Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Cayla Poteete Date

Maternal Concentrations of Perfluorinated Chemicals and Early Communication Development in British Girls

By

Cayla Poteete
Master of Science in Public Health

Environmental Health and Epidemiology

_____

Terry Hartman, PhD
Committee Chair

_____

Paige Tolbert, PhD
Committee Member

Maternal Concentrations of Perfluorinated Chemicals and Early Communication Development in British Girls

By

Cayla Poteete

B.A.
Boston University
2012

Thesis Committee Chair: Terry Hartman, Ph.D.

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science in Public Health
in Environmental Health and Epidemiology
2015

Abstract

Maternal Concentrations of Perfluorinated Chemicals and Early Communication Development in British Girls

By Cayla Poteete

Perfluorinated chemicals (PFCs) such as perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS) and perfluorononanoate (PFNA), are synthetic compounds designed for a wide range of industrial and consumer applications. Despite the phase-out of PFOS, PFOA and related PFCs, their indefinite persistence in the environment is a cause for health concerns. While animal studies suggest developmental toxicity, there is limited and inconsistent epidemiological evidence for their effects on development and more specifically, neurodevelopment, in humans.

This study utilized data from the large, well-characterized Avon Longitudinal Study of Parents and Children (ALSPAC) prospective birth cohort to further our understanding of how prenatal exposure to PFCs might affect neurodevelopment. Multiple linear regression models were constructed to assess associations between maternal serum concentrations of PFCs sampled during pregnancy and a summary total communication score and four communication sub-scores measured at 15 months of age in female offspring. Outcome assessment was based on an ALSPAC-adaptation of the MacArthur-Bates Communicative Development Inventories (MCDI).

None of the PFCs or the summary total PFC exposure were significantly associated with the total communication score ($p>0.05$). There were some significant positive associations between the PFCs and the summary total PFC exposure and the verbal comprehension, nonverbal communication, and social development sub-scores, suggesting a minimal yet beneficial effect. In addition, any self-reported maternal smoking during the first three months of pregnancy significantly and in general, positively, modified these associations. Other time frames of smoking were not investigated.

While many of the results of this study agree with findings of non-association, those that suggest positive, if minimal, associations could be due to chance or residual confounding. More research is needed to determine if there is an association between prenatal exposure to PFCs and early communication development.

Maternal Concentrations of Perfluorinated Chemicals and Early Communication Development in British Girls

By

Cayla Poteete

B.A.
Boston University
2012

Thesis Committee Chair: Terry Hartman, Ph.D.

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science in Public Health
in Environmental Health and Epidemiology
2015

Acknowledgements

I would like to thank my thesis advisor Dr. Terry Hartman for giving me this opportunity and for her guidance and patience throughout this process. I would also like to thank Dr. Paige Tolbert for allowing me to be a part of such a great program and school and for her support throughout my time at Rollins. I would also like to thank Dr. Kasia Kordas for her invaluable support and expertise on cognitive and neurobehavioral outcomes. Finally, I would like to thank Dr. Ethel Taylor and Dr. Adrienne Holmes and all at the Health Studies Branch at CDC that welcomed me into this fantastic research group and went above and beyond to support me in any way they could.

A very special thanks goes out to Dr. Sophie Godley, without whose passion I would have likely never discovered my love for public health. In addition, to one of the most inspiring and truly compassionate professors I have ever had, Dr. Fernando Ona—thank you for having a "vested interest" in my success. I promise to do everything in my power to continue your level of mentorship for future generations.

And last, but certainly not least, thank you to my parents who have supported my dreams wherever they have taken me and have been by my side, no matter the distance, every step of the way. And thank you to my close friends for always being there, near or far.

Table of Contents

Background

Perfluorinated Chemicals and Neurodevelopment

Perfluorinated compounds are organic chemicals with fluorine atoms, rather than hydrogen atoms, attached to the carbon chains (Lau 2012). Nearly all perfluorinated chemicals are synthetic compounds designed for a wide variety of industrial and consumer applications. Many are used in the production of everyday products to make them more resistant to stains, grease, and water. They are also useful in reducing friction so are often used in a variety of industries such as aerospace, automotive, building and construction, and electronics (National Institute of Environmental Health Sciences 2012). There are various classes of perfluorinated compounds, but one of the more prominently studied classes is the perfluoroalkyl acids (PFAAs) and their derivatives. There are approximately 30, but the most widely known and those that are of interest for the current study, are perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), and perfluorononanoic acid (PFNA).

Perfluorooctanoate (PFOA)

Perfluorooctanoate, also known as perfluorooctanoic acid, PFOA or simply as C8, has been around for nearly 70 years. The company 3M claims they began producing it in 1947 and DuPont began using it in the production of fluoropolymers in 1951 (3M 1995) as cited in (Buser and Morf 2009); (Buser and Morf 2009); (Emmett et al. 2006). The main use of PFOA is in the production of fluoropolymers [e.g. polytetrafluoroethylene (PTFE)] which are typically used in non-stick cookware [e.g. Teflon], electronics, textiles, wire and cables coating, and semiconductors (Buser and Morf 2009).

About 10 years after the beginning of its use, DuPont became aware of hepatotoxic effects on mice being fed PFOA (Arneson 1961) as cited in (Buser and Morf 2009); (Clapp et al. 2006) as cited in (Buser and Morf 2009). By 1976, it was found that blood serum of consumers was contaminated with PFOA, and by 1980, to be the primary organofluorine present in blood serum of fluorochemical production workers (Kennedy et al. 2004); (Ubel et al. 1980). In 1999, the United States Environmental Protection Agency (US EPA) began investigating perfluorooctane sulfonate (PFOS), a related compound (described below), and then expanded its investigation to PFOA and fluorinated telomers (Dominiak 2003). In May 2000, given the US EPA's conclusions on their global distribution and toxicity, 3M announced its intention to phase out PFOA- and PFOS-based products and did so within the following two years (3M 2008) as cited in (Buser and Morf 2009); (Buser and Morf 2009). Despite this phase-out, other companies, such as DuPont, began manufacturing them on their own (Lau 2012). This particular company is widely associated with a PFOA-contaminated community in Washington, West Virginia where a team of experts found a "probable link" between PFOA contamination from DuPont's manufacturing facility and adverse health effects, and in particular kidney and testicular cancers (Fletcher et al. 2012). The US EPA initiated the PFOA Stewardship Program with industry in 2006 with the goal of eliminating these chemicals from products and their emissions (Lau 2012).

The properties that make PFOA so useful and resistant to physical and chemical changes also make it problematic—its indefinite persistence in the environment and global distribution is especially of concern and begs the question of its effects on human health. The National Health and Nutrition Examination Survey (NHANES) conducted by

the National Center for Health Statistics has consistently found significant detection of PFOA and other PFAAs in the US population (Calafat et al. 2006); (Calafat et al. 2007a; Calafat et al. 2007b); (Kato et al. 2009); (Centers for Disease Control and Prevention 2009); (Centers for Disease Control and Prevention 2014). In addition, the levels of PFOS and PFOA appear to be higher in children than in adults, suggesting that children may be a vulnerable subpopulation for chemical exposure (Kato et al. 2009); (Lau 2012); (Zhang et al. 2010). Many studies have shown that exposure to PFAAs appears to begin early in life, as PFOS and PFOA in particular have been detected in umbilical cord and breast milk (Apelberg et al. 2007); (Fromme et al. 2010); (Inoue et al. 2004); (Kim et al. 2011); (Liu et al. 2010); (Monroy et al. 2008); (So et al. 2006); (Sundstrom et al. 2011); (Tao et al. 2008); (von Ehrenstein et al. 2009). The elimination half-life of PFOA is estimated to be 3.5 years (Olsen et al. 2007).

Lau (2012) asserts that PFAA exposure contributes to six major adverse health effects in laboratory animal studies: tumor induction, hepatotoxicity, developmental toxicity, immunotoxicity, endocrine disruption, and neuorotoxicity. However, human data presents much more varied and inconsistent conclusions. Given the numerous animal studies showing toxicity, PFAAs have garnered much attention from the field of epidemiology over the past few years. In particular, reproductive and developmental effects of PFOS and PFOA have become a main focus (Lau 2012). Considering the outcome of interest of this study, this section will focus on and present updates to a recent review of the neurodevelopmental and neurobehavorial effects of perfluorinated chemicals by Roth and Wilks (2014).

