Maternal levels of environmental toxicants and essential elements during pregnancy and associations with attention-deficit/hyperactivity disorder, autism spectrum disorder and cognitive functions in children

Thea Steen Skogheim

Department of Child Health and Development
Norwegian Institute of Public Health

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Summary

The developing brain is vulnerable to environmental insults, particularly from toxic compounds. Also, some essential elements that are necessary for normal brain development, have toxic effects at surplus levels. The knowledge about effects of toxicants and essential elements on neurodevelopment is still limited, and even more so whether prenatal exposure to toxicants is a risk factor for cognitive deficits and neurodevelopmental disorders such as attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD). In the current thesis, the relationships between gestational blood levels of environmental toxicants (PFASs and metals) and essential elements and ADHD symptoms, cognitive functions and diagnosis of ADHD and ASD in children were investigated. The maternal blood levels were measured mid-pregnancy and the associations with chemicals were examined individually and as mixtures. The project included 3,167 pregnant women and their children in the Norwegian Mother, Father and Child Cohort Study (MoBa) and data from the ADHD Study, the ABC Study (MoBa sub-studies), as well as linkages to the Norwegian Patient Registry and the Medical Birth Registry of Norway.

The findings in this thesis indicate that some of the toxicants and elements affect neurodevelopmental outcomes in a similar fashion. This was particularly evident for ASD and ADHD diagnosis, but also across other outcomes. This thesis revealed several significant associations between prenatal exposure to toxicants and elements and various neurodevelopmental outcomes. The most prominent were PFOA, cadmium, lead, arsenic, magnesium, manganese, and copper – with increased risk of ASD and/or ADHD diagnosis. The highest maternal levels of PFOA, PFNA, PFHxS, PFHpS, and PFOS were significantly associated with lowered scores on nonverbal working memory in preschoolers. There were also some non-linear relationships and several associations were modified by child sex and maternal education. Parallel findings for ASD and ADHD could indicate that the two disorders share some neurochemical and neurodevelopmental pathways. This may strengthen the view that ASD and ADHD co-exist on the same continuum of clinical expression, sharing environmental risk factors. The shared associations between prenatal exposures, particularly PFASs and ASD, ADHD and working memory could imply that the prenatal chemicals affect neurobehavioral domains mutual for ASD and ADHD, such as working memory. There were few significant results when assessing the joint effect of individual toxicants/elements in respective mixtures; only inverse associations between ASD and PFASs were identified. Nonetheless, the adverse impacts of some of the individual PFASs, metals, and essential

elements are of concern, as the sample is drawn from the general population with normal exposure level. Altogether, the results support that *in utero* exposure to some of the investigated toxicants and elements may represent overlapping environmental risk factors for neurodevelopmental disorders and cognitive deficits.

List of papers

I. Skogheim, T. S., Villanger, G. D., Weyde, K. V. F., Engel, S. M., Surén, P., Øie, M. G., Skogan, A. H., Biele, G., Zeiner, P., Øvergaard, K. R., Haug, L. S., Sabaredzovic, A., & Aase, H. (2020). Prenatal exposure to perfluoroalkyl substances and associations with symptoms of attention-deficit/hyperactivity disorder and cognitive functions in preschool children. *International Journal of Hygiene and Environmental Health, 223*(1), 80-92.

II. Skogheim, T. S., Weyde, K. V. F., Engel, S. M., Aase, H., Surén, P., Øie, M. G., Biele, G., Reichborn-Kjennerud, T., Caspersen, I. H., Hornig, M., Haug, L. S., & Villanger, G. D. Metal and essential element concentrations during pregnancy and associations with attention-deficit/hyperactivity disorder and autism spectrum disorder in children. (Accepted for publication in *Environment International*).

III. Skogheim, T. S., Weyde, K. V. F., Aase, H., Engel, S. M., Surén, P., Øie, M. G., Biele, G., Reichborn-Kjennerud, T., Brantsæter, A. L., Haug, L. S., Sabaredzovic, A., Auyeung, B., & Villanger, G. D. Prenatal exposure to per- and polyfluoroalkyl substances (PFAS) and associations with attention-deficit/hyperactivity disorder and autism spectrum disorder in children. (Under review in *Environmental Research*).

Abbreviations and explanations

ADD	Attention Deficit Disorder
ADHD	Attention-Deficit/Hyperactivity Disorder
As	Chemical symbol for arsenic
ASD	Autism Spectrum Disorder
Cd	Chemical symbol for cadmium
CDI	Child Development Inventory
Co	Chemical symbol for cobalt
Cs	Chemical symbol for cesium
Cu	Chemical symbol for copper
DOHaD	Developmental Origins of Health and Disease
DSM	Diagnostic and Statistical Manual for Mental Disorders
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
FFQ	Food Frequency Questionnaire
Hg	Chemical symbol for mercury
ICD	International Statistical Classification of Diseases
IQ	Intelligence Quotient
LOD/LOQ	Limit of Detection/Quantification
MBRN	The Medical Birth Registry of Norway
Mg	Chemical symbol for magnesium
MMR	Measles, mumps and rubella
Mn	Chemical symbol for manganese
Mo	Chemical symbol for molybdenum

MoBa	The Norwegian Mother, Father and Child Cohort Study
NPR	The Norwegian Patient Registry
PAPA	Preschool Age Psychiatric Assessment interview
Pb	Chemical symbol for lead
PBDEs	Polybrominated diphenyl ethers
PCA	Principal Component Analysis
PCBs	Polychlorinated biphenyls
PFASs	Perfluoroalkyl substances
PFCAs	Perfluoroalkyl carboxylates
PFDA	Perfluorodecanoic acid
PFHpS	Perfluoroheptane sulfonate
PFHxS	Perfluorohexane sulfonate
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PFSAs	Perfluoroalkyl sulfonates
PFUnDA	Perfluoroundecanoic acid
POP	Persistent organic pollutant
SB-5	Stanford-Binet 5th revision
Se	Chemical symbol for selenium
SPSS	Statistical Package for Social Sciences
WHO	World Health Organization
Zn	Chemical symbol for zinc

1. Introduction

Research on the impact of environmental chemicals on humans is more important than ever, as people nearly all over the world are exposed to a wide range of these chemicals. Since the 1950s, more than 140,000 chemicals have been manufactured (Landrigan et al., 2018). Many of them are suspected to be neurotoxic in humans, but only slightly more than 200 are confirmed (Grandjean & Landrigan, 2006, 2014). Neurotoxicity is the altering of the nervous system following exposure to a toxic substance (National Institute of Neurological Disorders and Stroke, 2019). Only a dozen of these chemicals is known to be developmental neurotoxicants that are harmful to the developing brain and nervous system (Grandjean & Landrigan, 2014). Many chemicals, however, remain untested, and we have little knowledge about their potential adverse effects on fetal and child brain development (Grandjean & Landrigan, 2006, 2014; Landrigan et al., 2018; Rauh & Margolis, 2016). Some essential elements can have toxic effects at surplus levels (Zoroddu et al., 2019) and will be referred to as chemicals herein.

In the late 1980s, the physician and epidemiologist David Barker noticed a higher risk of cardiovascular disease in poor regions (Barker, 2007; Suzuki, 2018). At that time, cardiovascular diseases were usually associated with wealth. He discovered a connection between small birth size due to fetal malnutrition and heart disease later in life. This led to the hypothesis that what fetuses are exposed to in the womb can have an impact on health later in life (Barker, 2007; Suzuki, 2018). This has later been termed as the Developmental Origins of Health and Disease (DOHaD) concept (Barker, 2007; Suzuki, 2018). The investigation of adverse neurodevelopment following prenatal exposure to chemicals can be viewed under the DOHaD paradigm. Chemicals can induce developmental neurotoxic effects that interfere with brain development of a growing fetus or child causing irreversible effects that affect later mental health and cognitive functions (Grandjean & Landrigan, 2014).

Prominent environmental epidemiologists have said: “strong evidence exists that industrial chemicals that are widely disseminated in the environment are important contributors to what we have called the global, silent pandemic of neurodevelopmental toxicity” (Grandjean & Landrigan, 2014, p. 330). Levels that may be safe for adults can be harmful to the developing brain of a fetus or child (Grandjean & Landrigan, 2014; Rauh & Margolis, 2016; Tran & Miyake, 2017). Furthermore, humans are not only exposed to one chemical at a time, but multiple, resulting in the so-called “cocktail effect” (Henn, Coull, & Wright, 2014; Rauh & Margolis, 2016; Svingen & Vinggaard, 2016). It has been suggested

that chemical mixtures can induce different health effects than single chemicals (Henn et al., 2014).

Most chemicals can cross the placenta and because the blood-brain barrier is underdeveloped in a fetus, toxins in the mothers' blood can reach the brain of the fetus (Gundacker & Hengstschläger, 2012; Gützkow et al., 2012; Kato et al., 2014; Osman et al., 2000). Some chemicals also have endocrine-disruptive abilities, which can harm the maternal and fetal thyroid hormone systems, both of which are essential for the normal development of the fetal nervous system and brain (De Cock, Maas, & Van De Bor, 2012; Mariussen, 2012; Tran & Miyake, 2017). Therefore, *in utero* exposure to environmental chemicals may disrupt normal brain development and increase the risks of neurocognitive deficits and neurodevelopment disorders such as attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD), intellectual disability, motor delays, and learning disabilities (Grandjean & Landrigan, 2006, 2014; Kajta & Wójtowicz, 2013; Liew, Goudarzi, & Oulhote, 2018; Rauh & Margolis, 2016; Vrijheid, Casas, Gascon, Valvi, & Nieuwenhuijsen, 2016). Hence, in a prevention perspective, research on environmental chemicals and neurodevelopment is important.

1.1 Neurodevelopment and neurodevelopmental disorders

The development of the nervous system is an ongoing process extending from the embryonic period through puberty (Rice & Barone, 2000). Numerous precisely timed and complex processes occur during fetal brain development (Heyer & Meredith, 2017). Figure 1 depicts several sensitive periods of prenatal development, including neurodevelopment during gestation. At these times, the nervous system is particularly vulnerable to exposure to environmental chemicals, which can cause developmental neurotoxic alterations of the brain development (Heyer & Meredith, 2017; Rice & Barone, 2000). These alterations can lead to deficits that will not appear until later in childhood (Rice & Barone, 2000).

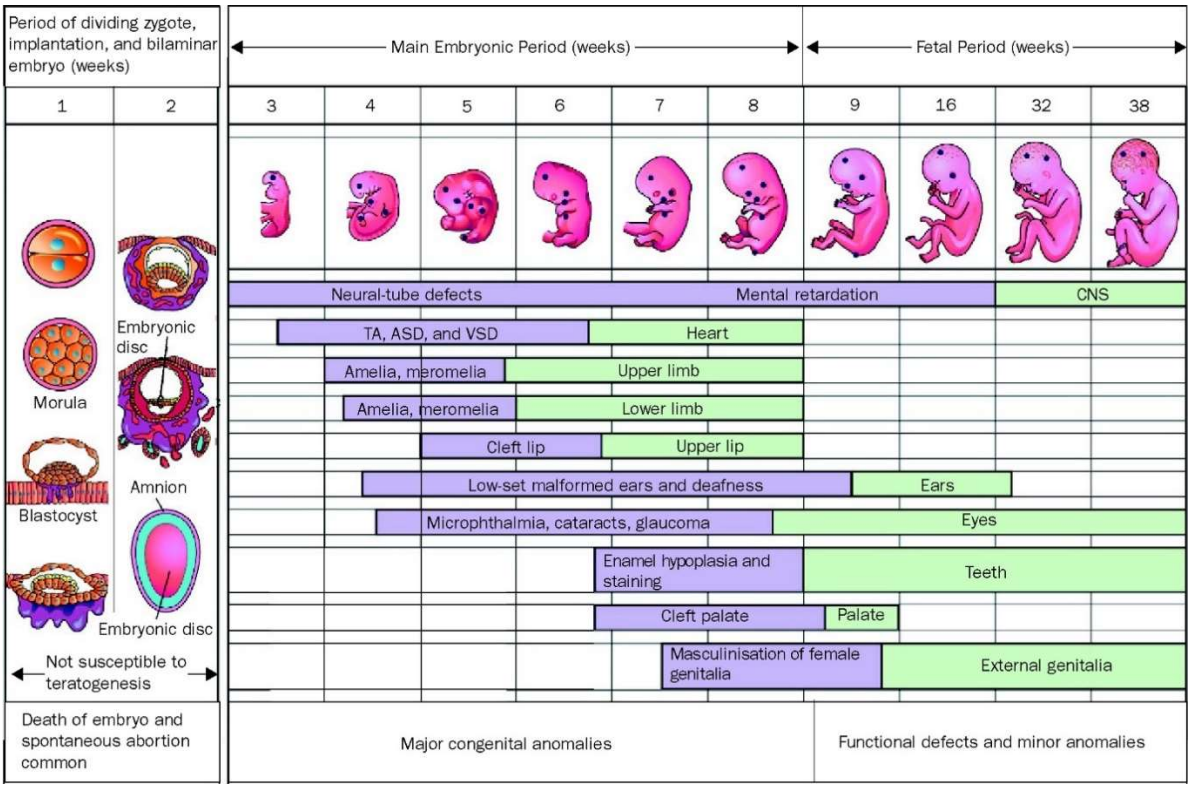

Figure 1. Crucial periods in prenatal development.

Dots on the developing fetus show common sites of action of teratogens (compounds that can disturb *in utero* development). Horizontal bars indicate fetal development during a highly sensitive period (purple) and a less sensitive period (green). Abbreviations: CNS, central nervous system; TA, truncus arteriosus; ASD, atrial septal defect; VSD, ventricular septal defect. Reprinted from Use of chemotherapy during human pregnancy, Vol 5/5, Cardonick & Iacobucci, Use of chemotherapy during human pregnancy, 283-291, copyright (2021), with permission from Elsevier.

Neurodevelopmental disorders such as ADHD and ASD are disabilities that have been subject to disruptions of brain development (Thapar, Cooper, & Rutter, 2017). Clinicians must base their diagnoses on symptoms and behavior, as there are no biomarkers of the disorders (Lord, Elsabbagh, Baird, & Veenstra-Vanderweele, 2018; Thapar & Cooper, 2016). Globally, there seems to have been an increase in the number of people diagnosed with ASD and ADHD (De Cock et al., 2012; Grandjean & Landrigan, 2014). Part of this increase can be attributed to greater awareness of the disorders and better use of diagnostic instruments, but it might also be related to increased exposure to environmental chemicals (De Cock et al., 2012; Grandjean & Landrigan, 2014; Rauh & Margolis, 2016).

1.1.1 Autism Spectrum Disorder (ASD)

ASD was assumingly first described in 1943 by physician Leo Kanner as "autistic aloneness" (Harris, 2018; Lai, Lombardo, & Baron-Cohen, 2014). Historically, several suspected causes of ASD have been discarded, including the "refrigerator mother theory" (Crowell, Keluskar, & Gorecki, 2019; Mandy & Lai, 2016) and the vaccine for measles, mumps and rubella (MMR) (Mandy & Lai, 2016; Rao & Andrade, 2011). The "refrigerator mother theory" was proposed in the 1960s by Bruno Bettelheim who claimed that cold and distant mothers were responsible for their children developing ASD (Mandy & Lai, 2016). This theory even went as far as recommending "parentectomy": the removal of children from their parents and placing them in foster care (Crowell et al., 2019). In 1998, *The Lancet* published an article by Andrew Wakefield and colleagues, suggesting that the MMR vaccine could cause ASD in children (Rao & Andrade, 2011). Part of the concern was caused by the fact that the MMR vaccine contained mercury as an adjuvant (DeStefano, 2007). It was eventually discovered that the results had been interpreted to fit the hypothesis, that some financial conflicts of interests had not been disclosed, and that there had been wrongful sampling reports (Rao & Andrade, 2011). Considering these suspicions about the conclusions of the paper, it was retracted in 2004. Nonetheless, the article made many parents skeptical about vaccinating their children (Rao & Andrade, 2011).

Initially, ASD was thought of as a categorical disorder, meaning that a person either had it or did not. In 1944, the German pediatrician Hans Asperger had applied the term "Asperger syndrome" to high-functioning children with some similar features to ASD (Hippler & Klicpera, 2003). However, it was not until 1981 that the term reached the English-speaking world through an article published by Dr. Lorna Wing (Harris, 2018). The term did not enter the Diagnostic and Statistical Manual of Mental Disorders (DSM) and International Classification of Diseases (ICD) until 1994. Today, ASD is described as a cluster of heterogeneous disorders characterized by persistent deficits in social communication and social interaction, in addition to restricted and repetitive patterns of behavior, interests, or activities (American Psychiatric Association, 2013).

In the American DSM, all subcategories of ASD have been collapsed into one large spectrum diagnosis ranging from mild to severe (Lord et al., 2018). In Europe, the standard diagnostic system is the ICD. In the ICD-10, ASD falls under "pervasive developmental disorders" with subcategories including childhood autism, atypical autism, Asperger syndrome and pervasive developmental disorder – not otherwise specified (WHO, 1992). However, in the upcoming ICD-11, the use of the umbrella term ASD, like the one used in

DSM, will be applied, where differentiation will be based on clinical features (Lord et al., 2020). In this thesis, the term "ASD" will be used, also when addressing diagnoses of pervasive developmental disorders.

1.1.2 Attention-Deficit/Hyperactivity Disorder (ADHD)

Several sources have claimed that ADHD was first described in 1902 by Dr. George Still, but it also seems to have been mentioned in a medical textbook written by Melchior Adam Weikard in 1775 (Barkley, 2015). Weikard described children with attention difficulties, impulsivity, distractibility, and overactivity, all of which resemble present-day criteria (Barkley, 2015). Like the controversial etiological theories for ASD, Weikard's work hypothesized that ADHD could be a result of poor childrearing (Barkley, 2015). However, he also postulated that biological factors could be likely causes (Barkley, 2015). Although Still was not the first to describe what later came to be known as ADHD, he had a detailed documentation of 43 children with attention problems, overactivity, and what he called "defect in moral control" (Barkley, 2015). He also noted that more boys than girls displayed the symptoms and that onset seemed to be before eight years of age (Barkley, 2015). He proposed that these problems could sometimes result from a hereditary disposition and sometimes from a pre- or postnatal injury (Barkley, 2015).

In the 20th century, ADHD was thought of as a brain dysfunction or a brain damage syndrome (Barkley, 2015). The idea that poor childrearing caused ADHD re-emerged in the 1970s, predominantly raised by the behaviorists (Barkley, 2015). Like the "refrigerator mothers" who were blamed for causing ASD, supposedly negligent mothers were accused of causing ADHD in their children (Barkley, 2015). In the late 1980s, attention deficit disorder (ADD) – the term that was previously used – was changed to ADHD in DSM-III-Revised. In the ICD, the clinical term for ADHD is hyperkinetic disorder (Barkley, 2015).

ADHD is characterized by inattention, impulsivity, and hyperactivity (Polanczyk, de Lima, Horta, Biederman, & Rohde, 2007). Hyperkinetic disorder diagnosis in the ICD-10 requires the combination of inattentiveness and hyperactivity, thus being analogous to the ADHD combined subtype in the DSM system (Thapar & Cooper, 2016). When DSM criteria are used, there are usually higher prevalence rates of ADHD than when ICD criteria are used (Faraone, Sergeant, Gillberg, & Biederman, 2003; Polanczyk et al., 2007). This is because diagnoses of ADHD can be based on inattention without hyperactivity, but for ICD, both symptoms need to be present (Polanczyk et al., 2007). In this thesis, the term "ADHD" will be used, also when addressing diagnoses of hyperkinetic disorder.

1.1.3 Cognitive functions

The preschool years are important for cognitive development (Garon, Bryson, & Smith, 2008; Rice, Taylor, & Zubrick, 2008). Cognition is a blanket term for mental processes that involve learning, remembering, decision-making, and problem solving (Roy, 2013). Executive functions are cognitive processes that govern the ability to regulate behavior (Carlson, 2005). Working memory, inhibition, cognitive flexibility, and planning are all measures of executive sub-functions (Geurts, Verté, Oosterlaan, Roeyers, & Sergeant, 2004). Different cognitive processes and skills are intertwined, so deficits in both language and working memory can co-occur (Leonard et al., 2007; Rose, Feldman, & Jankowski, 2009). Indeed, the verbal aspects of working memory are closely connected to language (Baddeley, 2003; Leonard et al., 2007).

Children with ASD and ADHD often have cognitive deficits, but there is a large variability (Thapar & Cooper, 2016). Impairment of executive functions is often prominent in these disorders (Geurts et al., 2004; Thapar & Cooper, 2016). For ADHD, particularly inhibition, working memory, vigilance, and planning are affected (Aguiar, Eubig, & Schantz, 2010; Thapar & Cooper, 2016). Other cognitive challenges include memory, reaction time, and decision-making (Thapar & Cooper, 2016). For children with ASD, problems with cognitive flexibility, working memory, and planning are common (Geurts et al., 2004). Impaired theory of mind is suggested as one of the core features of the social communication deficits that children with ASD experience (Lai et al., 2014). Deficits involving mentalizing and social perception are also common (Lai et al., 2014). The intelligence scores of children with ASD can range from high to severe intellectual disability (Lord et al., 2020).

1.1.4 Epidemiology

The prevalence of ASD in children has been found to be 1%–1.5% in the Nordic countries and in the USA (Hansen, Schendel, & Parner, 2015; Idring et al., 2015; Lyall et al., 2017; Surén et al., 2012). ADHD is slightly more common, affecting approximately 3%–4% of children globally (Polanczyk, Willcutt, Salum, Kieling, & Rohde, 2014; Surén et al., 2012; Thapar & Cooper, 2016). Both ASD and ADHD have onset in early childhood (Rutter, Caspi, & Moffitt, 2003). Symptoms of ASD usually occur before the age of three (Lyall et al., 2017). The more severe cases of ASD tend to be identified and diagnosed at a younger age compared to the milder cases (Lyall et al., 2017). For ADHD, the median age of onset was found in a review of epidemiological surveys to be between seven and nine years of age (Kessler et al., 2007).

ADHD in children has been associated with mothers who have lower education, even when accounting for shared familial risk factors (Torvik et al., 2020). For ASD, results have been mixed; some studies have reported associations with higher maternal education and other studies with lower education (Lung, Chiang, Lin, Lee, & Shu, 2018). For ASD, the association with maternal education seems to be closely related to societal factors, such as access to health care systems (Lung et al., 2018). Studies from the USA have reported a higher prevalence of children with ASD in families with higher socioeconomic status, but this might be due to the lack of universal health care as families with higher socioeconomic status will have better access (Lung et al., 2018; Rai et al., 2012). This is further supported by opposite findings in countries with universal health care (Lung et al., 2018; Rai et al., 2012).

1.1.5 Comorbidity

Comorbidity is common for both ASD and ADHD (Gillberg, 2010). Co-occurring symptoms across disorders during childhood has been viewed as the rule rather than the exception (Gillberg, 2010). Co-occurring disorders, such as anxiety, bipolar, and personality disorders have also been reported during adulthood, most prominently among people with both ASD and ADHD (Solberg et al., 2019). It has been estimated that 30%–80% of children with ASD meet the criteria for ADHD, while 20%–50% of children with ADHD meet the criteria for ASD (Rommelse, Geurts, Franke, Buitelaar, & Hartman, 2011). Due to the comorbidity and mutual risk factors, it has been proposed that ASD and ADHD are on the same continuum of clinical expression (Kern, Geier, Sykes, Geier, & Deth, 2015).

Between 67% and 80% of children with ADHD have comorbid psychiatric disorders (Pliszka, 2015). In addition to ASD, co-occurring deficits and disorders include disruptive behavior, anxiety, motor disorders, tic disorders, and intellectual disability (Pliszka, 2015; Thapar & Cooper, 2016). Oppositional defiant disorder and conduct disorder are among the behavioral problems that frequently co-occur with ADHD (Pliszka, 2015; Thapar & Cooper, 2016). Cognitive deficits, such as reading and learning problems, are more common among those with this type of comorbidity (Pliszka, 2015; Thapar & Cooper, 2016).

During the preschool years, children with ASD often have co-occurring epilepsy, language delay, motor problems, sleep and eating difficulties and high activity level (Lord et al., 2020). In school age, ADHD, intellectual disability, obsessive-compulsive disorder, anxiety, academic challenges, disruptive behaviors, and irritability occur more frequently in children with ASD (Lord et al., 2020).

1.1.6 Sex differences

For both ASD and ADHD, there is a male preponderance (Lord et al., 2020; Thapar & Cooper, 2016). The disorders with early age onset are typically more common among boys than girls, while emotional disorders with adolescent onset are more common among girls (Rutter et al., 2003). Boys seem to be three times more likely to meet the criteria for ASD compared to girls (Loomes, Hull, & Mandy, 2017). This persists even when considering possible underdiagnosing of girls (Loomes et al., 2017). Part of the issue with underdiagnosing of girls have been suggested to be due to girls with ASD "camouflaging" their symptoms (Lai et al., 2017). This "camouflaging" entails learning strategies that can conceal difficulties with social interaction (Lai et al., 2017). It has also been speculated whether there could be a male bias in the general perception of ASD (Dean, Harwood, & Kasari, 2017). The ratio of boys to girls is about 10:1 in high-functioning ASD or Asperger's syndrome, but 2:1 in children with additional intellectual disability (Dworzynski, Ronald, Bolton, & Happé, 2012).

Childhood ADHD has been reported to be from two to nine times more prevalent in boys, but there are smaller sex differences in population-based samples compared to clinical samples (Nussbaum, 2012; Thapar & Cooper, 2016). There is, however, a general difference in how ADHD symptoms are typically expressed among boys and girls. Girls tend to have more difficulties with inattention, whereas boys usually have more difficulties with hyperactivity and impulsivity, and females are usually older than males when they receive a diagnosis (Nussbaum, 2012). It has been hypothesized that a higher degree of externalizing behavior problems among boys with ADHD compared to girls may result in a sex-based referral bias (Biederman, 2005; Martin et al., 2018a; Nussbaum, 2012).

Sex steroid hormones have also been implicated in the sex differences in ASD and ADHD prevalence, particularly for ASD (Baron-Cohen et al., 2019). Brain sexual differentiation begins early in fetal development, and sex steroids have an important role at every stage of brain development (Weiss, 2012).

1.1.7 Treatment and prognosis

When it comes to treatment of ADHD, there is a difference between practices in the USA and in Europe (Thapar & Cooper, 2016). In the USA, pharmacological treatment can be given to preschoolers, while this is not recommended in Europe (Thapar & Cooper, 2016). When pharmacological treatment is put in place, it is usually in combination with behavioral interventions such as behavior management skills, classroom management strategies, and

parental psychoeducation (Thapar & Cooper, 2016). Unlike stimulants such as methylphenidate and dexamfetamine used to alleviate symptoms of ADHD (Thapar & Cooper, 2016), there are no such medicines for ASD symptoms alone (Lord et al., 2018). Still, some people with ASD can benefit from taking risperidone and aripiprazole to relieve symptoms of irritability and agitation or stimulants such as methylphenidate and dexamfetamine to relieve ADHD symptoms (Lord et al., 2018; Thapar & Cooper, 2016). Non-pharmacological interventions for ASD include parent-mediated interventions, and behavioral and social interventions (Lord et al., 2018). The parent-mediated intervention focuses on interaction with their children, which can have a positive impact on their children's social behavior and communication, whereas the behavior interventions focus more on cognition, language development, and adaptive skills (Lord et al., 2018). When children are in school age, behavioral interventions can also facilitate improved social skills (Lord et al., 2018).

For people with ASD and ADHD, difficulties can continue into adolescence and adulthood (Lord et al., 2020; Thapar & Cooper, 2016). Having a diagnoses of ADHD and/or ASD has been associated with a range of sub-optimal long-term outcomes including low educational levels, low life satisfaction, job insecurity, and mortality (Lai et al., 2014; Rauh & Margolis, 2016; Thapar, & Cooper, 2016). In addition, ADHD in adulthood has been associated with incarceration and substance abuse (Solberg et al., 2019; Thapar & Cooper, 2016).

1.1.8 Etiology of ASD and ADHD

Genetic predisposition is a major determinant of risk for ASD and ADHD (Sullivan et al., 2018). However, there is likely an interplay between genetic factors and environmental, psychosocial, and socioeconomic factors (Faraone et al., 2015; J. Martin, Taylor, & Lichtenstein, 2018b; Nuttall, 2017; Sandin, 2014; Thapar, Cooper, Eyre, & Langley, 2013). Heritability estimates for ASD and ADHD have ranged from 75% to 90% (Lai et al., 2014; Polderman et al., 2015). There seems to be no single gene explaining ADHD or ASD, with many genes explaining only a small part of the disorders (Lai et al., 2014; Thapar et al., 2013). Pleiotropy is likely, where one gene variant may be associated with multiple disorders (Rommelse et al., 2011).

Several pre- and postnatal environmental factors have been suggested to influence these disorders. Family stressors, diet, and psychosocial adversity have all been implicated as important environmental risk factors for ADHD and ASD (Thapar & Cooper, 2016; Tran &

Miyake, 2017). In addition, low birthweight, prematurity, maternal intake of alcohol, prescribed drugs, and illicit substances during pregnancy have been associated with ADHD (Thapar & Cooper, 2016). For ASD, birth trauma, older maternal age, maternal obesity, gestational diabetes, short intervals between pregnancies, and valproate use during gestation have all been implicated as risk factors (Lord et al., 2020).

Exposure to environmental chemicals has also been proposed as part of the etiology of ASD and ADHD, with adverse effects for chemicals such as lead, mercury, PCBs, and pesticides (Grandjean & Landrigan, 2006, 2014; Thapar et al., 2013; Vrijheid et al., 2016). Several other chemicals are suspected to interfere with human neurodevelopment, but more research is needed to elucidate their potential detrimental effects on brain development (Grandjean & Landrigan, 2014; Vrijheid et al., 2016).

1.2 Environmental toxicants and elements

The far-reaching distribution of harmful chemicals in the environment is mainly due to anthropogenic activities such as mining, burning of fossil fuels, and extensive chemical usage in agriculture and manufacturing (Järup, 2003; Tchounwou, Yedjou, Patlolla, & Sutton, 2012). This has more than local implications. Because of transportation by air and ocean currents, chemicals are ubiquitous in most places where people live (Järup, 2003; Zhao et al., 2012). In addition, many chemicals are highly persistent; they accumulate in organisms and magnify in concentration as they move up the food-chain (EFSA, 2018). Toxicants and elements can reach the human body through numerous sources, food being the most important (Caspersen et al., 2019; Haug et al., 2010; Papadopoulou et al., 2019). Humans are also exposed through sources such as drinking water, dust, cosmetics, and consumer products (Haug et al., 2018). Lead, mercury, arsenic, and cadmium are among the ten chemicals that the World Health Organization (WHO) has considered to be a major concern to public health (WHO, 2016). Per- and polyfluoroalkyl substances (PFASs) is a group of human-made contaminants that is reported to be among the most prominent pollutant groups found in human blood, including in pregnant women (Haug et al., 2018; Mariussen, 2012). Metals, elements, and PFASs have several shared properties; they can cross the placenta and they are either suspected or known as developmental neurotoxicants.

1.2.1 Per- and polyfluoroalkyl substances (PFASs)

PFASs is a large group of synthetic compounds used in products such as firefighting foam, cooking pans, food packaging, and textiles (Buck et al., 2011; Kissa, 2001). Results from

animal and *in vitro* studies have suggested that PFASs are developmental neurotoxicants, affecting several neurochemical targets in the developing brain and acting as endocrine disruptors (Johansson, Eriksson, & Viberg, 2009; Mariussen, 2012; Slotkin, MacKillop, Melnick, Thayer, & Seidler, 2008; Viberg, Lee, & Eriksson, 2013). Due to increased health concern about exposure to PFASs, some manufacturers have phased out production of some types of PFASs. Because of their reduced use, the environmental levels of some PFASs have declined the last ten to 15 years (EFSA, 2018; Land et al., 2018; Mariussen, 2012).

Two of the most well-known PFASs that are also closely associated with adverse health effects – perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) – are now subjected to international restrictions and regulations. In 2009, PFOS was listed in Annex B (as restricted) of the Stockholm Convention on Persistent Organic Pollutants (Stockholm Convention, 2019). As of 2020, PFOA was banned for use in the European Union and has been considered for restrictions under the Stockholm convention (ECHA, 2020; Stockholm Convention, 2019). PFOS and PFOA belong to two different PFAS groups; perfluoroalkyl sulfonates (PFSAs) and perfluoroalkyl carboxylates (PFCAs), respectively. PFASs are highly persistent in the environment and in humans, with PFOS and PFOA having estimated biological half-lives of two to five years in the human body (EFSA, 2018; Lau et al., 2007). There has been increased production and emission of PFCAs as well as some precursors with unknown health effects (Sunderland et al., 2019; Wang, Cousins, Scheringer, Buck, & Hungerbühler, 2014).

1.2.2 Metals and essential elements

Toxic metals and essential elements are naturally occurring in the environment but have elevated levels due to human activity (Järup, 2003; Tchounwou et al., 2012). Industrial pollution has led to metals and elements being released to air and disposed of in water and soil (Järup, 2003; Tchounwou et al., 2012). Essential elements are vital to humans and the developing fetal brain. However, both deficiency and excess of essential elements can have harmful effects (Tchounwou et al., 2012; Zoroddu et al., 2019). As the Renaissance physician and "father of toxicology" Paracelcus said, it is the dose that makes the poison: "What is there that is not poison? All things are poison and nothing is without poison. Solely the dose determines that a thing is not a poison" (cited in Grandjean, 2016, p. 126). Several metals and elements are known developmental neurotoxicants, including lead, mercury, arsenic, and manganese (Grandjean & Landrigan, 2006, 2014). In addition, some have been suspected as developmental neurotoxicants, such as cadmium (EFSA, 2009).

Blood concentrations of metals and essential elements in pregnant Norwegian women have been comparable to levels in other European countries (Caspersen et al., 2019; Haug et al., 2018), although the Norwegian levels seemed to be somewhat higher for arsenic and mercury (Haug et al., 2018). For mercury, arsenic, and selenium, the predominant sources in the Norwegian population are fish and shellfish (Birgisdottir et al., 2013; Papadopoulou et al., 2019), whereas multimineral supplements seem to be major sources for essential elements such as manganese copper, zinc as well as selenium (Caspersen et al., 2019).

1.3 Knowledge of exposure and outcomes

Knowledge of the health effects of environmental chemicals was historically based on findings from high-exposure-situations, such as handling of chemicals by industrial workers or chemicals being inadvertently released into the environment causing high local or regional exposures for inhabitants (Grandjean & Landrigan, 2006). An example of the latter is the Minamata Bay tragedy in Japan, where a chemical factory released wastewater containing methylmercury chloride into the Minamata Bay from 1950 to 1968 (Ekino, Susa, Ninomiya, Imamura, & Kitamura, 2007). Residents were chronically exposed to high doses of methylmercury through fish consumption (Ekino et al., 2007). The resulting neurological symptoms and syndromes were called Minamata disease (Ekino et al., 2007). Children that were exposed to methylmercury during gestation displayed severe neurodevelopmental deficits, although the mothers showed few or no symptoms of methylmercury poisoning (Heyer & Meredith, 2017). A lot of the knowledge on toxic chemicals also derives from experimental studies comparing animals exposed to high doses of toxicants to non-exposed (Grandjean & Landrigan, 2014; Mariussen, 2012). However, since animal studies usually focus on one chemical at a time and at high doses, such scenarios are not directly comparable to what most people are exposed to in their everyday life, implying simultaneous exposure to many different chemicals at lower (population) levels of exposures.

There has been increased attention to the potential risk of prenatal exposure to toxicants and elements and child neurodevelopment (Liew et al., 2018; Vrijheid et al., 2016). There have also been larger prospective studies using data from birth cohorts with normal population levels, as opposed to previous studies focusing more on high-exposed populations. In addition, there has been increased attention to mixtures of chemicals that fetuses are exposed to (Henn et al., 2014; Rauh & Margolis, 2016; Vrijheid et al., 2016). Still, there is a paucity of research due to lack of investigation of certain toxicants and elements and inconsistencies across studies (Forns et al., 2020; Liew, 2018; Vrijheid et al., 2016).

Most studies of prenatal metal/element exposure and ASD, ADHD, and cognitive functions have investigated the impacts of mercury and lead (Heyer & Meredith, 2017; Tran & Miyake, 2017; Vrijheid et al., 2016). Although some metals and elements need to be further investigated, the overall picture implies negative impacts from heavy metals on neurodevelopment (Heyer & Meredith, 2017; Tran & Miyake, 2017; Vrijheid et al., 2016; Yoshimasu, Kiyohara, Takemura, & Nakai, 2014). Research on lead, predominantly postnatally, have identified non-linear associations between low-level exposure and IQ (Vrijheid et al., 2016). Since some of the essential elements can have detrimental effects with both deficiency and surplus (Zoroddu et al., 2019), non-linear associations are to be expected.

Results from epidemiologic studies investigating prenatal exposure to PFASs and neurodevelopment, such as ASD, ADHD and cognitive functions, remain inconsistent and inconclusive (Forns et al., 2020; Liew et al., 2018; Rappazzo, Coffman, & Hines, 2017; Vrijheid et al., 2016). The inconsistencies for the PFAS studies could be due to the use of different measures of neurodevelopment, performing measurements at different developmental stages, or because of exposure assessment variations.

For some metals, elements, and PFASs, confounding by maternal seafood intake during pregnancy becomes a challenge. On the one hand, the polyunsaturated fatty acids found in seafood are important for normal fetal brain development (Budtz-Jørgensen, Grandjean, & Weihe, 2007; Choi, Cordier, Weihe, & Grandjean, 2008). On the other hand, seafood can also contain high levels of toxicants such as mercury, arsenic, and some PFASs (Budtz-Jørgensen et al., 2007; Haug et al., 2010). This makes seafood intake an example of negative confounding (Choi et al., 2008). However, many studies have not adjusted for maternal seafood consumption during pregnancy, which may have led to underestimation of effects from toxicants (Budtz-Jørgensen et al., 2007; Choi et al., 2008).

Some environmental chemicals are endocrine-disruptive, meaning that they can interfere with the endocrine/hormonal systems, and this can further impact the sexual differentiation (Weiss, 2012). Studies have showed sex differences in the relationships between prenatal PFASs, metals, and elements and neurodevelopmental outcomes. There are several potential explanations to these observed differences, as they could be related to sexually dimorphic placental transfer of toxicants, toxicant-induced alterations of sex steroid levels, and/or sex-specific neurodevelopmental vulnerabilities (Baron-Cohen et al., 2019; Kjeldsen & Bonefeld-Jørgensen, 2013; Mariussen, 2012; Werling & Geschwind, 2013). In addition, a meta-analysis on early-life exposure to PFASs, reported that associations with ADHD were stronger among girls than among boys (Forns et al., 2020). PFAS and metal

exposure have also been associated with socioeconomic position, such as education and employment (Brantsæter et al., 2013; Montazeri et al., 2019).

1.4 Literature gaps

Even though the general population is regularly exposed to a growing number of chemicals through food, air, water, and consumer products, there is remarkably limited knowledge of their impacts on the developing brain. Given the fact that disorders such as ASD and ADHD have profound consequences for the individuals affected and the society at large, it is important to investigate potential risk factors contributing to the etiologies of these disorders. This can be important contributions to prevention efforts, as exposures to environmental chemicals in pregnant women and their fetuses, are in theory modifiable.

As mentioned, most research has explored the better-known developmental neurotoxicants, such as lead and mercury. For some of the other metals and essential elements, there is still a is a lack of research on their effects on intrauterine brain development. As results for PFASs are inconsistent, there is a need for more research to elucidate the neurotoxicological potential of PFASs. Using prospective birth cohort studies is a suitable way to assess the potential effects of chemicals, with larger sample sizes, availability of confounding variables, and the possibility to investigate effect measure modifiers. As the dose-response relationships between toxicants and essential elements and neurodevelopmental outcomes may be non-linear, it is also necessary to inspect potential non-linearity of the relationships. Furthermore, there is a dearth of research investigating the joint effects of individual compounds in a mixture on intrauterine brain development (Liew et al., 2018; Vrijheid et al., 2016). In addition, few studies have investigated the potential modifying effects of child sex and maternal education on these exposure-outcome associations.

2. Research objectives

The main objective of this thesis was to investigate the associations between gestational levels of toxicants and elements and neurodevelopmental outcomes in children. This was explored with different methods; both of individual chemicals and the joint mixture effect. An over-arching aim was to compare differential and general associations of the dimensional and categorical outcomes observed in the different papers. This thesis included the following sub-aims:

- To investigate the associations between prenatal exposure to PFASs and ADHD symptoms and cognitive functions in preschool children (Paper I).

- To investigate the associations between gestational levels of metals and essential elements and childhood diagnoses of ADHD and ASD (Paper II).

- To investigate the associations between prenatal exposure to PFASs and childhood diagnoses of ADHD and ASD (Paper III).

- To investigate the functional form of the exposure-outcome relationships (Papers I-III).

- To investigate effect measure modification by child sex in the exposure-outcome relationships (Papers I-III).

- To investigate effect measure modification by maternal education (as a proxy for socioeconomic status) in the exposure-outcome relationships (Papers II and III).

3. Material and methods

3.1 Data sources and sample

This thesis was part of a project called NeuroTox and was based on data from the Norwegian Mother, Father and Child Cohort Study (MoBa) and two MoBa sub-studies; the Norwegian Longitudinal ADHD Cohort Study (The ADHD Study) and the Autism Birth Cohort Study (The ABC Study). In addition, information from the Norwegian Patient Registry (NPR) and the Medical Birth Registry of Norway (MBRN) were retrieved. Figure 2 illustrates how the participants from the ADHD Study (ADHD symptoms and cognitive functions) were selected to Paper I. Figure 3 shows how participants with ADHD and ASD diagnoses were selected to Paper II and III.

In this thesis, there were two different types of environmental chemicals; PFASs and metals/essential elements in maternal blood. Regarding the outcomes, there were two categorical/diagnostic outcomes; ADHD and ASD diagnoses. In addition, several dimensional outcomes were investigated; ADHD symptoms, language skills, working memory and estimated IQ in children of three and a half year of age (for PFASs only).

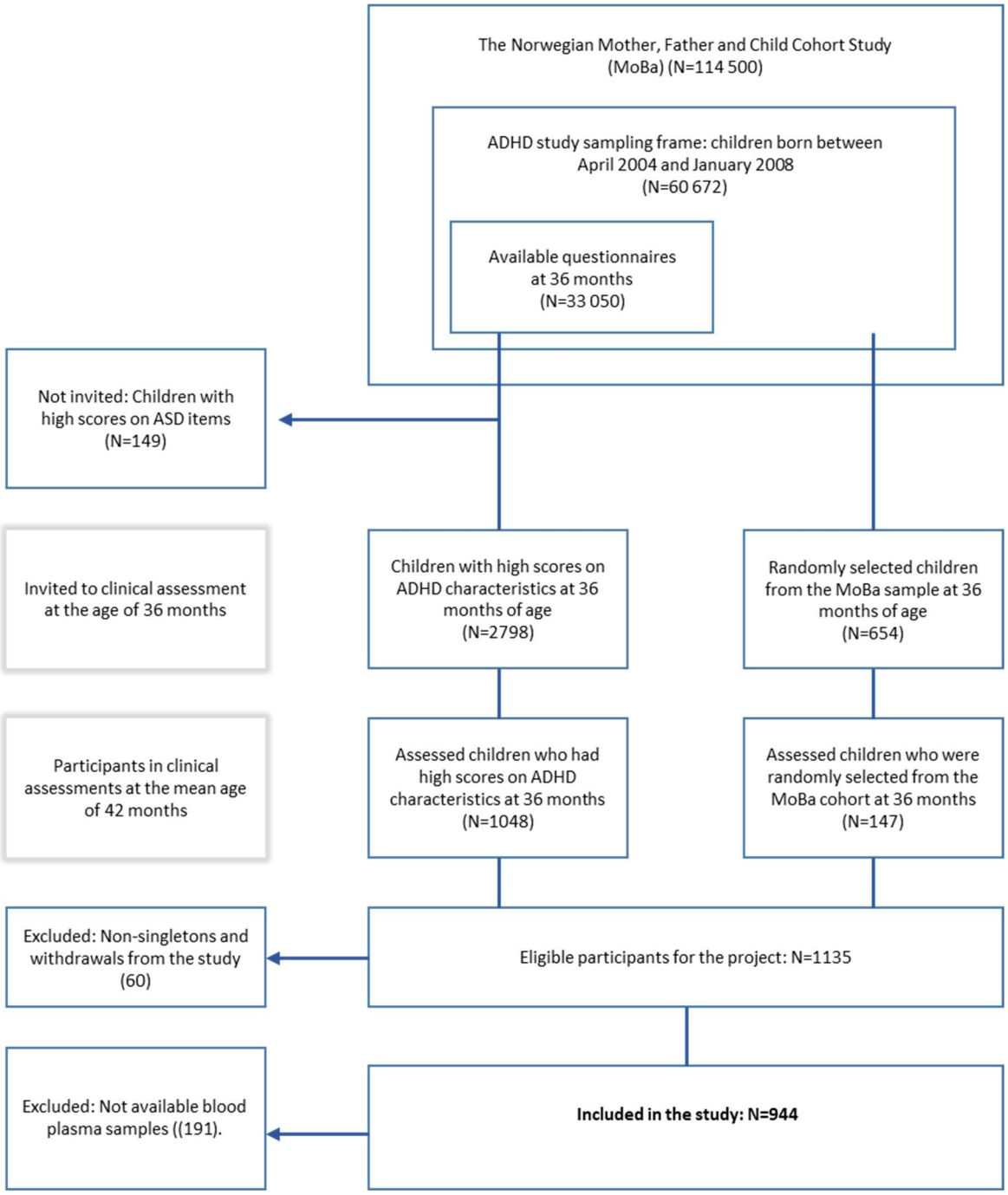

Figure 2. Flow chart for Paper I with participants from the ADHD Study.

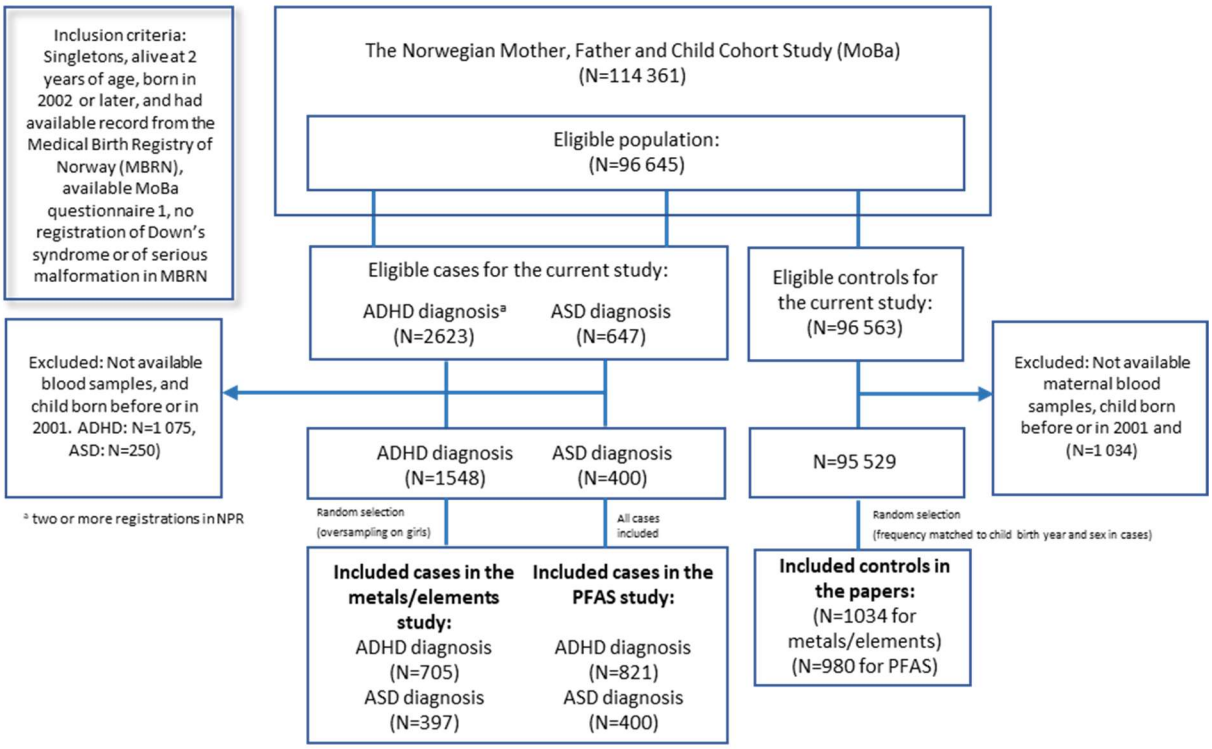

Figure 3. Flow chart for Papers II and III with participants with ASD and ADHD diagnoses.

3.1.1 The Norwegian Mother, Father and Child Cohort Study (MoBa)

MoBa is an ongoing prospective population-based cohort study conducted by the Norwegian Institute of Public Health (Magnus et al., 2016). Pregnant women from all over Norway were invited from 1999 to 2008 to participate when scheduling their first free ultrasound scanning in the 17th week of pregnancy, and 41% agreed to participate. The cohort now includes more than 114,000 children, 95,000 mothers, and 75,000 fathers. Blood samples were collected from both parents in mid-pregnancy and from the mother and child at birth (Magnus et al., 2016). So far, there are nine questionnaires for the mothers, two for the fathers and two for the children (at age 13 and 14). In addition, the children who have turned 16, have the possibility to participate in MoBa Young, where they are sent short questionnaires on their mobile phones.

3.1.2 The NeuroTox project

NeuroTox is short for "Prenatal exposure to toxicants and childhood neurodevelopmental disorders and cognitive functions". The primary aim of the project was to investigate whether prenatal exposure to environmental contaminants (measured) in maternal blood during pregnancy had a negative impact on the child's neurodevelopment. In addition to the

outcomes included in this thesis, cerebral palsy and epilepsy were investigated. The NeuroTox project is a nested case-cohort study, that retrieved case and control status of MoBa children via linkage with the NPR and retrieved and analyzed stored maternal blood samples from the MoBa biobank.

3.1.3 The Norwegian Longitudinal ADHD Cohort Study (The ADHD Study)

The ADHD Study is a nested case-cohort study within MoBa. It is a clinical sub-study that oversampled children at risk for ADHD, using data from the MoBa questionnaire that mothers completed when children were three years of age (Øvergaard et al., 2018). This questionnaire included 11 items about ADHD, of which six items were from the Child Behavior Checklist/1.5–5 (Achenbach, 2010) and five items from the DSM-IV-TR criteria for ADHD (Association, 2000) (American Psychiatric Association, 2000). Children with scores ≥90th percentile on these 11 items (n=2798) were invited to participate in a clinical assessment, along with randomly selected children from the MoBa cohort (n=654). In total, about 35% agreed to participate in the sub-study. From 2007 to 2011, 1195 children (mean age: 3.5 years, age range: 3.1–3.8 years) took part in a one-day clinical assessment including a neuropsychological assessment with the child and a diagnostic interview with one of the parents, usually the mother. In the overall sample, the proportions of girls and boys who met symptom criteria for ADHD diagnosis according to the parent interview were about 17% and 20%, respectively (Overgaard et al., 2019; Øvergaard et al., 2018). Further details about the ADHD Study have previously been published (Øvergaard et al., 2018).

3.1.4 The Autism Birth Cohort Study (The ABC Study)

The ABC Study is a case-cohort study of ASD nested within MoBa. Potential cases have been detected through questionnaire screening at ages three, five, and seven years, direct referrals of children suspected of having ASD, and linkages to the NPR. These children were invited to a clinical assessment that included the research-standard instruments for diagnosing ASD as well as assessment of cognitive skills. A randomly drawn control sample was also invited. Case identification is continuing through annual linkages to the NPR, where medical records are examined to assess whether the participants fit the diagnostic criteria for ASD (Norwegian Institute of Public Health 2020; Surén et al., 2019). Around 50% of the potential participants invited to the ABC clinical assessments accepted the invitation (Stoltenberg et al., 2010). In total, 1389 children have been identified with ASD diagnosis in MoBa with linkage to the NPR (Health, 2020) (Norwegian Institute of Public Health, 2020). Details about the ABC

Study have been described previously (Stoltenberg et al., 2010; Surén et al., 2019). In this thesis, only the NPR linkage was included to identify ASD case children within MoBa, not results from the clinical assessments.

3.1.5 The Norwegian Patient Registry (NPR) and the Medical Birth Registry of Norway (MBRN)

The NPR is a national health care registry that receives patient data on diagnoses reported from all hospitals and specialized health care services in Norway. The registry contains diagnoses for in- and outpatients recorded from 2008 onward (Bakken, Ariansen, Knudsen, Johansen, & Vollset, 2020). The diagnostic codes reported to the NPR are according to the ICD-10. Very few cases of ASD are diagnosed at private clinics, however, ADHD is more commonly diagnosed by private specialists, so the NPR captures an estimated 90–95% of ADHD diagnoses (Surén et al., 2012). The MBRN is a national health registry that contains information about all births in Norway. The registry provides information about maternal and child health during pregnancy and birth.

3.2 Participants

This thesis is based on data from MoBa and the MBRN. One of the papers (Paper I) use data from the ADHD Study (dimensional measures) and the two others (Papers II and III) use data from the NPR (diagnostic measures).

