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

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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 effects of the metal/element mixture on ASD and ADHD diagnoses were 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 and 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.

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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., 2017).

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 to 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 (Fig. 1). The total number of cases and controls in the present study was 705 ADHD cases, 397 ASD cases, and 1034 controls (Fig. 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 and Cooper, 2016). In the present study, we use the term ADHD.

For ASD, we selected cases if they had 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 (Fig. 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.

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

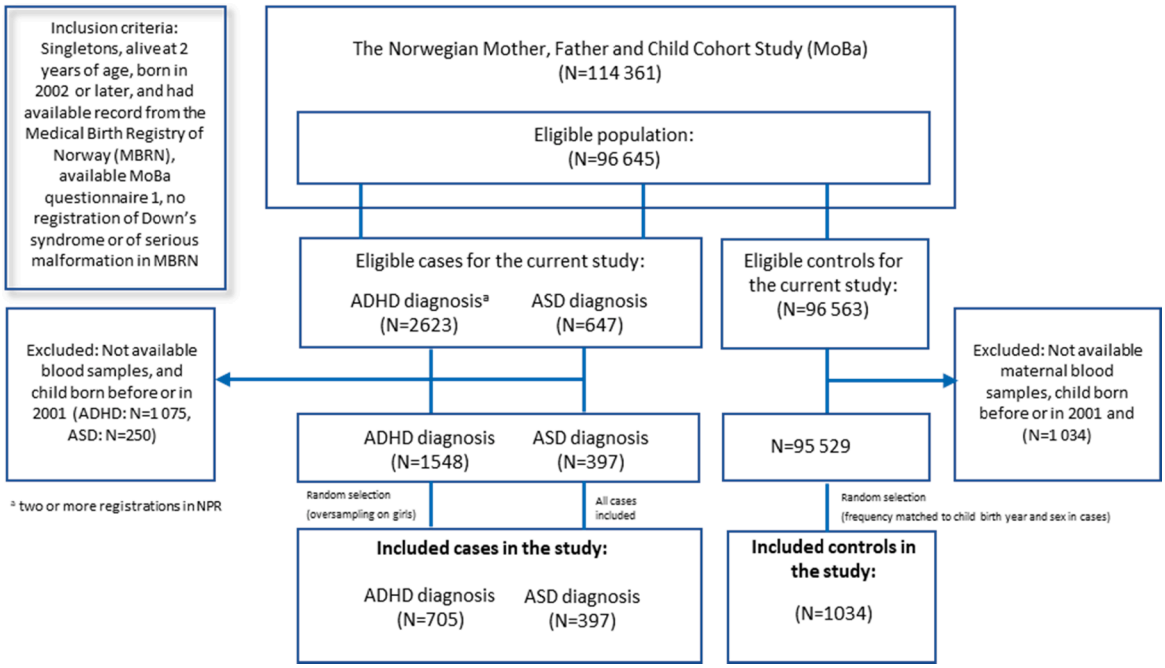

Fig. 1. Flow chart of recruitment of cases and controls in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009. Abbreviations: Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); The Medical Birth Registry of Norway (MBRN); The Norwegian Mother, Father and Child Cohort Study (MoBa); The Norwegian Patient Registry (NPR).

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 (Eq. (1)):

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

where $meanQC_l$ represents the geometric mean of metal/element i in reference samples across all analytical rounds (5 reference samples × 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/day), 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., 2005), were also included as a covariate in analyses with child ADHD as outcome.

2.5. Statistical analysis

Metal and element concentrations were natural log-transformed to approximate normal distributions. 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 $100(1\text{-}\alpha)^{1/k}$ % 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 10th, 50th, and 90th 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 1st percentile and greater or equal to the 99th percentile) by the values above or below the 1st and 99th 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 and Buckley, 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 and Buckley, 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 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.

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).

