HHS Public Access

Author manuscript

Environ Res. Author manuscript; available in PMC 2021 July 01.

Published in final edited form as:

Environ Res. 2020 July ; 186: 109518. doi:10.1016/j.envres.2020.109518.

Perfluoroalkyl substances and anthropomorphic measures in children (ages 3–11 years), NHANES 2013–2014.

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Abstract

Background: Perfluoroalkyl acids (PFAAs) are man-made compounds that are persistent in the environment and highly bioaccumulative in the body. Humans are exposed to a mixture of these substances, and the effects of these mixtures may be different than the effects noted for individual compounds. Prenatal exposure to PFAAs has been associated with decreased birth weight. The objective of the present study is to evaluate concurrent serum PFAA levels, as single compounds and as mixtures, in relation to anthropomorphic measures in children.

Methods: Using multivariate linear regression, we evaluated the association between single or PFAA mixtures and with height-for-age (HAZ), weight-for-age (WAZ), and BMI (BMIZ) z-scores in children (ages 3–11 years) participants of the National Health and Nutrition Examination Survey (NHANES) 2013–2014. Analyses were also stratified by sex. The PFAA mixture was based on relative potency factors express in terms of PFOA equivalency (CmixRPFi) or as molar sum of the PFAA congeners (ΣmolPFAA).

Results: There was a statistically significant association of PFHxS and PFOS with decreased HAZ in boys. The significantly decreased HAZ in boys was also found when the PFAAs were analyzed as mixtures: CmixRPFi (β = −0.33; 95%CI: 0.63, −0.04) or ΣmolPFAAs (β = −0.30; 95%CI: 0.56, −0.04). In boys, PFHxS was also associated with decreased WAZ and BMIZ. The only statistically significant association found in girls was between decreased HAZ and PFHxS.

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CRediT authorship contribution statement

Franco Scinicariello: Conceptualization, Methodology, Software, Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Supervision. **Melanie C. Buser:** Software, Writing - original draft, Visualization. **Henry G. Abadin:** Visualization, Writing - review & editing. **Roberta Attanasio:** Software, Visualization, Writing - review & editing.

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The findings and conclusion in this report are those of the authors and do not necessarily represent the official position of CDC/ATSDR.

Declaration of competing interests

The authors declare they have no actual or potential competing financial interests.

IRB approval: CDC/ATSDR has determined that our research did not meet the criteria for human research as per federal regulation and therefore did not require review.

Appendix A.: Supplementary data

Supplementary data to this article can be found online at http://doi.org/10.1016/j.envres.2020.109518.

Conclusions: We found sex differences in the association between concurrent serum PFAA levels and anthropomorphic measures in children 3–11 years old. PFAA levels, as single congeners or as mixture concentrations were associated with decreased height-for-age z-score in boys.

Keywords

Perfluoroalkyl and polyfluoroalkyl substances; Perfluoroalkyl acids; Height-for-age; Weight-for-age; Children; NHANES

1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are a large group of man-made chemicals that have been used extensively as ingredients or intermediates of surfactants and surface protectors for a wide range of industrial and consumer applications, as well as being used in fire-fighting foams (ATSDR. Agency, 2018; Buck et al., 2011). The general population is exposed to these substances through contaminated drinking water, food products, dust, and consumer products that contain PFAS (ATSDR. Agency, 2018; Calafat et al., 2007). A subset of PFAS, is represented by the perfluoroalkyl acids (PFAAs). More than 95% of the general U.S. population has detectable serum levels of PFAAs, such as perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorooctane sulfonic acid (PFOS) and perfluorohexane sulfonic acid (PFHxS) (ATSDR. Agency, 2018). The toxicity of these substances has been investigated in both human and laboratory animal studies. Evidence suggests that the aforementioned PFAAs may have similar targets of toxicity and act with similar mechanisms of action (ATSDR. Agency, 2018; Ballesteros et al. 2017; Liew et al. 2018; Rappazzo et al., 2017); however, there is still uncertainty about the mechanisms of action of PFAS especially for species other than PFOA and PFOS. One of the most studied endpoints is the effect of prenatal exposure to PFAAs on birth weight and early childhood growth (Andersen et al., 2010; Braun et al., 2016; reviewed in Kishi et al., 2017; Wang et al., 2016). In general, the results from these studies have been mixed; moreover, there have been suggestions of sex differences in these effects, though these too have been inconsistent. Timmermann et al. (2014) reported on the associations between serum PFAA levels and adiposity markers in children ages 8–10 years; they found no associations between PFOS or PFOA with BMI, waist circumference, skinfold thickness, adiponectin, or leptin.