Paper I included data from the ADHD Study to investigate prenatal exposure to PFASs in relation to ADHD symptoms and cognitive functions. Paper II and III used data from the NPR to investigate gestational levels of metals/elements (II) and PFASs (III) and associations with ADHD and ASD diagnoses. From the NPR, clinically diagnosed cases of children with ADHD and ASD were identified. The ADHD and ASD case group, and participants from the ADHD Study, were selected based on the following criteria: born in 2002 or later, singletons, alive at 2 years of age, had available record from the MBRN, available MoBa questionnaire 1, no registration of Down's syndrome or of serious malformation in MBRN and available maternal blood samples; whole blood for metal and element analyses and/or plasma for PFAS analyses. Control groups were randomly selected from the eligible population based on the same selection criteria as the case groups, and frequency-matched on child sex and birth year. The total number of mother-child pairs in each paper is displayed in Figure 2 and Figure 3. It should be noted that the participants in the

ADHD study were not the same sub-population as those identified with ADHD diagnoses from the NPR.

3.3 Diagnostic outcome measures

3.3.1 ASD diagnosis

Cases of ASD were selected if they had one or more registrations of "pervasive developmental disorders" meeting criteria for ASD (F84.0, F84.1, F84.5, F84.8 or F84.9) (WHO, 1992). Childhood autism (F84.0) is defined as "a pervasive developmental disorder defined by the presence of abnormal and/or impaired development which manifests before the age of 3 years, and by the characteristic type of abnormal functioning in all three areas of social interaction, communication, and restricted, repetitive behavior" (WHO, 1992).

3.3.2 ADHD diagnosis

Cases of ADHD were selected if they had at least two registrations of "hyperkinetic disorder" (ICD-10 codes F90, F90.0, F90.1, F90.8 or F90.9) (WHO, 1992). In order to exclude erroneous registrations or false diagnoses, two registrations of diagnosis were required. The ICD-10 criteria for hyperkinetic disorder/ADHD are "early onset; a combination of overactive, poorly modulated behavior with marked inattention and lack of persistent task involvement; and pervasiveness over situations and persistence over time of these behavioral characteristics" (WHO, 1992).

3.4 Outcomes from the ADHD Study

For the assessment of ADHD symptoms, psychologists, psychiatrists, or trained graduate psychology students conducted the interviews. When graduate students conducted the interviews with the parents, they were under supervision by a child psychologist or a psychiatrist. Experienced clinicians with competence in child neuropsychology conducted the tests of cognitive abilities of the children, including language skills, estimated IQ, and working memory.

3.4.1 ADHD symptoms

ADHD symptoms in the children were based on the Preschool Age Psychiatric Assessment (PAPA) interviews with the children's parents (Egger & Angold, 2004). This psychiatric interview has shown adequate test-retest reliability and validity in assessing psychiatric symptoms in preschoolers (Egger & Emde, 2011). The ADHD classification defined by

PAPA is not equivalent to clinical ADHD diagnoses that would require a broader assessment, including multiple sources of information and informants, as well as the children being too young (3.5 years) to be assigned a proper ADHD diagnosis. In the ADHD Study, only symptoms lasting ≥3 months were counted as present. As an inter-rater reliability check, a separate rater who was blind to the parent and teacher screen ratings, rescored audiotapes of 79 randomly selected assessment interviews. The average intra-class correlations (ICCs) were 0.97 for hyperactivity and impulsivity (HI) symptoms, 0.99 for inattention (IA) symptoms, and 0.98 for the total number of ADHD symptoms (Overgaard et al., 2015). In the present study, ADHD symptom sum scores were based on symptoms of inattention, hyperactivity, and impulsivity from the PAPA interview. Higher scores indicated more ADHD symptoms and higher severity.

3.4.2 Expressive language skills

Expressive language skills were measured with Child Development Inventory (CDI). The CDI is a questionnaire for assessment of children from 15 months to six years of age, where teachers and parents fill in the questionnaires (Ireton & Glascoe, 1995). The questionnaire is consistent with results from psychometric tests of children and has good sensitivity and specificity (> 80%) of identifying delayed development in children (Doig, Macias, Saylor, Craver, & Ingram, 1999). However, participants with delayed language development were sampled to other MoBa sub-studies, meaning that the language measure in this thesis was not very discriminative. In the CDI, delayed language is defined as at least 1.25 standard deviations below the mean (Rohrer-Baumgartner et al., 2016). In this thesis the language subscale that was filled in by the preschool teacher was used. The subscale contains 50 items that assess primarily expressive communication, from simple gestures to complex language expressions. The daycare teacher report was chosen instead of parental report, as preschool teachers generally are assumed to have a good reference base for the evaluations (Rohrer-Baumgartner et al., 2016). A higher score indicated better language skills.

3.4.3 Working memory

Working memory consists of a multicomponent cognitive system that allows for the rehearsal, storage and manipulation of information for a few seconds, and is a vital part of higher-order cognitive processes (Baddeley, 2012). Stanford Binet Intelligence scales (5th edition) was utilized to measure verbal and nonverbal working memory. Working memory assessed with SB-5, which has shown good psychometric properties (Roid, 2003). Verbal working memory

was assessed with the subtask "Memory for Sentences", where the child is asked to repeat sentences that increases gradually in length. Nonverbal working memory was measured with two subtasks; "Block Span" and "Delayed Response". In the Block Span test, the child is asked to tap blocks in the same order as the administrator. In the Delayed Response task, a small toy is placed under one of three cups when the child is watching; he or she is then asked to indicate where the toy is hidden after a short delay (Roid, 2003). A higher score indicated better working memory function.

3.4.4 Estimated IQ

Intelligence quotient (IQ) refers to performance on standardized tests measuring intellectual abilities (McGrath, 2011). The two subtests from SB-5, were used to assess estimated IQ. This test battery has good psychometric properties and is standardized for ages two to 85 (Roid, 2003). In the present study, estimated verbal IQ score was based on the "Vocabulary Task" where the child is requested to point at different body parts or name objects (toys) and explain the meaning of selected words. Estimated nonverbal IQ score was based on the "Object Matrices Task", that entails tasks such as detection of shapes that are alike and to fill in a missing shape on the basis of abstract reasoning. The verbal task is a measure of knowledge and the nonverbal task is a measure of fluid reasoning, which together is a good estimate of global ability (Roid, 2003). Both of these subtests have high loadings on the hierarchical g factor in cognitive ability batteries (Roid, 2003). The stop rule of discontinuing the test after four consecutive null scores was applied in all tests from this battery. A higher score indicated higher estimated IQ.

3.5 Exposures

3.5.1 PFASs

In this thesis PFAS levels were measured in maternal plasma samples from week 18 of gestation. Further details about the sampling procedure and handling and storage in the MoBa biobank is described in detail elsewhere (Paltiel et al., 2014). Nineteen PFASs were determined in maternal plasma samples, using liquid chromatography-triple quadruple mass spectrometry (LC-MS/MS) (Haug, Thomsen, & Becher, 2009). This method has been thoroughly validated and has been used for determination of more than 5000 serum/plasma samples, including approximately 2000 samples from MoBa (Singer et al., 2018). Only PFASs with levels above limit of quantification (LOQ) in more than 80% of the plasma samples were included in the present thesis. This included four carboxylates; PFOA,

perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA) and three sulfonates; perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS) and PFOS. To ensure high quality of the determinations throughout the project, internal quality control samples and procedure blanks were analyzed along with each batch of samples. Case and control samples were placed randomly across analytical batches (i.e. one unit of samples processed and analyzed as one group) and was blinded to the analyst. PFAS concentrations were given in (ng/mL). More information about LOD and LOQ can be found in Appendix I.

3.5.2 Metals and essential elements

In this thesis, maternal whole blood was measured in blood samples from week 18 of gestation. Twelve metals and essential elements were determined in maternal whole blood, using inductively coupled plasma-sector field mass spectrometry (ICP-SFMS). These included both toxic/non-essential metals; arsenic, cadmium, cesium, lead, mercury, and essential elements; cobalt, copper, magnesium, manganese, selenium, and zinc. Mercury and arsenic are measures of total mercury and total arsenic and contain both inorganic and organic forms. However, in the Norwegian population, these total measures will largely reflect organic forms (Brantsæter et al., 2010). The blood analysis was mainly conducted at ALS laboratory group of Norway, but a few samples were also analyzed at the University of Lund as part of another MoBa project. The Norwegian Institute of Public Health has a framework agreement with the ALS laboratory, and they have until now analyzed approximately 2000 samples of maternal whole blood from MoBa. Like for the PFASs, internal quality control samples and procedure blanks were analyzed along with each batch of samples to ensure high quality of the determinations throughout the project. In addition, reference samples were included (Seronorm Trace Elements whole blood L-1, SERO AS, Billingstad, Norway) that were used as project-specific quality control (QC) samples. Case, control and QC samples were randomized to batch and blinded to the analysist. More detailed information on determination of metal and element concentrations in maternal blood can be found in Appendix II. For most of the metals/elements, concentrations above LOQ were reported, except for arsenic, cadmium, lead, and mercury, for which concentrations above LOD were reported (more details in Appendix II). Metals/elements concentrations were given in µg/L, except for magnesium, which was given in mg/L.

The metals and elements measured in whole blood that were used for the ASD, ADHD, and control samples (Paper II), were pulled from the biobank and analyzed in three

separate analytical rounds. In addition, some samples were analyzed at the University of Lund (~4th round). To account for analytical variation across analytical rounds, the metal/element concentrations were normalized for each participant using our QC samples (Seronorm reference material) analyzed in each of the analytical rounds. This is described further in Appendix III.

3.6 Covariates

Information on covariates were obtained from the MBRN and from the MoBa questionnaires answered during pregnancy and up to child's age three years. The MoBa study also included a food frequency questionnaire (FFQ) completed at 22 weeks' gestation, which was designed to capture the average dietary intake during the first four to five months of pregnancy, providing good validity for estimates of foods and nutrients (Brantsæter, Haugen, Alexander, & Meltzer, 2008). Potential adjustment variables were selected *a priori* based on existing literature and directed acyclic graphs (DAGs). Dagitty.net was used to draw the DAGs to aid in choosing covariates and to estimate the total effect (Textor, Hardt, & Knüppel, 2011). Based on the DAGs, you get a minimal adjustment set (the minimal selection of variables to be adjusted for in order to avoid a biased result) that includes potential confounders. Figure 4 shows a simplified DAG illustrating the association between maternal levels of chemicals during gestation and child neurodevelopment and the covariates included in the thesis.

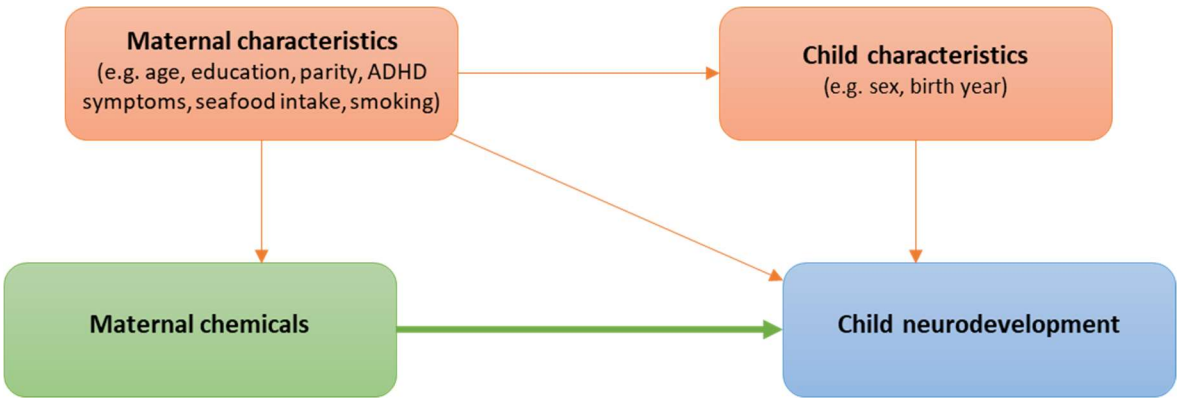

Figure 4. Simplified directed acyclic graph (DAG) illustrating the association between maternal levels of chemicals during gestation and child neurodevelopment.

3.7 Statistical analyses

Various statistical analyses have been used in this thesis which are described below. The quartile and quintile analyses were used to investigate whether there were dose-response

trends in our data. Investigation of dose-response relationships is important as it provides information on the shape of the exposure-outcome relationships. Categorizing into quartiles and quintiles, is the closest one can get to comparing different levels to an "unexposed" group (the lowest level). The doses represented in the categories can be useful in risk assessment of chemicals and in implementing prevention measures. The quartile and quintile models can be regarded as the main analyses across the papers. In addition, restricted cubic splines were included in two of the papers (Paper II and III) to assess non-linear relationships. Principal component analysis (PCA) or quantile-based g-computation approach was used to investigate mixture effects. When PCA was employed, it was used to reduce the number of variables to decide which analyses to run further in the quintile models. When quantile-based g-computation was used, it was after the main analyses to see whether any patterns among the metals/elements or PFASs would be revealed (e.g. toxic vs essential) and whether single compounds would drive potential associations. The metals, elements and PFASs were log-transformed if not specified otherwise.

Statistical analyses were conducted using Stata version 15 (StataCorp, 2019), the Statistical Package for the Social Sciences (SPSS) version 25 and 26 (Statistical Package for Social Sciences, 2020), and R version 3.6.2 and 4.0.0. In R, the "Amelia" (Honaker, King, & Blackwell, 2019), "qgcomp" (Keil, 2021), "foreign" (R Core Team, 2020), "psych" (Revelle, 2020), "readstata13" (Garbuszus et al., 2018), "ggplot2" (Wickham et al., 2020), and "tidyverse" (Wickham, 2019) packages were used.

3.7.1 Main models

In Paper I, the models included PFAS component scores as predictors derived from an exploratory PCA in which interaction analysis by child sex was performed. Oblimin rotation was chosen as this allows the components to be correlated, which can be the case when it comes to PFASs, independent of whether they are sulfonates or carboxylates. In Paper I, negative binomial regression analyses were done for ADHD symptoms and linear regression analyses for working memory, estimated IQ, and language skills. Based on the findings from the component models in Paper I, there was further investigation of the dose-response relationships between levels of individual PFASs categorized into quintiles and outcome variables (working memory) in separate linear regression models, with the lowest quintile as the reference group.

In Paper II and III, quartile models of the individual metals and elements or PFASs, also with the lowest quartile as the reference group, were investigated. The ASD and ADHD

diagnoses were investigated separately with multivariable logistic regression analysis. Interaction analyses by child sex and maternal education was performed in the quartile analyses.

In the main models in all the papers, results were evaluated with Šidák correction of confidence intervals to control for false discoveries/type 1 error. This was done using the following formula: $1-(1-\alpha)^{1/k}$ % confidence intervals. Where k = number of tests and $\alpha = 0.05$.

3.7.2 Restricted cubic splines

In Paper II and III, the metals, elements and PFASs were modelled using restricted cubic splines, with three knots at the 10^{th}, 50^{th}, and 90^{th} percentile of the metal, element, and PFAS distributions and tested against linear models. In order to reduce the influence of outliers, values below or at 1% were replaced with the value next to 1%, a method called winzorising (Glen, 2016). The same was done for values higher or at 99%. The models without outliers were compared to models where they were included.

3.7.3 Quantile-based g-computation

For Paper II and III, a quantile-based g-computation approach was used in order to analyze the joint effect of the metal/element or PFAS mixture on the outcomes. This method is developed to assess the effect of exposure mixtures on the outcome, giving estimates of the effect of simultaneous increase of all exposures in the mixture by one quantile (Keil, 2021; Keil et al., 2020). In this procedure weighted quantile sum regression and g-computation is combined, resulting in more flexible models. The metals/elements paper (Paper II) included the main mixture model (all 11 metals/elements), one that only contained essential elements (Se, Mn, Co, Cu, Zn, and Mg), and one mixture that only contained toxic/non-essential metals (As, Hg, Cd, Pb, and Cs). The PFAS and diagnoses paper (Paper III), included the main mixture model (all seven PFASs), one that contained sulfonates (PFOS, PFHxS, PFHpS), and one that contained carboxylates (PFOA, PFDA, PFUnDA, and PFNA). The sub-mixture groups were based on the structural groups (toxic vs essential and sulfonates vs carboxylates) and *a priori* assumptions. Weights, indicating the relative contribution of each metal/element/PFAS to the associated outcome, were computed for each mixture analysis.

3.7.4 Multiple imputation

In all the papers, multiple imputation by chained equations was used to replace missing data, both exposures and covariates. In Stata, 50 datasets were generated with the exposure and

outcome variables, covariates and auxiliary variables (Rubin, 1976; Sterne et al., 2009) using the mi ice command (Royston, 2007). The method for interval-censored data was used with specified upper and lower limits for imputed results for metals/elements and PFASs as limit of detection (LOD) and zero, respectively (Royston, 2007). The pooling procedure used in this thesis was mi estimate (Stata Press, 2017). Complete case analysis was performed for the quartile and quintile analyses, to compare with the imputed results. Before performing the quantile-based g-computation, multiple imputation generating 20 datasets was done.

3.8 Ethical considerations

This project was approved by The Regional Committee for Medical Research Ethics for Southeast Norway (ref. 2012/985–1). MoBa is regulated under the Health Registry Act and has conducted a Data Protection Impact Assessment (DPIA) in accordance with the new Personal Data Act, which has been approved by the Data Protection Officer at the Norwegian Institute of Public Health. Participation in MoBa is voluntary and based on written informed consent from the parents. By consenting, the participants agreed to donate biological material and information about themselves in addition to the permission of linkages to health registries and collection of data from medical records. Both parents consented to participation. The mother consented on behalf of the child until they reached 18 years of age. Participants have been able to withdraw from the study at any time. Version 9 and 12 of the MoBa quality-assured data files was used. There is additional approval from the NPR for the linkage between NPR and MoBa, identifying ADHD and ASD diagnostic cases. MoBa newsletters with research results are sent once or twice a year to current and previous MoBa participants.

The ADHD Study has approval from the Regional Committee for Health Research Ethics for Southeast Norway. Participation in the clinical assessments of the ADHD Study required an additional written informed consent. After the one-day assessment, the examiners went through the results with the parents of the assessed children. Parents were also offered a written report with information about the study, and a summary of the children's results. If requested, parents could also receive a written recommendation for further assessment at their local clinic.

The ABC Study has approval from the Regional Committee of Medical and Health Research Ethics for South-East Norway for retrieving register data and for the review of medical records. The clinical assessments conducted by the ABC Study and the review of the medical records were based on separate written informed consents.

4 Summary of papers

Paper I: Prenatal exposure to perfluoroalkyl substances and associations with symptoms of attention-deficit/hyperactivity disorder and cognitive functions in preschool children.

Aims: In Paper I, the objective was to investigate whether prenatal exposure to PFASs were associated with symptoms of ADHD and cognitive functioning (language skills, estimated IQ, and working memory) in preschool children.

Method: The study included 944 mother-child pairs from the ADHD Study, recruited from MoBa. Children aged three and a half years were assessed with; The Preschool Age Psychiatric Assessment interview, Child Development Inventory and Stanford-Binet (5th revision). Prenatal levels of seven PFASs included; PFOA; PFNA; PFDA; PFUnDA; PFHxS; PFHpS; and PFOS. Multivariable adjusted regression models examined exposure-outcome associations with two principal components extracted from the PFASs, followed by regression analyses of individual PFASs categorized into quintiles.

Results: PFAS component 1 consisted of PFHpS, PFOS, PFHxS and PFOA and PFAS component 2 consisted of PFDA, PFUnDA, and PFNA. There was a negative association between PFAS component 1 and nonverbal working memory [β=-0.08 (CI: -0.12, -0.03)] and a positive association between PFAS component 2 and verbal working memory [β=0.07 (CI: 0.01, 0.12)]. There were no significant associations with ADHD symptoms, language skills, or IQ. There was evidence for effect modification by child sex for verbal working memory and PFAS component 2, with associations only among boys. There were negative associations between nonverbal working memory and quintiles of PFOA, PFNA, PFHxS, PFHpS, and PFOS and positive associations between verbal working memory and quintiles of PFOA, PFNA, PFDA and PFUnDA. The associations were mainly in the highest concentration groups.

Conclusion: In this study there were no consistent evidence to conclude that prenatal exposure to PFASs are associated with ADHD symptoms or cognitive dysfunctions in preschool children aged three and a half years. There were some associations between PFASs and working memory, particularly negative relationships with nonverbal working memory, but also positive relationships with verbal working memory.

Paper II: Metal and essential element concentrations during pregnancy and associations with autism spectrum disorder in children and attention-deficit/hyperactivity disorder

Aims: The aim of Paper II was to investigate associations between gestational levels of toxic metals and essential elements and childhood diagnoses of ADHD and ASD.

Methods: The study was based on MoBa, with linkage to the Norwegian Patient Registry and included 705 ADHD cases, 397 ASD cases and 1034 controls. Gestational levels of 11 metals/elements included; arsenic; cadmium; cesium; cobalt; copper; lead; magnesium; manganese; mercury; selenium; and zinc. Multivariable adjusted logistic regression models examined associations between quartile levels of individual metals/elements and outcomes. Additionally, non-linear associations and the metal/element mixture on ASD and ADHD diagnoses were investigated.

Results: For ASD, there were increased risks in the second quartile of arsenic [OR = 1.77 (CI: 1.26, 2.49)] and the fourth quartiles of cadmium and manganese [OR = 1.57 (CI: 1.07 2.31); OR = 1.84 (CI: 1.30, 2.59)], respectively. In addition, there were negative associations between cesium, copper, mercury, and zinc with ASD. For ADHD, there was increased risk in the fourth quartiles of cadmium and magnesium [OR =1.59 (CI: 1.15, 2.18); [OR = 1.42 (CI: 1.06, 1.91)]. There were also some negative associations (e.g. mercury). In addition, there were non-linear associations between ASD and arsenic, mercury, magnesium, and lead, and between ADHD and arsenic, copper, manganese, and mercury. There were no significant findings in the mixture analyses.

Conclusion: There were several associations between levels of metals and elements during gestation and ASD and ADHD in children, including arsenic, cadmium, copper, mercury, manganese, magnesium, and lead. The results suggest that even population levels of these compounds may have negative impacts on neurodevelopment.

Paper III: Prenatal exposure to per- and polyfluoroalkyl substances (PFAS) and associations with attention-deficit/hyperactivity disorder and autism spectrum disorder in children.

Aims: The aim of Paper III was to investigate associations between prenatal levels of PFASs and childhood diagnoses of ADHD or ASD.

Methods: The study was based on MoBa with linkage to the Norwegian Patient Registry, and included 821 ADHD cases, 400 ASD cases and 980 controls. Prenatal levels of seven PFASs included; PFOA; PFNA; PFDA; PFUnDA; PFHxS; PFHpS; and PFOS. Multivariable adjusted logistic regression models examined exposure-outcome associations of the PFASs as quartiles. Additionally, non-linear associations and the PFAS mixture on ASD and ADHD diagnoses were investigated.

Results: For both ASD and ADHD, there was increased risk in the second quartile of PFOA [OR = 1.71 (CI: 1.20, 2.45); Q2: OR = 1.54 (CI: 1.16, 2.04)], respectively. There was also suggested evidence of non-linearity with an inverse U-shape. There were negative associations for both ASD and ADHD with PFDA and PFUnDA and between ASD and PFOS. In addition, some of the associations were modified by child sex and maternal education. The overall PFAS mixture was inversely associated with ASD [OR = 0.76 (95% CI: 0.64, 0.90)] as well as the carboxylate mixture [OR = 0.79 (95% CI: 0.68, 0.93)] and the sulfonate mixture [OR = 0.84 (95% CI: 0.73, 0.96)].

Conclusion: This study showed increased risk of ASD and ADHD in children prenatally exposed to PFOA. For some PFASs, as well as their mixtures, there were inverse associations with ASD and/or ADHD.

5. Discussion

5.1 Main findings

5.1.1 Summary of main findings

This thesis investigated associations between gestational levels of PFASs, metals, and elements and various outcomes of child neurodevelopment including ADHD symptoms and diagnosis, ASD diagnosis, and cognitive functions. This thesis compared the observed differential and general effects among the findings from three different papers. The main findings were that some of the chemicals were associated with neurodevelopment across domains and disorders, and that child sex and maternal education modified some of these relationships. In addition, this thesis identified non-monotonic associations between exposures and outcomes and investigated the relationships of chemical mixtures on neurodevelopment, separately for the metals/elements and the PFASs. PFOA, cadmium, lead, arsenic, magnesium, manganese, and copper were among the chemicals that were associated with ASD and ADHD. In addition, several of the PFASs were associated with nonverbal working memory. These associations will be the focus herein. Table 1 and 2 present an overview of the results.

Table 1. Overview of results for PFASs (Paper I and III).

	Higher score worse outcome			**Higher score better outcome**				
	ASD diagnosis	**ADHD diagnosis**	**ADHD symptoms**	**Language skills**	**Estimated Verbal IQ**	**Estimated nonverbal IQ**	**Verbal working memory**	**Nonverbal Working memory**
PFOA	Q2 ↗ ∩	Q2-4 ↗ ∩					Q3 ↗	Q5 ↘
PFNA							Q5 ↗	Q3 ↘
PFDA	Q3, Q4 ↘	Q4 ↘					Q5 ↗	
PFUnDA	Q3, Q4 ↘	Q3, Q4 ↘					Q5 ↗	
PFHxS								Q4 ↘
PFHpS								Q5 ↘
PFOS	Q3 ↘							Q5 ↘

Note: Q1 is reference in the quartile/quintile models. Quartiles (Q) with arrows indicates which quartiles and which directions that were significant. ∩ indicates non-linear inverse U-shape.

Abbreviations: arsenic (As); attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); quartile (Q); intelligence quotient (IQ); perfluorooctanoic acid (PFOA); perfluorononanoic acid (PFNA); perfluorodecanoic acid (PFDA); perfluoroundecanoic acid (PFUnDA); perfluorohexane sulfonate (PFHxS); perfluoroheptanesulfonic acid (PFHpS); and perfluorooctane sulfonate (PFOS).

Table 2. Overview of results for metals (Paper II).

	Higher score worse outcome	
	ASD diagnosis	**ADHD diagnosis**
As	Q2 ↗ ∩	Q3, Q4 ↘ _,
Cd	Q2, Q4 ↗	Q4 ↗
Co	-	Q4 ↘
Cs	Q4 ↘	Q4 ↘
Cu	Q2 ↘	Q2 ↘ U
Hg	Q2-4 ↘ __	Q3, Q4 ↘ __
Mg	Q4 ↗ U	Q4 ↗
Mn	Q4 ↗	Q3 ↘ U
Pb	U	
Se		
Zn	Q4 ↘	Q2 ↘

Note: Q1 is reference in the quartile/quintile models. Quartiles (Q) with arrows indicates which quartiles and which directions that were significant. Non-linear shapes are indicated with wither U, ∩, __ or _,

Abbrevations: arsenic (As); attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); cadmium (Cd); cesium (Cs); cobalt (Co); copper (Cu); quartile (Q); intelligence quotient (IQ); lead (Pb); magnesium (Mg); manganese (Mn); mercury (Hg); selenium (Se); zinc (Zn).

5.1.2 PFASs and neurodevelopment

In Paper III, prenatal PFOA exposure was found to be associated with increased risk of both ASD and ADHD. For ASD, there was higher risk among those who were exposed to mid-range levels of PFOA, compared to those with the lowest levels of exposure. For ADHD, there was increased risk across all exposure levels of PFOA (compared to the lowest level). The restricted cubic spline models (inverted U-shapes) indicated that mid-range levels of prenatal PFOA were associated with increased risk for diagnosis of ASD and ADHD. Some studies on prenatal PFOA have reported similar associations as observed herein. A study from the USA found increased risk of ASD with increased levels of prenatal PFOA (Oh et al., 2020). Likewise, a study based on pooled data from Greenland, Poland, and Ukraine reported increased levels of hyperactivity in children who had been prenatally exposed to the highest levels of PFOA (Høyer et al., 2015). Furthermore, results from a Danish study found a higher

risk of ADHD diagnosis in children exposed prenatally to the highest levels of PFOA compared to those exposed to the lowest levels (Liew et al., 2015a). In general, the results from epidemiological studies on prenatal exposure to PFASs and ASD and ADHD are inconsistent, as many studies have documented null effects (Forns et al., 2020; Liew et al., 2018; Rappazzo et al., 2017; Vrijheid et al., 2016). However, few studies have investigated these relationships non-linearly.

In Paper I, nonverbal working memory assessed among preschoolers was the most frequent cognitive outcome measurement associated with the different exposures. The associations were mainly identified for the highest exposure group of maternal PFAS levels, notably among the sulfonates; PFOS, PFHpS, and PFHxS, in addition to PFOA. Although PFOA is a carboxylate, it was found to be highly correlated with PFOS and was grouped in the same component as the sulfonates in the PCA. The findings of associations between the prenatal PFASs and nonverbal working memory appear to be fairly novel, as few studies have investigated these exposure-outcome associations. Nevertheless, the results are in line with a study of children aged five to eight years, reporting an association between increased levels of prenatal PFOS and lower working memory function and impaired metacognition, which is a measure of multiple executive functions (Vuong et al., 2016). Furthermore, two other studies measuring concurrent PFAS levels in children found associations with poorer executive function (Stein, Savitz, & Bellinger, 2014; Vuong et al., 2018a), although one reported adverse results only among girls (Stein et al., 2014).

5.1.3 Toxic metals and neurodevelopment

Lead is a well-known developmental neurotoxicant (EFSA, 2010; Grandjean & Landrigan, 2014) and postnatal lead exposure has been associated with detrimental effects on IQ in children, even at low blood levels (e.g. < 1 μg/dL) (Budtz-Jørgensen, Bellinger, Lanphear, Grandjean, & International Pooled Lead Study Investigators, 2013; Lanphear et al., 2005). Lead and neurodevelopment have been explored mostly in relation to IQ and ADHD (Kern et al., 2015; Vrijheid et al., 2016). Many studies have shown evidence for associations between childhood lead exposure and ADHD or related symptoms, but there are fewer prospective studies measuring lead prenatally (Kern et al., 2015; Vrijheid et al., 2016) or investigating associations with ASD (Bjørklund et al., 2018; Mason, Harp, & Han, 2014). In Paper II, no significant evidence for associations between prenatal lead exposure and diagnosis of ADHD was found. Still, there was a U-shaped association between lead and ASD diagnosis, indicating that both low and high prenatal exposures to lead were associated with increased

risk of ASD in children. This is in line with other studies reporting non-linear associations between lead exposure and neurodevelopmental outcomes, such as IQ (Vrijheid et al., 2016). Apparently, non-linear dose-response relationships, particularly with low levels of lead, is not uncommon (European Food Safety Authority, 2010).

Non-linear associations were seen between prenatal arsenic and ASD and ADHD diagnoses (Paper II). For ASD, there was an inverse U-shape, indicating higher risk at mid-range levels of arsenic; this was also observed in the quartile analyses, with mid-to-low range levels indicating higher risk compared to those with the lowest *in utero* exposure. For ADHD diagnosis, it was more of a U-shaped association, signifying increased risk/higher symptom load at both lower and higher levels of prenatal arsenic. Unpublished work from the NeuroTox group found the same U-shape associations between prenatal arsenic and ADHD symptoms in preschoolers (Villanger et al., in prep). As arsenic is a recognized developmental neurotoxicant, the increased risk observed herein is in line with the epidemiologic literature (Bjørklund et al., 2018; Grandjean & Landrigan, 2014). However, most studies have focused on cognitive functions, so there is still a lack of studies investigating ASD and ADHD (Rodríguez-Barranco et al., 2013), in addition to a lack of prospective studies measuring arsenic during gestation (Bjørklund et al., 2018). An important aspect to note is that the arsenic measure in the current thesis consists mainly of organic forms from fish consumption (e.g. arsenobetaines), as opposed to the inorganic form that has been documented as a neurotoxicant (Agency for Toxic Substances and Disease Registry, 2007). There are still uncertainties about the toxic properties of organic forms of arsenic (Molin, Ulven, Meltzer, & Alexander, 2015; Agency for Toxic Substances and Disease Registry, 2007). The increased risks of ASD and ADHD from prenatal exposure to mainly organic arsenic in the present thesis could thus be of importance.

Paper II also revealed increased risks of ASD and ADHD in children exposed to the highest maternal levels of cadmium compared to the lowest exposure levels, which is in line with the emerging epidemiological literature (Liu, Cai, Liu, Chen, & Wang, 2019; Sanders, Henn, & Wright, 2015). Cadmium is still only a suspected developmental neurotoxicant (EFSA, 2009), which makes these findings noteworthy. There is increasing evidence of adverse effects on cognitive development following prenatal and childhood cadmium exposure (Liu et al., 2019; Sanders et al., 2015). In support of this, results from unpublished work found a non-linear U-shaped association between cadmium and verbal IQ (Villanger et al., in prep). For ASD and ADHD, there are still few prospective studies on prenatal cadmium exposure (Rodríguez-Barranco et al., 2013; Vrijheid et al., 2016).

5.1.4 Essential elements and neurodevelopment

For magnesium, manganese, and copper, there were U-shaped associations with either ADHD and/or ASD. This U-shape detected for magnesium, manganese, and copper is not uncommon for associations between essential elements and neurodevelopmental outcomes, as both high and low levels can have harmful effects (Bjørklund, 2013; Lucchini et al., 2017).

For magnesium, the results (Paper II) indicated that children prenatally exposed to the highest levels had increased risk of both ASD and ADHD diagnoses. In addition, U-shaped non-linear relationships were seen for ASD. Seemingly, this thesis is one of the first to investigate gestational levels of magnesium and ADHD and ASD in children. Other studies have instead focused on concurrent measurement of magnesium levels in children with diagnoses (Botturi et al., 2020). Two meta-analyses found that children diagnosed with ADHD and ASD, respectively, had lower magnesium levels compared to neurotypical developing children (Huang et al., 2019; Saghazadeh, Ahangari, Hendi, Saleh, & Rezaei, 2017). However, unpublished results showed an inverse U-shape for verbal working memory, indicating higher risk/lower scores at both low and high levels of prenatal magnesium and a similar U-shape with ADHS symptoms as for ASD in this thesis (Villanger et al., in prep).

A U-shaped relationship was observed for maternal manganese with ADHD diagnosis in the present thesis (Paper II). In addition, there was an association between exposure to the highest gestational levels of manganese (compared to the lowest levels) and increased risk of ASD. Altogether, the results from previous studies on ASD and ADHD and manganese were inconclusive and have been based on cross-sectional studies in children, measuring manganese in different matrices (e.g., air distribution, tooth enamel, urine, hair, blood) (Lucchini et al., 2017). One prospective study detected no significant associations between manganese in umbilical cord serum and ADHD in the child (Ode et al., 2015).

This thesis also detected a U-shaped relationship for copper with ADHD diagnosis. This association between gestational levels of copper and ADHD diagnosis is fairly novel, as few other studies have investigated this. The findings are in line with Spanish research reporting adverse effects from elevated prenatal copper exposure on neuropsychological development in five-year-olds (Amorós et al., 2019). However, a study on prenatal metal exposure and ADHD symptoms in children did not detect any significant associations between copper and ADHD symptoms (Forns et al., 2014).

5.1.5 Observed similarities and differences in the results across outcomes

The developmental origins of ASD and ADHD are intertwined, and these disorders have strong genetic links (Dougherty, Evans, Myers, Moore, & Michael, 2016; Rommelse et al., 2011). In addition, comorbidity is common among children with ASD and ADHD (Gillberg, 2010; Rommelse et al., 2011). It has been suggested that ASD and ADHD share neurochemical and neurodevelopmental pathways and that the disorders co-exist on a continuum of clinical expression (Anttila et al., 2018; Jokiranta-Olkoniemi et al., 2016; Kern et al., 2015; Taylor et al., 2019; Wade, Prime, & Madigan, 2015). The findings in the present thesis appear to support that several chemicals *in utero* can represent overlapping environmental risk factors for ASD and ADHD and that the chemicals may target the neurodevelopmental pathways that are shared between these disorders.

Studies have shown that children with ASD and ADHD diagnoses often have deficits in executive function (Geurts et al., 2004). As working memory is a sub-component of executive functions, the findings of the PFASs' adverse effects on nonverbal working memory, in addition to the increased risks of ASD and ADHD, are particularly interesting. These results could suggest that chemicals, like some of the PFASs, may affect neurobehavioral domains mutual for ASD and ADHD, such as working memory.

Some differences were also observed in the relationships between the chemicals and the disorders. This could be attributed to differential vulnerability to the chemicals during fetal development (Kern et al., 2015). For metals and elements, specific features of prenatal dysregulation of metal and essential element metabolism may be present among children with ADHD and ASD (Arora et al., 2017; Austin et al., 2019). It has been proposed that uptake of toxic metals and deficiency of essential elements during fetal development can increase the risk of ASD in children (Arora et al., 2017; Austin et al., 2019).

5.1.6 Counterintuitive associations

Counterintuitive associations for mercury, arsenic, and some PFASs with working memory and diagnoses of ASD and ADHD were observed in this thesis. Several of the apparently "protective" associations may be related to maternal consumption of seafood as a main source of both PFASs and several toxic metals (e.g. mercury, arsenic), both containing polyunsaturated fatty acids and microelements. Chemicals from seafood consumption represent a challenge when investigating neurodevelopmental outcomes in epidemiologic studies. Even though the potential negative confounding was adjusted for in the regression models, it could be that some of these chemicals (e.g. mercury, arsenic, and PFASs) better

reflect seafood consumption than the estimated maternal intake of fish and seafood from the FFQ. If so, that could be a possible explanation for the inverse relationships observed in this thesis.

Inverse associations between prenatal exposure to PFASs and neurodevelopment have also been frequently reported in other studies (e.g. Lien et al., 2016; Liew et al., 2015a; Stein, Savitz, & Bellinger, 2013). The "live birth bias" has been proposed as a possible explanation of such associations (Liew, Olsen, Cui, Ritz, & Arah, 2015b). In short, live birth bias may occur when studying the relationship between prenatal exposure to environmental factors and later health outcomes only in live births (Liew et al., 2015b). If the exposure under scrutiny can contribute to pregnancy termination, the number of exposed live born children will be reduced, thereby affecting the results. Another suggested explanation has been that PFASs can activate the proliferator-activated receptors (PPARs) alpha and gamma, which have neuroprotective and central-nervous-system anti-inflammatory properties (Quaak et al., 2016; Stein et al., 2013).

5.1.7 Mixtures

This thesis investigated mixtures separately among the PFASs (Paper III) and the metals and elements (Paper II). Only the associations between PFAS mixtures and ASD diagnosis were significant. The total mixture, the sulfonate mixture and the carboxylate mixture, were all negatively (inversely) associated with ASD, indicating decreased risk of ASD diagnosis following prenatal exposure to PFASs. However, as many of the PFASs were inversely associated with ASD diagnosis in the quartile models, this pattern among the compounds was not that surprising. Regarding the lack of significant mixture associations for the other outcomes and metals/elements, both positive and negative relationships between exposure and outcomes were observed, possibly resulting from the chemicals cancelling each other out. The mixture approach; quantile-based g-computation utilized in this thesis, is a fairly new method (Keil et al., 2020) and has seemingly not been used before in this particular research context. In Paper I, PCA was used to investigate the joint effects of intercorrelated PFASs and to reduce the number of tests.

Although there is a lack of studies investigating multiple toxicants using mixture approaches, a few studies have investigated prenatal/perinatal chemical mixtures and similar outcomes such as the present thesis. Like the inverse associations observed in this thesis, research from the USA found associations between increasing levels of PFOA and lower autistic behavior scores in a multi-pollutant model (to reduce the number of exposures) with

several types of chemicals, including PFOS, PFNA, and PFHxS (Braun et al., 2014). Likewise, a Danish study using principal component analysis to reduce the number of toxicant exposures including PFASs found inverse associations between a component with PFASs (PFOS, PFOA, PFOSA) and ASD diagnosis (Long et al., 2019). In contrast, research from the USA showed an association between an overall measure of metals (antimony, arsenic, cadmium, chromium, lead, manganese, mercury, nickel) and increased ASD risk (Roberts et al., 2013). Furthermore, a study from the Faroe Islands reported positive associations between maternal levels of PFOA and PFOS and behavioral problems in children at age seven, in a multi-pollutant model investigating joint effects of multiple chemicals (Oulhote et al., 2019). In addition, a Norwegian study found associations between early-life exposure to PFOS and increased risk of ADHD, but not with the other PFASs, using a multi-pollutant model that was also adjusted for other chemicals (Lenters et al., 2019). A study from Arctic Quebec showed that an association between prenatal lead and impulsivity was stronger with higher levels of prenatal mercury and PCB (Boucher et al., 2012).

5.1.8 Sex differences and maternal education

While some previous studies report important modifying factors in the relationships between PFAS, metals, and elements and neurodevelopment, such as child sex, most studies lack the power to investigate these factors. All the papers in this thesis investigated effect measure modification by child sex, but interaction was established only for ASD diagnosis and verbal working memory. For both mercury and the majority of PFASs, the observed inverse associations (decreased risks/better scores) were found among boys, while for some of the relationships, girls showed an opposite pattern with increased risk/lower scores. However, for PFOA and ASD there was a higher risk of ASD among boys than among girls. Contrary to a meta-analysis on early-life-exposure to PFOS and PFOA (Forns et al., 2020), this thesis did not detect any modifying effect by child sex on the associations with ADHD. The meta-analysis did, however, report increased risk of ADHD among girls (Forns et al., 2020), which is consistent with the findings for ASD in this thesis. In this research context, few studies have investigated sex differences. Among the studies that have, there is a lack of consistent patterns of the exposure-outcome relationships in boys and girls. Some studies report higher risk/poorer scores among girls (e.g. Lenters et al., 2019; Oulhote, Steuerwald, Debes, Weihe, & Grandjean, 2016; Vuong et al., 2016; Vuong et al., 2018b) and others among boys (e.g.

Braun et al., 2014; Ryu et al., 2017; Shin, Bennett, Calafat, Tancredi, & Hertz-Picciotto, 2020), regardless of the chemical that was investigated.

Gestational levels of metals, elements, and PFASs have been related to socioeconomic status (Montazeri et al., 2019). ADHD and ASD among children have also been associated with parental socioeconomic status (Lung et al., 2018; Russell, Ford, Williams, & Russell, 2016). Socioeconomic factors and exposure to chemicals relate to nutritional regimens, exposures throughout a residential area, and occupational exposures. There are differences among countries as to which population segments have the greatest contaminant burden. In many countries, there is still a link between low socioeconomic status and high exposure to contaminants (Rauh & Margolis, 2016). The fact that some population groups seem to be disproportionately exposed to hazardous chemicals has been termed "environmental injustice" (Aschner et al., 2021; Rauh & Margolis, 2016). For example, exposure to lead is more widespread in poor communities in the United States (Rauh & Margolis, 2016). However, for compounds such as mercury and some PFASs, it has been reported that people in higher socioeconomic positions have higher exposure levels (Brantsæter et al., 2013; Montazeri et al., 2019; Tyrrell, Melzer, Henley, Galloway, & Osborne, 2013). Even though Norway is a nation with a lower degree of income disparities compared to other countries, research using MoBa has reported that levels of PFOA and PFOS increased with educational and household income (Brantsæter et al., 2013). In Norway, exposure to some of the metals and PFASs have occurred mainly through seafood. Seafood can also be used as a socioeconomic marker, because its intake is positively correlated with socioeconomic status (Touvier et al., 2010).

In two of the papers in this thesis (II and III), effect measure modification by maternal education was investigated as a proxy for socioeconomic status. For maternal education, different patterns in the chemicals and the neurodevelopmental outcomes were observed, which complicates the interpretation. For maternal manganese and cadmium, there were higher odds of ADHD among children of mothers with university/college education. It could be that the higher manganese levels resulted from intake of multivitamin supplements, leafy greens, or seafood, as these are dietary sources of manganese. Finding higher odds of ADHD among children of mothers with higher education in mid-range levels of cadmium appeared to contradict previous research, where higher levels of cadmium exposure in pregnant women was associated with lower education (Caspersen et al., 2019; Montazeri et al., 2019; Tyrrell et al., 2013). Smoking has been one of the most common sources of cadmium, and is related to lower socioeconomic status, but omission of smokers did not change the results herein. Perhaps other sources, such as seafood intake (Birgisdottir et al., 2013; Agency for Toxic

Substances and Disease Registry, 2012), contributed more to cadmium exposure revealed in this thesis.

For PFOA and PFOS, there were higher odds of the child having an ASD diagnosis among mothers with college/university education. This could be related to higher gestational levels of PFOA and PFOS in women with more years of education and higher household income, as have been reported in another Norwegian study using MoBa data (Brantsæter et al., 2013). However, for PFUnDA, PFNA, and PFHxS, there were higher odds of ASD diagnosis among children whose mothers had less education. The same pattern was observed for PFUnDA and ADHD diagnosis. The findings in this thesis for ADHD and PFUnDA and some of the results for ASD were similar to those of a study where stratified analyses indicated higher odds of ADHD with early-life exposure to PFASs among offspring of mothers with lower education (Forns et al., 2020).

5.2 Methodological considerations

Methodological considerations should be addressed when interpreting the results from this thesis. Herein, choices of statistical methods including handling of missing data and correction for multiple testing will be addressed. Potential violations to internal validity including selection bias, information bias, and confounding will also be discussed. In addition, considerations about external validity (generalizability) will be deliberated upon.

5.2.1 Missing data and correction for multiple testing

There are several ways to compensate for missing data. In all the papers included in this thesis, missing data for covariates and exposures were replaced with multiple imputation. The main analyses were compared to complete case analysis, to inspect that the patterns were not deviating substantially between the complete cases and the imputed ones. By just running complete case analysis, the sample size will be reduced and can therefore reduce statistical efficiency of estimates and be a source of bias (Sterne et al., 2009). Multiple imputation is one way to overcome bias when it is likely that the data are missing at random (Sterne et al., 2009). When conducting multiple imputation, several datasets are generated where missing values are imputed based on related information to the exposure-outcome relationship as well as the main variables, then the results from the datasets are combined (Sterne et al., 2009).

For the main analyses in all the papers, correction of multiple testing to compare with non-corrected analyses was performed. A method similar to the Šidák correction to control for familywise error rate (false discoveries or type 1 errors) was applied, generating more

conservative confidence intervals. Type 1 errors can lead to reporting associations that are false (false positives). This is more likely to occur when many analyses/tests are performed. However, type 2 errors mean reporting null effects when there actually are significant associations present (false negatives). Acknowledging both errors, results with and without correction of significance levels due to multiple testing were compared.

5.2.2 Selection bias

Selection bias can occur if certain variables influence the participation rate and/or follow-up rates (Lash, Fox, & Fink, 2009). Because of the initial participation rate (41%) in the MoBa cohort and subsequent attrition during follow-up (Magnus et al., 2006), potential selection bias cannot be ruled out in this thesis. Among the MoBa participants there was an underrepresentation of women living alone, young mothers, mothers with more than two previous births, with previous stillbirths, and smokers, while mothers taking multivitamin supplements and folic acid supplements were overrepresented, compared to the general Norwegian population (Nilsen et al., 2009).

Preceding studies of self-selection in MoBa have claimed that there has been little difference in the associations of exposure and outcomes, including neurodevelopmental outcomes, between the participants and the general population (Nilsen et al., 2013; Nilsen et al., 2009). However, a later study asserted that these differences might be larger than previously anticipated (Biele et al., 2019).

In Paper I, the sample consisted of participants from the ADHD Study. Of the eligible participants in MoBa, 35% agreed to participate in the sub-study (Øvergaard et al., 2018). Since most participants in the ADHD Study were recruited based on high scores on ADHD-related symptoms, it is a selected group and those included in the present project sample thus exhibit more symptoms than a general child population. This selection may have affected the estimates from the exposure-outcome associations. Selection bias can therefore not be ruled out in the paper with participants from the ADHD Study.

However, in Paper II and III, the sample consisted of diagnoses from the NPR (and random controls from MoBa). Using registry data is an advantageous way to account for attrition, as the mothers in this study only had to complete the first questionnaire and have available blood samples from pregnancy to be included. In addition, since Norway has universal healthcare, it ensures that economic disparity in healthcare access will not result in selection biases in clinical diagnoses. Therefore, in the papers with the NPR diagnoses, selection bias may be less of an issue.

5.2.3 Information bias

Information bias refers to measurement errors in the data. This will be discussed regarding assessment of exposures, outcomes, and covariates in the present thesis.

Exposures:

The exposures in this thesis were maternal concentration levels of metals, elements, and PFASs. When using measured levels of chemicals, there is always the possibility that there could be laboratory measurement errors. To overcome this issue, the laboratory conducted analysis of internal quality control samples and procedure blanks with each batch of samples. However, the blood samples included in Paper II were analyzed for metals and elements in three separate analytical rounds, in addition to some samples that were analyzed at a different laboratory, which could result in variations in concentrations across analytical rounds and/or laboratories. To account for analytical variation across analytical rounds, the metal/element concentrations were normalized for each participant using project-specific quality control samples analyzed in each of the analytical rounds (see Appendix I and II for further details). Additionally, a sensitivity analysis comparing models with and without the Lund sample was done, in order to check that laboratory did not substantially affect the results.

In the present thesis, maternal blood samples from mid-gestation was used as a proxy of prenatal toxicant/element exposures. Other measures of prenatal exposures can be done in for example hair, urine, amniotic fluid, umbilical cord blood, breast milk, and deciduous teeth (Heyer & Meredith, 2017). Most of the chemicals in the present thesis have relatively long half-lives, so the concentration levels of these are expected to remain relatively stable during pregnancy. A study using data from MoBa, in addition to other European birth cohorts, found moderate to high correlations between maternal levels and child levels of PFOS and mercury (Haug et al., 2018). In addition, a review reported strong correlations between PFASs measured in maternal blood samples during pregnancy and umbilical cord blood (Aylward et al., 2014). Regarding metals and essential elements, there is a larger variation concerning half-lives, depending on the chemical under scrutiny (Aylward et al., 2014). However, for some of the toxic metals, like lead and mercury, there were strong associations between maternal blood and umbilical cord blood (Aylward et al., 2014).

Outcomes:

In the papers with diagnostic case groups from the NPR (Paper II and III), the validity of diagnoses differed between ADHD and ASD. The clinical basis for the ADHD NPR registrations yields concern that alternative diagnoses should have been considered, as only

half of the diagnoses were reliably documented (Surén et al., 2018). However, for ASD the predictive value of ASD diagnoses was found to be high (Surén et al., 2012).

For the dimensional outcomes from the ADHD Study, other considerations must be discussed. Although the assessments of cognitive and neuropsychiatric symptoms were done with well-validated instruments, it can be a challenge to conduct these tests and interviews with preschoolers. Most cognitive functions begin to develop in early childhood, but there are several periods of development that must have occurred before the skills are fully functional and can be assessed adequately (Anderson, 2002). The development of cognitive skills goes through a developmental sequence starting with emerging (early acquisition, but not yet functional), developing (partially acquired, but not fully functional), and established (fully functional) functions (Anderson, 2002). Therefore, it could be that the impact of prenatal exposure to chemicals on cognitive functions only will be apparent when the child is older, and their abilities are fully functional and when the demands for performance (e.g. school) is higher. In addition, the test measures for cognitive functions may not only capture the trait, but also the ability of the child to perform on demand. Furthermore, as many cognitive functions are inter-dependent, it can be hard to isolate the measures. For example, language is closely related to the verbal features of working memory (Baddeley, 2003; Leonard et al., 2007).

Covariates:

A number of the included covariates in this thesis were based on self-report. With self-reported variables, response bias can be an issue. Response bias can lead to inaccurate information, that was either consciously or subconsciously done by the participant. Social desirability bias is a type of response bias that can be common when answering questionnaires about lifestyle and habits (Piedmont, 2014). For example, pregnant women may have been reluctant to give the correct information about smoking during pregnancy. Other variables such as income, education, mental health, or diet can also be affected by this type of bias.

5.2.4 Confounding

Some of the most important confounding factors in this thesis were variables such as maternal seafood consumption, age, parity, and education. As stated previously, maternal seafood consumption represents a challenge in observational studies as it is both a source of neurotoxicants like PFASs, mercury, arsenic, and selenium, but also a source of essential nutrients important for intrauterine brain development. This makes it an example of negative confounding (Choi et al., 2008). It is important to adjust for seafood intake to avoid

underestimation of effects from toxicants (Budtz-Jørgensen et al., 2007; Choi et al., 2008). Another issue is that, at least in MoBa-based studies, metals such as mercury, arsenic, and PFASs may better reflect maternal seafood consumption than the estimated seafood intake based on self-report in the FFQ (Brantsæter et al., 2010; Haug et al., 2010). Thus, disentangling the adverse effects of these environmental chemicals on brain development from the positive impacts of nutrients from fish consumption is challenging, as some of the counterintuitive results in this present thesis may indicate. Thus, even though it was adjusted for estimated fish intake, there could be residual confounding.

In studies with an epidemiological design, residual confounding – meaning confounding by factors that that were not included, not measured, or not accurately measured in the study, is always a possibility. In addition to fish intake, additional examples of residual confounding are other environmental contaminants contributing to the "cocktail effect" of chemicals, such as PCBs, PBDES, and phthalates, influencing both the maternal chemical levels and child neurodevelopment.

