Environment International 83 (2015) 176–182

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

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

J. Forns [a], N. Iszatt [a], R.A. White [a], S. Mandal [a], A. Sabaredzovic [b], M. Lamoree [c], C. Thomsen [b], L.S. Haug [b], H. Stigum [d], M. Eggesbø [a,*]

[a] Department of Genes and Environment, Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway
[b] Department of Exposure and Risk Assessment, Division of Environmental Medicine, Norwegian Institute of Public Health, Oslo, Norway
[c] Institute for Environmental Studies, Faculty of Earth and Life Sciences, VU University, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands
[d] Department of Chronic Diseases, Norwegian Institute of Public Health, Oslo, Norway

ARTICLE INFO

*Article history:*
Received 19 December 2014
Received in revised form 25 June 2015
Accepted 25 June 2015
Available online xxxx

*Keywords:*
Nervous system
Child development
Neuropsychology
Perfluorooctanoic acid
Perfluorooctane sulfonic acid
Epidemiology

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

* Corresponding author at: Department of Genes and Environment, Division of Epidemiology, Norwegian Institute of Public Health, P.O. Box 4404 Nydalen, N-0403 Oslo, Norway.
*E-mail address:* Merete.eggesbo@fhi.no (M. Eggesbø).

http://dx.doi.org/10.1016/j.envint.2015.06.013
0160-4120/

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

were quantified using high performance liquid chromatography/tandem mass spectrometry (LC-MS/MS) according to a described method (Haug et al., 2009a,b; Thomsen et al., 2010). In brief, after thawing and homogenization in a thermoshake incubator at 37 °C, 200 μL of breast-milk was transferred to a centrifugation tube, internal standards and acetonitrile added to make up a total volume of 600 μL for thorough precipitation of proteins, and mixed using a whirl mixer. The samples were then centrifuged, the supernatant transferred to a glass autosampler vial, and 500 μL 0.1 M formic acid was added. Four hundred μL of the extract was injected into a column switching LC–MS/MS system. Calibration solutions were prepared in unprocessed cow's milk, which has been shown to be an acceptable surrogate matrix for breast milk in a thorough method validation (Thomsen et al., 2010). High quality of the determinations was ensured by analyzing samples (n = 8) from a previous interlaboratory comparison study along with the samples (MTM research center, n.d.). The obtained concentrations were within ± 1 SD of the consensus value for all PFASs found above the limit of quantification (LOQ). The procedure blanks (n = 15) did not contain any PFASs above the LOQ. In addition, we detected 8% of PFOA values below the limit of detection (LOD) while all the PFOS values were above the LOD.

The analytical method used for the samples that were analyzed at the IVM was reported earlier (de Cock et al., 2014). This method was based on the method by Haug et al. (2009b), with adaptations derived from Tao et al. (2008). Briefly, after thawing and homogenizing the breast milk samples, 0.5 ml was taken for the analysis. After addition of the 13C4-PFOA and 13C4-PFOS internal standards (Wellington Laboratories) and 0.5 ml 1 M formic acid, the samples were sonicated for 30 min. Solid phase extraction was carried out using 1 $cm^3$, 30 mg Oasis WAX cartridges. After loading the whole sample mixture, the cartridges were washed with 1 ml 25 mM ammonium acetate pH4 and 0.5 ml 25% tetrahydrofuran in methanol. The PFASs were eluted from the cartridge with 0.4 ml 1% NH4OH in methanol and 0.4 ml 0.1 M formic acid was added to the eluate prior to injection onto a C8 trapping column in a column switching LC–MS/MS system. Like in the above method carried out at NIPH, the procedure blanks (n = 28) did not contain any PFASs above the LOQ. All breast milk samples had PFOS and PFOA concentrations above the LOD, for PFOS all levels were above LOQ while for PFOA 6% of the samples (n = 13) were below LOQ. Values below the LOQ were replaced with a randomly generated number between 0 and LOQ

### 2.4. Other variables

Information on child's sex, birth weight, gestational age and maternal pre-pregnancy body mass index (BMI) was obtained from the Medical Birth Registry of Norway (MBR) (Skjaerven et al., 2000). Demographic information (maternal age, education, parity, interpregnancy interval), the duration of breastfeeding of current child, and additional information (fish intake during pregnancy and duration of total breastfeeding of previous children), and child's age at milk sample collection were obtained from a questionnaire filled in by the mother after delivery (median 6 weeks).

