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Association of maternal prenatal urinary fluoride levels with ADHD symptoms in childhood.

Jesús Ibarluzea[a,b,c,d,1], Mikel Subiza-Pérez[b,c,d,e,1], Ane Arregi[b,d,*], Amaia Molinuevo[d], Enrique Arranz-Freijo[b,d], Manuel Sánchez-de Miguel[b,d], Ana Jiménez[a,c,d], Ainara Andiarena[b,d], Loreto Santa-Marina[a,c,d], Aitana Lertxundi[c,d,f]

[a] *Ministry of Health of the Basque Government, Sub-Directorate for Public Health and Addictions of Gipuzkoa, 20013, San Sebastián, Spain*
[b] *Faculty of Psychology of the University of the Basque Country (UPV-EHU), 20018, San Sebastian, Spain*
[c] *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), 28029, Madrid, Spain*
[d] *Biodonostia Health Research Institute, Environmental Epidemiology and Child Development Group, 20014, San Sebastian, Spain*
[e] *Bradford Institute for Health Research, Temple Bank House, Bradford Royal Infirmary, BD9 6RJ, Bradford, United Kingdom*
[f] *Faculty of Medicine and Nursery of the University of the Basque Country (UPV/EHU), 48940, Leioa, Spain*

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ABSTRACT

Background: Health concerns about the potential impact of exposure to fluoride via drinking water (DW) on neuropsychological development include behavioral outcomes such as ADHD.

Objective: We aimed to examine the association between prenatal maternal urinary fluoride and symptoms associated with attention-deficit/hyperactivity disorder (ADHD) at the age of 8 and 11 years.

Method: Data from 255 to 236 mother-child pairs from the "Infancia y Medio Ambiente" (INMA) birth cohort (Gipuzkoa; Spain) with maternal urinary F adjusted for creatinine (MUFcr) during pregnancy (first and third trimester) and child assessments of ADHD-like symptoms reported by Conners' Rating Scales-Revised at age of 8 and 11 years was available. Clinical approach was also used: cut off criteria ($T > 66$). Multiple linear regression models were fitted when outcomes were analyzed as continuous, and logistic regression models when the outcomes were analyzed with a categorical clinical approach. Covariates related to maternal characteristics, birth outcomes, childhood, quality of family context and biomarkers of neuro-toxicants were used.

Results: No association was found between MUFcr levels during pregnancy and cognitive problems-inattention, hyperactivity or ADHD index score of symptoms at 8 or 11 years. When results were analyzed from the perspective of a clinical approach, at the age of 11 years, there were significant inverse association between MUFcr and being categorized as a cognitive problems-inattention case. ORs were also indicative of a lower risk, although not significant, for ADHD index at age 11. Sensitivity analyses, taking into consideration quality of family context or the levels of other toxicants during pregnancy showed similar results.

Conclusions: Higher levels of MUFcr in pregnant women were associated with a lower risk of cognitive problems-inattention at 11 years. These findings are inconsistent with those from previous studies and indicate the need for other population-based studies to confirm or overturn these results.

Abbreviations: ADHD, attention-deficit and/or hyperactivity disorder; BW, bottled water; CRS-R, Conners' Rating Scale– Revised Short Form; MUFcr, maternal urinary fluoride adjusted by creatinine; DW, drinking water; CFDW, community fluoridated drinking water; CNFDW, community non-fluoridated drinking water; HOME, Home Observation for Measurement of the Environment; L, liter; dL, deciliter; mg, milligram; μg, microgram; M, Mean; SD, Standard Deviation; SE, Standard Error; USDA, U.S Department of Agriculture.

* Corresponding author. Biodonostia, Environmental Epidemiology and Child Development Group, 20014, San Sebastian, Spain.
E-mail address: ane.arregi@ehu.eus (A. Arregi).

[1] These authors contributed equally to this work and should be listed as the first authors.

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1. Introduction

1.1. The fluorination of drinking water

The addition of fluoride (F) to drinking water (DW) to generate community fluoridated drinking water (CFDW) has been used for decades to prevent dental caries. Although CFDW has been considered among the top-ten public health achievements of the 20th century, specifically for children from low social class (Jones et al., 2005; Khan et al., 2015), there is little evidence based on high quality studies to get a clear picture about the effectiveness of water fluoridation for the prevention of caries and about the reduction in disparities in caries levels across socioeconomic status (Iheozor-Ejiofor et al., 2015). Beside this debate, many scientific health authorities have endorsed the use of other applications of F (e.g. toothpastes, gels, and varnishes) due to the benefitial effect of its topical application, as well as safety and cost-effectiveness notions (NHS - National Health System, 2021; Veneri et al., 2023). It is well known that low F concentrations are present naturally in water in many geographies. However, raw waters in countries such as India, China, Iran or Mexico exceed the WHO recommendations (Grandjean, 2019). The main source of F in communities that fluoridate DW comes from the ingestion of CFDW or beverages prepared with this type of water -approximately 75% of F intake in the U.S. (U.S. Environmental Protection Agency, 2010; U.S. Department of Agriculture (USDA), 2005). Other sources of F are cooking salt, dental products, including toothpastes, mouth rinses, varnishes, supplements or processed foods prepared with fluoridated water (National Research Council, 2006). Drinking tea can also be a relevant source in countries where its ingestion is high (Waugh et al., 2017).

Given that oral hygiene has greatly improved in western countries in the last decades, the decision to maintain CFDW is nowadays controversial among public health practitioners, researchers, health professionals and the general population (Broadbent et al., 2015; Choi et al., 2012). Still in the dental health domain, a reason for concern is the association between high levels of this halogen and dental and skeletal fluorosis (Bronckers et al., 2009; Indermitte et al., 2009). It is well known that F crosses human placenta (Adinolfi, 1985) and accumulates in fetal brain tissues (Du et al., 2008; Narayanaswamy and Piler, 2010), thereby inducing neurotoxicity (Chen et al., 2018; Dong et al., 1993; Jiang et al., 2016; McPherson et al., 2018; Mullenix et al., 1995). Therefore, the most relevant health-related preoccupation nowadays is about the potential effects of F present in CFDW on neuropsychological development during childhood, like decrements in cognitive function, internalizing problems and learning problems (Wang et al., 2022; Barberio et al., 2017).

1.2. Epidemiological studies on F and neurodevelopment

Epidemiological studies have explored associations between early-life exposure to fluoride and decrements in cognitive function (i.e. IQ indexes). Some of these studies have detected such effects in areas where raw waters present high F levels (Bashash et al., 2017; Choi et al., 2012; Grandjean, 2019). However, when it comes to CFDW studies are not consistent and show toxic (Green et al., 2019; Valdez-Jiménez et al., 2017), null (B Broadbent et al., 2015; Aggeborn and Oehman, 2021) or even protective effects (Ibarluzea et al., 2022).

Attention-deficit and/or hyperactivity disorder (ADHD) is the most common neurodevelopmental disorder in school-aged children and adolescents, with an estimated worldwide prevalence of 5% (Polanczyk et al., 2017; American Psychiatric Association, 2013). Apart from the role played by genetics in the susceptibility to ADHD, different environmental exposures have been considered as potential risk factors. This is the case of exposure to tobacco and alcohol during pregnancy (Polańska et al., 2012), exposure to lead and mercury (Huang et al., 2016; Lozano et al., 2021), and other chemical exposures such as organochlorines (Xu et al., *2023*) or air pollution (Fuertes et al., 2016; Perera et al., 2018; Sentís et al., 2017). Nutritional (Polańska et al., 2012; Lertxundi et al., 2021), family (e.g. quality of the family context (Barreto-Zarza et al., 2022); and socio-economic factors (Markham and Spencer, 2022; Russell et al., 2016) have also been linked to ADHD.

The question of whether exposure to F could have negative implications for ADHD diagnosis and symptomatology as well has been addressed in a reduced number of studies which have also obtained heterogeneous results. For instance, an ecological study conducted in the United States reported a negative correlation between prevalence of FCDW and ADHD prevalence in youths (Malin and Till, 2015). On the contrary, two cross-sectional studies failed to find significant associations between urinary fluoride (UF) and ADHD symptoms or diagnoses in Chinese (Wang et al., 2022) and Canadian (Riddell et al., 2019) children and adolescents. Nevertheless, while the former study did not provide information about the potential association with F levels in DW, this latter study did show that higher levels of fluoride in DW was associated with an increased risk of ADHD symptoms and diagnosis of ADHD, particularly among adolescents. Another study conducted in Canada showed that UF was not associated with ADHD symptoms in participants 6–17 years old but F in tap water was (Riddell et al., 2019). Finally, a prospective study carried out in the ELEMENT birth cohort study (Mexico), concluded that higher levels of MUF during pregnancy were associated with higher ADHD and hyperactivity-inattention symptoms in children at age 6–12 (Bashash et al., 2018).

These inconsistencies, the vulnerability to environmental exposures of the fetus and the fact that population is widely exposed to F, require to further inquiry on the potential neuropsycholoical effects on childhood and pre-adolescence (Farmus et al., 2021; Fiore et al., 2023). To this end, we prospectively assessed the relationship between prenatal exposure to fluoride and parent-reported symptoms associated with ADHD at 8- and 11-years-old children in the INMA (INfancia y Medio Ambiente -Environment and Chilhood-) cohort from Gipuzkoa.