5.2.5 External validity

External validity refers to the extent in which results from studies can be generalized to other populations or the general population of which the sample was retrieved from (Kemper, 2017). In this thesis, this applies to the Norwegian population and the pregnant women specifically. As mentioned in the section about selection bias (section 5.2.2), there were several characteristics among the MoBa women that differed when comparing to the general population, with an underrepresentation of young mothers, low educated mothers, smokers, and minorities. Thus, generalizing the results in this thesis to these segments, must be done with caution.

Another concern with generalizability is the ability to compare the findings to those from other countries. Norway, like the other Scandinavian countries, is a relatively homogeneous nation with regards to ethnicity and socioeconomic factors, making global generalizations more challenging. In addition, Norway has universal access to health care. Together, this can make it difficult to compare results from MoBa studies to other countries, where there are for example larger socioeconomic differences.

5.3 Clinical and public health implications

Worldwide, mental disorders are among the leading causes of disability in children and youth (Erskine et al., 2015). Poor mental health has serious individual and social consequences

when people are unable to work, fulfill their potential, or contribute to their communities and societies (Rice & Barone, 2000; United Nations, 2021). Mental health has been raised as a neglected issue that needs attention if one is to reach the development goals for mental health set by the United Nations (United Nations, 2021). Identifying potential risk factors for neurodevelopmental disorders and deficits is important in prevention work and in understanding the etiologies. Findings from this thesis, together with other research in this field, can be used to map potential risk factors for ADHD, ASD, and cognitive deficits, and to support prevention efforts.

In this thesis, there were several associations between the chemicals and neurodevelopmental outcomes, some supporting previous research, and some making new contributions. Although the effect sizes of the findings in this thesis might not be large, they could have considerable impact in settings where people are more exposed. Small effects can also have a considerable impact on the society at large (Rice & Barone, 2000).

The fact that there were some similarities for the chemicals' associations with preschool working memory and older children's diagnoses of ASD and ADHD is of particular interest in a clinical perspective, especially since working memory deficits are common in children with ADHD and ASD. The sex differences observed herein are also noteworthy as ASD and ADHD have a male preponderance. Although there were some differences in directionality of the interactions, the general pattern observed in this thesis was that the inverse ("protective") associations were mostly observed among boys.

While the genetic components of neurodevelopmental disorders cannot be prevented, exposures to environmental chemicals can potentially be reduced. There is, however, little knowledge of the levels of the environmental chemicals that the Norwegian population at large, including pregnant women, are exposed to today. Most knowledge about chemical levels in Europe has been based on cohort studies dating back in time, implying that present-day levels and what the populations nowadays are being exposed to are largely unknown. Regular monitoring of population exposure levels is needed, not only considering the potential effects of chemicals on neurodevelopment, but also for other health aspects. For instance, the USA has a monitoring program for health and nutritional status; the National Health and Nutrition Examination Survey (NHANES) (Centers for Disease Control and Prevention, 2021). In the USA, they also screen children from vulnerable populations (e.g. those with low SES) for blood lead levels (Mayans, 2019). There is no such screening among Norwegian children. Although there are smaller differences in exposure due to SES in Norway, some population segments should perhaps be monitored. Among those are people

who consume large amounts of game meat hunted with lead bullets, and people who have a substantial intake of fish that often contain high levels of arsenic and mercury. Better monitoring of contaminant levels in fish and seafood, as well as in game meat, could also better inform risk management of exposures in vulnerable subpopulations, such as the dietary recommendations given to fertile and pregnant women.

5.4 Strengths and limitations

Some study limitations have been addressed in the section on methodological considerations. There are, however, other limitations that should be acknowledged. Although girls were oversampled when possible, there were still fewer girls in the ASD and ADHD case groups, making the sex-specific associations for girls less reliable. It is therefore possible that small numbers of girl cases across quartiles is resulting in the appearance of heterogeneity in estimates by child sex. In addition, there was no information about comorbidity, as ICD-10 does not allow comorbid diagnoses. However, there could still be an overlap of symptoms of ASD and ADHD across the diagnoses. Another limitation was the lack of possibility to adjust for maternal ASD, resulting in not accounting for familial confounding of risk in these exposure-outcome-relationships. Also, the self-reported maternal ADHD symptoms may not have been sufficient to account for familial confounding. Regarding the exposure to PFASs, a potential limitation includes not being able to adjust for glomerular filtration rate. This could be a source of residual bias in this thesis, as glomerular filtration rate influences the urinary excretion of PFASs (Verner et al., 2015). Regarding metals and elements, another limitation was not being able to adjust for maternal iron intake, as this can increase uptake of metals and elements (Meltzer et al., 2010).

The main strength of this thesis was the large sample size and the prospective case-cohort design. Additionally, the use of clinician-based diagnoses as well as clinical tests performed by specialized clinicians was a major advantage. Another major strength was the availability of a large number of relevant covariates collected prospectively during pregnancy, in order to account for residual confounding pathways and to investigate potential effect measure modifiers. Particularly investigating interaction by child sex and maternal education is a strength, which is lacking in several other studies. Investigating non-linear associations and chemical mixtures among the PFASs and metals/elements is also a strength.

5.5 Future research

Future studies should combine cohorts to increase their power to detect small effect sizes as well as investigating important potential modifiers between chemical exposures and neurodevelopmental outcomes. There should also be further investigation of the sex-specific associations depicted in this thesis, preferably with a more balanced sex distribution. Chemicals' interplay with sex steroids could offer an understanding of mechanistic underpinnings of sex-specific susceptibilities for early chemical exposures and later neurodevelopmental deficits in children and youth. Since socioeconomic factors seem to contribute to the understanding of the relationship between chemical exposure and neurodevelopment, this should be further elaborated upon. Employing genetically informed designs in combination with investigation of environmental factors should also be done in studies of potential etiological factors contributing to neurodevelopment. In addition, modelling of exposures is still in its infancy and could, if proved successful, allow for larger samples that are often hindered by high costs of pulling blood samples. As mentioned, there are uncertainties about the health effects of the new replacement compounds that are currently on the rise. This is something that also should be investigated. Including comorbid diagnoses/symptoms should be done, to further examine the shared etiological pathways for ASD and ADHD.

6. Conclusion

In this thesis, there were several significant associations between prenatal exposure to chemicals and various neurodevelopmental outcomes. The most prominent were PFOA, cadmium, lead, arsenic, magnesium, manganese, and copper, with increased risk of ASD and/or ADHD diagnosis. The highest maternal levels of PFOA, PFNA, PFHxS, PFHpS, and PFOS were significantly associated with lowered scores on nonverbal working memory in preschoolers. Several associations were modified by child sex and maternal education and some of the relationships were non-linear. There were few findings from the mixture analyses; only inverse associations between ASD and PFASs were apparent.

The observed parallel findings for ASD, ADHD and working memory for some of the exposures (e.g PFOA) could imply that some of the prenatal toxicants and elements affect neurobehavioral domains mutual for ASD and ADHD, such as working memory. Altogether, the results in the present thesis support that *in utero* exposure to some of the investigated toxicants and elements may represent overlapping environmental risk factors for neurodevelopmental disorders and cognitive deficits.

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Papers I-III

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Prenatal exposure to perfluoroalkyl substances and associations with symptoms of attention-deficit/hyperactivity disorder and cognitive functions in preschool children

Thea S. Skogheim[a,*], Gro D. Villanger[a], Kjell Vegard F. Weyde[a], Stephanie M. Engel[b], Pål Surén[a], Merete G. Øie[c,d], Annette H. Skogan[e], Guido Biele[a], Pål Zeiner[f,g], Kristin R. Øvergaard[f], Line S. Haug[a], Azemira Sabaredzovic[a], Heidi Aase[a]

[a] Norwegian Institute of Public Health, PO Box 222, Skøyen, N-0213, Oslo, Norway
[b] Gillings School of Global Public Health, University of North Carolina at Chapel Hill, 2104C McGavran-Greenberg Hall CB 7435, Chapel Hill, NC, 27599, USA
[c] Department of Psychology, University of Oslo, PO Box 1094, Blindern, N-0317, Oslo, Norway
[d] Research Department, Innlandet Hospital Trust, PO Box 104, N-2381, Brumunddal, Norway
[e] The National Centre for Epilepsy, PO Box 4956, Nydalen, N-0424, Oslo, Norway
[f] Division of Mental Health and Addiction, Oslo University Hospital, PO Box 4956, Nydalen, N-0424, Oslo, Norway
[g] Department of Clinical Medicine, University of Oslo, PO Box 1171, N-0318, Oslo, Norway

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ABSTRACT

Background: Perfluoroalkyl substances (PFASs) are persistent organic pollutants that are suspected to be neurodevelopmental toxicants, but epidemiological evidence on neurodevelopmental effects of PFAS exposure is inconsistent. We investigated the associations between prenatal exposure to PFASs and symptoms of attention-deficit/hyperactivity disorder (ADHD) and cognitive functioning (language skills, estimated IQ and working memory) in preschool children, as well as effect modification by child sex.

Material and methods: This study included 944 mother-child pairs enrolled in a longitudinal prospective study of ADHD symptoms (the ADHD Study), with participants recruited from The Norwegian Mother, Father and Child Cohort Study (MoBa). Boys and girls aged three and a half years, participated in extensive clinical assessments using well-validated tools; The Preschool Age Psychiatric Assessment interview, Child Development Inventory and Stanford-Binet (5th revision). Prenatal levels of 19 PFASs were measured in maternal blood at week 17 of gestation. Multivariable adjusted regression models were used to examine exposure-outcome associations with two principal components extracted from the seven detected PFASs. Based on these results, we performed regression analyses of individual PFASs categorized into quintiles.

Results: PFAS component 1 was mainly explained by perfluoroheptane sulfonate (PFHpS), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS) and perfluorooctanoic acid (PFOA). PFAS component 2 was mainly explained by perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA) and perfluorononanoic acid (PFNA). Regression models showed a negative association between PFAS component 1 and nonverbal working memory [β = -0.08 (CI: -0.12, -0.03)] and a positive association between PFAS component 2 and verbal working memory [β = 0.07 (CI: 0.01, 0.12)]. There were no associations with ADHD symptoms, language skills or IQ. For verbal working memory and PFAS component 2, we found evidence for effect modification by child sex, with associations only for boys. The results of quintile models with individual PFASs, showed the same pattern for working memory as the results in the component regression analyses. There were negative associations between nonverbal working memory and quintiles of PFOA, PFNA, PFHxS, PFHpS and PFOS and positive associations between verbal working memory and quintiles of PFOA, PFNA, PFDA and PFUnDA, with significant relationships mainly in the highest concentration groups.

Conclusions: Based on our results, we did not find consistent evidence to conclude that prenatal exposure to PFASs are associated with ADHD symptoms or cognitive dysfunctions in preschool children aged three and a half years, which is in line with the majority of studies in this area. Our results showed some associations between PFASs and working memory, particularly negative relationships with nonverbal working memory, but also

* Corresponding author. Department of Child Health and Development, NIPH, PO Box 222, Skøyen, N-0213, Oslo, Norway.
E-mail address: thea.skogheim@fhi.no (T.S. Skogheim).

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positive relationships with verbal working memory. The relationships were weak, as well as both positive and negative, which suggest no clear association – and need for replication.

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders, affecting approximately 5% of children worldwide (Polanczyk et al., 2007). ADHD is characterized by inattention, impulsivity and hyperactivity (American Psychiatric Association, 2013). Symptoms of ADHD are often present in the preschool years (Skogan et al., 2014), which is also an important period for the development of cognitive functions and language (Garon et al., 2008; Rice et al., 2008). Childhood ADHD is 2–9 times more prevalent in boys, but there are smaller sex differences in population-based samples compared with clinical samples (Nussbaum, 2012; Polanczyk et al., 2007). The reasons for sex differences are not known, but it has been hypothesized that a higher degree of externalizing behavior problems among boys with ADHD compared to girls may result in a sex-based referral bias (Biederman, 2005; Martin et al., 2018; Nussbaum, 2012). The underlying causes of ADHD are most likely interplays between genetic and non-genetic factors (Faraone et al., 2005; Thapar et al., 2013). While the role of heritability in the etiology of ADHD is well documented (Chang et al., 2013; Faraone et al., 2005), knowledge about how environmental factors may affect the development of ADHD is still scarce (Thapar et al., 2013). Exposure to environmental toxicants during pregnancy has gained increased interest as a risk factor for neurodevelopmental disorders (Grandjean and Landrigan, 2014). During pregnancy, toxicants can be transferred from mother to fetus via the placenta (Grandjean and Landrigan, 2014; Gützkow et al., 2012; Kato et al., 2014). The fetus has an undeveloped blood-brain barrier and limited ability to eliminate toxicants (Grandjean and Landrigan, 2014) therefore, exposure to toxicants *in utero* may disrupt normal brain development and hence be a potential risk factor for impaired cognitive functions and neurodevelopmental disorders such as ADHD or related symptoms (Grandjean and Landrigan, 2006, 2014; Kajta and Wójtowicz, 2013).

Compared to other environmental toxicants, poly- and perfluoroalkyl substances (PFASs) are among those with highest levels in human blood, including pregnant women (Haug et al., 2018; Mariussen, 2012). PFAS is a large group of synthetic compounds developed for use in a multitude of different products (e.g. firefighting foam, textiles, cooking pans, and food packaging) because of its water, oil and dirt repelling properties (Buck et al., 2011; Kissa, 2001). Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are the two most prevalent and extensively studied PFASs. Due to phase-out by major producers as well as international legislation and reduced use, levels of some PFASs have declined in the environment during the last 10–15 years (EFSA CONTAM Panel, 2018; Mariussen, 2012). However, several PFASs are highly persistent in the environment and in humans, and PFOS and PFOA have estimated biological half-lives of around two to five years in the human body (EFSA CONTAM Panel, 2018; Lau et al., 2007). Furthermore, new types of PFASs with longer half-lives have replaced PFOS and PFOA (Sunderland et al., 2019; Wang et al., 2017). Bound to protein-rich tissues, many of the PFASs will accumulate in animals and magnify up the food chain (Conder et al., 2008; Houde et al., 2006). In Norway, the major sources of exposure to these substances are food, especially seafood (Haug et al., 2010). Experimental rodent studies suggest that PFASs may be developmental neurotoxicants (Grandjean and Landrigan, 2014; Johansson et al., 2009; Mariussen, 2012; Viberg et al., 2013). Importantly, PFASs have endocrine-disruptive abilities and can affect the maternal and fetal thyroid hormone systems, which are essential for a normal development of the fetal nervous system and brain (De Cock et al., 2012; Mariussen, 2012; Tran and Miyake, 2017). Experimental animal studies have suggested that there are sex differences regarding the elimination of PFASs and that it is possibly linked to prenatal gonadal hormone levels (Lau et al., 2007). In addition, studies suggest interaction between PFAS exposure and sex hormone homeostasis (Kjeldsen and Bonefeld-Jørgensen, 2013; Mariussen, 2012).

Results from epidemiologic studies investigating prenatal exposure to PFASs and neurodevelopment, such as ADHD diagnosis/symptoms and cognitive functions, are inconsistent (Liew et al., 2018a; Rappazzo et al., 2017). Most studies on ADHD or related symptoms report no associations (Fei and Olsen, 2011; Lien et al., 2016; Liew et al., 2015; Ode et al., 2014; Oulhote et al., 2016; Quaak et al., 2016; Stein et al., 2013; Strøm et al., 2014; Vuong et al., 2018). Two studies report positive associations between prenatal PFAS exposure and hyperactivity symptoms (Høyer et al., 2015, 2018). Research on prenatal exposure to PFASs and offspring cognitive functions report weak or lack of associations, or report conflicting evidence (Chen et al., 2013; Harris et al., 2018; Jeddy et al., 2017; Liew et al., 2018b; Stein et al., 2013; Vuong et al., 2019; Zhang et al., 2018). However, one study did report negative associations between higher PFAS levels and lower IQ in the child at ages five and eight (Wang et al., 2015). In addition, another study reported associations between higher prenatal PFOS levels and increased impairments in metacognition (Vuong et al., 2016). Taken together, there is considerable uncertainty about the effect of PFASs as far as these types of neurodevelopmental outcomes are concerned. Among the studies, there is a large variety of different instruments and methods and several of them have small sample sizes. Furthermore, no previous studies have investigated prenatal PFAS exposure in relation to ADHD symptoms using neuropsychological assessments of three-year-old children.

The present study's overall aim is to investigate the associations between prenatal exposure to PFASs and ADHD symptoms, language skills, estimated IQ and working memory in preschool children, as well as to investigate effect modification by child sex of these associations.

2. Methods

2.1. Study design and participants

2.1.1. The Norwegian Mother, Father and Child Cohort Study

The Norwegian Mother, Father and Child Cohort Study (MoBa) is an ongoing prospective population-based cohort study conducted by the Norwegian Institute of Public Health (Magnus et al., 2016). The cohort now includes over 114,000 children, 95,000 mothers, and 75,000 fathers. Participants (41% participation rate) were recruited from all over Norway from 1999 to 2008. Pregnant women were invited to participate when scheduling their first free ultrasound scanning around the 17th week of pregnancy. Blood samples were collected from both parents in pregnancy and from the mother and child at birth (Magnus et al., 2016).

2.1.2. The ADHD study

The current paper is based on the ADHD Study, a sub-study of children with high levels of ADHD symptoms. The children were identified through the MoBa questionnaire that mothers completed when the child was three years of age (Overgaard et al., 2018). This questionnaire included 11 items about ADHD, of which six items were from the Child Behavior Checklist/1.5–5 (Achenbach and Rescorla, 2010) and five items from the DSM-IV-TR criteria for ADHD (American Psychiatric Association, 2000). Children with scores ≥ 90th percentile

on these 11 items (n = 2798) were invited to participate in a clinical assessment, along with randomly selected children from the MoBa cohort (n = 654). Among those eligible for the present sub-study, 149 children with high scores on autistic symptoms were sampled to another MoBa sub-study of autism (Fig. 1). In total, about 35% agreed to participate in the present sub-study. From 2007 to 2011, 1195 children (mean age: 3.5 years, age range: 3.1–3.8 years) took part in a one-day clinical assessment including a neuropsychological assessment with the child and a diagnostic interview with one of the parents, usually the mother. Details about the screening criteria are described elsewhere (Overgaard et al., 2018). In the overall sample, the proportions of girls and boys who met symptom criteria for ADHD diagnosis according to the parent interview were about 17% and 20%, respectively (Overgaard et al., 2018, 2019).

When excluding non-singleton pregnancies, withdrawals from the study, and those without available blood samples, the total number of mother-child pairs was 944 in the present study (Fig. 1). None of the children participating in this study had been or was medicated for ADHD at the time of assessment. We used version 9 of the MoBa quality-assured data files. MoBa is regulated under the Health Registry Act. Participation in MoBa is based on written informed consent from the parents. The ADHD Study has approval from the Regional Committee for Health Research Ethics for Southeast Norway. Participation in the clinical assessments of the ADHD Study required an additional written informed consent. This study was approved by The Regional Committee for Medical Research Ethics (ref. nu. 2012/985–1).

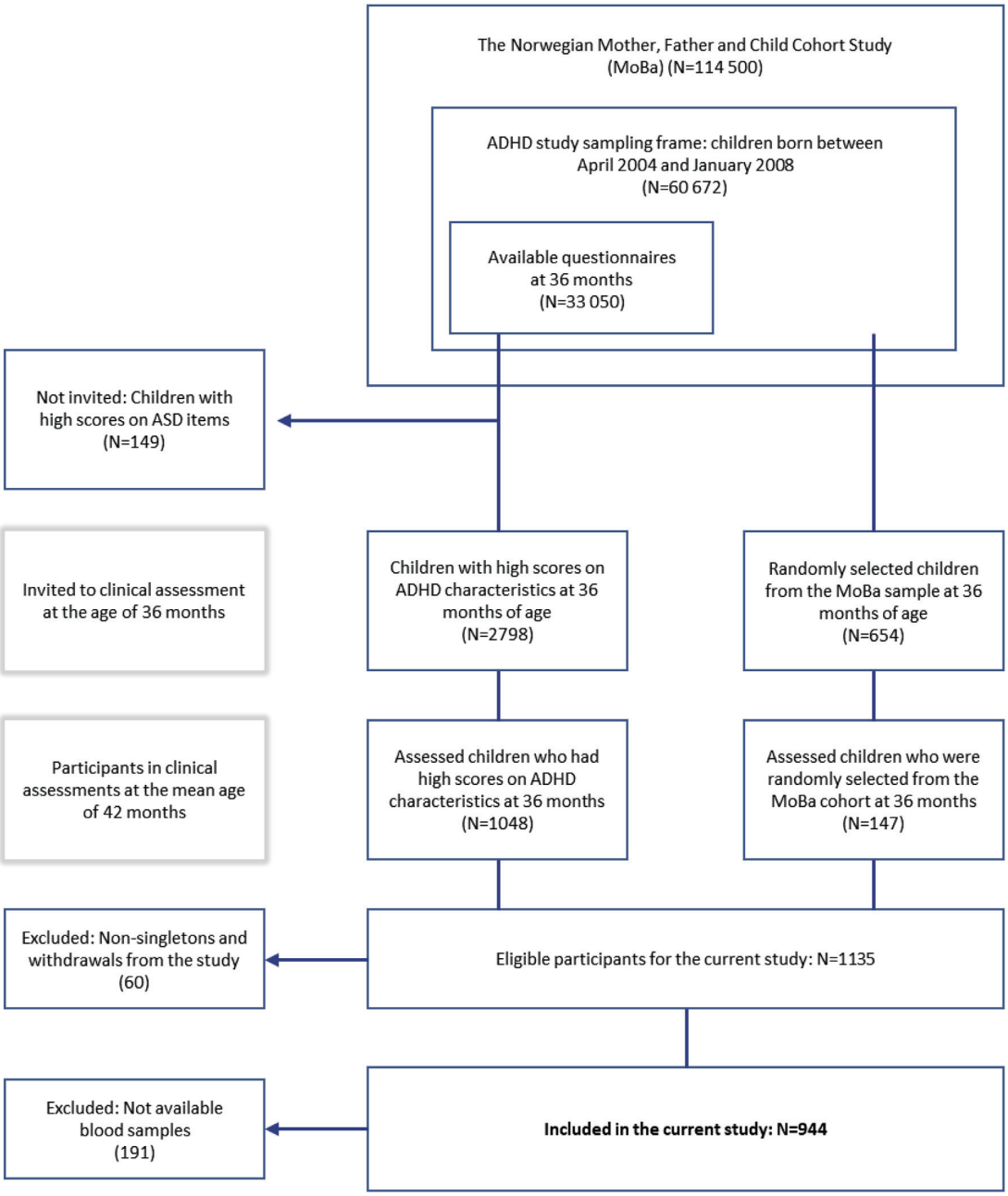


Fig. 1. Recruitment of participants and inclusion in the current study in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2004–2008. Abbreviations: Attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), The Norwegian Mother, Father and Child Cohort Study (MoBa).

2.2. Exposures

The present study used maternal plasma samples from week 17 of gestation to measure PFAS levels. Details about the sampling procedure and handling and storage in the MoBa biobank is described elsewhere (Paltiel et al., 2014). Nineteen PFASs were determined in maternal plasma (Table S1), using liquid chromatography-triple quadruple mass spectrometry (LC-MS/MS) as described previously (Haug et al., 2009). This method has been thoroughly validated and used for determination of more than 5000 serum/plasma samples so far, including approximately 2000 samples from MoBa (Singer et al., 2018). Only PFASs with levels above limit of quantification (LOQ) in > 80% of the plasma samples were included in the present study; PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS) and PFOS. Internal quality control samples and procedure blanks were analyzed along with each batch of samples to ensure high quality of the determinations throughout the project. The samples were also randomized to batch.

2.3. Outcomes

2.3.1. ADHD symptoms

Diagnostic assessments of the children were based on the Preschool Age Psychiatric Assessment (PAPA) interviews with their parents (Egger and Angold, 2004). The ADHD classification/diagnosis defined by PAPA is not equivalent to clinical ADHD diagnoses that would require a broader assessment, including multiple sources of information and informants. In the ADHD Study, only symptoms lasting ≥3 months were counted as present. Psychologists, psychiatrists, or trained graduate psychology students conducted the interviews. When graduate students conducted the interviews, they were under supervision by a child psychologist or a psychiatrist. As an inter-rater reliability check, a separate rater who was blind to the parent and teacher screen ratings, rescored audiotapes of 79 randomly selected assessment interviews. The average intra-class correlations (ICCs) were 0.97 for hyperactivity and impulsivity (HI) symptoms, 0.99 for inattention (IA) symptoms, and 0.98 for the total number of ADHD symptoms. In the present study, ADHD symptom sum scores were based on symptoms of inattention, hyperactivity, and impulsivity from the PAPA interview. Higher scores indicated more ADHD symptoms and higher severity.

2.3.2. Expressive language skills

Experienced clinicians with specialization in pediatric neuropsychological assessments conducted the tests of cognitive abilities of the children, including language skills, estimated IQ, and working memory. Expressive language skills were measured with Child Development Inventory (CDI). The CDI is a questionnaire for assessment of children from 15 months to six years of age, where teachers and parents fill in the questionnaires (Ireton and Glascoe, 1995). The questionnaire is consistent with results from psychometric tests of children and has good sensitivity and specificity (> 80%) of identifying delayed development in children (Doig et al., 1999). In the CDI, delayed language is defined as at least 1.25 standard deviations below the mean (Rohrer-Baumgartner et al., 2016). In the present study, we used the language subscale that was filled in by the preschool teacher. The subscale contains 50 items that assess primarily expressive communication, from simple gestures to complex language expressions. We used the daycare teacher report instead of parental report, as preschool teachers generally are assumed to have a good reference base for the evaluations (Rohrer-Baumgartner et al., 2016). A higher score indicated better language skills.

2.3.3. Estimated IQ (verbal and nonverbal)

Intelligence quotient (IQ) refers to performance on standardized tests measuring intellectual abilities (Rohrer-Baumgartner et al., 2014). Two subtests from Stanford Binet Intelligence scales (5th edition), were used to assess estimated IQ. This test battery has good psychometric properties and is standardized for ages two to 85 (Roid, 2003). In the present study, an estimated verbal IQ score was based on the "Vocabulary Task" where the child is requested to point at different body parts or name objects (toys) and explain the meaning of selected words. An estimated nonverbal IQ score was based on the "Object Matrices Task", that entails tasks such as detection of shapes that are alike and to fill in a missing shape on the basis of abstract reasoning. The verbal task is a measure of knowledge and the nonverbal task is a measure of fluid reasoning, which together is a good estimate of global ability (Roid, 2003). Both of these subtests have high loadings on the hierarchical g factor in cognitive ability batteries (Roid, 2003). The stop rule of discontinuing the test after four consecutive null scores was applied in all tests from this battery. A higher score indicated higher estimated IQ.

2.3.4. Working memory (verbal and nonverbal)

Working memory consists of a multicomponent cognitive system that allows for the rehearsal, storage and manipulation of information for a few seconds, and is a vital part of higher-order cognitive processes (Baddeley, 2012). Stanford Binet Intelligence scales (5th edition) was utilized to measure verbal and nonverbal working memory. Verbal working memory was assessed with the subtask "Memory for Sentences", where the child is asked to repeat sentences that increases gradually in length. Nonverbal working memory was measured with two subtasks; "Block Span" and "Delayed Response". In the Block Span test, the child is asked to tap blocks in the same order as the administrator. In the Delayed Response task, a small toy is placed under one of three cups when the child is watching; he or she is then asked to indicate where the toy is hidden after a short delay (Roid, 2003). A higher score indicated better working memory function.

2.4. Covariates

We obtained information on potential confounding variables from the Medical Birth Registry of Norway (MBRN) and MoBa questionnaires that were completed during pregnancy and up to child's age three years, as well as from questionnaires administered at three and a half years of age in the ADHD Study. Potential confounders were selected *a priori* based on existing literature and were guided by directed acyclic graphs (DAGs). Potential confounders included maternal age, maternal education, maternal fish intake, parity, maternal ADHD symptoms, child sex, premature birth, birth weight, maternal BMI, maternal smoking, maternal alcohol consumption, maternal anxiety/depression and maternal iodine intake. We did not include breastfeeding/breastfeeding duration because it temporally follows exposure, and therefore cannot confound prenatal PFAS concentrations. Based on the DAGs (Fig. S1 and Fig. S2), a minimal adjustment set (the minimal selection of variables to be adjusted for in order to avoid a biased result) was suggested to include maternal age, maternal education, maternal fish intake and parity using dagitty.net to estimate the total effect (Textor et al., 2011). We also included child sex in our final models as a confounder and effect measure modifier, because of the strong association between sex and the outcomes in question, and because effects of PFAS may be sexually dimorphic (Kjeldsen and Bonefeld-Jørgensen, 2013; Mariussen, 2012). When investigating ADHD symptoms and language skills as outcomes, the child's age at testing (in months) was also included as confounders, estimated IQ and working memory scores were already age-standardized. Maternal ADHD symptoms measured by the Adult ADHD Self-Report Scale (ASRS screener) (Kessler et al., 2007), was also included as a covariate in analyses of child ADHD symptoms as outcome.

2.5. Statistical analysis

Among the seven PFASs included in our study, four of them had

missing values due to levels below the LOQ. In addition, some of the covariates had missing values. To replace missing data, we ran multiple imputation by chained equations. In our analyses, we generated 50 datasets with the exposure and outcome variables, covariates and auxiliary variables (Rubin, 1976; Sterne et al., 2009) using the mi ice command in Stata (Royston, 2008). We used the method for interval-censored data and specified upper and lower limit for imputed results for PFASs as limit of detection (LOD) and zero, respectively (Royston, 2008). In the imputation model, we included the following (% missing): PFOA (0), PFNA (0.1), PFDA (17.5), PFUnDA (13.1), PFHxS (0), PFHpS (10.6), PFOS (0), child birth year (0), maternal age (0), maternal ADHD symptoms (1.0), maternal education (2.1), parity (0) maternal fish intake (1.6), child age at testing (0.5), child sex (0), maternal folate supplement (0), and the outcome variables. Some subjects were not included in the analyses due to missing values in an outcome variable (% missing): ADHD symptoms (0.1), estimated nonverbal IQ (1.0), estimated verbal IQ (0.8), nonverbal working memory (1.1), verbal working memory (18.6), and language (4.8). The pooling procedure used in the present article was mi estimate (Stata Press, 2017).

As a first step, we performed an exploratory principal component analysis (PCA) of log-transformed PFAS variables to investigate inter-correlation among the PFASs and to extract principal components. Oblimin rotation was chosen as this allows the components to be correlated, which can be the case when it comes to PFASs, independent of whether they are sulfonates or carboxylates. Delta was set at the default of zero. We performed multivariable analyses with negative binomial regression for ADHD symptoms and generalized linear regression analyses for language skills, nonverbal working memory, verbal working memory, estimated nonverbal IQ, and estimated verbal IQ with PFAS component scores as predictors, adjusting for the other component in the analyses. To optimize interpretation, the IQ and working memory scores were standardized into z-scores. For ADHD symptoms and language skills, sum scores were used. We also fitted models that included interaction terms of child sex and PFAS. In addition, we performed a sensitivity analysis in the models with PFASs as principal components, where we only included participants who were first-born. Based on significant findings from the component models, we further investigated the dose-response relationships between levels of individual PFASs categorized into quintiles and outcome variables in separate linear regression models, with the lowest quintile as the reference group. Investigation of dose-response relationships is important as this can give information on the function shape of PFAS-neurodevelopmental outcome relationships, which will not be interpretable by associations with component scores. We also performed a sensitivity analysis in the quintile models where fish intake was excluded as a covariate.

All regression models were expressed with regression coefficient (β) and accompanying 95% confidence intervals (CIs) or p-value (Wald's test, interaction term) with significance set at $p \leq 0.05$. The number of tests in this study was considerably reduced by using principal components as predictors in the regression models instead of single PFASs. Acknowledging that the number of tests performed is still fairly high ($n = 92$) and thus inflating the probability of type 1 error, we also evaluated the results with 99% CI and $p \leq 0.01$. This would correspond to Šidák correction to control for familywise error rate (false discoveries or type I errors) for $k = 92$ number of tests calculated by $100(1-\alpha)1/k$ % confidence intervals with $\alpha = 0.05$. Statistical analyses were performed in Stata version 15 (StataCorp, 2019).

3. Results

Characteristics of the study sample are displayed in Table 1. Mothers' mean age was 30.6 years. More than one third of the mothers had higher education (college or university) and almost all the mothers were married or cohabitating. The majority did not report smoking during pregnancy and most of them were primiparous. The sex distribution among the children was near equal with 51.4% boys. The sample characteristics by clinical symptoms are shown in the supplementary material (Table S2).

Table 2 shows the PFAS distribution of our sample including the mean, median and interquartile range of maternal PFAS concentrations during pregnancy. Three of the PFASs (PFOA, PFHxS and PFOS) were above LOQ in all measurements. These three also had the highest concentrations. The correlations among the PFASs are displayed in Table 3. The PFASs could largely be explained by two principal components and this model was chosen because it effectively captured the main correlation structure among the PFASs. Component one accounted for 42% of the covariation in the PFAS data with high loadings of PFOA, PFHxS, PFHpS and PFOS (Table S3). Component two accounted for 34% of the covariation and had high loadings of PFNA, PFDA and PFUnDA (Table S3). The distribution of the outcomes in the present study is presented in Table 4 and inter-correlations between the outcome variables are presented in Table 5.

The imputed and adjusted results are presented in this article, while complete case analyses (Table S4 and Fig. S3) and crude results (Table S5 and Fig. S4) are presented in supplementary material. The regression models showed a negative association between PFAS component one (mainly explained by PFOA, PFHxS, PFHpS and PFOS) and nonverbal working memory [β = -0.08 (95% CI: -0.12, -0.03)] (Table 6). This association remained with 99% confidence intervals. Between PFAS

Table 1
Characteristics of study population in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2004–2008.

Characteristic	Mean ± SD or n (%)
Total N	944
Maternal age at delivery (years)	30.58 ± 4.24
Missing (*n*)	0
Child sex	
Boy	485 (51.38)
Girl	459 (48.62)
Missing (*n*)	0
Maternal education	
< College/university	219 (23.70)
College/university	705 (76.30)
Missing (*n*)	20
Maternal marital status	
Married/Cohabitant	915 (96.93)
Single/Other	29 (3.07)
Missing (*n*)	0
Parity	
0	603 (63.88)
1 or more	341 (36.12)
Missing (*n*)	0
Maternal ADHD score	2.35 ± 0.62
Missing (*n*)	9
Maternal fish intake (g/day)	26.62 ± 17.73
Missing (*n*)	15
Child year of birth	
2004	109 (11.54)
2005	239 (25.32)
2006	303 (32.10)
2007–2008	293 (31.04)
Missing (*n*)	0
Smoking during pregnancy	
No	846 (89.62)
Yes	98 (10.38)
Missing (*n*)	0
Folate supplement	
No	166
Yes*	778
Missing (*n*)	0

Abbreviations: Attention-deficit/hyperactivity disorder (ADHD), standard deviation (SD). Note: *Any folate supplements between 4 weeks before and 8 weeks after conception.

Table 2
PFAS distribution in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

	N	% > LOQ	Mean	SD	Min	25%	50%	75%	Max
PFOA (ng/mL)	944	100%	2.61	1.18	0.33	1.77	2.50	3.21	9.81
PFNA (ng/mL)	943	99.89%	0.45	0.28	0.06	0.29	0.41	0.53	5.32
PFDA (ng/mL)	779	82.52%	0.19	0.14	0.05	0.10	0.15	0.23	1.77
PFUnDA (ng/mL)	820	86.86%	0.25	0.15	0.05	0.14	0.22	0.32	1.46
PFHxS (ng/mL)	944	100%	0.79	0.99	0.06	0.46	0.65	0.88	22.48
PFHpS (ng/mL)	844	89.41%	0.16	0.08	0.05	0.10	0.15	0.20	0.62
PFOS (ng/mL)	944	100%	12.32	5.38	2.38	8.77	11.51	14.84	42.23

Abbreviations: Perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorohexane sulfonate (PFHxS), perfluoroheptanesulfonic acid (PFHpS), perfluorooctane sulfonate (PFOS), limit of quantification (LOQ).

Table 3
Pearson correlations of PFASs in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

	PFOA	PFNA	PFDA	PFUnDA	PFHxS	PFHpS	PFOS
PFOA	1.00						
PFNA	0.67	1.00					
PFDA	0.50	0.77	1.00				
PFUnDA	0.26	0.57	0.71	1.00			
PFHxS	0.51	0.42	0.31	0.30	1.00		
PFHpS	0.64	0.47	0.39	0.32	0.61	1.00	
PFOS	0.62	0.51	0.44	0.42	0.54	0.80	1.00

Note: Correlation color coding goes from strong (green) to medium (yellow) and weak (red).

Table 4
Outcome distribution in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

	N	Mean	SD	Range
ADHD symptoms	943	5.54	6.10	0, 33
Language skills	899	49.31	7.02	15, 58
Nonverbal working memory	934	10.40	2.80	2, 17
Verbal working memory	768	7.60	1.91	5, 13
Nonverbal IQ	935	10.97	2.03	5, 18
Verbal IQ	936	9.72	2.11	2, 16

Note: Unscaled outcome variables. Abbreviation: Standard deviation (SD).

component two (mainly explained by PFNA, PFDA and PFUnDA) and verbal working memory there was a positive association [β = 0.07 (95% CI: 0.01, 0.12)] (Table 6). In the interaction models, we found effect modification by child sex (p = 0.01) for PFAS component two (PFNA, PFDA and PFUnDA) and verbal working memory. There was a stronger association in boys [β = 0.13 (95% CI: 0.06, 0.20)] compared to girls [β = 0.01 (95% CI: -0.06, 0.07)]. Among the girls, the confidence intervals contained the null (Table 6). The association for boys remained with 99% CI.

PFAS components were not associated with ADHD symptoms, language skills or estimated IQ (Tables 7 and 8). For ADHD symptoms, language skills, estimated IQ and nonverbal working memory, we observed no effect modification and no difference in the associations of PFAS component one and two by child sex (Tables 6–8).

The results from the sensitivity analysis, where we restricted our sample to first-born children, were fairly similar to the main analyses (Table S6). However, the association between component two (PFNA, PFDA and PFUnDA) and verbal working memory did not remain in the restricted models (Table S6).

We investigated dose-response relationships of significant relationships identified in the PFAS component models, using individual PFASs categorized into quintiles. For PFOA, PFHpS and PFOS there appeared to be a monotonic dose-dependent decrease in nonverbal working memory, however, only the fifth (highest) quintiles were significant; PFOA [β = -0.38 (95% CI: -0.61, -0.15)], PFHpS [β = -0.37 (95% CI: -0.58, -0.15)] and PFOS [β = -0.26 (95% CI: -0.47, -0.05)] (Fig. 2). A somewhat similar dose-response trend was observed for PFNA. In addition, there were negative associations between nonverbal working memory and the fourth quintile of PFHxS and the third quintile of PFNA (Fig. 2). The associations between PFOA and PFHpS with nonverbal working memory were also significant at 99% CI.

Quintile regression models with verbal working memory as outcome variable showed positive associations for the fifth quintiles of PFNA [β = 0.34 (95% CI: 0.10, 0.57)], PFDA [β = 0.32 (95% CI: 0.09, 0.55] and PFUnDA [β = 0.29 (95% CI: 0.05, 0.52)] (Fig. 3). In addition, there was a positive association between the third quintile of PFOA and verbal working memory (Fig. 3). With 99% CI, the associations with PFNA and PFDA remained. Excluding fish intake as a covariate in the sensitivity analyses did not change the association between PFASs and nonverbal and verbal working memory (Fig. S5).

Table 5
Pearson correlations of outcome variables in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

	ADHD symptoms	Language	Nonverbal IQ	Verbal IQ	Nonverbal WM	Verbal WM
ADHD symptoms	1.00					
Language	0.15	1.00				
Nonverbal IQ	-0.01	0.04	1.00			
Verbal IQ	0.09	0.23	0.10	1.00		
Nonverbal WM	0.15	0.12	0.02	0.22	1.00	
Verbal WM	0.06	0.16	0.02	0.26	0.13	1.00

Note: The variable ADHD symptoms is flipped. Correlation color coding goes from strong (green) to medium (yellow) and weak (red).

Table 6
Beta coefficients and 95% confidence intervals of adjusted regression models between PFAS components and working memory and interaction by child sex in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

PFAS components	Nonverbal working memory (n = 934)			Verbal working memory (n = 768)		
	All	Interaction term p = 0.863		All	Interaction term p = 0.105	
		Boys	Girls		Boys	Girls
Component 1: PFOA, PFHxS, PFHpS, PFOS	−0.08 (−0.12, −0.03)*	−0.08 (−0.14, −0.02)	−0.07 (−0.14, −0.01)	−0.01 (−0.06, 0.04)	0.02 (−0.04, 0.09)	−0.04 (−0.11, 0.02)
		Interaction term p = 0.662			Interaction term p = 0.012*	
Component 2: PFNA, PFDA, PFUnDA	0.03 (−0.02, 0.08)	0.02 (−0.05, 0.09)	0.04 (−0.03, 0.10)	0.07 (0.01, 0.12)	0.13 (0.06, 0.20)*	0.01 (−0.06, 0.08)

Note: A separate linear regression model (with multiple imputation) was conducted for each outcome with additional interaction analyses. The following PFASs most heavily loaded on component 1: PFHpS, PFOS, PFHxS and PFOA. The following PFASs most heavily loaded on component 2: PFDA, PFNA and PFUnDA. The PFASs were log transformed before computing principal components. Each regression model was adjusted for maternal education, age, parity, fish intake and child sex. Interaction term was tested with Wald's test. *Indicates significant results with 99% CIs.

4. Discussion

4.1. Main findings of the study

In the present study, we investigated the influence of prenatal exposure to seven PFASs (measured in maternal plasma in week 17 of pregnancy) on ADHD symptoms and cognitive functions in preschool children. With a sample of 944 mother-child pairs, this is one of the largest studies examining these exposure and outcome associations. In addition, few other studies have conducted neuropsychological tests of young preschool children in this particular research context. We accounted for the joint action of inter-correlated PFASs by extracting two principal components explaining 76% of the covariation in the PFAS data. Then we used component scores for component one and two as predictors in multivariable regression models with neurodevelopmental outcome variables. The results showed a negative association between component one (mainly explained by PFOA, PFHxS, PFHpS and PFOS) and nonverbal working memory. In quintile regression models, the individual PFASs of component one indicated a monotonic-like decrease in nonverbal working memory with increasing PFAS concentrations. Between PFAS component two (mainly explained by PFNA, PFDA and PFUnDA) and verbal working memory, there was a positive association. Quintile regression models with individual PFASs and working memory indicated some linear dose-response, but the relationships were both positive and negative, which complicates interpretation of these findings. There were no associations between PFAS components and ADHD symptoms, language skills or estimated IQ. Except for the weak negative associations between PFASs in component one, we did not find consistent evidence to suggest that prenatal exposure to PFASs is associated with ADHD symptoms or cognitive dysfunctions among preschool children.

4.2. Verbal and nonverbal working memory

In our study, we found some weak associations between PFAS components and working memory. Quintile models with individual PFAS concentrations and working memory were largely in agreement with the factor models. This also indicated that the use of PCA to reduce number of exposure variables was reasonable in this research context. However, we found results that were both positive (verbal working memory) and negative (nonverbal working memory), meaning that no clear pattern emerged. We found negative associations between nonverbal working memory and component one (PFOA, PFHxS, PFHpS and PFOS), where higher PFAS levels were associated with decreasing scores of nonverbal working memory. In the quintile models, the respective PFASs, in addition to PFNA, showed a similar pattern. Furthermore, there were indications of negative monotonic dose-response relationships for several of the PFASs (PFOS, PFOA and PFHpS), although only the fifth quintiles were significant. The associations between PFASs and nonverbal working memory appear to be somewhat novel, as few studies have investigated these particular exposure-outcome associations. Still, our results are partly in line with a study from the USA that analyzed exposure to prenatal PFASs and cognitive functions in children at the ages of five and eight years (Vuong et al., 2016).

Table 7
Beta coefficients and 95% confidence intervals of adjusted regression models between PFAS components and ADHD symptoms and language skills and interaction by child sex in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

PFAS components	ADHD symptoms (n = 943)			Language skills (n = 899)		
	All	Interaction term p = 0.212		All	Interaction term p = 0.078	
		Boys	Girls		Boys	Girls
Component 1: PFOA, PFHxS, PFHpS, PFOS	−0.01 (−0.07, 0.05)	−0.04 (−0.11, 0.03)	0.02 (−0.06, 0.09)	−0.09 (−0.42, 0.24)	0.12 (−0.29, 0.53)	−0.34 (−0.77, 0.09)
		Interaction term p = 0.526			Interaction term p = 0.024	
Component 2: PFNA, PFDA, PFUnDA	−0.00 (−0.06, 0.06)	−0.02 (−0.09, 0.06)	0.01 (−0.06, 0.09)	0.08 (−0.28, 0.43)	0.42 (−0.04, 0.89)	−0.24 (−0.69, 0.21)

Note: A separate regression model (with multiple imputation) was conducted for each outcome: negative binomial regression for ADHD symptoms and linear regression for language skills with additional interaction analyses. The following PFASs most heavily loaded on component 1: PFHpS, PFOS, PFHxS and PFOA. The following PFASs most heavily loaded on component 2: PFDA, PFNA and PFUnDA. The PFASs were log transformed before computing principal components. Each regression model was adjusted for maternal education, age, parity, fish intake, child sex and child age at testing. Child ADHD symptoms was adjusted for maternal ADHD symptoms. Interaction term was tested with Wald's test.

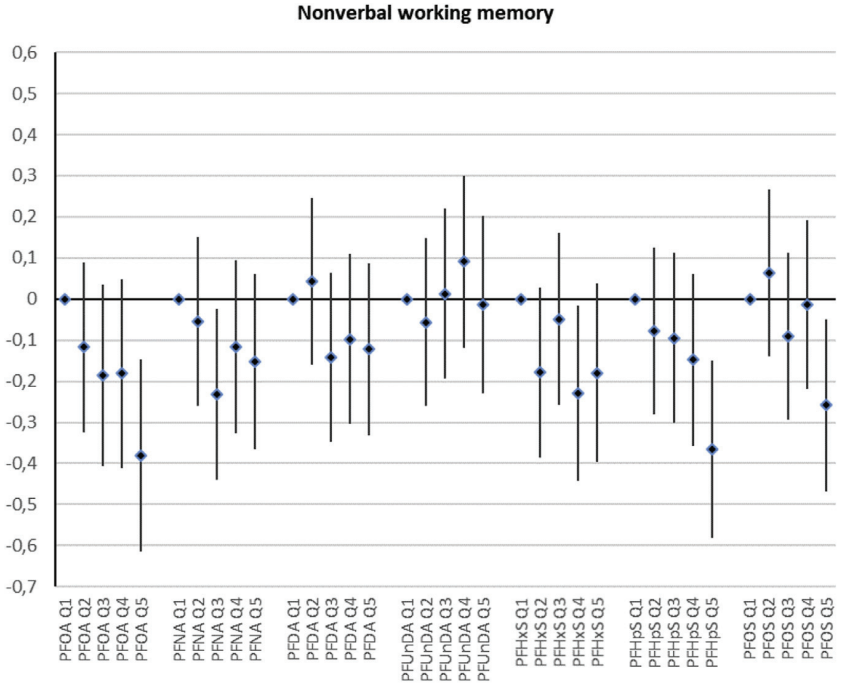
Table 8
Beta coefficients and 95% confidence intervals of adjusted regression models between PFAS components and estimated IQ and interaction by child sex in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

PFAS components	Nonverbal IQ (n = 935)			Verbal IQ (n = 936)		
	All	Interaction term p = 0.876		All	Interaction term p = 0.701	
		Boys	Girls		Boys	Girls
Component 1: PFOA, PFHxS, PFHpS, PFOS	0.00 (−0.05, 0.05)	0.00 (−0.05, 0.06)	−0.00 (−0.06, 0.06)	−0.02 (−0.07, 0.03)	−0.01 (−0.07, 0.04)	−0.03 (−0.09, 0.03)
		Interaction term p = 0.658			Interaction term p = 0.619	
Component 2: PFNA, PFDA, PFUnDA	−0.04 (−0.09, 0.01)	−0.03 (−0.10, 0.03)	−0.05 (−0.12, 0.01)	0.03 (−0.02, 0.08)	0.04 (−0.02, 0.11)	0.02 (−0.04, 0.09)

Note: A separate linear regression model (with multiple imputation) was conducted for each outcome with additional interaction analyses. The following PFASs most heavily loaded on component 1: PFHpS, PFOS, PFHxS and PFOA. The following PFASs most heavily loaded on component 2: PFDA, PFNA and PFUnDA. The PFASs were log transformed before computing principal components. Each regression model was adjusted for maternal education, age, parity, fish intake and child sex. Interaction term was tested with Wald's test.

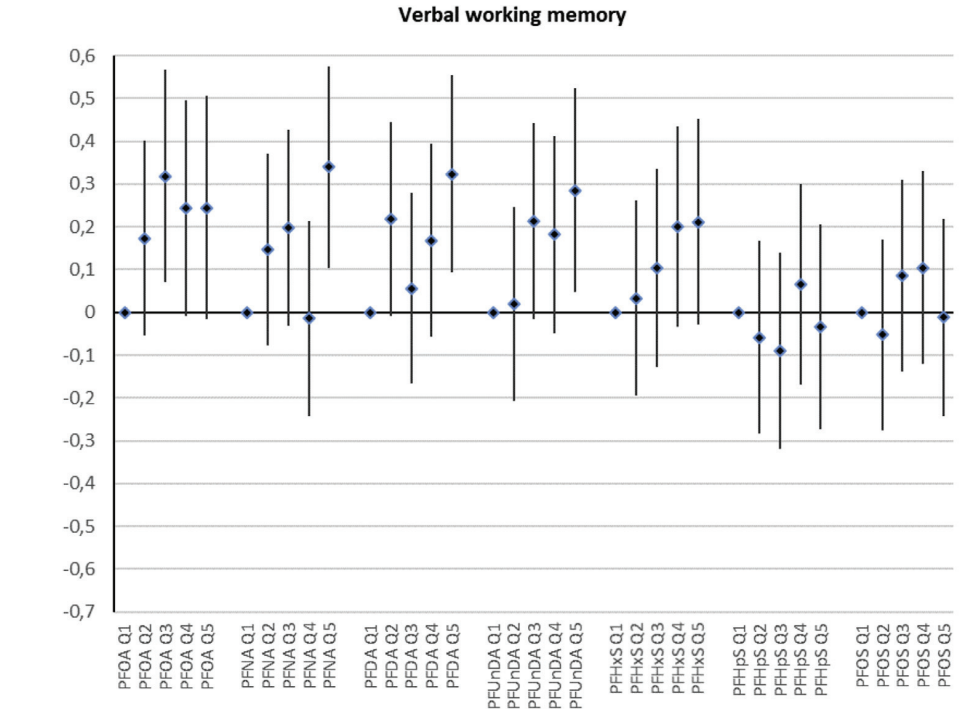
This study reported an association between increased levels of prenatal PFOS and impaired metacognition [β = 3.10 (95% CI: 0.62, 5.58)], which is dependent on multiple executive functions, such as working memory. However, there were no associations with the other investigated PFASs (PFOA, PFNA, PFHxS and PFDeA) and the sample size was quite small (n = 218) (Vuong et al., 2016).

Our study showed positive associations between verbal working memory and PFAS component two (PFNA, PFDA and PFUnDA) and the respective, individual PFAS quintiles, in addition to PFOA. Nevertheless, positive associations between PFASs and cognitive functions, such as language, IQ and memory have also been reported in other studies (e.g. Jeddy et al., 2017; Liew et al., 2018b; Stein et al., 2013; Vuong et al., 2019). Like our results, a recent study, reported positive associations between working memory and increases in prenatal levels of PFOA and PFNA (Vuong et al., 2019). That study used Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV) and assessed children at the age of 8 years (Vuong et al., 2019). A cohort study from the USA, investigating prenatal PFAS exposure and cognitive functions, reported both better and worse cognitive performance associated with prenatal PFAS exposure in three- and seven-year-olds (Harris et al., 2018). A possible mechanism behind the positive associations could be a result of PFASs that activate peroxisome



Note: Each PFAS by nonverbal working memory was modelled using a separate linear regression (with multiple imputation). The beta coefficient and 95% confidence intervals for each PFAS quintile are represented on the vertical axis (the reference level is the first quintile). Each regression model was adjusted for maternal education, age, parity, fish intake and child sex. Higher working memory score indicates better working memory function. Significant with 99% CIs: fifth quintiles of PFOA and PFHpS.

Fig. 2. Beta coefficients and 95% confidence intervals for regression models predicting nonverbal working memory (n = 934) from quintile categories of each PFAS in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.



Note: Each PFAS by verbal working memory was modelled using a separate linear regression (with multiple imputation). The beta coefficient and 95% confidence intervals for each PFAS quintile are represented on the vertical axis (the reference level is the first quintile). Each regression model was adjusted for maternal education, age, parity, fish intake and child sex. Higher working memory score indicates better working memory function. Significant with 99% CIs: fifth quintiles of PFNA and PFDA.