Head circumference at birth has classically been used to evaluate fetal brain development and to predict postnatal neurological outcomes and cognitive deficits (Roth and Wilks 2014). It has been proposed that the impact of neurotoxicants on the developing brain could be reflected by this measurement, though there is much hesitation to even use this as an endpoint due to numerous confounding risk factors (Apelberg et al. 2007); (Ivanovic et al. 2004); (Lagiou et al. 2005); (Leary et al. 2006); (Lindley et al. 1999); (Lindley et al. 2000); (Lunde et al. 2007); (Rushton and Ankney 1996). Apelberg et al. (2007) found PFOA to be significantly and positively associated with reduced head circumference among 293 American babies at birth, but only for vaginal deliveries after adjustment for delivery mode in the model. Fei et al. (2008b) found a non-significant positive association with PFOA and reduced head circumference among 1,399 Danish babies at birth. Chen et al. (2012), Lee et al. (2013), and Washino et al. (2009) found no association with PFOA and head circumference at birth in Taiwanese (n=429), Japanese (n=429), and Korean (n=70) birth cohorts, respectively. Chen et al. (2013) and two other questionnaire-based studies ((Fei et al. 2008a) and (Fei and Olsen 2011)) found no association with PFOA and motor function, cognition, or behavioral health in children of the same Taiwanese and Danish cohorts mentioned above at later ages. Stein et al. (2013) found no association between PFOA and cognition (IQ, reading, math skills, language, memory and learning, visual-spatial processing) or measures of attention and impulsivity among 320 6-12 year-old American children. Gump et al. (2011) found no association between PFOA and impulsivity among 83 9-11 year-old American children. Roth and Wilks (2014) concluded that the only consistent results were for PFOA in that none of the

studies they evaluated "have shown any developmental or behavioral effects on [any of] the different functional domains assessed."

Perfluorooctane Sulfonate (PFOS)

Perfluorooctane sulfonate, also known as perfluorooctanesulfonic acid, or simply PFOS has been around for a little over 50 years. The company 3M began producing PFOS in 1949, two years after it began production of PFOA (3M 1999) as cited in (Paul et al. 2009). It was the "key ingredient" in 3M's popular fabric protector Scotchgard but as mentioned above, beginning in 2000, the company began phasing out PFOA and PFOS from production and products. Due to its persistence, ability to bioaccumulate and biomagnify, affinity for long-range transport and toxicology in mammals and aquatic organisms, the Swiss government proposed that PFOS (and its salts and perfluorooctane sulfonyl fluoride (POSF)—its main feedstock) be added to Annex A (for elimination) of the Stockholm Convention on Persistent Organic Pollutants in June 2005 (Swedish Chemicals Inspectorate 2005). It was accepted to Annex B, which allows for some specific uses and exemptions, in May 2009 (Fourth Conference of the Parties 2009). These exemptions include processes for photo-imaging, coatings for semi-conductors, aviation hydraulic fluids, metal-plating, certain medical devices, fire-fighting foam, some insecticides, leather and apparel, textiles and upholstery, coatings and coating additives, etc. (Fourth Conference of the Parties 2009). Therefore, with this long list of exemptions, it is clear that this decision is more about accountability and encouraging the Parties of the Conference to phase-out and to seek safe alternatives to PFOS, its salts, and POSF when possible. Among the PFAAs, the level of PFOS is highest in the general population though there is a general trend for decline of PFAAs (with the exception of

PFNA, described in a later section) in NHANES data (Centers for Disease Control and Prevention 2009); (Centers for Disease Control and Prevention 2014). The estimated elimination half-life for PFOS is approximately a year longer, at 4.8 years, than its sister compound PFOA (Olsen et al. 2007). Also similar to PFOA, PFOS is widely regarded as toxic in animal studies but human data presents inconsistent conclusions (Lau 2012). As before, considering the outcome of interest of this study, this section will focus on and present updates to a recent review of the neurodevelopmental and neurobehavorial effects of perfluorinated chemicals by Roth and Wilks (2014).

Apelberg et al. (2007) found that PFOS was significantly and positively associated with reduced head circumference for vaginal deliveries after adjustment for delivery mode in the model (22). Fei et al. (2008b), Lee et al. (2013), and Washino et al. (2009) did not find a statistically significant association between PFOS and head circumference. Chen et al. (2012) was the only study reviewed by Roth and Wilks (2014) that found a significant positive dose-response for PFOS and reduced head circumference at birth. Chen et al. (2013) found a significant negative association between PFOS and motor coordination, particularly the gross motor domain. Fei et al. (2008a) and Fei and Olsen (2011) found no significant association between PFOS and maternal reported motor development among 1300-1400 Danish children at 6 months, 18 months, and 7 years of age. "Chen et al. (2013) found a significant negative dose-response association between exposure to PFOS and cognitive development, whereas an additional cross-sectional study by Stein and Savitz (2011) showed a similar trend for PFOS with learning problems, based on parental report of previous physician-diagnosed ADHD. A dose-response gradient was found by Chen et al. (2013) when PFOS levels

were categorized into quartiles" (Roth and Wilks 2014). However, Fei et al. (2008a) found no significant association between PFOS and maternal report of cognitive development. Unlike PFOA and the behavioral endpoints discussed in Roth and Wilks (2014), PFOS was found to have a statistically significant association with social competence and self-help skills (Chen et al. 2013) and impulsivity (Gump et al. 2011).

Perfluorohexane Sulfonate (PFHxS)

Perfluorohexane sulfonate, also known as perfluorohexane sulfonic acid, or simply PFHxS belongs to the same class of chemicals as PFOA and PFOS, and therefore shares a similar history and similar properties and applications. PFHxS is considered a "related compound" to PFOS and therefore was included in the 3M phase-out beginning in 2000. However, just as is the case for PFOA and PFOS, PFHxS is persistent and therefore can be found in the environment and general population despite this phase-out. Though it is found in lower levels in the general population than PFOS and PFOA, one unique property of PFHxS is its elimination half-life. Despite having a shorter carbon-chain, which is normally indicative of enhanced rates of elimination, PFHxS has the longest elimination half-life of the PFAAs of interest at 7.3 years (Olsen et al. 2007). Very little has been published in the literature regarding the toxicity of PFHxS specifically—though this is likely to change in the future especially given its longer elimination half-life. Viberg et al. (2013) found that exposure to PFHxS on postnatal day 10, during a vulnerable period of brain development in mice, can alter adult spontaneous behavior and cognitive function in both male and female mice and that these effects were both dose-response related and long-lasting/irreversible.

In focusing on neurodevelopment and neurobehavioral outcomes, the review by Roth and Wilks (2014) includes a study by Lee et al. (2013) that found no statistically significant association between exposure to PFHxS and head circumference at birth. Gump et al. (2011) found a significant positive association between PFHxS and impulsivity. Hoffman et al. (2010) found a statistically significant negative association with PFHxS and questionnaire-based maternal reports of ADHD. Stein and Savitz (2011) found a statistically significant negative association between PFHxS and parental or self-reported doctor-diagnosed cases of ADHD that do not use medication and a non-significant association between PFHxS and parental or self-reported doctor-diagnosed cases of ADHD that do use medication. These two studies, however, were included but not evaluated for quality by Roth and Wilks (2014) in their review primarily because both are questionnaire-based, cross-sectional studies, which did not meet their criteria and warrant caution in drawing conclusions. In addition, these studies use varying neurodevelopmental and neurobehavioral endpoints, making it difficult to come to any cohesive conclusion.

Perfluorononanoate (PFNA)

Perfluorononanoate, also known as perfluorononanoic acid or simply PFNA belongs to the same class of chemicals as PFOA, PFOS, and PFHxS and therefore shares a similar history and similar properties and applications. There is little to be added that has not already been described by the other compounds except for the fact that the levels of PFNA in NHANES data are increasing over time whereas the levels of the other PFAAs have been decreasing; in fact, the levels have nearly doubled in recent years compared to the 1999-2000 NHANES levels (Centers for Disease Control and Prevention

2014). PFNA is described as an "impurity in the process that produces PFOS[;]" therefore, it is likely that in the phase-out of PFOS in 2000-2002, PFNA was also phased-out or at least significantly reduced (Centers for Disease Control and Prevention 2013). There is little to be found regarding the toxicity of PFNA. Das et al. (2014) found that surviving mice neonates exposed to PFNA *in utero* exhibited dose-dependent delays in eye opening and onset of puberty and concluded that the developmental toxicity of PFNA in mice is comparable to that of PFOS and PFOA.

In focusing on neurodevelopment and neurobehavioral outcomes, the review by Roth and Wilks (2014) describes a study conducted by Chen et al. (2012) that found no significant association with PFNA and head circumference among 429 Taiwanese children at birth. Gump et al. (2011) found a significant positive association between PFNA and impulsivity. However, it is to be noted that these are two studies on different endpoints—conclusions cannot be drawn and make evident the limited availability of studies on these exposures and outcomes. Hoffman et al. (2010) found no significant association with PFNA and questionnaire-based maternal reports of ADHD. Stein and Savitz (2011) found no association between PFNA and parental or self-reported doctor-diagnosed cases of ADHD irrespective of medication usage. But as previously mentioned, these two studies were not evaluated for quality by Roth and Wilks (2014) primarily because both are questionnaire-based, cross-sectional studies, which did not meet their criteria and warrant caution in drawing conclusions.