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 (Fig. 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)] (Fig. 2, Table S3). We further identified negative associations with ASD (lowered risk) in some quartiles of cesium, copper, and zinc (Fig. 2, Table S3). For mercury, all three quartiles had significantly lowered risk of ASD compared to quartile 1 (Fig. 2, Table S3). The associations with arsenic, mercury, and manganese remained with 99.8% CIs, while the one with cadmium did not remain.

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, (Fig. 3). The association between arsenic and ASD showed an inverse U-shape (Fig. 3). For lead and magnesium, the splines were U-shaped (Fig. 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 (Fig. 3).

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 (Fig. 4, Table S3). This relationship was significantly modified by maternal education in quartile 3 only (p interaction = 0.02) with 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).

For children in the highest quartile of magnesium there was an elevated risk of ADHD [OR = 1.42 (CI: 1.06, 1.91)] (Fig. 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

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 (years)	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 (g/day)	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)
Other (single, divorced, widow)	29 (2.8)	56 (7.9)	20 (5.04)
Missing (*n*)	0	0	0
Child birth weight (grams)	3649 ± 519	3586 ± 638	3594 ± 670
Missing (*n*)	0	1	0
Length of gestation (weeks)	39.5 ± 1.67	39.3 ± 2.11	39.37 ± 2.25
Missing (*n*)	3	5	1
Maternal total folate intake (μg/day)	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); grams (g); micrograms (μg); Norwegian patient registry (NPR); standard deviation (SD).

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
	ASD case	393	99.0	2.12 (2.05, 2.19)	0.68	1.69	2.17	2.65	9.25
Cu	Control	1034	100	1562 (1548, 1577)	778	1426	1551	1737	3178
	ADHD case	705	100	1568 (1548, 1589)	774	1401	1573	1737	3629
	ASD case	397	100	1584 (1558, 1610)	901	1425	1584	1742	3583
Hg	Control	1034	100	1.39 (1.34, 1.45)	0.18	0.96	1.44	2.12	10.0
	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
Mg	Control	934	90.3	30.1 (29.9, 30.4)	18.5	28.0	30.3	32.6	45.0
	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
Mn	Control	1034	100	10.2 (9.97, 10.5)	3.38	8.04	9.91	12.30	164
	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
Pb	Control	1034	100	8.82 (8.60, 9.05)	1.96	6.61	8.68	11.21	82.4
	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
Se	Control	1034	100	93.04 (91.9, 94.2)	47.1	81.7	92.3	105	312
	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
Zn	Control	1034	100	5202 (5139, 5266)	1972	4643	5269	5896	9931
	ADHD case	705	100	5051 (5217)	1189	4495	5237	5875	11,186
	ASD case	397	100	4966 (4850, 5085)	1189	4393	5170	5707	10,743

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).

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).

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 (Fig. 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 (Fig. 5). The associations between ADHD and arsenic, copper, and manganese were slightly U-shaped (Fig. 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 (Fig. 5).

