HHS Public Access

Author manuscript

Epidemiology. Author manuscript; available in PMC 2022 July 01.

Published in final edited form as:

Epidemiology. 2021 July 01; 32(4): 573–582. doi:10.1097/EDE.0000000000001351.

Prenatal exposure to mixtures of persistent endocrine-disrupting chemicals and birth size in a population-based cohort of British girls

Kristin J. Marks[a,b,c], **Penelope P. Howards**[a], **Melissa M. Smarr**[d], **W. Dana Flanders**[a,b], **Kate Northstone**[e], **Johnni H. Daniel**[b], **Andreas Sjödin**[b], **Antonia M. Calafat**[b], **Terryl J. Hartman**[a,b]

[a]Department of Epidemiology, Rollins School of Public Health, Emory University, 1518 Clifton Rd NE, Atlanta, Georgia 30322, United States

[b]National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy, Atlanta, Georgia 30341, United States

[c]Oak Ridge Institute for Science and Education, 100 ORAU Way, Oak Ridge, Tennessee 37830, United States

[d]Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, 1518 Clifton Rd NE, Atlanta, Georgia 30322, United States

[e]Department of Population Health Sciences, Bristol Medical School, Oakfield House, Oakfield Grove, Bristol BS8 2BN, United Kingdom

Abstract

Background.—Previous studies of endocrine-disrupting chemicals have examined one of these chemicals at a time in association with an outcome; studying mixtures better approximates human experience. We investigated the association of prenatal exposure to mixtures of persistent endocrine disruptors [per- and polyfluoroalkyl substances (PFAS), polychlorinated biphenyls (PCBs), and organochlorine pesticides] with birth size among female offspring in the Avon Longitudinal Study of Parents and Children (ALSPAC), based in the United Kingdom in 1991–1992.

Methods.—We quantified concentrations of 52 endocrine-disrupting chemicals in maternal serum collected during pregnancy at median 15 weeks' gestation. Birth weight, crown-to-heel length, and head circumference were measured at birth; ponderal index and small for gestational age were calculated from these. We used repeated holdout weighted quantile sum regression and Bayesian kernel machine regression to examine mixtures in 313 mothers.

Corresponding author: Kristin Marks, MPH, 1518 Clifton Rd NE, Atlanta, GA, 30322, USA, (770)488-3414, kristin.marks@emory.edu.

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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

Study data and code must be requested from the University of Bristol and are subject to institutional data release policies.

Results.—Using weighted quantile sum regression, all mixtures (each chemical class separately and all three together) were inversely associated with birth weight. A one-unit increase in WQS index (a one-decile increase in chemical concentrations) for all three classes combined was associated with 55 g (β: −55 g, 95% CI: −89, −22 g) lower birth weight. Associations were weaker but still inverse using Bayesian kernel machine regression. Under both methods, PFAS were the most important contributors to the association with birth weight. We also observed inverse associations for crown-to-heel length.

Conclusions.—These results are consistent with the hypothesis that prenatal exposure to mixtures of persistent endocrine-disrupting chemicals affects birth size.

Keywords

ALSPAC; pregnancy; birth weight; endocrine disruptors; per- and polyfluoroalkyl substances; polychlorinated biphenyls; organochlorine pesticides

Introduction

An endocrine-disrupting chemical is defined as a chemical that may interfere with the body's endocrine system, potentially producing adverse developmental, reproductive, neurological, and immune effects.[1] Environmentally persistent endocrine disruptors, such as organochlorine pesticides, polychlorinated biphenyls (PCBs), and per- and polyfluoroalkyl substances (PFAS), used throughout the 20th and 21st centuries for a variety of purposes, are typically highly resistant to degradation, and tend to bioaccumulate in humans and animals.[2–4] Exposures to PFAS, PCBs, and organochlorine pesticides have declined in the general population following numerous countries banning or severely restricting the production, handling, and disposal of several organochlorine pesticides and PCBs, as well as certain PFAS. Still, almost all humans have detectable concentrations of some of these persistent chemicals.[5,6] Moreover, persistent endocrine-disrupting chemicals can cross the placental barrier, allowing for potential fetal exposure.[7–10]

Birth size is considered a relevant and sensitive marker of prenatal exposure to endocrine-disrupting chemicals and is an important predictor of future health.[11] Many previous studies (with select references cited here) of prenatal exposure to persistent endocrine disruptors and birth size suggest that they are associated with smaller birth size,[12–15] though others have shown somewhat mixed results.[16–19] A meta-analysis of maternal perfluorooctanoate (PFOA) exposure and infant birth weight estimated a 19 g reduction in birth weight for each 1 ng/mL increase in maternal serum PFOA concentration.[14] While these associations with birth size measures may not be considered large at the individual or clinical level, it is important to consider implications at the population level. A relatively modest and subclinical effect size may be associated with substantial population burden if the exposure is prevalent, like for PFAS.[14] Additionally, PFOA is just one of the many environmental chemicals that could affect birth weight. Examining the cumulative effect of several endocrine-disrupting chemicals may show an even larger effect size than reported in the meta-analysis and other previous studies that examined chemicals individually.

Historically, most studies have examined one endocrine-disrupting chemical at a time in relation to an outcome. Because humans are exposed to many chemicals, as opposed to one chemical in isolation, examining combined exposures or "mixtures" of chemicals would allow for a better approximation of the human experience.[20] A mixture is a combination of three or more independent chemicals or chemical groups.[21] Three previous studies have explored the use of mixture methods in relation to persistent endocrine-disruptors and birth size, though they used methods that accomplished different objectives. Generally speaking, these studies found that higher prenatal exposure to mixtures of PFAS[22,23] and organochlorine pesticides[23,24] was associated with smaller birth size measures (e.g., birth weight,[22,23] head circumference[24]).

While several studies have examined prenatal exposure to persistent endocrine-disrupting chemicals and birth size, few to our knowledge have explored persistent exposure to these chemicals as a mixture. Our aim was to investigate the association of maternal gestational concentrations of mixtures of persistent endocrine disruptors (PFAS, PCBs, and organochlorine pesticides) and birth-size measures (weight, crown-to-heel length, head circumference, ponderal index, small for gestational age) in a sub-study of the Avon Longitudinal Study of Parents and Children (ALSPAC). Specifically, we aimed to estimate the overall effect of the mixture and identify the chemicals contributing the most to the overall effect within the mixture.