Fig. 3. Beta coefficients and 95% confidence intervals for regression models predicting verbal working memory (n = 768) from quintile categories of each PFAS in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

proliferator-activated receptors (PPARs) alpha and gamma, which have neuroprotective and central-nervous-system anti-inflammatory properties (Quaak et al., 2016; Stein et al., 2013). Research on working memory and prenatal PFAS exposure is scarce, and the results so far have been unclear and inconsistent. Our study showed some potential effects, especially for nonverbal working memory that needs to be replicated.

4.3. ADHD symptoms

In our study, we did not find any significant associations between PFAS exposure and ADHD symptoms, which is in line with other studies that have investigated ADHD symptoms as outcomes (Fei and Olsen, 2011; Quaak et al., 2016) as well as studies with ADHD diagnosis in children (Liew et al., 2015; Ode et al., 2014; Strøm et al., 2014). However, some studies have reported inverse relationships between prenatal PFAS exposure and ADHD symptoms or diagnosis (Lien et al., 2016; Liew et al., 2015; Stein et al., 2013; Vuong et al., 2018), although they concluded that there is a lack of evidence to support these associations. Contrary to our findings, birth cohort studies from Greenland, Ukraine and Poland found that increasing levels of PFOA in maternal blood during pregnancy was associated with increasing levels of hyperactivity in children between the ages of seven and nine [odds ratio = 3.1 (95% CI: 1.3, 7.2)] (Høyer et al., 2015). Furthermore, a recent study using data from these cohorts reported that increasing prenatal exposure to PFNA [odds ratio = 1.8 (95% CI: 1.0, 3.2) and PFDA [odds ratio = 1.7 (95% CI: 1.0, 3.1)] was associated with increasing hyperactivity symptoms in children between five and nine years in Greenland and Ukraine (Høyer et al., 2018). However, the authors did not rule out that it could be spurious findings (Høyer et al., 2018). Additionally, a cohort study from the Faroe Islands found significant positive associations between increasing levels of postnatal PFOA, PFNA, and PFDA and more hyperactivity/inattention problems among seven-year-olds, but not with prenatal PFAS exposure (Oulhote et al., 2016). Taken together, there remain uncertainties regarding the effect of prenatal PFAS exposure on ADHD symptoms and diagnosis, although most studies, like ours, have reported lack of associations.

4.4. Language skills

In accordance with our results, two other studies did not find associations between PFASs and language among two-year-olds and children between six and 12, respectively (Chen et al., 2013; Stein et al., 2013). Both of these studies had quite small sample sizes (n = 239 and n = 320 respectively). A larger study (n = 631 to 971), examining children at age three and seven and language comprehension, found only associations between one type of PFAS; 2-(N-methyl-perfluorooctane sulfonamido) acetate (MeFOSAA), but not the other seven PFASs (Harris et al., 2018). In that study, the second quartile of MeFOSAA was associated with higher receptive vocabulary scores (Harris et al., 2018). Other studies have reported positive relationships. A small study, examining prenatal PFAS exposure and reading ability among children at age five and eight, reported that increasing levels of PFOA, PFNA and PFOS were associated with improved reading skills at five years and at eight years of age (Zhang et al., 2018). Likewise, a study exploring early communication development among children at the ages of 15 and 38 months found both positive and negative associations between various prenatal PFASs and communication development among girls (Jeddy et al., 2017). The authors did point out that the results showed an inconsistent pattern of association across the

measured PFASs (Jeddy et al., 2017). Regarding language skills, there is limited knowledge about potential effects of prenatal PFAS exposure. In line with previous studies, we also report lacking associations.

4.5. Estimated verbal and nonverbal IQ

Our results showed no association between prenatal PFAS exposure and estimated IQ, neither verbal nor nonverbal. A large study (n = 1592) using data from the Danish National Birth Cohort found no associations between prenatal PFAS levels and IQ (full-scale, verbal and nonverbal) in their total sample, but some inconsistent associations in sex-stratified quartile analyses (Liew et al., 2018b). They concluded that overall, there was no evidence of an effect from prenatal PFAS on IQ in their study sample (Liew et al., 2018b). One study found associations between higher PFUnDA and lower nonverbal IQ at age five and higher PFNA levels with lower verbal IQ at age eight (Wang et al., 2015), however, the sample size was quite small (n = 120). In contrast, some studies have found positive associations between PFAS exposure and IQ measures (e.g. Harris et al., 2018; Stein et al., 2013; Vuong et al., 2019). A study that assessed children between six and 12 years of age reported that elevated PFOA levels was associated with improved full-scale IQ score (Stein et al., 2013). The study had a fairly small sample size (n = 320) and the authors concluded that the positive associations were imprecise and inconsistent (Stein et al., 2013). Likewise, a larger study (n = 631 to 971) found that higher prenatal levels of PFOS were associated with better nonverbal IQ among seven-year-olds (Harris et al., 2018). Furthermore, a recent study found associations between increases in child PFNA concentrations and full scale IQ and perceptual reasoning (Vuong et al., 2019). Altogether, our study and the varied results from relatively few studies indicate no association between prenatal PFAS exposure and child IQ.

4.6. Sex specific effects

Our results suggest effect modification by child sex, where the positive association between component two (PFNA, PFDA and PFUnDA) and verbal working memory were mainly driven by boys. Other studies that are comparable to our study have examined effect modification by child sex, although none of them utilized the same sub-task from Stanford Binet as herein. Most of them report no effect modification (Harris et al., 2018; Høyer et al., 2015, 2018; Liew et al., 2015; Stein et al., 2013; Strøm et al., 2014). Still, one study that examined ADHD symptoms and prenatal PFAS exposure found different results by child sex; some associations were stronger for boys and some were stronger for girls depending on the specific PFAS investigated (Lien et al., 2016). In addition, three studies examining different cognitive or behavioral measures in children in the same cohort, report effect modification by child sex in some of the associations between prenatal and postnatal PFAS exposure and these outcomes (Vuong et al., 2016, 2018, 2019). The mechanistic underpinnings of these observed sex differences is a relatively unexplored area. It could be linked to sex-specific differences in toxicokinetics of PFASs and that PFASs have the potential to disrupt sex hormone homeostasis (Kjeldsen and Bonefeld-Jørgensen, 2013; Mariussen, 2012). A later cognitive development among boys compared to girls could also contribute to the observed difference in boys and girls.

4.7. Dose-response relationships and potential mechanisms

Our findings are in accordance with previous epidemiologic literature showing lack of associations and some inconclusive effects between prenatal PFAS exposure and adverse neurodevelopment. Although the reported associations in our sample are weak and difficult to interpret as clinically meaningful, these associations could be stronger and clearer in other populations where PFAS exposure levels are higher and with larger variability in the outcomes. Reasons for these inconsistencies across studies could be difference in PFAS exposure levels and patterns as well as timing of PFAS measurements during pregnancy. They could also be due to differences in study design and methodology. Another possible reason for the few significant results could be that exposure concentrations are below levels or at the threshold of neurodevelopmental toxicity, as indicated by our findings mainly in the group of highest PFAS exposure compared to the lowest group in the quintile models. This could indicate a dose-response relationship and that our population is in the lower part of this curve, while the top 20% of those with the highest PFAS exposure *in utero* could be at risk of adverse outcomes. Indeed, for some of the associations between the PFASs and nonverbal working memory, there were indications of negative linear dose-response trends in the quintile models, with a monotonic decrease of nonverbal working memory scores as the PFASs increased. Compared with previous studies of prenatal exposure and neurodevelopmental outcomes in children (e.g. Harris et al., 2018; Høyer et al., 2015; Liew et al., 2018b; Oulhote et al., 2016), the concentration levels of PFASs are generally lower in the present study. However, results from a study comparing PFAS levels in several European cohort studies showed that the PFAS levels (PFOA, PFNA, PFUnDA, PFHxS and PFOS) in a sample of pregnant women from the Norwegian MoBa cohort are equal or higher compared to the other cohorts (Haug et al., 2018). The levels reported from the MoBa sample in that study (Haug et al., 2018) are similar to the levels in the present sample.

It appears that effects of PFASs on neurodevelopment found in experimental rodent studies are not easily replicated in human studies. Experimental animal studies have shown that PFASs may be developmentally neurotoxic and endocrine disruptive and that PFAS exposure during critical phases of gestation can affect brain development (Johansson et al., 2008, 2009; Lau et al., 2003; Long et al., 2019; Mariussen, 2012). Mechanistic studies indicate that exposure to PFASs may potentially affect important factors or regulators of brain development such as the thyroid hormone system, calcium homeostasis, protein kinase C, synaptic plasticity, and cellular differentiation (Liew et al., 2018a; Mariussen, 2012). Findings from animal studies show that PFAS exposure may be connected to memory, learning and neuro-motor development and the results indicate that the critical windows of exposure are during early brain development (Mariussen, 2012). However, the exposure levels in animal studies are often higher than in human populations and the contaminants have shorter half-lives in for example rodents compared to humans (Fei and Olsen, 2011; Mariussen, 2012). The higher doses that the animals are exposed to, can cause other detrimental effects like increased mortality and birth defects (Mariussen, 2012). The real-life exposure scenario for the human fetus consists of a range of highly inter-correlated PFASs and other toxicants that can interfere with brain development in combination (Mariussen, 2012; Quaak et al., 2016). Species-specific differences in sensitivity of the various stages of brain development and ability to eliminate compounds in relation to the exposure timing and level, may in part explain these inconsistent findings in experimental versus epidemiological studies. In addition, it could be that only noticeable effects from prenatal PFAS exposure appear when the child is older and their cognitive functions are more developed.

4.8. Study limitations and strengths

Limitations to our study include potential selection bias. The participant rate in the MoBa cohort was 41%, and it was 35% for the clinical assessments of the ADHD Study. The participants in MoBa and the sub studies are in general older, have higher educational level and a healthier lifestyle compared with the general population (Nilsen et al., 2009). This might have led to underrepresentation of children with a higher exposure to risk factors or less variability of the cognitive test scores. Furthermore, since most participants in the ADHD Study were recruited based on high scores on ADHD-related symptoms, it is a

selected group and our study sample thus has more symptoms than a general child population. Another limitation is that we could not account for variation in maternal glomerular filtration rate (GFR), which may be a source of residual bias in our study. GFR influences the urinary excretion of PFASs, which can lead to the appearance of higher PFAS levels among people with lower GFR (Verner et al., 2015). Low GFR during pregnancy has also been associated with lower birthweights (Gibson, 1973; Morken et al., 2014), and lower birthweights with subsequent ADHD symptoms (Lim et al., 2018; Momany et al., 2018). Thus, the potential exists for residual confounding by GFR, which should be addressed in future studies. In addition, interactions between the outcome measures could impact the results, as interactions between ADHD symptoms and estimated IQ with language skills have been reported in another study using data from the ADHD Study (Rohrer-Baumgartner et al., 2014). It should be noted that the estimated nonverbal IQ measure employed in the present study were not significantly related to any of the other included variables (in the expected direction) in the full ADHD Study sample (data not presented). We cannot rule out the possibility that a considerable amount of random error in this variable has cancelled out potential associations between estimated nonverbal IQ and exposure to PFASs. Furthermore, participants with delayed language development were sampled to other sub studies in MoBa, meaning that our language measure is not very discriminative. Hence, regardless of our null findings, this does not prevent detection of associations between PFASs and language related outcomes in other studies.

Our study also has several strengths. Particularly, the use of clinical tests performed by specialized clinicians is a major advantage. In addition, we had a large sample size of 944 mother-child pairs, as well as a nearly equal sex distribution, meaning that we were able to explore potential sex-specific effects, which are lacking in several studies. Further, we investigated PFAS levels as principal components, which allowed us to investigate possible joint influence of correlated PFASs mutually adjusted for the other component. PCA is also a way to reduce the number of tests. To our knowledge, investigating prenatal PFAS exposure and ADHD symptoms with neuropsychological tests among preschoolers has not been done before. We also had the benefit of a large number of relevant covariates collected prospectively during pregnancy, in order to account for residual confounding pathways. Although certain other covariates, such as breastfeeding duration, may influence postnatal exposure and/or neurodevelopmental outcomes through other pathways, since breastfeeding occurs temporally after prenatal exposure, it could not confound prenatal estimates. Other studies have been conducted in MoBa to assess the neurodevelopmental impact of postnatal PFAS exposure (Forns et al., 2015; Lenters et al., 2019), however these studies have not considered prenatal exposure.

5. Conclusion

Based on our results, we did not find consistent evidence to conclude that prenatal exposure to PFASs are associated with ADHD symptoms or cognitive dysfunctions in preschool children aged three and a half years, which is in line with the majority of studies in this area. Our results did however, show some weak negative associations between PFASs and nonverbal working memory, we also observed weak positive relationships with verbal working memory. As exposure to PFASs can be high among small children, more studies measuring both postnatal and prenatal exposure to PFASs with regard to neurodevelopment and cognitive functioning, including measures of working memory, should be performed in future studies. Further studies should also investigate combined effects of the exposed PFAS mixture as well as together with other environmental contaminants. Additionally, there is an imminent need for studies investigating underlying mechanisms linking PFAS exposure to the suspected adverse effects on human brain development.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2019.10.003.

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Supplementary tables and figures

Table S1.

Overview of determined PFASs in a nested study of attention-deficit hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

PFAS compound, chain length (number of fluorinated carbons)	Acronym	LOD (ng/mL)	LOQ (ng/mL)
Perfluoriated carboxylic acids	PFCAs		
Perfluorobutanoate, C_4 (C_3)	PFBA	0.003	0.1
Perfluoropentanoate, C_5 (C_4)	PFPeA	0.008	0.05
Perfluorohexanoate, C_6 (C_5)	PFHxA	0.007	0.05
Perfluoroheptanoate, C_7 (C_6)	PFHpA	0.009	0.05
Perfluorooctanoate C_8 (C_7)	PFOA	0.006	0.05
Perfluorononanoate, C_9 (C_8)	PFNA	0.004	0.05
Perfluorodecanoate, C_{10} (C_9)	PFDA	0.002	0.05
Perfluoroundecanoate, C_{11} (C_{10})	PFUnDA	0.006	0.05
Perfluorododecanoate, C_{12} (C_{11})	PFDoDa	0.007	0.05
Perfluorotridecanoate, C_{13} (C_{12})	PFTrDa	0.004	0.05
Perfluorotetradecanoat, C_{14} (C_{13})	PFTeDa	0.05	0.20
Perfluorinated sulfonic acids	PFSAs		
Perfluorobutane sulfonate, C_4 (C_4)	PFBS	0.009	0.05
Perfluorohexane sulfonate, C_6 (C_6)	PFHxS	0.007	0.05
Perfluoroheptane sulfonate, C_7 (C_7)	PFHpS	0.01	0.05
Perfluorooctane sulfonate, C_8 (C_8)	PFOS	0.003	0.05
Perfluorodecane sulfonate, C_{10} (C_{10})	PFDS	0.05	0.20
Others			
Perfluorooctane sulfonamide, C_8 (C_8)	PFOSA	0.02	0.05
N-Methylperfluorooctane sulfonamide	MeFOSA	0.009	0.05
N-Ethylperfluorooctane sulfonamide	EtFOSA	0.009	0.05

Abbreviations: LOD: limit of detection, LOQ: limit of quantification.

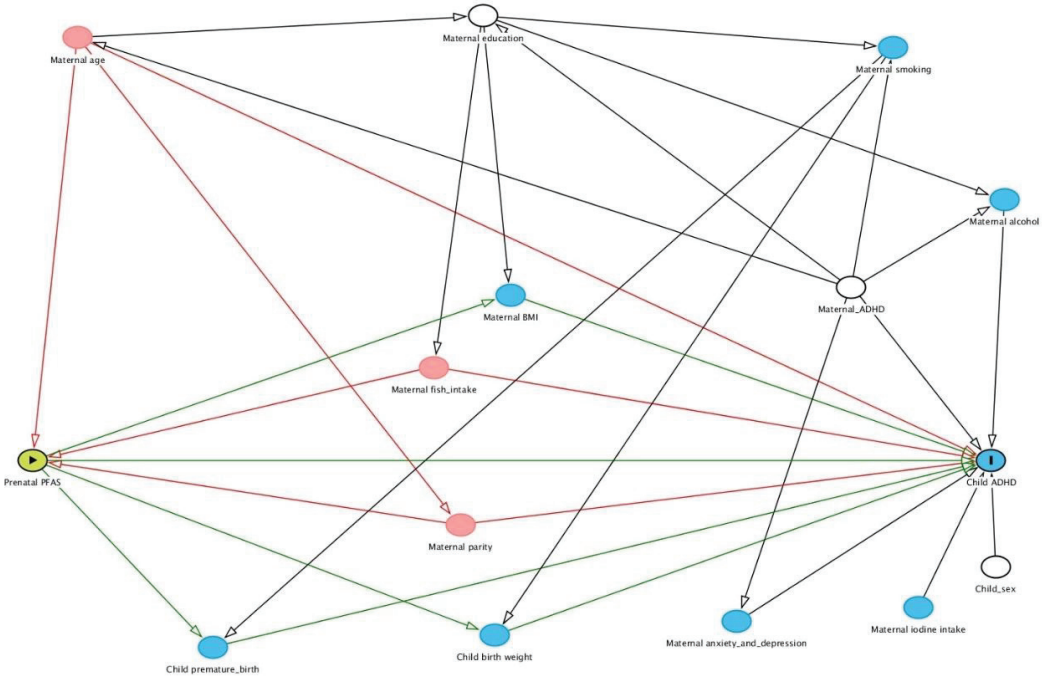

Figure S1. Directed acyclic graph (DAG) for prenatal PFAS exposure and child ADHD symptoms in a nested study of attention-deficit hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

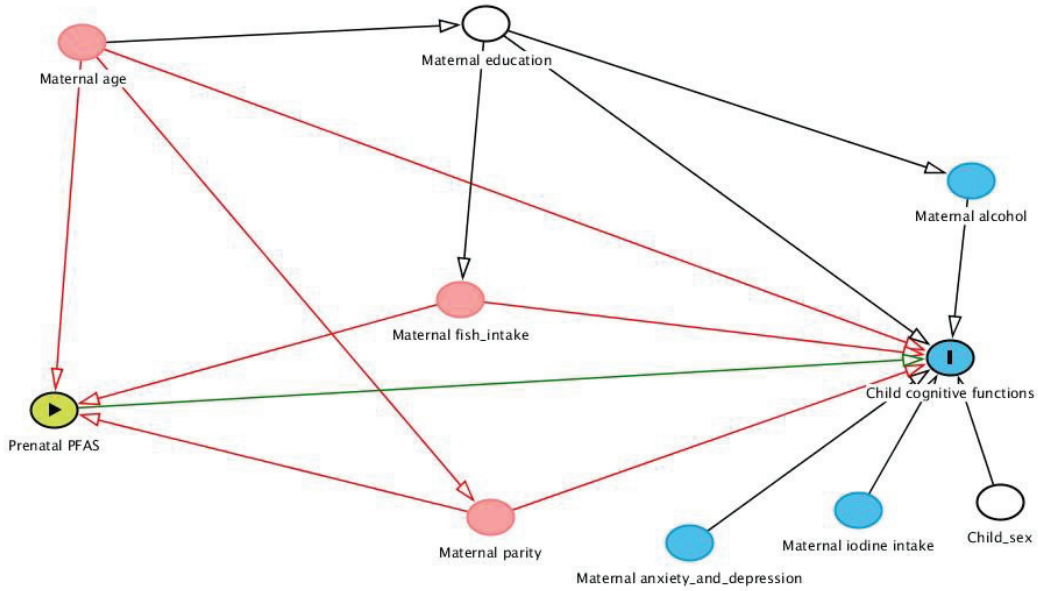

Figure S2. Directed acyclic graph (DAG) for prenatal PFAS exposure and child cognitive functions in a nested study of attention-deficit hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

Table S2.

Characteristics of study population divided by clinical group in a nested study of attention-deficit hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

	ADHD clinical symptoms	**ADHD sub-clinical symptoms**	**No ADHD symptoms**	***p***
Characteristic		**Mean ± SD or n (%)**		
Total N	138	169	637	
Maternal age	30.20 ± 4.39	29.98 ± 4.13	30.83 ± 4.22	0.165
Missing (*n*)	0	0	0	
Child sex				0.032
Boy	85 (61.59)	86 (50.89)	314 (49.29)	
Girl	53 (38.41)	83 (49.11)	323 (50.71)	
Missing (*n*)	0	0	0	
Maternal education				0.002
< College/university	48 (35.56)	39 (23.35)	132 (21.22)	
College/university	87 (64.44)	128 (76.65)	490 (78.78)	
Missing (*n*)	3	2	15	
Maternal marital status				0.097
Married/Cohabitant	130 (94.20)	163 (96.45)	622 (97.65)	
Single/Other	8 (5.80)	6 (3.55)	15 (2.35)	
Missing (*n*)	0	0	0	
Parity				0.010
0	75 (54.35)	114 (67.46)	414 (64.99)	
1 or more	63 (45.65)	55 (32.54)	223 (35.01)	
Missing (*n*)	0	0	0	
Maternal ADHD score	2.51 ± 0.67	2.47 ± 0.59	2.29 ± 0.60	0.007
Missing (*n*)	1	0	8	
Maternal fish intake (g/day)	26.48 ± 20.18	26.43 ± 17.17	26.70 ± 17.33	0.581
Missing (*n*)	1	3	11	
Child year of birth				0.712
2004	14 (10.15)	14 (8.29)	81 (12.71)	
2005	32 (23.19)	41 (24.26)	166 (26.06)	
2006	46 (33.33)	60 (35.50)	197 (30.93)	
2007-2008	46 (33.33)	54 (31.95)	193 (30.30)	
Missing (*n*)	0	0	0	
Smoking during pregnancy				0.068
No	116 (84.06)	153 (90.53)	577 (90.58)	
Yes	22 (15.94)	16 (9.47)	60 (9.42)	
Missing (*n*)	0	0	0	

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; SD, standard deviation. Note: p-values are based on chi-square test.

Table S3.

Rotated component loadings in a nested study of attention-deficit hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

PFAS	Component	
	1	2
PFHpS	**.56**	-.06
PFOS	**.49**	.04
PFHxS	**.48**	-.06
PFOA	**.44**	.09
PFDA	-.01	**.62**
PFUnDA	-.08	**.61**
PFNA	.13	**.48**

Note: The PFASs were log transformed before the PCA. Extraction Method: Principal Component Analysis. Rotation Method: Oblimin with Kaiser Normalization. Abbreviations: Perfluorooctane sulfonate (PFOS); perfluorooctanoic acid (PFOA); perfluorohexane sulfonate (PFHxS); perfluorononanoic acid (PFNA); perfluoroundecanoic acid (PFUnDA); perfluorodecanoic acid (PFDA); perfluoroheptanesulfonic acid (PFHpS).

Table S4.

Beta coefficients and 95% confidence intervals for complete cases of PFAS components by ADHD symptoms, language skills, nonverbal WM, verbal WM, nonverbal IQ, and verbal IQ in a nested study of attention-deficit hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

	ADHD symptoms (n = 642)	**Language skills (n = 616)**	**Nonverbal WM (n = 644)**	**Verbal WM (n = 540)**	**Nonverbal IQ (n = 644)**	**Verbal IQ (n = 646)**
Component 1: PFOA, PFHxS, PFHpS, PFOS	0.01 (-0.06, 0.07)	-0.04 (-0.40, 0.32)	-0.10 (-0.16, -0.05)	0.03 (-0.03, 0.09)	-0.02 (-0.08, 0.04)	0.01 (-0.04, 0.06)
Component 2: PFNA, PFDA, PFUnDA	0.03 (-0.04, 0.09)	0.09 (-0.28, 0.47)	0.05 (-0.01, 0.11)	0.05 (-0.01, 0.11)	-0.02 (-0.08, 0.03)	-0.02 (-0.08, 0.03)

Note: A separate regression model was conducted for each outcome: negative binomial regression for ADHD symptoms and linear regression for language skills. The following PFASs most heavily loaded on component 1: PFHpS, PFOS, PFHxS and PFOA. The following PFASs most heavily loaded on component 2: PFDA, PFNA and PFUnDA. The PFASs were log transformed before computing principal components. Each regression model was adjusted for maternal education, age, parity, fish intake, child sex and child age at testing. Child ADHD symptoms was adjusted for maternal ADHD symptoms.

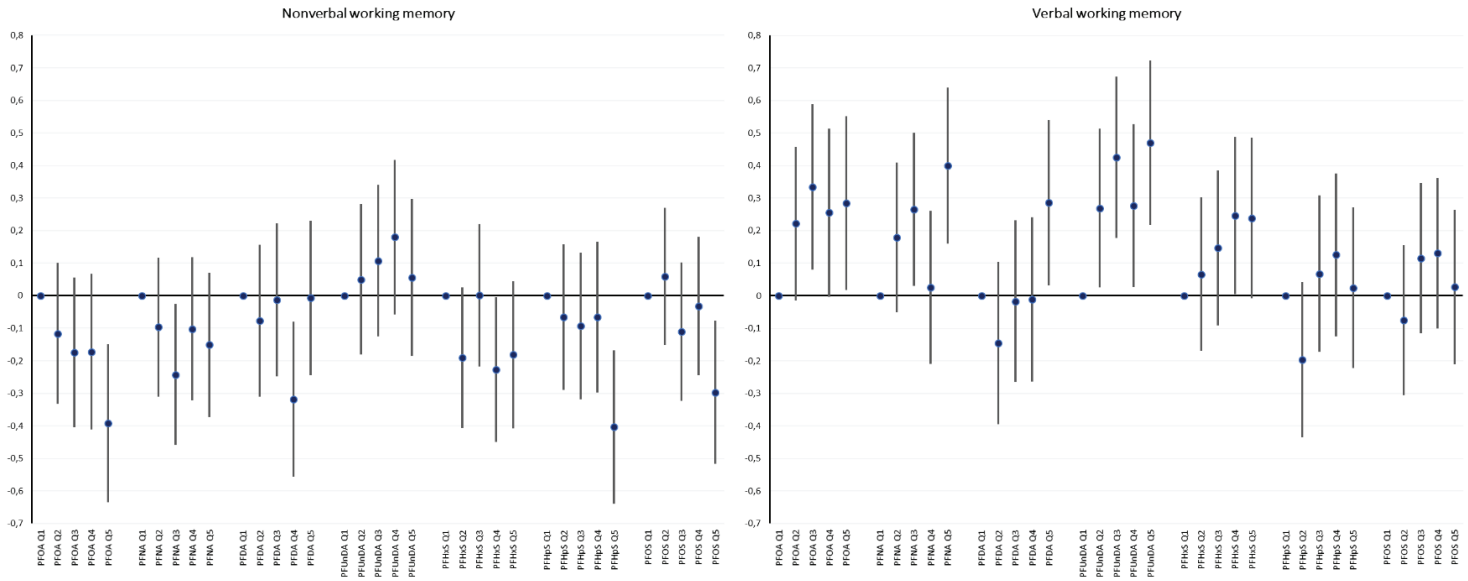

Figure S3. Beta coefficients and 95% confidence intervals for complete cases of regression models predicting nonverbal working memory and verbal working memory from quintile categories of each PFAS in a nested study of attention-deficit hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

Note: Each PFAS by nonverbal and verbal working memory combination was modelled using a separate linear regression adjusted for maternal education, age, parity, fish intake and child sex. The beta coefficient and 95% confidence intervals for each PFAS quintile are represented on the vertical axis (the reference level is the first quintile). Nonverbal working memory: PFOA (n = 901); PFNA (n = 900); PFDA (n = 743); PFUnDA (n = 782); PFHxS (n = 901); PFHpS (n = 807); PFOS (n = 901). Verbal working memory: PFOA (n = 740); PFNA (n = 739); PFDA (n = 615); PFUnDA (n = 646); PFHxS (n = 740); PFHpS (n = 670); PFOS (n = 740)

Table S5.

Crude beta coefficients and 95% confidence intervals for PFAS components by ADHD symptoms, language skills, nonverbal working memory, verbal working memory, nonverbal IQ, and verbal IQ in a nested study of attention-deficit hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

	ADHD symptoms (n = 943)	**Language skills (n = 899)**	**Nonverbal WM (n = 934)**	**Verbal WM (n = 768)**	**Nonverbal IQ (n = 935)**	**Verbal IQ (n = 936)**
Component 1: PFOA, PFHxS, PFHpS, PFOS	-0.04 (-0.08, 0.01)	0.19 (-0.08, 0.45)	-0.05 (-0.09, -0.02)	0.03 (-0.01, 0.08)	0.01 (-0.03, 0.04)	0.02 (-0.02, 0.06)
Component 2: PFNA, PFDA, PFUnDA	-0.04 (-0.09, 0.01)	0.27 (-0.03, 0.57)	-0.02 (-0.06, 0.03)	0.07 (0.02, 0.11)	-0.01 (-0.06, 0.03)	0.05 (0.00, 0.09)

Note: A separate regression model was conducted for each outcome: negative binomial regression for ADHD symptoms and linear regression for the other outcomes. The following PFASs most heavily loaded on component 1: PFHpS, PFOS, PFHxS and PFOA. The following PFASs most heavily loaded on component 2: PFDA, PFNA and PFUnDA. The PFASs were log transformed before computing principal components.

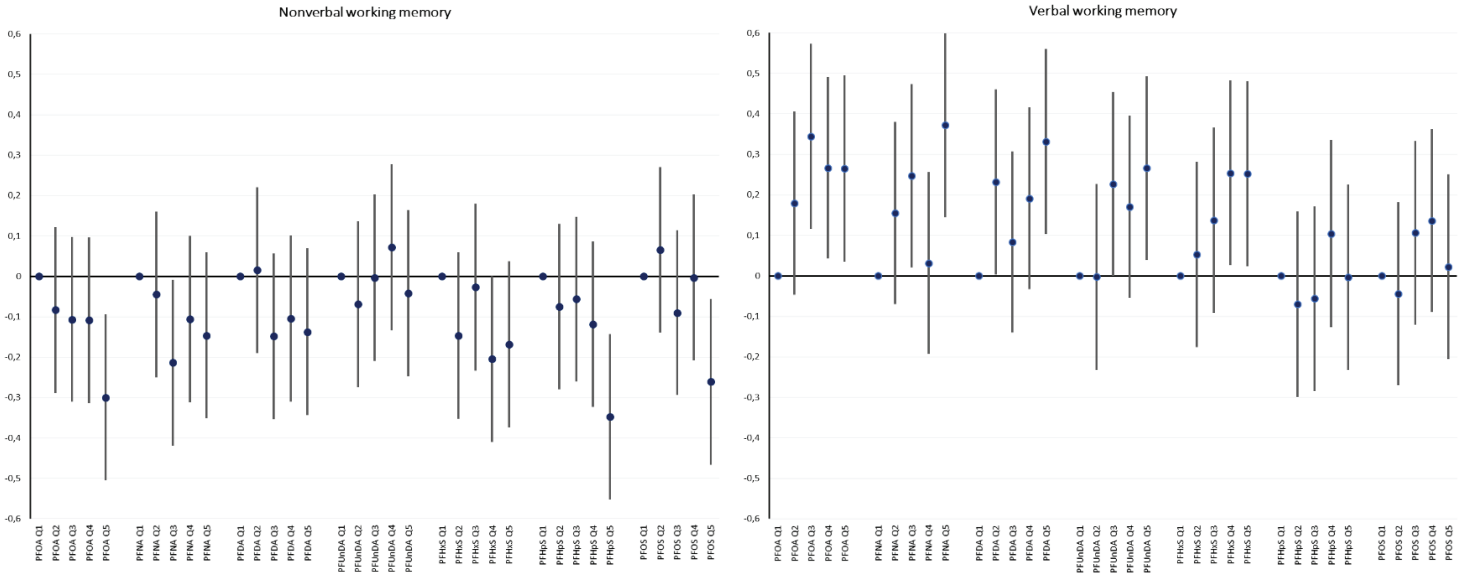

Figure S4. Crude beta coefficients and 95% confidence intervals for regression models predicting nonverbal working memory (n = 934) and verbal working memory (n = 768) from quintile categories of each PFAS in a nested study of attention-deficit hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

Note: Each PFAS by nonverbal and verbal working memory combination was modelled using a separate linear regression with multiple imputation. The beta coefficient and 95% confidence intervals for each PFAS quintile are represented on the vertical axis (the reference level is the first quintile).

Table S6.

Beta coefficients and 95% confidence intervals for PFAS components by ADHD symptoms, language skills, nonverbal WM, verbal WM, nonverbal IQ, and verbal IQ restricted to first-born children in a nested study of attention-deficit hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

	ADHD symptoms (n = 602)	**Language skills (n = 577)**	**Nonverbal WM (n = 597)**	**Verbal WM (n = 498)**	**Nonverbal IQ (n = 597)**	**Verbal IQ (n = 599)**
Component 1: PFOA, PFHxS, PFHpS, PFOS	-0.00 (-0.08, 0.08)	-0.09 (-0.51, 0.32)	-0.07 (-0.13, -0.01)	-0.01 (-0.07, 0.06)	0.03 (-0.03, 0.10)	-0.01 (-0.07, 0.05)
Component 2: PFNA, PFDA, PFUnDA	-0.01 (-0.08, 0.06)	-0.04 (-0.48, 0.39)	0.05 (-0.01, 0.12)	0.06 (-0.01, 0.13)	-0.06 (-0.13, -0.00)	0.02 (-0.05, 0.08)

Note: A separate regression model was conducted for each outcome: negative binomial regression for ADHD symptoms and linear regression for the other outcomes). The following PFASs most heavily loaded on component 1: PFHpS, PFOS, PFHxS and PFOA. The following PFASs most heavily loaded on component 2: PFDA, PFNA and PFUnDA. The PFASs were log transformed before computing principal components. Each regression model was adjusted for maternal education, age, parity, fish intake and child sex. Language skills and child ADHD symptoms were also adjusted for child age at testing and child ADHD symptoms was adjusted for maternal ADHD symptoms.

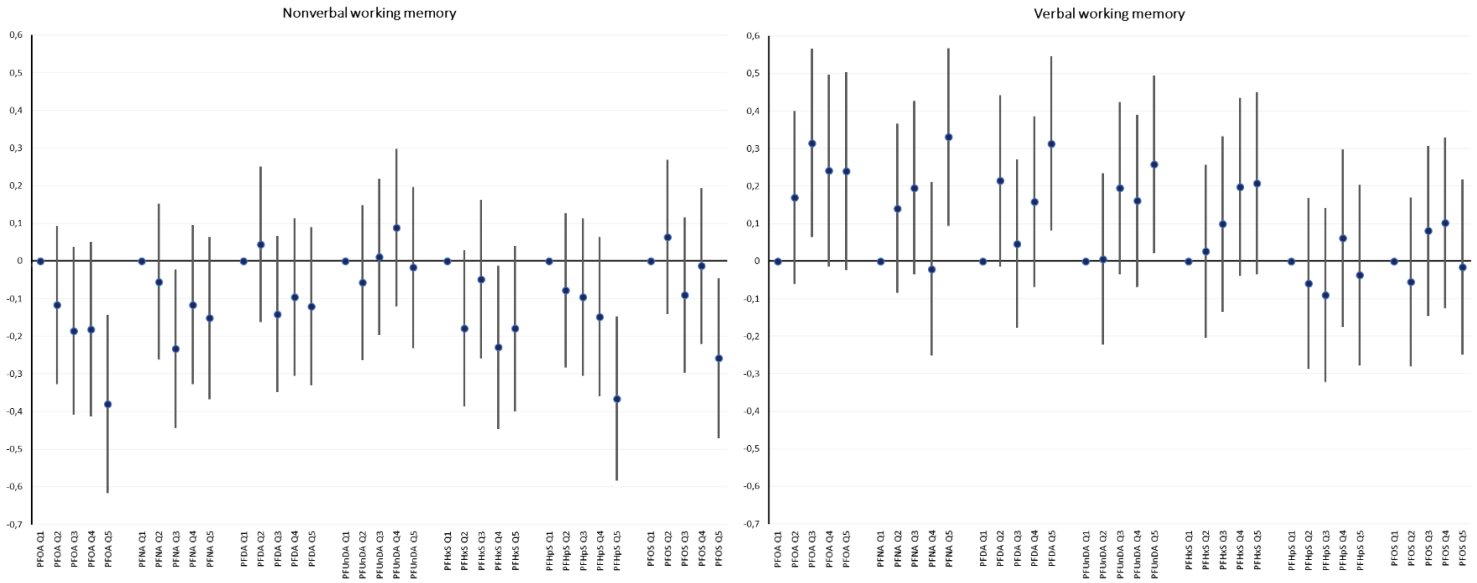

Figure S5. Beta coefficients and 95% confidence intervals for regression models predicting nonverbal working memory (n = 934) and verbal working memory (n = 768) from quintile categories of each PFAS without fish intake as a covariate in a nested study of attention-deficit hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

Note: Each PFAS by verbal working memory was modelled using a separate linear regression (with multiple imputation). The beta coefficient and 95% confidence intervals for each PFAS quintile are represented on the vertical axis (the reference level is the first quintile). Each regression model was adjusted for maternal education, age, parity and child sex. Higher working memory score indicates better working memory function.

II

Metal and essential element concentrations during pregnancy and associations with autism spectrum disorder and attention-deficit/hyperactivity disorder in children

Thea S. Skogheim[a*], Kjell Vegard F. Weyde[a], Stephanie M. Engel[b], Heidi Aase[a], Pål Surén[a], Merete G. Øie[c], Guido Biele[a], Ted Reichborn-Kjennerud[a,d], Ida H. Caspersen[e], Mady Hornig[f], Line S. Haug[g], Gro D. Villanger[a]

[a] Division of Mental and Physical Health, Norwegian Institute of Public Health, PO Box 222 Skøyen, 0213 Oslo, Norway

[b] Gillings School of Global Public Health, University of North Carolina at Chapel Hill, 135 Dauer Drive, Campus Box 7435, Chapel Hill, NC 27599-7435, USA

[c] Department of Psychology, University of Oslo, PO Box 1094 Blindern, 0317 Oslo, Norway

[d] Institute of Clinical Medicine, University of Oslo, PO Box 1171 Blindern, 0318 Oslo Norway

[e] Centre for Fertility and Health, Norwegian Institute of Public Health, PO Box 222 Skøyen, 0213 Oslo, Norway

[f] Department of Epidemiology, Columbia University, Mailman School of Public Health, 722 W 168th St, Rm. 736, New York, NY 10032, USA

[g] Division of Infection Control and Environmental Health, Norwegian Institute of Public Health, PO Box 222 Skøyen, 0213 Oslo, Norway

[*] Corresponding author at: Department of Child Health and Development, NIPH, PO Box 222 Skøyen, 0213 Oslo, Norway. E-mail address: thea.skogheim@fhi.no

Abstract

Background:

Prenatal exposure to toxic metals or variations in maternal levels of essential elements during pregnancy may be a risk factor for neurodevelopmental disorders such as attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) in offspring.

Objectives:

We investigated whether maternal levels of toxic metals and essential elements measured in mid-pregnancy, individually and as mixtures, were associated with childhood diagnosis of ADHD or ASD.

Methods:

This study is based on the Norwegian Mother, Father and Child Cohort Study and included 705 ADHD cases, 397 ASD cases and 1034 controls. Cases were identified through linkage with the Norwegian Patient Registry. Maternal concentrations of 11 metals/elements were measured in blood at week 17 of gestation; cadmium; cesium; cobalt; copper; lead; magnesium; manganese; selenium; zinc; total arsenic; and total mercury. Multivariable adjusted logistic regression models were used to examine associations between quartile levels of individual metals/elements and outcomes. We also investigated non-linear associations using restricted cubic spline models. The joint effect of the metal/element mixture on ASD and ADHD diagnoses was estimated using a quantile-based g-computation approach.

Results:

For ASD, we identified positive associations (increased risks) in the second quartile of arsenic [OR = 1.77 (CI: 1.26, 2.49)] and the fourth quartiles of cadmium and manganese [OR = 1.57 (CI: 1.07 2.31); OR = 1.84 (CI: 1.30, 2.59)], respectively. In addition, there were negative associations between cesium, copper, mercury, and zinc with ASD. For ADHD, we found increased risk in the fourth quartiles of cadmium and magnesium [OR =1.59 (CI: 1.15, 2.18); [OR = 1.42 (CI: 1.06, 1.91)]. There were also some negative associations, among others with mercury. In addition, we identified non-linear associations between ASD and arsenic, mercury, magnesium, and lead, and between ADHD and arsenic, copper, manganese, and mercury. There were no significant findings in the mixture approach analyses.

Conclusion:

Results from the present study show several associations between levels of metals and elements during gestation and ASD and ADHD in children. The most notable ones involved arsenic, cadmium, copper, mercury, manganese, magnesium, and lead. Our results suggest that even population levels of these compounds may have negative impacts on neurodevelopment. As we observed mainly similarities among the metals' and elements' impact on ASD and ADHD, it could be that the two disorders share some neurochemical and neurodevelopmental pathways. The results warrant further investigation and replication, as well as studies of combined effects of metals/elements and mechanistic underpinnings.

Keywords: Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); metal; essential element; The Norwegian Mother, Father and Child Cohort Study (MoBa); Medical Birth Registry of Norway (MBRN).

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) are neurodevelopmental disorders that interfere with learning and normal functioning during childhood and adolescence (Antshel et al., 2016; Kern et al., 2015). ADHD is one of the most common, affecting approximately 3–4% of children globally (Polanczyk et al., 2014). This disorder is characterized by inattention, impulsivity, and hyperactivity, with common additional dysfunctions like compromised motor skills and impaired cognitive functions (Polanczyk et al., 2007). ASD in children has a prevalence of around 1% in the Nordic countries and in the United States (Hansen et al., 2015; Idring et al., 2015; Sandin et al., 2014; Surén et al., 2012). ASD comprises heterogeneous disorders characterized by persistent deficits in social communication and social interaction, in addition to restricted and repetitive patterns of behavior, interests, or activities (American Psychiatric Association, 2013). Children with ASD have varied cognitive challenges and their intelligence scores can range from high levels to severe intellectual disability (Johnson & Myers, 2007). Childhood ADHD and ASD are more prevalent in boys compared to girls (Nussbaum, 2012; Polanczyk et al., 2007; Werling & Geschwind, 2013). While both disorders are to a large degree heritable, genetic factors are likely to interplay with environmental factors (Faraone et al., 2005; Nuttall, 2017; Sandin et al., 2014; Thapar et al., 2017a).

During pregnancy, toxic metals such as lead and mercury, are transferred from mother to fetus via the placenta (Grandjean & Landrigan, 2006). As fetal brain development is extraordinarily sensitive to toxicants, chemicals interfering with brain developmental processes may lead to neurodevelopmental deficits and related disorders during childhood, even at low exposure levels that may be considered safe for adults (Grandjean & Landrigan 2014; Heyer & Meredith, 2017; Tran & Miyake, 2017). For many of these chemicals (e.g. lead), a safe exposure level with regards to neurodevelopment is yet to be determined (Grandjean & Landrigan, 2014; Tran & Miyake, 2017).

Toxic metals such as mercury, lead, cadmium, and arsenic are naturally occurring in the environment (Järup, 2003; Tchounwou et al., 2012). In addition, there is a ubiquitous distribution of toxic metals in the environment due to anthropogenic activities such as mining, burning of fossil fuels and extensive use in agriculture and manufacturing of products (Järup, 2003; Tchounwou et al., 2012). A number of these elements are known developmental neurotoxicants, including lead, mercury, arsenic, manganese, and selenium (Grandjean & Landrigan, 2006). In addition, some are suspected as developmental neurotoxicants, for

example cadmium (European Food Safety Authority, 2009). Blood concentrations of metals and essential elements for pregnant Norwegian women are comparable to levels in other European countries (Caspersen et al., 2019; Haug et al., 2018), although the Norwegian levels seem to be somewhat higher for arsenic and mercury (Haug et al., 2018). For mercury, arsenic, and selenium, the predominant sources in the Norwegian population are fish and shellfish (Birgisdottir et al., 2013; Papadopoulou et al., 2019), whereas multimineral supplements seem to be major sources for some essential elements such as manganese, copper, zinc, and also selenium (Caspersen et al., 2019). Exposure to toxic metals in human populations seems to be associated with socioeconomic position, with higher concentrations of mercury in women with higher education (Montazeri et al., 2019). Fish and seafood intake, which is a source of mercury, is also related to socioeconomic position, with higher consumption among those with higher educational level (e.g. Touvier et al., 2010). Higher cadmium levels have been reported in women with lower education (Montazeri et al., 2019). There are also studies linking prenatal metal levels with sexually dimorphic placental transfer, potentially altered sex steroids, and/or sex-specific neurodevelopmental vulnerabilities (Baron-Cohen et al., 2019; Li et al., 2019; Wang et al., 2017; Werling & Geschwind, 2013).

Few studies have investigated gestational levels of metals and essential elements and ADHD in offspring, and these mainly report not on ADHD diagnosis, but rather on levels of ADHD-related symptoms such as inattention, impulsivity, and hyperactivity assessed through parent- or teacher-reported rating scales (Kalkbrenner et al., 2014; Vrijheid et al., 2016; Yoshimasu et al., 2014). Some studies report higher levels of ADHD symptoms in children with increased prenatal exposure to mercury, lead, or cadmium (Boucher et al., 2012; Kim et al., 2020; Neugebauer et al., 2015; Plusquellec et al., 2007; Sioen et al., 2013). However, other studies report no associations with metals/elements (e.g. Patel et al., 2019; Forns et al., 2014). One study investigated prenatal exposure to selenium and manganese and ADHD diagnosis in children and found increased risk with the highest levels of selenium (Ode et al., 2015). Most studies on prenatal metal/element exposure and ASD have investigated the impact of mercury exposure and report no associations (e.g. Golding et al., 2018; McKean et al., 2015; van Wijngaarden et al., 2013; Yau et al., 2014). One study measuring metals in amniotic fluids found no associations between ten different metals and ASD diagnosis in children when metals were assessed individually, except for an inverse association with a factor containing copper (amongst other compounds) (Long et al., 2019). Another study did identify a positive association between prenatal mercury and autistic behaviors in children at

five years of age (Ryu et al., 2017). Altogether, there is still limited knowledge on prenatal exposure to metals or variations of maternal levels of essential elements and clinician-based ASD and ADHD diagnoses in childhood. In addition, there are inconsistencies regarding study designs and findings.

The overall objective of the present study was to investigate associations between gestational levels of 11 metals and essential elements, individually and as mixtures, and childhood diagnosis of ADHD or ASD. In addition, we investigated effect measure modification by child sex and maternal education.

2. Methods

2.1 Study Design and Participants

The current study is based on data from the Norwegian Mother, Father and Child Cohort Study (MoBa) and the Medical Birth Registry of Norway (MBRN) with linkage to the Norwegian Patient Registry (NPR). MoBa is a population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health. Participants were recruited from all over Norway from 1999-2008 and are still being followed up. Among the invited women, 41% consented to participation. The cohort now includes 114,500 children, 95,200 mothers and 75,200 fathers (Magnus et al., 2016). Blood samples were obtained from both parents during pregnancy and from mothers and children (umbilical cord) at birth (Paltiel et al., 2014). The current study is based on version 12 of the quality-assured data files released for research in January 2019. The MoBa cohort is regulated under the Norwegian Health Registry Act. The NPR has approved the linkage between NPR and MoBa, identifying ADHD and ASD diagnostic cases. The current study was approved by The Regional Committees for Medical and Health Research Ethics (ref. no. 2012/985-1). MBRN is a national health registry containing information about all births in Norway. The NPR is a national health care registry that receives patient data on diagnoses reported from all hospitals and specialized health care services in Norway. The registry contains diagnoses for in- and outpatients recorded from 2008 and onward. The diagnostic codes reported to the NPR are according to the International Statistical Classification of Diseases, 10th Revision (ICD-10).

2.2 Cases and controls

For both cases and controls we included children that were singletons, born in 2002 or later and alive at 2 years of age (controls only), had records available from the MBRN and prenatal MoBa questionnaire 1 (~17 weeks' gestation), with no registration in MBRN of Down's syndrome or of serious malformation, and with available maternal whole blood sampled at week 17 of gestation (Figure 1). The total number of cases and controls in the present study was 705 ADHD cases, 397 ASD cases, and 1034 controls (Figure 1).

From the NPR, we obtained information on diagnosis of ADHD and ASD among children born in 2002 or later, because of the availability of data and biological samples. For ADHD, we selected cases if they had at least two registrations of "hyperkinetic disorder" (ICD-10 codes F90, F90.0, F90.1, F90.8, or F90.9) (World Health Organization, 1993). We required a minimum of two registrations in order to exclude erroneous registrations or false diagnoses. The ICD-10 criteria for hyperkinetic disorder/ADHD are "early onset; a combination of overactive, poorly modulated behavior with marked inattention and lack of persistent task involvement; and pervasiveness over situations and persistence over time of these behavioral characteristics" (World Health Organization, 1993). Hyperkinetic disorder requires the combination of inattentive and hyperactive symptoms and is thus similar to the ADHD combined subtype in the DSM system (Thapar et al., 2017b). In the present study, we use the term ADHD.

For ASD, we selected cases if they registrations of "pervasive developmental disorders", meeting criteria for ASD (F84.0, F84.1, F84.5, F84.8 or F84.9) (World Health Organization, 1993). Childhood autism (F84.0) is defined as "a pervasive developmental disorder defined by the presence of abnormal and/or impaired development that is manifest before the age of 3 years, and by the characteristic type of abnormal functioning in all three areas of social interaction, communication, and restricted, repetitive behavior" (World Health Organization, 1993). For both ADHD and ASD, girls were oversampled among cases if possible.

We selected a random sample of controls from the same eligible group of MoBa participants as the cases, fulfilling the same inclusion criteria as the cases (Figure 1). The controls were frequency-matched to case categories on sex and birth year. We used the same control group for ASD and ADHD analyses.

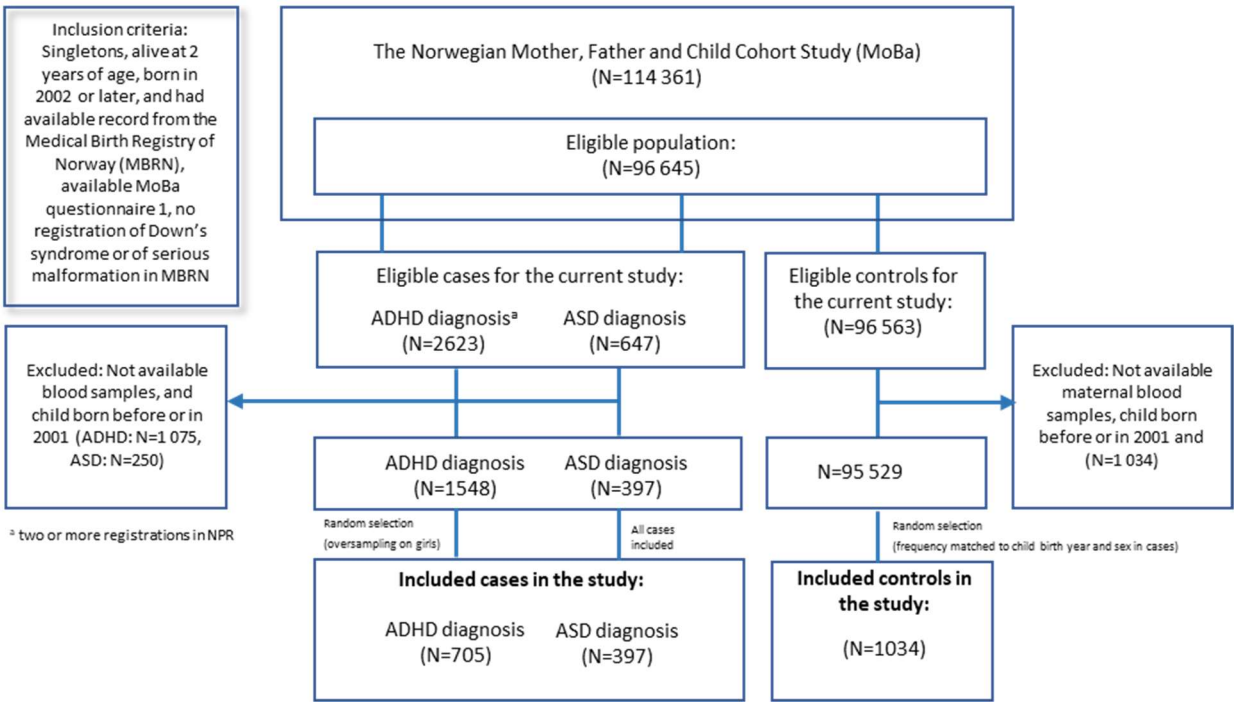

Figure 1. Flow chart of recruitment of cases and controls in in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Abbreviations: Attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), The Medical Birth Registry of Norway (MBRN); The Norwegian Mother, Father and Child Cohort Study (MoBa); The Norwegian Patient Registry (NPR).