Assessment of Communication Development

To assess communication development, researchers and clinicians typically use a variety of assessments that fall into three categories: structured tests, language samples, and

parent report (Fenson 2007). Structured tests elicit responses from children to assess their communication skills and have been found reliable and valid; however, structured tests are somewhat limited as a child can be scared or intimidated by strangers (e.g. the test administrators) and can have cognitive and emotional states that vary throughout the day (Fenson 2007). Language sampling is similar in that children are prompted to talk in a usual manner and the researcher is trained to follow the child's lead in conversation. Language sampling is training-intensive and resource-intensive which usually results in small samples with limited power. Parent reports started with diaries from parent-scientists that were trained to observe language and behavior and could be used to follow short-term progress in early communication development. Using this idea, modern-day parent-report instruments have been developed in a user-friendly format. Fenson (2007) discusses the various types of reliability and measures of validity of the parent-report-based MacArthur-Bates Communicative Development Inventories (MDCI), —while there are some particular difficulties with parent-report assessments, in general, "there is now a large body of evidence supporting the reliability, validity, clinical utility, and research potential of the MacArthur-Bates Communicative Development Inventories" (Fenson 2007). The MCDIs have been used in numerous studies looking at a wide range of research questions. For example, some have used the MCDIs to look at prenatal exposure to methylmercury from maternal fish consumption and cognitive outcomes in offspring while still others have focused more generally on a child's home environment from an early age to investigate how cognitive development progresses and differs between children (Camp et al. 2010); (Daniels et al. 2004); (Eriksson et al. 2012); (Paavola et al. 2005); (Strain et al. 2015).

Introduction

Perfluorinated compounds are organic chemicals where fluorine atoms, rather than hydrogen atoms, are attached to the carbon chains (Lau 2012). Nearly all perfluorinated chemicals are synthetic compounds designed for a wide variety of industrial and consumer applications. Many are used in the production of everyday products to make them more resistant to stains, grease, and water. They are also useful in reducing friction so are often used in a variety of industries such as aerospace, automotive, building and construction, and electronics (National Institute of Environmental Health Sciences 2012). There are several classes of perfluorinated compounds (PFCs), but one of the more commonly studied classes is the perfluoroalkyl acids (PFAAs) and their derivatives. There are approximately 30, but the most widely known and those that are of interest for the current study, are perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), and perfluorononanoate (PFNA).

In 1999, the United States Environmental Protection Agency (US EPA) began investigating PFOS as a potentially toxic compound and then expanded its investigation to PFOA and fluorinated telomers (Dominiak 2013). In May 2000, given the US EPA's conclusions on their global distribution and toxicity, the largest US-based manufacturer of these chemicals announced its intention to phase out PFOA- and PFOS-based products and did so within the following two years (3M 2008) (as cited in Buser and Morf 2009); (Buser and Morf 2009). However, the properties that make PFFAs so useful and resistant to physical and chemical changes also make them problematic—their indefinite persistence in the environment and global distribution are especially of concern and beg

the question of their effects on human health. The National Health and Nutrition Examination Survey (NHANES) conducted by the National Center for Health Statistics has consistently found detectable quantities of PFAAs in the US population (Calafat et al. 2006); (Calafat et al. 2007a; Calafat et al. 2007b); (Centers for Disease Control and Prevention 2009); (Centers for Disease Control and Prevention 2014); (Kato et al. 2009). While the levels of PFOA, PFOS, and PFHxs have been declining in recent surveys, most likely as a result of the phase-out mentioned above, the levels of PFNA have been increasing—in fact, the levels have nearly doubled in recent years compared to the 1999-2000 NHANES levels (Centers for Disease Control and Prevention 2014). Human exposure most likely occurs through consuming PFC-contaminated water or food, or by using products that contain these compounds. PFCs have been detected in human sera, breast milk, and cord blood (National Institute of Environmental Health Sciences 2012); (Apelberg et al. 2007); (Fromme et al. 2010); (Inoue et al. 2004); (Kim et al. 2011); (Liu et al. 2010); (Monroy et al. 2008); (So et al. 2006); (Sundstrom et al. 2011); (Tao et al. 2008); (von Ehrenstein et al. 2009).

In animal studies, rodent offspring exposed to PFAAs in the womb experience developmental problems. (Das et al. 2014); (Lindstrom et al. 2011); (Viberg et al. 2013); (White et al. 2011). Roth and Wilks (2014) conducted a quality-based systematic review of the epidemiological literature around neurodevelopmental and neurobehavioral effects of perfluorinated (and polybrominated) chemicals. They concluded that collectively, the epidemiological evidence does not currently support a strong causal association between PFCs and adverse neurodevelopmental and neurobehavioral outcomes in infants and children but also that many studies raise questions that warrant further investigation.

Only three of the eight prospective studies reviewed look at non-anthropometric neurodevelopmental and neurobehavioral endpoints and only one of the three were focused on infants and toddlers (Roth and Wilks 2014). Therefore, more prospective birth cohort studies are needed to determine if PFCs have meaningful associations with early communication development.

There is growing interest in studying whether intrauterine PFC exposure may deleteriously affect postnatal behavioral and cognitive outcomes. We report results from an ancillary study within the well-characterized Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort to further our understanding of prenatal exposure to PFOA, PFOS, PFHxS, and PFNA and early communication development.

Methods

Study Population

The parent study, the Avon Longitudinal Study of Parents and Children (ALSPAC), is an ongoing prospective birth cohort. The study enrolled pregnant women from three health districts in the county of Avon, Great Britain, with an expected delivery date between April 1st 1991 and December 31st 1992. The enrolled sample consisted of 14,775 live-born children from 15,247 pregnancies (Boyd et al. 2013). The ALSPAC Law and Ethics Committee, the local research ethics committees, and the U.S. Centers for Disease Control and Prevention (CDC) Institutional Review Board all assessed and approved human subject protection. Mothers provided informed consent at time of enrollment.

Data Collection

Exposure Measures

A sub-study was designed within ALSPAC to explore associations of maternal serum concentrations of PFCs and age at menarche. Girls that had at least two valid pubertal assessments were selected for analysis (Maisonet et al. 2012); (Christensen et al. 2011). This group was obtained from the larger group of 5,756 singleton females that were still active ALSPAC participants in 2004-2005. Of this group, 3,682 returned two valid pubertal assessments of pubertal status between the ages of 8 and 13 years. From this group, a sample of 448 girls was obtained for the study and included 218 girls that attained menarche before 11.5 years of age (cases) and a random sample of girls that attained menarche at or after 11.5 years of age (controls). PFOA, PFOS, PFHxS, and PFNA exposure data were available for 446 maternal serum samples.

Development Measures

The ALSPAC adaptation of the MacArthur-Bates Communicative Development Inventory (MCDI) questionnaire was completed by the mother when the child was 15 months old and returned via mail. The MacArthur-Bates Communicative Development Inventory (MCDI) is a validated parent-completed assessment used for clinical research on language and communication development (Fenson 2007). The ALSPAC adaptation created 4 derived sub-scores for different aspects of communication and then a total score based on the sum of the sub-scores. All component questions and details of scores are publicly available (Avon Longitudinal Study of Parents and Children 1996). The first sub-score is a measure for verbal comprehension. The mothers were asked if their child could understand 12 basic commands or questions such as “be quiet” and “are you

sleepy?" The child was scored on a dichotomous scale, 1 for yes and 0 for no, therefore the maximum verbal comprehension score was 12. The second sub-score was a measure of both vocabulary comprehension and production. The score was based on 134 questions and weighted. For example, if the child only understood the word, they received 1 point, if they both understood and used the word, they received 2 points, and if neither, 0 points; therefore, the maximum score was 268. The third sub-score was a measure for nonverbal communication and included questions regarding communicative gestures such as pointing or waving hello or goodbye. The child was scored based on frequency of this behavior; they received 2 points if they did the action often, 1 point if sometimes or 0 if not at all. There were 10 questions resulting in a maximum score of 20. The fourth sub-score was a measure of social development and was based on the mother's report of doing or attempting specific tasks or behaviors such as trying to comb their own hair or sniffing flowers. The child was scored based on ability or effort such that they received 2 points if the mother reported that the child engages in the activity or behavior successfully, 1 point if the child makes an effort or 0 if not at all. There were 16 questions resulting in a maximum score of 32. Therefore, based on the summation of the sub-scores, the total communication score had a maximum score of 332. The analysis was limited to those mother-daughter pairs for which all 4 PFC serum concentrations and for which all 5 ALSPAC-adapted MCDI scores were available, resulting in a final sample size of 417 dyads.

Covariates

Potential covariates and confounders to be considered in our analyses were identified a priori based on the previously published literature and biological plausibility.

This study considered the following as covariates: maternal age at delivery in years; maternal level of education (ordinally classified as lower than O level, O level, and higher than O level); maternal smoking (any/none) and maternal alcohol use during first three months of pregnancy (any/none); and duration of breastfeeding reported at 15 months (ordinally classified as never, < 3 months, 3-5 months, or 6+ months). We also considered variables describing maternal anxiety, depression, and somaticism at 8 months post-delivery measured using an adaptation of the Crown-Crisp Experimental Index (CCEI, continuous total based on validated sub-scales); parity (none/1 or more); ALSPAC adaptations of the Home Observation Measurement of Environment (HOME) Score at both 6 and 18 months (continuous); and the gestational age when the serum sample was obtained in weeks. All covariates except for maternal age at delivery and gestational age of serum sample collection had at least some missing data point; however, overall control variables were missing for less than 6% of the sample. Patterns of missingness were difficult to assess as the control variables came from different questionnaires and reports, but there appeared to be no meaningful pattern overall.