Methods

Study population

The Avon Longitudinal Study of Parents and Children (ALSPAC) is an ongoing prospective birth cohort of 14,541 pregnancies. ALSPAC enrolled pregnant women with an expected delivery date between 1 April 1991 and 31 December 1992 from three health districts in the former county of Avon, Great Britain. Information was collected on parents and children through clinic visits, interviews, and mailed questionnaires. Details on ALSPAC recruitment and study methods have been described elsewhere.[25,26] A nested case–control study was conducted within the ALSPAC cohort to explore associations of prenatal maternal concentrations of various suspected endocrine disruptors and early menarche among the daughters. Details of the nested case–control study are described elsewhere.[27] Cases were girls that obtained early menarche, defined as menarche prior to 11.5 years of age. Cases and controls were selected from singleton daughters who completed at least two (out of five) puberty staging questionnaires between 8 and 13 years old. To be eligible, cases needed to complete at least two staging questionnaires, with the second questionnaire returned after menarche had occurred. Controls had to complete the 13-year-old questionnaire to determine that menarche had not taken place before 11.5 years. The nested case–control study was reweighted to represent the full cohort. The weight for the cases (all girls who attained menarche before 11.5 years) was 1, and the weight for the controls (a random sample of girls who attained menarche at or after 11.5 years) was 15.1.

The study website contains details of all the data that are available through a fully searchable data dictionary and variable search tool (http://www.bris.ac.uk/alspac/researchers/our-data/). We obtained ethical approval for the study from the ALSPAC Ethics and Law Committee,

the Local Research Ethics Committees, and the Centers for Disease Control and Prevention (CDC) Institutional Review Board. Mothers provided written informed consent for participation in the study. Consent for biological samples has been collected in accordance with the Human Tissue Act (2004). Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time.

Exposure assessment

At enrollment in 1991–1992, study staff collected fasting blood samples from mothers at median 15 (interquartile range (IQR): 10–28) weeks gestation. Samples were processed and frozen for future analysis. Maternal serum samples were held in storage at the University of Bristol until they were transferred under controlled conditions and analyzed at the National Center for Environmental Health of the CDC (Atlanta, GA). Laboratory analyses included low- and high-concentration pooled quality control materials, standards, reagent blanks, and study samples. Prior to statistical analysis, concentrations below the limit of detection (LOD) were imputed by dividing the LOD by the square root of 2.

Per- and polyfluoroalkyl substances—We quantified eight PFAS (Table 1) in serum via on-line solid-phase extraction coupled to isotope dilution high-performance liquid chromatography-tandem mass spectrometry.[28] LODs were 0.20 ng/mL (EtFOSAA, PFDA, PFOS), 0.174 ng/mL (MeFOSAA), 0.10 ng/mL (FOSA, PFHxS, PFOA), and 0.082 ng/mL (PFNA). Coefficients of variation (CVs) for PFAS were largely below 10%. We included PFAS detected in greater than 75% of mothers in the main analyses.

Organochlorine pesticides and polychlorinated biphenyls—We measured nine organochlorine pesticides and 35 PCBs (Table 1) in serum using gas chromatography isotope dilution high resolution mass spectrometry.[29] For PCBs and organochlorine pesticides, LODs are dependent on the size of the sample available, thus an individual LOD was reported for each individual result instead of an overall LOD. CVs were generally below 10%. PCB and organochlorine pesticide concentrations were adjusted for lipids. As with PFAS, PCBs and organochlorine pesticides detected in greater than 75% of mothers were included in the main analyses.

Outcome assessment

Birth weight (g) was abstracted from infant medical records. Trained ALSPAC staff measured crown-to-heel length (cm) using a Harpenden neonatometer (Holtain Ltd., Crymych, United Kingdom) and head circumference (cm) using a lasso tape measure (median 1 day, IQR: 1–3 days).[30,31] Ponderal index was calculated using the following formula: (weight in g/height in cm^3) × 100. A ponderal index of <2.4 was considered low.[32] We defined small for gestational age (SGA) as below the 10^{th} percentile of the distribution for birth weight among female infants in the United Kingdom, adjusted for gestational age at birth. We calculated standard deviation scores of weight on the basis of the British growth reference centiles from 1990[33] with Excel macros provided on the internet (www.healthforallchildren.co.uk). In ALSPAC, the final clinical estimate of the expected

date of delivery was abstracted from the obstetric records and used to calculate gestational age at delivery.

Covariates

Covariate information was collected by clinical staff or through self-report on questionnaires completed by the mother during or immediately after pregnancy. Covariates under consideration included: gestational age at biological sample collection (weeks), maternal age (years), maternal pre-pregnancy body mass index (BMI) (kg/m^2), maternal race (white/nonwhite), maternal education (classified as <O-level (ordinary level: required, completed at age 16), O-level, or > O-level), parity (nulliparous/multiparous), smoking during pregnancy (any/none), and hours of physical activity (enough to work up a sweat) per week during pregnancy (>0 hours/0 hours).

Statistical analyses

We conducted descriptive analyses to compare mother–daughter dyad characteristics by median birth weight and select endocrine-disrupting chemicals. We reported the median and interquartile range, as well as percent below the LOD, for all measured endocrine-disrupting chemicals. We described correlations among endocrine-disrupting chemicals using Spearman correlation coefficients.

We modeled the chemical exposures under study as natural log-transformed continuous variables. We evaluated confounding using previous knowledge, which we assessed using a directed acyclic graph, and by taking into consideration the associations between persistent endocrine-disrupting chemicals and maternal characteristics. We ran single-chemical linear regression models to examine independent associations between each chemical and birth weight.

We used Bayesian kernel machine regression to visualize the exposure–response function and verify assumptions (linearity, no interaction) using the R package *bkmr*.[34–36] Assuming no identification of non-linearity and/or interaction within the mixture through Bayesian kernel machine regression, weighted quantile sum regression was used to estimate associations of maternal endocrine-disrupting mixtures with birth size using the R package *gWQS*.[37] Mixtures under study were each chemical class separately (PFAS, PCBs, and organochlorine pesticides) and all three chemicals classes combined.

Weighted quantile sum regression creates a weighted linear index of correlated predictors that are weighted according to their strength of association with the outcome of interest.[38] Specifically, we used the following equation to calculate the weights of c set of correlated variables:

$$g(\mu) = \beta_0 + \beta_1\left(\sum_{i=1}^{c} w_i q_i\right) + z'\varphi$$

The sum term was the index for the c items, scored into quantiles (denoted q_i), and weights were represented by the sum of w_i. Each w_i was constrained between 0 and 1. Within each

bootstrap sample, we estimated the w_i by maximum likelihood and constrained them to sum to 1. All covariates were represented by $z'\varphi$. Prior to analysis, we split the data into two datasets at random: a training dataset (40%) and validation dataset (60%). Using the training dataset, we selected 100 bootstrap samples and determined the strength of the associations for each c item by the beta coefficient. The index was calculated based on the mean w_is across all bootstrap samples and was interpreted as an estimation of the overall mixture effect.[38–42] To improve stability of the estimates of weights across training and test data partitions, we applied repeated holdout validation; this approach combines cross-validation and bootstrap resampling.[43] We generated a distribution of results by repeating weighted quantile sum regression 100 times on data split randomly into training (40%) and validation (60%) sets and the mean was taken as the final estimate.

