Environmental Research 207 (2022) 112181

Contents lists available at ScienceDirect

Environmental Research

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

Prenatal exposure to fluoride and neuropsychological development in early childhood: 1-to 4 years old children

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ARTICLE INFO

Keywords:
Fluoride
Pregnancy
Neuropsychological development
Intelligence
Children

ABSTRACT

Background: Cross-sectional and prospective studies have provided evidence of the neurotoxic effect of early exposure to fluoride (F) in pregnancy. It has been negatively associated with cognitive development during childhood, with most research conducted in areas with high F levels in community drinking water (CDW).

Method: Data from 316 to 248 mother-child pairs from the Infancia y Medio Ambiente (Childhood and Environment, INMA) birth cohort project with maternal urinary F level adjusted for creatinine (MUFcr) measurements in the first and third trimesters of pregnancy. Children's cognitive domains and intelligence indexes were evaluated using the Bayley Scales (age of 1) and the McCarthy Scales (age of 4). Multiple linear regression analyses were carried out adjusting for a wide range of covariates related to the child, mother, family context and other potential neurotoxicants.

Results: No association was found between MUFcr levels and Bayley Mental Development Index score. Nevertheless, regarding the McCarthy scales, it was found that per unit (mg/g) of MUFcr across the whole pregnancy, scores in boys were greater for the verbal, performance, numeric and memory domains ($\beta = 13.86$, CI 95%: 3.91, 23.82), ($\beta = 5.86$, CI 95%: 0.32, 11.39), ($\beta = 6.22$, CI 95%: 0.65, 11.79) and ($\beta = 11.63$, CI 95%: 2.62, 20.63) respectively and for General Cognitive Index ($\beta = 15.4$, CI 95%: 6.32, 24.48). For girls there was not any cognitive score significantly associated with MUFcr, being the sex-F interactions significant (P interaction <0.05). Including other toxicants levels, quality of family context or deprivation index did not substantially change the results.

Abbreviations: As, Arsenic; BSID, Bayley Scales of Infant Development; CI, Confidence interval; CDW, Community Drinking water; DW, drinking water; F, Fluoride; FCDW, Fluoridated community drinking water; GCI, General cognitive index; HES, Haezi-Etxadi Scale; Hg, Mercury; INMA, INfancia y Medio Ambiente -Environment and Childhood- Project; IQ, intelligence quotient; MDI, Mental development index; Mn, Manganese; MSCA, McCarthy Scales of Children's Abilities; MUF, maternal urinary fluoride; MUFcr, maternal urinary Fluoride adjusted by creatinine; NFCDW, Non-Fluoridated community drinking water; Pb, Lead; SD, Standard deviation.

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https://doi.org/10.1016/j.envres.2021.112181
Received 11 April 2021; Received in revised form 21 September 2021; Accepted 3 October 2021
Available online 8 October 2021
0013-9351/

Conclusions: In boys, positive associations were observed between MUFcr and scores in cognitive domains at the age of 4. These findings are inconsistent with those from some previous studies and indicate the need for other population-based studies to confirm or overturn these results at low levels of F in CDW.

1. Introduction

Fluoride (F^-), the ionic form of the chemical element fluorine, is found in soil, air and water, especially in waters containing elements like sodium, potassium or chlorine that solubilize fluorine-bearing minerals, such as fluorite (Mondal et al., 2016). It is also intentionally added to toothpaste, salt, milk or drinking water in some areas where natural F content is low, as a public health measure for the prevention of dental caries. The addition of F to drinking water has been considered among the top-ten public health achievements of the 20th century, as it considerably decreased caries in children, especially those from a low social class (Jones et al., 2005; Khan et al., 2015). Nonetheless, given that oral hygiene has improved in the general population since the adoption of this measure, the decision to maintain community water fluoridation is controversial among public health practitioners, because of the possible health implications of high doses. Indeed, high F doses can induce dental or skeletal fluorosis (Bronckers et al., 2009; Indermitte et al., 2009; Liteplo, 2002). Other effects, such as a potential impact on neurodevelopment, need to be further investigated, especially in child populations. To date, animal models inform about moderate and small behavioral and developmental (learning and memory) effects (U.S. National Toxicology Program, 2016).

Maternal F exposure during pregnancy may be relevant for children's future neurodevelopment, given that the F can cross the placenta and penetrate the fetal blood-brain barrier (ASTDR (Agency for Toxic Substances and Disease Registry), 2003; Goasdoué et al., 2017), and then accumulate in cerebral tissues (Khan et al., 2015; Razdan et al., 2017). It is important to note that structural and functional changes in the central nervous system, specifically in the fetal period and during the first years of life, may lead to cognitive impairments (Choi et al., 2015). Most epidemiologic studies assessing neurodevelopmental effects have been ecological and cross-sectional and have estimated F exposure by measuring F concentration in drinking water and assessing water consumption habits rather than measuring F in individuals (Aravind et al., 2016; Choi et al., 2012; Grandjean, 2019; Khan et al., 2015; Razdan et al., 2017; Sebastian and Sunitha, 2015). The majority of these studies have been conducted in endemic areas, where a high F content in water (0.9–11.0 mg/L) occurs naturally, such as in Mexico, India, Iran and China. Systematic reviews conducted by Choi et al. (2012) and Grandjean (2019) concluded that the available research data points towards a possible adverse effect of high F exposure on children's neurodevelopment. Nonetheless, given that most of the studies available are based on intake of high vs low F in water, there is a clear need to carry out studies in non-endemic areas, for example, to obtain information about the safety of community water fluoridation. The range of F levels recommended in public drinking water by the World Health Organization is: 0.7–1.2 mg/L (2004). Further, many of the aforementioned cross-sectional studies show no or poor control of potential confounders in the statistical analyses performed.

New approaches, employing a prospective design, using mother and child cohorts, and individual biological measurements of F, provide data on early exposure during pregnancy, relevant to critical-phases of neurodevelopment and information of a wide range of variables of interest. A Mexican birth cohort recruiting pregnant women in Jalisco, an area with endemic hydrofluorosis (2.6–3.7 mg/L), found a negative association between cognitive development and maternal urinary fluoride (MUF) in samples collected during the three trimesters of pregnancy in children from 3 to 15 months of age (Valdez-Jiménez et al., 2017). A second study also from Mexico (Mexico City) in which F levels in community drinking water (CDW) were lower than in the former study (0.15–1.38 mg/L), but where the general population was also exposed to fluoridated salt, also showed an inverse association with cognitive function at 6 and 6–12 years of age (Bashash et al., 2017). A third longitudinal study included mother-child pairs living in communities supplied with fluoridated water and others supplied with non-fluoridated water in six cities across Canada. In this case and in contrast to the Mexican studies, the inverse association was observed in boys but not in girls (Green et al., 2019). On the contrary, a recent Swedish retrospective cohort study did not found a negative association between F levels and cognitive ability measured at 18–20 years old men, from the national military conscription (Aggeborn and Oehman, 2021). Similarly, a New Zealand study, based on a birth cohort followed until the age of 38 years, neither found significant association in IQ related to water fluoridation levels (Broadbent et al., 2015).

Other factors potentially influencing neuropsychological development are the co-exposure to pollutants (from water or other environmental sources) with neurotoxic effects during pregnancy. Specifically, sex-dependent adverse effects on neuropsychological outcomes related to early environmental exposure have been reported for other environmental neurotoxicants (Gochfeld, 2017; Evans et al., 2014: Lertxundi et al., 2019) but are not routinely explored. Animal, ecological and cross-sectional studies have provided some evidence for an earlier and latter sexual maturation in females and males respectively related to F exposure (NRC , 2006; Liu et al., 2019). Besides, there is a paucity of information about sex-dependent neurocognitive effects which is inconsistent and mostly limited to ecological studies. Co-exposure to F and other toxic metalloids and metals from the same or different sources have recently been considered. For instance, co-exposure to F and arsenic (As) in water has generated great interest in recent epidemiological research, although exposure to other co-pollutants such as lead (Pb) and mercury (Hg) has also been explored (Bashash et al., 2017; Green et al., 2019).

A true negative fluoride-neurodevelopment association in children would have major health implications not only in endemic areas but also in areas with community water fluoridation. Therefore, the aim of this study was to assess the association between maternal F exposure during pregnancy and neurodevelopmental outcomes in 1- and 4-year-old children in the INMA-Gipuzkoa cohort (from the Spanish for Environment and Childhood: *INfancia y Medio Ambiente*).

2. Methods

2.1. Study population

This study draws on data from a mother and child birth cohort established in Gipuzkoa, Spain (Guxens et al., 2012). It should be noted that, among the different subcohorts of the INMA project, only Gipuzkoa had an active community fluoridated drinking water (CFDW) program in place across drinking water treatment plants serving more than 30,000 inhabitants. In the distribution systems with less than 30,000 inhabitants community drinking water was not fluoridated. The recruitment of mother-child pairs took place during the first antenatal visit to the gynecologist in the public referral hospital. 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 trimester of pregnancy, pregnancy achieved without assisted reproduction techniques, planned birth in the referral hospital and no communication problems in Spanish or

Basque (Guxens et al., 2012). In total, 638 pregnant women met the inclusion criteria and agreed to be enrolled in the INMA study. Of the 612 children born, 483 (78.9%) underwent neuropsychological testing at the age of 1 and 379 (61.9%) at the age of 4.