### 2.5. Statistical analysis

We selected confounders based on a directed acyclic graph (DAG) representing associations reported in the existing literature: child's age at milk sample collection (days), fish consumption during pregnancy (serving per year), maternal age (years), maternal education (years of education), parity (0/1/2/3/4), pre-pregnancy BMI (continuous score), maternal smoking during pregnancy (no/occasional/yes), interpregnancy interval (years), duration of total breastfeeding of previous children (weeks) and child's sex (male/female). The minimally sufficient adjustment set was identified using DAGitty v2.0 (www.dagitty.net) (Supplementary Fig. 2).

We used weighted logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CIs) to study the risk of having an abnormal score in ASQ domain score at 6 and at 24 months associated with the PFOS and PFOA exposure. In addition, weighted negative binomial regression models were used to assess the association between PFOS and PFOA and behavioral development (ITSC total score) in both assessments, at 12 and 24 months, to account for over-dispersion. We used weighted regression analysis to account for the oversampling of SGA, preterm and rapid grower infants. We included PFOS and PFOA concentrations in the different models as (1) a continuous variable (presented per interquartile range, IQR, increase); and (2) a categorical variable (median, below the median as the referent category).

We assessed the sensitivity of our results to missing data by performing multiple imputations of missing values for covariates on the subjects for which information on neuropsychological tests and PFASs was available. Multiple imputations by chained equations were implemented using Stata 13 (Supplementary table 1). Twenty imputed data sets were generated and analyzed separately, and the results were combined using the Rubin's method (Royston, 2004). We then re-ran our main analyses on the imputed data sets and compared them with estimates from the complete case analyses. Several sensitivity analyses were conducted to test the influence of child's age at milk sample collection because it is well known that PFASs have a fast depuration rate during breastfeeding (Thomsen et al., 2010). Therefore, we repeated the main analysis restricting to subjects with milk sample collection date before 90, 60 and 30 days. In addition, we also repeated the main analysis adjusting by the amount of breast milk given to the infant in the period from delivery until sample collection (cumulative proportion of breastfeeding) to explore possible exposure misclassification. A further sensitivity analysis was performed by adjusting our main models for the two different laboratories where PFAS was analyzed. Finally, we repeated the main analysis restricting to those participants which milk samples were immediately frozen after collection to test the possible effects of milk storage methods.

## 3. Results

A description of the characteristics of the study population is shown in Table 1. More than 65% of mothers had more than 12 years of education and 40% of the participating mothers were primiparous. Almost 70% of the mothers were between 25 and 35 years old and 10% were active smokers during pregnancy. With regard to neuropsychological development, 14% and 17% of children had an abnormal score in ASQ domain score at 6 and at 24 months, respectively. The median ITSC total score at 12 months was 2 (interquartile range (IQR) = 4), whereas the mean ITSC total score at 24 months was 4 (IQR = 2).

Table 2 lists the bivariate association between a priori selected determinants and PFOS and PFOA exposure. The median concentration at milk sample collection time was 110 ng/L for PFOS and 40 ng/L for PFOA. Child's birth weight, parity, smoking during pregnancy, interpregnancy interval and duration of total breastfeeding in previous births were the main determinants of PFOS concentrations in milk. The same determinants were identified for PFOA, which in addition was associated with prematurity.