2.3 Exposures

In this study, we used maternal blood samples from approximately week 17 of gestation. Details about the sampling procedure and handling and storage in the MoBa biobank are described in detail elsewhere (Paltiel et al., 2014). Eleven metals and essential elements were determined in maternal whole blood, using inductively coupled plasma-sector field mass spectrometry (ICP-SFMS). These included both toxic/non-essential metals; arsenic, cadmium, cesium, lead, mercury, and essential elements; cobalt, copper, magnesium, manganese, selenium, and zinc. Mercury and arsenic are measures of total mercury and total arsenic, containing both inorganic and organic forms. However, in the Norwegian population, these measures will largely reflect organic forms (Brantsæter et al., 2010). The analysis was mainly conducted at ALS laboratory group of Norway; a few samples were analyzed at the University of Lund as part of another MoBa project. The Norwegian Institute of Public Health

has a framework agreement with ALS, and they have until now analyzed ~2000 samples of maternal whole blood from MoBa. Internal quality control samples and procedure blanks were analyzed along with each batch of samples to ensure high quality of the determinations throughout the project. We additionally included reference samples (Seronorm Trace Elements whole blood L-1, SERO AS, Billingstad, Norway) that were used as project-specific quality control (QC) samples. Case, control and QC samples were randomized to batch and blinded to the analysist. More detailed information on analytical procedures, limits of detection (LOD), limits of quantification (LOQ) and quality control can be found in Appendix A and Table S1. For most metals/elements, concentrations above LOQ are reported, except for arsenic, cadmium, lead, and mercury, for which concentrations above LOD are reported. Metals/elements concentrations are given in μg/L, except for magnesium, which is given in mg/L.

The blood samples of our maternal participants were pulled from the Biobank and analyzed for metals and elements in three separate analytical rounds (see Appendix A). In addition, some samples were analyzed at the University of Lund (~4th round). To account for analytical variation across analytical rounds, we normalized the metal/element concentrations for each participant using our QC samples (Seronorm reference material) analyzed in each of the analytical rounds. We used a similar approach with scaled variation of the Ratio-G batch adjustment as described in Luo et al. (2010). Suppose M represents the measured concentrations of metal/element *i* for each participant *j*. M^*_{ij} represents the analytical round-adjusted metal/element concentration, which is calculated using the following equation (Equation 1):

$$M^*_{ij} = M_{ij} \; x \; (meanQC_l/meanQC_{lk}), \qquad (1)$$

where $meanQC_l$ represents the geometric mean of metal/element *i* in reference samples across all analytical rounds (5 reference samples x 4 rounds), and $meanQC_{lk}$ represents the geometric mean of metal/element *i* in reference samples from analytical round *k* (i.e. in the analytical round in which sample of the participant *j* was measured).

2.4 Covariates and other variables

We obtained information on covariates from the MBRN and the MoBa questionnaires completed during pregnancy and up to child's age three years. The MoBa study included a

food frequency questionnaire (FFQ) that the participants completed at 22 weeks' gestation, providing good validity for estimates of intake of foods and nutrients (Brantsæter et al., 2008). Potential adjustment variables were selected *a priori* based on existing literature using a directed acyclic graph (DAG) approach (Greenland et al., 1999). We considered these as interdependent variables relevant for the current analysis: child sex, birth weight, birth year, and small for gestational age (SGA), maternal age at delivery, education, parity, pre-pregnancy body mass index (BMI, kg/m^2), self-reported smoking and alcohol intake during pregnancy, as well as FFQ-based estimates of seafood intake (g/week), and dietary iodine intake (µg/day). We used dagitty.net (Textor et al., 2011) to determine the minimal adjustment set, i.e. the minimal set of adjustment variables to obtain an unbiased causal effect under the assumption of no unmeasured confounders, for estimating the total effect of a metal/element given our hypothesized causal model (c.f. DAGs in Figures S1 and S2). This set included maternal age, seafood intake, smoking and parity. In addition, we adjusted for maternal education, child sex, and birth year in our analyses. Maternal ADHD symptoms, measured by the Adult ADHD Self-Report Scale (ASRS screener) (Kessler et al., 2007), were also included as a covariate in analyses with child ADHD as outcome.

2.5 Statistical analysis

Among the 11 metals/elements included in our study, arsenic, cadmium, and cobalt had missing values due to levels below LOD or LOQ. Cesium and magnesium were not included in the analyses at the University of Lund and had missing values for this reason. In addition, some of the covariates had missing values. To replace missing data, we ran multiple imputation by chained equations, separately for the ADHD sample (with cases and controls) and for the ASD sample (with cases and controls). We generated 50 datasets with the exposure and outcome variables, covariates, and auxiliary variables (Rubin, 1976; Sterne et al., 2009) using the mi ice command in Stata (Royston, 2009). We used the method for interval-censored data and specified upper and lower limit for imputed results for metals/elements as LOD (arsenic and cadmium) or LOQ (cobalt) and zero, respectively (Royston, 2009). The pooling procedure used in this article was mi estimate (Stata Press, 2017). Details about the missing data and the imputation model are displayed in supplemental material (Appendix B).

As a first approach, logistic regression analyses were performed for ADHD and ASD diagnoses, separately, to investigate dose-response relationships between outcome variables and quartile levels of individual metals/elements. The lowest quartile was the reference group. We also explored effect measure modification (significance at $p < 0.10$) by child sex and maternal education (as a measure of socioeconomic position). As many metals/elements were tested individually in quartile plots, we acknowledge that the number of tests performed is fairly high (n = 33 for each outcome), thus inflating the probability of type 1 error. Therefore, we also evaluated the results with 99.8% confidence intervals (CIs) and $p < 0.002$ for the quartile analyses. This would correspond to Šidák correction to control for familywise error rate (false discoveries or type I errors) for k = 33 number of tests calculated by $100(1-\alpha)^{1/k}$ % confidence intervals with $\alpha = 0.05$.

Secondly, to further investigate and test if the shape of the dose-response relationships between individual elements/metals and ADHD/ASD deviated from a monotonic function, we modeled the association between single metal/element as restricted cubic splines with knots at the 10^{th}, 50^{th}, and 90^{th} percentile of the metal/element distributions (with baseline at the median). Prior to the spline analyses, metal/element outliers were replaced (less or equal to the 1^{st} percentile and greater or equal to the 99^{th} percentile) by the values above or below the 1^{st} and 99^{th} percentile. We tested if the spline association significantly differed from a linear, logistic regression model association using likelihood ratio test (LRT; significance for non-linearity at $p < 0.05$). These analyses were performed in one of the imputed data sets.

Finally, we analyzed the joint effect of metals and essential elements on ASD and ADHD diagnoses. The effect of individual metals or essential elements may be small and thus more challenging to identify. This makes it difficult to predict the joint (total) effect of the mixture based on modelling of individual metals/elements. For the mixture analyses we used a quantile-based g-computation approach (R package qgcomp; Keil et al. 2019). This novel method, combining weighted quantile sum (WQS) regression and g-computation, is developed to assess effect of mixtures, giving estimates of the simultaneous effect on the outcome of an increase of all exposures in the mixture by one quantile (Keil et al. 2019; Niehoff et al. 2020). In our study the quantile was set to one quartile increase in log-metal/element concentrations. We investigated three different mixtures a priori based on the literature (Tchounwou et al. 2012; Zoroddu et al. 2019): A mixture containing all 11 metals and essential elements, a mixture containing essential elements (selenium, manganese, cobalt,

copper, zinc, and magnesium) and a mixture containing toxic/non-essential metals (arsenic, mercury, cadmium, lead, and cesium).

We performed several sensitivity analyses. In the quartile models we restricted the sample to non-smokers (during pregnancy) for cadmium and lead. We also ran the quartile models without seafood intake as a covariate. We performed an additional interaction analysis for maternal education and cadmium, excluding children of mothers who smoked during pregnancy. Additionally, as maternal zinc and magnesium levels may be a marker of pregnancy multivitamin supplement intake, we ran the quartile models for zinc and magnesium with only children of mothers who took folate supplement during pregnancy. Finally, we did a sensitivity analysis where we removed those with blood samples measured at Lund and compared the results to our main results. This was done in order to ensure that laboratory did not substantially affect our results.

All logistic regression models were expressed with odds ratios (ORs) and accompanying 95% CIs. Significance for non-linearity was set at $p < 0.05$ and interaction at $p < 0.01$. Most statistical analyses were performed in Stata version 15 (StataCorp, 2019). In addition, we used R version 3.6.2 (R Core Team, 2018) with the "foreign" (R Core Team, 2020), "Amelia" (Honaker et al., 2019), "psych" (Revelle, 2020), "readstata13" (Garbuszus & Jeworutzki, 2018), "qgcomp" (Keil, 2020), "ggplot2" (Wickham et al., 2020), and "tidyverse" (Wickham, 2019) packages. The imputed and adjusted results for the logistic regression models are presented in this article, while complete case analyses (Figures S3 and S4) are presented in the supplementary material. Metals/element concentrations before and after normalization to analytical round are presented in supplementary material (Table S2) as well as the quartile levels, ORs and CIs from the adjusted quartile models (Table S3).

3. Results

3.1 Study sample characteristics and metal/element distribution

Study sample characteristics are displayed in Table 1. Mothers of cases were slightly younger than mothers of controls. Among controls and ASD cases, the majority of the mothers had higher education (university/college), whereas the majority of the mothers of the ADHD cases had lower education (less than university/college). Most of the mothers of controls and ADHD cases were multiparous, whereas the majority of mothers of ASD cases

were primiparous. Mothers of ADHD cases were more likely to have reported smoking during pregnancy than mothers of controls and ASD cases.

Table 1. Characteristics of study population in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Characteristic	MoBa Controls Mean ± SD or n (%)	NPR ADHD Cases Mean ± SD or n (%)	NPR ASD Cases Mean ± SD or n (%)
Total N	1034	705	397
Maternal ADHD sum score	2.09 ± 0.56	2.24 ± 0.71	2.24 ± 0.59
Missing (*n*)	397	258	170
Maternal education			
< University/college	336 (33.3)	356 (51.8)	163 (42.1)
University/college	673 (66.7)	331 (48.2)	224 (57.9)
Missing (*n*)	25	18	10
Maternal age	30.06 ± 4.43	29.0 ± 4.97	29.6 ± 4.94
Missing (*n*)	0	0	0
Parity			
0	440 (42.6)	340 (48.2)	228 (57.4)
1 or more	594 (57.4)	365 (51.8)	169 (42.6)
Missing (*n*)	0	0	0
Maternal total seafood intake	36.7 ± 21.87	37.4 ± 27.7	36.3 ± 25.1
Missing (*n*)	123	82	26
Child sex			
Girl	329 (31.8)	185 (26.2)	61 (15.4)
Boy	705 (68.2)	520 (73.8)	336 (84.6)
Missing (*n*)	0	0	0
Child year of birth			
2002	243 (23.5)	93 (13.2)	35 (8.82)
2003	149 (14.4)	149 (21.2)	68 (17.1)
2004	188 (18.2)	152 (21.6)	55 (13.9)
2005	249 (24.1)	142 (20.1)	70 (17.6)
2006	84 (8.1)	93 (13.2)	69 (17.4)
2007	67 (6.5)	63 (8.9)	55 (13.9)
2008	49 (4.7)	13 (1.8)	38 (9.6)
2009	5 (0.5)	-	7 (1.8)
Missing (*n*)	0	0	0
Alcohol during pregnancy			
No	680 (68.6)	486 (71.6)	269 (70.2)
Yes	311 (31.4)	193 (28.4)	114 (29.8)
Missing (*n*)	43	26	14
Smoking during pregnancy			
No	901 (87.1)	545 (77.4)	332 (83.6)
Yes	133 (12.9)	159 (22.6)	65 (16.4)
Missing (*n*)	0	1	0
Maternal marital status			
Married/cohabitant	1005 (97.2)	649 (92.1)	377 (95.0)

Characteristic	MoBa Controls Mean ± SD or n (%)	NPR ADHD Cases Mean ± SD or n (%)	NPR ASD Cases Mean ± SD or n (%)
Other (single, divorced, widow)	29 (2.8)	56 (7.9)	20 (5.04)
Missing (*n*)	0	0	0
Child birth weight	3649 ± 519	3586 ± 638	3594 ± 670
Missing (*n*)	0	1	0
Length of gestation	39.5 ± 1.67	39.3 ± 2.11	39.37 ± 2.25
Missing (*n*)	3	5	1
Maternal total folate intake	240 ± 233	260 ± 260	262 ± 306
Missing (*n*)	78	42	26
Maternal folate supplement			
No	395 (38.2)	260 (36.9)	127 (32.0)
Yes*	639 (61.8)	445 (63.1)	270 (68.0)
Missing (*n*)	0	0	0

Note: *Any folate supplements between 4wk before and 8 wk after conception. Abbreviations: Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); Norwegian patient registry (NPR); standard deviation (SD).

Table 2 shows the distribution of maternal blood concentrations of toxic metals and essential elements in our sample including the geometric mean, median, and interquartile range of maternal metal/element concentrations during pregnancy. Six of the metals/elements (copper, lead, manganese, mercury, selenium, and zinc) were above LOD/LOQ in all measurements.

Table 2. Metal/essential element distribution (µg/L or mg/L) in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002-2009.

Metal/ element	Case/ control	N	% > LOQ	Geometric mean (95% CI)	Min	25%	50%	75%	Max
As	Control	1022	98.8	1.76 (1.67, 1.85)	0.12	1.00	1.68	2.85	54.2
	ADHD case	687	97.4	1.59 (1.49, 1.70)	0.09	0.88	1.50	2.71	27.0
	ASD case	396	99.7	1.65 (1.55, 1.77)	0.33	1.03	1.43	2.40	27.0
Cd	Control	1012	97.9	0.19 (0.18, 0.20)	0.01	0.12	0.18	0.28	3.05
	ADHD case	696	98.7	0.24 (0.22, 0.25)	0.02	0.14	0.20	0.40	3.14
	ASD case	397	100	0.22 (0.20, 0.24)	0.02	0.13	0.19	0.32	142
Co	Control	1003	97.0	0.19 (0.18, 0.20)	0.04	0.12	0.18	0.28	29.5
	ADHD case	681	96.6	0.18 (0.17, 0.18)	0.04	0.11	0.17	0.25	1.77
	ASD case	397	100	0.18 (0.17, 0.19)	0.04	0.12	0.18	0.27	1.31
Cs	Control	934	90.3	2.28 (2.23, 2.33)	0.91	1.83	2.26	2.82	8.45
	ADHD case	628	89.1	2.13 (2.06, 2.19)	0.72	1.67	2.12	2.62	9.49

Metal/ element	Case/ control	N	% > LOQ	Geometric mean (95% CI)	Min	25%	50%	75%	Max
	ASD case	393	99.0	2.12 (2.05, 2.19)	0.68	1.69	2.17	2.65	9.25
	Control	1034	100	1562 (1548, 1577)	778	1426	1551	1737	3178
Cu	ADHD case	705	100	1568 (1548, 1589)	774	1401	1573	1737	3629
	ASD case	397	100	1584 (1558, 1610)	901	1425	1584	1742	3583
	Control	1034	100	1.39 (1.34, 1.45)	0.18	0.96	1.44	2.12	10.0
Hg	ADHD case	705	100	1.17 (1.11, 1.23)	0.08	0.77	1.25	1.91	7.86
	ASD case	397	100	1.17 (1.09, 1.26)	0.12	0.73	1.20	1.93	10.1
	Control	934	90.3	30.1 (29.9, 30.4)	18.5	28.0	30.3	32.6	45.0
Mg	ADHD case	628	89.1	30.2 (29.9, 30.6)	15.2	28.0	30.4	33.2	74.45
	ASD case	393	99.0	30.1 (29.7, 30.5)	15.2	27.8	30.6	33.0	42.7
	Control	1034	100	10.2 (9.97, 10.5)	3.38	8.04	9.91	12.30	164
Mn	ADHD case	705	100	9.97 (9.63, 10.3)	2.06	7.60	9.63	12.0	221
	ASD case	397	100	11.1 (10.5, 11.6)	2.06	8.33	10.2	13.5	128
	Control	1034	100	8.82 (8.60, 9.05)	1.96	6.61	8.68	11.21	82.4
Pb	ADHD case	705	100	8.74 (8.46, 9.02)	1.88	6.74	8.57	11.53	57.4
	ASD case	397	100	8.35 (7.97, 8.75)	1.59	6.22	8.30	11.08	57.4
	Control	1034	100	93.04 (91.9, 94.2)	47.1	81.7	92.3	105	312
Se	ADHD case	705	100	90.1 (88.8, 91.5)	41.7	79.7	88.9	102	223
	ASD case	397	100	93.3 (91.3, 95.3)	44.4	81.6	93.0	108	182
	Control	1034	100	5202 (5139, 5266)	1972	4643	5269	5896	9931
Zn	ADHD case	705	100	5051 (5217)	1189	4495	5237	5875	11186
	ASD case	397	100	4966 (4850, 5085)	1189	4393	5170	5707	10743

Note: Mg values are in mg/L, all others are in µg/L. Abbreviations: Arsenic (As); attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); cadmium (Cd); cesium (Cs); cobalt (Co); copper (Cu); lead (Pb); magnesium (Mg); manganese (Mn); mercury (Hg); selenium (Se); zinc (Zn).

The correlations among the metals/elements are displayed in Table 3. The strongest correlations were between mercury and arsenic (r = 0.61), zinc and magnesium (r = 0.51), mercury and selenium (r = 0.38), and selenium and arsenic (r = 0.33).

Table 3. Spearman correlations between metals and essential elements (µg/L or mg/L) in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

	As	Cd	Co	Cs	Cu	Hg	Mg	Mn	Pb	Se	Zn
As	1.00										
Cd	0.00	1.00									
Co	0.07	0.13	1.00								
Cs	0.20	-0.02	0.07	1.00							
Cu	-0.01	0.01	0.10	0.01	1.00						
Hg	0.61	0.05	0.04	0.29	-0.05	1.00					
Mg	0.05	0.13	0.03	0.21	0.27	0.08	1.00				
Mn	0.06	0.09	0.19	0.00	0.15	0.05	0.25	1.00			
Pb	0.11	0.23	0.07	0.17	0.01	0.22	0.23	0.13	1.00		
Se	0.33	0.01	-0.04	0.26	0.09	0.38	0.29	0.12	0.15	1.00	
Zn	0.09	0.07	0.14	0.17	0.11	0.12	0.51	0.20	0.26	0.25	1.00

Abbreviations: Arsenic (As); cadmium (Cd); cesium (Cs); cobalt (Co); copper (Cu); lead (Pb); magnesium (Mg); manganese (Mn); mercury (Hg); selenium (Se); zinc (Zn).

3.2 Quartile models with effect measure modification and restricted cubic splines

3.2.1 ASD

For ASD, the quartile models showed an elevated risk for children in quartile 2 of arsenic [OR = 1.77 (CI: 1.26, 2.49)] compared to quartile 1 (reference) and with a decreasing monotonic trend in the next two quartiles (Figure 2, Table S3). There was an elevated ASD risk for children in the highest quartiles of cadmium [OR = 1.57 (CI: 1.07, 2.31)] and manganese [OR = 1.84 (CI: 1.30, 2.59)] (Figure 2, Table S3). We further identified negative associations with ASD (lowered risk) in some quartiles of cesium, copper, and zinc (Figure 2, Table S3). For mercury, all three quartiles had significantly lowered risk of ASD compared to quartile 1 (Figure 2, Table S3). The associations with arsenic, mercury, and manganese remained with 99.8% CIs, while the one with cadmium did not remain.

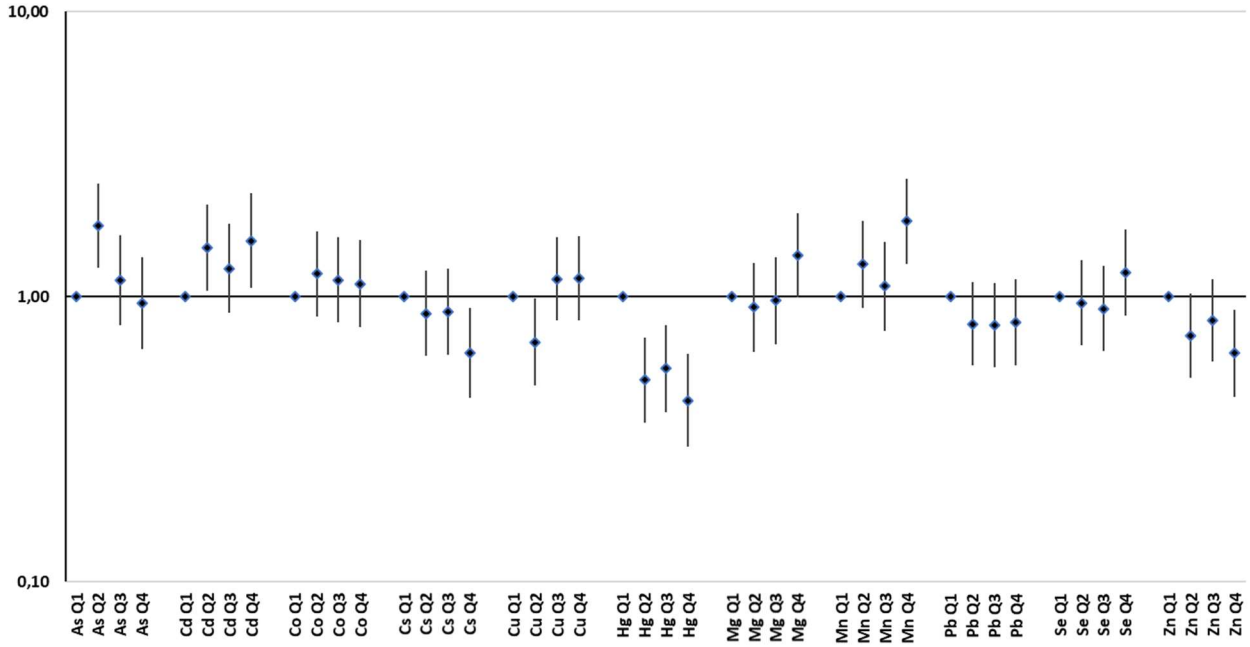

Figure 2. Odds ratios and 95% confidence intervals of logistic regression models predicting autism spectrum disorder in quartile categories of gestational metal/essential element levels in a nested case–control study of autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009 (n=1431).

Note: Logistic regression with multiple imputed datasets (n=50). All metals/element concentrations were normalized to analytical round and natural log transformed. The odds ratio and 95% confidence intervals for each metal/element quartile are represented on the vertical axis (the reference level is the first quartile). Each regression model was adjusted for maternal age, education, parity, seafood intake, smoking, child sex, and child birth year. Abbreviations: Arsenic (As); cadmium (Cd); cesium (Cs); cobalt (Co); copper (Cu); lead (Pb); magnesium (Mg); manganese (Mn); mercury (Hg); selenium (Se); zinc (Zn).

There was evidence of effect measure modification by child sex in the quartile models of mercury for ASD (overall p interaction < 0.10; Table S4). For mercury, the interaction with child sex was limited to quartile 2 (p interaction = 0.02) and quartile 4 (p interaction = 0.07), such that boys were driving the negative relationships with mercury in these quartiles [Q2: OR = 0.41 (CI: 0.28, 0.60); Q4: OR = 0.37 (CI: 0.25, 0.56)] and not the girls [Q2: OR = 1.19 (CI: 0.55, 2.58); Q4: OR = 0.86 (CI: 0.37, 1.98)] (Table S4).

The restricted cubic splines showed significant non-linear associations for ASD with arsenic, lead, magnesium, and mercury, (Figure 3). The association between arsenic and ASD showed an inverse u-shape (Figure 3). For lead and magnesium, the splines were u-shaped

(Figure 3). For mercury there was a non-linear shape with elevated ASD risk among the lower mercury concentrations and no apparent risk at higher concentrations (Figure 3).

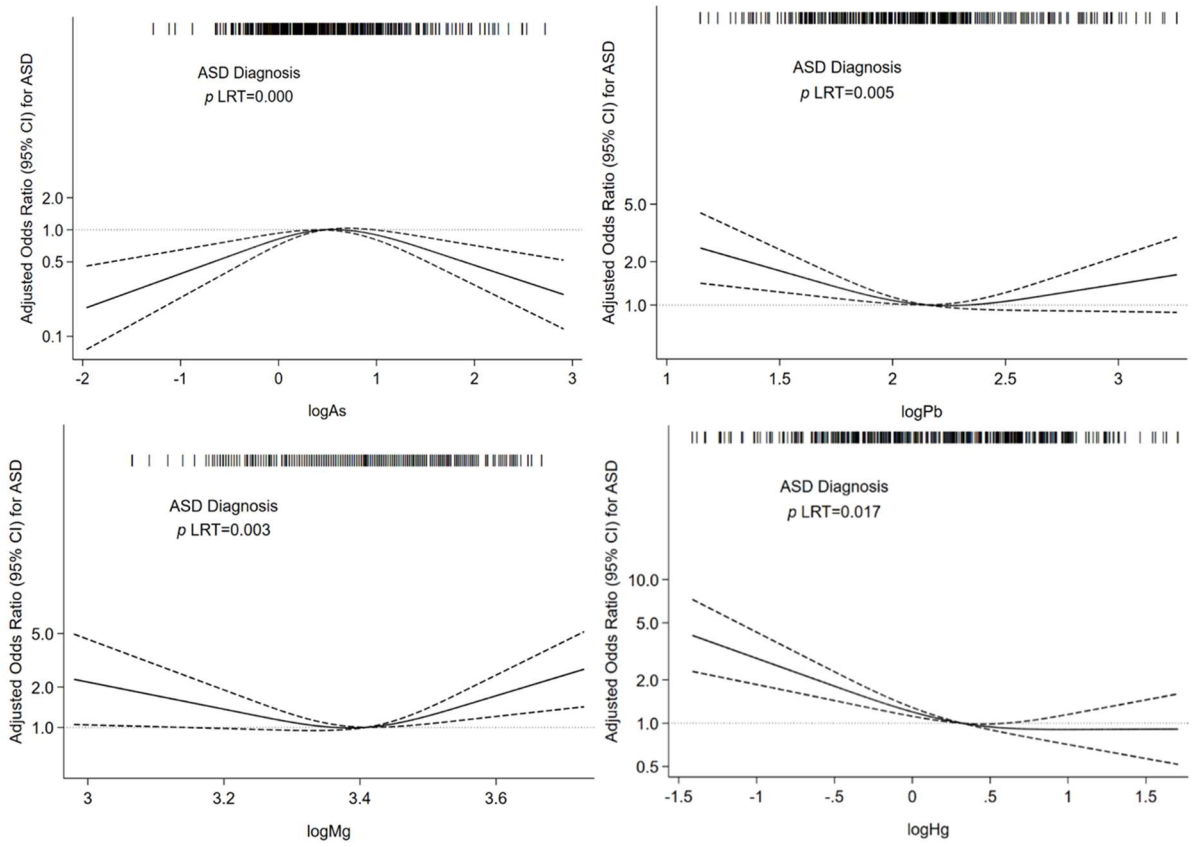

Figure 3. Restricted cubic spline predicting the odds of autism spectrum disorder in children associated with gestational levels of arsenic, lead, magnesium, and mercury in a nested case–control study of autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009 (n=1431).

Note: Three knot positions at 10th, 50th and 90th percentiles of arsenic, lead, magnesium, and mercury. Solid lines represent estimated odds ratios, and the dashed lines represent 95% confidence intervals. Hashing along the top horizontal axis represents the distribution of cases. Analyses was performed in one imputed dataset. The model was adjusted for maternal age, education, parity, seafood intake, smoking, child sex, and child birth year. Abbreviations: Arsenic (As); autism spectrum disorder (ASD); confidence intervals (CI); lead (Pb); likelihood ratio test (LRT); magnesium (Mg); mercury (Hg).

3.2.2 ADHD

For ADHD there was an increasing risk with increasing cadmium quartiles in a monotonic dose response pattern, although this was only significant for children in highest

cadmium quartile [OR =1.59 (CI: 1.15, 2.18)] compared to the lowest quartile (Figure 4, Table S3). This relationship was significantly modified by maternal education in quartile 3 only (p interaction = 0.02) with a higher odds of ADHD among children with mothers of university/college education [OR = 1.54 (CI: 1.08, 2.20)] compared to those with less than university/college [OR = 0.77 (CI: 0.48, 1.24) (Table S5). This relationship also persisted when children of mothers who smoked during pregnancy were excluded (Table S6).

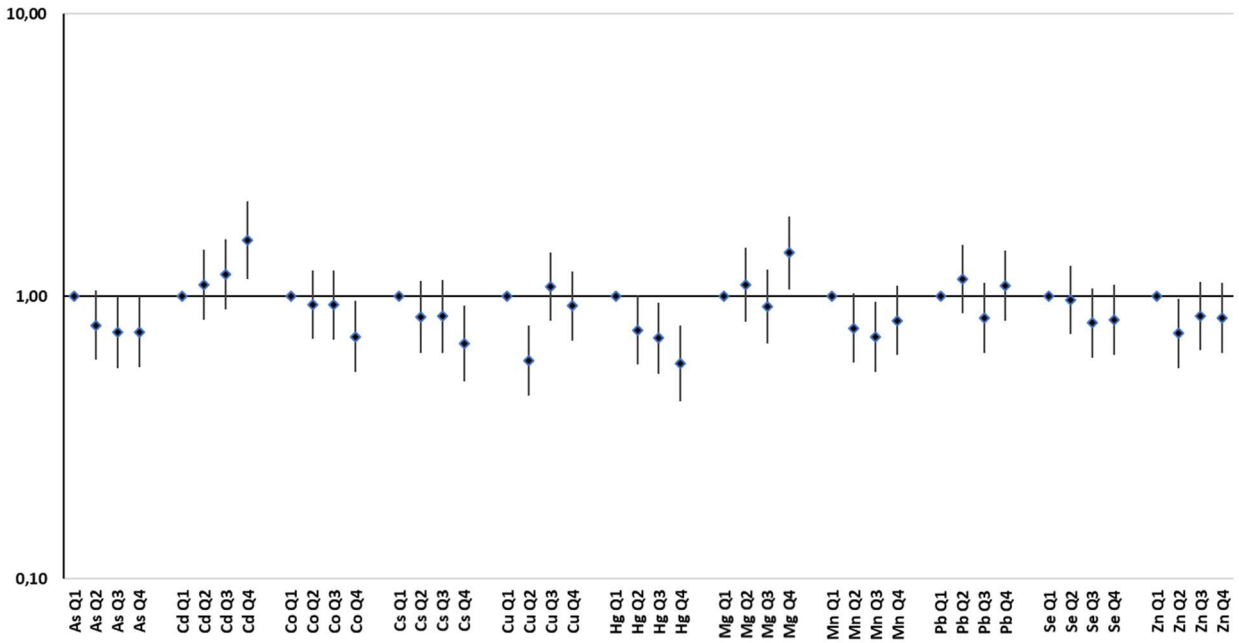

Figure 4. Odds ratios and 95% confidence intervals of logistic regression models predicting attention-deficit/hyperactivity disorder in quartile categories of gestational metal/essential element levels in a nested case–control study of attention-deficit hyperactivity disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009 (n=1739).

Note: Logistic regression with multiple imputed datasets (n=50). All metals/element concentrations were normalized to analytical round and natural log transformed. The odds ratio and 95% confidence intervals for each metal/element quartile are represented on the vertical axis (the reference level is the first quartile). Each regression model was adjusted for maternal age, education, parity, ADHD symptoms, seafood intake, smoking, child sex, and child birth year. Abbreviations: Arsenic (As); attention-deficit/hyperactivity disorder (ADHD); cadmium (Cd); cesium (Cs); cobalt (Co); copper (Cu); lead (Pb); magnesium (Mg); manganese (Mn); mercury (Hg); selenium (Se); zinc (Zn).

For children in the highest quartile of magnesium there was an elevated risk of ADHD [OR = 1.42 (CI: 1.06, 1.91)] (Figure 4, Table S3). Although the main models of manganese did not identify any significant relationship with ADHD, apart from a weak negative association with quartile 3, this relationship was significantly modified by maternal education (p interaction = 0.02). There were higher odds among children in the highest quartile of manganese whose mothers had college/university education [OR = 1.08 (CI: 0.75, 1.56)] compared to those with less than college/university [OR = 0.55 (CI: 0.35, 0.86)]. There were several negative associations with ADHD, among others with copper and mercury (Figure 4, Table S3). The associations with copper and mercury remained with 99.8% CIs, but not the relationships with cadmium and magnesium.

For ADHD there were non-linear associations with arsenic, copper, manganese, and mercury (Figure 5). The associations between ADHD and arsenic, copper, and manganese were slightly u-shaped (Figure 5). The association between ADHD and mercury had a similar shape to the one between ASD and mercury, with higher risk at the lowest levels (Figure 5).

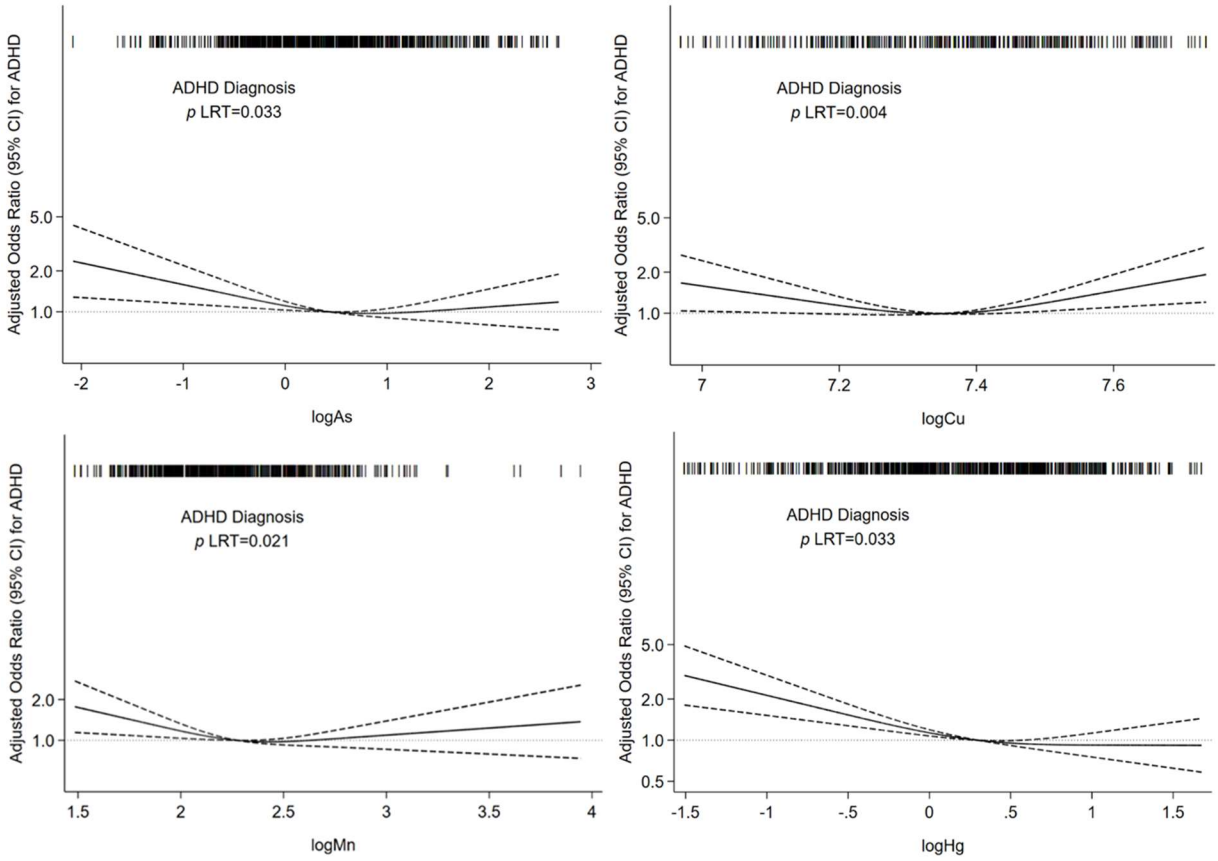

Figure 5. Restricted cubic spline predicting the odds of attention-deficit/hyperactivity disorder in children associated with gestational levels of arsenic, copper, manganese, and mercury in a nested case–control study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009 (n=1739).

Note: Three knot positions at 10th, 50th and 90th percentiles of arsenic, copper, manganese, and mercury. Solid lines represent estimated odds ratios, and the dashed lines represent 95% confidence intervals. Hashing along the top horizontal axis represents the distribution of cases. Analyses was performed in one imputed dataset. The model was adjusted for maternal age, education, parity, ADHD symptoms, seafood intake, smoking, child sex, and child birth year. Abbreviations: Arsenic (As); attention-deficit/hyperactivity disorder (ADHD); confidence intervals (CI); copper (Cu); likelihood ratio test (LRT); manganese (Mn); mercury (Hg).

3.3 Sensitivity analyses in the quartile models

The sensitivity analysis for zinc with ASD, where we only included mother-child pairs who took folate supplement during pregnancy (Table S7), did not change the estimates considerably. For magnesium and ASD, the estimates increased for the fourth quartile (Table S7). For ADHD, the sensitivity analyses for zinc and magnesium were similar to the main analyses (Table S7). The quartile analyses where we omitted seafood intake as a covariate did

not differ considerably from our main models (data not shown). The sensitivity analysis where we omitted smokers from the quartile models of cadmium and lead, did neither differ to a considerable degree from the main models (data not shown). We compared the main quartile models to models without blood samples analyzed at Lund, and the results were similar (data not shown).

3.4 Quantile-based g-computation

None of the results from the quantile-based g-computation of the total metal/element mixture were significant, for neither ASD [OR = 0.98 (CI: 0.76, 1.27)] nor ADHD [OR = 0.83 (CI: 0.67, 1.03)] (Table S8, Figure S5). The separate analyses with either essential elements or toxic/non-essential elements, were neither significant for ASD nor for ADHD (Table S8, Figure S6 and S7).

4. Discussion

In this large, prospective study, we found associations indicating increased risk of ASD in children with increased maternal levels of arsenic, cadmium, and manganese and increased risk of ADHD with increased maternal levels of cadmium and magnesium. In addition, there were negative (inverse) associations , between mercury and ASD and between mercury and copper with ADHD. Several of the associations were significantly non-linear or non-monotonic when dose-response relationships were modeled using restricted cubic splines. Neither of the mixtures from the quantile-based g-computation analyses were significantly associated with either ASD or ADHD.

4.1 Gestational toxic metals and ASD and ADHD in children

4.1.1 Cadmium

In the present study, we found 1.6 times higher odds for both ASD and ADHD in children of the highest cadmium exposure groups compared to the lowest exposure group. This is noteworthy, as cadmium is yet to be verified (i.e. suspected) as a developmental neurotoxicant (European Food Safety Authority, 2009). Our findings add to the emerging evidence from human epidemiological and experimental animal studies that cadmium can

interfere with important functions of brain development (Liu et al., 2019; Sanders et al., 2015). The results in the present study are in line with a recent study that reported associations between prenatal cadmium exposure and increased ADHD symptoms in girls (Kim et al., 2020). Another study reported an association between prenatal exposure to cadmium and an increased risk of emotional problems among boys (Sioen et al., 2013), but no effects on hyperactivity. However, two other studies investigating prenatal cadmium exposure, reported no adverse effects on neurodevelopmental outcomes (Forns et al., 2014; Long et al., 2019). There are still few prospective studies on prenatal cadmium exposure and ADHD or ASD in children (Rodríguez-Barranco et al., 2013; Vrijheid et al., 2016).

The association between cadmium and ADHD was modified by maternal education in the mid-to-high-exposure groups, with higher odds of ADHD diagnosis in children of mothers with university/college education compared to those with less than university/college education. Since smoking is a source of cadmium exposure (Agency for Toxic Substances and Disease Registry, 2012), and often related to lower SES (e.g. Magnus et al., 2015), we ran the analyses without mothers who smoked. However, our results remained when we omitted children of mothers who smoked during pregnancy. These results appear to contrast previous findings where higher cadmium exposure in pregnant women were associated with lower education (Caspersen et al., 2019; Montazeri et al., 2019; Tyrrell et al., 2013). Perhaps other sources of cadmium than smoking, such as intake of seafood (Agency for Toxic Substances and Disease Registry, 2012; Birgisdottir et al., 2013), contributed more to the cadmium exposure in the current study. Indeed, higher intake of seafood is related to higher educational level (Montazeri et al., 2019).

4.1.2 Arsenic

Our findings of increased risk of ASD and ADHD associated with prenatal arsenic exposure are in line with the epidemiologic literature, with numerous studies documenting the developmental neurotoxic effect of arsenic (as reviewed in Bjørklund et al., 2018; Grandjean & Landrigan, 2014). Arsenic (mainly inorganic) has been associated with adverse effects on cognitive functions, such as IQ, but there is still a lack of studies examining prenatal arsenic exposure and ADHD or ASD diagnosis (Rodríguez-Barranco et al., 2013). However, two prospective studies found no associations between prenatal arsenic exposure and ADHD and ASD, respectively (Forns et al., 2014; Long et al., 2019). Most studies on ASD have

measured arsenic in hair, by proximity to industrial facilities, or by levels in drinking water, as well as measured arsenic at the same time as outcome assessment (Bjørklund et al., 2018). Further, the majority of studies have measured exposure to inorganic arsenic species, whereas for the Norwegian population, including the participants herein, the main source of arsenic is organic forms (e.g. arsenobetaines) from fish and seafood intake (European Food Safety Authority, 2014; Molin et al., 2015). However, it is mainly the inorganic arsenic form that is recognized as a neurotoxicant (Agency for Toxic Substances and Disease Registry, 2007). Still, there are uncertainties about the toxicity of organic forms (Agency for Toxic Substances and Disease Registry, 2007; Molin et al., 2015). The increased risk of ASD and ADHD with prenatal exposure to mainly organic arsenic in the present study could thus be of importance and should be investigated further.

4.1.3 Mercury

Mercury, particularly methylmercury, is a well-established developmental neurotoxicant (Grandjean & Landrigan, 2006). Early-life (mainly childhood) exposure has been shown to adversely affect neurodevelopment in children (Grandjean & Landrigan, 2006; Vrijheid et al., 2016), although there are some inconsistencies in findings due to the confounding effects of seafood intake (Vrijheid et al., 2016). Still, there is a lack of prospective studies investigating prenatal exposure to mercury and diagnoses of ADHD, and particularly, ASD, where most studies are cross-sectional (Kern et al., 2016; Sanders et al., 2015; Vrijheid et al., 2016). Prenatal mercury levels have been associated with risk of ADHD or related symptoms in a few previous studies (e.g. Boucher et al., 2012; Grandjean et al., 1997; Sagiv et al., 2012). In the present study, however, increasing gestational mercury was associated with lowered risk of both ASD and ADHD in children, which are unexpected findings. Although we adjusted for estimated maternal seafood intake (fish and shellfish) during pregnancy in our analyses, this had little impact on the estimates as our results remained when we excluded maternal seafood intake as a covariate. A study of metals as biomarkers for fish and seafood intake has been performed using data from MoBa (Brantsæter et al., 2010). Blood levels of mercury were associated with total fish and seafood intake, as well as several sub-categories (Brantsæter et al., 2010). Although our findings may reflect some other unknown biases we did not adjust for, it could be that mercury concentrations in maternal blood serve as a better marker for seafood intake than FFQ-based estimates. Thus, mercury levels could be a proxy measure for the intake of polyunsaturated fatty acids and

other beneficial nutrients for brain development that is found in seafood (Avella-Garcia & Julvez, 2014). This could explain the observed lowered odds of ASD and ADHD in relation to increased prenatal mercury. Furthermore, this protective effect seemed, at least for ASD, to be sex-specific and found primarily in boys. A comparable study on prenatal mercury exposure and symptoms of ASD in children also reported interaction with child sex with significant results for boys, although they found a positive association (Ryu et al., 2017). Further studies are needed to disentangle the potential negative impact of mercury exposure from fish intake on neurodevelopment from the positive effect of beneficial nutrients from the same source.

4.1.4 Lead

Lead is another well-known developmental neurotoxicant with no known safe exposure level for neurodevelopment (European Food Safety Authority, 2010; Grandjean & Landrigan, 2014). Postnatal lead exposure has been associated with detrimental effects on IQ in children, even at low blood levels (e.g. < 10 µg/dL) (Budtz-Jørgensen et al., 2013; Lanphear et al., 2005). We identified a non-linear (u-shaped) association with prenatal lead exposure and ASD, while there were no such findings for ADHD diagnosis in children. The non-linear/u-shape observed in this study, indicate that both low-level and higher prenatal exposures to lead are associated with increased risk of ASD in children. Non-linear dose-response relationships have been shown in several studies of lead exposure in childhood and neurodevelopmental outcomes, such as IQ (Vrijheid et al., 2016). According to the European Food Safety Authority, dose-response relationships appear to be non-linear, with greater impact at lower levels of lead (European Food Safety Authority, 2010). We also detected increased risk at higher levels of lead, which is in line with the literature, although prospective studies are lacking when it comes to prenatal lead exposure and ASD (reviewed in Bjørklund et al., 2018; Mason et al., 2014). However, one study on lead in amniotic fluid and ASD did not report any associations between lead exposure and ASD diagnosis (Long et al., 2019), although non-linearity was not investigated. Regarding ADHD, there are many studies showing evidence for associations between childhood lead exposure and ADHD or related symptoms (reviewed in Kern et al., 2015), as well as a few prospective studies (lead measured in cord blood or maternal blood) (e.g. Boucher et al., 2012; Neugebauer et al., 2015; Plusquellec et al., 2007; Sioen et al., 2013).

4.2 Gestational essential elements and ASD and ADHD in children

4.2.1 Copper

Copper is an essential element that is important in several biological processes, and necessary for a normal fetal development (Uriu-Adams et al., 2010; Zoroddu et al., 2019). Copper is also a suspected neurotoxicant when surplus exposure occurs due to copper's highly reactive nature and thus ability to cause oxidative stress (Amorós et al., 2019; Zoroddu et al., 2019). Furthermore, copper deficiency in pregnancy has been linked with abnormal human perinatal development (Zoroddu et al., 2019). We identified a non-monotonic association between ADHD and copper, showing a u-shaped pattern with higher risk at both lower and higher levels. This association between gestational levels of copper and ADHD diagnosis is novel, as few other studies have investigated this. Our findings of increased risk of ADHD in children with increasing maternal levels of copper are in line with one study reporting adverse effects from elevated prenatal copper exposure on neuropsychological development in 12 months old infants and five-year-olds (Amorós et al., 2019). It was also consistent with a study reporting dysregulation of copper amongst ADHD cases (Austin et al., 2019). In contrast, a study on ADHD symptoms in children did not detect any associations with copper levels during pregnancy (Forns et al., 2014). Neither did a study on prenatal levels of copper and neurodevelopmental outcomes (cognitive, language, and motor functions) (Polanska et al., 2017). There are however some recent cross-sectional studies that have reported associations between higher copper levels in childhood and increased risk of ADHD (e.g. Li et al., 2020; Skalny et al., 2020). In addition, results from human and animal studies suggest that prenatal copper toxicity can be a contributor to ASD (Nuttall, 2017), although we did not detect any (noteworthy) associations between copper and ASD herein.

4.2.2 Magnesium

Magnesium is an essential element that is vital for fetal development, and deficiency of magnesium during pregnancy has been associated with increased neonatal mortality and morbidity (Pathak & Kapil, 2004; Zhang et al., 2013). In the present study, there was an association with magnesium in the highest exposure group with increased risk of ADHD diagnosis in children. In addition, we identified a non-monotonic u-shaped pattern between gestational magnesium and ASD in children with higher risk at both lower and higher levels. In the sensitivity analysis where we only included mothers who took folate supplement during

pregnancy, the estimates for the highest exposure group of magnesium and ASD increased. This could indicate that multivitamin supplements are important sources of magnesium for pregnant women. Two meta-analyses found that children diagnosed with ADHD and ASD, respectively, had lower magnesium levels compared to neurotypical developing children (Huang et al., 2019; Saghazadeh et al., 2017). However, the results from the cross-sectional studies are inconsistent and still based on few studies (Botturi et al., 2020). These studies have, nonetheless, hypothesized that sufficient magnesium levels can counteract or protect against development of ADHD through increased synaptic plasticity and dopaminergic and serotonergic signaling (Huang et al., 2019; Skalny et al., 2020). To our knowledge, the present study is one of the first that have prospectively investigated maternal blood levels of magnesium in pregnancy and ADHD and ASD diagnosis in offspring. Our results on the lower levels of magnesium and ASD, are in line with the studies reporting lower levels among ADHD cases, still the literature on magnesium and ASD is scarce and results are inconsistent (Botturi et al., 2020).

4.2.3 Manganese

There was increased risk of ASD among children whose mothers had the highest levels of manganese compared to those with the lowest levels. In addition, we observed a non-monotonic, slightly u-shaped pattern between gestational manganese and ADHD in children. Manganese is an essential element that is vital for brain growth and development, but in excess it is recognized as a developmental neurotoxicant as well as having a u-shaped dose-response relationship with outcomes: both insufficiency and excess levels can adversely affect neurodevelopment (Grandjean & Landrigan, 2014; Lucchini et al., 2017). Previous epidemiologic studies on ASD have however, reported conflicting results (Lucchini et al., 2017), but most of these studies are cross-sectional and have measured manganese in different matrices (e.g. air distribution, tooth enamel, urine, hair, blood). For ADHD, there seems to be more consensus in the literature that high levels of manganese in childhood contributes to increased risk, but this is still based on few and cross-sectional studies (Lucchini et al., 2017). However, one prospective study did not detect any associations between manganese in umbilical cord serum and child ADHD (Ode et al., 2015). In the present study, there was evidence of effect measure modification by maternal education within the highest levels of maternal manganese and child ADHD, with higher odds of ADHD among children of mothers with university/college education. To our knowledge, manganese has not been studied to a

large degree in relation to socioeconomic factors. One previous study of pregnant women did not detect any associations between manganese and maternal education (Montazeri et al., 2019).

4.3 Potential mechanisms and mixtures

Developmental neurotoxicants such as lead, mercury, and arsenic may act on several cellular, molecular, and biochemical targets in the developing brain and induce structural changes or affect neural plasticity (Grandjean & Landrigan, 2006; Grandjean & Landrigan, 2014). Some essential nutrients, such as selenium, manganese, and zinc, are vital for various biochemical and physiological functions (Tchounwou et al., 2012), and both insufficient and excess prenatal levels can adversely affect fetal brain development (Amorós et al., 2018a; Amorós et al., 2018b). Metals and essential elements can be developmentally neurotoxic through several overlapping mechanisms. One of the hypothesized mechanisms is abnormal methylation during fetal development (Kern et al., 2015; Tran & Miyake, 2017). This can affect DNA methylation homoeostasis, which again may negatively impact brain development (Alvarado-Cruz et al., 2018; Tran & Miyake, 2017). Other mutual mechanisms disrupting normal brain development, include alterations of maternal and fetal thyroid and immune functions, oxidative stress, and induced changes in fetal neurotransmitter systems (Heyer & Meredith, 2017). Alterations of neurotransmitter systems can lead to deficits in the central nervous system structure (Heyer & Meredith, 2017) indeed, dysfunction of the dopaminergic system has been linked to ADHD, and increased levels of serotonin to ASD (Heyer & Meredith, 2017).

There is a lack of studies investigating toxic metals and essential elements and neurodevelopment using mixture approaches (Tran & Miyake, 2017; Vrijheid et al., 2016). Toxic metals can, in combination with other metals, as well as nutrients, including essential elements, produce interactive effects (additive, synergistic or antagonistic) that adversely impact neurodevelopment (Tchounwou et al. 2012; Wu et al. 2016), where the net effect cannot be predicted by analyzing only single compounds. In the present study, the metals and elements were both positively and negatively associated with ASD or ADHD; negative associations with mercury, cesium, and zinc and positive associations with cadmium and magnesium. It is therefore likely that they cancelled each other out, and thus the overall estimation was null. Nonetheless, in other populations, where exposure to toxic metals are

higher and/or where essential elements or other micronutrients show a greater variation, these mixtures may have a stronger impact on neurodevelopmental outcomes. Quantile-based g-computation has to our knowledge not been done before in this particular research context, making comparisons to other studies challenging. Similar studies have mainly used mixture approaches for variable selection (e.g. Lenters et al., 2019; Long et al., 2019), as opposed to estimating the overall effect of the mixture.

4.4 Observed similarities and differences in the results for ASD and ADHD

We observed several similarities in gestational levels of metals/elements and their associations with ADHD and ASD cases compared to neurotypical developing children; including cadmium, copper, magnesium, mercury, and zinc, as well as arsenic and manganese, although the two latter metals/elements were related to ASD and ADHD in opposite directions. The developmental origins of ASD and ADHD are intertwined, and the disorders have strong genetic correlates (Dougherty et al.,2016; Rommelse et al., 2011). It has been proposed that the two disorders share some neurochemical and neurodevelopmental pathways (Kern et al., 2015). Additionally, comorbidity among ASD and ADHD has been reported in several studies (Brookman-Frazee et al., 2018; Hansen et al., 2018; Surén et al., 2012). The overlapping neural features, comorbidity patterns and the similarities in risk factors, support the hypothesis that ASD and ADHD might exist on a continuum of clinical expression (Anttila et al., 2018; Jokiranta-Olkoniemi et al., 2016; Kern et al., 2015; Taylor et al., 2019; Wade et al., 2015), and may share genetic and environmental causes. The findings in the present study appear to support that several toxic or essential elements *in utero* represent overlapping environmental factors risk factors for ASD and ADHD.