Participants were required to meet the following criteria: 1) children with data on neuropsychological assessment at 1 year of age, 2) children with data on neuropsychological assessment at 4 years of age provided they also had data on this assessment at 1 year of age (criterion 1), and 3) mothers with data on maternal urinary F level adjusted for creatinine (MUFcr) at the first and third trimesters of pregnancy. Additional analyses were also performed including, besides the former women, those with an impaired MUFcr sample.

MUFcr was analyzed in all the mothers with urine samples available. In total, there were 393 maternal samples with data on MUFcr levels for both trimesters. 316 and 248 mother-child pairs had complete information on respectively MUFcr for both trimesters and neuropsychological outcomes at 1 and 4 years of age. Fig. 1 shows the flowchart. All participating mothers gave written informed consent for themselves and on behalf of their children after the Ethics Committee of Donostia Hospital (Gipuzkoa) approved the protocol.

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

Data on drinking water source (tap or bottled) and the amount of water consumed was obtained through food and drink questionnaires (Vioque et al., 2013), administered in the first and third trimesters of pregnancy. These questionnaires collected data about the brand of bottled water (BW) consumed but not its quantity, and hence, neither the amount of water nor the amount of F intake could be calculated for women who consumed BW. 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. The study area covers the regions of Goierri and Urola in the province of Gipuzkoa (Basque Country) and had a population of 89,000 inhabitants distributed in 25 municipalities, with a population ranging from 127 to 13,900 inhabitants. In 18 municipalities the distributed drinking water comes from 3 conventional treatment plants (pretreatment, coagulation, sedimentation, filtration and disinfection) that also included fluoridation in 2 of them. The remaining 7 municipalities were supplied with water treated with filtration and disinfection. Three reservoirs were the source of raw water for the 3 conventional treatment plants; spring water for the rest 7 villages or small towns. In all, 15 municipalities are supplied with CFDW (62% of the population) and 10 with CNFDW (38%). Raw and treated water quality is soft and in low in minerals in all the municipalities (Gobierno, 2020). From the food and drink questionnaires administered during pregnancy, we identified the ten most consumed BW brands and then ascertained that F levels in these brands ranged from 0.07 to 0.48 mg/L.

Among the 316 pregnant women with information on MUFcr, whose children underwent neuropsychological testing at the age of 1, 36.7% consumed CFDW, 37.3% CNFDW and 24.4% BW. Among the 248 pregnant women with information on MUFcr, whose children underwent neuropsychological testing at age 4, 35.5% consumed FDW, 38.3% NFDW and 24.2% BW (Table 1). The percentage of pregnant women that used BW for cooking was extremely low (1.1%).

2.3. Biomarker of F exposure

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 concentration of F in urine samples was quantified by potentiometry using an ion-selective electrode (DX219-F, Mettler Toledo). Total ionic strength adjustment buffer (TISAB) II was used to adjust the pH and ionic strength of the urine samples. This solution was prepared from 58 mg/mL of NaCl (Panreac), 10 mg/mL of trans-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid monohydrate (DCTA, Fluka) and 57 μL/mL of glacial acetic acid (Panreac). The TISAB II pH was adjusted to between 4.8 and 5.2 with a 7% (w/v) solution of NaOH (Prolabo). TISAB II was added at a final concentration of 20% (v/v). The quantification was performed with an F calibration curve (0.010–10 mg/L) prepared from NaF (1000 mg/L as F, Panreac) in 20% (v/v) TISAB II. Quality

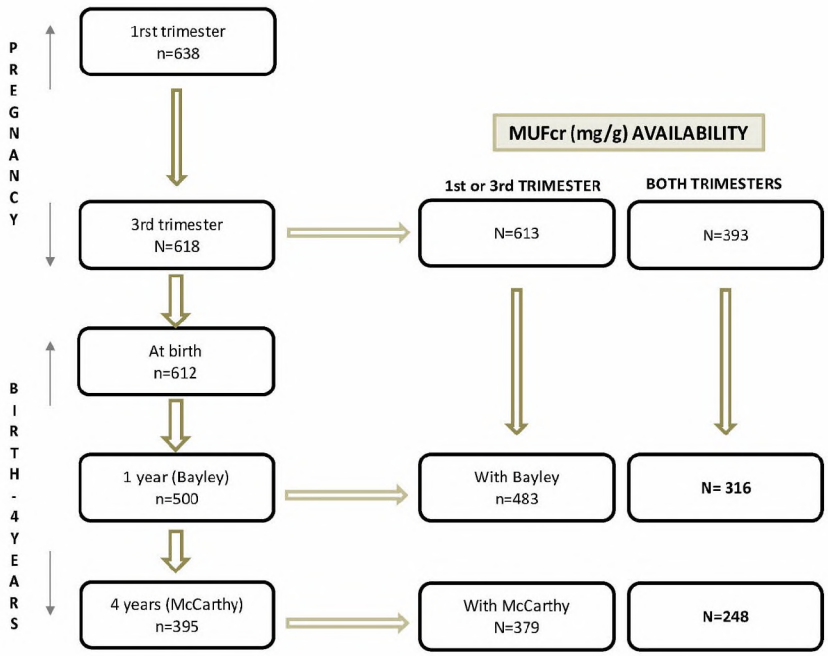

Fig. 1. Flowchart showing mother-child pairs included in the analyses described in this paper.

Table 1
Characteristics of the study sample.

N (%)/mean (sd)*	Bayley	McCarthy
	N = 316	N = 248
Mother		
Maternal age	31.2 (3.4)	31.5 (3.4)
BMI	22.9 (3.3)	22.8 (3.4)
Educational level		
Primary or without education	39 (12.3%)	33 (13.3%)
Secondary	112 (35.4%)	89 (35.9%)
University	164 (51.9%)	126 (50.8%)
Missing	1 (0.3%)	
Social class		
No manual	177 (56.0%)	147 (59.3%)
Manual	139 (44.0%)	101 (40.7%)
MEDEA Index of deprivation (4 years)**		
1 Very low	22 (7.0%)	19 (7.7%)
2 Low	100 (31.6%)	78 (31.4%)
3 Moderate	117 (37.0%)	85 (34.3%)
4 High	57 (18.0%)	52 (21.0%)
5 Very high	20 (6.3%)	14 (5.6%)
Maternal IQ	9.90 (2.8)	9.91 (2.7)
Smoking in pregnancy		
No	252 (79.7%)	199 (80.2%)
Yes	61 (19.3%)	45 (18.1%)
Missing	3 (1.0%)	4 (1.6%)
Zone		
Non fluoridated zone	153 (48.4%)	123 (49.6%)
Fluoridated zone	160 (50.6%)	124 (50.0%)
Missing	3 (1.0%)	1 (0.4%)
Type of drinking water		
Community fluoridated drinking water (CFDW)	116 (36.7%)	88 (35.5%)
Community not fluoridated drinking water (CNFDW)	118 (37.3%)	95 (38.3%)
Bottled water	77 (24.4%)	60 (24.2%)
Missing	5 (1.6%)	5 (2.0%)
Parity		
0	186 (58.9%)	146 (58.9%)
>0	130 (41.1%)	102 (41.1%)
Missing		
Child		
Order between brothers/sisters		
Not first	129 (40.8%)	100 (40.3%)
First	187 (59.2%)	148 (59.7%)
Missing		
Sex		
Female	170 (53.8%)	123 (49.6%)
Male	146 (46.2%)	125 (50.4%)
Nursery 1 year		
No	159 (50.3%)	123 (49.6%)
Yes	139 (44.0%)	113 (45.6%)
Missing	18 (5.7%)	12 (4.8%)
Preterm		
No	305 (96.5%)	240 (96.8%)
Yes	8 (2.5%)	6 (2.4%)
Missing	3 (1.0%)	2 (0.8%)
Small for gestational age		
No	288 (91.1%)	222 (89.5%)
Yes	24 (7.6%)	22 (8.9%)
Missing	4 (1.3%)	4 (1.6%)
Breastfeeding weeks	29.2 (19.9%)	28.5 (19.8%)

*Number (N) of subjects and the proportion in each category have been used for categorical variables and mean and standard deviation (sd) for continuous variables. **Deprivation index: MEDEA project.

control was carried out by analyzing a urine reference sample (Medisafe® Metalle U, LGC Standards) with a certified F concentration (assigned value: 10 mg/L; confidence interval: 7.6–12.4 mg/L). 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), with Roche reagents, in a Hitachi 911 auto-analyzer.

2.4. Child neurodevelopment evaluation

The neuropsychological development of the children was assessed at 14.6 ± 0.8 months and 4.4 ± 0.1 years (mean ± SD) using the Bayley Scales of Infant Development (BSID) (Bayley, 1977) and a standardized version of the McCarthy Scales of Children's Abilities (MSCA) adapted to the Spanish population (McCarthy, 2009) respectively.