2. Methods

2.1. Study population

This study was carried out with data from the INMA-Gipuzkoa cohort (from the Spanish for Environment and Childhood: *INfancia y Medio Ambiente*), a mother and child birth cohort (Guxens et al., 2012). Among the cohorts involved in INMA, only the Gipuzkoa cohort is exposed to an active CFDW program that affects to those treatment plants that serve to populations larger than 30,000 inhabitants and therefore to a remarkable proportion of participants (c. 30%). The recruitment of pregnant mothers took place during the first antenatal visit to the gynecologist in the public referral hospital of Zumarraga. Participating mothers received antenatal follow-up towards the end of the first trimester or early in the second trimester (mean ± standard deviation) (13.9 ± 1.5 weeks) and towards the end of the third trimester (32.8 ± 2.6 weeks). The inclusion criteria were: maternal age ≥16 years old, singleton pregnancy, recruitment during the first antenatal visit, pregnancy achieved without assisted reproduction techniques, planned to give birth in the referral hospital and no communication problems in Spanish or Basque (Guxens et al., 2012). Mothers and children have been followed-up in several times after delivery and for this study we will analyze data from the 8 and 11 years follow-ups. Mothers gave written informed consent for themselves and on behalf of their children after the Ethics Committee of Donostia Hospital (Gipuzkoa) approved the protocol. Children also provided written informed consent at the age of 11. The following criteria were established to select mother-child pairs for the analyses presented in this manuscript: 1) children with data on neuropsychological assessment at 8 or 11 year of age, and 2) mothers with data on maternal urinary F level adjusted for creatinine (MUFcr) at the first and third trimesters of pregnancy.

At the recruitment phase 638 pregnant women met the inclusion criteria and started their participation in the INMA study. Of the 612 children born, 397 (64.9%), had data on ADHD symptoms provided by parents at the age of 8 and 372 (60.8%) 372 (60.8%) at the age of 11. MUFcr was determined in all the mothers with urine samples available. In total, there were 393 maternal samples with data on MUFcr levels for both trimesters. Fig. 1 shows the flowchart describing this procedure.

2.2. *Fluoride in drinking water and type of drinking water consumed*

Data on Community Drinkign Water (CDW) source and the amount of water consumed was obtained through food and drink questionnaires, administered in the first and third trimesters of pregnancy (Vioque et al., 2013). The CFDW and community non-fluoridated drinking water systems (CNFDW) supplied, during pregnancy, water with F levels of 0.81 ± 0.15 mg/L (mean ± standard deviation) vs < 0.1 mg/L. If bottled water (BW) was participants' main choice for water consumption, information was also collected on the brand of bottled water (BW) consumed, but not about amount of water consumption. The study area covers the counties of Goierri and Urola in the province of Gipuzkoa (Basque Country) with a population of 89,000 inhabitants distributed in 25 municipalities. More detailed information can be found in Ibarluzea et al. (2022). In all, 15 municipalities are supplied with CFDW and 10 with CNFDW. The ten most consumed BW brands had low to moderate F levels that range from 0.07 to 0.48 mg/L.

Approximately half of the women lived in fluoridated municipalities. Depending on the age of children who underwent neuropsychological testing, pregnant women consumed CFDW (31.8–32.6%), CNFDW (40.0–37.1%) or BW (26.7–28.4%) respectively (Table 1). The percentage of pregnant women that used BW for cooking was around 1.0%.

2.3. *Urinary F measurements*

F measured in maternal urine spot samples was used as biomarker of prenatal exposure. F in urine has been considered a good biomarker for F levels as excretion is in equilibrium with F intake (Aylward et al., 2015; Bashash et al., 2017). Maternal urine samples from the first and third trimesters were aliquoted and stored in 10-mL glass vials at −20 °C. The analyses were carried out in the laboratory of the Institute of Agrochemistry and Food Technology (IATA-CSIC) in Valencia. The description of the analytical technique and quality control can be found elsewhere (Ibarluzea et al., 2022). The limit of quantification (LOQ), calculated as 10 times the standard deviation of the F concentration in 20 blanks, was found to be 0.0052 mg/L. MUF levels were adjusted for creatinine and reported as F mg/g creatinine (MUFcr). Urinary creatinine was determined at the Normative Public Health Laboratory of Bilbao, Basque Country, by the Jaffé method (compensated kinetic with target measurement).

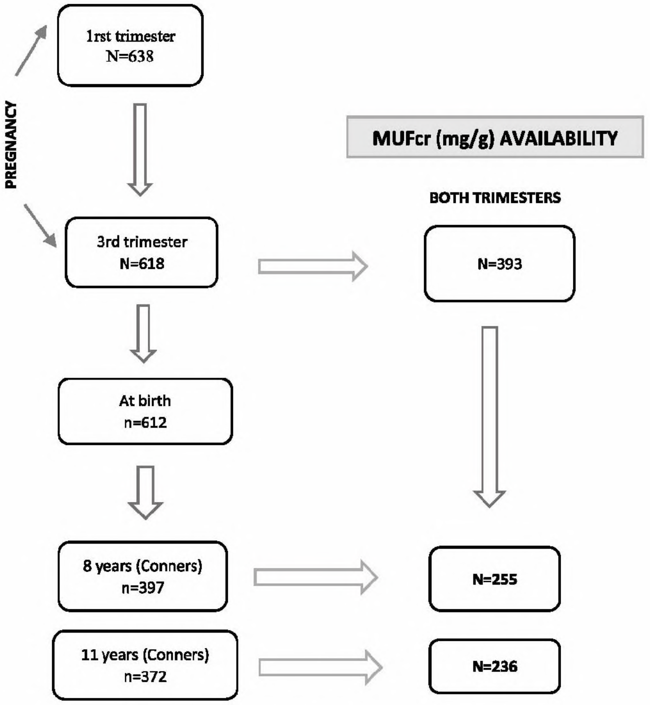

Fig. 1. Flowchart; mother-child pairs included in the study.

Table 1
Characteristics of the mother and child pairs at different follow-ups and MUFcr (mg/g).

	8 years (Conners') N = 255	11 years (Conners`) N = 236
Mother		
Maternal age	31.38 (3.31)	31.63 (3.27)
BMI		
Less than 18.5	7 (2.8%)	7 (3.0%)
18.5–25	197 (77.3%)	184 (78.0%)
25 - 30	40 (15.7%)	35 (14.8%)
More than 30	11 (4.3%)	10 (4.2%)
Social class		
No manual	152 (59.6%)	141 (59.8%)
Manual	103 (40.4%)	95 (40.3%)
Maternal IQ *	9.99 (2.75)	10.04 (2.75)
Smoking in pregnancy		
No	227 (89.0%)	212 (89.8%)
Yes	24 (9.4%)	21 (8.9%)
Zone		
Non fluoridated zone	134 (52.6%)	120 (50.9%)
Fluoridated zone	121 (47.5%)	116 (49.2%)
Type of drinking water		
Community fluoridated drinking water	81 (31.8%)	77 (32.6%)
Community non-fluoridated drinking water	102 (40.0%)	89 (37.7%)
Bottled Water	68 (26.7%)	67 (28.4%)
Parity		
0	153 (60.0%)	139 (58.9%)
>0	102 (40.0%)	97 (41.1%)
Child		
Order between brothers/sisters		
Not first	100 (39.2%)	95 (40.3%)
First	155 (60.8%)	141 (59.8%)
Age at the moment of the test*	7.76 (0.09)	10.72 (0.23)
Sex		
Female	127 (49.8%)	128 (54.2%)
Male	128 (50.2%)	108 (45.8%)
Daycare 1 year*		
No	125 (49.0%)	112 (47.5%)
Yes	114 (44.7%)	110 (46.6%)
Preterm		
No	246 (96.5%)	229 (97.0%)
Yes	7 (2.8%)	6 (2.5%)
Small for gestational age		
No	230 (90.2%)	214 (90.7%)
Yes	20 (7.8%)	19 (8.1%)
Breastfeeding weeks*	28.53 (19.73)	28.48 (19.85)
MUFcr (mg/g) pregnancy		
Period of pregnancy		
Whole pregnancy (mean value)	0.62 (0.58, 0.67)	0.64 (0.60, 0.69)]
First trimester	0.54 (0.49, 0.59)	0.55 (0.50, 0.61)
Third trimester	0.71 (0.65, 0.77)	0.74 (0.67, 0.80)
Zone		
Non fluoridated zone	0.46 (0.42, 0.51)	0.45 (0.41, 0.50)
Fluoridated zone	0.81 (0.74, 0.87)	0.84 (0.77, 0.91)
Type of drinking water		
Community fluoridated drinking water	0.88 (0.79, 0.97)	0.93 (0.84, 1.03)
Community non-fluoridated drinking water	0.42 (0.37, 0.46)	0.42 (0.38, 0.47)
Bottled Water	0.63 (0.56, 0.70)	0.61 (0.55, 0.68)

Note:* Variables with number of missings more than 5%.