We employed Bayesian kernel machine regression as a complementary mixture method to weighted quantile sum regression. Bayesian kernel machine regression is a flexible semi-parametric technique that models the combined effects of different chemicals, while allowing for nonlinearity and interactions among chemicals.[44] This approach enables the examination of independent effects of mixture members, interactions among them, and the overall mixture effect. Within Bayesian kernel machine regression, we used hierarchical variable selection, which provided group importance scores [posterior inclusion probabilities (PIPs)] for pre-defined mutually exclusive groups of variables. Further, we estimated the importance of a chemical given that the group containing the chemical was important (conditional PIPs).[34–36] Within Bayesian kernel machine regression, we standardized all continuous variables to improve computational efficiency. Currently, the *bkmr* package does not allow for weighting, so we were unable to weight our nested case–control data back to the full cohort. SAS software 9.4 (Cary, NC) was used for descriptive analyses. We used R software 3.5.0 (Vienna, Austria) for weighted quantile sum and Bayesian kernel machine regression analyses.

Results

Descriptive statistics

Most mothers in this subsample of ALSPAC were white (98%), well-educated (82% completed secondary education or higher), and above the age of 25 (79%). PCB153 and p,p'-DDE were highest among women with greater than a secondary education and higher among women who drank alcohol during pregnancy (Table 2). PCB153 and p,p'-DDE were higher among older women and PFOA was higher among nulliparous women. Very few infants were born with low birth weight (4%) and 13% of infants had a low ponderal index (Table 2).

Of the 52 chemicals measured, 31 were detected in more than 75% of mothers (Table 3). Correlation was high among the subset of chemicals detected in most mothers (eFigure 1). Among the 31 chemicals, PCBs and organochlorine pesticides showed high correlation within and between classes. PFAS chemicals exhibited lower correlation within the class, but were still positively correlated with some strong correlations.

Single-chemical models

In adjusted single-chemical models of maternal serum concentrations of endocrine-disrupting chemicals with birth weight, almost all chemicals were inversely associated with birth weight (eTable 1). Among PFAS, a 10% higher PFOA concentration was associated with 25 g lower birth weight (β: −25 g, 95% CI: −36, −14 g); PFOS and EtFOSAA were also strongly inversely associated with birth weight. PCB105, PCB138, PCB153, PCB170, PCB180, PCB196, and PCB206 were strongly inversely associated with birth weight, while no organochlorine pesticides were strongly associated with birth weight.

Weighted Quantile Sum Regression

In repeated holdout weighted quantile sum regression models, the weighted quantile sum indices for mixtures (PFAS, PCBs, organochlorine pesticides, and all three classes combined) were inversely associated with birth weight, head circumference, and crown-to-heel length (Table 4 and eTable 2). For example, one-unit higher of the weighted quantile sum index (representing a one-decile increase in chemical concentrations) for all three classes combined was associated with 55 g (β: −55 g, 95% CI: −89, −22 g) lower birth weight (Table 4). We identified EtFOSAA, PFOA, and MeFOSAA as contributing the most to the weighted quantile sum index (weights: 0.16, 0.11, and 0.10, respectively). Inverse associations were also seen for head circumference and crown-to-heel length: one-unit higher of the weighted quantile sum index for all three classes combined was associated with 0.11 cm (β: −0.11 cm, 95% CI: −0.21, −0.01 cm) smaller head circumference and 0.29 cm (β: −0.29 cm, 95% CI: −0.44, −0.13 cm) shorter crown-to-heel length. Associations with ponderal index were null for all mixtures under consideration. All mixtures showed weak associations with SGA using weighted quantile sum regression. For the mixture of all three classes combined, one-unit higher of the weighted quantile sum index (representing a one-decile increase in chemical concentrations) was associated with 21% higher odds of SGA (odds ratio: 1.21; 95% CI: 0.88, 1.66) (eTable 3).

Bayesian Kernel Machine Regression

Bayesian kernel machine regression results for the overall effect of the mixtures on birth size outcomes are presented in eFigure 2. Associations were strongest and in the inverse direction for the overall effect of the PFAS mixture and the mixture of all three classes combined on birth weight and crown-to-heel length. We observed some weak inverse associations for the PCB mixture on birth weight and crown-to-heel length. Associations of all mixtures with head circumference and SGA were weak, while associations with ponderal index were null.

For the 31-chemical mixture of all three classes combined, we estimated a weak overall mixture effect using Bayesian kernel machine regression, with higher exposure to the mixture associated with lower birth weight (eFigure 2). Holding all 31 endocrine-disrupting chemicals in the mixture at the 75th percentile compared to the 50th percentile was associated with 0.13 lower birth weight z-score (estimate: −0.13, 95% credible interval: −0.34, 0.07), which translates to 61 g lower birth weight (estimate: −61, 95% credible interval: −156, 33). PFAS had the highest PIP (0.76), making it the most important group in

the mixture (eTable 4). The independent chemical associations all appear relatively linear (eFigure 3A) and we observed no interaction among mixture members (eFigure 3B).

Sensitivity analyses

We conducted a sensitivity analysis to explore differences in birth weight among those with detectable versus those with non-detectable concentrations (coded as a dichotomous variable) (eTable 5). We found that for FOSA, infants born to mothers with detectable concentrations were 120 g smaller (β: −120 g, 95% CI: −218, −23 g) than those with non-detectable concentrations. We conducted another sensitivity analysis to examine the effect of the timing biological sample collection during pregnancy on the association of persistent endocrine-disrupting chemicals with birth size. We observed no differences when restricting our analyses to samples collected during the first half of pregnancy (≤20 weeks gestation) (eTable 6 and eFigure 4).

Discussion

In this study, we observed an inverse association of prenatal exposure to mixtures of PFAS, PCBs, and organochlorine pesticides with birth size among British girls using weighted quantile sum regression. We observed suggestions of an inverse association of prenatal exposure to mixtures of persistent endocrine-disrupting chemicals with birth weight and crown-to-heel length using Bayesian kernel machine regression; associations were strongest for the PFAS mixture. Taken together, these results support previous findings under the single-chemical paradigm: higher prenatal concentrations of persistent endocrine-disrupting chemicals are associated with small decreases in birth weight and crown-to-heel length.

Comparing our results across single-chemical linear, weighted quantile sum, and Bayesian kernel machine regression models, there are similarities and differences. First, all models suggested that higher prenatal exposure to persistent endocrine-disrupting chemicals was associated with lower birth size measures, though the strength of the association varied by model. Generally speaking, associations were stronger in weighted quantile sum regression than in Bayesian kernel machine regression. Weighted quantile sum regression assumes that all associations are in the same direction (in this case, negative); if this assumption is not met, results can be biased away from the null.[45] While we saw inverse associations in all but one single-chemical model (PCB172), the potential for bias could explain the differences in magnitude between weighted quantile sum regression and Bayesian kernel machine regression. Second, we frequently identified PFAS chemicals as the most important class of chemicals. Across models, PFOA, PFHxS, MeFOSAA, and EtFOSAA were often the top contributors to the outcome. Third, across mixture methods, we saw that birth weight and crown-to-heel length were more strongly inversely associated with prenatal exposure to mixtures of endocrine-disrupting chemicals than other outcomes.