Laboratory Analyses

PFOA, PFOS, PFHxS and PFNA were measured in 446 stored maternal serum samples collected at median gestation age 15 weeks (interquartile range 10-28 weeks) in 1991-1992. The maternal serum samples were analyzed at the National Center for Environmental Health of the Centers for Disease Control and Prevention (Atlanta, GA). The analytical methods used have been described elsewhere (Kuklenyik et al. 2005).

Statistical Analyses

All data analysis was performed using SAS 9.3 (Cary, NC). Descriptive statistics for all study variables were calculated for the sample compromised of daughter-mother pairs for which exposure and outcome data were available across all PFCs and all communication scores. Correlation analysis was conducted among the PFCs and between all exposure-control variable and control variable-outcome combinations to examine bivariate associations. Multiple linear regression models were constructed for each PFC-communication score combination with the full model adjusted for all of the aforementioned control variables. Models using log-transformed exposures were compared with models using non-transformed exposures, and as there were minimal differences, the non-transformed exposures were used to facilitate interpretability. Based on hierarchical backward selection with $p < 0.05$ as the cutoff for retention, nine best predictive models were also identified for the 20 exposure-outcome relationships. The exposure was retained regardless of significance. Interaction was assessed for all exposure-covariate relationships at $\alpha=0.05$. The predictive models were then evaluated in comparison to the literature and one final best explanatory model that included either statistically significant and/or biologically important predictors was constructed. The final model included the ALSPAC-adapted HOME scores at 6 and 18 months, maternal age at delivery, maternal smoking and alcohol use during the first three months of pregnancy, parity, duration of breastfeeding at 15 months, and maternal education level. Interaction assessment was repeated for the final model again at $\alpha=0.05$. This model was also used with a standardized total PFC exposure that was obtained by subtracting the mean of each PFC from the raw PFC values and then dividing by the standard deviation

of the respective PFC. These standardized exposures were then summed to represent the total PFC exposure in an effort to estimate the overall effect of PFCs in comparison to the effect of each PFC.

Results

Among the 417 girls, median total communication (min-max) score was 132.0 (29.0-311.0) (Table 1). Among the PFCs, in univariate analyses there appeared to be mostly insignificant, and when significant, weak correlation with the MCDI sub-scores and total communication score (r <.15). PFOS had the highest median maternal serum concentration, followed by PFOA, PFHxS, and PFNA, respectively (Table 2). Spearman correlation coefficients showed a high level of correlation between PFOA and PFOS (r=0.71), moderate levels between PFOA and PFHxS (r=0.44), PFOA and PFNA (r=0.51), PFOS and PFHxS (r=0.53) and PFOS and PFNA (r=0.60) and a weak relationship between PFHxS and PFNA (r=0.26). There were significant weak-to-moderate correlations between PFOA and parity (r=-0.42, p <0.0001) and the gestational age when the serum sample was obtained (r=-0.27, p<0.0001). However, gestational age at sample collection was not significantly correlated with any of the sub-scores or total communication score and was not included in the final adjusted model.

The sample size was 367 mother-daughter pairs in the multivariate analyses due to missing data across control variables. There did not appear to be a meaningful pattern in missing data. None of the PFCs or the standardized total PFC exposure were significantly associated with the total communication score, which is the summation of the sub-scores, in either the crude or the adjusted models (Table 3). Maternal smoking during the first three months of pregnancy significantly modified the associations

between several of the PFCs and various MCDI sub-scores; therefore, the estimates are stratified on maternal smoking for all PFC-score combinations for consistency (Table 4). For example, the verbal comprehension score increased by 0.44 (p=0.0424) for each one unit increase (ng/ml) of PFOA among the daughters of mothers that reported smoking during the first three months of pregnancy compared to a 0.22 increase (p=0.0036) for the corresponding change among those of mothers that reported not smoking during this time (Table 4). PFOS was significantly associated with verbal comprehension, though had a minimal effect (a 0.03 increase for a one unit (ng/ml) increase in PFOS), and does not appear to be modified by maternal smoking. In addition, the verbal comprehension score increased by 0.99 (p=0.0203) for each unit increase (ng/ml) of PFNA (Table 4). The verbal comprehension score significantly increased by 0.11 for each unit (ng/ml) increase in the standardized total PFC exposure but only among those girls of mothers not reporting smoking during the first three months of pregnancy. There were no statistically significant associations for any of the individual analytes or the summary measure of total exposure with the vocabulary comprehension and production score (p>0.05). The nonverbal communication score increased by 0.62 for a one unit increase (ng/ml) in both PFOA and PFHxs, but only among girls of mothers that reported smoking during the first three months of pregnancy (p=0.0363, p=0.0352, respectively) (Table 4). Similarly, the nonverbal communication score increased by 0.37 for a one unit increase (ng/ml) in the standardized total PFC exposure, but only among girls of mothers that reported smoking during the first trimester (p=0.0410). Maternal smoking during the first three months of pregnancy also significantly modified associations between PFCs and the social development score; the score increased by 1.15, 4.43 and 0.74 for PFOA (p=0.0213),

PFNA (p=0.0438), and the standardized total PFC exposure (p=0.0159), respectively, but only among girls of mothers that reported smoking during the first three months of pregnancy. There were no other consistent and meaningful interactions observed.

Discussion

Multiple linear regression models were constructed to determine if there is an association between maternal serum concentrations of PFOA, PFOS, PFHxS, PFNA and a summary measure of total PFC maternal serum load with communication development in their 15-month old female offspring. After adjusting for potential confounders there were no statistically significant associations between PFOA, PFOS, PFHxS, PFNA, or total PFC maternal serum load and the total communication score. Maternal smoking appeared to be an effect modifier for the association between several PFCs and the nonverbal communication and social development scores, as well as for PFOA and total PFC maternal serum load and the verbal comprehension score. However, despite statistical significance between PFCs and the sub-scores, most of the effects appear to be small to negligible from a clinical perspective although they could be of importance at the population level. An unexpected finding is that all significant PFC-sub-score effect estimates are positive, and therefore suggest a protective or beneficial effect of prenatal exposure to PFCs and various aspects of communication development in female offspring. In addition, it appears that where maternal smoking during the first three months significantly modified the association between a PFC and sub-score, that maternal smoking also confers a protective or beneficial effect on early communication development. While we acknowledge the possibility that these compounds may have negligible effects or no effect on early communication development after being exposed

in utero, we find it unlikely that it is truly protective and that this finding is likely due to chance or some unknown and uncontrolled confounding factor. Furthermore, multiple significant associations were likely found due to the strong correlations among the PFCs.

Studies looking at these associations are limited. To our knowledge, Fei et al. (2008a) is the only other study that looks at neurodevelopmental and neurobehavioral endpoints at the same stage of development as this analysis (6-18 months) and with analytes (PFOA and PFOS) measured in maternal blood. Consistent with the findings for our overall total communication score, Fei and colleagues found no significant association between maternal blood concentrations of PFOA or PFOS with maternal reporting of cognitive and motor development among 1,400 Danish children assessed at 6 and 18 months of age (Fei et al. 2008a). The majority of other studies on this association includes a wide age range for outcome assessment and utilize a variety of study designs, but in general have found no significant associations between PFOA and motor development and cognition. In contrast, Chen et al. (2013), which looked at 239 2 year-olds, found consistent significant adverse effects of umbilical cord blood concentrations of PFOS on similar aspects of communication development, such as language and social behavior. The outcome assessment in Chen et al. (2013) was similar to ours in that it relied on a development inventory but it utilized specially trained physical therapists to score the children rather than maternal reporting. In addition, their analysis allowed for the use of cotinine measured in umbilical cord blood as an estimate of prenatal exposure to environmental tobacco smoke and also had trained professionals to assess and generate the HOME score at time of assessment. Their analysis also adjusted for family income whereas, given our source population, it was assumed that maternal education was an

adequate proxy for socioeconomic status. While the outcome assessment in our study is validated, the more direct measurements of both the outcome and covariates could provide insight to the differing conclusions of these studies.

This analysis involved a sample of mothers and their female offspring that were selected for a nested case-control study on pubertal development. This sampling scheme was not addressed in the current analysis and could introduce bias if those in the sample are not representative of the source population. In a subsequent analysis, we plan to address this issue. Median values of the ALSPAC-adapted MCDI scores and maternal characteristics of the girls included in the main analyses were similar to those enrolled in the larger cohort suggesting that selection bias is an unlikely explanation for our results.

The dichotomous variable for maternal smoking during the first three months of pregnancy, which was a significant effect modifier in our analysis, was derived from another categorical variable based on the type of product smoked (cigarette, cigar, other, or none) and may not represent maternal smoking as well as if it had been based on a measure of how often the mother reported smoking during the time frame of interest. The initial intent was to use the biomarker cotinine from the maternal serum sample but this information was missing for a large proportion of our sample.