Most research to date has evaluated the effects of a single PFAA on a health outcome; however, mixtures of PFAAs are commonly present in environmental media, and the exposure to PFAAs is typically to a mixture of the individual compounds. The few *in vitro* studies using equimolar mixtures of PFAAs indicate that the combined effect may be additive. Wolf et al. (2014) found that the activation of PPARα by a mixture of several concentrations of PFOA (1–1.28 μM) combined with concentrations of PFNA (1–128 μM), perfluorohexanoic acid (PFHxA) (8–1024 μM), PFOS (4–384 μM), or PFHxS (8–2084 μM) behaved in an additive manner at lower concentration ranges of PFOA (1–32 μM, which corresponds to 0.41–13.25 ng/mL). Among the most used methods to predict toxicity and risk of mixtures, hazard index (HI) and relative potency factors (RPF) are two examples of cumulative addition. These methods are based on the concept of concentration addition

where the individual compounds act via a similar mode of action, only differing in their relative potency to elicit a toxic effect (Backhaus et al. 2010). The concept of RPF assumes that the chemical substances that make up the mixture are toxicologically similar and act in a similar fashion with the same mechanism of action. In this type of analysis, the concentrations of mixture components are scaled relative to the concentration of an index compound and then summed up. A recent report from the Netherlands National Institute for Public Health and the Environment suggested that the RPF method is suitable for analyzing mixtures of PFAS (Zeilmaker et al., 2018).

In this study, using data from the National Health and Nutrition Examination Survey (NHANES) 2013–2014, we investigated the relationship between concurrent serum PFAA levels and anthropomorphic measures in children (ages 3–11 years) both as single PFAA compounds and as a mixture of the PFAAs. In this study, we used two simple and practical different ways to estimate PFAA mixtures: 1) an RPF method based on the intermediate oral Minimal Risk Levels (MRLs) for PFOA, PFOS, PFNA and PFHxS established by the Agency for Toxic Substances and Disease Registry (ATSDR), and; 2) a cumulative molar sum of the PFAA congeners.

2. Methods

2.1. Study population

NHANES is a cross-sectional, nationally representative survey of the non-institutionalized civilian population of the United States conducted annually by the National Center for Health Statistics (CDC/NCHS) with data released in 2-year cycles (Johnson et al., 2013). For our study, we used the publicly available files for the NHANES 2013–2014 cycle. The survey employs a multistage stratified probability sample based on selected counties, blocks, households, and persons within households.

NCHS-trained professionals performed interviews in participants' homes; extensive physical examinations, including blood and urine collection, were conducted at mobile exam centers. All procedures were approved by the NCHS Research Ethics Review Board (Continuation of Protocol #2011–17 http://www.cdc.gov/nchs/nhanes/irba98.htm), and all participants provided written informed consent. PFAS have been evaluated in NHANES participants 12 years of age and older since the 1999–2000 cycle. Recognizing the persistence of these chemicals, that childhood exposures may impact health later in life, and the dearth of data on general exposure to PFAS among children, analyses were expanded in the 2013–2014 data set to include serum PFAS measurements in a randomly selected one-third subsample of children participants (ages 3–11 years). Analysis of the samples was performed by the CDC's National Center for Environmental Health (NCEH), Division of Laboratory Sciences (DLS). For our analysis, we included children (ages 3–11 years) who had biological measurements for PFAAs and information regarding the covariates included in the models (n = 600).

2.2. Perfluoroalkyl substances (PFAS) measurements

NCEH/DLS analyzed the serum levels of fourteen different PFAS. We included the following PFAAs: PFOA (both linear and branched isomers), PFNA, PFOS (both linear and branched isomers), and PFHxS. These compounds were selected because they were detected in 95% of the samples. We summed the concentrations of the branched and linear isomers to obtain the total PFOA and PFOS (Ye et al., 2018). These compounds were measured using automated solid-phase extraction coupled to reverse-phase high-performance liquid chromatography/tandem mass spectrometry (https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/labmethods/PFAS_H_MET.pdf). The limit of detection (LOD) was 0.1 ng/mL for all four PFAA included in the analysis. For concentrations less than the LOD, a value equal to the limit of detection divided by the square root of two was used.

2.3. Mixture analyses

An RPF approach uses a health guidance value such as the draft intermediate oral Minimal Risk Levels (MRLs) for PFOA, PFOS, PFNA and PFHxS proposed by the Agency for Toxic Substances and Disease Registry (ATSDR. Agency, 2018). The points of departure (PODs) for PFOA (Koskela et al., 2016), PFOS (Luebker et al., 2005), and PFNA (Das et al., 2015) are based on developmental effects, while the POD for PFHxS (Butenhoff et al., 2009) is based on thyroid histological alterations, which the authors suggested to be secondary to the liver effects in F0 male rats in a developmental toxicity study. Despite slight differences in the POD effects, evidence suggests that PFAAs have similar targets of toxicity and act with similar mechanisms of action (ATSDR. Agency, 2018; Ballesteros et al. 2017; Liew et al. 2018; Rappazzo et al., 2017; Zeilmaker et al., 2018). This is the critical underlying assumption of the RPF approach. Risk assessment for PFAAs begins with the assumption that a serum concentration resulting in an effect in a laboratory animal would also result in an effect in humans. From these PODs, human equivalent doses (HEDs) were calculated to represent the continuous ingestion dose (mg/kg/day) that would result in steady-state serum concentrations of the PFAA species equal to the serum concentration (μg/mL) selected as the POD. MRLs were then calculated by dividing the HEDs by uncertainty and/or modifying factors. The RPF method normalizes the dose of each chemical according to its potency to a referent compound (RC), with the RC having an RPF equal to 1. We selected PFOA as the referent compound in our analyses. Therefore, the RPF were calculated as the following:

$$RPFi = \frac{\mathrm{POD pfoa}/\mathrm{UF pfoa}}{\mathrm{POD}i/\mathrm{UF}i} \quad (1)$$

where POD*i* and UF*i* represent each single PFAA compound. Applying the formula, the exposure level of each PFAA mixture component will be multiplied by its specific RPF. These products will then be summed up to express the serum mixture exposure in terms of PFOA equivalents (CmixRPFi).:

$$CmixrPFi = S_{PFOA} + (S_{PFNA} * 1.22) + (S_{PFOS} * 1.12) + (S_{PFHxS} * 0.11) \quad (2)$$

where S represents the serum concentration of the individual PFAA compounds. Additionally, we created a mixture variable based on the sum of the individually calculated

molar mass of the PFAA compounds metabolites (ΣmolPFAA). The rationale for this second mixture variable is because *in vitro* studies of mixtures typically employ molar sums.

2.4. Outcomes

Early childhood growth measures – including weight, height, and BMI – depend on the age and sex of the child. Therefore, it is recommended to calculate z-scores when evaluating these variables in children. Z-scores calculate the number of standard deviations (SDs) by which a child differs from the mean value of children of the same age and sex. Thus, these z-scores allow comparison of children of different ages and both sexes. For our analysis, we focused on the following z-scores as our outcomes of interest: height-for-age (HAZ), weight-for-age (WAZ), and BMI (BMIZ). The age and sex dependent z-scores were calculated using the methodology provided by the CDC (https://www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm).

2.5. Covariates

The following *a priori* covariates were included in the analyses: age, race/ethnicity, sex, poverty income ratio (PIR), serum cotinine, birthweight, and maternal smoking during pregnancy. Race/ethnicity was categorized as “non-Hispanic white,” “non-Hispanic black,” “Hispanic,” “non-Hispanic Asian,” and “non-Hispanic other race and multiracial.” PIR is a measure of socioeconomic status and represents the calculated ratio of household income to the poverty threshold after accounting for inflation and family size, with income values < 1 representing those below the poverty line. PIR was categorized by weighted tertiles, Serum cotinine was log-natural transformed in the analyses. Hematocrit and birthweight (ounces) were entered as continuous variables. Maternal smoking during pregnancy was assessed from the self-report Early Childhood questionnaire.

2.6. Statistical methods

All analyses were performed using the special “surplus specimen 3–11 years old subsample weight” as recommended by NCHS, to account for the complex sampling design and non-response of NHANES. SAS 9.3 (SAS Institute, Cary, NC) was used for all statistical analyses, and SAS-Callable SUDAAN 10 (Research Triangle Institute, Research Triangle Park, NC) was used to account for the NHANES complex sample design. P-values were presented at the significance level < 0.05. We used multivariable linear regression to calculate adjusted β-coefficients and 95% confidence intervals (CIs) for the associations between HAZ, WAZ, and BMIZ with each individual serum PFAA compound (PFOA, PFOS, PFNA, and PFHxS) and as a mixture (CmixRPFi). In the analyses, the individual compounds and the mixture CmixRPFi were categorized as weighted tertiles, with cutoffs based on the weighted distribution of the PFAA in the study population. Analyses were conducted for the whole sample and after stratification by sex. As complementary analyses, we also evaluated the association between the log-transformed PFAA compounds and anthropomorphic measures.

3. Results

Table 1 illustrates the weighted characteristics of children participants (ages 3–11 years) from NHANES 2013–2014 who were included in this analysis. Among the study population (n = 600), the geometric mean (GM) age of the participants was 6.6 years and 50.9% were male. Non-Hispanic whites (NHW) accounted for 52.4% of the total study group; 13.5% were non-Hispanic blacks and 24.6% were Hispanic. The GM of serum cotinine was 0.05 ng/mL, and the GM of hematocrit was 0.03%. The GM [standard error (SE)] BMIZ, HAZ, and WAZ in our population were 0.76 (0.03), 0.61 (0.05), and 0.73 (0.06), respectively. Girls had higher HAZ compared to boys (0.65 vs 0.56), but the difference was not statistically significant (Table 1). The geometric mean PFOA and PFNA were 1.92 ng/mL and 0.80 ng/mL, respectively. The geometric mean of PFOS and PFHxS were 3.90 ng/mL and 0.85 ng/mL, respectively. (Table 1). In general, male participants had higher concentrations of serum PFAAs, with statistically significant differences for PFOS and PFHxS (Table 1). The GM of the mixture CmixRPFi was 7.76 ng/mL PFOA equivalent (Table 1). Table 1 also presents the descriptive statistics for our study sample stratified by sex.