The Bayley Mental Development Index (MDI) consists of 163 items that assesses cognitive development and communication skills by evaluating skills such as performance abilities, memory, and early verbal communication. All testing was carried out in local health centers in the presence of the mother, father or another caregiver by specially trained neuropsychologists who were blinded to the child's F exposure status. To limit inter-observer variability, we applied a strict protocol, including training sessions in which inter-observer differences were discussed (Guxens et al., 2012). The scores according to the age were standardized to a mean of 100 points with a standard deviation of 15 points. Higher scores indicated better general cognitive development.

The MSCA were designed to evaluate children's general intellectual abilities and their strengths and weaknesses. They comprise 18 subtests that yield standardized test scores for 6 scales. The verbal scale refers to cognitive tasks related to the processing of verbal information; the performance scale refers to cognitive tasks related to perceptual information processing, including manual skills; the quantitative scale assesses quantitative abilities; the global memory scale considers short-term retention of information (verbal, visual or quantitative), and the General Cognitive Index (GCI) calculated using the first three scales (McCarthy, 2009). Lastly, there is a motor scale, but this was not included in the study. Raw MSCA scores were normalized to a mean of 100 (SD 15), higher scores indicating better neuropsychological abilities.

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), alcohol consumption (no or occasional, more than occasional), diet (drinking water sources -tap or brands of bottled water-, and the amount of water consumed only from the tap was estimated through a food and drink questionnaire (Vioque et al., 2013) and duration (weeks) of breastfeeding. Furthermore, when the child reached around 15 months of age, maternal scores on the Similarities subtest of the Wechsler Adult Intelligence-Third Edition (WAIS-III) (Wechsler and Kaufman, 2001) were assessed as a proxy for maternal IQ, given that this subtest has been shown to be a good predictor of global IQ (Wechsler, 1997). In order to control the possible confounding effects of the socioeconomic level, in addition to the social class of the mother, the deprivation index at the census section level was used (Dominguez-Berjon et al., 2008) in the sensitivity analysis.

In addition, we collected data on characteristics of the children: sex, birth order, whether they were premature (born before 37 weeks of pregnancy) (World Health Organization (WHO), 2015), whether they

were 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 kindergarten before 2 years of age. All questionnaires were administered face-to-face by trained interviewers.

Recent research provides empirical support for the view that the family context has a significant impact on children's cognitive development. Scores on the Haezi-Etxadi Scale (HES) (Arranz et al., 2014; Barreto et al., 2017), assessing the influence of the quality of family context on children's cognitive development in early childhood, were available at the age of 4 year. This scale 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), but also incorporates new variables gathered by observational procedures.

Additional analyses included neurotoxic substances detected in maternal urine samples during pregnancy, such as total As (μg/g) and manganese (Mn, μg/g), both adjusted for creatinine, and Hg in umbilical cord blood (μg/l). Following the type of analyses carried out in previous cohort studies (Bashash et al., 2017; Green et al., 2019), we decided to include also Pb levels. In our case Pb had been measured in cord blood, although the levels of the samples with levels above the limit of quantification (2 μg/dl) were very low (5.9%). Iodine was also included in the main model due to, on the one hand, the association between urinary iodine (μg/g adjusted for creatinine) and cognitive scores in childhood (Levie et al., 2019), and on the other, the potential effect of F, as an endocrine disruptor, on thyroid function (NRC , 2006; Peckham et al., 2015).

2.6. Data handling and statistical analysis

The study sample was described in terms of the characteristics of mothers and children using percentages, means and 95% confidence intervals (CIs). Bivariate analyses of the mean MUFcr levels and Bayley and McCarthy scale scores by: 1) maternal characteristics (including sociodemographic, behavioral and reproductive) and habits (type of water consumed) and 2) child characteristics (including sex, breastfed, small for gestational age, and prematurity) and habits (nursery attendance), were analyzed by Student's t tests or one-way analysis of variance. Pearson correlations were calculated between urinary F levels and children's scores on each of the Bayley and McCarthy scales.

Based on this analysis, the variables with a p value below 0.2 were included in the multiple linear regression models, which were conducted for each of the Bayley and McCarthy cognitive scales, in order to explore the association of the F exposure during pregnancy on the performance of neuropsychological tests. In the multiple linear regression, the criterion for statistical significance was $p < 0.05$. 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 model. For scales for which the interaction between sex and F was significant, results in the tables are presented separately for girls and boys. The association with GCI and the other cognitive scale scores appears to be linear across the range of MUFcr during pregnancy. GAM models are shown in Supplementary Fig. 1.

Further, analyses were performed including in the main regression model data available on the HES score and other pollutants that have been considered neurotoxic, namely, total As, Mn, Pb and Hg. Iodine, which is a bioelement essential for life, was also included. All statistical analyses were performed using R (version 3.6.1).

3. Results

The characteristics of the mother-child pairs samples analyzed at the age of 1 (Bayley scales) and 4 (McCarthy scales) respectively (Table 1) can be summarized as follows (only one figure is reported when both values in the consecutive follow ups are the same or very similar): the mean age of the mothers when they gave birth was 31 years old, had a BMI of 22.8 kg/m^2, most of them were nulliparous (58.8%), half of them had a university degree (51.9% and 50.8%) and more than half came from a non-manual social class (56.0 and 59.2%). 19.3% and 18.1% of the pregnant women smoked at some time during pregnancy. In relation to the characteristics of the samples of children included in the analyses, at the 1- and 4-year follow-ups respectively, 53.8% and 49.6% were females, 2.5% and 2.4% were preterm and 7.6% and 8.9% were small for gestational age. Around 44% of the children started kindergarten before the first follow-up. The percentages of the different types of drinking water consumed in pregnancy are reported in Section 2.2. The differences between the study sample and all women that gave birth in the INMA-Gipuzkoa cohort are shown in Supplementary Table 1. In general, there were not significant differences in relation to mother's and child's characteristics with the exception of smoking during pregnancy. Among the participating mothers at any of the follow ups, the percentage of ever smoking in pregnancy was lower than among non-participating mothers.

MUFcr levels (mg F/g creatinine) for the whole pregnancy (mean of the two samples) varied according to the source of drinking water consumed (mean and 95% CI) in each data collection wave, being 0.91 (0.83, 0.99) in mothers drinking CFDW, 0.43 (0.39, 0.47) in those drinking CNFDW and 0.62 (0.56, 0.69) in those drinking BW ($p = 0.04$) among the sample involved in the first wave of data collection (Bayley scales administered), and 0.89 (0.81, 0.98) in the mothers drinking CFDW, 0.41 (0.36, 0.45) in those drinking CNFDW and 0.63 (0.55, 0.71) in those drinking BW ($p = 0{,}078$) among those involved in the second wave (McCarthy scales administered) (Supplementary Table 2). Mean MUFcr levels (mg F/g creatinine) also varied by trimester of pregnancy, levels being lower in the first trimester than the third at both waves: respectively 0.57 (0.52, 0.62) vs 0.74 (0.69, 0.79) among the first-wave sample ($p < 0.001$) and 0.55 (0.50, 0.60) vs 0.73 (0.67, 0.79) among the second-wave sample ($p < 0.001$). Levels of MUF, MUFcr, Mn, As, and iodine in urine, and of Hg and Pb in cord blood are described in Supplementary Table 3. Differences between those living in municipalities with fluoridated water vs those living in non-fluoridated municipalities were only seen for those variables related to F (participants living in fluoridated or not fluoridated zone, type of drinking water, MUF, and MUFcr), age of the mother (follow up at the age 4) and attending nursery (follow up at the age 1)". No differences in toxin levels between zones were observed (only whole pregnancy levels are shown) (Supplementary Table 4), including variables potentially related to neurodevelopment such as maternal education, social class or maternal IQ. There were no significant differences in MUFcr levels between women living in fluoridated and non-fluoridated municipalities according to categories of social class indexes (Supplementary Table 5).

While no significant association was observed between MUFcr levels and Bayley MDI scores, MUFcr (third trimester and whole pregnancy) was weakly ($0.13 \leq r \leq 0.20$) but significantly ($p < 0.05$) and positively correlated with all the McCarthy's cognitive domains scores (third trimester) and with all, except the verbal domain, for the whole pregnancy. Besides, Hg levels at birth were also associated with numeric and memory domains ($r = 0.25$ and 0.15 respectively) and As (third trimester) with the numeric domain ($r = 0.16$). Correlations between MUFcr with iodine, Mn and As in urine were low-to-moderate ($r = 0.12$ and 0.30), but significant. Hg cord blood levels were not associated with MUFcr (Supplementary Table 6). Correlation analyzes between Pb and cognitive functions could not be done due to the small number of samples (<7%) with lead levels ≥ LOQ.