Due to differing regulations and phase-outs of these compounds around the world, these findings may not be generalizable to other populations. In addition, due to the sampling scheme and a lack of male offspring data at the time of this analysis, the findings are limited to female offspring and may miss significant differences between sexes, though this has not been suggested in the literature. Similar to the challenge of not being able to use cotinine to control for maternal smoking, there was very limited data on

maternal serum concentrations of known neurotoxicants such lead and mercury and therefore could not be controlled in this analysis.

Though there may be residual confounding, this analysis controlled for a multitude of potential confounders. Provided there is no selection bias, the underlying large population, prospective study design adds strength to this analysis as many other studies of these associations have relied on cross-sectional designs or smaller sample sizes. Other strengths of this analysis include the quality of the exposure data given the well-characterized methodologies performed by the National Center for Environmental Health of the CDC (Kuklenyik et al. 2005).

Conclusions and Recommendations

In conclusion, we found little evidence to suggest an association between prenatal exposure to PFOA, PFOS, PFHxS, PFNA, and total PFC maternal serum load and early communication development at 15 months in British girls. There were some significant associations between PFOS and PFNA and the sub-score for verbal comprehension after adjusting for potential confounders. PFOA and the standardized total PFC maternal serum load were also associated with the verbal comprehension sub-score as well as those for nonverbal communication and social development but were significantly modified by maternal smoking during the first three months of pregnancy. Significant associations were observed between PFHxS and nonverbal communication development and between PFNA and social development but only among girls whose mothers reported smoking during the first three months of pregnancy. For all significant associations, including those among girls with mothers that smoked during the first three months of

pregnancy, the effect of exposure appears to be protective or beneficial on the specified domain of communication development, though small in magnitude.

The results of this study present many opportunities for future research. This is the first study that has shown a positive association between specific domains of communication development and prenatal exposure to PFCs. In addition, this is the first study to investigate the association between in utero exposure to PFHxS and PFNA and the communication-focused endpoints of neurodevelopment. Therefore, given the limited number of studies on the relationship of these compounds and early communication development and the lack of consistency across studies, there is a need for more studies in a variety of different populations to further explore this association. In addition, future studies would do well to control for potential confounding by direct measurements such as biomarkers rather than self-reported data when possible. A longitudinal approach using scores of communication development at multiple time points throughout the child's life could provide insight to if this association changes in magnitude or direction throughout childhood.

References

Apelberg BJ, Witter FR, Herbstman JB, Calafat AM, Halden RU, Needham LL, et al. 2007. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environmental Health Perspectives 115:1670-1676.

Avon Longitudinal Study of Parents and Children. 1996. KC Files: Data Collected from the Questionnaire 'My Infant Son/Daughter.' Available: http://www.bristol.ac.uk/alspac/external/documents/ALSPAC-documentation-21Mar2015.zip [accessed November 11 2014].

Boyd A, Golding J, Macleod J, Lawlor DA, Fraser A, Henderson J, et al. 2013. Cohort profile: The 'children of the 90s'--the index offspring of the Avon Longitudinal Study of Parents and children. International Journal of Epidemiology 42:111-127.

Buser A, Morf L. 2009. Substance flow analysis of PFOS and PFOA. Perfluorinated surfactants perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA) in Switzerland. Bern:Swiss Confederation.

Calafat AM, Kuklenyik Z, Caudill SP, Reidy JA, Needham LL. 2006. Perfluorochemicals in pooled serum samples from United Sates residents in 2001 and 2002. Environmental Science & Technology 40:2128-2134.

Calafat AM, Kuklenyik Z, Reidy JA, Caudill SP, Tully JS, Needham LL. 2007a. Serum concentrations of 11 polyfluoroalkyl compounds in the U.S. Population: Data from the National Health and Nutrition Examination Survey (NHANES). Environmental Science & Technology 41:2237-2242.

Calafat AM, Wong LY, Kuklenyik Z, Reidy JA, Needham LL. 2007b. Polyfluoroalkyl chemicals in the U.S. Population: Data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. Environmental Health Perspectives 115:1596-1602.

Camp BW, Cunningham M, Berman S. 2010. Relationship between the cognitive environment and vocabulary development during the second year of life. Archives of Pediatrics & Adolescent Medicine 164:950-956.

Centers for Disease Control and Prevention. 2009. Fourth national report on human exposure to environmental chemicals.

Centers for Disease Control and Prevention. 2013. Biomonitoring summary: Perfluorochemicals. Available: http://www.cdc.gov/biomonitoring/PFCs_BiomonitoringSummary.html [accessed April 9 2015].

Centers for Disease Control and Prevention. 2014. Fourth national report on human exposure to environmental chemicals, updated tables, August 2014.

Chen MH, Ha EH, Wen TW, Su YN, Lien GW, Chen CY, et al. 2012. Perfluorinated compounds in umbilical cord blood and adverse birth outcomes. PloS One 7:e42474.

Chen MH, Ha EH, Liao HF, Jeng SF, Su YN, Wen TW, et al. 2013. Perfluorinated compound levels in cord blood and neurodevelopment at 2 years of age. Epidemiology (Cambridge, Mass) 24:800-808.

Christensen KY, Maisonet M, Rubin C, Holmes A, Calafat AM, Kato K, et al. 2011. Exposure to polyfluoroalkyl chemicals during pregnancy is not associated with

offspring age at menarche in a contemporary British cohort. Environment International 37:129-135.

Daniels JL, Longnecker MP, Rowland AS, Golding J. 2004. Fish intake during pregnancy and early cognitive development of offspring. Epidemiology (Cambridge, Mass) 15:394-402.

Das KP, Grey BE, Rosen MB, Wood CR, Tatum-Gibbs KR, Zehr RD, et al. 2014. Developmental toxicity of perfluorononanoic acid in mice. Reproductive Toxicology (Elmsford, NY) 51c:133-144.

Dominiak MF. PFOS and PFOA program office update. In: Proceedings of the Region/ORD Emerging Pollutants Workshop, 2003. Chicago, IL, United States Environmental Protection Agency.

Emmett EA, Shofer FS, Zhang H, Freeman D, Desai C, Shaw LM. 2006. Community exposure to perfluorooctanoate: Relationships between serum concentrations and exposure sources. Journal of Occupational and Environmental Medicine / American College of Occupational and Environmental Medicine 48:759-770.

Eriksson M, Marschik PB, Tulviste T, Almgren M, Perez Pereira M, Wehberg S, et al. 2012. Differences between girls and boys in emerging language skills: Evidence from 10 language communities. The British Journal of Developmental Psychology 30:326-343.

Fei C, McLaughlin JK, Lipworth L, Olsen J. 2008a. Prenatal exposure to perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and maternally reported developmental milestones in infancy. Environmental Health Perspectives 116:1391-1395.

Fei C, McLaughlin JK, Tarone RE, Olsen J. 2008b. Fetal growth indicators and perfluorinated chemicals: A study in the Danish national birth cohort. American Journal of Epidemiology 168:66-72.

Fei C, Olsen J. 2011. Prenatal exposure to perfluorinated chemicals and behavioral or coordination problems at age 7 years. Environmental Health Perspectives 119:573-578.

Fenson L. 2007. Macarthur-Bates Communicative Development Inventories : User's guide and technical manual. 2nd ed. Baltimore, Md.:Paul H. Brookes Pub. Co.

Fletcher T, Savitz D, Steenland K. 2012. Probable link evaluation of cancer.C8 Science Panel.

Fourth Conference of the Parties (of the Stockholm Convention on Persistent Organic Pollutants). 2009. SC-4/17: Listing of perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride. Fed Reg 17.

Fromme H, Mosch C, Morovitz M, Alba-Alejandre I, Boehmer S, Kiranoglu M, et al. 2010. Pre- and postnatal exposure to perfluorinated compounds (PFCs). Environmental Science & Technology 44:7123-7129.

Gump BB, Wu Q, Dumas AK, Kannan K. 2011. Perfluorochemical (PFC) exposure in children: Associations with impaired response inhibition. Environmental Science & Technology 45:8151-8159.

Hoffman K, Webster TF, Weisskopf MG, Weinberg J, Vieira VM. 2010. Exposure to polyfluoroalkyl chemicals and attention deficit/hyperactivity disorder in U.S. Children 12-15 years of age. Environmental Health Perspectives 118:1762-1767.

Inoue K, Okada F, Ito R, Kato S, Sasaki S, Nakajima S, et al. 2004. Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: Assessment of PFOS exposure in a susceptible population during pregnancy. Environmental Health Perspectives 112:1204-1207.

Ivanovic DM, Leiva BP, Perez HT, Olivares MG, Diaz NS, Urrutia MS, et al. 2004. Head size and intelligence, learning, nutritional status and brain development. Head, IQ, learning, nutrition and brain. Neuropsychologia 42:1118-1131.