The results of the multivariate linear analyses of PFAAs with HAZ, WAZ, and BMIZ outcomes are presented in Tables 2–4, respectively. Analyses performed on the whole group showed that higher level of PFAAs were consistently associated with decreased HAZ (Table 2), decreased WAZ (Table 3) and decreased BMIZ (Table 4). The associations were statistically significant for PFOS, PFHxS, ΣmolPFAAs, and CmixRPFi with HAZ and WAZ. Stratified analyses by sex showed similar consistent inverse associations. The only statistically significant association found in girls was between HAZ and the highest tertile of PFHxS (β = −0.37; 95% CI: 0.74, −0.01) (Table 2). In boys, the highest tertile of PFHxS was statistically significantly inversely associated with HAZ (β = −0.40; 95% CI: 0.61, −0.19), WAZ (β = −0.49; 95% CI: 0.76, −0.22), and BMIZ (β = −0.38; 95% CI: 0.72, −0.05). Additionally, in boys, decreased HAZ were associated with high levels of PFOS (β = −0.28; 95% CI: 0.56, −0.01), PFNA (β = −0.28; 95% CI: 0.53, −0.03), as well as with the highest tertile of the PFAA mixtures, both as CmixRPFi (β = −0.33; 95% CI: 0.63, −0.04) and as ΣmolPFAAs (β = −0.30; 95% CI: 0.56, −0.04). Complementary analyses using the chemicals as continuous log-natural transformed variables yielded similar results (Supplemental Table 1).

4. Discussion

The most striking result of our analyses is the sex differences we observed between the concurrent PFAAs and the anthropomorphic measurement in children (ages 3–11) participants of a nationally-representative survey dataset of the general US population.

PFOS, PFHxS, and PFAAs mixtures (CmixRPFi or ΣmolPFAA) were statistically significantly inversely associated with HAZ in boys but not in girls. Additionally, PFHxS was found to be statistically significantly inversely associated with WAZ and BMIZ in boys but not in girls.

The sex differences reported here are consistent with many studies that use maternal PFAAs exposure. Andersen et al. (2010), in a study conducted in 1010 infants, found an association between maternal PFOA levels and decreased body weight and BMI in boys at age 5 months and between maternal PFOA and PFOS with these outcomes in boys at age 12 months; these associations were not found in girls. On the other hand, Maisonet et al. (2012) reported an association of higher body weight in girls at 20 months of age with higher maternal PFOS levels but not PFHxS. Furthermore, Halldorsson et al. (2012) reported an association of maternal PFOA levels with an increased risk of being overweight and having a high waist circumference in 20-year-old females, in a cohort of 665 persons.

Exposure to PFAAs in the general population is typically to a mixture of the individual compounds. To evaluate the effects of mixtures, we used two methods: 1) an RPF approach (CmixRPFi), or 2) the cumulative sum of the individually calculated molar mass of the PFAA compounds (ΣmolPFAA). Both CmixRPFi and ΣmolPFAA are based on the concept of concentration addition; this concept assumes that the chemical substances are toxicologically similar and act in a similar fashion, with the same mechanism of action. This additive effect is supported by *in vitro* studies on the combined effects of PFAAs. Wolf et al. (2014) assessed binary combinations of PFAS using monkey-derived COS-1 cells and found that the species activated PPARα in an additive manner at lower concentration ranges. Another study reported a slightly less than additive relationship when looking at binary combinations of four PFAAs (Carr et al., 2013). The authors concluded that the most conservative interpretation would be to assume additivity for these species. In fact, when the results were restricted only to the response region (i.e., before the plateau region where it can be assumed that the receptors are saturated), they found that the mixtures were in fact additive (Carr et al., 2013). Further support of the additive effect of PFAA mixtures comes from the *in vitro* study of Hu and Hu (2009), which found that PFOA (50–200 μmol/L) and PFOS (50–200 μmol/L) induced and promoted cell apoptosis in an additive manner, at all concentration levels tested. These results may suggest the additive assumption as a conservative and protective approach when investigating the effects of PFAAs in the general population.