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

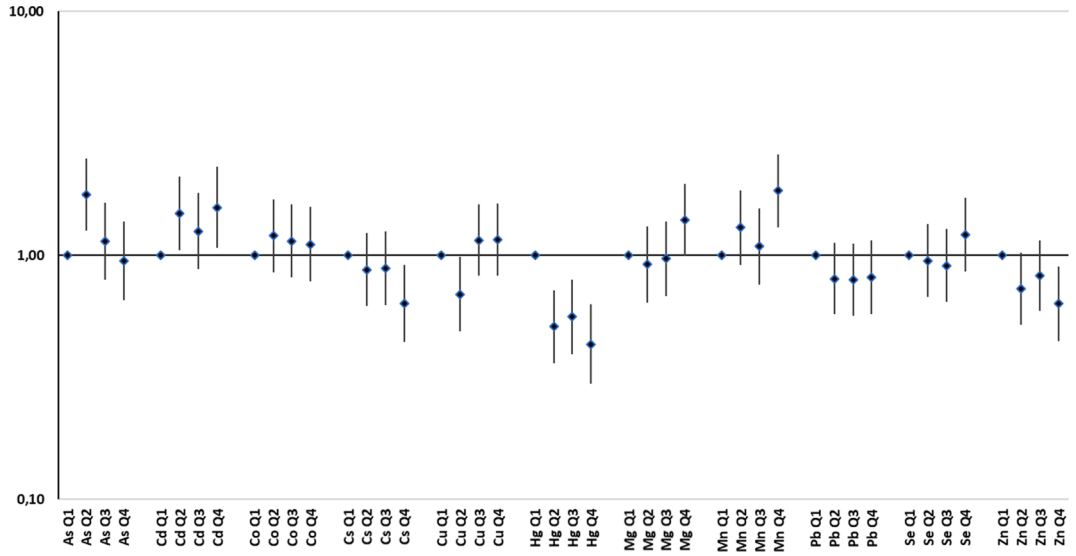

Fig. 2. Odds ratios and 95% confidence intervals of logistic regression models predicting autism spectrum disorder in quartile categories of gestational metal/essential element levels in a nested case–control study of autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009 (n = 1431). Note: Logistic regression with multiple imputed datasets (n = 50). All metal/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).

