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Perfluoroalkyl substances measured in breast milk and child neuropsychological development in a Norwegian birth cohort study

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ABSTRACT

Perfluoroalkyl substances (PFASs) are chemicals with potential neurotoxic effects although the current evidence is still limited. This study investigated the association between perinatal exposure to perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) and neuropsychological development assessed at 6, 12 and 24 months. We measured PFOS and PFOA in breast milk samples collected one month after delivery by mothers of children participating in the HUMIS study (Norway). Cognitive and psychomotor development was measured at 6 and at 24 months using the Ages and Stages Questionnaire (ASQ-II). Behavioral development was assessed using the infant–toddler symptom checklist (ITSC) at 12 and at 24 months. Weighted logistic regression and weighted negative binomial regression models were applied to analyze the associations between PFASs and ASQ-II and ITSC, respectively. The median concentration of PFOS was 110 ng/L, while the median for PFOA was 40 ng/L. We did not detect an increased risk of having an abnormal score in ASQ-II at 6 months or 24 months. Moreover, no consistent increase in behavioral problems assessed at 12 and 24 months by ITSC questionnaire was detected. We observed no association between perinatal PFOS and PFOA exposure and early neuropsychological development. Further longitudinal studies are needed to confirm the effects of these compounds on neuropsychological development in older children.

1. Introduction

Perfluoroalkyl substances (PFASs) are a group of chemicals with surface-active properties that have been used in the industry during the last 50 years. PFASs are widely used as surfactants, emulsifiers, and in consumer products such as food packaging, nonstick pan coatings, fire extinguishers, textiles and paper (Calafat et al., 2007; Renner, 2001). Among the PFASs, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are two of the most common compounds. PFASs are persistent environmental pollutants detected in higher levels in populations in industrialized and urbanized areas (Houde et al., 2006). In contrast to the classical lipophilic persistent organic pollutants, PFASs do not typically accumulate in lipids, instead bind to serum proteins, particularly albumin (Han et al., 2003; Jones et al., 2003). Long elimination half-lives have been observed for PFOS (~5 years) and PFOA (~4 years) in humans (Olsen et al., 2007; Seals et al., 2011). The use of PFOS was restricted in 2009 under the Stockholm Convention on Persistent Organic Pollutants (http://chm.pops.int) and the US Environmental Protection Agency (US EPA) requested eight manufacturers to voluntarily eliminate their production and use of PFOA, its precursors and related chemicals (http://www.epa.gov/oppt/pfoa/pubs/stewardship/). Correspondingly, studies have reported a decrease in body burdens of PFOS and PFOA in two studies conducted in Norway and Sweden (Haug et al., 2009a,b; Sundström et al., 2011). However, exposure will continue for a long time as a consequence of long half-lives as well as degradation of other fluorinated compounds still in use (D'eon and Mabury, 2011; Martin et al., 2010). Thus the potential toxic effects on human health associated with low-level PFASs exposure remains a global concern.

Interest in the potential developmental neurotoxic effects of PFASs has increased in recent years. Several toxicological studies have reported negative effects in cognitive and behavior development in animals prenatally exposed to PFASs (Fuentes et al., 2007; Luebker et al., 2005; Slotkin et al., 2008). Human evidence is limited with only eight epidemiological studies published investigating the possible effects of PFASs on child neuropsychological development. Three were cross-sectional studies of highly exposed populations (Gump et al., 2011; Hoffman

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et al., 2010; Stein and Savitz, 2011). Among these studies, two reported increased attention deficit and hyperactivity disorder (ADHD) prevalence and symptomatology associated with PFASs exposure (Gump et al., 2011; Hoffman et al., 2010). The third study reported increased prevalence of ADHD associated with PFOS exposure (and other PFASs), but a non-monotonic dose response curve with a marked decreased risk in the highest exposure concentrations of PFOA (Stein and Savitz, 2011). Five longitudinal studies were based on the general population in Denmark, Taiwan and United States (Chen et al., 2013; Fei and Olsen, 2011; Fei et al., 2008; Stein et al., 2013; Strøm et al., 2014). In contrast to the cross-sectional studies, the longitudinal studies generally reported no statistically significant negative effects of PFASs on early neuropsychological development neither in outcomes assessed later in life such as ADHD, depression and school achievement. Only the recent Taiwanese study found that prenatal exposure to PFOS, but not PFOA, may be negatively associated with neuropsychological development at the age of 2 years, especially gross-motor development (Chen et al., 2013). Most of these studies assessed the effects of prenatal PFASs exposure (measured in maternal and cord blood samples or estimated) on child neuropsychological development (Chen et al., 2013; Fei and Olsen, 2011; Fei et al., 2008) and clinical outcomes (such as ADHD, depression and school achievement) (Strøm et al., 2014), while one assessed postnatal exposure to PFASs in child blood samples during childhood (Stein et al., 2013). While the sample size in the three studies conducted in Denmark (Fei and Olsen, 2011; Fei et al., 2008; Strøm et al., 2014) was considerable ($n = 1400$, $n = 700$, and $n = 800$, respectively), the sample size in the other two studies (Chen et al., 2013; Stein et al., 2013) was small in comparison ($n = 239$ and $n = 320$, respectively).