Kato K, Calafat AM, Wong LY, Wanigatunga AA, Caudill SP, Needham LL. 2009. Polyfluoroalkyl compounds in pooled sera from children participating in the National Health and Nutrition Examination Survey 2001-2002. Environmental Science & Technology 43:2641-2647.

Kennedy GL, Jr., Butenhoff JL, Olsen GW, O'Connor JC, Seacat AM, Perkins RG, et al. 2004. The toxicology of perfluorooctanoate. Critical Reviews in Toxicology 34:351-384.

Kim SK, Lee KT, Kang CS, Tao L, Kannan K, Kim KR, et al. 2011. Distribution of perfluorochemicals between sera and milk from the same mothers and implications for prenatal and postnatal exposures. Environmental Pollution (Barking, Essex : 1987) 159:169-174.

Kuklenyik Z, Needham LL, Calafat AM. 2005. Measurement of 18 perfluorinated organic acids and amides in human serum using on-line solid-phase extraction. Analytical Chemistry 77:6085-6091.

Lagiou P, Mucci L, Tamimi R, Kuper H, Lagiou A, Hsieh CC, et al. 2005. Micronutrient intake during pregnancy in relation to birth size. European Journal of Nutrition 44:52-59.

Lau C. 2012. Perfluorinated compounds. In: Molecular, clinical and environmental toxicology, Vol. 3: Environmental Toxicology, (Luch A, ed). Basel:Springer.

Leary S, Fall C, Osmond C, Lovel H, Campbell D, Eriksson J, et al. 2006. Geographical variation in relationships between parental body size and offspring phenotype at birth. Acta obstetricia et gynecologica Scandinavica 85:1066-1079.

Lee YJ, Kim MK, Bae J, Yang JH. 2013. Concentrations of perfluoroalkyl compounds in maternal and umbilical cord sera and birth outcomes in korea. Chemosphere 90:1603-1609.

Lindley AA, Benson JE, Grimes C, Cole TM, 3rd, Herman AA. 1999. The relationship in neonates between clinically measured head circumference and brain volume estimated from head CT-scans. Early Human Development 56:17-29.

Lindley AA, Becker S, Gray RH, Herman AA. 2000. Effect of continuing or stopping smoking during pregnancy on infant birth weight, crown-heel length, head circumference, ponderal index, and brain:Body weight ratio. American Journal of Epidemiology 152:219-225.

Lindstrom AB, Strynar MJ, Libelo EL. 2011. Polyfluorinated compounds: Past, present, and future. Environmental Science & Technology 45:7954-7961.

Liu J, Li J, Zhao Y, Wang Y, Zhang L, Wu Y. 2010. The occurrence of perfluorinated alkyl compounds in human milk from different regions of China. Environment International 36:433-438.

Lunde A, Melve KK, Gjessing HK, Skjaerven R, Irgens LM. 2007. Genetic and environmental influences on birth weight, birth length, head circumference, and gestational age by use of population-based parent-offspring data. American Journal of Epidemiology 165:734-741.

Maisonet M, Terrell ML, McGeehin MA, Christensen KY, Holmes A, Calafat AM, et al. 2012. Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls. Environmental Health Perspectives 120:1432-1437.

Monroy R, Morrison K, Teo K, Atkinson S, Kubwabo C, Stewart B, et al. 2008. Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. Environmental Research 108:56-62.

National Institute of Environmental Health Sciences. 2012. Perfluorinated chemicals (PFCs). Research Triangle Park, 4.

Olsen GW, Burris JM, Ehresman DJ, Froehlich JW, Seacat AM, Butenhoff JL, et al. 2007. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environmental Health Perspectives 115:1298-1305.

Paavola L, Kunnari S, Moilanen I. 2005. Maternal responsiveness and infant intentional communication: Implications for the early communicative and linguistic development. Child: Care, Health and Development 31:727-735.

Paul AG, Jones KC, Sweetman AJ. 2009. A first global production, emission, and environmental inventory for perfluorooctane sulfonate. Environmental Science & Technology 43:386-392.

Roth N, Wilks MF. 2014. Neurodevelopmental and neurobehavioural effects of polybrominated and perfluorinated chemicals: A systematic review of the epidemiological literature using a quality assessment scheme. Toxicology Letters 230:271-281.

Rushton JP, Ankney CD. 1996. Brain size and cognitive ability: Correlations with age, sex, social class, and race. Psychonomic Bulletin & Review 3:21-36.

So MK, Yamashita N, Taniyasu S, Jiang Q, Giesy JP, Chen K, et al. 2006. Health risks in infants associated with exposure to perfluorinated compounds in human breast milk from Zhoushan, China. Environmental Science & Technology 40:2924-2929.

Stein CR, Savitz DA. 2011. Serum perfluorinated compound concentration and attention deficit/hyperactivity disorder in children 5-18 years of age. Environmental Health Perspectives 119:1466-1471.

Stein CR, Savitz DA, Bellinger DC. 2013. Perfluorooctanoate and neuropsychological outcomes in children. Epidemiology (Cambridge, Mass) 24:590-599.

Strain J, Yeates AJ, van Wijngaarden E, Thurston SW, Mulhern MS, McSorley EM, et al. 2015. Prenatal exposure to methyl mercury from fish consumption and polyunsaturated fatty acids: Associations with child development at 20 mo of age in an observational study in the republic of seychelles. The American Journal of Clinical Nutrition 101:530-537.

Sundstrom M, Ehresman DJ, Bignert A, Butenhoff JL, Olsen GW, Chang SC, et al. 2011. A temporal trend study (1972-2008) of perfluorooctanesulfonate,

perfluorohexanesulfonate, and perfluorooctanoate in pooled human milk samples from Stockholm, Sweden. Environment International 37:178-183.

Swedish Chemicals Inspectorate. 2005. Proposal for listing perfluorooctane sulfonate (PFOS) in Annex A of the Stockholm Convention on Persistent Organic Pollutants.

Tao L, Ma J, Kunisue T, Libelo EL, Tanabe S, Kannan K. 2008. Perfluorinated compounds in human breast milk from several Asian countries, and in infant formula and dairy milk from the United States. Environmental Science & Technology 42:8597-8602.

Ubel FA, Sorenson SD, Roach DE. 1980. Health status of plant workers exposed to fluorochemicals--a preliminary report. American Industrial Hygiene Association Journal 41:584-589.

Viberg H, Lee I, Eriksson P. 2013. Adult dose-dependent behavioral and cognitive disturbances after a single neonatal PFHxS dose. Toxicology 304:185-191.

von Ehrenstein OS, Fenton SE, Kato K, Kuklenyik Z, Calafat AM, Hines EP. 2009. Polyfluoroalkyl chemicals in the serum and milk of breastfeeding women. Reproductive Toxicology (Elmsford, NY) 27:239-245.

Washino N, Saijo Y, Sasaki S, Kato S, Ban S, Konishi K, et al. 2009. Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. Environmental Health Perspectives 117:660-667.

White SS, Stanko JP, Kato K, Calafat AM, Hines EP, Fenton SE. 2011. Gestational and chronic low-dose pfoa exposures and mammary gland growth and differentiation

in three generations of cd-1 mice. Environmental Health Perspectives 119:1070-1076.

Zhang T, Wu Q, Sun HW, Zhang XZ, Yun SH, Kannan K. 2010. Perfluorinated compounds in whole blood samples from infants, children, and adults in China. Environmental Science & Technology 44:4341-4347.

Tables

Table 1. Characteristics of selected continuous study variables in a sample of British girls, ALSPAC.

Covariates	Median	Min-Max	n
Maternal Age at Delivery, years	29.0	(17.0-43.0)	417
CCEI Score at 8 Months[a]	8.0	(0.0-44.0)	392
Gestational age when sample was obtained, weeks	15.0	(2.0-41.0)	417
HOME Score at 6 Months	8.0	(0.0-12.0)	405
HOME Score at 18 Months	11.0	(6.0-12.0)	401
Adapted-MCDI Scores at 15 Months			
Total Communication Score	132.0	(29.0-311.0)	417
Nonverbal Communication Score	15.0	(5.0-20.0)	417
Verbal Comprehension Score	10.0	(0.0-12.0)	417
Vocabulary Comprehension and Production Score	18.0	(6.0-32.0)	417
Social Development Score	87.0	(7.0-254.0)	417

Abbreviations: max, maximum; min, minimum; CCEI, Crown-Crisp Experimental Index; HOME, Home Observation for Measurement of the Environment; MCDI, MacArthur-Bates Communicative Development Inventory

[a]CCEI is a validated self-reported measure of depression, anxiety, and somaticism

Table 2. Frequency distribution and maternal serum concentrations (in ng/ml) for selected categorical study variables in a sample of British girls, ALSPAC (n=417).