No association was found between MUFcr levels and Bayley MDI in

multiple regression models. In the same way, no association was found between MUFcr levels at first trimester and cognitive functions at the age of 4. Nevertheless, per unit (mg/g) of MUFcr in the third trimester, significantly greater verbal (β [CI 95%]: 12.01 [(4.82, 19.19]), memory (9.20 ([2.67, 15.73]) and GCI (11.48 ([4.88, 18.08]) scores were observed in boys, while non-significant effects were seen in girls (p for interaction <0.05). When referring to the MUFcr levels across the whole pregnancy, per unit (mg/g) of MUFcr, significant associations were observed with all the cognitive domains: performance (β [CI 95%]: 5.86 [0.32, 11.39]), numeric (6.22 ([0.65, 11.79]), verbal (13.86 ([3.91, 23.82]), memory (11.63 ([2.62, 20.63]) and GCI (15.40 ([6.32, 24.48]), being the latter three, again only significant for boys (*p* for interaction <0.05) (Table 2; Supplementary Fig. 2). In stratified analysis, the effects were significant only for boys and for all the cognitive domains at the age of 4 (Supplementary Table 7).

Additional analyses including other variables like other neurotoxicants (As, Mn, Pb, Hg and As x Pb), iodine, quality child's family context (HES) and deprivation index instead of maternal social class, were carried out. All of them, except Hg, showed results that did not substantially change the overall picture of associations (Supplementary Tables 8–14). However, adjusting for Hg lowered the β values of cognitive functions for the third trimester and whole pregnancy. Nevertheless, at the third trimester of pregnancy significant associations, only in boys, were observed for verbal and GCI (9.74 ([1.75, 17.74]) and (8.15 (0.69, 15.61'+) respectively)), and across whole pregnancy for GCI (10.54 ([0.19, 20.80]) (Table 3). Verbal, numeric and performance domains were no longer significant at whole pregnancy, but showed positive and moderate β values. When all neurotoxic pollutants together were included in the analysis, the beta values remained positives and significant for boys for GCI and numeric domain in whole pregnancy and verbal function in the third trimester of pregnancy (Supplementary Table 15).

The traditional approach used in the cross-sectional studies, comparing the scores of the different functions in those living and nonliving in fluoridated areas, regardless MUF levels, was also carried out, but not significant differences were observed, unless for the numeric domain; favoring those children living in the fluoridated area (Supplementary Table 16). Sensitivity analyses including women with availability of only one sample of urine (first or third trimester), adjusting by zone (fluoridated vs non-fluoridated or excluding extreme low scores of cognitive functions (less than 2 SD) (Supplementary Tables 17–19) were carried out, but results were basically not modified from those observed in Table 2.

Finally, the association between MUFcr and cognitive functions was analyzed by zone (Supplementary Table 20). The results showed that positive associations were mainly observed in non-fluoridated zones. When stratification analyzes among boys were carried out, results showed that: 1) the association was significant only in the non-fluoridated zones, although the beta values were also high, but non-significant, in the fluoridated zone (Supplementary Tables 21) and 2) more positive and significant associations were observed in children of mothers with a better social position (Supplementary Tables 22) and 3) the association between MUFcr and HES was only statistically significant in families with a lower quality of the family context, although again, the beta values were also high, but non-significant, in high quality context families (Supplementary Table 23).

4. Discussion

In this study, we assessed the association between prenatal F exposure, measured as MUFcr, and neuropsychological development at 1 and 4 years old of the children. We consider that this kind of study is necessary to gather evidence which could be crucial for public health policy making. For instance, it might help to decide, based on scientific data, whether community fluoridation programs should continue. We observed no negative effects on children's cognition and even found positive associations for verbal, performance, numeric, memory scores and GCI, in boys at the age of 4 years, although when Hg levels were included in the model only verbal and GCI at week 32 and whole pregnancy remained significant or marginally significant. The positive associations between MUFcr and cognitive functions seemed to be more evident in children of mothers who lived their pregnancy in the non-fluoridated zones. The reduced sample size after stratification and the high beta values in both zones recommend to be prudent about this last association.

The associations have been seen with MUFcr of the third trimester and not with those of the first one. Higher levels of MUFcr have been reported in the third trimester of this study and other prospective studies (Till et al., 2018; Valdez-Jiménez et al., 2017). As there is not information of MUFcr of the second trimester of pregnancy, it is difficult to identify a window of exposure related to the effect, but the lack of associations in the first trimester indicate that the effects are associated with later periods in pregnancy.

The general conclusion drawn from the 27 cross-sectional (ecological) studies included in a meta-analysis (Choi et al., 2012) was that children at school age living in areas with high-F (from 1.0 to more than 11.0 mg/L F in water) and/or high dental fluorosis index had significantly lower IQ scores than those children in the reference group (Choi et al., 2012; Kumar et al., 2020; Li et al., 2010; Li et al., 2003). A recent review of these cross-sectional studies carried out by Grandjean (2019) including 14 new studies, all of them from endemic areas, provided further evidence of cognitive deficits in children with elevated F exposure. The major limitation of these cross-sectional studies is the small number of the covariates included in their design and the lack of

Table 2
Multiple lineal regression models for the association between MUFcr (mg/g) levels during pregnancy and cognitive domains scores (Bayley and McCarthy).

Beta (IC95%)		Bayley N = 316	McCarthy N = 248				
		Mental	Verbal	Performance	Numeric	Memory	General cognitive
MUFcr (mg/g) at pregnancy	*All/ Boys Girls*	1.48 (−4.2, 7.16)	**13.86 (3.91, 23.82)** † ** −1.48 (−9.29, 6.32)	**5.86 (0.32, 11.39)***	**6.22 (0.65, 11.79)***	**11.63 (2.62, 20.63)** † * −1.77 (−8.82, 5.29)	**15.4 (6.32, 24.48)** † *** −0.19 (−7.31, 6.93)
MUFcr (mg/g) at week 12	*All*	0.55 (−4.64, 5.74)	1.11 (−4.86, 7.07)	4.63 (−0.57, 9.82)	4.47 (−0.79, 9.73)	1.71 (−3.66, 7.09)	3.37 (−2.09, 8.83)
MUFcr (mg/g) at week 32	*All/ Boys Girls*	1.52 (−2.92, 5.97)	**12.01 (4.82, 19.19)** † *** −1.09 (−7.01, 4.83)	3.68 (−0.49, 7.85)	4.13 (−0.07, 8.32)	**9.2 (2.67, 15.73)** † ** −1.87 (−7.24, 3.51)	**11.48 (4.88, 18.08)** † *** −0.54 (−5.97, 4.9)

Adjusted by age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, maternal social class, IQ and smoking.
Bayley (n = 316): 170 girls and 146 boys; McCarthy (n = 248): 123 girls and 125 boys.
† 004DUFcr and sex interaction statistically significant at the $p < 0.05$ level. When this interaction is significant, first coefficient indicates the effect found in boys and the second in girls. When there is no interaction, the first and only coefficient is indicative of the effect detected for the whole sample (All). * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$ in beta coefficients.

Table 3
Multiple lineal regression models for the association between MUFcr (mg/g) levels during pregnancy and cognitive domains scores (Bayley and McCarthy) adjusted by cord blood Hg levels.

Beta (IC95%)		Bayley N = 316	McCarthy N = 248				
		Mental	Verbal	Performance	Numeric	Memory	General cognitive
MUFcr (mg/g) at pregnancy	*All/*	2.67 (−3.46, 8.81)	*9.4 (-1.78, 20.57)* †	4.41 (−1.59, 10.41)	5.28 (−0.54, 11.1)	0.8 (−5.3, 6.9)	**10.54 (0.19, 20.89)** †*
	Boys						
	Girls		*−2.07 (-10, 5.87)*				−0.83 (−8.18, 6.52)
MUFcr (mg/g) at week 12	*All*	0.89 (−4.55, 6.32)	−1.5 (−7.53, 4.54)	3.85 (−1.62, 9.33)	3.38 (−1.96, 8.71)	−0.52 (−6.06, 5.02)	1 (−4.61, 6.61)
MUFcr (mg/g) at week 32	*All/*	2.65 (−2.14, 7.45)	**9.74 (1.75, 17.74)** †*	2.33 (−2.15, 6.82)	3.47 (−0.88, 7.82)	1.15 (−3.4, 5.69)	**8.15 (0.69, 15.61)** ǂ*
	Boys						
	Girls		−0.74 (−6.72, 5.25)				−0.46 (−6.04, 5.12)

Adjusted by age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, maternal social class, IQ and smoking.
Bayley (n = 316): 170 girls and 146 boys; McCarthy (n = 248): 123 girls and 125 boys.
ǂ MUFcr and sex interaction statistically significant at the $p < 0.05$ level.
† MUFcr and sex interaction statistically significant at the $p < 0.10$ level. When this interaction is significant, first coefficient indicates the effect found in boys and the second in girls. When there is no interaction, the first and only coefficient is indicative of the effect detected for the whole sample (All).
* = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$ in beta coefficients.

measures of prenatal F exposure. Our results are opposite to those of the ecological studies mentioned above.