Previous studies of prenatal exposure to mixtures of persistent endocrine disruptors have used different methods with differing goals. One study used elastic net regression to identify the chemicals that contribute the most to the outcome from a correlated mixture of chemicals, and identified PFOA and p,p’-DDE as being inversely associated with birth weight.[23] While not the primary goal, weighted quantile sum and Bayesian kernel machine

regression can also identify important chemical contributors within a mixture. In our birth weight analyses using these regression models (our study examined a different mixture of chemicals than Lenters et al., 2016),[23] PFOA was one of the most important contributors to the overall mixture effect using both methods, but p,p'-DDE was not identified as an important contributor. Another previously published study of prenatal concentrations of endocrine-disrupting mixtures and birth weight used Bayesian hierarchical linear models, which takes a priori defined groups and estimates their overall effect on the outcome. Woods et al. reported PFAS, PCBs, and organochlorine pesticides had null or small associations with birth weight; PFAS were most strongly associated with lower birth weight.[22] While our study found differences of a larger effect size, the findings of Woods et al. are in line with what we found in this study in terms of the most important class, PFAS. Last, a study employing Bayesian kernel machine regression found that prenatal exposure to a mixture of organochlorine pesticides was inversely associated with most fetal growth measures, including head circumference.[24] In our analyses, birth weight, head circumference, and crown-to-heel length associations were in the inverse direction, though associations were comparatively the weakest for the mixture of four organochlorine pesticides in both weighted quantile sum and Bayesian kernel machine regression (compared to PFAS and PCB mixtures). Overall, the maternal serum concentrations seen in our study of British mothers from 1991–1992 tended to be higher than the concentrations seen in Ohio mothers from 2003–2006[22] and American mothers from 2009–2013,[24] and similar to concentrations among mothers from Greenland, Poland, and Ukraine in 2002–2004.[23] These varied concentrations, alongside the use of different mixture methods with differing goals, could explain some differences observed in these studies of prenatal exposure to mixtures of endocrine-disrupting chemicals with birth size.

Birth size is an important predictor of future health. Low birth weight infants face more immediate health problems than their normal weight counterparts and may be more likely to develop certain health conditions later in life such as intellectual and developmental delays, obesity, diabetes, and heart disease.[46] Any progress that could be made in reducing the incidence of low birth weight, such as through the reduction of exposure to endocrine disruptors, would have a profound public health impact. Analyzing endocrine-disrupting chemicals as mixtures will aid in meeting this goal: here, we gain a clearer picture of the cumulative estimated effect of prenatal exposure to persistent endocrine disruptors on birth size and we identify the PFAS class as the most important contributor to the association. Such results could help guide public health efforts by quantifying the risk of disease from cumulative chemical exposure to identify exposures that may be amenable to public health interventions.[47] Further, analyzing chemicals as mixtures instead of as single chemicals allows us to determine whether public health strategies to reduce chemical exposures should target the entire mixture or simply components of it.[48]

This study has several strengths, including its prospective study design within a population-based birth cohort with frequent and thorough longitudinal data collection. Additionally, we have reliable biomonitoring measurements of more than 50 persistent endocrine disruptors, a number of outcomes measured by health professionals at birth, and extensive covariate data available for mothers and daughters. Lastly, we were able to compare results across two complementary mixture methods.

This study also has limitations. We only examined mother–daughter dyads in this sub-study that was originally intended to investigate early menarche. There is some evidence to suggest that associations of endocrine-disrupting chemicals and birth size are modified by infant sex,[49,50] so restricting to daughters may be a prudent choice. Additionally, we were unable to weight Bayesian kernel machine regression analyses of this nested case–control study back to the full cohort, which limits generalizability. Nevertheless, results of the unweighted analyses were similar to single-chemical and weighted quantile sum regression results. Further, while we have detailed covariate data, there is always the possibility that we were not able to completely control for confounding by certain sensitive or self-reported variables, such as smoking, alcohol use, and socioeconomic status. Approaches to mixture analyses that involve regressing the outcome on several correlated exposures simultaneously can in some cases amplify rather than reduce confounding bias ("coexposure amplification bias"), particularly in cases of residual confounding.[51] As discussed previously, there is the potential for bias away from the null in weighted quantile sum regression models due to the assumption that associations of all mixture components are in the same direction[45] (here, negative), which is why we also used Bayesian kernel machine regression for all mixture analyses. Further, due to the large number of variables used in mixture analyses, we were missing data on roughly 30% of the subsample (eFigure 5). We compared mother–daughter characteristics for those with complete data included in mixture analyses (n=313) to those in the nested case–control study (n=448) and to the population from which the case–control study was drawn (n=3338) (eTable 7). Characteristics were similar across subsets, though low birth weight infants were slightly underrepresented in the subset with complete data. Finally, there is the possibility of reverse causality and confounding because the outcome of interest, birth size, may affect the measured biomarker concentrations and there may be shared determinants, such as hemodynamics, of the biomarker and pregnancy outcome.[52] Studies have demonstrated that reverse causality and confounding are less of a concern when the range of concentrations is wide and when blood samples are collected early in pregnancy.[12,53] In our study, 66% of samples were collected in the first half of pregnancy (≤20 weeks gestation) and we adjusted for gestational age (in weeks) of biological sample collection. Sensitivity analyses showed little to no difference when restricting to mother–daughter dyads with biological samples collected in the first half of pregnancy (eTable 6 and eFigure 4).

In conclusion, we found inverse associations between prenatal concentrations of mixtures of persistent endocrine-disrupting chemicals and birth size, namely birth weight and crown-to-heel length. While this study reaches a similar conclusion as previous studies published on this topic under the single-chemical paradigm, it fills a gap relating to mixtures of endocrine-disrupting chemicals and birth size and comes closer to replicating the human experience.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, and nurses.

Sources of financial support:

The UK Medical Research Council and the Wellcome Trust (Grant ref: 102215/2/13/2) and the University of Bristol provide core support for ALSPAC. A comprehensive list of grant funding is available on the ALSPAC website. This work was specifically funded by the Centers for Disease Control and Prevention (AY5350).