The inverse relationship we observed between PFAAs and HAZ and WAZ may potentially be mediated through thyroid hormones. Thyroid hormones are essential for growth and development, and there is evidence that PFAAs are associated with alterations in thyroid hormone levels both *in vitro* and *in vivo.* Using binding assays, it has been demonstrated that several individual PFAAs have the ability to bind to thyroid hormone transport proteins, thus displacing the binding of thyroxine to the transport protein (Ren et al., 2016; Weiss et al., 2009). Furthermore, Weiss et al. (2009) reported that PFHxS had the highest binding potency, followed by PFOS and PFOA. Alterations in thyroid hormone levels have been reported in laboratory animal studies following exposure to PFOS (Chang et al., 2008). In rats, exposure to PFHxS resulted in thyroid follicular epithelial hypertrophy/hyperplasia (Butenhoff et al., 2009). Another potential mechanism through which PFAAs may affect normal growth is through skeletal effects. A few studies in laboratory animals have reported reduced or delayed ossification following *in utero* exposure to PFOA (Lau et al., 2006; Koskela et al., 2016) or to PFOS (Case et al., 2001; Yahia et al., 2008).

Most of the epidemiological studies on growth development endpoints have investigated the association of prenatal exposure to PFAAs. A meta-analysis conducted by Johnson et al. (2014) evaluated the association of PFOA exposure and growth. The authors found that a 1 ng/mL increase in serum or plasma PFOA was associated with a −18.9 g (95% CI: 29.8, −7.9) change in birth weight and −0.1 cm (95% CI: 0.1, −0.02) change in birth length. A second meta-analysis by Verner et al. (2015) found similar associations of PFOA and PFOS with decreased birth weight. In this study, the researchers applied a PBPK model to simulate PFAA concentrations in maternal and cord plasma to evaluate the influence of glomerular filtration rate on the associations, and results with simulated concentrations were compared to a meta-analysis of epidemiological data with measured PFAA concentrations. The meta-analysis of epidemiological studies reported that each 1-ng/mL increase in blood PFOA and PFOS was associated with a change in birth weight of −14.72 g (95% CI: 8.92, −1.09) and −5.00 g (95% CI: 21.66, −7.78), respectively. Similarly, simulated maternal PFOA and PFOS concentrations were associated with a 7.9 g (95% CI: 9.4, −6.4) and 1.5 g (95% CI: 1.8, −1.1) decrease in birth weight, respectively. Recently, Pinney et al. (2019) investigated the relationship between serum PFOA collected in girls age 6–8 years and the longitudinal changes in BMI z-score and waist to height ratio, and waist to hip ratio at age 6–18 years in girls of the Greater Cincinnati (n = 353) and San Francisco bay area (n = 351) participants of the female puberty cohort of the Breast Cancer and the Environment Research Program. The authors reported an inverse association of PFOA level with BMI z-score in the Cincinnati cohort as well as in the combined two -site data. Moreover, in the analyses of the Cincinnati cohort, the authors found that the strength of the inverse relationship between PFOA and BMI z-score decreased with age (Pinney et al., 2019). Further analyses of the Cincinnati cohort using structural equation models (Fassler et al. 2019), showed not only the inverse association of PFOA and BMI z-score and fat mass percent, also an effect of PFOA on HOMA both as direct effect and as indirect effect through BMI (Fassler et al. 2019).

There are several important limitations of this analysis. This is a cross-sectional study that uses concurrent measures of exposure and outcome, so causality cannot be established. Furthermore, the use of a single serum measure may not reflect past exposure, and this exposure measure does not allow for assessment of prenatal exposures, which may be more etiologically relevant to the outcomes of interest. However, these substances have long half-lives in humans, which may be upwards of 20 years (ATSDR. Agency, 2018), so exposure misclassification is less likely. There are some limitations to using the molar sum for this mixture analysis including that this assumes that PFAAs included are additive on the outcome and that the sum may be unduly influenced by the contribution of one or more individual species. While there is still some uncertainty surrounding the mechanism of action of these compounds, *in vitro* studies have suggested that additivity should be the default when assess mixtures. Moreover, by also including the relative potency factor approach to the mixtures analysis, the concern of too much influence by a given species is abated. This study focused on PFAAs exposure, thus the potential for co-exposure to other environmental factors that may affect these outcomes is possible. Finally, because many associations were being evaluated, we cannot dismiss the possibility that the significant associations were observed by chance.

5. Conclusion

This study may provide support for analyzing the adverse effects of the investigated PFAA compounds as mixtures by either summing the molar concentrations of the individual compounds or by using the Relative Potency Factor methodology. These approaches are similar to the hazard index method utilized by the Environmental Protection Agency when analyzing different pollutants that may produce similar adverse health effects. The few *in vitro* studies cited above indicate that mixtures of PFAAs behave in an additive manner, both at lower concentration ranges similar to those seen in the general population and at the higher concentrations tested. From a risk assessment perspective, this has important public health implications because it may allow for analysis of multiple PFAA contaminants at once. Moreover, this may provide support for the development of health-based guidelines for PFAS using a class-based approach rather than a chemical-by-chemical approach, allowing for more rapid development of these values. In the absence of health guidance values for individual PFAA congeners, either approach suggests that as long as the molar sum of all congeners is below the established health guidance value for an individual species, then the exposure to the mixture of PFAAs (at least for the four PFAA compounds investigated) will be without an appreciable risk of an adverse effect. PFAAs are of public health concern due to their persistence in the environment and long biological half-lives.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Weighted Characteristics of NHANES 2013–2014 participants aged 3–11 years.