In general, our results are inconsistent with those of the prospective studies with information of prenatal exposure. Valdez-Jiménez et al. (2017) studied Mexican children and including a sample of similar age to our first assessment, exposure to F during pregnancy, and neurodevelopmental tests. The Bayley Scales were used in a sample of 65 mother-infant pairs when offspring were aged 3–15 months. The mean F levels in CDW ranged from 2.6 to 3.7 mg/L, being 3–4 times higher than F levels in the CFDW area of this study. MUFcr levels were also higher (4 times) for Mexican women. Another relevant difference with our study is the high proportion of children below the cutoff for normal mental development. These results are quite different to ours: while the MDI showed a strong inverse association with MUF in the Mexican study, our results did not show any significant association. The second prospective study, also from Mexico, reported data on the McCarthy's GCI and the full-scale IQ from the Wechsler Abbreviated Scale of Intelligence, administered at the ages of 4 and 6–12 years respectively (Bashash et al., 2017). The mean F level in CDW in Mexico City, where the children lived (ranged from 0.15 to 1.3 mg/L), this is, with an upper range above levels in our study. As the authors indicated there was another relevant source of F for the children in Mexico and this was the consumption of fluoridated salt. The mean levels of MUF in Mexican women were similar to the levels found in our group of women consuming CFDW and higher than those consuming CNFDW: 0.82–0.90 vs 0.89–0.91 and 0.41–0.43 mg/g respectively (the ranges of mean values are due to different woman-child pairs being included at different follow-up assessments). The results again differ between the two studies; specifically, while the GCI at the age of 4 and at 6–12 years was inversely and significantly associated with higher levels of MUFcr in pregnancy (Bashash et al., 2017), our results indicated positive associations and a direct and significant association with GCI and the verbal domain, although only for boys. We did not observe any significant association in girls.

The third prospective study analyzed the effect of F in women living in cities in Canada who were supplied with CFDW or CNFDW. The mean levels of F in CDW ranged from 0.13 to 0.59 mg/L, the upper limit of this range being below the levels in CFDW in our study, and the mean MUF (adjusted for specific gravity) was 0.51 mg/L, similar to our mean value (values adjusted for creatinine and gravity usually being similar). Also in this case, a significant and inverse association was observed, although only in boys (Green et al., 2019). Besides the latter three prospective studies with prenatal exposure data, there are two other cohort studies that do not confirm the former negative association. A New Zealander birth cohort followed up 3-year-old children, until they became adults (38 years old) administering IQ tests repeatedly, and did not find any significant difference in IQ by water fluoridation levels (Broadbent et al., 2015). This study was considered the first comprehensive study evidencing the lack of negative effect on IQ (Grandjean, 2019). Besides, a recent Swedish retrospective men cohort study using registry data of F levels and cognitive ability measured at 18–20 years old, did not show either a negative association between both variables (Aggeborn and Oehman, 2021).

Neurotoxicity appeared to be dose-dependent and data from the prospective studies suggest that safe exposure could be below currently recommended F concentrations in drinking water (Grandjean, 2019). Nevertheless, there is no general consensus on this, as while Bashash et al. (2017) consider that the effects in Mexican children could be limited to exposure above 0.8 mg/L of F in drinking water, Green et al. (2019), based on their findings in Canadian children, propose the reduction of maternal intake with respect to the optimal F level of 0.7 mg/L currently recommended for drinking water in US and Canada. A recent cross-sectional study (Xu et al., 2020), presented results that indicated a nonlinear relationship with MUF, with a positive association between MUF levels and IQ, below 1.7 mg/L of F, and a negative association for F levels above that threshold. Our sample's prenatal MUF concentrations were well below 1.7 mg/L (mean and 95% CI of MUF of mothers involved in the second wave (McCarthy scales), were (0.48; 0.17, 0.98 in mg/L); this is, in the positive association side of the curve.

As in most of the literature on this subject, measurements in urine are adjusted by creatinine or gravity to correct for variations in urine dilution (Bashash et al., 2017; Green et al., 2019; Thomas et al., 2016). Given that kidney function has been associated with lower IQ (Elias et al., 2009), this potential source of bias should be considered. Nevertheless, the mean age of mothers at delivery was 31 years old. Mother/child pairs belong to the general population and the expected number of cases with kidney function problems was probably very low; according to the prevalence of moderate chronic kidney disease (0.8%) observed by Hailpern et al. (2007) in a random general population sample, aged 20–59 years old. Accordingly, and added to the fact that the outcomes are assessed in childhood, the potential bias derived from the association between kidney function with IQ (Elias et al., 2009) should be low in this study.

There is not much evidence of the differential effect of F by sex in either toxicological or epidemiological studies, the potential differential effect not having been studied because most experiments have been carried out in male rats. Nevertheless, differential adverse effects of early environmental exposure have been reported in epidemiological studies in boys and girls for F (Green et al., 2019) and other environmental neurotoxins (Gochfeld, 2017; Evans et al., 2014: Lertxundi et al., 2019).

Experimental studies, most of them using high F concentrations, show 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). Nevertheless, there is still debate about the toxicity of F in experimental studies, the National Toxicology Program (NPT) concluding that evidence was low in animals exposed during development (NTP , 2016). Some authors have suggested that F exposure in utero may impair attention, memory and visuospatial organization (Basha et al., 2011; Calderón et al., 2003; Jiang et al., 2014) and might explain cognitive deficits observed in preschool and school age children (Rocha-Amador et al., 2007; Poureslami et al., 2011), as well as attention problems and hyperactivity in adolescents (Malin and Till, 2015). Another mechanism of action proposed for F is changes in the levels of neurotransmitter, as have been observed in experimental studies (Faraone et al., 2015) and a study of aborted fetuses of mothers living in endemic vs non endemic hydrofluorosis areas (Yu et al., 2008).

One of the main limitations of the epidemiological cross-sectional studies on the effects of F is that the presence of other pollutants that may be present in water supplies has rarely been considered (Rocha-Amador et al., 2007; Wang et al., 2007). It is well known that F and As are common co-contaminants in ground water in arid and semi-arid regions of the world, and that more than 300 million people worldwide use groundwater contaminated by one or both of them (Limón-Pacheco et al., 2018). When As, Mn, Pb or iodine levels were included in the model of the association between MUF and cognitive domains, the associations were not substantially modified. The reason to include iodine was that its deficiency has been identified as a cause of hypothyroidism (Levie et al., 2019) and also has been suggested that F exposure from CDW may also contribute to the development of hypothyroidism (Peckham et al., 2015). The inclusion of cord blood Hg, however, changed the number of significant associations found with cognitive functions reducing these to the verbal and GCI, and leaving the rest of the cognitive domain positive but not significant. Contrary to the change in the association shown in our study, the associations observed in the cohort studies of Mexico (Bashash et al., 2017) and Canada (Green et al., 2019) remained, in general, unchanged after being adjusted by Hg or other neurotoxins as As, Pb, and Mn. Our results do not show any interaction between MUF and Hg (cord blood) with cognitive functions. It could be considered that the change in the observed effect could be attributed to: 1) the fact that the toxic effect of Hg is produced at concentrations, a couple of orders of magnitude lower than the toxic effect of F (Mahaffey et al., 2009), and 2) the high levels of Hg in our children, 65% had levels above the United States Environmental Protection Agency (US EPA) reference dose at birth (Llop et al., 2012).

4.1. Study strengths and limitations

Among the strengths of this study are that the F was measured in urine during pregnancy, in two different trimesters, and hence, the exposure relates to early phases of brain development. We measured levels in only two spot samples from the first and third trimester, but morning spot sample F levels have shown a good correlation with 24-h F concentration and intake (Zohouri et al., 2006). Nevertheless, it remains unknown whether such a small number of samples represents real fetal exposure throughout pregnancy for this and other pollutants. A relevant strength relates to the data gathered in the study on wide range of covariates related to the child and maternal characteristics described in the method section. However, we are not able to exclude the possibility of unmeasured residual confounding. The stressors with the greatest impact on cognition 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 (Nilsen et al., 2020). In conducting these analyses, we attempted to consider combined exposure to both chemical and non-chemical stressors at early developmental life stages. In this sense, all the analyses were controlled for a comprehensive group of the aforementioned covariates. Additional sensibility analyses included the quality of the family context, deprivation index instead of maternal social class, and the co-exposure to all the toxic chemicals together. In general, although a smoothness in the association was found, there were not relevant changes in the direction of the associations previously observed. Further stratification analyzes among boys were carried out, by type of zone (fluoridated vs non-fluoridated), maternal social class and quality of family context.

Among the limitations, we should mention that, unlike other prospective studies (Bashash et al., 2017; Green et al., 2019), we did not use F intake from CDW as a proxy for internal exposure and as the independent variable to assess the potential effect in neurocognitive development. Water intake was not included in the analyses due to the lack of information on the amount of each brand of bottled water consumed in the food and drink questionnaire. Nonetheless, BW is a relevant source of DW: around 24% of our pregnant women consumed this type of water, and what is more, BW may be a considerable source of F and the results of MUF clearly showed that women drinking BW had intermediate levels between those found in women consuming CFDW and those consuming CNFDW. Hence, the inclusion of the consumption of BW can also be considered a strength of this study. As far as we know, no other studies include this source of F or type of DW in their epidemiological analysis, at least not in an explicit way.