References

1. National Institute of Environmental Health Sciences. Endocrine Disruptors. https://www.niehs.nih.gov/health/topics/agents/endocrine/index.cfm Accessed November 25, 2019.
2. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Polychlorinated Biphenyls (PCBs). https://www.atsdr.cdc.gov/toxprofiles/tp17.pdf.
3. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for DDT, DDE, and DDD. In: Department of Health and Human Services PHS, ed. Atlanta, GA, 2002.
4. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for Perfluoroalkyls. (Draft for Public Comment). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, 2009.
5. Schoeters G, Govarts E, Bruckers L, et al. Three cycles of human biomonitoring in Flanders - Time trends observed in the Flemish Environment and Health Study. Int J Hyg Environ Health 2017;220(2 Pt A):36–45. [PubMed: 28160993]
6. Centers for Disease Control and Prevention, National Center for Health Statistics (CDC). National Report on Human Exposure to Environmental Chemicals. Atlanta, GA: Department of Health and Human Services, 2017.
7. Inoue K, Okada F, Ito R, et al. Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of PFOS exposure in a susceptible population during pregnancy. Environ Health Perspect 2004;112(11):1204–7. [PubMed: 15289168]
8. Kezios KL, Liu X, Cirillio PM, et al. Prenatal polychlorinated biphenyl exposure is associated with decreased gestational length but not birth weight: archived samples from the Child Health and Development Studies pregnancy cohort. Environ Health 2012;11:49. [PubMed: 22817616]
9. Jacobson JL, Fein GG, Jacobson SW, Schwartz PM, Dowler JK. The transfer of polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) across the human placenta and into maternal milk. Am J Public Health 1984;74(4):378–9. [PubMed: 6322600]
10. Sala M, Ribas-Fito N, Cardo E, et al. Levels of hexachlorobenzene and other organochlorine compounds in cord blood: exposure across placenta. Chemosphere 2001;43(4-7):895–901. [PubMed: 11372882]
11. Barker DJ. The developmental origins of chronic adult disease. Acta Paediatr Suppl 2004;93(446):26–33.
12. Sagiv SK, Rifas-Shiman SL, Fleisch AF, et al. Early-Pregnancy Plasma Concentrations of Perfluoroalkyl Substances and Birth Outcomes in Project Viva: Confounded by Pregnancy Hemodynamics? Am J Epidemiol 2018;187(4):793–802. [PubMed: 29155920]
13. Maisonet M, Terrell ML, McGeehin MA, et al. Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls. Environmental health perspectives 2012;120(10):1432. [PubMed: 22935244]
14. Johnson PI, Sutton P, Atchley DS, et al. The Navigation Guide - evidence-based medicine meets environmental health: systematic review of human evidence for PFOA effects on fetal growth. Environ Health Perspect 2014;122(10):1028–39. [PubMed: 24968388]
15. Govarts E, Nieuwenhuijsen M, Schoeters G, et al. Birth weight and prenatal exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE): a meta-analysis

within 12 European Birth Cohorts. Environ Health Perspect 2012;120(2):162–70. [PubMed: 21997443]

16. Whitworth KW, Haug LS, Baird DD, et al. Perfluorinated compounds in relation to birth weight in the Norwegian Mother and Child Cohort Study. Am J Epidemiol 2012;175(12):1209–16. [PubMed: 22517810]
17. Longnecker MP, Klebanoff MA, Zhou H, Brock JW. Association between maternal serum concentration of the DDT metabolite DDE and preterm and small-for-gestational-age babies at birth. Lancet 2001;358(9276):110–4. [PubMed: 11463412]
18. Longnecker MP, Klebanoff MA, Brock JW, Guo X. Maternal levels of polychlorinated biphenyls in relation to preterm and small-for-gestational-age birth. Epidemiology 2005;16(5):641–7. [PubMed: 16135940]
19. Bjerregaard P, Hansen JC. Organochlorines and heavy metals in pregnant women from the Disko Bay area in Greenland. Sci Total Environ 2000;245(1-3):195–202. [PubMed: 10682367]
20. National Institute of Environmental Health Sciences. 2018-2023 Strategic Plan: Advancing Environmental Health Science, Improving Health 2.0. In: Department of Health and Human Services, ed. Durham, NC 2018.
21. National Institute of Environmental Health Sciences. Powering Research through Innovative Methods for mixtures in Epidemiology (PRIME) (R01) RFA-ES-17-001. https://grants.nih.gov/grants/guide/rfa-files/RFA-ES-17-001.html.
22. Woods MM, Lanphear BP, Braun JM, McCandless LC. Gestational exposure to endocrine disrupting chemicals in relation to infant birth weight: a Bayesian analysis of the HOME Study. Environmental Health 2017;16(1):115. [PubMed: 29078782]
23. Lenters V, Portengen L, Rignell-Hydbom A, et al. Prenatal Phthalate, Perfluoroalkyl Acid, and Organochlorine Exposures and Term Birth Weight in Three Birth Cohorts: Multi-Pollutant Models Based on Elastic Net Regression. Environ Health Perspect 2016;124(3):365–72. [PubMed: 26115335]
24. Ouidir M, Buck Louis GM, Kanner J, et al. Association of Maternal Exposure to Persistent Organic Pollutants in Early Pregnancy With Fetal Growth. JAMA Pediatr 2020;174(2):149–161. [PubMed: 31886849]
25. Boyd A, Golding J, Macleod J, et al. Cohort Profile: the 'children of the 90s'--the index offspring of the Avon Longitudinal Study of Parents and Children. Int J Epidemiol 2013;42(1):111–27. [PubMed: 22507743]
26. Fraser A, Macdonald-Wallis C, Tilling K, et al. Cohort Profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. Int J Epidemiol 2013;42(1):97–110. [PubMed: 22507742]
27. Christensen KY, Maisonet M, Rubin C, et al. Exposure to polyfluoroalkyl chemicals during pregnancy is not associated with offspring age at menarche in a contemporary British cohort. Environment international 2011;37(1):129–135. [PubMed: 20843552]
28. Kuklenyik Z, Needham LL, Calafat AM. Measurement of 18 perfluorinated organic acids and amides in human serum using on-line solid-phase extraction. Anal Chem 2005;77(18):6085–91. [PubMed: 16159145]
29. Sjödin A, Jones RS, Lapeza CR, et al. Semiautomated high-throughput extraction and cleanup method for the measurement of polybrominated diphenyl ethers, polybrominated biphenyls, and polychlorinated biphenyls in human serum. Analytical chemistry 2004;76(7):1921–1927. [PubMed: 15053652]
30. Dunger DB, Ong KK, Huxtable SJ, et al. Association of the INS VNTR with size at birth. ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. Nat Genet 1998;19(1):98–100. [PubMed: 9590300]
31. Taylor CM, Golding J, Emond AM. Blood mercury levels and fish consumption in pregnancy: Risks and benefits for birth outcomes in a prospective observational birth cohort. Int J Hyg Environ Health 2016;219(6):513–20. [PubMed: 27252152]
32. Puccio G, Giuffré M, Piccione M, et al. Intrauterine growth restriction and congenital malformations: a retrospective epidemiological study. Ital J Pediatr 2013;39:23. [PubMed: 23578323]