2.4. Measurement of attention and hyperactivity related outcomes

At 8 (7.8 ± 0.1) and 11 (10.8 ± 0.3) years of age, ADHD symptoms and probable diagnosis were assessed using the Conners' Parent Rating Scale-Revised: Short Form (CPRS-R:S). This test is formed of 4 subscales comprising 27 items, in which the items for the Cognitive problems/Inattention (CI), Hyperactivity-Impulsivity (HI), Oppositional (OP), and the ADHD Index (AD) are 6, 6, 6, and 12 respectively. Each item was rated on a four-point scale (0 = never or rarely, 1 = sometimes, 2 = often, or 3 = very often). ADHD index score ranges between 0 and 36, and the other subscales range between 0 and 18. The Spanish version of the Conners' Rating Scales-Revised (CRS-R:S-27; Conners, 1997; Gianarris et al., 2001) was validated in a previous study (Ortiz-Luna and Acle-Tomasini, 2006). The Conners' ADHD Index shows favorable specificity and sensitivity in ADHD assessment (Chang et al., 2016). The crude scores of the test were transformed into sex and age specific T scores (mean of 50, SD of 10). Higher T-scores indicate greater symptomatology. Conners' subscales were analyzed as a continuous variables (T score of each subscale) and as a dichotomous variable of ADHD symptom diagnostic criteria, using T score >66 as cut off point (Conners, 1997).

2.5. Covariates

Data on maternal sociodemographic characteristics were gathered using questionnaires completed during the first and third trimester of pregnancy. These included: age of the mother, maternal social class (based on occupation, derived from the longest-held occupation reported during pregnancy or for mothers not working during their pregnancy, their most recent occupation, regrouped into two categories: non-manual workers [I + II + III for managers, technicians, associate professionals and other non-manual workers], and manual workers [IV + V for skilled, semi-skilled and unskilled manual workers] (Domingo-Salvany et al., 2013), educational level (secondary or less, university), country of birth (Spain, another country), body mass index (BMI), parity (0, ≥1), smoking during pregnancy (yes/no, reported at the at first and third trimesters of pregnancy) and alcohol consumption (no or occasional, at least one unit per week). Diet information estimated through a food and drink questionnaire (Vioque et al., 2013) included DW consumption, source -tap or brands of bottled water-, and the amount of tap water consumed. Breastfeeding and its duration in weeks were included in the questionnaire of the follow up of children at age of 15 months. Furthermore, said follow-up questionnaire contained the Similarities subtest of the Wechsler Adult Intelligence-Third Edition (WAIS-III:Wechsler and Kaufman, 2001), which was used as a proxy for maternal IQ, given that it has been shown to be a good predictor of global IQ (Wechsler, 1997).

In addition, we also collected information from children regarding: sex, birth order, premature birth (<37 weeks of pregnancy; World Health Organization, 2015), small for gestational age (birth weight below 10th percentile for gestational age and sex considering national reference values (Carrascosa et al., 2004) and whether they attended a daycare center before 2 years of age (Aranbarri et al., 2023). All questionnaires were administered face-to-face by trained interviewers.

Family context was measured with the Haezi-Etxadi Scale (HES; Arranz et al., 2014; Barreto-Zarza et al., 2022) when the participants were 4 years of age, This tool assesses the quality of family context for children's cognitive development in and is based on the Home Observation for Measurement of the Environment Inventory (Bradley, 2009; Caldwell and Bradley, 1984) and the developmental history interview proposed by Pettit et al. (1997). It also incorporates new variables gathered by observational procedures related for example with the socio-emotional quality of the interactions.

Additional neurotoxic substances detected in maternal urine samples during pregnancy were included: total As (μg/g) and manganese (Mn, μg/g), both adjusted for creatinine, and Hg in umbilical cord blood (μg/l). Pb was included in the analyses although the samples with levels above the limit of quantification (2 μg/dl) were low (5.9–7.4%, depending on the wave). The analytical methods used to analyze these metals/metaloids in urine/blood samples have been described elsewhere: Hg (Ramon et al., 2011), Pb (Llop et al., 2011), As (Lozano et al., 2021) and Mn (Soler-Blasco et al., 2020).

2.6. Statistical analysis

The study sample was described in terms of the characteristics of mothers and children using percentages, means and 95% confidence intervals (CIs). The characteristics included in the description were: 1) maternal characteristics (including sociodemographic, behavioral and reproductive) and habits (type of water consumed and smoking during pregnancy), 2) child characteristics (including sex, breastfeeding, daycare center attendance, small for gestational age, and prematurity) and 3) mean MUFcr levels, type of DW consumed and residence in fluorinated or non-fluorinated zone. Data analysis was restricted to mother-child pairs with F measurements at the first and third trimester of pregnancy. MUFcr was averaged from the first and third trimester measurement.

Covariates were selected a priori based on previous studies carried out in this birth cohort in relation to cognitive effects of early exposure to F during pregnancy (Ibarluzea et al., 2022), their theoretical relevance or observed associations with fluoride exposure, and/or the analyzed neurobehavioral outcomes. As such, models were adjusted for the following child characteristics: sex, age at neurobehavioral measurement, birth order (first born vs. others), and for the maternal social class, IQ, breastfeeding and smoking (ever vs. never).

In order to explore the association of the F exposure during pregnancy with the performance of neuropsychological tests, linear regression models were fitted for the continuous scales and logistic regression models for the categorized scales (as not risk ≤66 and probable risk >66). In the multiple linear and logistic regression models, the criterion for statistical significance was set at $p < 0.05$. Parameter estimates were expressed as regression coefficients and their 95% CIs associated with one-unit increase in MUFcr level. Residuals from each model had approximately normal distributions, and their Q-Q plots revealed no extreme outliers. Plots of residuals against fitted values did not suggest any assumption violations. Effect modification by sex was studied by including an interaction term in the regression models, but these interactions between sex and F were not significant. The association with the scale scores at the different waves appears to be linear across the range of MUFcr during pregnancy. GAM models are shown in Supplementary Fig. 1. Additionally, we fitted linear and logistic regression models specifying splines with a knot at the 75th percentile to study whether the association between fluoride exposure and the outcomes here considered varied in those with the highest fluoride levels.

Sensitivity analyses were carried out using these neurotoxicants as they have also been considered by other authors in previous studies (Bashash et al., 2017, 2018; Green et al., 2019; Ibarluzea et al., 2022). Further, sensitivity analyses were run including in the regression models the following covariates: alcohol consumption during pregnancy, mothers with only one urinary sample during pregnancy instead of two, living in municipalities supplied or not with CFDW, HES score and other pollutants that are neurotoxic, namely, total As, Mn, Pb and Hg. All statistical analyses were performed using R (version 4.1.3).

3. Results

3.1. Maternal and children characteristics

The characteristics of the mother at pregnancy and of the children participating at the age of 8 and 11 years (see Table 1) can be summarized as follows: the mean age of the mothers when they gave birth was 31 years old, they had a pre-pregnancy BMI of 22.8–23.0 kg/m^2, most of them were nulliparous (58.9–60.0%), half of them had a university degree (49.3–51.7%) and two thirds belonged from a non-manual social class (59.6–59.7%). Around 9% of the pregnant women smoked at some time during pregnancy. In relation to the characteristics of the children included in the analyses, at the 8 and 11 years follow-ups respectively, 49.8% and 54.2% were females, 2.7% and 2.5% were preterm and 7.8% and 8.0% were small for gestational age. The characteristics of the participating and non-participating women and children at the different waves are shown in Supplementary Table 1. In general, there were not significant differences between the follow-ups, with the exception to the zone of residence (fluoridated vs non-fluoridated), type of drinking water consumed, parity and order between brother/sister at the age of 8.

3.2. *Maternal fluoride levels*

MUFcr levels (mg F/g creatinine; mean and 95% CI) for the whole pregnancy (mean of the two samples) varied according to the source of drinking water consumed and according to the type of municipality where they lived (CFDW vs CNFDW) in each data collection wave (Table 1). For example, at the 8 years follow up, MUFcr levels were higher in mothers drinking CFDW, 0.88 (0.79, 0.97), lower in those drinking CNFDW, 0.42 (0.37, 0.46) and intermediate in mothers drinking bottled water, 0.63 (0.56, 0.70) ($p < 0.001$). In the same sense, mothers living in fluoridated zones also had significantly higher MUFcr values 0.81 (0.74, 0.87) than those living in non-fluoridated zones 0.46 (0.74, 0.87) ($p < 0.001$). Besides, mean MUFcr levels varied by trimester of pregnancy, levels being statistically lower in the first 0.54 (0.49, 0.59) than in the third trimester at both, 0.71 (0.65, 0.77) ($p < 0.001$). Levels of Mn and As in urine, and of Hg and Pb in cord blood are described at each follow-up in Supplementary Table 2.