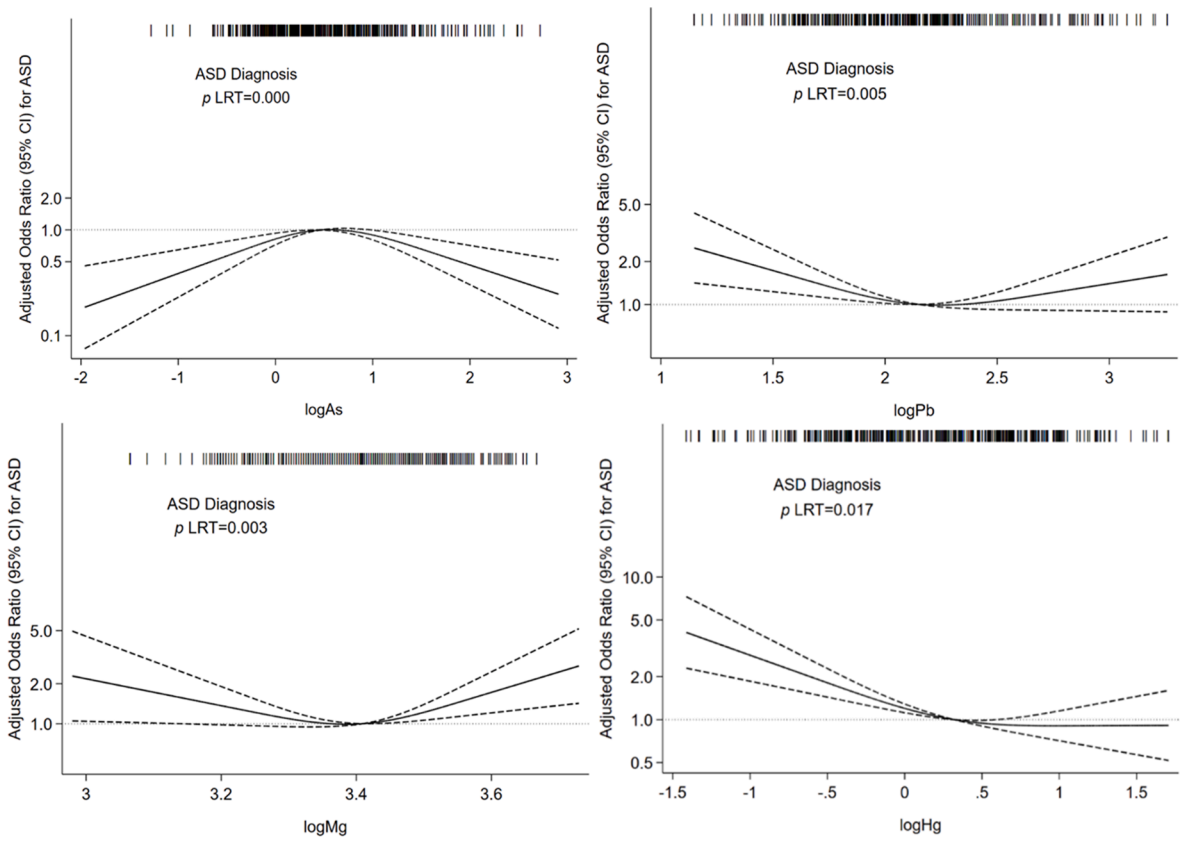

Fig. 3. Restricted cubic spline predicting the odds of autism spectrum disorder in children associated with gestational levels of arsenic, lead, magnesium, and mercury in a nested case–control study of autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009 (n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

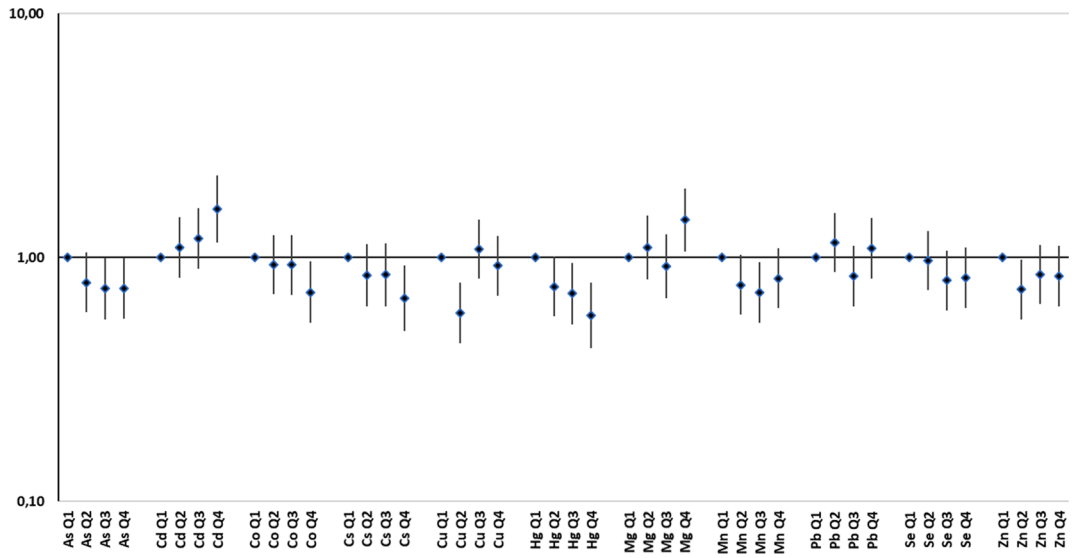

Fig. 4. Odds ratios and 95% confidence intervals of logistic regression models predicting attention-deficit/hyperactivity disorder in quartile categories of gestational metal/essential element levels in a nested case–control study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009 (n = 1739). Note: Logistic regression with multiple imputed datasets (n = 50). All metal/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).