Even so, we did observe some differences in the present study, e.g. lead increasing risk for ASD (but not for ADHD), arsenic with a u-shaped pattern for ADHD and an inverse u-shape for ASD, and manganese with increased risk for ASD in the highest levels while a non-monotonic relationship with ADHD was observed. This could be attributed to differential vulnerability to metals/elements during fetal development (Kern et al., 2015) caused by specific features in prenatal (dys)regulation of metal and essential element metabolism among children with ADHD and ASD (Arora et al., 2017; Austin et al., 2019). Seemingly, metal toxicant uptake and deficiency of essential elements during fetal development can increase ASD risk (Arora et al., 2017; Austin et al., 2019). One study showed that children with ASD

had lower uptake of essential elements (manganese and zinc) and a higher uptake of neurotoxic metals (lead) compared to controls (Arora et al., 2017). Similarly, in a study of ADHD cases, regularity and complexity of elemental cycles were reduced for lead, copper, cobalt and vanadium (Austin et al., 2019). Although this does not fit entirely with the differential patterns in ASD and ADHD cases observed herein, it is important to note that we did not measure metal/element levels in the children. Overall, both similarities and differences in the roles of toxic metals or essential elements during development and later development of ASD and ADHD in children, could point to important aspects in the etiologies of these two disorders. Future studies on this subject, using a prospective design, should also include comorbid ASD-ADHD case groups.

4.5 Limitations and strengths

Our study has some limitations. Despite our efforts to oversample girls, there were fewer girls than boys in the present study, especially in the ASD case groups. The estimates for girls were less precise and reliable than for boys and this may have influenced the interaction analyses. While it would have been interesting to explore iron deficiency, as it can increase uptake of other metals (Meltzer et al., 2010), measures of iron status were not available for the present analyses. In our sample, there was no information of overlap between ASD and ADHD cases, as coding according to ICD-10 does not allow comorbid primary diagnoses (F84 and F90). However, this does not exclude the possibility of overlap regarding symptoms. Another potential limitation concerns the clinical basis for the ADHD NPR registrations and the possibility that alternative diagnoses should have been considered (Surén et al., 2018). The validity of the ASD diagnoses in NPR was found to be very high in a study involving participants in MoBa (Surén et al., 2014). Lastly, limitations in our study also include potential self-selection bias. The participant rate in the MoBa cohort was 41% and the participants in MoBa are in general older, have higher educational level and a healthier lifestyle compared with the general population (Nilsen et al., 2009).

Our research also has several strengths. This is one of the first studies to investigate the impact of gestational levels of 11 metals and essential elements, individually and as mixtures, on the risk of clinician-based ADHD and ASD diagnoses in children in a large, population-based sample. The large sample enabled exploration of potential effect measure modifiers. Further, the use of a prospective study design is more informative on risks than

cross-sectional studies. Moreover, our approach benefitted from a large number of relevant covariates collected prospectively during pregnancy, used to account for residual confounding pathways. In addition, most of the associations among the quartiles remained when we adjusted for multiple testing, even when using a relatively conservative method.

5. Conclusion

Results from the present study show several associations between levels of metals and elements during gestation and ASD and ADHD in children. The most notable ones involved arsenic, cadmium, copper, mercury, manganese, magnesium, and lead. The measured blood levels of toxic metals were in line with previous studies of pregnant women in Norway and in other European countries (Haug et al., 2018), indicating that even population levels of these compounds may have a negative impact on neurodevelopment. As we observed mainly similarities among the metals and elements' impact on ASD and ADHD, it could be that the two disorders share some neurochemical and neurodevelopmental pathways. The results of this study warrant further investigation and replication, as well as studies of combined effects of metals/elements and mechanistic underpinnings.

Declarations of interest

None.

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Supplementary material

Appendix A. Detailed description of analytical procedures of biospeciemens

Determination of metal and element concentrations in maternal blood

Maternal whole blood sampled in MoBa at approximately week 17 of pregnancy in 3 mL trace free sampling tubes and transported to the biobank at the Norwegian Institute of Public Health for storage at – 80°C. Before analyses, the blood was thawed and 500 µl was aliquoted in matrix tubes (8 x 16) and re-frozen. The samples were shipped frozen on dry-ice to the respective analytical laboratories.

The majority of the maternal blood samples (n=1847) were analysed by ALS Laboratory group, at the ALS laboratory in Luleå, Sweden in 2015-2016. In addition to the maternal blood samples, five reference samples consisting of standard reference material (Seronorm Urine L-1: Sero AS, Billingstad, Norway) were randomly placed within the sample batches, blinded to the analyst.

The analytical method at ALS laboratory group is accredited according to the standard EN ISO 17294-2:2016. The analyses included the following toxic or non-essential metals/elements: Lead (Pb), total Mercury (Hg), Cadmium (Cd), Thallium (Tl), total Arsenic (As), and Cesium (Cs), and as well as the following essential metals/elements: Cobolt (Co), Magnesium (Mg), Copper (Cu), Selenium (Se), Manganese (Mg), and Zink (Zn). The method have been described previously [1,2]. Briefly, it included microwave-assisted sample decomposition followed by inductively coupled plasma-sector field mass spectrometry (ICP-SFMS). Sample blanks (200 µl of high purity water), control samples (Seronorm Urine L-2: Sero AS, Billingstad, Norway) and calibration standards were subjected to the same handling as samples, including the addition of internal standards. The concentration of metals/elements in blood and blind samples, blanks and laboratory controls were calculated based on calibration curves. Limit of quantifications (LOQ) was calculated as ten times the standard deviation of the blank samples, whereas limit of detection (LOD) was approximately one third of the LOQ. The LOD and LOQ for the various elements are presented in Table S1. All reported concentrations in this study were above LOQ, except for As, Hg, Cd and Pb where concentrations above LOD were reported.

Results of maternal whole blood concentrations of metals and essential elements for some of our participants (n=179) were provided by The Norwegian Environmental Biobank, a sub-study of MoBa. These samples were analysed 2015-2016 at the Department of Occupational and Environmental Medicine at Lund University, Sweden, using comparable analytical methods as the ALS Laboratory group, as described in Caspersen et al. (2019)[9], however, these analyses did not include Mg or Cs. All metals and elements, except Total Hg, were analysed using ICP-MS (iCap Q Thermo Fisher Scientific, Bremen, GmbH) according to previous method descriptions [3,4]. Total Hg was determined by cold vapor fluorescence spectrometry after acid-digestion of samples [3,5]. The LOD and LOQ of the metals/elements measured by Lund University is given in Caspersen et al. [3]

Certified reference material (Seronorm Trace Elements whole blood L-1, SERO AS, Billingstad, Norway) were used to ensure the analytical accuracy at both laboratories. The intra-laboratory coefficients of variation were within acceptable ranges for ALS Laboratory group (Table S1) and Lund University (see Caspersen et al. 2019 [3]). 110 participants were registered with both an ASD and an ADHD diagnosis, which explains why the total number of samples are 2026 and not 2136.

Table S1. Limit of detection (LOD) and quantification (LOQ), and quality control data for the analyses of metals and elements in whole blood at ALS Laboratory group. Concentrations are given in microgram per milliliter (µg/mL)

ALS Laboratory group – MoBa NeuroTox project

			Reference samples [a,b]		
Metal/ element	~LOD	LOQ	Measured Mean±SD (RSD)	Target Mean±SD	Mean deviation from target
As	0.3	1.0	2.7±0.5 (19%)	2.4±0.5	12%
Pb	0.15	0.5	10.1±0.7 (7%)	10.2±2.1	0.9%
Cd	0.02	0.05	0.35±0.03 (8%)	0.36 ± 0.02	2.2%
Co	0.02	0.05	0.21±0.03 (14%)	0.16±0.03	33%
Cu	0.3	1.0	656±49.7(8%)	680±140	3.5%
Hg	0.07	0.2	1.8±0.1 (5%)	1.5±0.3	21%
Mn	0.15	0.5	22.4± 5.3 (24%)	20.7±4.2	8.2%
Se	1.7	5.0	60.9±7.4 (12%)	59.0±12.0	3.2%
Zn	3.3	10	4056±147.7 (4%)	4400±200	7.8%
Cs	0.02	0.05	2.52±0.11 (4%)	2.5±0.03	0.8%
Mg	0.07	0.2	14.6±1.52 (10%)	16.2±1,6	9.9%

[a] n = 5 blind samples, [b] Seronorm.

Abbreviations: Arsenic (As); cadmium (Cd); cesium (Cs); cobalt (Co); copper (Cu); lead (Pb); limit of detection (LOD); limit of quantification (LOQ); magnesium (Mg); manganese (Mn); mercury (Hg); selenium (Se); standard deviations (SD); zinc (Zn).

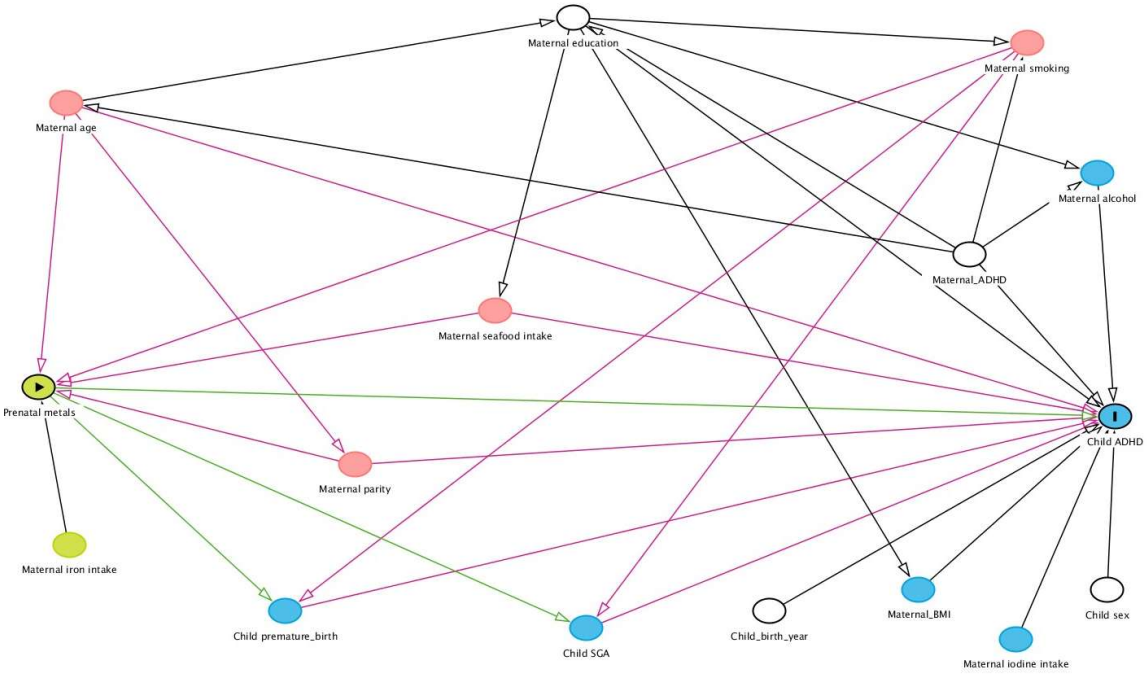

Figure S1. Directed acyclic graph (DAG) for prenatal metal/element exposure and child attention-deficit/hyperactivity disorder in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Note: Abbreviations: Attention-deficit/hyperactivity disorder (ADHD); body mass index (BMI); small for gestational age (SGA).

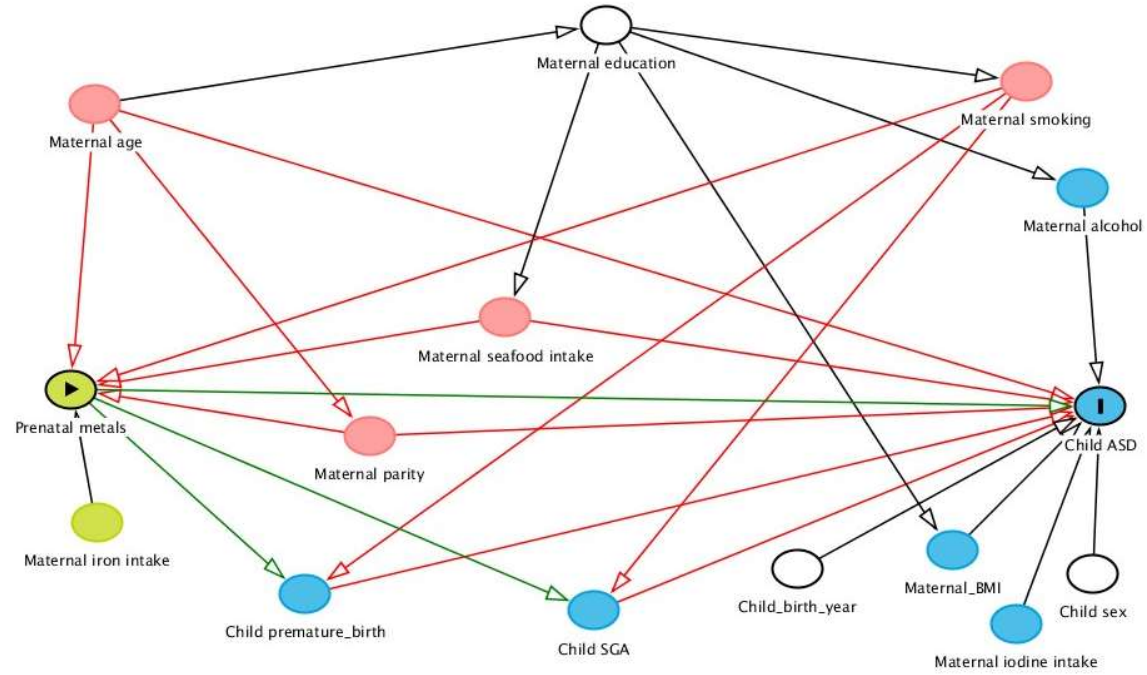

Figure S2. Directed acyclic graph (DAG) for prenatal metal/element exposure and child autism spectrum disorder in a nested study of autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Note: Abbreviations: Autism spectrum disorder (ASD); body mass index (BMI); small for gestational age (SGA).

Appendix B. From statistical analysis

In the ADHD imputation model, we included the following (% missing): arsenic (1.7), cadmium (1.8), cesium (10.2), cobalt (3.2), copper (0), lead (0), magnesium (10.2), manganese (0), mercury (0), selenium (0), zinc (0), child birth year (0), child sex (0), child birth weight (0), maternal age (0), maternal ADHD symptoms (37.7), maternal education (2.5), parity (0) maternal seafood intake (11.8), maternal smoking (0.1), length of gestation (0.46) and maternal folate intake (6.9).

In the ASD imputation model, we included the following (% missing): arsenic (0.9), cadmium (1.5), cesium (7.3), cobalt (2.2), copper (0), lead (0), magnesium (7.3), manganese (0), mercury (0), selenium (0), zinc (0), child birth year (0), child sex (0), child birth weight (0), maternal age (0), maternal education (2.4), parity (0) maternal seafood intake (10.4), maternal smoking (0), length of gestation (0.3) and maternal folate intake (7.3).

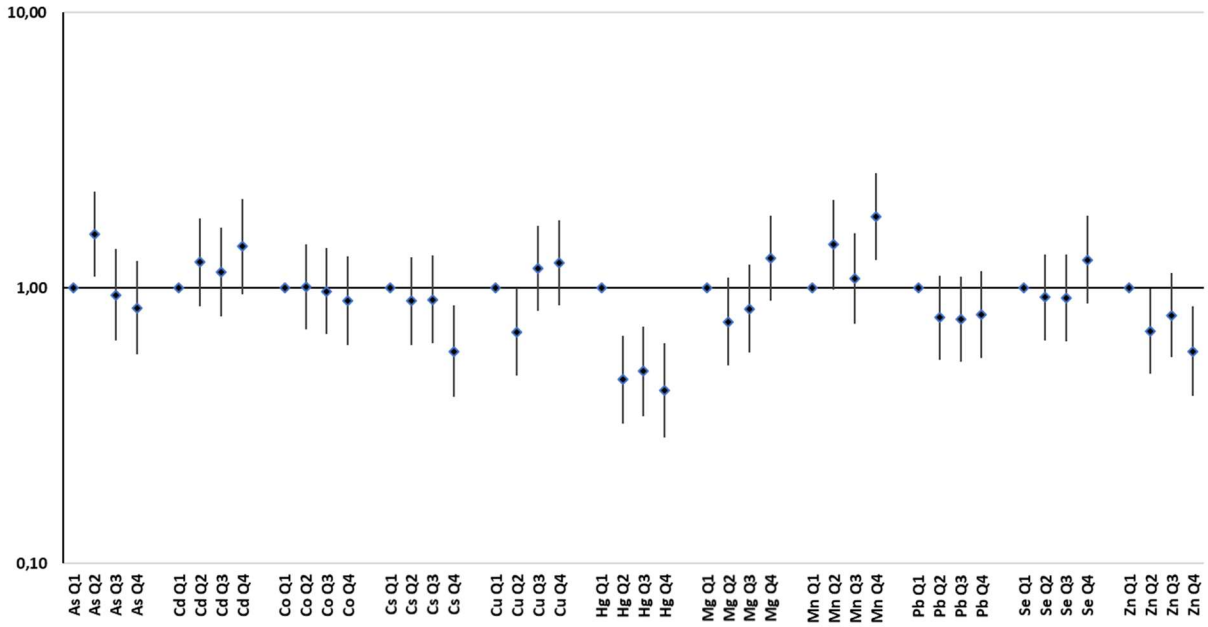

Figure S3. Odds ratios and 95% confidence intervals for complete cases of logistic regression models predicting autism spectrum disorder in quartile categories of gestational metal/essential element levels in a nested case–control study of autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Note: Each metal/element was modelled using a separate logistic regression adjusted for maternal age, education, parity, seafood intake, smoking, child sex and child birth year. All metals/element concentrations were normalized to analytical round and natural log transformed. The odds ratio and 95% confidence intervals for each metal/element quartile are represented on the vertical axis (the reference level is the first quartile). As (n=1240); Cd (n=1233); Cs and Mg (n=1193); Co (n=1223); Cu, Pb, Mn, Hg, Se, and Zn (n=1251). Abbreviations: Arsenic (As); cadmium (Cd); cesium (Cs); cobalt (Co); copper (Cu); lead (Pb); magnesium (Mg); manganese (Mn); mercury (Hg); selenium (Se); zinc (Zn).

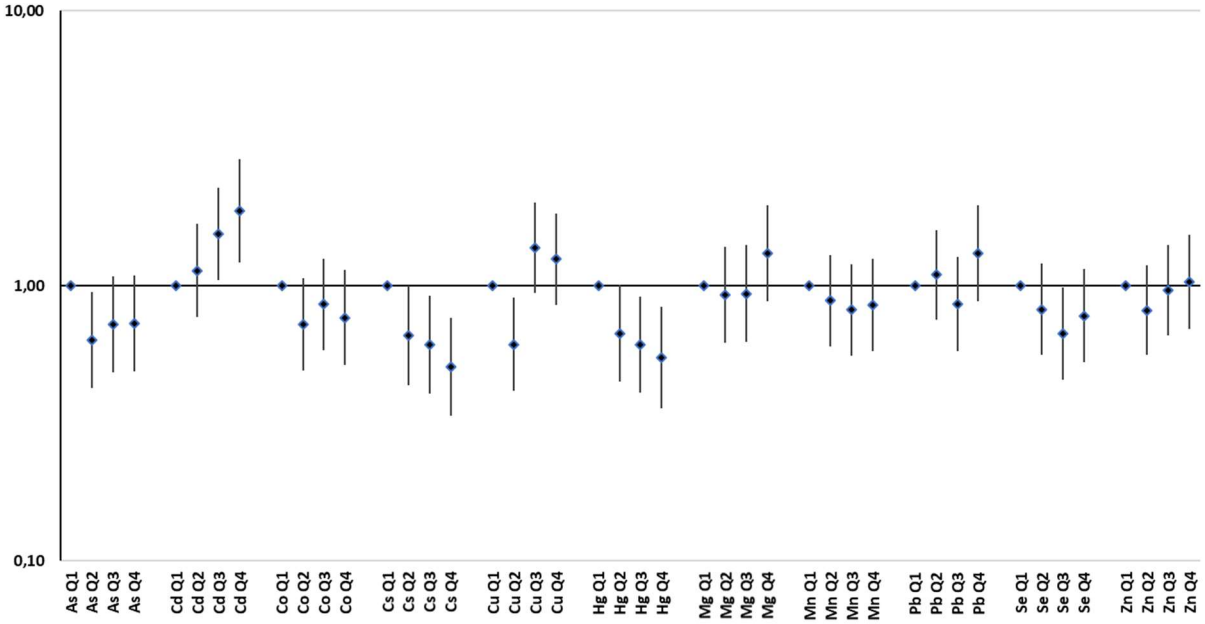

Figure S4. Odds ratios and 95% confidence intervals for complete cases of logistic regression models predicting attention-deficit/hyperactivity disorder in quartile categories of gestational metal/essential element levels in a nested case–control study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Note: Each metal/element was modelled using a separate logistic regression adjusted for maternal age, education, parity, ADHD symptoms, seafood intake, smoking, child sex and child birth year. All metals/element concentrations were normalized to analytical round and natural log transformed. The odds ratio and 95% confidence intervals for each metal/element quartile are represented on the vertical axis (the reference level is the first quartile). As (n=908); Cd (n=913); Cs and Mg (n=837); Co (n=895); Cu, Pb, Mn, Hg, Se, and Zn (n=925). Arsenic (As); cadmium (Cd); cesium (Cs); cobalt (Co); copper (Cu); lead (Pb); magnesium (Mg); manganese (Mn); mercury (Hg); selenium (Se); zinc (Zn).

Table S2. Concentration levels of metals/elements before normalization to analytical round in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Metal/ element	Case/ control	Mean (SD)
As	Before normalization	2.37 (2.85)
	After normalization	2.42 (2.96)
Cd	Before normalization	0.36 (3.15)
	After normalization	0.36 (3.18)
Co	Before normalization	0.24 (0.63)
	After normalization	0.25 (0.70)
Cs	Before normalization	2.33 (0.94)
	After normalization	2.35 (0.95)
Cu	Before normalization	1525 (262)
	After normalization	1589 (261)
Hg	Before normalization	1.57 (1.08)
	After normalization	1.57 (1.06)
Mg	Before normalization	29.8 (3.73)
	After normalization	30.4 (3.85)
Mn	Before normalization	11.5 (10.3)
	After normalization	11.7 (10.6)
Pb	Before normalization	9.24 (4.82)
	After normalization	9.58 (5.04)
Se	Before normalization	96.3 (21.0)
	After normalization	94.0 (20.7)
Zn	Before normalization	5228 (1046)
	After normalization	5261 (1081)

Note: Mg values are in mg/L, all others are in µg/L.
Abbreviations: Arsenic (As); cadmium (Cd); cesium (Cs); cobalt (Co); copper (Cu); lead (Pb); magnesium (Mg); manganese (Mn); mercury (Hg); selenium (Se); standard deviation (SD); zinc (Zn).

Table S3. Quartile concentration levels and odds ratios and 95% confidence intervals for logistic regression models predicting autism spectrum disorder and attention-deficit/hyperactivity disorder in quartile categories of gestational metal/essential element levels in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Metal/ element	ASD and controls			ADHD and controls		
	Concentration	OR	95% CI	Concentration	OR	95% CI
As Q1 (ref.)	0.12-1.01	1.00		0.09-0.96	1.00	
As Q2	1.01-1.59	1.77	1.26, 2.49	0.96-1.58	0.79	0.59, 1.05
As Q3	1.59-2.76	1.14	0.80, 1.64	1.59-2.81	0.75	0.56, 1.00
As Q4	2.77-54.2	0.95	0.66, 1.38	2.82-54.2	0.75	0.56, 1.00
Cd Q1 (ref.)	0.01-0.12	1.00		0.01-0.13	1.00	
Cd Q2	0.12-.018	1.48	1.05, 2.10	0.13-0.19	1.10	0.83, 1.46
Cd Q3	018-0.30	1.26	0.88, 1.80	0.19-0.31	1.20	0.90, 1.59
Cd Q4	0.30-142	1.57	1.07, 2.31	0.31-3.14	1.59	1.15, 2.18
Co Q1 (ref.)	0.04-0.12	1.00		0.04-0.12	1.00	
Co Q2	0.12-0.18	1.20	0.85, 1.69	0.12-0.18	0.94	0.71, 1.24
Co Q3	0.18-0.27	1.15	0.81, 1.62	0.18-0.27	0.93	0.70, 1.24
Co Q4	0.28-29.5	1.11	0.78, 1.58	0.27-29.5	0.72	0.54, 0.96
Cs Q1 (ref.)	0.68-1.78	1.00		0.72-1.75	1.00	
Cs Q2	1.78-2.23	0.87	0.62, 1.23	1.75-2.21	0.84	0.63, 1.14
Cs Q3	2.23-2.78	0.88	0.62, 1.25	2.21-2.74	0.85	0.63, 1.15
Cs Q4	2.78-9.25	0.63	0.44, 0.91	2.75-9.49	0.68	0.50, 0.93
Cu Q1 (ref.)	778-1425	1.00		774-1420	1.00	
Cu Q2	1425-1554	0.69	0.49, 0.98	1420-1561	0.59	0.44, 0.79
Cu Q3	1554-1737	1.15	0.83, 1.61	1562-1737	1.08	0.82, 1.42
Cu Q4	1737-3583	1.16	0.82, 1.63	1737-3629	0.92	0.70, 1.23
Hg Q1 (ref.)	0.12-0.87	1.00		0.08-0.87	1.00	
Hg Q2	0.87-1.38	0.51	0.36, 0.72	0.87-1.35	0.76	0.57, 1.01
Hg Q3	1.38-2.07	0.56	0.39, 0.80	1.35-2.04	0.71	0.53, 0.95
Hg Q4	2.08-10.1	0.43	0.30, 0.63	2.04-10.0	0.58	0.42, 0.79
Mg Q1 (ref.)	15.2-27.9	1.00		15.2-28.0	1.00	
Mg Q2	27.9-30.3	0.92	0.64, 1.31	28.0-30.3	1.10	0.81, 1.48
Mg Q3	30.3-32.7	0.97	0.68, 1.38	30.3-32.8	0.92	0.68, 1.25
Mg Q4	32.7-45.0	1.40	0.99, 1.97	32.8-74.5	1.42	1.06, 1.91
Mn Q1 (ref.)	2.06-8.13	1.00		2.06-7.83	1.00	
Mn Q2	8.14-9.97	1.30	0.91, 1.85	7.84-9.81	0.77	0.58, 1.02
Mn Q3	9.98-12.60	1.09	0.76, 1.56	9.82-12.3	0.72	0.54, 0.95
Mn Q4	12.60-164	1.84	1.30, 2.59	12.3-221	0.82	0.62, 1.09
Pb Q1 (ref.)	1.59-6.50	1.00		1.88-6.64	1.00	
Pb Q2	6.51-8.55	0.80	0.57, 1.12	6.64-8.61	1.15	0.87, 1.52
Pb Q3	8.55-11.2	0.79	0.56, 1.12	8.62-11.3	0.84	0.63, 1.12
Pb Q4	11.2-82.4	0.81	0.57, 1.15	11.3-82.4	1.09	0.82, 1.45
Se Q1 (ref.)	44.4-81.7	1.00		41.7-80.7	1.00	
Se Q2	81.7-92.4	0.95	0.67, 1.34	80.8-90.9	0.97	0.74, 1.28
Se Q3	92.4-106	0.91	0.64, 1.28	90.9-104	0.80	0.61, 1.07
Se Q4	106-312	1.21	0.86, 1.72	104-312	0.83	0.62, 1.10
Zn Q1 (ref.)	1189-4585	1.00		1189-4585	1.00	
Zn Q2	4587-5227	0.73	0.52, 1.02	4587-5258	0.74	0.56, 0.98
Zn Q3	5227-5833	0.82	0.59, 1.15	5266-5886	0.85	0.64, 1.13
Zn Q4	5833-10743	0.63	0.45, 0.90	5886-11186	0.84	0.63, 1.11

Note: Mg values are in mg/L, all others are in µg/L. The models were adjusted for maternal age, education, parity, seafood intake, smoking, child sex and, child birth year. For ADHD, maternal ADHD symptoms was also included as a covariate. Arsenic (As); attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); cadmium (Cd); cesium (Cs); cobalt (Co); confidence intervals (CI); copper (Cu); lead (Pb); magnesium (Mg); manganese (Mn); mercury (Hg); odds ratio (OR); selenium (Se); zinc (Zn).

Table S4. Odds ratios and 95% confidence intervals and interaction terms for child sex from logistic regression models predicting autism spectrum disorder from quartile categories of mercury in a nested case–control study of and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Metal	ASD			
	All	Boys	Girls	Interaction term p
Hg		Interaction term p = 0.076		
Hg Q1 (ref.)	1.00	1.00	1.00	
Hg Q2	0.51 (0.36, 0.72)	0.41 (0.28, 0.60)	1.19 (0.55, 2.58)	0.015
Hg Q3	0.56 (0.39, 0.80)	0.52 (0.36, 0.77)	0.76 (0.32, 1.81)	0.433
Hg Q4	0.43 (0.30, 0.63)	0.37 (0.25, 0.56)	0.86 (0.37, 1.98)	0.071

Note: Logistic regression with multiple imputation with additional interaction analyses. All metals/elements were normalized according to batch. Each regression model was adjusted for maternal age, education, parity, seafood intake, smoking, child sex, and child birth year. Interaction term was tested with Wald's test. Abbreviations: Autism spectrum disorder (ASD); mercury (Hg).

Table S5. Odds ratios and 95% confidence intervals and interaction terms for maternal education from logistic regression models predicting attention-deficit/hyperactivity disorder from quartile categories of cadmium and manganese in a nested case–control study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Metal	ADHD			
	All	College/ university	< college/ university	Interaction term p
Cd		Interaction term p = 0.141		
Cd Q1 (ref.)	1.00	1.00	1.00	
Cd Q2	1.10 (0.83, 1.46)	1.23 (0.85, 1.78)	0.90 (0.56, 1.42)	0.297
Cd Q3	1.20 (0.90, 1.59)	1.54 (1.08, 2.20)	0.77 (0.48, 1.24)	0.022
Cd Q4	1.59 (1.15, 2.18)	1.69 (1.12, 2.56)	1.33 (0.84, 2.12)	0.428
Mn		Interaction term p = 0.137		
Mn Q1 (ref.)	1.00	1.00	1.00	
Mn Q2	0.77 (0.58, 1.02)	0.86 (0.59, 1.25)	0.65 (0.42, 1.00)	0.330
Mn Q3	0.72 (0.54, 0.95)	0.86 (0.60, 1.25)	0.55 (0.35, 0.85)	0.118
Mn Q4	0.82 (0.62, 1.09)	1.08 (0.75, 1.56)	0.55 (0.35, 0.86)	0.023

Note: Logistic regression with multiple imputation with additional interaction analyses. All metals/elements were normalized according to batch. Each regression model was adjusted for maternal ADHD symptoms, age, education, parity, seafood intake, smoking, child sex, and child birth year. Interaction term was tested with Wald's test. Abbreviations: Attention-deficit/hyperactivity disorder (ADHD); cadmium (Cd); manganese (Mn).

Table S6. Odds ratios and 95% confidence intervals and interaction terms for maternal education from logistic regression models predicting attention-deficit/hyperactivity disorder from quartile categories of cadmium without mothers who smoked in a nested case–control study of and attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Metal	ADHD (without mothers who smoked)		
	College/ university	< college/ university	Interaction term p
Cd	Interaction term p = 0.128		
Cd Q1 (ref.)	1.00	1.00	
Cd Q2	1.24 (0.85, 1.81)	0.89 (0.55, 1.44)	0.287
Cd Q3	1.57 (1.09, 2.26)	0.73 (0.44, 1.22)	0.018
Cd Q4	1.70 (1.07, 2.70)	1.13 (0.66, 1.94)	0.262

Note: Logistic regression with multiple imputation with additional interaction analyses. Mothers who smoked were excluded from the analyses. Cadmium was normalized according to batch. The regression model was adjusted for maternal ADHD symptoms, age, education, parity, seafood intake, child sex, and child birth year. Interaction term was tested with Wald's test. Abbreviations: Attention-deficit/hyperactivity disorder (ADHD); cadmium (Cd).

Table S7. Odds ratios and 95% confidence intervals from logistic regression models predicting autism spectrum disorder from quartile categories of zinc and magnesium with only mothers who took folate supplement in a nested case–control study of autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Metal	ASD			
	All		Only mothers who took folate supplement	
	OR	95% CI	OR	95% CI
Zn Q1 (ref.)	1.00	1.00	1.00	1.00
Zn Q2	0.73	0.52, 1.02	0.84	0.56, 1.27
Zn Q3	0.82	0.59, 1.15	0.94	0.62, 1.43
Zn Q4	0.63	0.45, 0.90	0.78	0.50, 1.21
Mg Q1 (ref)	1.00	1.00	1.00	1.00
Mg Q2	0.92	0.64, 1.31	1.08	0.70, 1.68
Mg Q3	0.97	0.68, 1.38	1.01	0.65, 1.57
Mg Q4	1.40	0.99, 1.97	1.87	1.22, 2.87
	ADHD			
	All		Only mothers who took folate supplement	
	OR	95% CI	OR	95% CI
Zn Q1 (ref.)	1.00	1.00	1.00	1.00
Zn Q2	0.74	0.56, 0.98	0.68	0.48, 0.95
Zn Q3	0.85	0.64, 1.13	0.87	0.62, 1.22
Zn Q4	0.84	0.63, 1.11	0.79	0.55, 1.13
Mg Q1 (ref)	1.00	1.00	1.00	1.00
Mg Q2	1.10	0.81, 1.48	1.20	0.83, 1.73
Mg Q3	0.92	0.68, 1.25	0.85	0.59, 1.24
Mg Q4	1.42	1.06, 1.91	1.49	1.02, 2.18

Note: Logistic regression with multiple imputation. Only mothers who took folate supplement were included in the analyses. Zinc was normalized according to batch. The regression model was adjusted for maternal age, education, parity, seafood intake, child sex, and child birth year. For ADHD, maternal ADHD symptoms was also included as a covariate Abbreviations: Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); confidence intervals (CI); magnesium (Mg); odds ratio (OR); zinc (Zn).

Table S8. Odds ratios and 95% confidence intervals for quantile-based g-computation approach of metals/elements on autism spectrum disorder and attention-deficit/hyperactivity disorder in a nested case–control study of autism spectrum disorder and attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Metal/element mix	ASD and controls		ADHD and controls	
	OR	95% CI	OR	95% CI
Total mix	0.98	0.76, 1.27	0.83	0.67, 1.03
Essential elements mix	1.21	0.98, 1.49	0.87	0.73, 1.03
Toxic/non-essential elements mix	0.83	0.68, 1.02	0.90	0.76, 1.06

Note: The models were adjusted for maternal age, education, parity, seafood intake, smoking, child sex and child birth year. For ADHD, maternal ADHD symptoms was also included as a covariate. Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); confidence intervals (CI); odds ratio (OR).

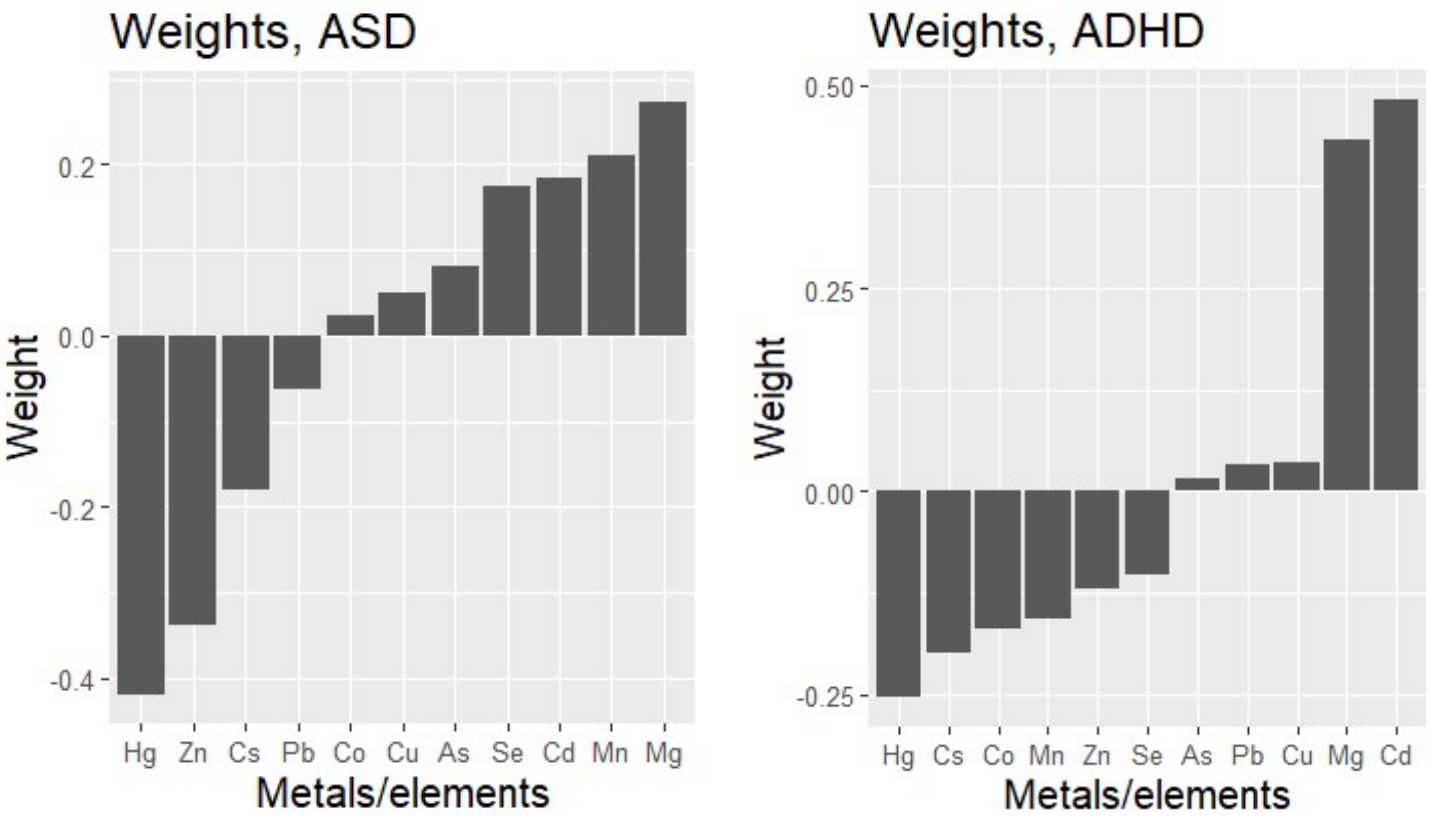

Figure S5. Quantile-based g-computation approach of metals/elements on autism spectrum disorder and attention-deficit/hyperactivity disorder in a nested case–control study of autism spectrum disorder and attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009 (n=1431 and n=1739).

Note: Missing data imputed. All metals/elements were normalized according to batch. The metals/element were log transformed. The model was adjusted for maternal age, education, parity, seafood intake, smoking, child sex, and child birth year. For ADHD, maternal ADHD symptoms was also included as a covariate. Abbreviations: Arsenic (As); attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); cadmium (Cd); cesium (Cs); cobalt (Co); copper (Cu); lead (Pb); magnesium (Mg); manganese (Mn); mercury (Hg); selenium (Se); zinc (Zn).

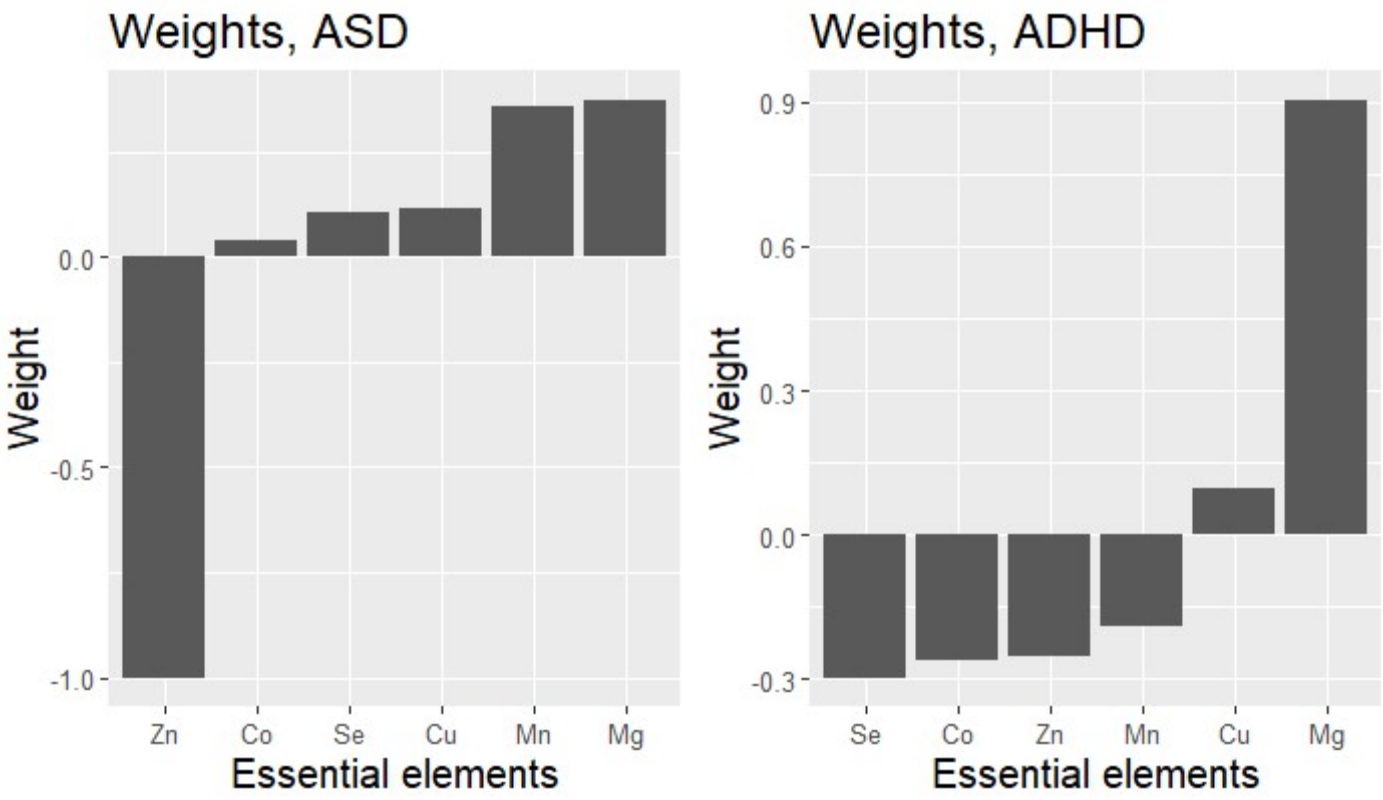

Figure S6. Quantile-based g-computation approach of essential elements on autism spectrum disorder and attention-deficit/hyperactivity disorder in a nested case–control study of autism spectrum disorder and attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Note: Missing data imputed. All metals/elements were normalized according to batch. The metals/element were log transformed. The model was adjusted for maternal age, education, parity, seafood intake, smoking, child sex, and child birth year. For ADHD, maternal ADHD symptoms was also included as a covariate. Abbreviations: Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); cobalt (Co); copper (Cu); magnesium (Mg); manganese (Mn); selenium (Se); zinc (Zn).

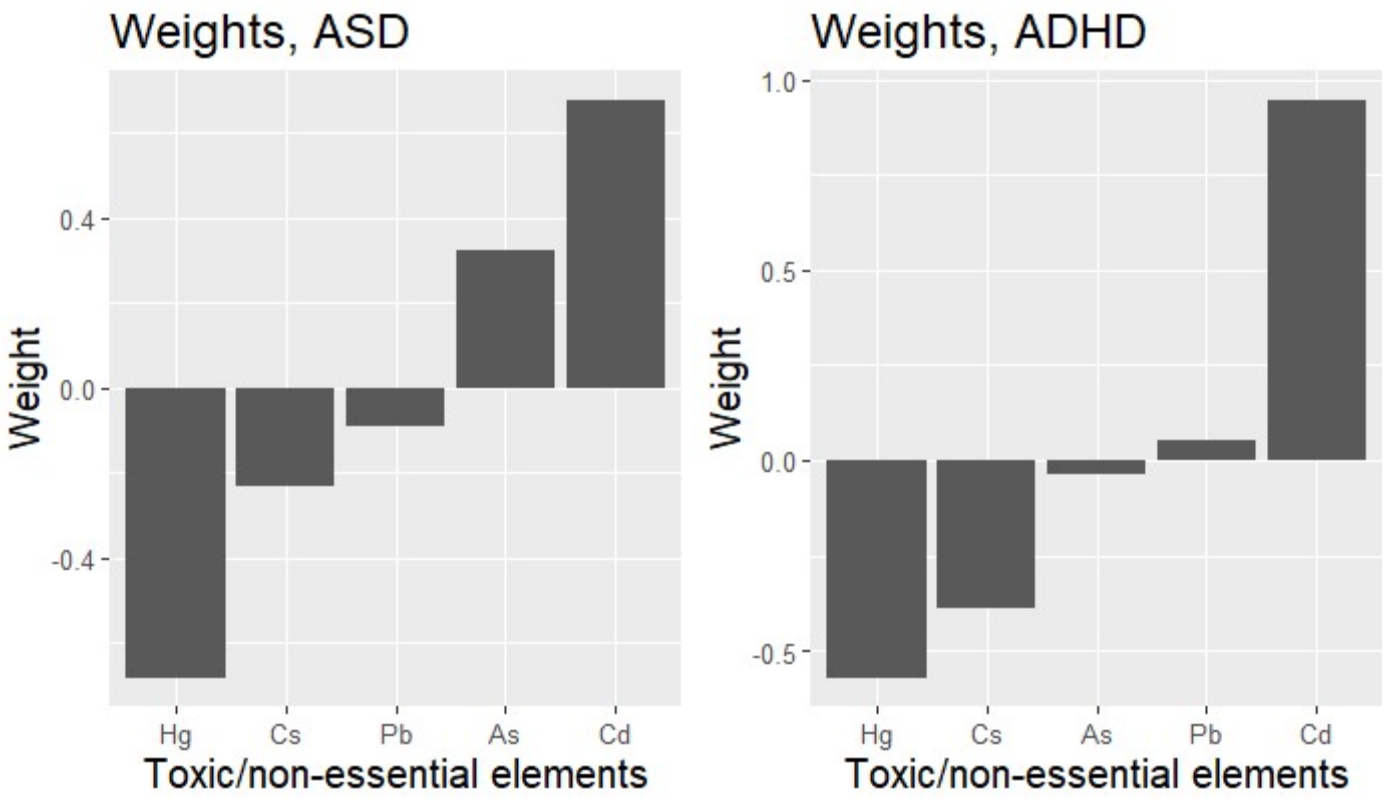

Figure S7. Quantile-based g-computation approach of toxic/non-essential elements on autism spectrum disorder and attention-deficit/hyperactivity disorder in a nested case–control study of autism spectrum disorder and attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Note: Missing data imputed. All metals/elements were normalized according to batch. The metals/element were log transformed. The model was adjusted for maternal age, education, parity, seafood intake, smoking, child sex, and child birth year. For ADHD, maternal ADHD symptoms was also included as a covariate. Abbreviations: Arsenic (As); attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); cadmium (Cd); cesium (Cs); lead (Pb); mercury (Hg).

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III

Prenatal exposure to per- and polyfluoroalkyl substances (PFAS) and associations with attention-deficit/hyperactivity disorder and autism spectrum disorder in children

Thea S. Skogheim[a*], Kjell Vegard F. Weyde[a], Heidi Aase[a], Stephanie M. Engel[b], Pål Surén[a], Merete G. Øie[c], Guido Biele[a], Ted Reichborn-Kjennerud[a,d], Anne Lise Brantsæter[e], Line S. Haug[e], Azemira Sabaredzovic[e], Bonnie Auyeung[f,g], Gro D. Villanger[a]

[a] Division of Mental and Physical Health, Norwegian Institute of Public Health, PO Box 222 Skøyen, N-0213 Oslo, Norway

[b] Gillings School of Global Public Health, University of North Carolina at Chapel Hill, 135 Dauer Drive, Campus Box 7435, Chapel Hill, NC 27599-7435, USA

[c] Department of Psychology, University of Oslo, PO Box 1094 Blindern, N-0317 Oslo, Norway

[d] Institute of Clinical Medicine, University of Oslo, PO Box 1171 Blindern, N-0318 Oslo, Norway

[e] Division of Infection Control and Environmental Health, Norwegian Institute of Public Health, PO Box 222 Skøyen, N-0213 Oslo, Norway

[f] Department of Psychology, School of Philosophy, Psychology and Language Sciences, University of Edinburgh, 7 George Square, Edinburgh, EH8 9JZ, UK

[g] Department of Psychiatry, Autism Research Centre, University of Cambridge, Douglas House, 18b Trumpington Road, Cambridge, CB2 8AH, UK

[*] Corresponding author at: Department of Child Health and Development, NIPH, PO Box 222 Skøyen, 0213 Oslo, Norway. E-mail address: thea.skogheim@fhi.no

Abstract

Background:

Prenatal exposure to per- and polyfluoroalkyl substances (PFASs) may be a risk factor for neurodevelopmental deficits and disorders, but evidence is inconsistent.

Objectives:

We investigated whether prenatal exposure to PFASs were associated with childhood diagnosis of attention-deficit/hyperactivity disorder (ADHD) or autism spectrum disorder (ASD).

Methods:

This study was based on the Norwegian Mother, Father and Child Cohort Study and included n=821 ADHD cases, n=400 ASD cases and n=980 controls. Diagnostic cases were identified by linkage with the Norwegian Patient Registry. In addition, we used data from the Medical Birth Registry of Norway. The study included the following PFASs measured in maternal plasma sampled mid-pregnancy: Perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorohexane sulfonate (PFHxS), perfluoroheptanesulfonic acid (PFHpS), and perfluorooctane sulfonate (PFOS). Relationships between individual PFASs and ADHD or ASD diagnoses were examined using multivariable adjusted logistic regression models. We also tested for possible non-linear exposure-outcome associations. Further, we investigated the PFAS mixture associations with ASD and ADHD diagnoses using a quantile-based g-computation approach.

Results:

Odds of ASD was significantly elevated in PFOA quartile 2 [OR = 1.71 (95% CI: 1.20, 2.45)], and PFOA appeared to have a non-linear, inverted U-shaped dose-response relationship with ASD. PFOA was also associated with increased odds of ADHD, mainly in quartile 2 [OR = 1.54 (95% CI: 1.16, 2.04)] and a similar non-linear relationship as shown for ASD. Several PFASs (e.g. PFUnDA and PFDA) were inversely associated with odds of ADHD and/or ASD. Some of the associations were modified by child sex and maternal education. The overall PFAS mixture was inversely associated with ASD [OR = 0.76 (95% CI: 0.64, 0.90)] as well as the carboxylate mixture [OR = 0.79 (95% CI: 0.68, 0.93)] and the sulfonate mixture [OR = 0.84 (95% CI: 0.73, 0.96)].

Conclusion:

Prenatal exposure to PFOA was associated with increased risk of ASD and ADHD in children. For some PFASs, as well as their mixtures, there were inverse associations with ASD and/or ADHD. The epidemiologic literature linking PFAS exposures with neurodevelopmental outcomes is still inconclusive, suggesting the need for more research to elucidate the neurotoxicological potential of PFAS during early development.

Keywords: Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); per- and polyfluoroalkyl substance (PFAS); The Norwegian Mother, Father and Child Cohort Study (MoBa); Medical Birth Registry of Norway (MBRN).

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) refer to a large group of synthetic compounds developed for use in various industrial processes and in a multitude of different products such as firefighting foam, textiles, cooking pans, and food packaging (Buck et al., 2011; Kissa, 2001). Due to extensive production and usage of PFASs after being produced in the late 1940s, it is now a globally spread environmental contaminant group (Wang et al., 2017a). Because of its high persistence in the environment, PFASs bioaccumulate in organisms (including humans) and in food chains (EFSA, 2018). Fish and seafood are the most important sources of exposure for human populations (Brantsæter et al., 2013; Haug et al., 2010; Papadopoulou et al., 2019). In humans, the two predominating PFASs, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), have estimated biological half-lives of around two to five years (EFSA, 2018; Lau et al., 2007). Both PFOS and PFOA have been subjected to international restrictions and regulations due to their toxicity and adverse health effects, affecting among other things; immune function, endocrine systems, and liver function (Fenton et al., 2020; Sunderland et al., 2019). PFOS was in 2009 listed in Annex B (as restricted) of the Stockholm Convention on Persistent Organic Pollutants (Stockholm Convention, 2019). Due to phase-out by major producers as well as international restrictions, levels of some PFASs, particularly PFOS, have declined in the environment and in humans during the last ten to 15 years (EFSA, 2018; Land et al., 2018; Mariussen, 2012). As of 2020, PFOA was banned for use in the European Union (unless specific exemption) and is being considered for restrictions under the Stockholm Convention (ECHA, 2020; Stockholm Convention, 2019). However, there has been an increase in production and emission of perfluoroalkyl carboxylic acids (PFCAs) and some precursors from China and other countries in Asia (Wang et al., 2014). There are uncertainties about the health effects of these new replacement compounds that are currently on the rise (Sunderland et al., 2019; Wang et al., 2017a).