3.3. *CPRS-R:S scores by follow-up*

The scores of the CPRS-R:S subscales and the number and percentage of children meeting the clinical criteria for ADHD (Probable problem) are shown in Table 2. At the age of 8 children with probable cognitive problems/inattention, Hyperactivity-Impulsivity or ADHD were 9.8%, 9.8% and 6.7% respectively; at the age of 11 the percentages of probable cases were of 6.8%, 8.5% and 5.5%. T values for the different subscales were close to the expected values (mean = 50 and SD = 10) (Table 2).

3.4. *Association between fluoride exposure and ADHD symptoms score or clinical approach*

Non-significant correlations were observed between MUFcr or MUF levels of the whole pregnancy and the scores of CRS-R-S (Supplementary Table 3). For the rest of the neurotoxics (Mn, As and Hg), no statistically significant correlations with said scores were observed either. Correlation analyses between Pb and inattention or hyperactivity were not calculated due to the small number of samples with lead levels ≥ LOQ (<7.2%).

Table 2
Descriptive results of Cognitive Problems-Inattention, Hyperactivity-Impulsivity and ADHD index (CPRS-R:S).

	8 years N (%)	11 years N (%)
Cognitive Problems- Inattention		
Not risk	230 (90.2)	220 (93.2)
Probable problem	25 (9.8)	16 (6.8)
Hyperactivity-Impulsivity		
Not risk	230 (90.2)	216 (91.5)
Probable problem	25 (9.8)	20 (8.5)
ADHD Index		
Not risk	238 (93.3)	223 (94.5)
Probable problem	17 (6.7)	13 (5.5)
T score: Mean (sd)		
Cognitive Problems- Inattention	50.3 (9.3)	49.8 (8.0)
Hyperactivity-Impulsivity	50.9 (8.7)	52.6 (9.9)
ADHD Index	49.9 (8.6)	49.8 (8.0)

No significant associations were found between MUFcr levels (beta values per unit increase in MUFcr (1 mg/g) and cognitive problems-inattention, hyperactivity-Impulsivity and ADHD index scores of the CRS-R-S in the adjusted continuous models for the 8 and 11 years samples. Following the established cut-off point ($T > 66$), there was an inverse association between MUFcr and being probably categorized with cognitive problems/Inattention at the age of 11. OR for whole, first and third trimester were 0.08 (0.00, 0.87), 0.32 (0.03, 1.83) and 0.05 (0.00, 0.64) respectively, indicating that higher MUFcr levels for the whole pregnancy and third trimester were significantly associated with a lower risk of cognitive problems/inattention. ORs values for whole and first trimester of pregnancy were also indicative of a lower risk, although not significant, for hyperactivity or ADHD index (Table 3). At the age of 8, no significant association was observed. Results, in general, did not show interaction by sex at both waves (8 an 11 years) for any of the subscales (Supplementary Table 4). Finally, the study of the association in low exposed versus high exposed (>75th percentile) participants did not reveal statistically significant differences between said groups (see Supplementary Table 5).

3.5. *Sensitivity analyses*

Additional analyses including other neurotoxicants (lead, arsenic, mercury and manganese) levels (Supplementary Table 6), quality child's family context (HES) (Supplementary Table 7), alcohol consumption during pregnancy (Supplementary Table 8) and CFDW (Supplementary Table 9) were carried out. The inclusion of those variables did not change substantially the overall picture and OR values for cognitive problems-inattention showed low OR values, specifically at the age of 11 years, but in the case of CFDW, in which the statistically significant effects detected at the age of 11 disappeared. Although not significant, in general, ORs for hyperactivity and ADHD index at age of 8 and 11 years were also suggestive of a reduced risk.

4. Discussion

ADHD is a neuropsychological disorder characterized by symptoms of impulsivity-hyperactivity, inattention, or both that commonly debuts in childhood. Our study did not show any significant association between fluoride exposure, MUFcr, during pregnancy and the ADHD symptoms using CPRS-R:S at different ages in childhood, but showed a lower significant risk of probable diagnosis for cognitive problems-inattention at the age of 11 after accounting for potential confounding variables. Although not significant, reduced risk were also observed for hyperactivity-impulsivity and ADHD Index at 8 and 11 years. In general terms, these results did not change after sensitivity analyses.

The first study suggesting that fluoride exposure could increase the prevalence of ADHD in children and adolescents aged 3–17 years was carried out by Malin and Till (2015) in the United States using an ecological design. State prevalence of artificial water fluoridation significantly positively predicted state prevalence of ADHD after controlling for socioeconomic status. This study had relevant limitations; its ecological design did not provide individual information of exposure and outcomes and F exposure was operationalized following the drinking water modality present at each location (CFDW vs CNFDW). Information about other potential covariates or neurotoxicants was neither available. Taking into consideration conclusions and critics to the extent of the diffusion of the results of this latter study, Perrott (2018) indicated that the relationship between fluoride exposure and ADHD should be explored by epidemiological studies with individual information and data for other probable risk-modifying factors. Other associations were found using aggregate data at state level, for example with the mean altitude of the states, showing the weakness of the former

Table 3
Association between MUFcr (mg/g) levels during pregnancy and beta and OR values for the ADHD symptoms or probable ADHD (T > 66).

8 years **(N=255)**	OR (CI 95%)			Beta (CI 95%)		
	Cognitive problems-Inattention	Hyperactivity-Impulsivity	ADHD index	Cognitive problems-Inattention	Hyperactivity-Impulsivity	ADHD index
MUFcr pregnancy	0.87 (0.21, 2.88)	0.56 (0.09, 2.49)	0.50 (0.06, 2.55)	−1.20 (−4.90, 2.50)	−1.07 (−4.46, 2.32)	−1.96 (−5.37, 1.46)
MUFcr week 12	1.06 (0.29, 3.10)	0.63 (0.09, 2.53)	0.37 (0.04, 1.85)	−0.61 (−4.15, 2.92)	−0.63 (−3.87, 2.61)	−1.6 (−4.86, 1.66)
MUFcr week 32	0.79 (0.23, 2.06)	0.70 (0.17, 2.05)	0.85 (0.20, 2.56)	−1.00 (−3.81, 1.81)	−0.83 (−3.41, 1.74)	−1.25 (−3.84, 1.35)
11 years **(N=236)**	Cognitive problems-Inattention	Hyperactivity-Impulsivity	ADHD index	Cognitive problems-Inattention	Hyperactivity-Impulsivity	ADHD index
MUFcr pregnancy	**0.08 (0.00, 0.87)**	0.42 (0.04, 2.55)	0.26 (0.02, 1.96)	−0.70 (−3.95, 2.54)	−1.22 (−5.31, 2.86)	−0.57 (−3.82, 2.67)
MUFcr week 12	0.32 (0.03, 1.83)	1.44 (0.27, 5.77)	0.57 (0.06, 2.92)	−0.03 (−3.17, 3.11)	0.06 (−3.90, 4.02)	0.75 (−2.39, 3.90)
MUFcr week 32	**0.05 (0.00, 0.64)**	0.16 (0.01, 1.04)	0.27 (0.02, 1.49)	−0.74 (−3.12, 1.64)	−1.35 (−4.34, 1.65)	−1.05 (−3.43, 1.33)

Note: Adjusted by sex, age in the moment of the test, order between sisters/brothers, breastfeeding, breastfeeding (weeks), smoking at pregnancy, social class, nursery and mother's CI.

association (Huber et al., 2015).

Three cross-sectional studies have been carried out in Canada and China respectively. In the first one, data from 1877 youth, 6–17 years of age, from the Canadian Health Measures Survey showed that urinary F did not predict ADHD diagnosis or hyperactive/inattentive symptoms (Riddell et al., 2019). An increase in 1 mg/L in F in DW, instead, was associated with a 6.1 times higher odds of an ADHD diagnosis, being the effects stronger among older youth. The second cross-sectional study's aim was to examine the relationship between F exposure and parental- or self-reported diagnosis of a learning disability among a population-based sample of Canadian children aged 3–12 years. Authors reported that no significant association was observed between urinary F exposure and self-reported: learning disability, diagnosis of ADHD or diagnosis of attention deficit disorder, although a marginal inverse association between urinary F and attention deficit was observed (Barberio et al., 2017). Finally, Chinese (Henan Province) school-age children (7–13 years old) took part in a study aiming at assessing the association between urinary F exposure and children's behavioral outcomes using CPRS-R. Authors did not find any significant association between urinary F exposure and conduct problems, hyperactive-impulsive-or ADHD index (Wang et al., 2022). There was not a clear evidence of association between urinary F levels and ADHD among these cross-sectional studies, but our results pointed to an inverse association with cognitive problems-inattention symptoms.

A prospective study carried out with the birth Mexican cohort (ELEMENT), showed that an increase of 0.5 mg/L in MUFcr levels during pregnancy were significantly associated with scores of cognitive problems and inattention and ADHD index in children at age 6–12 (Bashash et al., 2018). In this birth cohort, lower scores in cognitive functions like IQ were also associated with higher MUFcr in children of the same age (Bashash et al., 2017). Our results, as previously shown (Ibarluzea et al., 2022), strongly differ from those showed by Bashash et al. (2018).