Table 3 shows the adjusted associations between PFOS and PFOA concentrations in breast milk and ASQ domain score at 6 and at 24 months. We did not observe an increased risk of abnormal scores in the ASQ domain score in association with PFOS or PFOA. We did not observe any increase in the risk of having higher scores in behavioral problems (ITSC total score) at 12 and 24 months associated with PFOS and PFOA exposure (Table 4).

Results from the imputed models (Supplementary Tables 2 and 3) were similar to those in the complete-case analysis. Restricting the main analysis to subjects with milk collection dates before 90, 60 or 30 days after birth did not change the results (data not shown). There were also no changes in the main results after adjusting by the amount

**Table 1**
Characteristics of the study population (n = 843).

| Child characteristics | N | (%) |
|---|---|---|
| Sex | | |
| Female | 379 | (45.1) |
| Male | 462 | (54.9) |
| Prematurity | | |
| No | 777 | (92.2) |
| Yes | 66 | (7.8) |
| Parity | | |
| 0 | 334 | (39.7) |
| >1 | 508 | (60.7) |
| Maternal characteristics | | |
| Maternal age | | |
| <25 yrs | 118 | (14.0) |
| >25–35 yrs | 587 | (69.6) |
| >35 yrs | 138 | (16.4) |
| Maternal education | | |
| <12 years education | 107 | (12.3) |
| 12 years education | 179 | (21.6) |
| 13–16 years education | 333 | (40.2) |
| >16 years education | 209 | (25.2) |
| Maternal BMI prepregnancy | | |
| Normal | 533 | (65.5) |
| Overweight (>25) | 194 | (23.8) |
| Obesity (>30) | 87 | (10.7) |
| Smoking during pregnancy | | |
| No | 530 | (62.3) |
| Occasional | 216 | (25.6) |
| Yes | 89 | (10.6) |
| Outcomes | | |
| ASQ-II at 6 months, abnormal score | 116 | (13.8) |
| ASQ-II at 24 months, abnormal score | 145 | (17.2) |
| ITSC at 12 months (total score), *median* (*iqr*) | 2 | (4.0) |
| ITSC at 24 months (total score), median (iqr) | 4 | (2.0) |

ASQ-II: Ages and Stages Questionnaire-second edition.
Abnormal scores in ASQ-II was defined as follows: if the child's score falls below the established cut-off score in one or more of the ASQ-II sub-scales.
ITSC: Infant–toddler symptom checklist.

**Table 2**
Concentrations of PFOS and PFOA (ng/L) according to characteristics of the study subjects.

| | PFOS (ng/L) | PFOA (ng/L) |
|---|---|---|
| | P50 (IQR) | P50 (IQR) |
| All sample, median (IQR) | 110 (77) | 40 (37) |
| Sex | | |
| Female | 114 (70) | 41 (40) |
| Male | 114 (82) | 40 (38) |
| Birth weight (gr) | | |
| <2500 g | 135 (94)* | 52 (47)* |
| >2500–3999 g | 115 (78) | 41 (40) |
| >4000 g | 109 (76) | 36 (33) |
| Prematurity | | |
| No | 114 (73) | 40 (38)* |
| Yes | 128 (89) | 46 (47) |
| Parity | | |
| 0 | 137 (90)* | 58 (41)* |
| >1 | 100 (70) | 32 (30) |
| Maternal characteristics | | |
| Maternal age | | |
| <25 yrs | 114 (74) | 42 (48) |
| >25–35 yrs | 114 (80) | 40 (39) |
| >35 yrs | 115 (72) | 37 (36) |
| Maternal education | | |
| <12 years education | 110 (65) | 40 (33) |
| 12 years education | 120 (83) | 42 (37) |
| 13–16 years education | 109 (74) | 39 (41) |
| >16 years education | 118 (77) | 45 (39) |
| Maternal BMI prepregnancy | | |
| Normal | 114 (80) | 40 (39) |
| Overweight (>25) | 114 (70) | 41 (36) |
| Obesity (>30) | 113 (70) | 40 (45) |
| Smoking during pregnancy | | |
| No | 110 (75)* | 39 (36)* |
| Occasional | 124 (87) | 42 (42) |
| Yes | 120 (56) | 50 (45) |
| Interpregnancy interval (yrs) | | |
| First born | 137 (90)* | 59 (41)* |
| <1 to 2 years | 97 (63) | 28 (26) |
| >2 to 5 years | 99 (70) | 33 (28) |
| >5 years | 120 (65) | 46 (37) |
| Duration of breastfeeding in previous births (months) | | |
| 0 months | 133 (87)* | 58 (41)* |
| 1 to 12 months | 108 (65) | 34 (34) |
| >12 months | 96 (68) | 30 (27) |
| Fish intake during pregnancy† (serves/year) | | |
| <60 serves/year | 110 (61) | 42 (38) |
| >60–108 serves/year | 118 (83) | 40 (40) |
| >108 serves/year | 123 (82) | 40 (36) |