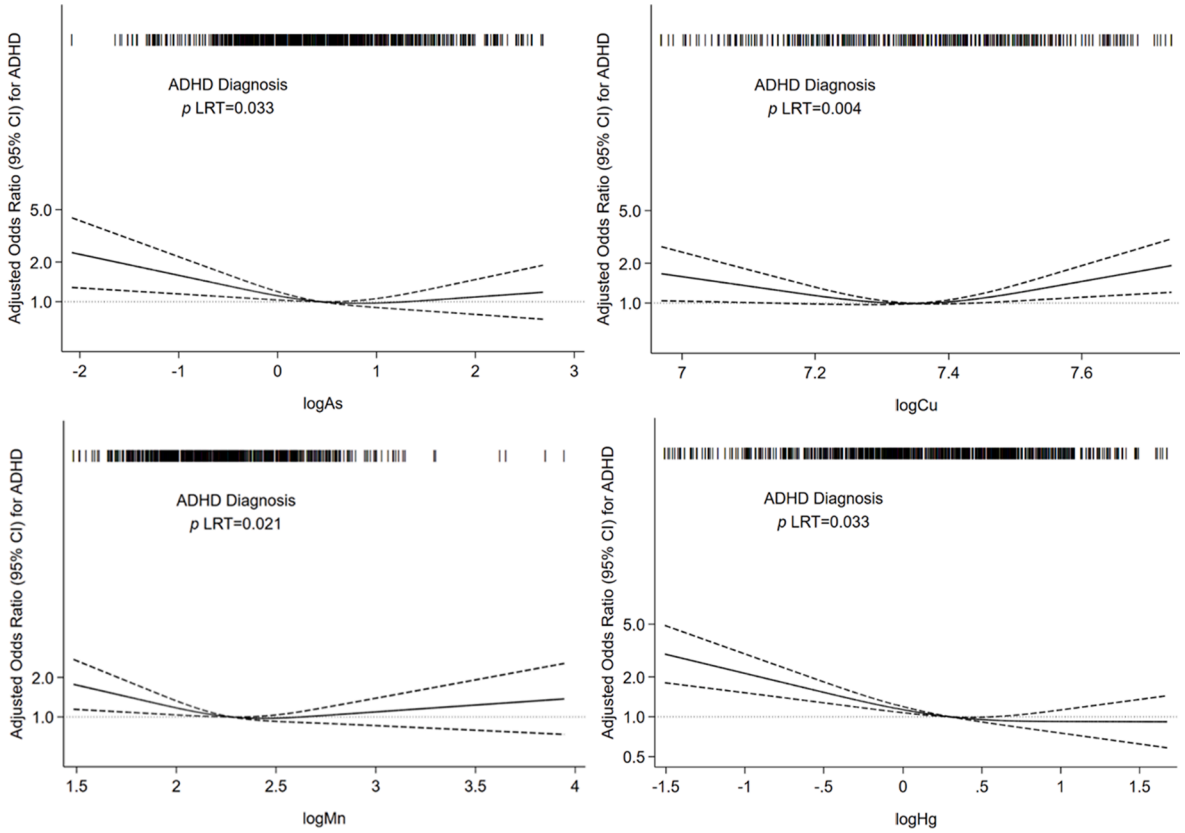

Fig. 5. Restricted cubic spline predicting the odds of attention-deficit/hyperactivity disorder in children associated with gestational levels of arsenic, copper, manganese, and mercury in a nested case–control study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child 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).

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; 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 significant 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 significant 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 significant 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, 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, 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.

CRediT authorship contribution statement

Thea S. Skogheim: Conceptualization, Methodology, Formal analysis, Writing - original draft, Visualization. **Kjell Vegard F. Weyde:**

Methodology, Writing - review & editing. **Stephanie M. Engel:** Conceptualization, Methodology, Writing - review & editing. **Heidi Aase:** Conceptualization, Writing - review & editing, Project administration, Funding acquisition. **Pål Surén:** Conceptualization, Writing - review & editing, Supervision. **Merete G. Øie:** Writing - review & editing, Supervision. **Guido Biele:** Conceptualization, Writing - review & editing. **Ted Reichborn-Kjennerud:** Resources, Writing - review & editing. **Ida H. Caspersen:** Writing - review & editing. **Mady Hornig:** Writing - review & editing. **Line S. Haug:** Resources, Writing - review & editing. **Gro D. Villanger:** Conceptualization, Methodology, Writing - review & editing, Supervision.

Declaration of Competing 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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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106468.

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