Although PFASs such as PFOS and PFOA have been shown to adversely affect several aspects of human health, there is still limited knowledge concerning potential adverse effects on intrauterine brain development (Liew et al., 2018; Vrijheid et al., 2016). The fetus is especially vulnerable to toxic chemicals, including PFASs, which are transferred from mother to fetus via the placenta and enter the fetal brain via an undeveloped blood-brain barrier and may disrupt the finely tuned brain developmental processes and timing (Grandjean and Landrigan, 2014; Gützkow et al., 2012; Kato et al., 2014). Results from animal and *in*

vitro studies suggest that PFASs are developmental neurotoxicants, affecting several neurochemical targets in the developing brain (Johansson et al., 2009; Mariussen, 2012; Slotkin et al., 2008; Viberg et al., 2013). Additionally, PFASs have endocrine-disruptive abilities and can affect the maternal and fetal thyroid or sex steroid hormones, which are important for a normal development of the fetal nervous system and brain (De Cock et al., 2012; Mariussen, 2012; Tran & Miyake, 2017). Furthermore, humans are not only exposed to one chemical at a time, but multiple compounds. Chemical mixtures can induce different or stronger health effects than single chemicals (Henn et al., 2014; Rauh et al., 2016). Accordingly, *in utero* exposure to multiple PFASs may disrupt normal brain development and thereby increase risk of neurocognitive deficits, behavioral problems, and neurodevelopmental disorders such as autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD).

ADHD and ASD interfere with normal functioning and learning during childhood and adolescence (Antshel et al., 2016; Kern et al., 2015). Childhood ASD has a prevalence of around 1% in the Nordic countries and in the United States (Hansen, Schendel, & Parner, 2015; Idring et al., 2015; Sandin et al., 2014; Surén et al., 2012). It comprises heterogeneous disorders that are characterized by persistent deficits in social communication and social interaction, in addition to restricted and repetitive patterns of behavior, interests, or activities (American Psychiatric Association, 2013). Children with ASD have various cognitive challenges and their intelligence can range from high intellectual functioning to severe intellectual disability (Lord et al., 2020). ADHD is one of the most common neurodevelopmental disorders characterized by inattention, impulsivity, and hyperactivity (American Psychiatric Association, 2013) and associated with a range of sub-optimal long-term outcomes, including low educational levels and low life satisfaction (Rauh & Margolis, 2016). Globally, ADHD affects approximately 3–4% of children (Polanczyk et al., 2007). Both ADHD and ASD are more prevalent in boys compared to girls (Nussbaum, 2012; Polanczyk et al., 2007; Werling & Geschwind, 2013). The etiologies of these disorders are multifactorial in which heredity is thought to play a major role (Faraone et al., 2005; Kern et al., 2015; Sandin et al., 2014; Thapar et al., 2017). However, environmental risk factors, including *in utero* toxicant exposure, may interact with genetic factors (Nuttall, 2017; Sandin et al., 2014; Thapar et al., 2013). Children with ADHD and ASD share several overlapping genetic and psychopathological traits indicating that there may be common risk factors or etiological mechanisms at play (Kern et al., 2015; Martin et al., 2018). As toxicant exposure

in pregnant women and their fetuses are potentially modifiable, it is of high importance to investigate their contribution to the risks of these disorders.

Compared to other environmental toxicants, PFASs have some of the highest concentration levels in human blood, including in pregnant women (Haug et al., 2018; Mariussen, 2012). Several studies have reported higher levels of some PFASs among those with higher socioeconomic status (SES), such as higher education and income (Brantsæter et al., 2013; Montazeri et al., 2019; Tyrrell et al., 2013). This is probably to some degree related to the higher consumption of fish and seafood among those with higher educational levels (e.g. Touvier et al., 2010). In addition, maternal education has been associated with ASD and ADHD in children (Lung et al., 2018; Torvik et al., 2020). There are studies reporting differential relationships by child sex, which could be a consequence of altered prenatal sex steroid levels, and/or sex-specific neurodevelopmental vulnerabilities (Baron-Cohen et al., 2019; Wang et al., 2017b; Werling & Geschwind, 2013). Although the experimental literature has demonstrated PFASs' potential for developmental neurotoxicity in the human brain (Mariussen et al., 2012), the epidemiological literature has been mixed, with some studies finding increased risks of adverse outcomes, whereas others found none or even report inverse associations (Forns et al., 2020; Liew et al., 2018; Rappazzo et al., 2017; Vrijheid et al., 2016). There is a lack of investigation of prenatal exposure to PFAS and their mixtures, as well as investigating the risk of childhood ADHD or ASD diagnoses as outcomes. Even if child sex and factors related to parental SES (e.g. maternal education) often are included as covariates in studies of PFASs and neurodevelopmental outcomes, very few investigate if these relationships differ by these important variables.

Against this background, the overall aim of the present study was to investigate the associations between prenatal exposure to seven PFASs, individually and as mixtures, and childhood diagnosis of ADHD and ASD. An additional goal was to explore whether these relationships were modified by child sex or maternal education (as a proxy for SES).

2. Methods

2.1 Study Design and Participants

The present study is based on data from the Norwegian Mother, Father and Child Cohort Study (MoBa) and the Medical Birth Registry of Norway (MBRN) with linkage to the

Norwegian Patient Registry (NPR) (Bakken et al., 2020). MoBa is a population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health. Participants were recruited from all over Norway from 1999-2008. The women consented to participation in 41% of the pregnancies. The cohort now includes 114,500 children, 95,200 mothers and 75,200 fathers (Magnus et al., 2016). Differences between those who consented vs non-consented is described elsewhere (Biele et al., 2019; Nilsen et al., 2009). The current study is based on version 12 of the quality-assured data files released for research in January 2019. The establishment of MoBa and initial data collection was based on a license from the Norwegian Data Protection Agency and approval from The Regional Committees for Medical and Health Research Ethics. The MoBa cohort is now based on regulations related to the Norwegian Health Registry Act. The current study was approved by The Regional Committee for Medical and Health Research Ethics (ref. no. 2012/985-1). The NPR has approved the linkage between NPR and MoBa, identifying ADHD and ASD diagnostic cases. The NPR is a national health care registry that receives patient data on diagnoses reported from all hospitals and specialized health care services in Norway. The registry contains diagnoses for in- and outpatients recorded from 2008 onward (Bakken et al., 2020). The diagnostic codes reported to the NPR are according to the International Statistical Classification of Diseases, 10th Revision (ICD-10). The Medical Birth Registry (MBRN) is a national health registry containing information about all births in Norway. Blood samples were obtained from both parents at the free routine ultrasound examination around gestational week 18 and from mothers and children (umbilical cord) at birth.

2.2 Cases and controls

Based on available data, information on diagnosis of ADHD and ASD among children born in 2002 or later were obtained from the NPR. ADHD is a term used in the DSM system, while hyperkinetic disorder is the term used in ICD-10 (Thapar & Cooper, 2016). In the present study, we will use the term ADHD. For ADHD, we selected cases if they had at least two registrations of "hyperkinetic disorder" according to ICD-10 codes F90, F90.0, F90.1, F90.8 or F90.9 (World Health Organization, 1993). We required two registrations in order to exclude erroneous or false diagnoses (Surén et al., 2018). The ICD-10 criteria for hyperkinetic disorder are "early onset; a combination of overactive, poorly modulated behavior with marked inattention and lack of persistent task involvement; and pervasiveness over situations

and persistence over time of these behavioral characteristics" (World Health Organization, 1993). Hyperkinetic disorder requires the combination of inattentive and hyperactive symptoms and is thus similar to the ADHD combined subtype in the DSM system (Thapar & Cooper, 2016). We selected cases of ASD if they had registrations of "pervasive developmental disorders" according to ICD-10 codes F84.0, F84.1, F84.5, F84.8 or F84.9 (World Health Organization, 1993). We could include all cases because the positive predictive value was high (Surén et al., 2012). For both ADHD and ASD, girls were oversampled among cases if possible. We retrieved a random sample of controls from MoBa that were frequency-matched to case categories on sex and birth year. In the present study, we included children that were: singletons, alive at 2 years of age (controls only), born in 2002 or later, had available record from the MBRN and available MoBa questionnaire 1, had no registration of Down's syndrome or of serious malformation in MBRN, and had available maternal blood samples (Figure 1). The present study included 821 ADHD cases, 400 ASD cases, 980 controls, and their mothers (Figure 1).

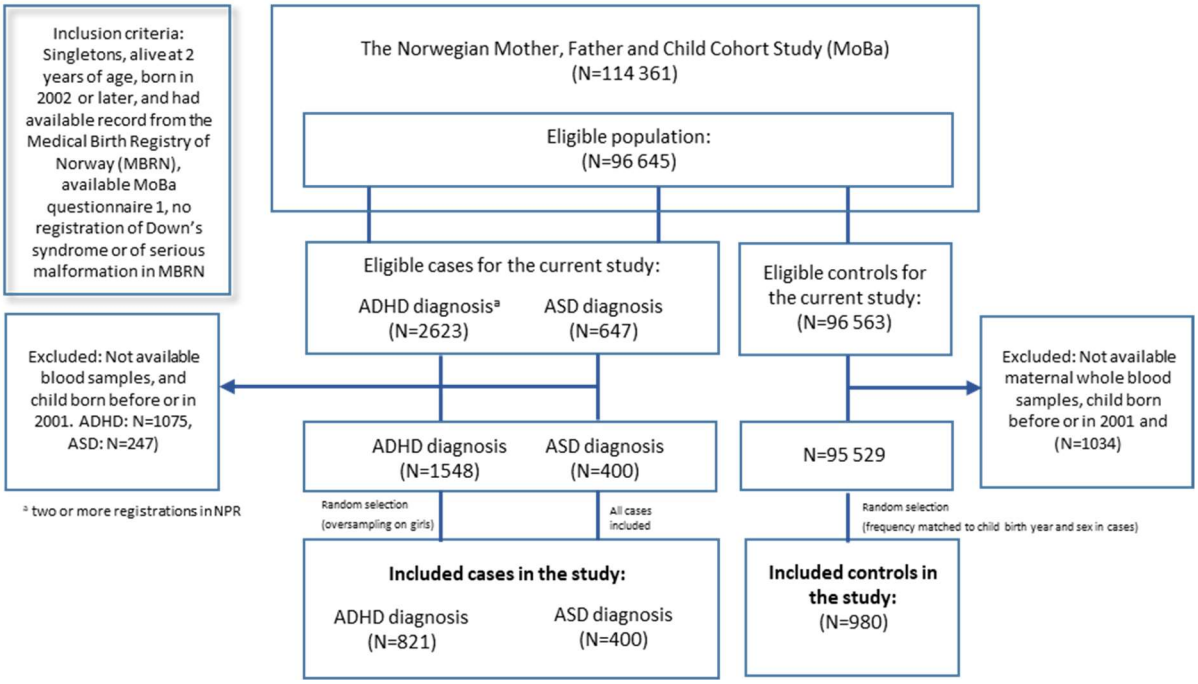

Figure 1. Flow chart of recruitment of cases and controls in in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Note: Abbreviations: Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); Norwegian Medical Birth Registry (MBRN); The Norwegian Mother, Father and Child Cohort Study (MoBa).

2.3 Exposures

We used maternal plasma sampled in gestational week 18 to measure PFAS concentrations. Details about the sampling procedure and handling and storage in the MoBa biobank is described in detail elsewhere (Paltiel et al., 2014). Nineteen PFASs were determined in maternal plasma (Table S1), using liquid chromatography-triple quadruple mass spectrometry (LC-MS/MS) as described previously (Haug et al., 2009). This method has been thoroughly validated and used for determination of more than 5000 serum/plasma samples so far, including approximately 2000 samples from MoBa (Singer et al., 2018). Only PFASs with levels above limit of quantification (LOQ) in > 80% of the plasma samples were included in the present study; PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS) and PFOS. Internal quality control samples and procedure blanks were analyzed along with each batch of samples to ensure high quality of the determinations throughout the project. Case and control status were randomized to batch and blinded to the analysist. More detailed information on limits of detection (LOD) and limits of quantification (LOQ) can be found in Table S1.

2.4 Covariates and other variables

Potential covariates were obtained from the MBRN and MoBa questionnaires completed during pregnancy and up to child's age three years. The MoBa study also included a food frequency questionnaire (FFQ) completed at 22 weeks' gestation, which was designed to capture the average dietary intake during the first four to five months of pregnancy, providing good validity for estimates of foods and nutrients (Brantsæter et al., 2008). Potential adjustment variables were selected *a priori* based on existing literature using a directed acyclic graph (DAG) approach (Greenland et al., 1999; Textor Hardt, & Knüppel, 2011). We considered child sex, birth weight, and small for gestational age (SGA); maternal age at delivery, education, parity, pre-pregnancy body mass index (BMI, kg/m2), self-reported smoking and alcohol intake during pregnancy, as well as FFQ-based estimates of seafood (g/day), and dietary iodine intake (µg/day). Figures illustrating the assumed causal structures are shown in supplementary material (Figures S1 and S2). The final adjustment set included: maternal age, seafood intake, education, parity, and child sex and birth year. We additionally adjusted for maternal ADHD symptoms with ADHD diagnosis as the outcome,

measured by the Adult ADHD Self-Report Scale (ASRS screener) in the questionnaire at child age three years (Kessler et al., 2007).

2.5 Statistical analysis

PFAS concentrations were natural log-transformed to approximate normal distributions. Among the seven PFASs included here, four of them had missing values due to levels below LOQ (PFNA, PFDA, PFUnDA, and PFHpS). In addition, some of the covariates had missing values. To replace missing data, we ran multiple imputation by chained equations, separately for the ADHD sample (cases and controls) and the ASD sample (cases and controls). We generated 50 datasets with the exposure and outcome variables, covariates, and auxiliary variables (Rubin, 1976; Sterne et al., 2009) using the mi ice command in Stata (Royston, 2009). We employed the method for interval-censored data and specified upper and lower limit for imputed results for metals as limit of detection (LOD) and zero, respectively (Royston, 2009). The pooling procedure utilized in the present article was mi estimate (Stata Press, 2017). Details about the missing data in the imputation model are included in supplemental material (Appendix A).

As a first approach, we performed logistic regression analyses separately for ADHD and ASD diagnoses to investigate dose-response relationships between outcome variables and levels of individual PFASs categorized into quartiles, with the lowest quartile as the reference group. Results are presented as odds ratios (ORs) with 95 % confidence intervals (CIs). We also explored effect measure modification (Wald test; significance at $p < 0.10$) by child sex and maternal education (as a measure of SES).

As many PFASs were tested individually in quartile plots, the number of tests performed is fairly high (n=21 for each outcome), thus inflating the probability of type 1 error. Therefore, we also evaluated the results with 99.7% CIs and $p < 0.003$ for the quartile analyses. This would correspond to Šidák correction to control for familywise error rate (false discoveries or type 1 errors) for $k = 21$ number of tests calculated by $100(1-\alpha)^{1/k}$ % confidence intervals with $\alpha = 0.05$.

Secondly, we modeled the associations between PFASs and ASD and ADHD with restricted cubic splines with knots at 10^{th}, 50^{th}, and 90^{th} percentiles. We tested if this model described the data better than a basic logistic regression model, using likelihood ratio tests

(LRT; significance for non-linearity at $p < 0.05$). These analyses were performed in one of the imputed data sets. Prior to these analyses, PFAS outliers were replaced (less or equal to the 1st percentile and greater or equal to the 99th percentile) by the values above or below the 1st and 99th percentile (e.g. Liao et al., 2017). As a sensitivity analysis, we compared splines with and without outliers.

Finally, in order to address the inter-correlations among PFAS chemicals, we analyzed the joint effect of the PFAS mixture on ASD and ADHD diagnoses separately. The effects of individual chemicals may be small and thus more challenging to identify. This makes it difficult to predict the joint (total) effect of the mixture based on modelling of individual PFASs. For the mixture analyses we used a quantile-based g-computation approach (Keil et al. 2020; Keil, 2020). This novel method, combining weighted quantile sum regression and g-computation, was developed to assess the effect of mixtures, giving estimates of the simultaneous effect on the outcome of an increase of all exposures in the mixture by one quantile (Keil et al., 2020; Niehoff et al., 2020). In our study, the quantile was set to one quartile increase in log-PFAS concentrations. We investigated three different mixtures *a priori* based on PFAS structural groups: a mixture containing all seven PFASs, a mixture containing carboxylates (PFOA, PFNA, PFDA, PFUnDA), and a mixture containing sulfonates (PFHxS, PFHpS, PFOS). Results are presented as ORs with 95% CIs associated with one quartile increase in all compounds of the respective mixtures. We also calculated weights; indicating the relative contribution of each PFAS to the associated outcome estimate, for each mixture. The weights are useful to identify compounds contributing most to the mixture effect and the direction of association for each compound.

Most statistical analyses were performed in Stata version 15 (StataCorp, 2019). In addition, we used R version 3.6.2 (R Core Team, 2018) with the "foreign" (R Core Team, 2020), "Amelia" (Honaker et al., 2019), "psych" (Revelle, 2020), "readstata13" (Garbuszus & Jeworutzki, 2018), "qgcomp" (Keil, 2020), "ggplot2" (Wickham et al., 2020), and "tidyverse" (Wickham, 2019) packages. The imputed and adjusted results for the logistic regression models are presented in the main text, while complete case analyses are presented in the supplementary material (Figures S3 and S4). Also, the PFAS quartile boundaries and corresponding ORs with 95% CIs from the quartile models are presented in supplementary material (Table S2).

3. Results

3.1 Study sample characteristics and PFAS distribution

The study sample characteristics are displayed in Table 1. Mothers of both ASD and ADHD cases were slightly younger than mothers of controls. The majority of mothers had higher education (university/college) among controls and ASD cases, and lower education (less than university/college) among the ADHD cases. Most of the mothers of controls and ADHD cases were multiparous, whereas most of the mothers of ASD cases were primiparous. Mothers of ADHD cases were more likely to have reported smoking during pregnancy than mothers of controls and ASD cases.

Table 1. Characteristics of study population in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Characteristic	MoBa Controls Mean ± SD or n (%)	NPR ADHD Cases Mean ± SD or n (%)	NPR ASD Cases Mean ± SD or n (%)
Total N	980	821	400
Maternal ADHD sum score	2.10 ± 0.56	2.27 ± 0.70	2.23 ± 0.58
Missing (*n*)	400	321	171
Maternal education			
< University/college	319 (33.4)	429 (53.9)	164 (42.1)
University/college	636 (66.6)	367 (46.1)	226 (57.9)
Missing (*n*)	25	25	10
Maternal age (years)	30.1 ± 4.45	28.9 ± 4.98	29.5 ± 4.92
Missing (*n*)	0	0	0
Parity			
0	420 (42.9)	394 (48.0)	231 (57.8)
1 or more	560 (57.1)	427 (52.0)	169 (42.2)
Missing (*n*)	0	0	0
Maternal total seafood intake (g/day)	36.9 ± 21.8	36.84 ± 26.7	36.6 ± 25.1
Missing (*n*)	117	112	27
Child sex			
Girl	306 (31.2)	231 (28.1)	62 (15.5)
Boy	674 (68.8)	590 (71.9)	338 (84.5)
Missing (*n*)	0	0	0
Child year of birth			
2002	240 (24.5)	152 (18.5)	35 (8.8)
2003	146 (14.9)	196 (23.9)	70 (17.5)
2004	172 (17.6)	173 (21.1)	55 (13.75)
2005	228 (23.3)	144 (17.5)	71 (17.75)
2006	77 (7.9)	83 (10.1)	69 (17.25)
2007	64 (6.5)	61 (7.4)	55 (13.75)

Characteristic	MoBa Controls Mean ± SD or n (%)	NPR ADHD Cases Mean ± SD or n (%)	NPR ASD Cases Mean ± SD or n (%)
2008	48 (4.9)	12 (1.5)	38 (9.50)
2009	5 (0.5)	-	7 (1.75)
Missing (*n*)	0	0	0
Smoking during pregnancy			
No	854 (87.1)	617 (75.2)	335 (83.7)
Yes	126 (12.9)	203 (24.8)	65 (16.3)
Missing (*n*)	0	1	0
Alcohol during pregnancy			
No	638 (67.9)	579 (73.0)	272 (70.5)
Yes	301 (32.1)	214 (27.0)	114 (29.5)
Missing (*n*)	41	28	14
Maternal marital status			
Married/cohabitant	952 (97.1)	751 (91.5)	380 (95.0)
Other (single, divorced, widow)	28 (2.9	70 (8.5)	20 (5.0)
Missing (*n*)	0	0	0
Maternal folate supplement			
No	384 (39.2)	334 (40.7)	129 (32.3)
Yes*	596 (60.8)	487 (59.3)	271 (67.7)
Missing (*n*)	0	0	0

Note: *Any folate supplements between 4wk before and 8 wk after conception. Abbreviations: Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); Norwegian Patient Registry (NPR); standard deviation (SD); The Norwegian Mother, Father and Child Cohort Study (MoBa).

Table 2 shows the distribution of maternal blood concentrations of PFAS in our sample, including the mean, median and interquartile range of maternal PFAS concentrations during pregnancy. Three of the PFASs (PFOA, PFHxS and PFOS) were above LOQ in all measurements. The spearman correlations among the PFASs for the whole study population (cases and controls) are displayed in Table 3. The strongest correlations were between PFOS and PFHpS (r = 0.81), PFDA and PFUnDA (r = 0.74), PFDA and PFNA (r = 0.73), PFHxS and PFHpS (r = 0.69), PFOA and PFHpS (r = 0.65), PFOA and PFOS (r = 0.61), and PFOS and PFHxS (r = 0.61).

Table 2. PFAS distribution (ng/mL) in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002-2009.

Metal	Case/ control	N	% > LOQ	Mean (SD)	Min	25%	50%	75%	Max
PFOA	Control	980	100	2.31 (1.19)	0.37	1.42	2.12	2.95	16.1
	ADHD case	821	100	2.39 (1.06)	0.47	1.65	2.23	2.99	7.01
	ASD case	400	100	2.46 (3.46)	0.28	1.60	2.11	2.86	67.8
PFNA	Control	979	99.9	0.40 (0.19)	0.05	0.28	0.37	0.49	1.78
	ADHD case	821	100	0.39 (0.24)	0.09	0.27	0.35	0.45	4.40
	ASD case	400	100	0.42 (0.20)	0.10	0.29	0.38	0.49	1.64
PFDA	Control	963	98.3	0.20 (0.10)	0.02	0.13	0.18	0.24	1.05
	ADHD case	778	94.8	0.19 (0.11)	0.05	0.12	0.17	0.22	1.47
	ASD case	399	99.8	0.19 (0.15)	0.01	0.12	0.16	0.23	2.09
PFUnDA	Control	975	99.5	0.26 (0.13)	0.05	0.17	0.24	0.32	0.96
	ADHD case	797	97.1	0.23 (0.12)	0.05	0.14	0.20	0.28	1.02
	ASD case	399	99.8	0.24 (0.15)	0.01	0.14	0.21	0.30	1.10
PFHxS	Control	980	100	0.79 (0.73)	0.15	0.46	0.64	0.91	12.1
	ADHD case	821	100	0.87 (1.18)	0.12	0.47	0.63	0.87	15.2
	ASD case	400	100	0.81 (0.94)	0.10	0.45	0.61	0.87	9.33
PFHpS	Control	969	98.9	0.19 (0.27)	0.02	0.12	0.16	0.22	7.90
	ADHD case	804	97.9	0.19 (0.10)	0.02	0.12	0.17	0.23	1.67
	ASD case	398	99.5	0.17 (0.08)	0.01	0.11	0.15	0.21	0.64
PFOS	Control	980	100	14.2 (9.45)	1.85	9.29	12.9	16.9	203
	ADHD case	821	100	14.1 (6.68)	2.96	9.66	13.0	17.0	89.2
	ASD case	400	100	12.42 (5.83)	1.21	8.10	11.5	16.2	33.5

Abbreviations: Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); limit of quantification (LOQ); perfluorooctanoic acid (PFOA); perfluorononanoic acid (PFNA); perfluorodecanoic acid (PFDA); perfluoroundecanoic acid (PFUnDA); perfluorohexane sulfonate (PFHxS); perfluoroheptanesulfonic acid (PFHpS); perfluorooctane sulfonate (PFOS); standard deviation (SD).

Table 3. Spearman correlations between PFASs (ng/mL) in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002-2009.

	PFOA	PFNA	PFDA	PFUnDA	PFHxS	PFHpS	PFOS
PFOA	1.00						
PFNA	0.55	1.00					
PFDA	0.39	0.73	1.00				
PFUnDA	0.23	0.56	0.74	1.00			
PFHxS	0.57	0.47	0.36	0.36	1.00		
PFHpS	0.65	0.44	0.34	0.30	0.69	1.00	
PFOS	0.61	0.41	0.39	0.41	0.61	0.81	1.00

Abbreviations: Perfluorooctanoic acid (PFOA); perfluorononanoic acid (PFNA); perfluorodecanoic acid (PFDA); perfluoroundecanoic acid (PFUnDA); perfluorohexane sulfonate (PFHxS); perfluoroheptanesulfonic acid (PFHpS); perfluorooctane sulfonate (PFOS).

3.2 Associations between maternal PFAS concentrations and odds of ADHD in children

The quartile models showed an elevated risk for ADHD in children across all quartiles of PFOA [Q2: OR = 1.54 (95% CI: 1.16, 2.04); Q3: OR = 1.41 (95% CI: 1.05, 1.89); Q4: OR = 1.38 (95% CI: 1.01, 1.89)] compared to quartile 1 (reference) and with a decreasing monotonic trend (Figure 2, Table S2). The second quartile of PFOA remained with 99.7% CIs (Table S3). The restricted cubic splines suggested evidence of non-linearity for PFOA and ADHD, with a shape resembling an inverse U-shape (Figure 3).

We identified negative associations with ADHD (lowered risk) in the third and fourth quartiles of PFUnDA [Q3: OR = 0.58 (95% CI: 0.43, 0.77); Q4: OR = 0.49 (95% CI: 0.36, 0.66)] (Figure 2, Table S2). These associations remained with 99.7% CIs (Table S3). There was evidence of effect measure modification by maternal education for the second quartile of PFUnDA (p interaction = 0.02), where those with lower education had higher ORs [OR = 1.05 (95% CI: 0.71, 1.55)] and those with higher education had lower ORs [OR = 0.56 (95% CI: 0.37, 0.83)] (Table S4).

For PFDA, there was a negative association with ADHD in the fourth quartile [Q4: OR = 0.61 (95% CI: 0.46, 0.81)] (Figure 2, Table S2). This association remained with 99.7% CIs (Table S3).

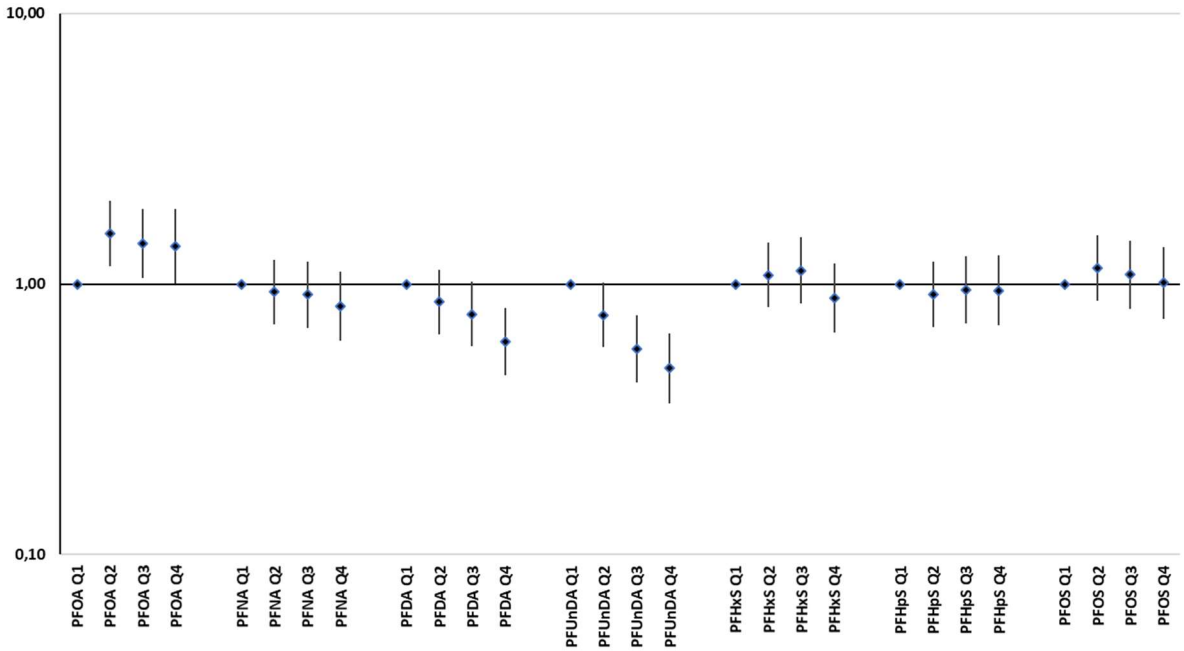

Figure 2. Odds ratios and 95% confidence intervals of logistic regression models predicting attention-deficit/hyperactivity disorder from quartile categories of each PFAS in a nested case–control study of attention-deficit hyperactivity disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009 (n=1801).

Note: Logistic regression with multiple imputation. The PFASs were log transformed. The odds ratio and 95% confidence intervals for each PFAS quartile are represented on the vertical axis (the reference level is the first quartile). Each regression model was adjusted for maternal ADHD symptoms, age, education, parity, seafood intake, child sex, and child birth year. Abbreviations: Perfluorooctanoic acid (PFOA); perfluorononanoic acid (PFNA); perfluorodecanoic acid (PFDA); perfluoroundecanoic acid (PFUnDA); perfluorohexane sulfonate (PFHxS); perfluoroheptanesulfonic acid (PFHpS); perfluorooctane sulfonate (PFOS).

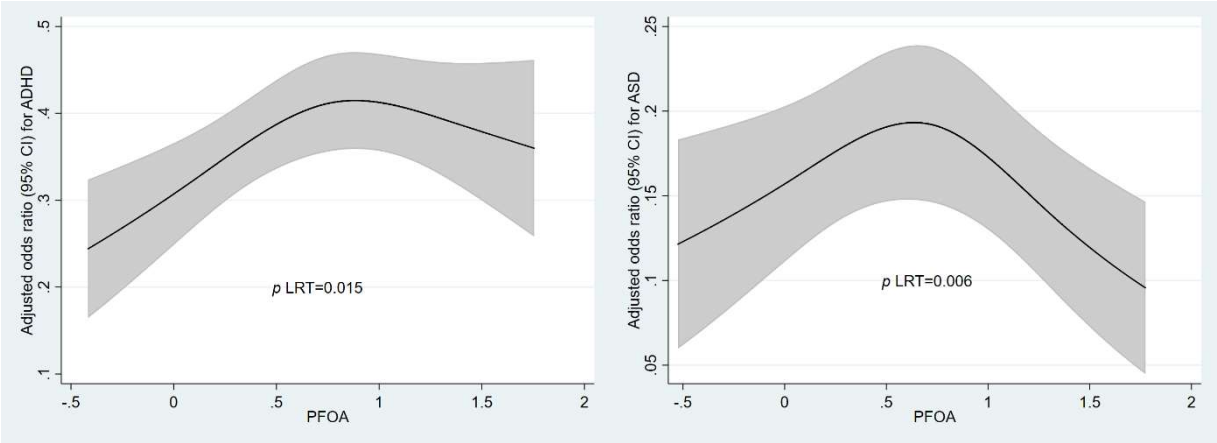

Figure 3. Logistic regression models predicting attention-deficit/hyperactivity disorder and autism spectrum disorder from restricted cubic splines of PFOA in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Note: Three knot positions at 10th, 50th and 90th percentiles of PFOA. Solid lines represent estimated odds ratios, and the grey areas illustrate 95% confidence intervals. P-values were calculated with likelihood ratio test, comparing a model with a log-linear exposure to a restricted cubic spline exposure. PFOA was log transformed, outliers less or equal to the 1st percentile and greater or equal to the 99th percentile were replaced with the value above/below 1st and 99th percentile and missing data imputed. The model was adjusted for maternal ADHD symptoms (ADHD diagnosis only), age, education, parity, seafood intake, child sex, and child birth year. Abbreviations: Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); confidence intervals (CI); likelihood ratio test (LRT); perfluorooctanoic acid (PFOA).

3.3 Associations between maternal PFAS concentrations and odds of ASD in children

The quartile models showed an elevated risk for ASD in children in quartile 2 of PFOA [OR = 1.71 (95% CI: 1.20, 2.45)] compared to quartile 1 (reference) and with a decreasing monotonic trend in the next two quartiles (Figure 4, Table S2). This association persisted with 99.7% CIs (Table S3). The restricted cubic spline suggested a non-linear dose-response relationship between PFOA and odds of ASD, with an inverse U-shape (Figure 3).

For PFOA, there was evidence of effect measure modification by child sex in quartile 3 (p interaction = 0.05), such that the positive relationship in this quartile was greater for boys [OR = 1.47 (95% CI: 0.97, 2.23)] than for girls [OR = 0.56 (95% CI: 0.23, 1.39)] (Table S5). There was also evidence of effect measure modification by maternal education in quartile 2 of PFOA (p interaction = 0.01), with higher ORs for children of mothers with higher education

[OR = 2.58 (95% CI: 1.61, 4.12)] compared to those with lower education [OR = 0.97 (95% CI: 0.56, 1.68)] (Table S4).

We further identified negative associations with ASD (lowered risk) in the third and fourth quartiles of PFDA [Q3: OR = 0.63 (95% CI: 0.45, 0.89); Q4: OR = 0.60 (95% CI: 0.42, 0.86)] and PFUnDA [Q3: OR = 0.59 (95% CI: 0.41, 0.85); Q4: OR = 0.57 (95% CI: 0.39, 0.82)] (Figure 4, Table S2). For PFUnDA, there was also an interaction with maternal education in the second quartile (p interaction = 0.02), where children of mothers with lower education had higher ORs [OR = 1.42 (95% CI: 0.86, 2.34)] compared to those with higher education [OR = 0.64 (95% CI: 0.40, 1.02)] (Table S4).

There was a negative association between PFOS and ASD in quartile 3 [OR = 0.63 (95% CI: 0.44, 0.91) (Figure 4, Table S2). We observed an interaction by child sex in the fourth quartile of PFOS (p interaction = 0.02), with elevated odds for girls [OR = 1.80 (95% CI: 0.79, 4.11)] and lowered odds boys [OR = 0.62 (95% CI: 0.40, 0.95)] (Table S5). There was also an interaction by maternal education in the second quartile of PFOS (p interaction = 0.09), where children of mothers with higher education had higher ORs [OR = 1.20 (95% CI: 0.79, 1.84)] compared to those with lower education [OR = 0.65 (95% CI: 0.37, 1.15)] (Table S4).

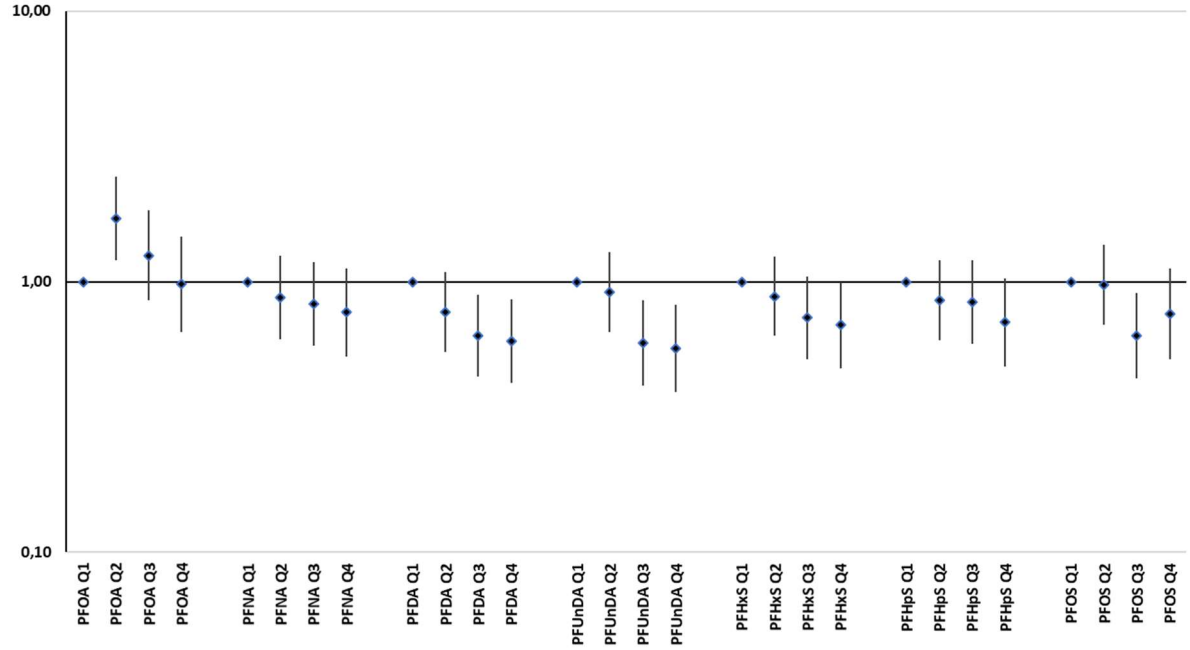

Figure 4. Odds ratios and 95% confidence intervals of logistic regression models predicting autism spectrum disorder from quartile categories of each PFAS in a nested case–control study of autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009 (n=1380).

Note: Logistic regression with multiple imputation. The PFASs were log transformed. The odds ratio and 95% confidence intervals for each PFAS quartile are represented on the vertical axis (the reference level is the first quartile). Each regression model was adjusted for maternal age, education, parity, seafood intake, child sex, and child birth year. Abbreviations: Perfluorooctanoic acid (PFOA); perfluorononanoic acid (PFNA); perfluorodecanoic acid (PFDA); perfluoroundecanoic acid (PFUnDA); perfluorohexane sulfonate (PFHxS); perfluoroheptanesulfonic acid (PFHpS); perfluorooctane sulfonate (PFOS).

We observed effect measure modification by child sex for PFHpS, where the girls were driving the relationship in the third quartile (p interaction = 0.09), with higher ORs for girls [OR = 1.53 (95% CI: 0.69, 3.40)] compared to boys [OR = 0.73 (95% CI: 0.49, 1.08)] (Table S5). There was also evidence of an interaction by child sex in the second quartile of PFHxS (p interaction = 0.06), where the girls were driving the relationship [OR = 1.80 (95% CI: 0.79, 4.12)], while the boys had lower ORs [OR = 0.76 (95% CI: 0.52, 1.11)] (Table S5). We also identified an interaction by maternal education in the fourth quartile of PFHxS (p interaction = 0.04), where those with lower education had higher ORs [OR = 1.12 (95% CI: 0.63, 1.98)] compared to those with higher education [OR = 0.52 (95% CI: 0.33, 0.83)] (Table S4).

3.4 Total mixture effect by quantile-based g-computation

The PFASs demonstrated strong inter-correlations (Table 3), making it difficult to ascertain chemical-specific effects. We therefore used quantile-based g-computation to investigate the joint effects of PFASs on ASD and ADHD. We observed an inverse association, such that a one-quantile increase in the overall PFAS mixture was associated with lower odds of ASD [OR = 0.76 (95% CI: 0.64, 0.90)] (Table 4 and Figure S5). However individual PFAS constituents had both adverse and inverse influences on the outcome. By weight (in decreasing order), PFNA, PFOA, and PFOS had the highest adverse influence in the total PFAS mixture estimate on ASD, while PFDA, PFUnDA, PFHpS, and PFHxS had the highest inverse influence (Figure S5). The carboxylate and sulfonate mixtures were negatively associated with ASD [OR = 0.79 (95% CI: 0.68, 0.93) and OR = 0.84 (95% CI: 0.73, 0.96), respectively] (Table 4, Figures S6 and S7). By weight, PFNA in the carboxylate mixture had a positive influence while PFDA, PFUnDA, and PFOA (in decreasing order) had a negative influence on ASD estimates (Figure S6). In the sulfonate mixture, PFHxS, PFHpS, and PFOS (in decreasing order) were inversely associated with ASD (Figure S7). None of the PFAS mixtures were significantly associated with risk of ADHD (Table 3, Figure S5-S7).

Table 4. Odds ratios and 95% confidence intervals for quantile-based g-computation approach of PFASs on attention-deficit/hyperactivity disorder and autism spectrum disorder in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

PFAS mix	ADHD and controls		ASD and controls	
	OR	95% CI	OR	95% CI
Total mix	0.93	0.82, 1.07	0.76	0.64, 0.90
Carboxylates	0.94	0.83, 1.06	0.79	0.68, 0.93
Sulfonates	0.85	0.88, 1.11	0.84	0.73, 0.96

Note: The models were adjusted for maternal age, education, parity, seafood intake, child sex and child birth year. For child ADHD, maternal ADHD symptoms was also included as a covariate. Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); confidence intervals (CI); odds ratio (OR).

4. Discussion

In this large prospective population-based study, we found increased risk of ASD and ADHD diagnoses in children prenatally exposed to PFOA, however associations were not monotonic, with the strongest association found at mid-range (2nd quartile) of exposure.

PFOA exhibited non-linear, inverted U-shaped dose-response relationships with odds of ASD and ADHD. We also found several inverse associations (decreased risk) between individual PFASs as well as between the total, carboxylate, and sulfonate mixtures with odds of childhood ASD and ADHD.

4.1 Associations between prenatal PFOA and ASD and ADHD in children

Although PFOA is one of the most studied PFASs, there is still insufficient evidence for associations between prenatal exposure and neurodevelopment, particularly regarding ASD (EFSA, 2018; Liew et al., 2018). In the current study we found almost two times higher odds of ASD in children in the second quartile PFOA exposure group (1.47-2.11 ng/ml) compared to the lowest exposure group (0.28-1.47 ng/ml), which also persisted after adjusting for multiple comparisons. In addition, there appeared to be an inverse U-shaped pattern between PFOA and ASD diagnosis in children, with highest risk in the mid-range levels of PFOA. Our findings are in line with a study from the USA that reported positive associations between prenatal PFOA exposure and ASD diagnosis in children (Oh et al., 2020). Another study from the USA reported higher odds of ASD diagnosis in children in the highest prenatal exposure group of PFHxS, but not for PFOA (Shin et al., 2020). Furthermore, research from the Faroe Islands reported a positive association between PFOA measured in blood at child age five years with autism symptoms at seven years of age (Oulhote et al., 2016). The Faroese study did, however, not find any significant associations between prenatal PFAS concentrations (including PFOA) and autism symptoms (Oulhote et al., 2016). Some studies have reported null findings or inverse associations for prenatal exposure to various PFASs, including PFOA, and ASD diagnoses or related symptoms in children (Braun et al., 2014; Liew et al., 2015; Long et al., 2019; Lyall et al., 2018).

The C8 Science Panel is among the most known PFOA studies. Among the various health effects following PFOA-contaminated drinking water in Mid-Ohio Valley communities, Vest-Virginia (USA) they investigated children's neurodevelopmental outcomes in relation to estimated fetal exposure and concurrent childhood PFOA levels measured in blood (Steenland et al., 2020). Altogether, they found no evidence of adverse effects of PFOA exposure on child ADHD (diagnosis and symptoms) or cognitive functions (Steenland et al., 2020). Likewise, several studies have also reported null findings between prenatal exposure to PFASs and ADHD diagnosis or symptoms (Fei & Olsen, 2011; Ode et

al., 2014; Quaak et al., 2016; Strøm et al., 2014; Vuong et al., 2018) and a recent meta-analysis showed little support for an association between early-life exposure to PFOS or PFOA and ADHD (Forns et al., 2020). In contrast, the results herein indicated increased risk of ADHD in children prenatally exposed to mid-range PFOA levels (1.52-2.17 ng/ml) compared to the lowest exposure group (0.37-1.51 ng/ml), which also persisted after adjusting for multiple comparisons. And there are other studies that support our findings. A study based on the Danish National Birth Cohort found higher risk of ADHD diagnosis in children exposed prenatally to the highest levels of PFOA compared to the lowest exposure levels (although they questioned the consistency across results) (Liew et al., 2015a). Another study based on pooled data from Poland, Ukraine, and Greenland found an association between the highest levels of prenatal PFOA exposure (compared to the lowest levels) and increased levels of hyperactivity in children of 5-9 years of age (Høyer et al., 2015). Furthermore, a study from the Faroe Islands reported that postnatal (but not prenatal) exposure to several PFASs was associated with an increase in behavioral difficulties which consisted of hyperactivity problems, inattention, conduct problems, and peer relationship problems among seven-year-olds (Oulhote et al. 2016).

In the present study we observed that prenatal PFOA exposure increased risk of both ADHD and ASD diagnosis with similar (non-linear) dose-response-relationships. This may suggest that PFOA is a common risk factor for both disorders, perhaps affecting shared neurochemical and neurodevelopmental pathways (Kern et al., 2015). Interestingly, we reported in a previous study based on MoBa data that increasing prenatal PFOA exposure was related to poorer nonverbal working memory (Skogheim et al., 2020). Deficits in working memory is a common executive function impairment often found in children with ADHD and ASD (Geurts et al., 2004; Thapar & Cooper, 2016). We thus speculate if PFOA could affect neurobehavioral domains mutual for ASD and ADHD, such as working memory.

4.2 Inverse associations between prenatal exposure to some PFASs and ASD and ADHD in children

In the present study, we observed some counter-intuitive associations between prenatal exposure to PFASs and ASD and ADHD diagnosis in children. Although there is no plausible biological reason for these inverse associations, this phenomenon has also been reported in previous research (e.g. Lien et al., 2016; Liew et al., 2015a; Stein et al., 2013). It has been

speculated that the "live birth bias" might explain these inverse associations between prenatal PFAS exposure and neurodevelopmental outcomes observed in children (Liew et al., 2015b). This entails that bias is introduced when one studies the effects of prenatal exposure to environmental factors and later health outcomes only in live births (Liew et al., 2015b). If the contaminant being investigated can contribute to termination of the pregnancy, the number of exposed live born children will be reduced, which can influence the surviving children in this group and thus the results. Others have also proposed that PFASs can activate the proliferator-activated receptors (PPARs) alpha and gamma, which is known to have neuroprotective and central-nervous-system anti-inflammatory properties (Quaak et al., 2016; Stein et al., 2013). As many PFASs have seafood consumption as the main dietary source for humans (Haug et al., 2010), it may be that the inverse associations reflect beneficial consumption of polyunsaturated fatty acids and micronutrients from eating seafood rather than the possibly adverse effects from PFASs. Although we adjusted for self-reported seafood intake in the present study, the adjustment may not have been sufficient. This could also be due to unmeasured confounding, as we were not able to account for several (other) sources of PFASs, such as exposure via consumer products.

4.3 Sex-specific effects and socioeconomic factors

Experimental animal studies have suggested that there are sex differences in both exposure and capacity to metabolize and eliminate PFOS and other PFASs and that this is possibly linked to prenatal gonadal hormone levels (Kjeldsen & Bonefeld-Jørgensen, 2013; Lau et al., 2007; Mariussen, 2012). A recent meta-analysis showed that early life-exposure to PFASs and associations with ADHD was more prominent among girls compared to boys (Forns et al., 2020). In the present study, we observed effect measure modification by child sex in several associations between PFASs and ASD. For PFOA, it was the boys who accounted for the positive association, with higher odds of ASD compared to girls. For the remaining associations; PFOS, PFHxS, and PFHpS, increased odds ratios for ASD were mainly found in girls, while odds for ASD were decreased in boys. This could indicate that overall, the girls had a higher risk of ASD with higher PFAS levels. Likewise, a study from Norway reported a positive association between early-life exposure to PFOS and ADHD diagnosis, with stronger associations among girls compared to boys (Lenters et al., 2019). Furthermore, a study from the Faroe Islands, reported consistently positive scores on

behavioral problems and autistic screening scores among girls compared to boys with postnatal PFAS exposure (Oulhote et al., 2016). While there is scarce research on prenatal PFASs and ASD, two studies investigated sex differences and found an opposite pattern; with higher risk among boys exposed prenatally to PFOS and PFNA, respectively (Braun et al., 2014; Shin et al., 2020).

PFAS exposure levels have been associated with SES, such as education, income, and employment (Brantsæter et al., 2013; Montazeri et al, 2019; Tyrrell, 2013). In addition, ADHD in children is associated with mothers who have lower education (Torvik et al., 2020), while for ASD, results are mixed with some reporting associations with higher maternal education and some with lower (Lung et al., 2018). In the present study, we observed that several associations between PFAS and ASD diagnosis were modified by maternal education, however inconsistencies regarding the direction complicates interpretation. For PFOA and PFOS, there were higher odds for the child having an ASD diagnosis among those with mothers who had higher education (college or higher). This is in line with a study on determinants of PFASs, where they also used data from MoBa, where they found that levels of PFOA and PFOS increased with educational level and household income, respectively (Brantsæter et al, 2013). A similar finding was reported in a study on environmental contaminants and SES, showing higher concentrations of PFASs among employed pregnant women (Montazeri et al., 2019). In the present study we found the opposite pattern for PFUnDA, PFNA, and PFHxS, with higher odds of ASD diagnosis among children whose mothers had lower education. For ADHD, education appeared to be a modifier only in relation to PFUnDA exposure, as we found higher odds of ADHD among children whose mothers had lower education. Our findings for ADHD and PFUnDA, are in line with a study where stratified analyses indicated higher odds of ADHD with early-life exposure to PFASs among offspring of mothers with lower education (Forns et al., 2020).

4.4 Mixtures

The human fetus is exposed to a range of highly inter-correlated PFASs and other toxicants that can interfere in combination with brain development (Mariussen, 2012; Quaak et al., 2016; Virjheid et al., 2016s). There are, however, still only a few studies that have investigated prenatal exposure to the total mixture of PFASs and neurodevelopmental outcomes (Vrijheid et al., 2016). In the present study, the total mixture of PFASs as well as

the carboxylate and the sulfonate mixtures were negatively associated with ASD. Although we would expect an overall adverse effect from prenatal exposure to PFASs, the general pattern in the present study consisted of many negative associations between individual PFAS and ASD. Similarly, research from the USA found associations between increasing levels of PFOA and lower autistic behavior scores in a multi-pollutant model with several types of chemicals, including PFOS, PFNA, and PFHxS (Braun et al., 2014). Likewise, a Danish study using principal component analysis to reduce the number of toxicant exposures including PFASs, found inverse associations between a component with PFASs (PFOS, PFOA, PFOSA) and ASD diagnosis (Long et al., 2019). In contrast, a study from the Faroe Islands, reported positive associations between maternal levels of PFOA and PFOS and behavioral problems in children at age seven in a multi-pollutant model (Oulhote et al., 2019). In addition, a Norwegian study found associations between early-life exposure to PFOS and ADHD diagnoses, but not with the other PFASs, using a multi-pollutant model that was also adjusted for other chemicals (Lenters et al., 2019).

4.5 Public health implications and regulations

Although the reported PFAS levels in the present study were lower than what has been reported in other studies that investigated neurodevelopment (e.g. Liew et al., 2015a; Oulhote et al., 2016; Stein et al., 2013), results from a study comparing PFAS levels in several European cohort studies showed that the PFAS levels in a subsample from MoBa were equal to or higher than in the other cohorts (Haug et al., 2018). The PFAS levels in that MoBa subsample (Haug et al., 2018) were similar to the levels in the present MoBa-based study population. Most of the knowledge about PFAS levels in Europe is based on cohort studies dating back in time, meaning that we do not know the present-day levels and what the population is exposed to with regards to novel PFASs. Although the production of some PFASs, such as PFOS and PFOA, has declined, several new replacement compounds are on the rise, with unknown health effects (Sunderland et al., 2019; Wang et al., 2017a). Therefore, it is of high importance to perform studies measuring the present levels of PFAS exposure and other environmental toxicants in pregnant women and assess potential adverse effects on health and development in children.

The results from epidemiological research on prenatal PFAS exposure and neurodevelopment are inconsistent, with many studies showing null findings. However,

different methods and measures to assess neurodevelopmental outcomes complicate comparison across studies. Even so, there are other health outcomes with more consistent findings on adverse effects following PFAS exposure, such as fetal growth and immune function (Liew et al., 2018; Vrijheid et al., 2016). We urge for more research to investigate potential neurotoxicological effects of PFASs and their mixtures on human brain development, both epidemiological and experimental, to elucidate mechanistic underpinnings and assess risk of adverse neurodevelopment associated with *in utero* exposure. The paradoxical findings of seemingly improved neurodevelopmental outcome measures with PFAS exposure that is regularly reported in epidemiological studies, including the present study, also need to be resolved.

4.6 Limitations and strengths

Despite our efforts to oversample girls, our study included fewer girls than boys, especially in the ASD case group. Although all analyses were adjusted for child sex, the estimates for girls were less precise and less reliable than for boys and this may have influenced the interaction analyses. Another potential limitation is that we could not account for variation in maternal glomerular filtration rate, which may be a source of residual bias. In our sample, there was no information of overlap between ASD and ADHD cases, as coding according to ICD-10 does not allow comorbid primary diagnoses (F84 and F90). However, this does not exclude the possibility of overlap regarding symptoms, but only diagnostic codes were available for our research. Another potential limitation concerns the clinical basis for the ADHD NPR registrations and the possibility that alternative diagnoses should have been considered (Surén et al., 2018). Limitations also include potential self-selection bias. Participants in MoBa were generally older, had higher educational level, and reported less smoking compared with the general population (Nilsen et al., 2009). However, our study may be less prone to self-selection bias, as the mothers only had to complete the first questionnaire and because we nested case groups based on registries that are mandatory in Norway.