p-value based on Kruskal–Wallis test.
* $p < 0.05$.
† Recategorized in tertiles.

of breast milk given to the infant in the period from delivery until sample collection as a cumulative proportion of breastfeeding (data not shown). Adjusting for the two different analytical laboratories, did not affect the results (data not shown). Finally, we did not observe meaningful changes after restricting our main analysis those participants whose milk samples were immediately frozen after collection (data not shown).

## 4. Discussion

In this Norwegian study based on 843 infants, we did not observe an increased risk of abnormal neuropsychological development at 6 and at 24 months associated with PFOS and PFOA exposure. In addition, there was no consistent increment of behavioral problems at 12 and at 24 months associated with measured exposure to PFASs.

Our results are in accordance with the most prevalent conclusion in the published epidemiological studies showing no associations between PFASs and early neuropsychological development (Fei and Olsen, 2011; Fei et al., 2008; Stein et al., 2013). In a very recent longitudinal study conducted in Denmark, the authors reported no association between prenatal exposure to PFASs and ADHD, depression and school achievement after 20 years of follow-up (Strøm et al., 2014). However, in that study, the authors did not assess any cognitive domain. The absence of consistent negative effects of PFASs on the different domains of child neuropsychological development could be due to the fact that most of the studies, including the present, are studying very young children. The only longitudinal study testing the effects of PFASs on neuropsychological development in school-aged children (6 to 12 years old) was performed by Stein et al. (Stein et al., 2013). In that study, the authors tested the effects of estimated in utero and measured childhood PFOA exposure in a considerable battery of neuropsychological assessments, including functions such as IQ, memory, and perceptual-performance. However, there were no adverse associations between PFOA exposure and these outcomes. The effects of PFASs on child neuropsychological development could become evident only with age, when the cognitive repertoire is higher and our ability to detect any subtle changes associated with PFASs exposure is enhanced.

There is little existing epidemiologic research with which to compare these results. Only the Taiwanese study reported a negative association between PFOS levels measured in cord blood and global neuropsychological development, gross motor, fine motor and self-help areas (Chen et al., 2013). One possible explanation for the discrepancy between the Taiwanese study and our results is the difference in the neuropsychological tests used. While the Taiwan study used the Comprehensive Developmental Inventory for Infant and Toddlers (CDIIT) with a total of 343 items, we used the ASQ-II questionnaire with a total of 24 items and ITSC with a total of 28 and 33 items in the two different assessments. In addition, there are also differences in the matrix used to quantify PFOS and PFOA concentrations. While in the Taiwanese study PFASs were measured in cord blood, in our study

**Table 3**
ORs (95% CIs) for Ages and Stages Questionnaire-II administered at 6 and at 24 months according to maternal PFOS and PFOA levels (ng/L) as continuous (per IQR increase) and as categorical variable (by median).