33. Cole TJ, Freeman JV, Preece MA. British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. Statistics in medicine 1998;17(4):407–429. [PubMed: 9496720]
34. Coull B, Bobb J, Wellenius G, et al. Part 1. Statistical Learning Methods for the Effects of Multiple Air Pollution Constituents. Research report (Health Effects Institute) 2015(183 Pt 1-2):5–50. [PubMed: 26333238]
35. Bobb JF, Valeri L, Claus Henn B, et al. Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. Biostatistics 2015;16(3):493–508. [PubMed: 25532525]
36. Bobb JF, Claus Henn B, Valeri L, Coull BA. Statistical software for analyzing the health effects of multiple concurrent exposures via Bayesian kernel machine regression. Environ Health 2018;17(1):67. [PubMed: 30126431]
37. Renzetti S, Curtin P, Just AC, Bello G, Gennings C. gWQS: An R Package for Linear and Generalized Weighted Quantile Sum (WQS) Regression. https://cran.r-project.org/web/packages/gWQS/vignettes/gwqs-vignette.html.
38. Carrico C, Gennings C, Wheeler DC, Factor-Litvak P. Characterization of Weighted Quantile Sum Regression for Highly Correlated Data in a Risk Analysis Setting. J Agric Biol Environ Stat 2015;20(1):100–120. [PubMed: 30505142]
39. Christensen KLY, Carrico CK, Sanyal AJ, Gennings C. Multiple classes of environmental chemicals are associated with liver disease: NHANES 2003-2004. Int J Hyg Environ Health 2013;216(6):703–9. [PubMed: 23491026]
40. Czarnota J, Gennings C, Colt JS, et al. Analysis of Environmental Chemical Mixtures and Non-Hodgkin Lymphoma Risk in the NCI-SEER NHL Study. Environ Health Perspect 2015;123(10):965–70. [PubMed: 25748701]
41. Gennings C, Sabo R, Carney E. Identifying subsets of complex mixtures most associated with complex diseases: polychlorinated biphenyls and endometriosis as a case study. Epidemiology 2010;21 Suppl 4:S77–84. [PubMed: 21422968]
42. Zilversmit Pao L, Harville EW, Wickliffe JK, Shankar A, Buekens P. The Cumulative Risk of Chemical and Nonchemical Exposures on Birth Outcomes in Healthy Women: The Fetal Growth Study. Int J Environ Res Public Health 2019;16(19).
43. Tanner EM, Bornehag CG, Gennings C. Repeated holdout validation for weighted quantile sum regression. MethodsX 2019;6:2855–2860. [PubMed: 31871919]
44. Gibson EA, Nunez Y, Abuawad A, et al. An overview of methods to address distinct research questions on environmental mixtures: an application to persistent organic pollutants and leukocyte telomere length. Environ Health 2019;18(1):76. [PubMed: 31462251]
45. Keil AP, Buckley JP, O'Brien KM, et al. A Quantile-Based g-Computation Approach to Addressing the Effects of Exposure Mixtures. Environ Health Perspect 2020;128(4):47004. [PubMed: 32255670]
46. March of Dimes. Low Birthweight. https://www.marchofdimes.org/complications/low-birthweight.aspx# Accessed 15 May 2020.
47. Braun JM, Gennings C, Hauser R, Webster TF. What Can Epidemiological Studies Tell Us about the Impact of Chemical Mixtures on Human Health? Environ Health Perspect 2016;124(1):A6–9. [PubMed: 26720830]
48. Billionnet C, Sherrill D, Annesi-Maesano I. Estimating the health effects of exposure to multi-pollutant mixture. Ann Epidemiol 2012;22(2):126–41. [PubMed: 22226033]
49. Lind DV, Priskorn L, Lassen TH, et al. Prenatal exposure to perfluoroalkyl substances and anogenital distance at 3 months of age in a Danish mother-child cohort. Reproductive Toxicology 2017;68:200–206. [PubMed: 27592399]
50. Andersen CS, Fei C, Gamborg M, et al. Prenatal exposures to perfluorinated chemicals and anthropometric measures in infancy. American journal of epidemiology 2010;172(11):1230–1237. [PubMed: 20940176]
51. Weisskopf MG, Seals RM, Webster TF. Bias Amplification in Epidemiologic Analysis of Exposure to Mixtures. Environ Health Perspect 2018;126(4):047003. [PubMed: 29624292]
52. Savitz DA. Invited commentary: interpreting associations between exposure biomarkers and pregnancy outcome. Am J Epidemiol 2014;179(5):545–7. [PubMed: 24401560]

53. Steenland K, Barry V, Savitz D. Serum Perfluorooctanoic Acid and Birthweight: An Updated Meta-analysis With Bias Analysis. Epidemiology 2018;29(6):765–776. [PubMed: 30063543]

Table 1.

Persistent endocrine-disrupting chemicals quantified in maternal serum in the ALSPAC nested case–control study.

Chemical Name	Abbreviated Name
Per- and polyfluoroalkyl substances	
Perfluorooctane sulfonamide	FOSA
2-(N-ethylperfluorooctanesulfonamido) acetate	EtFOSAA
2-(N-methyl-perfluorooctanesulfonamido) acetate	MeFOSAA
Perfluorohexane sulfonate	PFHxS
Perfluorooctane sulfonate	PFOS
Perfluorooctanoate	PFOA
Perfluorononanoate	PFNA
Perfluorodecanoate	PFDA
Polychlorinated Biphenyls	
2,4,4′-trichlorobiphenyl	PCB28
2,2′,3,5′-tetrachlorobiphenyl	PCB44
2,2′,4,5′-tetrachlorobiphenyl	PCB49
2,2′,5,5′-tetrachlorobiphenyl	PCB52
2,3′,4,4′-tetrachlorobiphenyl	PCB66
2,4,4′,5-tetrachlorobiphenyl	PCB74
2,2′,3,4,5′-pentachlorobiphenyl	PCB87
2,2′,4,4′,5-pentachlorobiphenyl	PCB99
2,2′,4,5,5′-pentachlorobiphenyl	PCB101
2,3,3′,4,4′-pentachlorobiphenyl	PCB105
2,3,3′,4′,6-pentachlorobiphenyl	PCB110
2,3′,4,4′,5-pentachlorobiphenyl	PCB118
2,2′,3,3′,4,4′-hexachlorobiphenyl	PCB128
2,2′,3,4,4′,5′-hexachlorobiphenyl and 2,3,3′,4,4′,6-hexachlorobiphenyl	PCB138-158
2,2′,3,4′,5,5′-hexachlorobiphenyl	PCB146
2,2′,3,4′,5′,6-hexachlorobiphenyl	PCB149
2,2′,3,5,5′,6-hexachlorobiphenyl	PCB151
2,2′,4,4′,5,5′-hexachlorobiphenyl	PCB153
2,3,3′,4,4′,5-hexachlorobiphenyl	PCB156
2,3,3′,4,4′,5′-hexachlorobiphenyl	PCB157
2,3′,4,4′,5,5′-hexachlorobiphenyl	PCB167
2,2′,3,3′,4,4′,5-heptachlorobiphenyl	PCB170
2,2′,3,3′,4,5,5′-heptachlorobiphenyl	PCB172
2,2′,3,3′,4′,5,6-heptachlorobiphenyl	PCB177
2,2′,3,3′,5,5′,6-heptachlorobiphenyl	PCB178
2,2′,3,4,4′,5,5′-heptachlorobiphenyl	PCB180
2,2′,3,4,4′,5′,6-heptachlorobiphenyl	PCB183