Our study also has several strengths. We had a large sample size of mother-child pairs, meaning that we could investigate potential effect measure modification by child sex and maternal education as well as non-monotonic relationships, in addition to the main association analyses. Another strength was that this study was nested within a prospective birth cohort, meaning that our analyses benefitted from a large number of relevant covariates to account for

residual confounding pathways. Furthermore, we examined several PFASs, both individually and as mixtures. To our knowledge, only one other study has investigated prenatal PFAS exposure and compared ADHD and ASD diagnosis in children in the same study (Liew et al., 2015a). Particularly studying ASD is a strength, as there is a lack of research on prenatal PFAS exposure and ASD in children. Furthermore, the validity of the ASD diagnoses in NPR was found to be very high in a study involving participants in MoBa (Surén et al., 2012).

5. Conclusion

Results from the present study showed elevated risk of both ASD and ADHD in children prenatally exposed to mid-range levels of PFOA and that this relationship was non-linear showing inverted U-shapes in both case groups. For ASD, this risk was mainly found for boys. For other carboxylates, sulfonates, and their mixtures, there appeared to be inverse associations with ASD. Also, SES appeared to modify some of the relationships. Overall, the literature linking PFAS exposures with neurodevelopmental outcomes is still inconsistent, suggesting the need for more research to elucidate the neurotoxicological potential of PFAS, both epidemiological and experimental.

Declarations of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Availability of data and material

The consent given by the participants does not open for storage of data on an individual level in repositories or journals. Researchers who want access to data sets for replication should submit an application to datatilgang@fhi.no. Access to data sets requires approval from The Regional Committees for medical and health research ethics in Norway and a formal contract with MoBa.

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Supplementary material

Table S1.

Overview of determined PFASs in a nested case–control study of attention-deficit hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2002–2009[1].

PFAS compound, chain length (number of fluorinated carbons)	Acronym	LOD (ng/mL)	LOQ (ng/mL)
Perfluoriated carboxylic acids	PFCAs		
Perfluorobutanoate, C_4 (C_3)	PFBA	0.003	0.10
Perfluoropentanoate, C_5 (C_4)	PFPeA	0.008	0.05
Perfluorohexanoate, C_6 (C_5)	PFHxA	0.007	0.05
Perfluoroheptanoate, C_7 (C_6)	PFHpA	0.009	0.05
Perfluorooctanoate C_8 (C_7)	PFOA	0.006	0.05
Perfluorononanoate, C_9 (C_8)	PFNA	0.004	0.05
Perfluorodecanoate, C_{10} (C_9)	PFDA	0.002	0.05
Perfluoroundecanoate, C_{11} (C_{10})	PFUnDA	0.006	0.05
Perfluorododecanoate, C_{12} (C_{11})	PFDoDa	0.007	0.05
Perfluorotridecanoate, C_{13} (C_{12})	PFTrDa	0.004	0.05
Perfluorotetradecanoat, C_{14} (C_{13})	PFTeDa	0.050	0.20
Perfluorinated sulfonic acids	PFSAs		
Perfluorobutane sulfonate, C_4 (C_4)	PFBS	0.009	0.05
Perfluorohexane sulfonate, C_6 (C_6)	PFHxS	0.007	0.05
Perfluoroheptane sulfonate, C_7 (C_7)	PFHpS	0.010	0.05
Perfluorooctane sulfonate, C_8 (C_8)	PFOS	0.003	0.05
Perfluorodecane sulfonate, C_{10} (C_{10})	PFDS	0.050	0.20
Others			
Perfluorooctane sulfonamide, C_8 (C_8)	PFOSA	0.020	0.05
N-Methylperfluorooctane sulfonamide	MeFOSA	0.009	0.05
N-Ethylperfluorooctane sulfonamide	EtFOSA	0.009	0.05

Abbreviations: LOD: limit of detection, LOQ: limit of quantification.

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Appendix A. From statistical analysis

In the ADHD imputation model we included the following (% missing): PFOA (0), PFNA (0.1), PFDA (3), PFUnDA (1.2), PFHxS (0), PFHpS (1.4), PFOS (0), maternal age (0), maternal ADHD symptoms (41.6), maternal education (2.9), parity (0) maternal seafood intake (13.2), child sex (0), child birth year (0), length of gestation (0.3), child birth weight (0), maternal smoking (0.1), maternal iodine intake (13.2), and maternal folate intake (8.8).

In the ASD imputation model we included the following (% missing): PFOA (0), PFNA (0.1), PFDA (1.4), PFUnDA (0.4), PFHxS (0), PFHpS (1.1), PFOS (0), maternal age (0), maternal education (2.6), parity (0) maternal seafood intake (10.5), child sex (0), child birth year (0), length of gestation (0.2), child birth weight (0), maternal smoking (0), maternal iodine intake (10.5), and maternal folate intake (7.5).

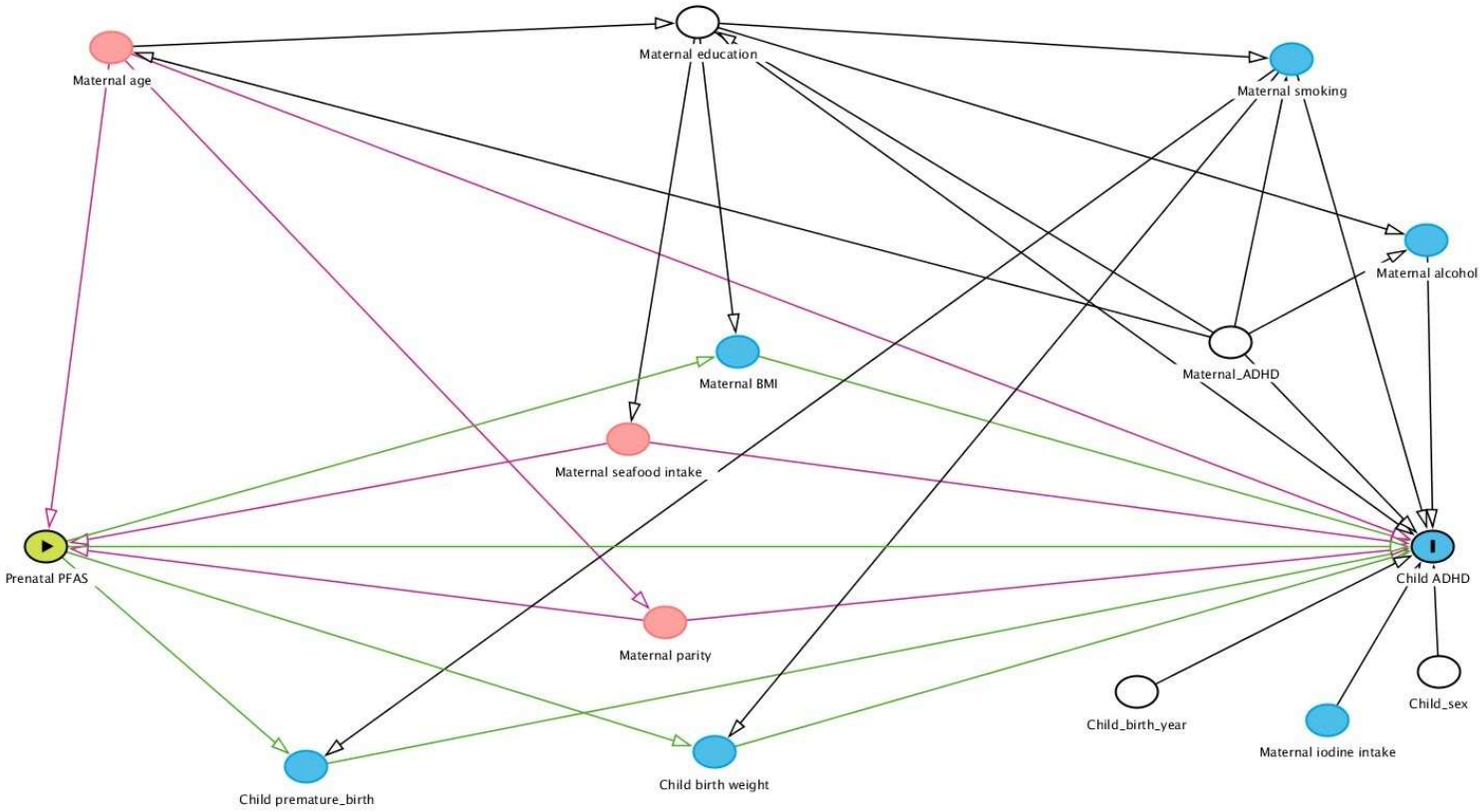

Figure S1. Directed acyclic graph (DAG) for prenatal PFAS exposure and child attention-deficit/hyperactivity disorder in a nested case–control study of attention-deficit/hyperactivity disorder (ADHD) in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

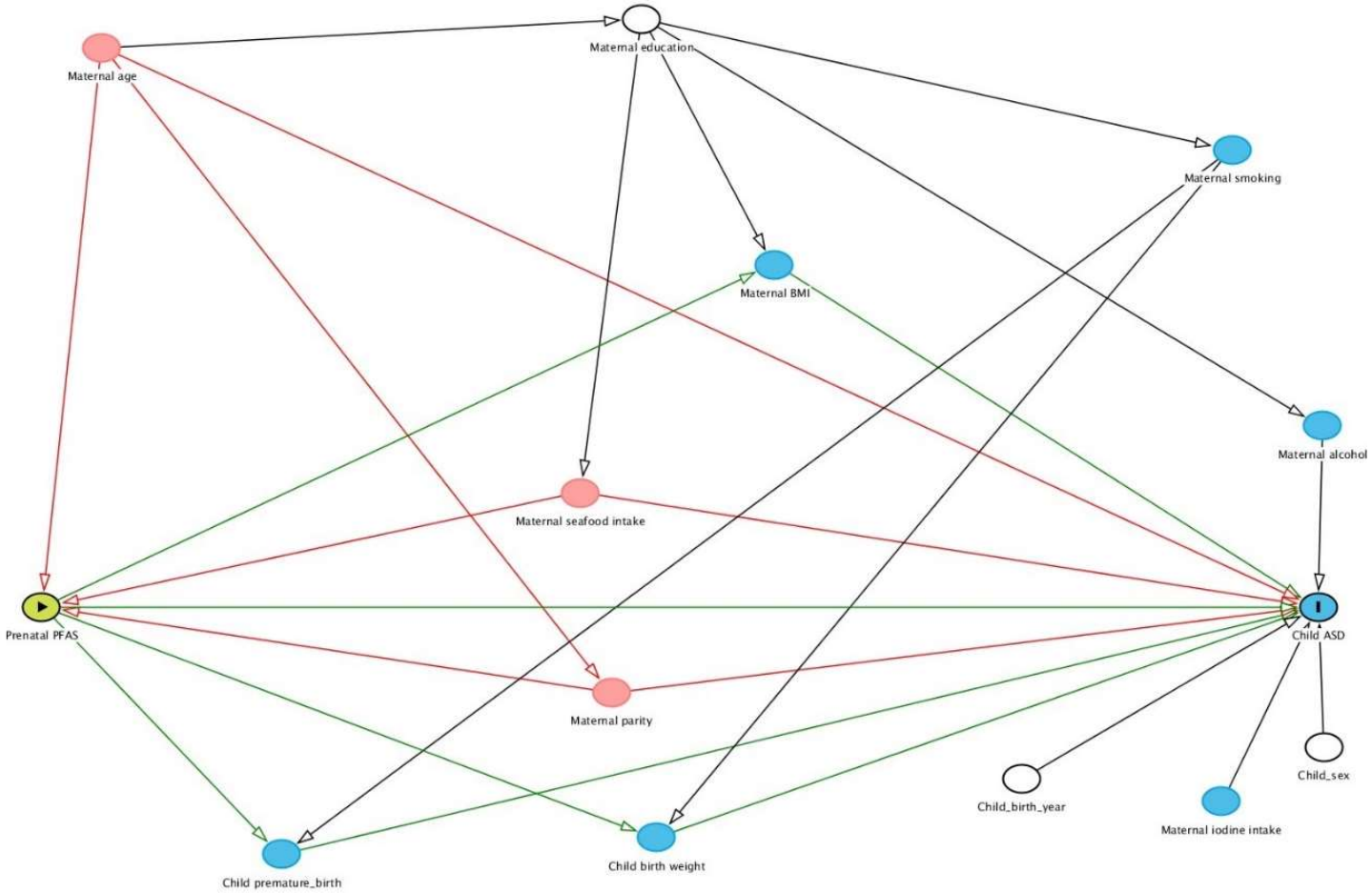

Figure S2. Directed acyclic graph (DAG) for prenatal PFAS exposure and child autism spectrum disorder in a nested case–control study of autism spectrum disorder (ASD) in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

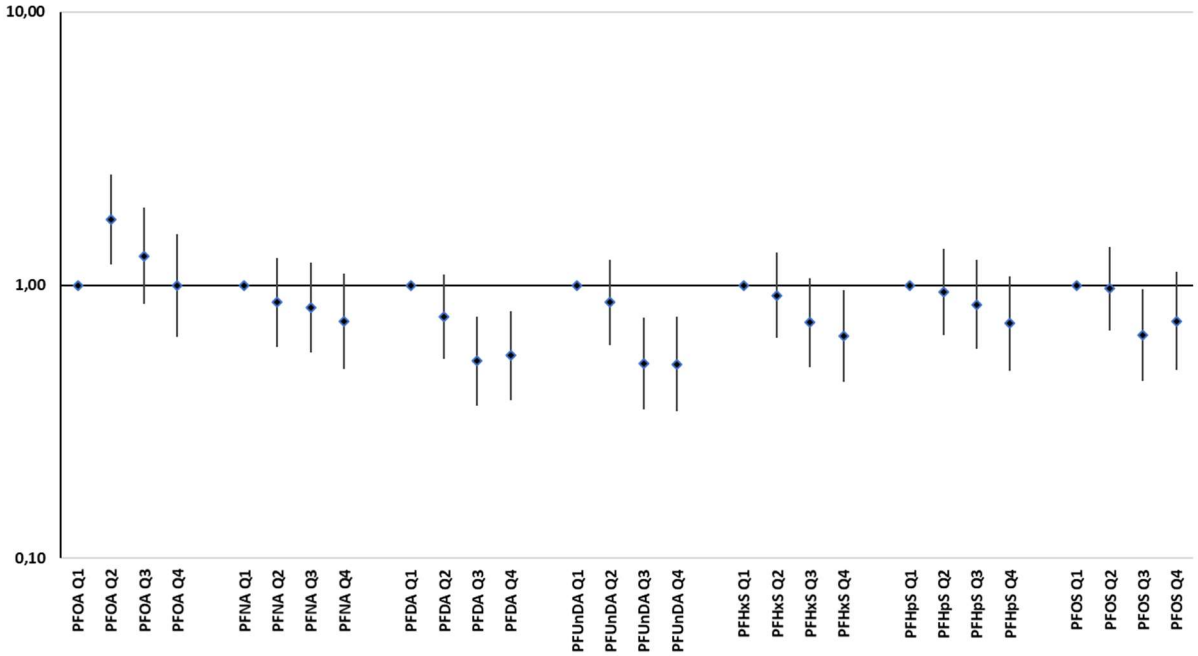

Figure S3. Odds ratios and 95% confidence intervals for complete cases of logistic regression models predicting attention-deficit/hyperactivity disorder from quartile categories of each PFAS in a nested case–control study of attention-deficit hyperactivity disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Note: Logistic regression with multiple imputation. The PFASs were log transformed. The odds ratio and 95% confidence intervals for each PFAS quartile are represented on the vertical axis (the reference level is the first quartile). Each regression model was adjusted for maternal ADHD symptoms, age, education, parity, seafood intake, child sex, and child birth year. Abbreviations: Perfluorooctanoic acid (PFOA); perfluorononanoic acid (PFNA); perfluorodecanoic acid (PFDA); perfluoroundecanoic acid (PFUnDA); perfluorohexane sulfonate (PFHxS); perfluoroheptanesulfonic acid (PFHpS); perfluorooctane sulfonate (PFOS).

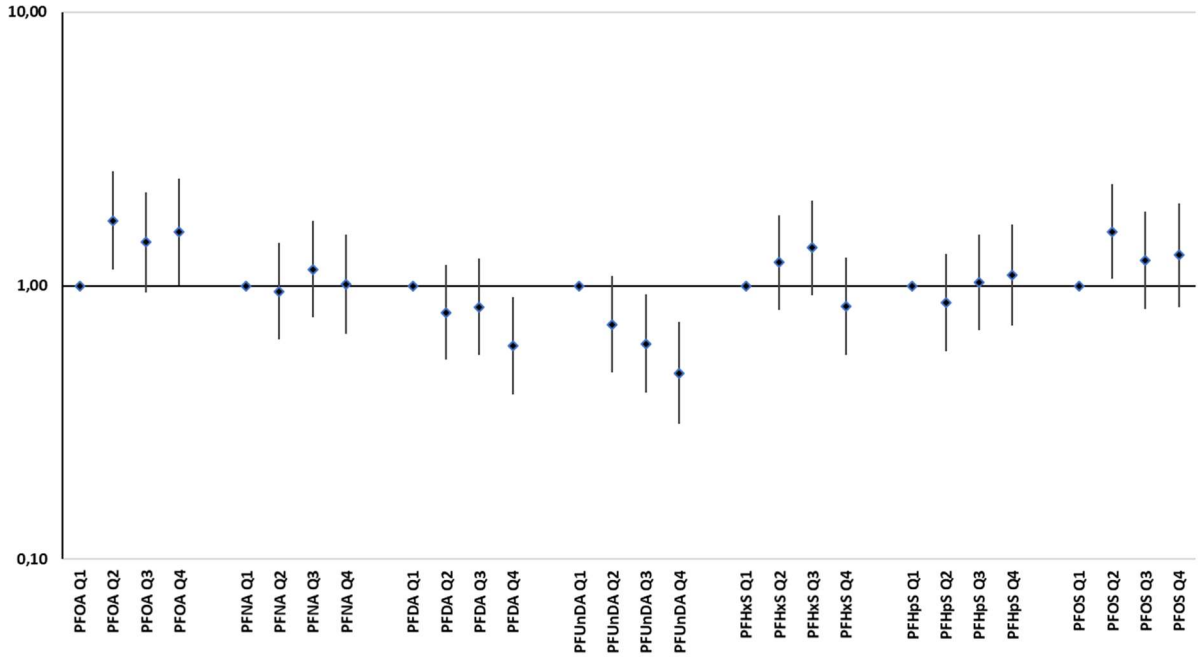

Figure S4. Odds ratios and 95% confidence intervals for complete cases of logistic regression models predicting autism spectrum disorder from quartile categories of each PFAS in a nested case–control study of autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Note: Logistic regression with multiple imputation. The PFASs were log transformed. The odds ratio and 95% confidence intervals for each PFAS quartile are represented on the vertical axis (the reference level is the first quartile). Each regression model was adjusted for maternal age, education, parity, seafood intake, child sex, and child birth year. Abbreviations: Perfluorooctanoic acid (PFOA); perfluorononanoic acid (PFNA); perfluorodecanoic acid (PFDA); perfluoroundecanoic acid (PFUnDA); perfluorohexane sulfonate (PFHxS); perfluoroheptanesulfonic acid (PFHpS); perfluorooctane sulfonate (PFOS).

Table S2. Quartile concentration levels and odds ratios and 95% confidence intervals for logistic regression models predicting attention-deficit/hyperactivity disorder and autism spectrum disorder from quartile categories of each PFAS in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Metal/ element	ADHD and controls			ASD and controls		
	Concentration	OR	95% CI	Concentration	OR	95% CI
PFOA Q1 (ref.)	0.37-1.51			0.28-1.47		
PFOA Q2	1.52-2.17	1.54	1.16, 2.04	1.47-2.11	1.71	1.20, 2.45
PFOA Q3	2.17-2.95	1.41	1.05, 1.89	2.11- 2.15	1.25	0.85, 1.83
PFOA Q4	2.96-16.1	1.38	1.01, 1.89	2.92- 67.8	0.98	0.65, 1.47
PFNA Q1 (ref.)	0.05-0.27			0.05-0.28		
PFNA Q2	0.27-0.36	0.93	0.71, 1.23	0.28-0.37	0.87	0.61, 1.24
PFNA Q3	0.36-0.47	0.91	0.69, 1.21	0.37-0.49	0.83	0.58, 1.18
PFNA Q4	0.47-4.40	0.82	0.62, 1.11	0.49-1.78	0.77	0.53, 1.12
PFDA Q1 (ref.)	0.02-0.13			0.02-0.13		
PFDA Q2	0.13-0.17	0.86	0.65, 1.13	0.13-0.17	0.77	0.55, 1.08
PFDA Q3	0.17-0.23	0.77	0.59, 1.02	0.18-0.23	0.63	0.45, 0.89
PFDA Q4	0.23-1.47	0.61	0.46, 0.81	0.24-2.09	0.60	0.42, 0.86
PFUnDA Q1 (ref.)	0.05-0.16			0.01-0.16		
PFUnDA Q2	0.16-0.22	0.77	0.58, 1.01	0.16-0.23	0.91	0.65, 1.28
PFUnDA Q3	0.22-0.30	0.58	0.43, 0.77	0.23-0.32	0.59	0.41, 0.85
PFUnDA Q4	0.30-1.02	0.49	0.36, 0.66	0.32-1.10	0.57	0.39, 0.82
PFHxS Q1 (ref.)	0.12-0.47			0.10-0.46		
PFHxS Q2	0.47-0.64	1.08	0.82, 1.42	0.46-0.63	0.88	0.63, 1.24
PFHxS Q3	0.64-0.89	1.12	0.85, 1.49	0.63-0.90	0.74	0.52, 1.05
PFHxS Q4	0.89-15.2	0.89	0.66, 1.19	0.91-12.1	0.69	0.48, 1.00
PFHpS Q1 (ref.)	0.02-0.12			0.02-0.12		
PFHpS Q2	0.12-0.17	0.92	0.69, 1.21	0.12-0.16	0.85	0.61, 1.20
PFHpS Q3	0.17-0.23	0.95	0.72, 1.27	0.16-0.22	0.84	0.59, 1.20
PFHpS Q4	0.23-7.90	0.95	0.70, 1.28	0.22-7.90	0.71	0.49, 1.03
PFOS Q1 (ref.)	1.85-9.45			1.21-8.83		
PFOS Q2	9.46-13.0	1.14	0.87, 1.51	8.83-12.55	0.97	0.69, 1.36
PFOS Q3	13.0-17.0	1.08	0.81, 1.45	12.55-16.7	0.63	0.44, 0.91
PFOS Q4	17.0-203	1.01	0.74, 1.37	16.7-203	0.76	0.52, 1.12

Note: PFAS concentration levels are in ng/mL. The quartile models were adjusted for maternal age, education, parity, seafood intake, child sex, and child birth year. For child ADHD, maternal ADHD symptoms was also included as a covariate. Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); confidence interval (CI); odds ratio (OR); perfluorooctanoic acid (PFOA); perfluorononanoic acid (PFNA); perfluorodecanoic acid (PFDA); perfluoroundecanoic acid (PFUnDA); perfluorohexane sulfonate (PFHxS); perfluoroheptanesulfonic acid (PFHpS); perfluorooctane sulfonate (PFOS).

Table S3. Odds ratios and 99.7% confidence intervals for logistic regression models predicting attention-deficit/hyperactivity disorder and autism spectrum disorder from quartile categories of each PFAS in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Metal/ element	ADHD and controls		ASD and controls	
	OR	99.7% CI	OR	99.7% CI
PFOA Q2	1.54	1.01, 2.35	1.71	1.00, 2.94
PFOA Q3	1.41	0.90, 2.20	1.25	0.70, 2.23
PFOA Q4	1.38	0.86, 2.22	0.98	0.53, 1.81
PFNA Q2	0.93	0.62, 1.42	0.87	0.51, 1.49
PFNA Q3	0.91	0.60, 1.40	0.83	0.48, 1.42
PFNA Q4	0.82	0.53, 1.29	0.77	0.44, 1.36
PFDA Q2	0.86	0.57, 1.29	0.77	0.46, 1.29
PFDA Q3	0.77	0.51, 1.18	0.63	0.37, 1.07
PFDA Q4	0.61	0.40, 0.94	0.60	0.35, 1.03
PFUnDA Q2	0.77	0.51, 1.17	0.91	0.55, 1.53
PFUnDA Q3	0.58	0.37, 0.89	0.59	0.34, 1.03
PFUnDA Q4	0.49	0.31, 0.78	0.57	0.32, 1.00
PFHxS Q2	1.08	0.71, 1.63	0.88	0.53, 1.48
PFHxS Q3	1.12	0.73, 1.72	0.74	0.43, 1.26
PFHxS Q4	0.89	0.57, 1.38	0.69	0.40, 1.20
PFHpS Q2	0.92	0.60, 1.39	0.85	0.51, 1.44
PFHpS Q3	0.95	0.62, 1.47	0.84	0.49, 1.44
PFHpS Q4	0.95	0.60, 1.49	0.71	0.40, 1.25
PFOS Q2	1.14	0.75, 1.74	0.97	0.58, 1.63
PFOS Q3	1.08	0.70, 1.68	0.63	0.36, 1.10
PFOS Q4	1.01	0.64, 1.61	0.76	0.42, 1.37

Note: The quartile models were adjusted for maternal age, education, parity, seafood intake, child sex, and child birth year. For child ADHD, maternal ADHD symptoms was also included as a covariate. Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); confidence interval (CI); odds ratio (OR); perfluorooctanoic acid (PFOA); perfluorononanoic acid (PFNA); perfluorodecanoic acid (PFDA); perfluoroundecanoic acid (PFUnDA); perfluorohexane sulfonate (PFHxS); perfluoroheptanesulfonic acid (PFHpS); perfluorooctane sulfonate (PFOS).

Table S4. Odds ratios and 95% confidence intervals and interaction terms for maternal education from logistic regression models predicting attention-deficit/hyperactivity disorder and autism spectrum disorder from quartile categories of PFASs in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

PFAS	ADHD			
	All	College/ university	< college/ university	Interaction term p
PFUnDA		Interaction term p = 0.09		
PFUnDA Q1	1.00	1.00	1.00	
PFUnDA Q2	0.77 (0.58, 1.01)	0.56 (0.37, 0.83)	1.05 (0.71, 1.55)	0.02
PFUnDA Q3	0.58 (0.43, 0.77)	0.55 (0.38, 0.81)	0.56 (0.37, 0.85)	0.95
PFUnDA Q4	0.49 (0.36, 0.66)	0.43 (0.29, 0.63)	0.54 (0.34, 0.85)	0.45
		ASD		
PFOA		Interaction term p = 0.03		
PFOA Q1 (ref.)	1.00	1.00	1.00	
PFOA Q2	1.71 (1.20, 2.45	2.58 (1.61, 4.12)	0.97 (0.56, 1.68)	0.01
PFOA Q3	1.25 (0.85, 1.83)	1.54 (0.93, 2.53)	0.98 (0.56, 1.72)	0.22
PFOA Q4	0.98 (0.65, 1.47)	1.06 (0.64, 1.77)	0.99 (0.54, 1.81)	0.85
PFOS		Interaction term p = 0.17		
PFOS Q1 (ref.)	1.00	1.00	1.00	
PFOS Q2	0.97 (0.69, 1.36)	1.20 (0.79, 1.84)	0.65 (0.37, 1.15)	0.09
PFOS Q3	0.63 (0.44, 0.91)	0.59 (0.37, 0.94)	0.69 (0.39, 1.21)	0.67
PFOS Q4	0.76 (0.52, 1.12)	0.76 (0.47, 1.23)	0.75 (0.42, 1.32)	0.96
PFHxS		Interaction term p = 0.15		
PFHxS Q1	1.00	1.00	1.00	
PFHxS Q2	0.88 (0.63, 1.24)	0.84 (0.54, 1.32)	0.94 (0.56, 1.60)	0.74
PFHxS Q3	0.74 (0.52, 1.05)	0.70 (0.45, 1.09)	0.76 (0.43, 1.35)	0.83
PFHxS Q4	0.69 (0.48, 1.00)	0.52 (0.33, 0.83)	1.12 (0.63, 1.98)	0.04
PFUnDA		Interaction term p = 0.02		
PFUnDA Q1	1.00	1.00	1.00	
PFUnDA Q2	0.91 (0.65, 1.28)	0.64 (0.40, 1.02)	1.42 (0.86, 2.34)	0.02
PFUnDA Q3	0.59 (0.41, 0.85)	0.63 (0.40, 1.00)	0.46 (0.26, 0.83)	0.40
PFUnDA Q4	0.57 (0.39, 0.82)	0.49 (0.31, 0.77)	0.73 (0.39, 1.35)	0.30
PFNA		Interaction term p = 0.17		
PFNA Q1	1.00	1.00	1.00	
PFNA Q2	0.87 (0.61, 1.24)	0.91 (0.57, 1.46)	0.79 (0.47, 1.35)	0.71
PFNA Q3	0.83 (0.58, 1.18)	0.63 (0.39, 1.02)	1.18 (0.70, 1.99)	0.08
PFNA Q4	0.77 (0.53, 1.12)	0.72 (0.45, 1.14)	0.81 (0.44, 1.49)	0.76

Note: Logistic regression with multiple imputation with additional interaction analyses. Each regression model was adjusted for maternal age, education, parity, seafood intake, child sex, and child birth year. For child ADHD, maternal ADHD symptoms was also included as a covariate. Interaction term was tested with Wald's test. Abbreviations: Attention-deficit/hyperactivity (ADHD); autism spectrum disorder (ASD); perfluorooctanoic acid (PFOA); perfluorononanoic acid (PFNA); perfluoroundecanoic acid (PFUnDA); perfluorohexane sulfonate (PFHxS); perfluorooctane sulfonate (PFOS).

Table S5. Odds ratios and 95% confidence intervals and interaction terms for child sex from logistic regression models predicting autism spectrum disorder from quartile categories of PFASs in a nested case–control study of autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

PFAS	ASD			
	All	Boys	Girls	Interaction term p
PFOA		Interaction term p = 0.01		
PFOA Q1 (ref.)	1.00	1.00	1.00	
PFOA Q2	1.71 (1.20, 2.45	1.89 (1.27, 2.80)	1.14 (0.51, 2.56)	0.27
PFOA Q3	1.25 (0.85, 1.83)	1.47 (0.97, 2.23)	0.56 (0.23, 1.39)	0.05
PFOA Q4	0.98 (0.65, 1.47)	0.88 (0.56, 1.38)	1.38 (0.62, 3.06)	0.32
PFOS		Interaction term p = 0.12		
PFOS Q1 (ref.)	1.00	1.00	1.00	
PFOS Q2	0.97 (0.69, 1.36)	0.90 (0.62, 1.31)	1.47 (0.63, 3.46)	0.30
PFOS Q3	0.63 (0.44, 0.91)	0.59 (0.40, 0.88)	0.94 (0.39, 2.26)	0.35
PFOS Q4	0.76 (0.52, 1.12)	0.62 (0.40, 0.95)	1.80 (0.79, 4.11)	0.02
PFHxS		Interaction term p = 0.32		
PFHxS Q1 (ref.)	1.00	1.00	1.00	
PFHxS Q2	0.88 (0.63, 1.24)	0.76 (0.52, 1.11)	1.80 (0.79, 4.12)	0.06
PFHxS Q3	0.74 (0.52, 1.05)	0.67 (0.45, 0.98)	1.23 (0.52, 2.92)	0.19
PFHxS Q4	0.69 (0.48, 1.00)	0.64 (0.43, 0.96)	1.06 (0.46, 2.46)	0.28
PFHpS		Interaction term p = 0.29		
PFHpS Q1 (ref.)	1.00	1.00	1.00	
PFHpS Q2	0.85 (0.61, 1.20)	0.84 (0.58, 1.23)	0.93 (0.39, 2.24)	0.84
PFHpS Q3	0.84 (0.59, 1.20)	0.73 (0.49, 1.08)	1.53 (0.69, 3.40)	0.09
PFHpS Q4	0.71 (0.49, 1.03)	0.65 (0.43, 0.98)	1.10 (0.47, 2.56)	0.26

Note: Logistic regression with multiple imputation with additional interaction analyses. All metals/elements were normalized according to batch. Each regression model was adjusted for maternal age, education, parity, seafood intake, child sex, and child birth year. Interaction term was tested with Wald's test. Abbreviations: Autism spectrum disorder (ASD); perfluorooctanoic acid (PFOA); perfluorohexane sulfonate (PFHxS); perfluoroheptanesulfonic acid (PFHpS); perfluorooctane sulfonate (PFOS).

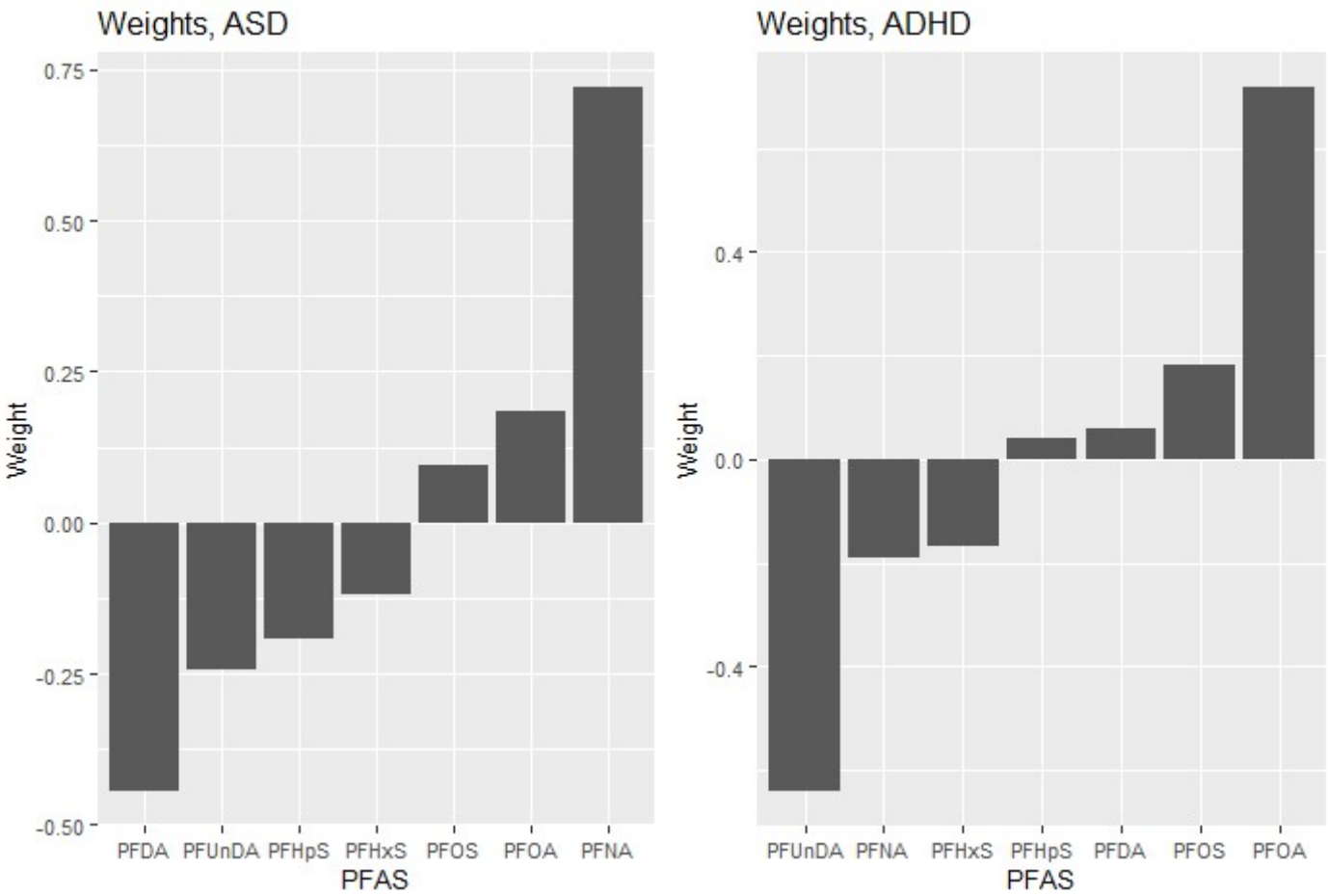

Figure S5. Quantile-based g-computation approach of PFASs on attention-deficit/hyperactivity disorder and autism spectrum disorder in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009 (n=1380 and n=1801).

Note: PFASs were log transformed and missing data imputed. The model was adjusted for maternal age, education, parity, seafood intake, child sex, and child birth year. For child ADHD, maternal ADHD symptoms was also included as a covariate. Abbreviations: Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); perfluorooctanoic acid (PFOA); perfluorononanoic acid (PFNA); perfluorodecanoic acid (PFDA); perfluoroundecanoic acid (PFUnDA); perfluorohexane sulfonate (PFHxS); perfluoroheptanesulfonic acid (PFHpS); perfluorooctane sulfonate (PFOS).

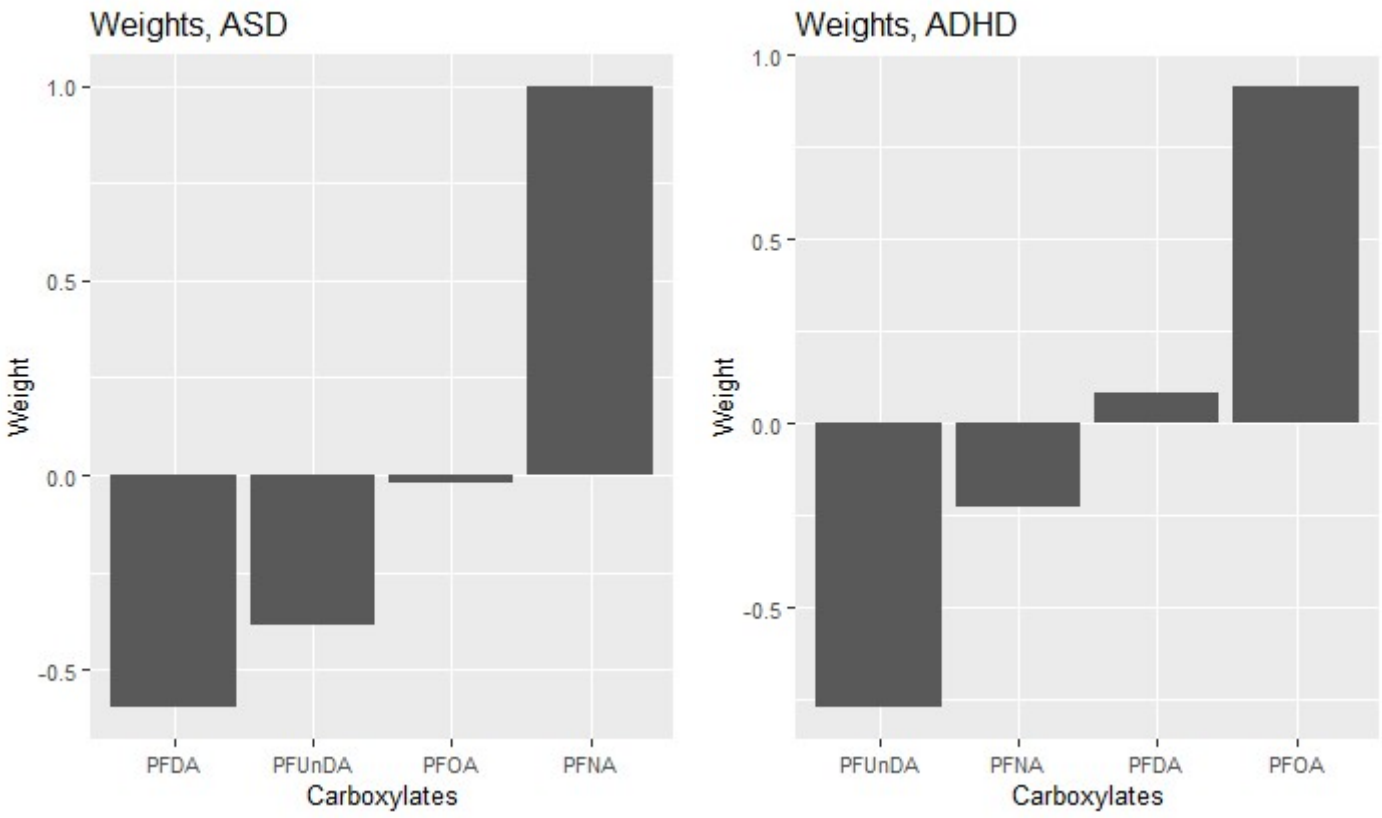

Figure S6. Quantile-based g-computation approach of carboxylates on attention-deficit/hyperactivity disorder and autism spectrum disorder in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009 (n=1380 and n=1801).

Note: PFASs were log transformed and missing data imputed. The model was adjusted for maternal age, education, parity, seafood intake, child sex and child birth year. For child ADHD, maternal ADHD symptoms was also included as a covariate. Abbreviations: Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); perfluorononanoic acid (PFNA); perfluorodecanoic acid (PFDA); perfluoroundecanoic acid (PFUnDA).

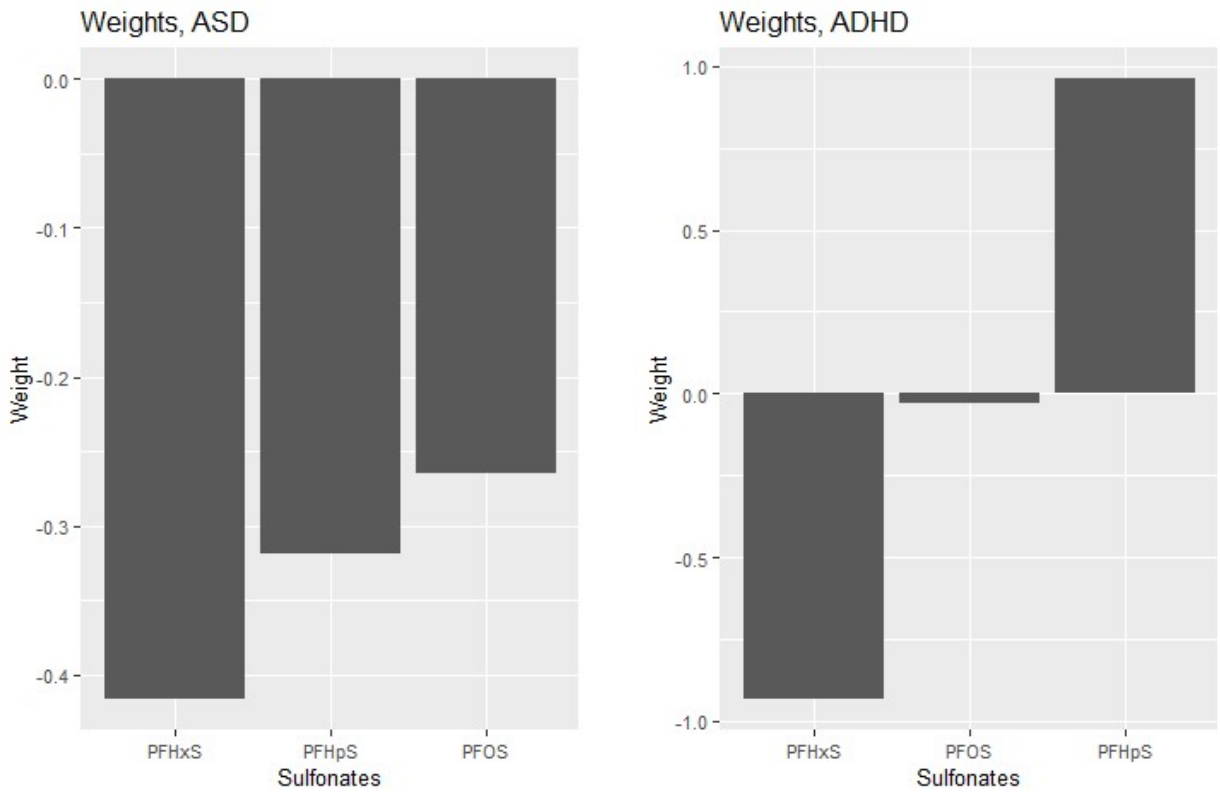

Figure S7. Quantile-based g-computation approach of sulfonates on attention-deficit/hyperactivity disorder and autism spectrum disorder in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009 (n=1380 and n=1801).

Note: PFASs were log transformed and missing data imputed. The model was adjusted for maternal age, education, parity, seafood intake, child sex and child birth year. For child ADHD, maternal ADHD symptoms was also included as a covariate. Abbreviations: Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); perfluorooctanoic acid (PFOA); perfluorohexane sulfonate (PFHxS); perfluoroheptanesulfonic acid (PFHpS); perfluorooctane sulfonate (PFOS).

Appendix I.

Overview of PFASs analysed in maternal plasma from gestation week 18 in a nested case–control study of attention-deficit hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2002–2009[1].

PFAS compound, chain length (number of fluorinated carbons)	Acronym	LOD (ng/mL)	LOQ (ng/mL)
Perfluoriated carboxylic acids	PFCAs		
Perfluorobutanoate, C_4 (C_3)	PFBA	0.003	0.10
Perfluoropentanoate, C_5 (C_4)	PFPeA	0.008	0.05
Perfluorohexanoate, C_6 (C_5)	PFHxA	0.007	0.05
Perfluoroheptanoate, C_7 (C_6)	PFHpA	0.009	0.05
Perfluorooctanoate C_8 (C_7)	**PFOA**	**0.006**	**0.05**
Perfluorononanoate, C_9 (C_8)	**PFNA**	**0.004**	**0.05**
Perfluorodecanoate, C_{10} (C_9)	**PFDA**	**0.002**	**0.05**
Perfluoroundecanoate, C_{11} (C_{10})	**PFUnDA**	**0.006**	**0.05**
Perfluorododecanoate, C_{12} (C_{11})	PFDoDa	0.007	0.05
Perfluorotridecanoate, C_{13} (C_{12})	PFTrDa	0.004	0.05
Perfluorotetradecanoat, C_{14} (C_{13})	PFTeDa	0.050	0.20
Perfluorinated sulfonic acids	PFSAs		
Perfluorobutane sulfonate, C_4 (C_4)	PFBS	0.009	0.05
Perfluorohexane sulfonate, C_6 (C_6)	**PFHxS**	**0.007**	**0.05**
Perfluoroheptane sulfonate, C_7 (C_7)	**PFHpS**	**0.010**	**0.05**
Perfluorooctane sulfonate, C_8 (C_8)	**PFOS**	**0.003**	**0.05**
Perfluorodecane sulfonate, C_{10} (C_{10})	PFDS	0.050	0.20
Others			
Perfluorooctane sulfonamide, C_8 (C_8)	PFOSA	0.020	0.05
N-Methylperfluorooctane sulfonamide	MeFOSA	0.009	0.05
N-Ethylperfluorooctane sulfonamide	EtFOSA	0.009	0.05

Abbreviations: perfluoroalkyl substances, PFAS: LOD: limit of detection, LOQ: limit of quantification: nanogram per milliliter (ng/mL). Bald letters denote PFAS results above LOQ in more than 80% of the samples and used in the present thesis.

[1] Haug, L.S., Thomsen, C., & Becher, G., 2009. A sensitive method for determination of a broad range of perfluorinated compounds in serum suitable for large-scale human biomonitoring. J Chromatogr A. 1216 (3), 385-393.

Appendix II. Analytical procedure for determination of metal and element concentrations in maternal blood (for paper II)

Maternal whole blood sampled in MoBa at week 18 of pregnancy in 3 mL trace free sampling tubes and transported to the biobank at the Norwegian Institute of Public Health for storage at – 80°C. Before analyses, the blood was thawed and 500 µl was aliquoted in matrix tubes (8 x 16) and re-frozen. The samples were shipped frozen on dry-ice to the respective analytical laboratories.

The majority of the maternal blood samples (n=1847) were analyzed by ALS Laboratory group, at the ALS laboratory in Luleå, Sweden in 2015-2016. In addition to the maternal blood samples, five reference samples consisting of standard reference material (Seronorm Urine L-1: Sero AS, Billingstad, Norway) were randomly placed within the sample batches, blinded to the analyst.

The analytical method at ALS laboratory group is accredited according to the standard EN ISO 17294-2:2016. The analyses included the following toxic or non-essential metals/elements: Lead (Pb), total Mercury (Hg), Cadmium (Cd), Thallium (Tl), total Arsenic (As), and Cesium (Cs), and as well as the following essential metals/elements: Cobolt (Co), Magnesium (Mg), Copper (Cu), Selenium (Se), Manganese (Mg), and Zink (Zn). The method have been described previously. Briefly, it included microwave-assisted sample decomposition followed by inductively coupled plasma-sector field mass spectrometry (ICP-SFMS). Sample blanks (200 µl of high purity water), control samples (Seronorm Urine L-2: Sero AS, Billingstad, Norway) and calibration standards were subjected to the same handling as samples, including the addition of internal standards. The concentration of metals/elements in blood and blind samples, blanks and laboratory controls were calculated based on calibration curves. Limit of quantifications (LOQ) was calculated as ten times the standard deviation of the blank samples, whereas limit of detection (LOD) was approximately one third of the LOQ. The LOD and LOQ for the various elements are presented in the table below. All reported concentrations in this study were above LOQ, except for As, Hg, Cd and Pb where concentrations above LOD were reported.

Results of maternal whole blood concentrations of metals and essential elements for some of our participants (n=179) were provided by The Norwegian Environmental Biobank, a sub-study of MoBa. These samples were analyzed in 2015-2016 at the Department of Occupational and Environmental Medicine at Lund University, Sweden, using comparable

analytical methods as the ALS Laboratory group, however, these analyses did not include Mg or Cs. All metals and elements, except Total Hg, were analysed using ICP-MS (iCap Q Thermo Fisher Scientific, Bremen, GmbH) according to previous method descriptions. Total Hg was determined by cold vapor fluorescence spectrometry after acid-digestion of samples. The LOD and LOQ of the metals/elements measured by Lund University is given in Caspersen et al., 2019[2].

Certified reference material (Seronorm Trace Elements whole blood L-1, SERO AS, Billingstad, Norway) were used to ensure the analytical accuracy at both laboratories. The intra-laboratory coefficients of variation were within acceptable ranges for ALS Laboratory group (see table below) and Lund University. An overlap of 110 participants registered with both an ASD and an ADHD diagnosis, explains why the total number of analyzed blood samples are 2026 and not 2136.

Limit of detection (LOD) and quantification (LOQ), and quality control data for the analyses of metals and elements in whole blood at ALS Laboratory group. Concentrations are given in microgram per liter (µg/L) for all metals/elements, except for Mg, which is given in milligram per liter (mg/L).

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			Reference samples [a,b]		
Metal/ element	**~LOD**	**LOQ**	**Measured Mean±SD (RSD)**	**Target Mean±SD**	**Mean deviation from target**
As	0.3	1.0	2.6±0.5 (18%)	2.4±0.5	6.6%
Pb	0.15	0.5	9.6±0.6 (7%)	10.2±2.1	6.2%
Cd	0.02	0.05	0.34±0.05 (14%)	0.36 ± 0.02	5.7%
Co	0.02	0.05	0.19±0.04 (18%)	0.16±0.03	17.9%
Cu	0.3	1.0	625±46.9 (8%)	680±140	8.0%
Hg	0.06	0.2	1.7±0.2 (10%)	1.5±0.3	14.7%
Mn	0.02	0.5	20.8±3.4 (17%)	20.7±4.2	0.3%
Se	1.7	5.0	59.9±4.4 (7%)	59.0±12.0	1.6%
Zn	3.3	10	4016±199 (5%)	4400±200	8.7%
Cs	0.02	0.05	2.4±0.1 (5%)	2.5±0.03	4.0%
Mg	0.06	0.2	14.0± (7%)	16.2±1,6	13.6%
Mo	0.06	0.2	1.2±0.3 (24%)	0.94±0.22	26%

[a] n = 5 blind samples, [b] Seronorm.

Abbreviations: Arsenic (As); cadmium (Cd); cesium (Cs); cobalt (Co); copper (Cu); lead (Pb); limit of detection (LOD); limit of quantification (LOQ); magnesium (Mg); manganese (Mn); mercury (Hg); selenium (Se); standard deviations (SD); zinc (Zn); relative standard deviation (RSD).

[2] Caspersen, I. H., Thomsen, C., Haug, L. S., Knutsen, H. K., Brantsæter, A. L., Papadopoulou, E., ... & Meltzer, H. M. (2019). Patterns and dietary determinants of essential and toxic elements in blood measured in mid-pregnancy: The Norwegian Environmental Biobank. Science of the Total Environment, 671, 299-308.

Appendix III. Normalization of the metal/element concentrations across analytical rounds

To normalize measured blood concentrations of metals and elements across analytical rounds, similar approach with scaled variation of the Ratio-G batch adjustment as described in Luo et al. (2010)[3] was used. Suppose M represents the measured concentrations of metal/element i for each participant j. M*ij represents the analytical round-adjusted metal/element concentration, which is calculated using the following equation (Equation 1):

$$M^*ij = Mij \times (meanQCl/meanQClk), \quad (1)$$

where meanQCl represents the geometric mean of metal/element i in reference samples (Seronorm L-1) across all analytical rounds (5 reference samples x 4 rounds), and meanQClk represents the geometric mean of metal/element i in reference samples from analytical round k (i.e. in the analytical round in which sample of the participant j was measured).

[3] Luo, J., Schumacher, M., Scherer, A., Sanoudou, D., Megherbi, D., Davison, T., ... & Zhang, J. (2010). A comparison of batch effect removal methods for enhancement of prediction performance using MAQC-II microarray gene expression data. The pharmacogenomics journal, 10(4), 278-291.