| | ASQ6 | | | ASQ24 | | |
|---|---|---|---|---|---|---|
| | AS/total | OR | 95%CI | AS/total | OR | 95%CI |
| PFOS (ng/L) as continuous (based on an IQR increase) | 116/734 | 0.96 | (076, 1.20) | 113/687 | 0.93 | (0.74, 1.17) |
| PFOS as categorical (Ref. <median) < median | 58/369 | 0.93 | (0.60, 1.44) | 58/355 | 0.99 | (0.64, 1.52) |
| PFOA (ng/L) as continuous (based on an IQR increase) | 116/734 | 1.05 | (0.77, 1.44) | 113/687 | 1.00 | (0.78, 1.28) |
| PFOA as categorical (Ref. <median) > median | 62/361 | 1.14 | (0.71, 1.80) | 60/336 | 1.25 | (0.81, 1.95) |

AS = Abnormal scores.
Models were adjusted for child's age at milk sample collection, fish consumption during pregnancy, maternal age, maternal education, parity, pre-pregnancy BMI, maternal smoking during pregnancy, interpregnancy interval, duration of total breastfeeding of previous children and child's sex.

the used matrix was milk. It has been pointed out that PFAS concentrations in milk samples are frequently detected in lower concentrations compared to plasma samples (Kärrman et al., 2007).

Despite the current evidence, it is still precipitate to determine that PFASs are not associated with neuropsychological development. A recent review suggested that PFASs are suspected developmental neurotoxicants (Grandjean and Landrigan, 2014). Furthermore, the neurotoxic effects of PFASs have been documented in animals (Mariussen, 2012). In vivo studies, using rats and mice, concluded that animals prenatally exposed to PFOS and PFOA showed delays on motor development. Postnatal exposure to PFOS and PFOA at the time of high neuronal growth induced later behavioral problems in mice involving the cholinergic system (Johansson et al., 2008). There is some evidence from in vitro models (using P12 cells) that PFOS can trigger developmental neurotoxicity by direct effects on the replication and differentiation of neurons (Slotkin et al., 2008). An effect on the regulation of thyroid hormone function, essential for normal brain development, was also associated with PFOS exposure in animal studies (Cheng et al., 2011). Finally, oxidative stress may also be induced as a consequence of inflammatory responses in brain as a consequence of prenatal exposure to PFOS (Zeng et al., 2011).

This study has a number of strengths. We had information on a variety of important potential socio-demographic confounders, including maternal education, parity, duration of total breastfeeding of previous children and interpregnancy interval were also available. In addition, the amount of breast milk given to the infant in the period from delivery until sample collection as a cumulative variable of proportion of breastfeeding was also available, leading to improved exposure estimates. Although the matrix used in the present study differs from the previous published papers (mainly, maternal blood samples), there are some reasons to consider breast milk a good exposure biomarker to study the effects of PFASs on neuropsychological development. Firstly, postnatal exposure via breast milk is important in its own right because some crucial processes for brain development (such as synaptogenesis, synaptic pruning and myelination) will occur during the postnatal period (Casey et al., 2005). Secondly, concentrations measured in breast milk may also serve as a marker for prenatal exposure. Several studies have reported high correlations between concentrations in breast milk, cord blood and maternal serum (Haug et al., 2011; Kim et al., 2011; Liu et al., 2011), although the correlation may be slightly lower between maternal serum and breast milk for PFOS (r = .71) compared with PFOA (r = .97) (Haug et al., 2011). Thus, even though concentrations of PFASs in breast milk are not as commonly used as a proxy for prenatal exposure, breast milk concentrations together with information on breast feeding practices may appropriately represent perinatal exposure. Another important thing is that postnatal exposure to PFOS and PFOA is higher than prenatal exposure, even though the concentrations detected in milk are usually lower than the concentrations detected in plasma (Liu et al., 2011). Finally, the PFASs concentrations observed in the present study were comparable to those reported from other Western countries (Fromme et al., 2009; Haug et al., 2009a,b).