Environmental exposures can play a relevant role as risk factors for ADHD. The result of the studies suggest a different degree of evidence for different environmental agents like air pollutants (Aghaei et al., 2019; Zhang et al., 2015; Fuertes et al., 2016; Sentís et al., 2017; Perera et al., 2018), the exposure to different toxic substances (Cheslack-Postava et al., 2022; Sussman et al., 2022; Roy et al., 2011; Huang et al., 2016; Nigg et al., 2010; Donzelli et al., 2019) or habits, such as smoking, alcohol exposure and diet (Polańska et al., 2012; Lertxundi et al., 2021). Besides the roles of the different types of exposures considered, it has been observed that the family and psychosocial context, material hardship and, in general, socioeconomic stressors, show a role as risk factors of ADHD in combination with classical environmental exposures, like air pollution or lead (Perera et al., 2018; Vishnevetsky et al., 2015).

There are many physiological mechanisms that might be behind the detrimental neuropsychological effects of early exposure to F. Exposure to high concentrations of F has shown that F can cross both the placental barrier and the blood-brain barrier, and accumulate in the brain, specifically in the hippocampus, (ASTDR -Agency for Toxic Substances and Disease Registry-, 2003), an area associated with effects on memory, attention and learning (Mullenix et al., 1995; Bera et al., 2007). Animal studies found positive correlations between fluoride intake and toxicity phenotypes closely associated with neurotoxicity (Ottappilakkil et al., 2022), probably consequence of biochemical and celular changes in oxidative stress (Akinrinade et al., 2015; Zhang et al., 2015), structural changes in mitochondria (Zhao et al., 2019) and apoptotic neurons (Bhatnagar et al., 2011; Lou et al., 2013). Further, some studies have suggested that fluoride may contribute to thyroid hormone insufficiency (Dugbartey, 1998) and that subclinical hypothyroidism during pregnancy could be linked with an increased risk for attention disorders in the offspring (Modesto et al., 2015; Päkkilä et al., 2014). Other potential mechanisms through which fluoride may contribute to neuropsychological impairments or conditions relate to: 1) the dopamine system (Pal and Sarkar, 2014), 2) the modulation of this neurotransmitter in the planning and initiation of motor responses, activation, switching, reaction to novelty and processing of reward (Faraone et al., 2015), and, 3) alterations in acetylcholine or cholinergic receptors (Chouhan et al., 2010; Liu et al., 2010; Reddy et al., 2021). Nevertheless, there is no evidence of the beneficial neuropsychological effect or protective mechanism of F.

4.1. Study strengths and limitations

The design of this study presents some qualities that might make it a relevant contribution to the field. First, it has been conducted in a geographical area having a balanced variety of DW modalities (CFDW and CNFDW), being this unique within the INMA cohort study.

Besides its prospective design, the fact that MUF was measured in urine in two different trimesters during pregnancy is a key value of the study. We measured levels in only two spot samples from the first and third trimester, but as it has been previously documented, morning spot sample F levels have shown a good correlation with 24-h F concentration and intake (Zohouri et al., 2006). Stressors with high impact on neuropsychological development during the prenatal and early childhood period have been related to the health of the mother, her diet and lifestyle, the quality of family and social interaction, and exposure to toxic substances (Ibarluzea et al., 2022; Nilsen et al., 2020). Even though we were not able to exclude the possibility of unmeasured residual confounding we have used information of socioeconomic position, psychosocial characteristics and lifestyle that have been gathered since pregnancy for the mothers and therefore think that we have covered a wide set of potential confounders. Information from the children is also available starting from pregnancy outcomes, family characteristics and quality of the family context. This way, we considered combined exposure to both chemical and non-chemical stressors at early developmental life stages. Finally, one of the main strengths of the study is the ability to run sensitivity analyses including other neurotoxicants (lead, arsenic, mercury and manganese), alcohol consumption, living or not in a CFDW municipality or the quality of family context. These analyses have shown the robustness of our findings.

Among the limitations of the study, we must point out that we have only used MUF as potential predictor of ADHD and were not able to measure F concentrations in DW or the intake of F from DW (Riddell et al., 2019). The reduced sample size of the study could have shadowed some of the potential associations between MUF levels and the presence of symptoms or the probable diagnosis of ADHD disorder.

5. Conclusion

An inverse association between MUF and probable diagnosis of cognitive problems-inattention at 11 years of age was observed in a prospective birth cohort in Spain. Obtained results contrast partially with those observed by other authors in cross-sectional and prospective studies. Despite difficulties in identifying other studies and biological mechanisms that support these results, other population-based studies are warranted to confirm these results at low levels of F in drinking water.

Author contribution

Jesús Ibarluzea and Mikel Subiza-Pérez (These authors contributed equally to this work and should be listed as the first authors): Conceptualization, Methodology, Formal analysis, Writing - original draft. Ane Arregi: Resources, Validation, Writing - Review & editing, Amaia Molinuevo: Data curation, Formal analysis, Methodology, Enrique Arranz-Freijo: Writing - Review & editing, Manuel Sánchez de Miguel: Writing - Review & editing, Ana Jiménez: Writing - Review & editing, Ainara Andiarena: Resources, Writing - Review & editing, Loreto Santa Marina: Funding acquisition, Supervision. Aitana Lertxundi: Funding acquisition, Conceptualization, Supervision.

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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.

Data availability

The authors do not have permission to share data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.116705.

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SUPPLEMENTARY MATERIAL.

Association of maternal prenatal urinary fluoride levels with ADHD symptoms in childhood.

Ibarluzea[a,b,c,d]†, Mikel Subiza-Pérez[b,c,d,e]†, Ane Arregi[b,d]*. Amaia Molinuevo[d], Enrique Arranz-Freijo[b,d], Manuel Sánchez de Miguel [b,d], Ana Jiménez[a,c,d], Ainara Andiarena[b,d,], Loreto Santa-Marina[a,c,d], Aitana Lertxundi[c,d,f]

a Ministry of Health of the Basque Government, Sub-Directorate for Public Health and Addictions of Gipuzkoa, 20013, San Sebastián, Spain

b Faculty of Psychology of the University of the Basque Country (UPV-EHU), 20018, San Sebastian, Spain

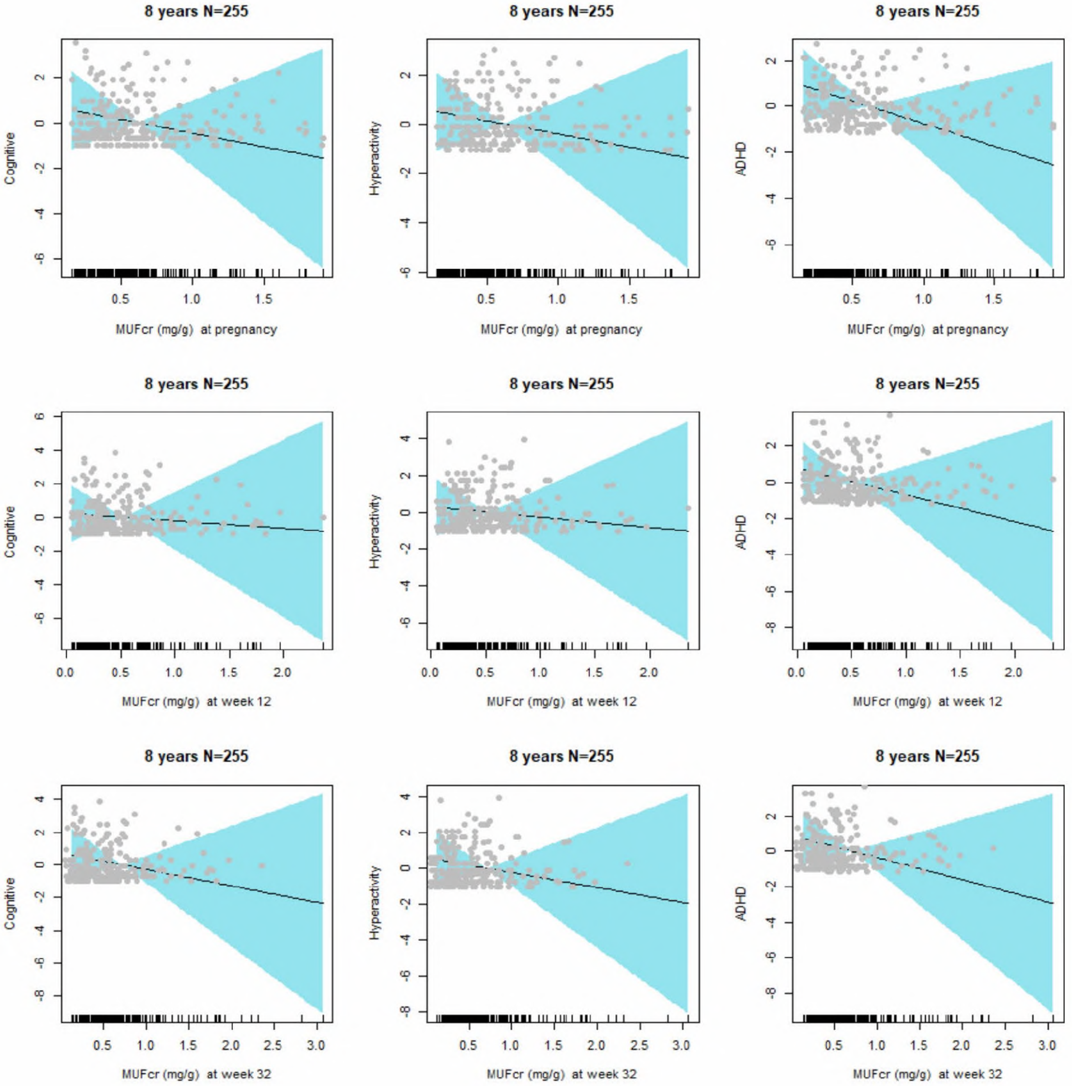
c Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), 28029, Madrid, Spain