Chemical Name	Abbreviated Name
2,2′,3,4′,5,5′,6-heptachlorobiphenyl	PCB187
2,3,3′,4,4′,5,5′-heptachlorobiphenyl	PCB189
2,2′,3,3′,4,4′,5,5′-octachlorobiphenyl	PCB194
2,2′,3,3′,4,4′,5,6-octachlorobiphenyl	PCB195
2,2′,3,3′,4,4′,5′,6-octachlorobiphenyl and 2,2′,3,4,4′,5,5′,6-octachlorobiphenyl	PCB196-203
2,2′,3,3′,4,5,6,6′-octachlorobiphenyl	PCB199
2,2′,3,3′,4,4′,5,5′,6-nonachlorobiphenyl	PCB206
Decachlorobiphenyl	PCB209
Organochlorine Pesticides	
Hexachlorobenzene	HCB
β-Hexachlorocyclohexane	β-HCH
ϒ-Hexachlorocyclohexane (Lindane)	γ-HCH
Oxychlordane	Oxychlordane
Trans-Nonachlor	Trans-nonachlor
2,2-Bis(4-chlorophenyl)-1,1-dichloroethene	p,p’-DDE
2-(4-chlorophenyl)-2-(2-chlorophenyl)-1,1,1-trichloroethan	o,p’-DDT
2,2-Bis(4-chlorophenyl)-1,1,1-trichloroethan	p,p’-DDT
Mirex	Mirex

Table 2.

Characteristics of the Avon Longitudinal Study of Parents and Children (ALSPAC) sub-study population (N=448 mother-daughter dyads) by select persistent endocrine-disrupting chemicals.

		Birth weight (g)	PFOA (ng/mL)	PCB153 (ng/g lipid)	p,p'-DDE (ng/g lipid)
Characteristic	**n (%)**[a]	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**
Maternal race					
White	423 (98)	3420 (3140–3700)	3.8 (2.9–4.8)	64.8 (48.6–85.8)	308 (193–490)
Non-white	8 (2)	2985 (2530–3740)	2.3 (1.6–2.9)	67.7 (47.4–95.1)	620 (363–1635)
Maternal education[b]					
<O-level	75 (18)	3490 (3180–3650)	3.6 (2.8–4.5)	59.7 (45.5–78.6)	298 (184–472)
O-level	140 (34)	3420 (3100–3680)	3.7 (2.9–5.0)	55.9 (44.2–72.3)	257 (166–460)
>O-level	200 (48)	3400 (3140–3720)	3.9 (2.8–4.8)	74.4 (57.8–95.6)	384 (227–536)
Maternal pre-pregnancy BMI					
<25 kg/m^2 (under/normal weight)	313 (78)	3400 (3100–3620)	3.8 (2.8–4.8)	69.0 (51.2–88.1)	329 (194–513)
≥25 kg/m^2 (overweight/obese)	89 (22)	3560 (3200–3870)	3.7 (3.0–4.8)	57.2 (44.0–77.6)	306 (211–541)
Prenatal smoking					
Any	79 (19)	3300 (2880–3560)	3.4 (2.9–4.4)	59.8 (46.0–74.3)	283 (170–412)
None	348 (81)	3460 (3180–3740)	3.8 (2.8–4.9)	65.7 (48.9–87.5)	323 (200–504)
Prenatal alcohol use					
Any	215 (51)	3420 (3140–3680)	3.7 (2.8–4.6)	71.5 (49.6–94.3)	352 (218–549)
None	208 (49)	3400 (3080–3720)	3.8 (2.9–4.9)	60.5 (46.4–80.8)	278 (176–469)
Physical activity					
Any	252 (65)	3420 (3120–3740)	3.8 (2.9–5.0)	65.2 (49.9–88.4)	322 (203–504)
None	133 (35)	3390 (3100–3625)	3.7 (2.9–4.7)	66.3 (46.1–85.2)	316 (187–533)
Maternal age at delivery					
<25 years	92 (21)	3360 (3070–3605)	3.9 (3.0–4.8)	44.2 (35.0–56.5)	178 (136–292
25–29 years	164 (37)	3460 (3100–3760)	3.8 (3.0–4.9)	59.8 (48.1–74.1)	289 (198–422)
≥30 years	189 (42)	3420 (3140–3700)	3.6 (2.5–4.6)	81.9 (64.3–105.4)	451 (283–620)
Child birth order					
First born	208 (50)	3320 (3015–3580)	4.4 (3.4–5.4)	63.9 (46.3–84.3)	316 (198–513)

Epidemiology. Author manuscript; available in PMC 2022 July 01.

Characteristic	n (%)[a]	Birth weight (g) Median (IQR)	PFOA (ng/mL) Median (IQR)	PCB153 (ng/g lipid) Median (IQR)	p,p'-DDE (ng/g lipid) Median (IQR)
Second born or later	211 (50)	3500 (3240–3790)	3.1 (2.4–4.0)	66.9 (50.2–87.5)	323 (193–497)
Child birth weight					
<2500 g	17(4)	2220 (2020–2340)	4.1 (3.3–5.6)	74.4 (60.0–102.8)	461 (329–1390)
≥2500 g	423 (96)	3430 (3160–3720)	3.7 (2.8–4.8)	63.7 (47.5–84.5)	302 (185–487)
Preterm birth					
<37 weeks	14(3)	2421 (2100–2780)	4.7 (2.8–5.6)	69.7 (64.3–110.4)	299 (226–614)
≥37 weeks	431 (97)	3430 (3160–3720)	3.7 (2.8–4.8)	63.6 (47.9–85.4)	311 (188–494)
Ponderal index					
<2.4	47 (13)	3080 (2720–3440)	3.4 (2.9–4.6)	60.3 (44.9–87.2)	283 (165–517)
≥2.4	328 (87)	3460 (3180–3740)	3.8 (2.8–4.9)	63.7 (48.6–84.5)	311 (194–493)
Small for Gestational Age					
<10th percentile	40 (9)	2715 (2570–2920)	4.1 (3.1–4.9)	68.0 (47.6–96.6)	368 (211–513)
≥10th percentile	400 (91)	3460 (3200–3740)	3.7 (2.8–4.7)	64.0 (48.1–84.1)	299 (187–491)

Abbreviations: IQR, interquartile range; PFOA, perfluorooctanoate; PCB153, 2,2′,4,4′,5,5′-hexachlorobiphenyl; p,p'-DDE, 2,2-bis(4-chlorophenyl)-1,1-dichloroethene; g, grams; kg/m^2, kilograms per meter-squared

[a]Missing data not represented

[b]<O-level=none, Certificate of Secondary Education, and vocational education, which are equivalent to no diploma or a GED in the United States. O-levels (ordinary levels) are required and completed at the age of 16. >O-level=A-levels (advanced levels) completed at 18, which are optional, but required to get into university; and a university degree.

Table 3.