In summary, our study shows a direct positive effect for boys and no significant effect for girls. The results of our study could support the view that F has a detrimental effect with exposure through CDW at levels above 0.8 mg/l as previously suggested (Bashash et al., 2017) and may even have a positive effect at lower levels. The highest levels of F in CDW to which pregnant mothers were exposed in our study were slightly above 0.8 mg/L and the lowest levels were below 0.10 mg/l. A question that emerges from these results is if F could have a dose-response effect like those of other chemical elements essential for life, showing a different behavior at levels in the range of or lower than those recommended by agencies such as the World Health Organization (Marthaler, 1999) and the U.S. Department of Health and Human Services Federal Panel on Community Water Fluoridation (2015). In this line, a recent cross-sectional publication suggests a positive relationship between MUF and the score of cognitive functions under a specific level of MUF, clearly lower than those found in our women (Xu et al., 2020). Further, studies should be carried out before ruling out a potential beneficial effect of F at low levels in natural or FCDW.

5. Conclusion

A positive association between MUF and GCI scores and other measures of cognitive functions at 4 years of age is observed among boys in a prospective birth cohort in Spain. The current findings contradict, with a few exceptions, results obtained previously in cross-sectional and prospective studies. Despite difficulties in interpreting and identifying other studies and biological mechanisms that support these results, other population-based studies are warranted to confirm or overturn these results at low levels of F in drinking water.

Credit author statement

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Funding

This study was funded by grants from Spanish Institute of Health Carlos III-Ministry of Economy and Competitiveness (INMA Network G03/176, CB06/02/0041, and FIS-European Regional Development Fund (ERDF): PI03/1615, PI04/1436, PI08/1151, PI04/2018, PI04/1509, PI04/1112, PI04/1931, PI05/1079, PI05/1052, PI06/1213, PI06/0867, PI07/0314, PI09/02647, PS09/00090, PI09/02311, and MS11/0178); Miguel Servet-ERDF (MSII16/00051, CP14/00108 & PI16/00261 [Co-funded by the ERDF "A way to make Europe"], and MS13/00054); Generalitat de Catalunya- Interministerial Council for Research and Technological Innovation (CIRIT) 1999SGR 00241; Ministry of Science and Innovation (MICINN) (JCI- 2011–09771); Generalitat Valenciana (Department of Health [Conselleria de Sanitat]-048/2010 and 060/2010 and FISABIO-UGP 15–230, 15–244, and 15–249); Fundació La Marató de TV3 (090430); Alicia Koplowitz Foundation (2017); University of Oviedo (Uniovi); Fundación Cajastur-Liberbank; Department of Health of the Basque Government (2005111093, 2009111069 and 2015111065); the Provincial Government of Gipuzkoa (DFG06/004 DFG08/001 and DF2015/221); and the Fundación Roger Torné. ISGlobal is a member of the CERCA Programme, Generalitat de Catalunya. Funding sources played no role in the design or execution of the study, including collection, management, analysis and interpretation of the data; or the preparation, review, and approval of the manuscript. All authors contributed to the design of the study, securing funding, and execution of the study, interpretation of the data and preparation of the final manuscript.

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.

Acknowledgements

The authors would like to acknowledge all the INMA study participants for their generous collaboration, and the interviewers for their assistance in contacting the families and administering the questionnaires.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.112181.

References

Aggeborn, L., Oehman, M., 2021. The effects of fluoride in drinking water. J. Polit. Econ. 129, 465–491. https://doi.org/10.1086/711915.

Aravind, A., Dhanya, R., Narayan, A., Sam, G., Adarsh, V., Kiran, M., 2016. Effect of fluoridated water on intelligence in 10-12-year-old school children. J. Int. Soc. Prev. Community Dent. 6, S237–S242. https://doi.org/10.4103/2231-0762.197204.

Arranz, E.B., Olabarrieta, F., Manzano, A., Martín Ayala, J.L., Galende, N., 2014. Etxadi-Gangoiti scale: a proposal to evaluate the family contexts of two-year-oldchildren. Early Child. Dev. Care 184, 933–948. https://doi.org/10.1080/03004430.2013.829823.

ASTDR (Agency for Toxic Substances and Disease Registry), 2003. Toxicological Profile for Fluorides, Hydrogen Fluoride, and Fluorine (Atlanta, Georgia).

Aylward, L.L., Hays, S.M., Vezina, A., Deveau, M., St Amand, A., Nong, A., 2015. Biomonitoring Equivalents for interpretation of urinary fluoride. Regul. Toxicol. Pharmacol. 2, 158–167. https://doi.org/10.1016/j.yrtph.2015.04.005.

Barreto, F.B., Sánchez de Miguel, M.l., Ibarluzea, J., Andiarena, A., Arranz, E., 2017. Family context and cognitive development in early childhood: a longitudinal study. Intelligence 65, 11–22. https://doi.org/10.1016/j.intell.2017.09.006.

Basha, P.M., Rai, P., Begum, S., 2011. Fluoride toxicity and status of serum thyroid hormones, brain histopathology, and learning memory in rats: a multigenerational assessment. Biol. Trace Elem. Res. 144, 1083–1094. https://doi.org/10.1007/s12011-011-9137-3.

Bashash, M., Thomas, D., Hu, H., Martinez-Mier, E.A., Sanchez, B.N., Basu, N., et al., 2017. Prenatal fluoride exposure and cognitive outcomes in children at 4 and 6 12 Years of age in Mexico. Enviromental Heal Perspect 1, 1–12. https://doi.org/10.1289/EHP655.

Bayley, N., 1977. Manual for the Bayley Scales of Infant Development. Escalas Bayley de Desarrollo Infantil, TEA Ediciones, Madrid.

Bera, I., Sabatini, R., Auteri, P., Flace, P., Sisto, G., Montagnani, M., et al., 2007. Neurofunctional effects of developmental sodium fluoride exposure in rats. Eur. Rev. Med. Pharmacol. Sci. 11, 211–224.

Bradley, R.H., 2009. The HOME environment. The handbook of cultural developmental science. In: Bornstein, M.H. (Ed.), Part 2. Development in Different Places on Earth. Taylor & Francis., New York, pp. 505–530.

Broadbent, J.M., Thomson, W.M., Ramrakha, S., Moffitt, T.E., Zeng, J., Foster Page, L.A., et al., 2015. Community water fluoridation and intelligence: prospective study in New Zealand. Am. J. Publ. Health 105, 72–76. https://doi.org/10.2105/AJPH.2013.301857.

Bronckers, A.L.J.J., Lyaruu, D.M., Denbesten, P.K., 2009. Critical review in oral biology and medicine: the impact of fluoride on ameloblasts and the mechanisms of enamel fluorosis. J. Dent. Res. 88, 877–893. https://doi.org/10.1177/0022034509343280.

Calderón, J., Ortiz-Pérez, D., Yáñez, L., Díaz-Barriga, F., 2003. Human exposure to metals. Pathways of exposure biomarkers of effect, and host factors. Ecotoxicol. Environ. Saf. 56, 93–103. https://doi.org/10.1016/S0147-6513(03)00053-8.

Caldwell, B.M., Bradley, R.H., 1984. HOME Observation for Measurement of the Environment. University of Arkansas. Little Rock.

Carrascosa, A., Yeste, D., Copil, A., Almar, A., Salcedo, S., Gussinyé, M., 2004. [Anthropometric growth patterns of preterm and full-term newborns (24-42 weeks' gestational age) at the Hospital Materno-Infantil Vall d'Hebron (Barcelona)(1997-2002)]. An. Pediatr. (Barc). 60, 406–416. https://doi.org/10.1016/s1695-4033(04)78299-5.

Choi, A.L., Sun, G., Zhang, Y., Grandjean, P., 2012. Developmental fluoride neurotoxicity: a systematic review and meta-analysis. Enviromental Heal. Perspect. 120, 1362–1368. https://doi.org/10.1016/j.placenta.2016.12.005.

Choi, A.L., Zhang, Y., Sun, G., Bellinger, D.C., Wang, K., Yang, X.J., et al., 2015. Association of lifetime exposure to fluoride and cognitive functions in Chinese children: a pilot study. Neurotoxicol. Teratol. 47, 96–101. https://doi.org/10.1016/j.ntt.2014.11.001.

Domingo-Salvany, A., Bacigalupe, A., Carrasco, J.M., Espelt, A., Ferrando, J., Borrell, C., 2013. Proposals for social class classification based on the Spanish National Classification of Occupations using neo-Weberian and neo-Marxist approaches. Gac. Sanit. 27, 263–272. https://doi.org/10.1016/j.gaceta.2012.12.009.

Dominguez-Berjon, M.F., Borrell, C., Cano-Serral, G., Esnaola, S., Nolasco, A., Pasarin, M. I., et al., 2008. Constructing a deprivation index based on census data in large Spanish cities(the MEDEA project). Gac. Sanit. 22, 179–187. https://doi.org/10.1157/13123961.