The present study is also affected by a number of limitations. ASQ-II is a questionnaire completed by parents, and therefore, some random misclassification of the outcome might influence our results. Another potential limitation of the present study is the age of assessment of neuropsychological outcomes (6, 12 and 24 months). A further assessment at older ages when the cognitive repertoire is more developed using a complete neuropsychological development battery administered by an expert neuropsychologist is warranted. This would allow a stronger measure of neuropsychological development, perhaps enabling detection of subclinical effects. We did not include the problem-solving scale of the ASQ-II in the questionnaire in order to minimize the non-response of the parents. The problem-solving scale would have provided us with more information on cognitive development, particularly as a component of executive function. In addition, we did not collect information on some potentially important confounders such as maternal intelligence and home environment, although inclusion of psychosocial covariates such as mother's age and education partly controls for this, residual confounding of home environment cannot be excluded. The critical window for exposure to PFASs and neuropsychological development is unknown, although most of the previous studies focused on the prenatal period. We argue above that perinatal exposure from breast milk might be highly relevant. However, different exposure assessment timing reduces the comparability between our results and previous reports (Chen et al., 2013; Fei and Olsen, 2011; Fei et al., 2008; Stein et al., 2013; Strøm et al., 2014). Finally, as breastfeeding might be a predictor of neuropsychological development (Der et al., 2006), our study might be affected by selection bias. This could be due to the fact that the mothers who did not breastfeed or did not deliver enough milk

**Table 4**
IRRs (95% CIs) for Infant–toddler symptom checklist (ITSC) administered at 12 and at 24 months according to maternal PFOS and PFOA levels (ng/L) as continuous (per IQR increase) and as categorical variable (by median):.

| | itsc12 | | itsc24 | |
|---|---|---|---|---|
| | IRR | 95%CI | IRR | 95%CI |
| PFOS (ng/L) as continuous (based on an IQR increase) | 0.95 | (0.85, 1.05) | 0.98 | (0.91, 1.05) |
| PFOS as categorical (Ref. <median) > median | 1.00 | (0.83, 1.22) | 0.96 | (0.85, 1.08) |
| PFOA (ng/L) as continuous (based on an IQR increase) | 0.99 | (0.87, 1.13) | 0.97 | (0.89, 1.05) |
| PFOA as categorical (Ref. <median) > median | 0.89 | (0.74, 1.09) | 0.92 | (0.82, 1.04) |

Models were adjusted for child's age at milk sample collection, fish consumption during pregnancy, maternal age, maternal education, parity, pre-pregnancy BMI, maternal smoking during pregnancy, interpregnancy interval, duration of total breastfeeding of previous children and child's sex.

could not participate in the study. However, breastfeeding was not set as an eligibility criterion of our study because nearly all Norwegian women initiate breastfeeding (96–99%) (Heiberg Endresen and Helsing, 1995; Lande et al., 2003). In addition, we found no differences in socioeconomic characteristics between our study population (n = 843), the full HUMIS cohort (n = 2.606) and the general population of birth-giving mothers in Norway (Supplementary Table 4 suggesting that our study is not affected by selection bias.

## 5. Conclusion

The present study provides further support that perinatal PFASs exposure does not hamper early neuropsychological development. Further longitudinal studies assessing children at older ages are warranted to disentangle the effects of these compounds on neuropsychological development.

## Funding and approvals

This research was primarily supported by a grant from the European Community's Seventh Framework Programme [FP7/2007–2013] under grant agreement DENAMIC no.282957, a grant from the Norwegian Research Council, under the NEVRINOR program grant agreement no. 226402, and in part from grant agreement OBELIX no. 227391.

The study was approved by the Regional Ethics Committee for Medical Research in Norway (ref. S-02122) and the Norwegian Data Inspectorate (refs 2002/1398-2 and 02/01398-7), and participation did not occur until after informed consent was obtained.

## Acknowledgments

The authors would particularly like to thank all the participants for their generous collaboration. We also acknowledge the Public Health nurses for recruitment of mothers.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2015.06.013.

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