Serum concentrations of persistent endocrine-disrupting chemical exposure among mothers of the Avon Longitudinal Study of Parents and Children (ALSPAC) during pregnancy (median gestational age at sample collection: 15 weeks) (N=448 mother-daughter dyads).

	Serum concentrations			
	Q1	**Median**	**Q3**	**% <LOD**
Per- and polyfluoroalkyl substances (PFAS) (ng/mL)				
PFOA	2.8	3.7	4.8	0.0
PFOS	15.1	19.8	24.9	0.0
PFHxS	1.2	1.6	2.2	0.2
PFNA	0.41	0.49	0.66	0.2
FOSA	<LOD	0.20	0.30	30.6
MeFOSAA	0.26	0.35	0.65	14.5
EtFOSAA	0.40	0.60	0.90	2.5
PFDA	<LOD	<LOD	<LOD	97.3
Polychlorinated biphenyls (PCBs) (ng/g lipid)				
PCB28	3.5	5.3	8.3	8.7
PCB44	<LOD	1.9	4.0	30.4
PCB49	<LOD	<LOD	1.9	58.3
PCB52	<LOD	3.3	7.6	30.1
PCB66	<LOD	1.6	2.5	30.4
PCB74	8.6	11.1	15.1	0.22
PCB87	<LOD	<LOD	1.7	59.6
PCB99	7.0	9.4	12.1	0.9
PCB101	<LOD	2.2	5.4	30.4
PCB105	2.0	2.9	4.0	7.4
PCB110	<LOD	<LOD	2.8	53.6
PCB118	10.9	14.9	20.6	0.22
PCB128	<LOD	<LOD	<LOD	89.5
PCB138[a]	30.4	41.5	54.0	0.2
PCB146	4.6	6.0	8.1	2.5
PCB149	<LOD	<LOD	1.8	60.9
PCB151	<LOD	<LOD	<LOD	79.5
PCB153	48.3	64.5	85.8	0.0
PCB156	4.8	6.3	8.4	1.8
PCB157	<LOD	1.3	1.9	33.9
PCB167	<LOD	2.0	2.8	26.1
PCB170	14.5	18.9	24.9	0.0
PCB172	1.1	1.9	2.7	23.0
PCB177	2.3	3.1	4.1	8.9
PCB178	1.8	2.7	3.7	14.3
PCB180	33.5	45.2	60.1	0.0

	Serum concentrations			
	Q1	**Median**	**Q3**	**% <LOD**
PCB183	4.6	6.2	8.2	3.4
PCB187	8.6	11.3	15.2	1.1
PCB189	<LOD	<LOD	0.7	74.3
PCB194	5.5	7.5	10.4	3.4
PCB195	1.5	2.2	3.0	19.0
PCB196[a]	5.7	7.7	10.5	2.0
PCB199	3.9	5.5	7.8	2.7
PCB206	1.7	2.3	3.2	10.3
PCB209	<LOD	1.5	2.0	27.7
Organochlorine pesticides (ng/g lipid)				
HCB	37.9	50.2	63.4	0.0
β-HCH	34.6	47.1	62.4	1.8
γ-HCH	<LOD	<LOD	<LOD	79.0
Oxychlordane	<LOD	<LOD	4.2	71.9
Trans-nonachlor	<LOD	<LOD	4.6	67.0
p,p'-DDE	193	311	499	0.2
o,p'-DDT	<LOD	<LOD	<LOD	98.4
p,p'-DDT	7.7	11.0	16.2	11.4
Mirex	<LOD	<LOD	<LOD	99.3

Abbreviations: Q1, quartile 1; Q3, quartile 3; LOD, limit of detection; ng/mL, nanogram per milliliter; ng/g lipid, nanogram per gram lipid

[a]PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration, referred to as PCB138. Similarly, PCB congeners 196 and 203 could not be separated and were quantified as a summed concentration, referred to as PCB196

Table 4.

Adjusted[a] associations for the 31-chemical mixture[b] with accompanying weights of maternal serum concentrations of persistent endocrine-disrupting chemical (EDC) exposure with birth size measures in the Avon Longitudinal Study of Parents and Children (ALSPAC) sub-study using repeated holdout weighted quantile sum regression (N=313).

	Birth weight (g)			Head circumference (cm)			Crown-to-heel length (cm)		
	β[c]	95% CI	Weight	β[c]	95% CI	Weight	β[c]	95% CI	Weight
Overall[b]	−55	−89, −22		−0.11	−0.21, −0.01		−0.29	−0.44, −0.13	
PFOA			0.11[e]			0.01			0.11[e]
PFOS			0.04[e]			0.01			0.06[e]
PFHxS			0.04[e]			0.08[e]			0.07[e]
PFNA			0.04[e]			0.03			0.03
MeFOSAA			0.10[e]			0.14[e]			0.13[e]
EtFOSAA			0.16[e]			0.13[e]			0.17[e]
PCB28			0.02			0.08[e]			0.02
PCB74			0.01			0.03			0.01
PCB99			0.01			0.02			0.01
PCB105			0.01			0.01			0.00
PCB118			0.01			0.01			0.00
PCB138[d]			0.01			0.01			0.00
PCB146			0.04[e]			0.02			0.05[e]
PCB153			0.03			0.03			0.01
PCB156			0.03[e]			0.01			0.01
PCB170			0.02			0.02			0.01
PCB172			0.04[e]			0.05[e]			0.04[e]
PCB177			0.03			0.02			0.02
PCB178			0.02			0.05[e]			0.01

	Birth weight (g)			Head circumference (cm)			Crown-to-heel length (cm)		
	β[c]	95% CI	Weight	β[c]	95% CI	Weight	β[c]	95% CI	Weight
PCB180			0.04[e]			0.03			0.02
PCB183			0.01			0.01			0.01
PCB187			0.01			0.01			0.01
PCB194			0.02			0.02			0.04[e]
PCB195			0.03			0.02			0.04[e]
PCB196[d]			0.01			0.01			0.01
PCB199			0.04[e]			0.04[e]			0.03
PCB206			0.02			0.02			0.01
HCB			0.01			0.01			0.03
β-HCH			0.02			0.05[e]			0.02
p,p'-DDE			0.01			0.02			0.01
p,p'-DDT			0.02			0.02			0.01

Abbreviations: CI, confidence interval; PFAS, per- and polyfluoroalkyl substances; PCBs, polychlorinated biphenyls

[a]Adjusted for maternal education, parity, pre-pregnancy body mass index, maternal age, prenatal smoking, and gestational week at sample collection

[b]Overall mixture includes PFAS, PCB, and organochlorine pesticide classes

[c]β for one-unit higher of the WQS index (representing a one-decile increase in chemical concentrations)

[d]PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration, referred to as PCB138. Similarly, PCB congeners 196 and 203 could not be separated and were quantified as a summed concentration, referred to as PCB196

[e]Significant contributor to the overall mixture effect (>1/number of chemicals in mixture, or 0.032 in this analysis)