Elias, M.F., Elias, P.K., Seliger, S.L., Narsipur, S.S., Dore, G.A., Robbins, M.A., 2009. Chronic kidney disease, creatinine and cognitive functioning. Nephrol. Dial. Transplant. 24, 2446–2452. https://doi.org/10.1093/ndt/gfp107.

Evans, S.F., Kobrosly, R.W., Barrett, E.S., Thurston, S.W., Calafat, A.M., Weiss, B., 2014. Prenatal bisphenol A exposure and maternally reported behavior in boys and girls. Neurotoxicology 91–99. https://doi.org/10.1016/j.neuro.2014.10.003, 2014.

Faraone, S.V., Asherson, P., Banaschewski, T., Biederman, J., Buitelaar, J.K., Ramos-Quiroga, J.A., et al., 2015. Attention-deficit/hyperactivity disorder. Nat. Rev. Dis. Prim. 1, 15020. https://doi.org/10.1038/nrdp.2015.20.

Goasdoué, K., Miller, S.M., Colditz, P.B., Björkman, S.T., 2017. Review: the blood-brain barrier; protecting the developing fetal brain. Placenta 54, 111–116. https://doi.org/10.1016/j.placenta.2016.12.005.

Gobierno, E.K.U.I.S.. Vasco. Departamento de Salud. Sistema de Información de Aguas de Consumo de la CAPV. http://www.osakidetza.euskadi.net/r85-cksalu10/es/j15aWar/j15aindex.jsp. (Accessed 20 February 2021).

Gochfeld, M., 2017. Sex differences in human and animal toxicology. Toxicol. Pathol. 45, 172–189. https://doi.org/10.1177/0192623316677327.

Grandjean, P., 2019. Developmental fluoride neurotoxicity: an updated review. Environ. Health 18, 110. https://doi.org/10.1186/s12940-019-0551-x.

Green, R., Lanphear, B., Hornung, R., Flora, D., Martinez-Mier, E.A., Neufeld, R., et al., 2019. Association between maternal fluoride exposure during pregnancy and IQ scores in offspring in Canada. JAMA Pediatr 173, 940–948. https://doi.org/10.1001/jamapediatrics.2019.1729.

Guxens, M., Ballester, F., Espada, M., Fernández, M.F., Grimalt, J.O., Ibarluzea, J., et al., 2012. Cohort profile: the INMA-INfancia y Medio Ambiente-(environment and childhood) project. Int. J. Epidemiol. 41, 930–940. https://doi.org/10.1093/ije/dyr054.

Hailpern, S.M., Melamed, M.L., Cohen, H.W., Hostetter, T.H., 2007 Jul. Moderate chronic kidney disease and cognitive function in adults 20 to 59 years of age: third National Health and Nutrition Examination Survey (NHANES III). J. Am. Soc. Nephrol. 18 (7), 2205–2213. https://doi: 10.1681/ASN.2006101165.

Indermitte, E., Saava, A., Karro, E., 2009. Exposure to high fluoride drinking water and risk of dental fluorosis in Estonia. Int. J. Environ. Res. Publ. Health 6, 710–721. https://doi.org/10.3390/ijerph6020710.

Jiang, S., Su, J., Yao, S., Zhang, Y., Cao, F., Wang, F., Wang, H., Li, J., Xi, S., 2014. Fluoride and arsenic exposure impairs learning and memory and decreases mGluR 5 expression in the hippocampus and cortex in rats. PLoS One 23 (4). https://doi.org/10.1371/journal.pone.0096041.

Jones, S., Burt, B.A., Petersen, P.E., Lennon, M.A., 2005. The effective use of fluorides in public health. Bull. World Health Organ. 83, 670–676.

Khan, S.A., Singh, R.K., Navit, S., Chadha, D., Johri, N., Navit, P., et al., 2015. Relationship between dental fluorosis and intelligence quotient of school going children in and around lucknow district: a cross sectional study. J. Clin. Diagn. Res. 9, ZC10–ZC15. https://doi.org/10.7860/JCDR/2015/15518.6726.

Kumar, R.K., Reddy, K.S., Reddy, N.V., Karthik, T., Reddy, M.A., Nagakishore, 2020. Relationship between dental fluorosis and I.Q of school going children aged 10-12 years in and around Nalgonda district-A cross-sectional study. J. Indian Soc. Pedod. Prev. Dent. 38, 332–337. https://doi.org/10.4103/JISPPD.JISPPD_160_20.

Lertxundi, A., Andiarena, A., Martínez, M.D., Ayerdi, M., Murcia, M., Estarlich, M., et al., 2019. Prenatal exposure to PM2.5 and NO2 and sex-dependent infant cognitive and motor development. Environ. Res. 174, 114–121. https://doi.org/10.1016/j.envres.2019.04.001.

Levie, D., Korevaar, T.I.M., Bath, S.C., Murcia, M., Dineva, M., Llop, S., et al., 2019. Association of maternal iodine status with child IQ: a meta-analysis of individual participant data. J. Clin. Endocrinol. Metab. 104, 5957. https://doi.org/10.1210/jc.2018-02559, 596.

Li, Y., Jing, X., Chen, D., Lin, L., Wang, Z., 2003. The effects of endemic fluoride poisoning on the intellectual development of children in Baotou. Chin. J. Public Health Mangement 19, 337–338.

Li, X.H., Hou, G.Q., Yu, B., Yuan, C.S., Liu, Y., Zhang, L., et al., 2010. Investigation and analysis of children's intelligence and dental fluorosis in high fluoride area. J Med Pest Control 26, 230–231.

Limón-Pacheco, J.H., Jiménez-Córdova, M.I., Cárdenas-González, M., Sánchez Retana, I. M., Gonsebatt, M.E., Del Razo, L.M., 2018. Potential Co-exposure to arsenic and fluoride an.d biomonitoring equivalents for Mexican children. Ann Glob IIealth 27, 257–273. https://doi.org/10.29024/aogh.913.

Liteplo, R., 2002. World health organization., united nations environment Programme., international labour organisation., inter-organization Programme for the sound management of chemicals., international program on chemical safety. In: WHO Task Group on Environmental Health Criteria for Fluorides. World Health Organization, Fluorides.

Liu, Y., Téllez-Rojo, M., Howard Hu, H., Sánchez B, N., Martinez-Mier, E.A., Bas, N., et al., 2019. Fluoride exposure and pubertal development in children living in Mexico City. Environ. Health 18, 26. https://doi.org/10.1186/s12940-019-0465-7, 2019.

Llop, S., Guxens, M., Murcia, M., Lertxundi, A., Ramon, R., Riaño, I., et al., 2012. Prenatal exposure to mercury and infant neurodevelopment in a multicenter cohort in Spain: study of potential modifiers. Am. J. Epidemiol. 175, 451–465. https://doi.org/10.1093/aje/kwr328.

Mahaffey, K.R., Clickner, R.P., Jeffries, R.A., 2009. Adult women's blood mercury concentrations vary regionally in the United States: association with patterns of fish consumption (NHANES 1999–2004). Environ. Health Perspect. 117, 47–53. https://doi.org/10.1289/ehp.11674.

Malin, A.J., Till, C., 2015. Exposure to fluoridated water and attention deficit hyperactivity disorder prevalence among children and adolescents in the United States: an ecological association. Environ. Health 14, 17. https://doi.org/10.1186/s12940-015-0003-1.

Marthaler, T., 1999. Monitoring of Renal Fluoride Excretion in Community Preventive Programmes on Oral Health. World Health Organization, Geneva, Switzerland.

McCarthy, D., 2009. MSCA. Escalas McCarthy de Aptitudes y Psicomotricidad para Niños. TEA aditions, Madrid.

Mondal, D., Dutta, G., Gupta, S., 2016. Inferring the fluoride hydrogeochemistry and effect of consuming fluoride-contaminated drinking water on human health in some endemic areas of Birbhum district, West Bengal. Environ. Geochem. Health 38, 557–576. https://doi.org/10.1007/s10653-015-9743-7.

Mullenix, P.J., Denbesten, P.K., Schunior, A., Kernan, W.J., 1995. Neurotoxicity of sodium fluoride in rats. Neurotoxicol. Teratol. 17, 169–177. https://doi.org/10.1016/0892-0362(94)00070-t.

Nilsen, F.M., Ruiz, J.D.C., Tulve, N.S., 2020. A meta-analysis of stressors from the total environment associated with children's general cognitive ability. International Journal of Environmental Research and Public Health 2020 17. https://doi.org/10.3390/ijerph17155451.

NRC (National Research Council), 2006. Fluoride in Drinking Water: a Scientific Review of EPA's Standards. National Academies Press, Washington DC.

NTP (National Toxicology Program), 2016. Systematic Literature Review on the Effects of Fluoride on Learning and Memory in Animal Studies. National Institute of Environmental Health Sciences, Research Triangle Park.

