2018; Zhu et al. 2017).

^cPFAS in serum measured by California Department of Toxic Substances Control (Erick et al. 2021)

Table 2.

Descriptive Characteristics of the Pooled Cohort Overall and Participants Contributing to SRS and ASD Analyses, NIH ECHO

	Overall	SRS sample	ASD sample
	(n=1429)	(n=1224)	(n=987)
Maternal age			
<25	151 (10.6%)	103 (8.4%)	124 (12.6%)
25–29	302 (21.1%)	256 (20.9%)	180 (18.2%)
30–34	571 (40.0%)	512 (41.8%)	376 (38.1%)
>=35	405 (28.3%)	353 (28.8%)	307 (31.1%)
Maternal education			
Up to high school degree, GED, or Equivalent	205 (14.3%)	149 (12.2%)	149 (15.1%)
Some college, no degree	278 (19.5%)	223 (18.2%)	202 (20.5%)
Bachelor's and above	912 (63.8%)	824 (67.3%)	623 (63.1%)
Missing	34 (2.4%)	28 (2.3%)	13 (1.3%)
Maternal race			
Hispanic All	149 (10.4%)	121 (9.9%)	124 (12.6%)
Non-Hispanic Black	232 (16.2%)	139 (11.4%)	215 (21.8%)
Non-Hispanic Other	123 (8.6%)	106 (8.7%)	107 (10.8%)
Non-Hispanic White	918 (64.2%)	851 (69.5%)	<540
Missing/Unknown	7 (0.5%)	7 (0.6%)	<5
Parity			
Nulliparous	687 (48.1%)	593 (48.4%)	516 (52.3%)
Multiparous	715 (50.0%)	604 (49.3%)	<470
Missing	27 (1.9%)	27 (2.2%)	<5
Infant sex			
Female	734 (51.4%)	624 (51.0%)	504 (51.1%)
Male	695 (48.6%)	600 (49.0%)	483 (48.9%)
Year of birth			
1991–2000	181 (12.7%)	149 (12.2%)	181 (18.3%)
2001–2010	374 (26.2%)	345 (28.2%)	329 (33.3%)
2011–2018	874 (61.2%)	730 (59.6%)	477 (48.3%)
Total SRS raw score			
Mean (SD)	28.8 (18.1)	28.8 (18.1)	
Median [Min, Max]	25.0 [2.00, 133]	25.0 [2.00, 133]	NA
Missing	1224 (85.7%)	0 (0%)	
Total SRS T-score			
Mean (SD)	47.4 (7.33)	47.4 (7.33)	
Median [Min, Max]	46.0 [35.0, 89.0]	46.0 [35.0, 89.0]	NA
Missing	1224 (85.7%)	0 (0%)	
SRS Second Edition (SRS-2) Version			

	Overall	SRS sample	ASD sample
	(n=1429)	(n=1224)	(n=987)
SRS-2 Preschool (ages 2.5–4.5y)	491 (34.4%)	491 (40.1%)	
SRS-2 School-Age (ages 4–18y)	733 (51.3%)	733 (59.9%)	NA
Missing	205 (14.3%)	0 (0%)	
Clinical ASD diagnosis			
No	949 (66.4%)	749 (61.2%)	949 (96.1%)
Yes	38 (2.7%)	33 (2.7%)	38 (3.9%)
Missing	442 (30.9%)	442 (36.1%)	0 (0%)

ASD, autism spectrum disorder; ECHO, Environmental influences on Child Health Outcomes; GED, Certification of completion of General Educational Development Test (High school equivalency diploma); NIH, National Institutes of Health; SRS, Social Responsiveness Scale.

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Table 3.

Associations of PFAS and SRS T-score from Single-Pollutant Models

PFAS analyte	n _{crude} /n _{adj}	SRS total T-score	
		β_{crude} (95% CI) ^a	β_{adj} (95% CI) ^b
EtFOSAA	694/677	0.8 (-1.2, 2.8)	0.8 (-1.2, 2.8)
NMFOSAA_MeFOSAA	1087/1057	1.0 (-0.4, 2.3)	0.6 (-0.8, 1.9)
PFDA	1151/1121	0.9 (-0.4, 2.2)	0.9 (-0.5, 2.2)
PFHXS	1223/1193	-0.7 (-2.0, 0.5)	-0.8 (-2.0, 0.5)
PFNA	1223/1193	1.6 (0.1, 3.1)	1.5 (-0.1, 3.0)
PFOA	1224/1194	0.5 (-1.0, 2.0)	0.3 (-1.3, 1.9)
PFOS	1223/1193	0.8 (-0.8, 2.3)	0.3 (-1.3, 1.9)
PFuNDA	618/592	-0.7 (-2.5, 1.0)	-0.1 (-1.8, 1.7)

^a Adjusted for cohort.^b Adjusted models included ECHO cohort, maternal age, race/ethnicity, parity, and educational attainment. T-score is standardized for SRS version (preschool, school-age) and child's sex.

CI, confidence interval; EtFOSAA, 2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid; NMFOSAA_MeFOSAA, N-methyl perfluorooctane sulfonamido acetic acid; PFDA, perfluorodecanoic acid; PFHXS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; PFuNDA, perfluoroundecanoic acid; SRS, Social Responsiveness Scale.

Table 4:

Associations of PFAS and SRS T-score in mixture models using Bayesian Weighted Sums (n=1057)

Weighted sums	SRS total T-score ^a	
	β_{adj}	95% HPD
Summed association	0.7	(-1.4, 3.0)
w(PFOA) ^b	0.16	(0.00, 0.43)
w(PFOS) ^b	0.19	(0.00, 0.49)
w(PFNA) ^b	0.18	(0.00, 0.47)
w(PFHxS) ^b	0.14	(0.00, 0.41)
w(NMFOSAA_MeFOSAA) ^b	0.17	(0.00, 0.45)
w(PFDA) ^b	0.16	(0.00, 0.42)
Individual association^c		
PFOA	-0.5	(-2.7, 1.1)
PFOS	1.1	(-0.6, 3.5)
PFNA	0.9	(-0.7, 2.8)
PFHxS	-0.7	(-2.3, 0.5)
NMFOSAA_MeFOSAA	0.2	(-1.0, 1.3)
PFDA	-0.1	(-1.5, 1.2)

HPD, Highest Posterior Density; NMFOSAA_MeFOSAA, N-methyl perfluorooctane sulfonamido acetic acid; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate;; SRS, Social Responsiveness Scale

^a Adjusted models included ECHO cohort, maternal age, race/ethnicity, educational attainment, and parity. T-score is standardized for SRS version (preschool, school-age) and child's sex.

^b Percent (%) weight contributed by each mixture component to the summed association. Weight and 95% HPD reported to two decimal places.

^c Co-adjusted for other PFAS using semi-Bayesian Shared Means approach.