Characteristics	All	Boys	Girls	p-value
n,	600	319	281	
Boys, GM (SE)	50.8 (2.3)			
Girls, GM (SE)	49.2 (2.3)			
Perfluorononanoic acid (PFNA), (ng/mL), GM (SE)	0.80 (0.06)	0.83 (0.08)	0.76 (0.05)	0.12
Perfluorooctanoic acid (PFOA), (ng/mL), GM (SE)	1.92 (0.08)	1.95 (0.09)	1.90 (0.10)	0.49
Perfluorooctane sulfonic acid (PFOS), (ng/mL), GM (SE)	3.90 (0.17)	4.12 (0.27)	3.69 (0.15)	0.03
Perfluorohexane sulfonic acid (PFHxS), (ng/mL), GM (SE)	0.85 (0.04)	0.95 (0.06)	0.77 (0.04)	0.007
CMIXRPFi[a] PFOA equivalent, (ng/mL), GM (SE)	7.77 (0.33)	8.19 (0.43)	7.36 (0.34)	0.02
Σmolar PFASs[a], (nmol/L), GM (SE)	17.44 (0.75)	18.51 (0.98)	16.41 (0.75)	0.005
Age, GM (SE)	6.59 (0.17)	6.52 (0.17)	6.66 (0.23)	0.57
BMI z-score (BMIZ), GM (SE)	0.76 (0.03)	0.75 (0.07)	0.76 (0.08)	0.79
Height-for-age z-score (HAZ), GM (SE)	0.61 (0.05)	0.56 (0.05)	0.65 (0.07)	0.61
Weight-for-age z-score (WAZ), GM (SE)	0.73 (0.06)	0.74 (0.09)	0.72 (0.08)	0.42
Ratio of family income to poverty (PIR), GM (SE)	1.61 (0.17)	1.64 (0.17)	1.57 (0.23)	0.98
Serum cotinine, (ng/mL), GM (SE)	0.05 (0.01)	0.06 (0.01)	0.04 (0.01)	0.70
Hematocrit (%), GM (SE)	0.03 (0.0)	0.03 (0.00)	0.02 (0.00)	0.53
Birthweight (ounces), GM (SE)	135.41 (4.85)	138.29 (7.02)	132.49 (5.13)	0.68
Race/ethnicity:				
White (Non-Hispanic), n (% [SE])	52.4 [5.8])	88 (54.2 [5.6])	72 (50.5 [6.9])	0.89
Non-Hispanic Black, n (% [SE])	147 (13.5 [2.3])	80 (12.5 [2.5])	67 (14.5 [3.4])	
Hispanic, n (% [SE])	205 (24.6 [4.0])	103 (24.6 [3.8])	102 (24.6 [4.7])	
Non-Hispanic Asian, n (% [SE])	45 (4.7 [1.1])	24 (4.6 [1.1])	21 (4.8 [1.7])	
Other and Multi-race, n (% [SE])	43 (4.8 [1.0])	24 (4.2 [1.0])	19 (5.5 [1.7])	
Mother smoked when pregnant				
Yes, n (% [SE])	74 (13.9 [2.3])	34 (13.0 [2.9])	40 (14.7 [2.5])	0.55
No, n (% [SE])	526 (86.1 [2.3])	285 (87.0 [2.9])	241 (85.3 [2.5])	

Table 2

Adjusted* β Coefficient (95% CI) for height-for-age z-score (HAZ) by PFAA level in child participants (ages 3–11 years) in NHANES 2013–2014.