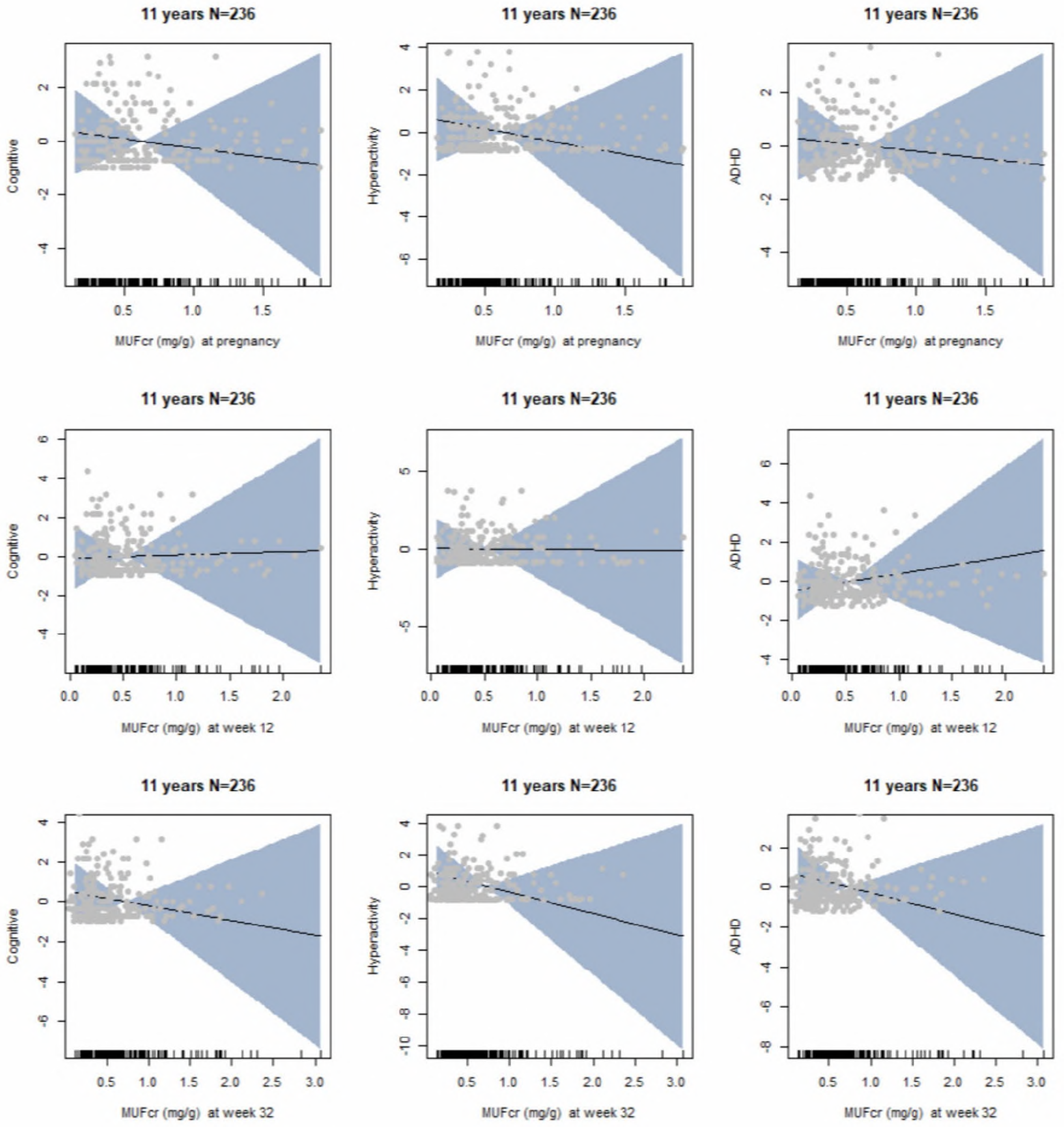
d Biodonostia Health Research Institute, Environmental Epidemiology and Child Development Group, 20014, San Sebastian, Spain

e Bradford Institute for Health Research, Temple Bank House, Bradford Royal Infirmary, BD9 6RJ, Bradford, United Kingdom.

f Department of Preventive Medicine and Public Health, Faculty of Medicine, University of the Basque Country (UPV/EHU), 48940 Leioa, Spain

Supplementary Figure 1. GAM models. Association between MUFcr and ADHD related outcomes at the follow-ups at 8 and 11 years.





11 years N=236
Cognitive
Hyperactivity
ADHD
MUFcr (mg/g) at pregnancy
MUFcr (mg/g) at week 12
MUFcr (mg/g) at week 32

Supplementary Table 1

Characteristics of the participating and non-participating women and children in the INMA-Gipuzkoa cohort.

	Sample at birth N=612	**Excluded N=357**	**8 YEARS (Conners) N=255**	***p***	**Excluded N=376**	**11 YEARS (Conners) N=236**	***p***
Mother							
Maternal age	31.35 (3.61)	31.34 (3.82)	31.38 (3.31)	*0.89*	31.18 (3.81)	31.63 (3.27)	*0.12*
BMI				*0.75*			*0.86*
Less than 18.5	22 (3.6%)	15 (4.2%)	7 (2.8%)		15 (4.00%)	7 (3.00%)	
18.5 - 25	469 (76.6%)	272 (76.2%)	197 (77.3%)		285 (75.8%)	184 (77.80%)	
25 - 30	92 (15.0%)	52 (14.6%)	40 (15.7%)		57 (15.2%)	35 (14.8%)	
More than 30	29 (4.7%)	18 (5.0%)	11 (4.3%)		19 (5.1%)	10 (4.2%)	
Social class				*0.05*			*0.05*
No manual	354 (57.84%)	202 (56.6%)	152 (59.6%)		213 (56.7%)	141 (59.8%)	
Manual	258 (42.2%)	155 (43.4%)	103 (40.4%)		163 (43.4%)	95 (40.3%)	
Maternal IQ	9.84 (2.74)	9.63 (2.72)	9.99 (2.75)	*0.21*	9.62 (2.72)	10.04 (2.75)	*0.15*
Smoking in pregnancy				*0.18*			*0,12*
No	523 (85.5%)	296 (82.9%)	227 (89.0%)		311 (82.7%)	212 (89.8%)	
Yes	70 (11.4%)	46 (12.9%)	24 (9.4%)		49 (13.0%)	21 (8.9%)	
'Missing'	19 (3.1%)	15 (4.2%)	4 (1.6%)		16 (4.3%)	3 (1.3%)	
Alcohol consumption							
No	547 (89.38%)	315 (88.24%)	232 (90.98%)	*0.852*	333 (88.56%)	214 (90.68%)	*0.406*
Yes (at least 1 unit/week)	34 (5.56%)	18 (5.04%)	16 (6.27%)		17 (4.52%)	17 (7.20%)	
'Missing'	31 (5.07%)	24 (6.72%)	7 (2.75%)		26 (6.92%)	5 (1.44%)	
Zone				***0.006***			*0.07*
Non fluoridated zone	277 (45.8%)	143 (40.9%)	134 (52.6%)		157 (42.6%)	120 (50.9%)	
Fluoridated zone	328 (54.2%)	207 (59.1%)	121 (47.5%)		212 (57.5%)	116 (49.2%)	
Type of drinking water				***0.02***			*0.11*
Community fluoridated drinking water	226 (36.9%)	145 (40.6%)	81 (31.8%)		149 (39.6%)	77 (32.6%)	
Community non-fluoridated drinking water	209 (34.2%)	106 (29.7%)	103 (40.4%)		119 (31.7%)	90 (38.1%)	
Bottled water	155 (25.3%)	88 (24.7%)	67 (26.3%)		89 (23.7%)	66 (28.0%)	
'Missing'	22 (3.6%)	18 (5.0%)	4 (1.6%)		19 (5.1%)	3 (1.23%)	
Parity				***0.02***			*0.07*
0	331 (54.1%)	178 (49.9%)	153 (60.0%)		192 (51.1%)	139 (58.9%)	
>0	281 (45.9%)	179 (50.1%)	102 (40.0%)		184 (48.9%)	97 (41.1%)	
Child							
Order between brothers/sisters				***0.02***			*0.07*
Not first	276 (45.1%)	176 (49.3%)	100 (39.2%)		181 (48.1%)	95 (40.3%)	
First	336 (54.9%)	181 (50.7%)	155 (60.8%)		195 (51.9%)	141 (59.8%)	
Sex				*1*			*0.11*
Female	305 (49.8%)	178 (49.9%)	127 (49.8%)		177 (47.1%)	128 (54.2%)	
Male	306 (50.0%)	178 (49.9%)	128 (50.2%)		198 (52.7%)	108 (45.8%)	
'Missing'	1 (0.2%)	1 (0.3%)	0 (0.0%)		1 (0.3%)	0 (0.0%)	
Nursery 1 year				*0.86*			*0.38*
No	288 (47.1%)	163 (45.1%)	125 (49.0%)		176 (46.8%)	112 (47.5%)	