The aim of this study was to assess any potential detrimental effects of perinatal exposure to PFASs on child neuropsychological development at 6, 12 and at 24 months in a Norwegian birth cohort. Specifically, we measured PFASs concentrations in milk to assess their possible association with different areas of early child neuropsychological development: cognitive, psychomotor and behavioral development in a sample of 843 children.

2. Methods

2.1. Study population

The "Norwegian Human Milk Study" (HUMIS) is a multi-center cohort of mother–child pairs conducted in Norway. Recruitment started in 2003 and was completed in 2009. Within approximately two weeks of giving birth mothers were recruited by public health nurses during a routine home visit to all new mothers in Norway. Participants were asked to collect 25 ml breast milk sample from each morning for eight consecutive days, although milk sampled otherwise was also accepted. The milk was kept in a 250 ml container kept in the freezer. Minor changes in sampling protocol and milk samples collected by pump were accepted. Date and time of collection were recorded for each sample, as well as whether a breast pump had been used. When the container had been filled, participants mailed it by regular mail to the Norwegian Institute of Public Health in Oslo, where it was stored at −20 °C upon arrival. This procedure was different for these mothers from the county of Oestfold where they were collected by study personnel and kept frozen during transport. Further details have been published elsewhere (Eggesbø et al., 2011). Among the 2606 participants in the HUMIS study, to date, 989 women in total have had their milk samples analyzed for PFASs (due to financial constraints not everyone could be analyzed at once): 828 were randomly selected; 31 due to small for gestational age (SGA) infants (Eggesbø et al., 2009), 69 were oversampled due to rapid growth of their infant (Iszatt et al., in preparation) and 51 were oversampled based on preterm status. Supplementary Fig. 1 details this process further. Among the 989 subjects with information on PFAS, there were 86, 79 and 123 who had not sent in the 6, 12 and 24 month questionnaires, respectively. In addition there were some with missing values due to not filling out some of the specific questions needed for the neuropsychological assessments (ranging from 11 to 49 depending on the neuropsychological questionnaire) (Supplementary Fig. 1). Therefore, our analyses were based on a sample size ranging between 843 and 896 (depending on the lost to follow-up in the different neuropsychological questionnaires) mother–child pairs.

Informed consent was obtained prior to the study and the study was approved by the Norwegian Data Inspectorate (refs 2002/1398-2 and 02/01398-7) and the Regional Committees for Medical and Health Research Ethics (ref. S-02122).

2.2. Neuropsychological assessment

Early cognitive and psychomotor development was assessed at 6 and at 24 months using the Ages and Stages Questionnaire (ASQ-II), a parent-completed screening test to identify children at risk for developmental delay (Squires et al., 1999). Each questionnaire contains thirty items designed to assess the infant's neuropsychological development in children aged 2 to 60 months, covering five developmental areas: communication, gross motor, fine motor, problem solving, and personal–social skills. Parents or other caregivers are asked whether the child performs the described behavior based on three alternatives: 'yes' (10 points), 'sometimes' (5 points) and 'not yet' (0 points). The ASQ-II was validated for the Norwegian population with good results in terms of construct validity (Richter and Janson, 2007). For the present study, we only assessed four developmental areas of the ASQ-II due to time and space constraints in the questionnaire: communication, fine and gross motor and personal–social development. The ASQ-II subscales clearly had a skewed distribution with approximately 60% of scores in the perfect performance (max score = 60), and therefore, the 4 ASQ-II sub-scales were dichotomized, considering as abnormal scores 2 standard deviations (SD) below the mean (Boucher et al., 2013; Lindsay et al., 2008). As final outcomes, we used the "ASQ domain score" at 6 and at 24 months: a child's neuropsychological development is considered suspect, if the child's score falls below the established cut-off score in one or more of the ASQ-II sub-scales (Hornman et al., 2013; Squires et al., 1997).

In addition, we assessed the behavioral problems at 12 and 24 months using a subset of items from the Infant/Toddler Symptoms Checklist (ITSC): long version (De Gangi and Poisson, 2000). ITSC was filled out by mothers. The ITSC is a questionnaire that assesses self-regulation and aspects of temperament, and identifies any regulatory problems that may be arising, such as fussiness, going quickly from a whimper to a loud cry, and sleeping and eating difficulties in children aged 7 to 30 months. For the present study, we included questions on self-regulation, attention, sleep, eating or feeding, dressing-bathing-touch, and listening-language-sound subscales. For each item, the child is rated as "never" or "sometimes" fits the description (0); "fitted the description in the past" (1); or "fits the description most of the time" (2). The scores for each item are summed obtaining a total score. Higher score indicates worse behavior development. In the present study, the ITSC at 12 months included a total of 28 items, whereas the ITSC at 24 months included a total of 33 items. The internal consistency of ITSC total scores at 12 and 24 months was adequate for research use (Cronbach alpha, 0.7) (Bland and Altman, 1997). The Spearman correlation between ITSC total score at 12 and at 24 months is 0.34 (p-value < 0.001).

2.3. Exposure measurement

PFOS and PFOA concentrations were measured in breast milk sampled one month after delivery (median 32 days, min 2, max 177 days). PFOS and PFOA concentrations were measured in two different laboratories: 789 samples were analyzed at the Norwegian Institute of Public Health (NIPH) and 200 samples were analyzed at the Institute for Environmental Studies (IVM), the Netherlands. At NIPH, PFOS and PFOA