	All n = 600	**Boys n = 319**	**Girls n = 281**
PFOA T1	0.00	0.00	0.00
PFOA T2	−0.17 (−0.38, 0.03)	−0.20 (−0.53, 0.13)	−0.25 (−0.45, −0.05)
PFOA T3	−0.28 (−0.66, 0.11)	−0.23 (−0.64, 0.19)	−0.35 (−0.88, 0.17)
p-value trend	0.19	0.30	0.05
PFNA T1	0.00	0.00	0.00
PFNA T2	−0.12 (−0.29, 0.06)	−0.17 (−0.44, 0.10)	−0.07 (−0.41, 0.27)
PFNA T3	−0.18 (−0.38, 0.02)	−0.28 (−0.56, −0.01)	0.02 (−0.33, 0.37)
p-value trend	0.13	0.12	0.85
PFHxS T1	0.00	0.00	0.00
PFHxS T2	−0.32 (−0.68, 0.04)	−0.37 (−0.74, −0.01)	−0.28 (−0.80, 0.24)
PFHxS T3	−0.41 (−0.63, −0.19)	−0.40 (−0.61, −0.19)	−0.37 (−0.74, −0.01)
p-value trend	0.004	0.004	0.13
PFOS T1	0.00	0.00	0.00
PFOS T2	−0.32 (−0.60, −0.04)	−0.27 (−0.56, 0.02)	−0.35 (−0.84, 0.14)
PFOS T3	−0.39 (−0.72, −0.06)	−0.28 (−0.53, −0.03)	−0.46 (−1.06, 0.14)
p-value trend	0.06	0.08	0.29
CmixRPFi T1	0.00	0.00	0.00
CmixRPFi T2	−0.27 (−0.55, 0.01)	−0.13 (−0.49, 0.22)	−0.31 (−0.70, 0.09)
CmixRPFi T3	−0.42 (−0.69, −0.154)	−0.33 (−0.63, −0.04)	−0.45 (−0.98, 0.09)
p-value trend	0.02	0.04	0.19
Σ Molar PFAS T1	0.00	0.00	0.00
Σ Molar PFAS T2	−0.20 (−0.47, 0.07)	−0.03 (−0.41, 0.35)	−0.28 (−0.64, 0.08)
Σ Molar PFAS T3	−0.39 (−0.63, −0.15)	−0.30 (−0.56, −0.04)	−0.44 (−0.91, 0.04)
Trend p-value	0.01	0.04	0.14

*Adjusted for: age (continuous), quadratic age, race/ethnicity, PIR, serum cotinine, birthweight, maternal smoking during pregnancy, hematocrit, and sex. Models stratified by sex were not adjusted for sex. Tertiles PFOA (ng/mL): T1: <1.53; T2: 1.54–2.39; T3: > 2.39. Tertiles PFNA (ng/mL): T1: ≤0.55; T2: 0.56–0.91; T3: > 0.91. Tertiles PFHxS (ng/mL): T1: ≤0.58; T2: 0.58–1.03; T3: > 1.03. Tertiles PFOS (ng/mL): T1: ≤2.95; T2: 2.96–4.79; T3: > 4.79. Tertiles ΣMolar PFAS (nmol/L): T1: <13.85; T2: 13.86–21.07; T3: > 21.07. Tertiles CMIXRPFi- PFOA equivalent, (ng/mL): T1: ≤6.17; T2: 6.17–9.25; T3: > 9.25.

Table 3

Adjusted * β Coefficient (95% CI) for weight-for-age z-score (WAZ) by PFAA level in child participants 3–11 in NHANES 2013–2014.

	All n = 600	Boys n = 319	Girls n = 281
PFOA T1	0.00	0.00	0.00
PFOA T2	−0.33 (−0.63, −0.04)	−0.42 (−0.77, −0.07)	−0.28 (−0.73, 0.16)
PFOA T3	−0.28 (−0.65, 0.08)	−0.21 (−0.56, 0.15)	−0.43 (−1.08, 0.23)
p-value trend	0.08	0.06	0.34
PFNA T1	0.00	0.00	0.00
PFNA T2	−0.05 (−0.33, 0.23)	−0.11 (−0.51, 0.29)	−0.03 (−0.48, 0.43)
PFNA T3	−0.09 (−0.42, 0.24)	−0.06 (−0.37, 0.24)	−0.06 (−0.59, 0.46)
p-value trend	0.84	0.84	0.95
PFHxS T1	0.00	0.00	0.00
PFHxS T2	−0.30 (−0.67, 0.07)	−0.38 (−0.79, 0.02)	−0.22 (−0.82, 0.39)
PFHxS T3	−0.42 (−0.76, −0.08)	−0.49 (−0.76, −0.22)	−0.31 (−0.84, 0.23)
p-value trend	0.05	0.005	0.48
PFOS T1	0.00	0.00	0.00
PFOS T2	−0.32 (−0.60, −0.04)	−0.25 (−0.66, 0.15)	−0.39 (−0.84, 0.06)
PFOS T3	−0.40 (−0.76, −0.04)	−0.26 (−0.57, 0.05)	−0.58 (−1.25, 0.08)
p-value trend	0.06	0.23	0.17
CmixRPFi T1	0.00	0.00	0.00
CmixRPFi T2	−0.32 (−0.63, −0.00)	−0.16 (−0.45, 0.13)	−0.41 (−0.97, 0.15)
CmixRPFi T3	−0.37 (−0.66, −0.08)	−0.28 (−0.58, 0.03)	−0.47 (−1.00, 0.07)
p-value trend	0.03	0.19	0.19
Σ Molar PFAS T1	0.00	0.00	0.00
Σ Molar PFAS T2	−0.26 (−0.61, 0.09)	−0.07 (−0.39, 0.25)	−0.39 (−0.96, 0.19)
Σ Molar PFAS T3	−0.37 (−0.67, −0.07)	−0.26 (−0.60, 0.08)	−0.49 (−0.99, 0.01)
Trend p-value	0.05	0.30	0.15