Yes	256 (41.8%)	142 (39.8%)	114 (44.7%)		146 (38.8%)	110 (46.6%)	
'Missing'	68 (11.1%)	52 (14.6%)	16 (6.3%)		54 (14.4%)	14 (5.9%)	
Preterm				*0.56*			*0.45*
No	584 (95.4%)	338 (94.7%)	246 (96.5%)		355 (94.4%)	229 (97.0%)	
Yes	21 (3.4%)	14 (3.9%)	7 (2.8%)		15 (4.0%)	6 (2.5%)	
'Missing'	7 (1.1%)	5 (1.4%)	2 (0.8%)		6 (1.6%)	1 (0.4%)	
Small for gestational age				*1*			*1*
No	549 (89.7%)	319 (89.4%)	230 (90.2%)		335 (89.1%)	214 (90.7%)	
Yes	48 (7.8%)	28 (7.8%)	20 (7.8%)		29 (7.7%)	19 (8.1%)	
'Missing'	15 (2.5%)	10 (2.8%)	5 (2.0%)		12 (3.2%)	3 (1.3%)	
Breastfeeding weeks	28.95 (20.57)	29.28 (21.23)	28.53 (19.73)	*0.67*	29.27 (21.07)	28.48 (19.85)	*0.65*

Supplementary Table 2

Descriptive levels of the Mn, As (urine pregnancy), Hg and Pb (cord blood).

	N	min	mean	sd	median	IQR	max
***8 years* (N=255)**							
Manganese level at pregnancy *(ng/mL) adjusted by creatinine (g/L)	250	0.03	0.30	0.88	0.11	0.15	9.47
Arsenic level at pregnancy *(ng/mL)adjusted by creatinine (g/L)	250	4.59	114.63	156.51	68.60	83.10	1546.23
Mercury (mg/l)	226	1.41	9.73	5.70	8.50	6.17	38.00
Lead (µg/dL)**	226	1 (½ LOQ)	-	-	-	-	3.5
***11 years* (N=236)**							
Manganese level at pregnancy *(ng/mL) adjusted by creatinine (g/L)	229	0.03	0.34	1.01	0.11	0.16	9.47
Arsenic level at pregnancy *(ng/mL)adjusted by creatinine (g/L)	229	4.59	120.02	164.02	69.23	90.54	1546.23
Mercury (mg/l)	208	1.41	9.70	5.59	8.55	6.12	38.00
Lead (µg/dL)**	210	1 (½ LOQ)	-	-	-	-	3.7

* mean between measurements at weeks 12 and 32 of pregnancy; ** 93.0% <LOQ at age of 8 and 92.8% <LOQ at age of 11, being LOQ =2 µg/dL

Supplementary Table 3.

Pearson correlation coefficients between MUFcr or MUF levels of the whole pregnancy and the scores of CRS-R-S

	MUFcr pregnancy	MUFcr week 12	MUFcr week 32	MUF pregnancy	MUF week 12	MUF week 32
4 years						
Inattention	0.039	0.03	0.036	0.046	0.047	0.029
Hyperactivity	0.085	0.072	0.073	0.083	0.109	0.032
ADHD	0.068	0.055	0.059	0.07	0.085	0.033
8 years						
Cognitive	-0.061	-0.044	-0.058	-0.016	-0.024	-0.003
Hyperactivity	-0.054	-0.035	-0.056	0.032	0.047	0.005
ADHD	-0.084	-0.072	-0.071	-0.032	-0.045	-0.008
11 years						
Cognitive	-0.05	-0.024	-0.059	-0.036	-0.042	-0.017
Hyperactivity	-0.044	-0.003	-0.066	0.012	0.037	-0.017
ADHD	-0.064	-0.005	-0.095	-0.043	-0.027	-0.044

* *$p<0.05$*

Supplementary Table 4

P-values for the interaction between MUF and sex in the logistic and linear models fit for this study.

	Logistic models			**Linear models**		
8 years **(N=255)**	**Cognitive**	**Hyperactivity**	**ADHD**	**Cognitive**	**Hyperactivity**	**ADHD**
MUFcr pregnancy	0.767	0.129	0.073	0.783	0.768	0.641
MUFcr week 12	0.649	0.049	0.170	0.805	0.616	0.575
MUFcr week 32	0.938	0.392	0.090	0.807	0.409	0.752
11 years **(N=236)**	**Cognitive**	**Hyperactivity**	**ADHD**	**Cognitive**	**Hyperactivity**	**ADHD**
MUFcr pregnancy	0.560	0.239	0.643	0.609	0.578	0.586
MUFcr week 12	0.705	0.128	0.279	0.837	0.259	0.246
MUFcr week 32	0.733	0.727	0.729	0.395	0.962	0.975

Note: Adjusted by sex, age in the moment of the test, order between sisters/brothers, breastfeeding, breastfeeding (weeks), smoking at pregnancy, social class, nursery and mother's CI.

Supplementary Table 5

Analyses of splines fixed at the 75th percentile of MUFCr levels in the logistic and linear models fitted for the 8 and 11 years participants.

				Logistic models				Linear models			
Age	**Exposure**	**Cutpoint**	**Outcome**	**Estimate**	**Std.Error**	**z**	**p**	**Estimate**	**Std.Error**	**z**	**p**
8 years	MUFcr pregnancy	0.79	Cognitive problems-Inattention	0.57	1.33	0.43	0.667	-0.71	3.83	-0.19	0.853
			Hyperactivity-Impulsivity	2.04	2.06	0.99	0.324	-0.36	3.52	-0.10	0.919
			ADHD index	4.04	2.30	1.75	0.080	-3.14	3.54	-0.89	0.376
	MUFcr 12 weeks	0.68	Cognitive problems-Inattention	1.54	1.72	0.90	0.370	0.34	4.48	0.08	0.939
			Hyperactivity-Impulsivity	1.08	2.33	0.47	0.642	-0.23	4.11	-0.06	0.956
			ADHD index	5.38	3.65	1.47	0.141	-3.00	4.13	-0.73	0.468
	MUFcr 32 weeks	0.88	Cognitive problems-Inattention	3.23	2.09	1.54	0.123	-1.60	4.46	-0.36	0.721
			Hyperactivity-Impulsivity	1.00	2.23	0.45	0.653	-1.63	4.11	-0.40	0.692
			ADHD index	3.12	2.39	1.31	0.190	-1.55	4.11	-0.38	0.706
11 years	MUFcr pregnancy	0.81	Cognitive problems-Inattention	-2.71	5.87	-0.46	0.644	-2.01	3.37	-0.60	0.551
			Hyperactivity-Impulsivity	3.59	3.56	1.01	0.312	-0.77	4.24	-0.18	0.856
			ADHD index	3.27	4.08	0.80	0.423	-0.62	3.36	-0.18	0.855
	MUFcr 12 weeks	0.70	Cognitive problems-Inattention	3.20	4.10	0.78	0.435	-0.41	3.89	-0.11	0.917
			Hyperactivity-Impulsivity	3.61	2.40	1.51	0.132	2.03	4.89	0.42	0.678
			ADHD index	2.67	3.46	0.77	0.441	1.08	3.89	0.28	0.782
	MUFcr 32 weeks	0.91	Cognitive problems-Inattention	-17.49	14.68	-1.19	0.234	-6.27	3.82	-1.64	0.103
			Hyperactivity-Impulsivity	-7.46	10.07	-0.74	0.459	0.26	4.85	0.05	0.957
			ADHD index	-1.77	4.24	-0.42	0.676	-3.33	3.85	-0.87	0.388

Supplementary Table 6.

Association between MUFcr (mg/g) levels during pregnancy and OR values for probable ADHD (beta values). adjusted by Mn. As. Hg and Pb.