			PFOA		PFOS		PFHxS		PFNA	
	n	%	Median	(min-max)	Median	(min-max)	Median	(min-max)	Median	min-max)
Overall	417	(100.0)	3.8	(1.1-16.4)	19.8	(6.5-112.0)	1.6	(0.2-54.8)	0.6	(0.2-3.9)
Mother's highest educational qualification[a]										
< O Level	77	(19.1)	3.7	(1.3-16.4)	18.9	(8.0-94.5)	1.6	(0.4-54.8)	0.7	(0.2-2.3)
O Level	136	(33.7)	3.7	(1.6-8.6)	19.5	(8.5-112.0)	1.6	(0.5-37.3)	0.7	(0.2-2.1)
> O Level	191	(47.3)	3.9	(1.1-15.7)	20.4	(6.5-69.2)	1.7	(0.2-54.1)	0.6	(0.2-3.9)
Missing	13		3.6	(2.4-8.6)	16.5	(10.7-24.4)	1.6	(0.9-2.9)	0.6	(0.3-1.1)
Mother smoked during first 3 months of pregnancy										
Any	90	(22.3)	3.4	(1.2-7.1)	17.5	(6.5-39.6)	1.7	(0.2-9.5)	0.6	(0.2-2.3)
None	313	(77.7)	3.8	(1.1-16.4)	21.0	(7.1-94.5)	1.6	(0.4-54.8)	0.7	(0.2-3.9)
Missing	14		3.9	(1.9-15.7)	17.8	(9.2-112.0)	1.7	(0.9-37.3)	0.7	(0.3-1.2)
Mother's alcohol consumption in first 3 months of pregnancy										
Any	217	(53.9)	3.7	(1.2-14.6)	19.4	(7.6-74.2)	1.6	(0.2-54.1)	0.6	(0.2-3.9)
None	186	(46.2)	3.8	(1.1-16.4)	20.6	(6.5-94.5)	1.6	(0.5-54.8)	0.6	(0.2-2.1)
Missing	14		3.9	(1.9-15.7)	17.8	(9.2-112.0)	1.7	(0.9-37.3)	0.7	(0.3-1.2)

Abbreviations: max, maximum; min, minimum; O level, ordinary level

[a] <O = none, Certificate of Secondary Education, and vocational; >O = Advanced level and degree. U.S. equivalents of these levels are as follows: <O = none, G.E.D.; O level = High school diploma and/or associate's degree/other vocational training; >O level = bachelor's degree and higher

Table 2 (Continued). Frequency distribution and maternal serum concentrations (in ng/ml) for selected categorical study variables in a sample of British girls, ALSPAC (n=417).

			PFOA		PFOS		PFHxS		PFNA	
	n	%	Median	(min-max)	Median	(min-max)	Median	(min-max)	Median	(min-max)
Duration of Breastfeeding at 15 months										
Never	81	(19.7)	4.1	(2.1-15.7)	20.0	(8.5-39.6)	1.6	(0.4-7.1)	0.6	(0.3-1.6)
<3 Months	102	(24.8)	3.8	(1.1-8.6)	19.3	(7.1-41.2)	1.7	(0.5-9.5)	0.7	(0.2-2.3)
3-5 Months	62	(15.1)	4.0	(1.5-14.6)	19.8	(6.5-74.2)	1.5	(0.6-54.1)	0.7	(0.2-2.1)
6+ Months	166	(40.4)	3.6	(1.1-16.4)	20.4	(7.6-112.0)	1.6	(0.2-54.8)	0.6	(0.2-3.9)
Missing	6		3.5	(2.5-4.6)	20.1	(10.0-25.5)	1.3	(0.6-2.1)	1.0	(0.6-1.6)
Parity										
0	201	(50.4)	4.4	(1.8-16.4)	21.4	(8.0-94.5)	1.8	(0.4-54.8)	0.7	(0.2-3.9)
1+	198	(49.6)	3.1	(1.1-13.8)	18.3	(6.5-74.2)	1.5	(0.2-49.8)	0.6	(0.2-2.1)
Missing	18		4.1	(1.3-15.7)	19.7	(9.2-112.0)	1.7	(0.9-37.3)	0.7	(0.3-1.6)

Abbreviations: max, maximum; min, minimum; O level, ordinary level

[a] <O = none, Certificate of Secondary Education, and vocational; >O = Advanced level and degree. U.S. equivalents of these levels are as follows: <O = none, G.E.D.; O level = High school diploma and/or associate's degree/other vocational training; >O level = bachelor's degree and higher

Table 3. Association between maternal serum polyfluoroalkyl concentrations and ALSPAC-adapted MacArthur Bates Communicative Development Inventory Total Communication Score for 15 month old British girls, ALSPAC.

	Total Communication Score		
Analyte	**β**	**95% CI**	***p* value[4]**
PFOA			
Unadjusted[1]	1.87	(-0.80 , 4.54)	
Full[2]	2.11	(-0.95 , 5.16)	
Stratified[3]			
Smoker	7.58	(-1.51 , 16.67)	
Non-Smoker	1.34	(-1.84 , 4.52)	
PFOS			
Unadjusted[1]	0.07	(-0.41 , 0.56)	
Full[2]	0.27	(-0.29 , 0.82)	
Stratified[3]			
Smoker	-0.35	(-2.10 , 1.39)	
Non-Smoker	0.30	(-0.28 , 0.88)	
PFHxS			
Unadjusted[1]	0.17	(-0.80 , 1.15)	
Full[2]	0.31	(-0.68 , 1.31)	
Stratified[3]			
Smoker	-1.06	(-10.17 , 8.06)	
Non-Smoker	0.32	(-0.68 , 1.33)	
PFNA			
Unadjusted[1]	12.51	(-2.07 , 27.08)	
Full[2]	9.49	(-7.97 , 26.95)	
Stratified[3]			
Smoker	-7.89	(-48.19 , 32.40)	
Non-Smoker	11.31	(-8.49 , 31.10)	
Total PFC Exposure			
Unadjusted[1]	1.02	(-0.62 , 2.65)	
Full[2]	1.16	(-0.64 , 2.96)	
Stratified[3]			
Smoker	0.83	(-4.77 , 6.43)	
Non-Smoker	1.08	(-0.81 , 2.97)	

Abbreviations: CI, confidence interval; [1] n=417 for unadjusted estimates; [2]Model is adjusted for ALSPAC-adapted HOME Scores at 6 and 18 months (continuous), maternal age at delivery (continuous), maternal smoking and alcohol use during the first three months of pregnancy (any/none), parity (0/1+), maternal highest educational achievement (<O level/ O level/ >O level), breastfeeding duration (Never/<3 Months/3-5 Months/6+ Months); n=367; [3]Model is adjusted for ALSPAC-adapted HOME Scores at 6 and 18 months (continuous), maternal age at delivery (continuous), maternal smoking and alcohol use during the first three months of pregnancy (any/none), parity (0/1+), maternal highest educational achievement (<O level/ O level/ >O level), breastfeeding duration (Never/<3 Months/3-5 Months/6+ Months) and stratified on maternal smoking during the first three months of pregnancy; Smoker represents any smoking and Non-smoker represents no smoking; n=367; [4] *: $p < 0.05$; **: $p<0.01$

Table 4. Association between maternal serum polyfluoroalkyl concentrations and ALSPAC-adapted MacArthur Bates Communicative Development Inventory Sub-Scores for15 month old British girls, ALSPAC.

	Verbal Comprehension Score		Vocabulary Comprehension and Production Score		Nonverbal Communication Score		Social Development Score	
Analyte	**β**	***p* value[4]**	**β**	***p* value[4]**	**β**	***p* value[4]**	**β**	***p* value[4]**
PFOA								
Unadjusted[1]	0.16	*	1.56		0.10		0.05	
Full[2]	0.27	**	1.58		0.14		0.11	
Stratified[3]								
Smoker	0.44	*	5.37		0.62	*	1.15	*
Non-Smoker	0.22	**	1.04		0.08		0.00	
PFOS								
Unadjusted[1]	0.02		0.02		0.02		0.02	
Full[2]	0.03	*	0.17		0.03		0.03	
Stratified[3]								
Smoker	0.05		-0.65		0.08		0.16	
Non-Smoker	0.03		0.24		0.02		0.01	

[1] n=417 for unadjusted estimates

[2]Model is adjusted for ALSPAC-adapted HOME Scores at 6 and 18 months (continuous), maternal age at delivery (continuous), maternal smoking and alcohol use during the first three months of pregnancy (any/none), parity (0/1+), maternal highest educational achievement (<O level/ O level/ >O level), breastfeeding duration (Never/<3 Months/3-5 Months/6+ Months); n=367

[3]Model is adjusted for ALSPAC-adapted HOME Scores at 6 and 18 months (continuous), maternal age at delivery (continuous), maternal smoking and alcohol use during the first three months of pregnancy (any/none), parity (0/1+), maternal highest educational achievement (<O level/ O level/ >O level), breastfeeding duration (Never/<3 Months/3-5 Months/6+ Months) and stratified on maternal smoking during the first three months of pregnancy; Smoker represents any smoking and Non-smoker represents no smoking; n=367

[4] *: $p < 0.05$; **: $p<0.01$

Table 4 (Continued). Association between maternal serum polyfluoroalkyl concentrations and ALSPAC-adapted MacArthur Bates Communicative Development Inventory Sub-Scores for 15 month old British girls, ALSPAC.