*Adjusted for: age (continuous), quadratic age, race/ethnicity, PIR, serum cotinine, birthweight, maternal smoking during pregnancy, hematocrit, and sex. Models stratified by sex were not adjusted for sex. Tertiles PFOA (ng/mL): T1: ≤1.53; T2: 1.54–2.39; T3: > 2.39. Tertiles PFNA (ng/mL): T1: ≤0.55; T2: 0.56–0.91; T3: > 0.91. Tertiles PFHxS (ng/mL): T1: ≤0.58; T2: 0.58–1.03; T3: > 1.03. Tertiles PFOS (ng/mL): T1: ≤2.95; T2: 2.96–4.79; T3: > 4.79. Tertiles ΣMolar PFAS (nmol/L): T1: ≤13.85; T2: 13.86–21.07; T3: > 21.07. Tertiles CMIXRPFi- PFOA equivalent, (ng/mL): T1: ≤6.17; T2: 6.17–9.25; T3: > 9.25.

Table 4

Adjusted* β Coefficient (95% CI) for BMI z-score (BMIZ) by PFAA level in child participants (ages 3–11 years) in NHANES 2013–2014.

	All n = 600	Boys n = 319	Girls n = 281
PFOA T1	0.00	0.00	0.00
PFOA T2	−0.30 (−0.60, 0.01)	−0.38 (−0.70, −0.05)	−0.20 (−0.68, 0.29)
PFOA T3	−0.15 (−0.49, 0.20)	−0.07 (−0.50, 0.37)	−0.31 (−0.90, 0.28)
p-value trend	0.14	0.06	0.54
PFNA T1	0.00	0.00	0.00
PFNA T2	0.05 (−0.28, 0.38)	−0.01 (−0.45, 0.44)	0.07 (−0.29, 0.53)
PFNA T3	0.02 (−0.36, 0.39)	0.12 (−0.23, 0.48)	−0.09 (−0.60, 0.41)
p-value trend	0.93	0.69	0.43
PFHxS T1	0.00	0.00	0.00
PFHxS T2	−0.17 (−0.47, 0.13)	−0.28 (−0.66, 0.09)	−0.07 (−0.60, 0.46)
PFHxS T3	−0.26 (−0.57, 0.04)	−0.38 (−0.72, −0.05)	−0.15 (−0.68, 0.38)
p-value trend	0.22	0.08	0.83
PFOS T1	0.00	0.00	0.00
PFOS T2	−0.19 (−0.41, 0.03)	−0.14 (−0.55, 0.27)	−0.28 (−0.60, 0.05)
PFOS T3	−0.21 (−0.53, 0.11)	−0.11 (−0.63, 0.40)	−0.40 (−0.88, 0.08)
p-value trend	0.17	0.76	0.17
CmixRPFi T1	0.00	0.00	0.00
CmixRPFi T2	−0.20 (−0.50, 0.09)	−0.08 (−0.38, 0.22)	−0.30 (−0.83, 0.24)
CmixRPFi T3	−0.17 (−0.50, 0.16)	−0.10 (−0.59, 0.39)	−0.29 (−0.70, 0.12)
p-value trend	0.33	0.84	0.35
Σ Molar PFAS T1	0.00	0.00	0.00
Σ Molar PFAS T2	−0.18 (−0.49, 0.13)	−0.04 (−0.41, 0.33)	−0.30 (−0.83, 0.23)
Σ Molar PFAS T3	−0.19 (−0.49, 0.12)	−0.09 (−0.58, 0.40)	−0.34 (−0.75, 0.07)
p-value trend	0.34	0.92	0.24

*Adjusted for: age (continuous), quadratic age, race/ethnicity, PIR, serum cotinine, birthweight, maternal smoking during pregnancy, hematocrit, and sex. Models stratified by sex were not adjusted for sex. Tertiles PFOA (ng/mL): T1: ≤1.53; T2: 1.54–2.39; T3: > 2.39. Tertiles PFNA (ng/mL): T1: ≤0.55; T2: 0.56–0.91; T3: > 0.91. Tertiles PFHxS (ng/mL): T1: ≤0.58; T2: 0.58–1.03; T3: > 1.03. Tertiles PFOS (ng/mL): T1: ≤2.95; T2: 2.96–4.79; T3: > 4.79. Tertiles ΣMolar PFAS (nmol/L): T1: ≤13.85; T2: 13.86–21.07; T3: > 21.07. Tertiles CMIXRPFi- PFOA equivalent, (ng/mL): T1: ≤6.17; T2: 6.17–9.25; T3: > 9.25.