	Logistic regression models: OR (CI 95%)			Linear regression models: Beta (CI 95%)		
8 years **(N=255)**	Cognitive problems-Inattention	Hyperactivity-impulsivity	ADHD index	Cognitive problems-Inattention	Hyperactivity-impulsivity	ADHD index
MUFcr pregnancy	0.86 (0.20 . 2.95)	0.48 (0.06 . 2.37)	0.78 (0.10 . 4.17)	-1.63 (-5.86 , 2.59)	-0.55 (-4.40 , 3.30)	-2.17 (-6.01 , 1.68)
MUFcr week 12	1.03 (0.28 . 3.05)	0.55 (0.08 . 2.23)	0.55 (0.06 . 2.73)	-1.05 (-4.99 , 2.89)	-0.23 (-3.82 , 3.36)	-1.76 (-5.35 , 1.82)
MUFcr week 32	0.80 (0.23 . 2.11)	0.66 (0.14 . 2.17)	1.10 (0.25 . 3.77)	-1.15 (-4.32 , 2.01)	-0.47 (-3.36 , 2.41)	-1.29 (-4.17 , 1.6)
11 years **(N=236)**	Cognitive problems-Inattention	Hyperactivity-impulsivity	ADHD index	Cognitive problems-Inattention	Hyperactivity-impulsivity	ADHD index
MUFcr pregnancy	**0.08 (0.00 . 1.02)**	0.44 (0.03 . 3.85)	0.28 (0.01 . 3.08)	-1.18 (-4.94 , 2.57)	-1.7 (-6.34 , 2.94)	-1.36 (-5.09 , 2.37)
MUFcr week 12	0.37 (0.03 . 2.29)	1.72 (0.23 . 8.80)	0.54 (0.03 . 3.87)	-0.81 (-4.36 , 2.74)	-0.61 (-5.01 , 3.78)	-0.19 (-3.72 , 3.35)
MUFcr week 32	**0.03 (0.00 . 0.58)**	0.16 (0.01 . 1.33)	0.34 (0.02 . 2.24)	-0.74 (-3.42 , 1.93)	-1.38 (-4.68 , 1.93)	-1.27 (-3.93 , 1.38)

Note: Adjusted by sex, age in the moment of the test, order between sisters/brothers, breastfeeding (weeks), smoking at pregnancy, social class, nursery and mother CI and Mn, As, Hb And Pb(</≥ LOD). Statistically significant associations in bold (p-value <0.05).

Supplementary Table 7.

Association between MUFcr (mg/g) levels during pregnancy and OR (beta values) for probable ADHD. adjusted by Total Score of Etxadi Gangoiti Scale.

	Logistic regression models: OR (CI 95%)			Linear regression models: Beta (CI 95%)		
8 years **(N=255)**	Cognitive problems-Inattention	Hyperactivity-impulsivity	ADHD index	Cognitive problems-Inattention	Hyperactivity-impulsivity	ADHD index
MUFcr pregnancy	0.79 (0.16 . 3.06)	0.45 (0.04 . 2.89)	0.37 (0.03 . 2.28)	-1.07 (-4.8 , 2.67)	-1.31 (-4.57 , 1.95)	-2.17 (-5.63 , 1.28)
MUFcr week 12	0.80 (0.17 . 2.87)	0.18 (0.01 . 1.65)	0.13 (0.01 . 1.13)	-1.61 (-5.33 , 2.11)	-2.19 (-5.43 , 1.05)	-2.85 (-6.29 , 0.58)
MUFcr week 32	0.86 (0.22 . 2.54)	0.88 (0.17 . 3.18)	0.89 (0.16 . 3.22)	-0.29 (-3.12 , 2.54)	-0.24 (-2.71 , 2.24)	-0.85 (-3.47 , 1.78)
11 years **(N=236)**	Cognitive problems-Inattention	Hyperactivity-impulsivity	ADHD index	Cognitive problems-Inattention	Hyperactivity-impulsivity	ADHD index
MUFcr pregnancy	**0.02 (0.00 . 0.96)**	0.16 (0.00 . 1.85)	0.14 (0.00 . 1.59)	-0.18 (-3.12 , 2.76)	-2.03 (-5.99 , 1.93)	-0.50 (-3.5 , 2.51)
MUFcr week 12	**0.02 (0.00 . 0.91)**	0.45 (0.03 . 3.32)	0.12 (0.00 . 1.53)	-0.94 (-3.92 , 2.04)	-2.15 (-6.18 , 1.87)	-0.32 (-3.37 , 2.74)
MUFcr week 32	0.08 (0.00 . 1.50)	0.15 (0.01 . 1.34)	0.33 (0.02 . 1.86)	0.29 (-1.84 , 2.42)	-1.04 (-3.92 , 1.84)	-0.36 (-2.54 , 1.82)

Note: Adjusted by sex, age in the moment of the test. order between sisters/brothers, breastfeeding (weeks), smoking at pregnancy, social class, nursery and mother CI and Total Score Etxadi Gangoiti Scale. Statistically significant associations in bold (p-value <0.05)

Supplementary table 8

Association between MUFcr (mg/g) levels during pregnancy and beta and OR values for the ADHD symptoms or probable ADHD (T >66), adjusted by alcohol consumption during pregnancy.

	OR (CI 95%)			Beta (CI 95%)		
8 years **(N=255)**	Cognitive problems-Inattention	Hyperactivity-Impulsivity	ADHD index	Cognitive problems-Inattention	Hyperactivity-Impulsivity	ADHD index
MUFcr pregnancy	0.84 (0.20 , 2.84)	0.61 (0.09 , 2.74)	0.43 (0.05 , 2.33)	-1.22 (-4.96 , 2.53)	-0.85 (-4.28 , 2.59)	-1.90 (-5.36 , 1.55)
MUFcr week 12	1.05 (0.29 , 3.08)	0.55 (0.08 , 2.29)	0.37 (0.04 , 1.85)	-0.59 (-4.13 , 2.96)	-0.58 (-3.83 , 2.67)	-1.56 (-4.83 , 1.71)
MUFcr week 32	0.77 (0.22 , 2.03)	0.82 (0.19 , 2.58)	0.76 (0.17 , 2.38)	-1.05 (-3.92 , 1.82)	-0.61 (-3.24 , 2.02)	-1.22 (-3.87 , 1.43)
11 years **(N=236)**	Cognitive problems-Inattention	Hyperactivity-Impulsivity	ADHD index	Cognitive problems-Inattention	Hyperactivity-Impulsivity	ADHD index
MUFcr pregnancy	**0.04 (0.00 , 0.60)**	0.37 (0.04 , 2.38)	0.25 (0.02 , 2.00)	-0.81 (-4.09 , 2.47)	-0.85 (-4.98 , 3.28)	-0.65 (-3.93 , 2.63)
MUFcr week 12	0.29 (0.02 , 1.69)	1.43 (0.27 , 5.74)	0.58 (0.06 , 2.94)	0.01 (-3.14 , 3.16)	0.12 (-3.85 , 4.08)	0.80 (-2.35 , 3.95)
MUFcr week 32	**0.02 (0.00 , 0.40)**	0.13 (0.01 , 0.92)	0.26 (0.02 , 1.51)	-0.89 (-3.32 , 1.53)	-1.00 (-4.05 , 2.05)	-1.19 (-3.61 , 1.24)

Note: Adjusted by sex, age in the moment of the test, order between sisters/brothers, breastfeeding, breastfeeding (weeks), smoking at pregnancy, social class, nursery, mother's CI and alcohol consumption during pregnancy.

Supplementary table 9

Association between MUFcr (mg/g) levels during pregnancy and beta and OR values for the ADHD symptoms or probable ADHD (T >66), adjusted by CFDW.

	OR (CI 95%)			Beta (CI 95%)		
8 years **(N=255)**	Cognitive problems-Inattention	Hyperactivity-Impulsivity	ADHD index	Cognitive problems-Inattention	Hyperactivity-Impulsivity	ADHD index
MUFcr pregnancy	1.11 (0.23 , 4.46)	0.44 (0.05 , 2.40)	0.42 (0.04 , 2.56)	-0.89 (-5.10 , 3.32)	-1.26 (-5.12 , 2.60)	-1.28 (-5.16 , 2.60)
MUFcr week 12	1.30 (0.33 , 4.20)	0.53 (0.07 , 2.50)	0.32 (0.03 , 1.80)	-0.26 (-4.09 , 3.58)	-0.66 (-4.17 , 2.86)	-1.00 (-4.54, 2.53)
MUFcr week 32	0.91 (0.24 , 2.58)	0.63 (0.13 , 2.02)	0.81 (0.16 , 2.68)	-0.79 (-3.89 , 2.30)	-0.94 (-3.78 , 1.91)	-0.73 (-3.59 , 2.13)
11 years **(N=236)**	Cognitive problems-Inattention	Hyperactivity-Impulsivity	ADHD index	Cognitive problems-Inattention	Hyperactivity-Impulsivity	ADHD index
MUFcr pregnancy	0.14 (0.00 , 1.71)	0.53 (0.05 , 3.59)	0.41 (0.02 , 3.83)	0.41 (-3.22 , 4.05)	-0.19 (-4.78 , 4.41)	0.37 (-3.28 , 4.02)
MUFcr week 12	0.49 (0.04 , 2.99)	1.75 (0.32 , 7.69)	0.83 (0.08 , 4.54)	0.85 (-2.5 , 4.19)	0.98 (-3.24 , 5.21)	1.60 (-1.75, 4.95)
MUFcr week 32	0.08 (0.00 , 1.12)	0.17 (0.01 , 1.24)	0.39 (0.02 , 2.29)	-0.09 (-2.68 , 2.51)	-0.78 (-4.05 , 2.50)	-0.58 (-3.18 , 2.02)

Note: Adjusted by sex, age in the moment of the test, order between sisters/brothers, breastfeeding, breastfeeding (weeks), smoking at pregnancy, social class, nursery and mother's CI and CFDW.