	Verbal Comprehension Score		Vocabulary Comprehension and Production Score		Nonverbal Communication Score		Social Development Score	
Analyte	**β**	***p* value[4]**	**β**	***p* value[4]**	**β**	***p* value[4]**	**β**	***p* value[4]**
PFHxS								
Unadjusted[1]	0.02		0.10		0.01		0.04	
Full[2]	0.03		0.23		0.02		0.04	
Stratified[3]								
Smoker	0.35		-2.70		0.62	*	0.67	
Non-Smoker	0.02		0.26		0.01		0.03	
PFNA								
Unadjusted[1]	0.71	*	9.65		0.56		1.58	*
Full[2]	0.99	*	5.99		0.75		1.76	
Stratified[3]								
Smoker	0.53		-14.51		1.66		4.43	*
Non-Smoker	0.87		9.34		0.37		0.74	
Total PFC Exposure								
Unadjusted[1]	0.09	*	0.75		0.07		0.11	
Full[2]	0.13	**	0.80		0.09		0.13	
Stratified[3]								
Smoker	0.22		-0.50		0.37	*	0.74	*
Non-Smoker	0.11	*	0.86		0.06		0.06	

[1] n=417 for unadjusted estimates; [2]Model is adjusted for ALSPAC-adapted HOME Scores at 6 and 18 months (continuous), maternal age at delivery (continuous), maternal smoking and alcohol use during the first three months of pregnancy (any/none), parity (0/1+), maternal highest education(<O level/ O level/ >O level), breastfeeding duration (Never/<3 Months/3-5 Months/6+ Months); n=367; [3]Model is adjusted for ALSPAC-adapted HOME Scores at 6 and 18 months (continuous), maternal age at delivery (continuous), maternal smoking and alcohol use during the first three months of pregnancy (any/none), parity (0/1+), maternal highest educational achievement (<O level/ O level/ >O level), breastfeeding duration (Never/<3 Months/3-5 Months/6+ Months) and stratified on maternal smoking during the first three months of pregnancy; Smoker represents any smoking and Non-smoker; represents no smoking; n=367; [4] *: $p < 0.05$; **: $p<0.01$

Appendix

Table S1. Frequency distribution and adapted-MCDI Total Communication Score at 15 months post-delivery for selected categorical variables in a sample of British girls, ALSPAC (n=417).

			Total Communication Score	
	n	%	Median	(min-max)
Overall	417	(100.0)	132.0	(29.0-311.0)
Mother's highest educational qualification[a]				
< O Level	77	(19.1)	125.0	(44.0-270.0)
O Level	136	(33.7)	129.0	(32.0-311.0)
> O Level	191	(47.3)	134.0	(29.0-299.0)
Missing	13		166.0	(68.0-235.0)
Mother smoked during first 3 months of pregnancy				
Any	90	(22.3)	138.0	(32.0-311.0)
None	313	(77.7)	130.0	(29.0-293.0)
Missing	14		171.5	(68.0-277.0)
Mother's alcohol consumption in first 3 months of pregnancy				
Any	217	(53.9)	133.0	(32.0-299.0)
None	186	(46.2)	128.0	(29.0-311.0)
Missing	14		171.5	(68.0-277.0)
Duration of Breastfeeding at 15 months				
Never	81	(19.7)	132.0	(52.0-311.0)
<3 Months	102	(24.8)	135.5	(32.0-291.0)
3-5 Months	62	(15.1)	144.5	(29.0-256.0)
6+ Months	166	(40.4)	126.0	(44.0-293.0)
Missing	6		101.0	(91.0-223.0)
Parity				
0	201	(50.4)	136.0	(29.0-311.0)
1+	198	(49.6)	125.5	(38.0-293.0)
Missing	18		164.5	(68.0-277.0)

Abbreviations: max, maximum; min, minimum; MCDI, MacArthur-Bates Communicative Development Inventory

[a] <O = none, Certificate of Secondary Education, and vocational; >O = Advanced level and degree. U.S. equivalents of these levels are as follows: <O = none, G.E.D.; O level = High school diploma and/or associate's degree/other vocational training; >O level = bachelor's degree and higher

Table S2. Frequency distribution and adapted-MCDI Sub-Scores at 15 months post-delivery for selected categorical variables in a sample of British girls, ALSPAC (n=417).

			Verbal Comprehension Score		Vocabulary Comprehension and Production Score		Nonverbal Communication Score		Social Development Score	
	n	%	Median	(min-max)	Median	(min-max)	Median	(min-max)	Median	(min-max)
Overall	417	(100.0)	10.0	(0.0-12.0)	87.0	(7.0-254.0)	15.0	(5.0-20.0)	18.0	(6.0-32.0)
Mother's highest educational qualification[a]										
< O Level	77	(19.1)	10.0	(2.0-12.0)	82.0	(20.0-214.0)	16.0	(7.0-20.0)	17.0	(7.0-29.0)
O Level	136	(33.7)	10.0	(3.0-12.0)	84.5	(7.0-254.0)	15.0	(5.0-20.0)	18.0	(8.0-32.0)
> O Level	191	(47.3)	10.0	(0.0-12.0)	91.0	(10.0-239.0)	15.0	(6.0-20.0)	19.0	(6.0-32.0)
Missing	13		11.0	(2.0-12.0)	116.0	(37.0-185.0)	16.0	(11.0-20.0)	18.0	(9.0-26.0)
Mother smoked during first 3 months of pregnancy										
Any	90	(22.3)	10.0	(3.0-12.0)	94.0	(7.0-254.0)	16.0	(8.0-20.0)	19.0	(9.0-31.0)
None	313	(77.7)	10.0	(0.0-12.0)	87.0	(10.0-238.0)	15.0	(5.0-20.0)	18.0	(6.0-32.0)
Missing	14		10.0	(5.0-12.0)	118.5	(37.0-227.0)	16.5	(11.0-20.0)	20.5	(12.0-26.0)
Mother's alcohol consumption in first 3 months of pregnancy										
Any	217	(53.9)	10.0	(0.0-12.0)	87.0	(7.0-239.0)	15.0	(6.0-20.0)	19.0	(7.0-32.0)
None	186	(46.2)	10.0	(2.0-12.0)	86.5	(10.0-254.0)	15.0	(5.0-20.0)	18.0	(6.0-32.0)
Missing	14		10.0	(5.0-12.0)	118.5	(37.0-227.0)	16.5	(11.0-20.0)	20.5	(12.0-26.0)

Abbreviations: max, maximum; min, minimum; MCDI, MacArthur-Bates Communicative Development Inventory

[a] <O = none, Certificate of Secondary Education, and vocational; >O = Advanced level and degree. U.S. equivalents of these levels are as follows: <O = none, G.E.D.; O level = High school diploma and/or associate's degree/other vocational training; >O level = bachelor's degree and higher

Table S2 (Continued). Frequency distribution and adapted-MCDI Scores at 15 months post-delivery for selected categorical variables in a sample of British girls, ALSPAC (n=417).

			Verbal Comprehension Score		Vocabulary Comprehension and Production Score		Nonverbal Communication Score		Social Development Score	
	n	%	Median	(min-max)	Median	(min-max)	Median	(min-max)	Median	(min-max)
Duration of Breastfeeding at 15 months										
Never	81	(19.7)	10.0	(3.0-12.0)	84.0	(29.0-254.0)	15.0	(5.0-20.0)	18.0	(7.0-29.0)
<3 Months	102	(24.8)	10.0	(2.0-12.0)	92.0	(7.0-238.0)	15.0	(7.0-20.0)	19.0	(8.0-31.0)
3-5 Months	62	(15.1)	10.0	(2.0-12.0)	95.5	(10.0-206.0)	16.0	(7.0-20.0)	19.0	(6.0-32.0)
6+ Months	166	(40.4)	10.0	(0.0-12.0)	86.0	(18.0-235.0)	15.0	(6.0-20.0)	19.0	(7.0-32.0)
Missing	6		10.0	(8.0-12.0)	66.0	(52.0-166.0)	16.0	(10.0-20.0)	15.0	(10.0-26.0)
Parity										
0	201	(50.4)	10.0	(0.0-12.0)	94.0	(7.0-254.0)	15.0	(6.0-20.0)	18.0	(6.0-31.0)
1+	198	(49.6)	10.0	(2.0-12.0)	82.0	(10.0-235.0)	15.5	(5.0-20.0)	19.0	(7.0-32.0)
Missing	18		10.0	(5.0-12.0)	110.5	(37.0-227.0)	16.0	(10.0-20.0)	21.0	(12.0-26.0)

Abbreviations: max, maximum; min, minimum; MCDI, MacArthur-Bates Communicative Development Inventory

[a] <O = none, Certificate of Secondary Education, and vocational; >O = Advanced level and degree. U.S. equivalents of these levels are as follows: <O = none, G.E.D.; O level = High school diploma and/or associate's degree/other vocational training; >O level = bachelor's degree and higher