Peckham, S., Lowery, D., Spencer, S., 2015. Are fluoride levels in drinking water associated with hypothyroidism prevalence in England? A large observational study of GP practice data and fluoride levels in drinking water. J. Epidemiol. Community Health 69, 619 624. https://doi.org/10.1136/jech 2014 204971.

Pettit, G.S., Bates, J.E., Dodge, K.A., 1997. Supportive parenting, ecological context,and children's adjustment: a seven-year longitudinal study. Child Dev. 68, 908–923. https://doi.org/10.1111/j.1467-8624.1997.tb01970.x.

Poureslami, H.R., Horri, A., Khoramian, S., Garrusi, B., 2011. Intelligence quotient of 7 to 9 year-old children from an area with high fluoride in drinking water. J. Dent. Oral Hyg. 3, 61–64.

Razdan, P., Patthi, B., Kumar, J.K., Agnihotri, N., Chaudhari, P., Prasad, M., 2017. Effect of fluoride concentration in drinking water on intelligence quotient of 12–14-year-old children in mathura district: a cross-sectional study. J. Int. Soc. Prev. Community Dent. 7, 252. https://doi.org/10.4103/JISPCD.JISPCD_201_17.

Rocha-Amador, D., Navarro, M.E., Carrizales, L., Morales, R., Calderón, J., 2007. Decreased intelligence in children and exposure to fluoride and arsenic in drinking water. Cad. Saúde Pública 23, S579–S587. https://doi.org/10.1590/s0102-311x2007001600018.

Sebastian, S., Sunitha, S., 2015. A cross-sectional study to assess the intelligence quotient (IQ) of school going children aged 10-12 years in villages of Mysore district, India with different fluoride levels. J. Indian Soc. Pedod. Prev. Dent. 33, 307–311. https://doi.org/10.4103/0970-4388.165682.

Thomas, D.B., Basu, N., Martinez-Mier, E.A., Sánchez, B.N., Zhang, Z., Liu, Y., et al., 2016. Urinary and plasma fluoride levels in pregnant women from Mexico City. Environ. Res. 150, 489–495. https://doi.org/10.1016/j.envres.2016.06.046. Epub 2016 Jul 14. PMID: 27423051.

Till, C., Green, R., Grundy, J.G., Hornung, R., Neufeld, R., Martinez-Mier, E.A., et al., 2018. Community water fluoridation and urinary fluoride concentrations in a national sample of pregnant women in Canada. Environ. Health Perspect. 126, 107001. https://doi.org/10.1289/EHP3546.

U.S. Department of Health and Human Services Federal Panel on Community Water Fluoridation, 2015. U.S. public health service recommendation for fluoride concentration in drinking water for the prevention of dental caries. Publ. Health Rep. 130, 21–28.

U.S. National Toxicology Program, N., 2016. NTP Research Report 1: Systematic Literature Review on the Effects of Fluoride on Learning and Memory in Animal Studies.

Valdez-Jiménez, L., López Guzmán, O.D., Cervantes Flores, M., Costilla-Salazar, R., Calderón Hernández, J., Alcaraz Contreras, Y., et al., 2017. In utero exposure to fluoride and cognitive development delay in infants. Neurotoxicology 59, 65–70. https://doi.org/10.1016/j.neuro.2016.12.011.

Vioque, J., Navarrete-Muñoz, E.M., Gimenez-Monzó, D., García-de-la-Hera, M., Granado, F., Young, I.S., et al., 2013. Reproducibility and validity of a food frequency questionnaire among pregnant women in a Mediterranean area. Nutr. J. 19, 12–26. https://doi.org/10.1186/1475-2891-12-26.

Wang, S.X., Wang, Z.H., Cheng, X.T., Li, J., Sang, Z.P., Zhang, X.D., 2007. Arsenic and fluoride exposure in drinking water: children's IQ and growth in Shanyin county, Shanxi province, China. Environ. Health Perspect. 115 (4), 643–647. https://doi.org/10.1289/ehp.9270.

Wechsler, D., 1997. WAIS-III Escala intelectual de Wechsler para adultos. TEA ediciones, Madrid, Spain.

Wechsler, D., Kaufman, A., 2001. WAIS-III. Escala de inteligencia de Wechsler para adultos (III). Tea Ediciones, Madrid, Spain.

World Health Organization (WHO), 2015. What Is a Preterm Baby?. WHO Team Maternal Health. World Health Organization.

Xu, K., An, N., Huang, H., Duan, L., Ma, Jun, Ding, J., et al., 2020. Fluoride exposure and intelligence in school-age children: evidence from different windows of exposure susceptibility. BMC Publ. Health 20, 1657. https://doi.org/10.1186/s12889-020-09765-4.

Yu, Y., Dong, W., Zhang, L., Xiao, H.L., 2008. Neurotransmitter and receptor changes in the brains of fetuses from areas of endemic fluorosis. Fluoride 41 (2), 134–138.

Zohouri, F., Swinbank, C., Maguire, A., Moynihan, P., 2006. Is the fluoride/creatinine ratio of a spot urine sample indicative of 24-h urinary fluoride? Community Dent. Oral Epidemiol. 34, 130–138. https://doi.org/10.1111/j.1600-0528.2006.00269.x.

Supplementary Table S1. Differences between the study sample and the women that gave birth (N=612) in Gipuzkoa cohort

	Bayley			McCarthy		
	Excluded; N=296	Incuded; N:316		Excludad: N=364	Included: N=248	
Mother	N (%) / mean (sd)		***p-value***	N (%) / mean (sd)		***p-value***
Maternal age	31.5 (3.83)	31.2 (3.40)	*0.246*	31.2 (3.75)	31.5 (3.40)	*0.341*
BMI	23.0 (3.88)	22.9 (3.31)	*0.556*	23.0 (3.73)	22.8 (3.39)	*0.537*
Educational level			*0.778*			*0.989*
Primary or without education	42 (14.2%)	39 (12.3%)		48 (13.2%)	33 (13.3%)	
Secondary	105 (35.5%)	112 (35.4%)		128 (35.2%)	89 (35.9%)	
University	148 (50.0%)	164 (51.9%)		186 (51.1%)	126 (50.8%)	
Missing	1 (0.3%)	1 (0.3%)		2 (0.5%)	0 (0.0%)	
Social class			*0.387*			*0.611*
No manual	177 (59.8%)	177 (56.0%)		207 (56.9%)	147 (59.23%)	
Manual	119 (40.2%)	139 (44.0%)		157 (43.1%)	101 (40.7%)	
Missing						
Mathernal IQ	9.7 (2.7)	9.9 (2.7)	*0.642*	9.7 (2.7)	9.9 (2.73)	*0.542*
Smoking in pregnancy			***0.010***			***0.011***
No	199 (67.2%)	252 (79.7%)		252 (69.2%)	199 (80.2%)	
Yes	81 (27.3%)	61 (19.3%)		97 (26.6%)	45 (18.1%)	
Missing	16 (5.4%)	3 (0.9%)		15 (4.1%)	4 (1.6%)	
Living zone			*0.133*			*0.118*
Non-fluoridated zone	124 (42.4%)	153 (48.8%)		154 (43.0%)	123 (49.80%)	
Fluoridated zone	168 (57.5%)	160 (51.1%)		204 (57.0%)	124 (50.2%)	
Missing	4 (1.3%)	3 (0.9%)		6 (1.6%)	1 (0.4%)	
Type of drinking water			*0.382*			*0.296*
Community fluoridated drinking water (CFDW)	110 (37.2%)	116 (36.7%)		138 (37.9%)	88 (35.5%)	
Community non-fluoridated drinking water (CNFDW)	91 (30.7%)	118 (37.3%)		114 (31.3%)	95 (38.3%)	
Bottled water	78 (26.3%)	77 (24.3%)		95 (26.1%)	60 (24.2%)	
Missing	17 (5.7%)	5 (1.5%)		17 (4.7%)	5 (2.0%)	
Parity			*0.018*			*0.06*
0	145 (490%)	186 (58.9%)		185 (50.8%)	146 (58.9%)	
>0	151 (51.0%)	130 (41.1%)		179 (49.2%)	102 (41.1%)	
Missing						
Child						
Order between brothers/sisters			*0.034*			*0.061*
Not first	147 (49.7%)	129 (40.8%)		176 (48.3%)	100 (40.3%)	
First	149 (50.3%)	187 (5928%)		188 (51.65%)	148 (59.7%)	
Sex			*0.057*			*0.961*
Female	135 (45.6%)	170 (53.8%)		182 (50.0%)	123 (49.6%)	
Male	160 (54.05)	146 (46.2%)		181 (49.7%)	125 (50.4%)	
Missing	1 (0.3%)	0 (0.0%)		1 (0.3%)	0 (0.0%)	
Nursery 1 year			*0.899*			*0.803*
No	129 (43.6%)	159 (50.3%)		165 (45.3%)	123 (49.6%)	

Yes	117 (39.5%)	139 (44.0%)		143 (39.3%)	113 (45.5%)	
Missing	50 (16.9%)	18 (5.70)		56 (15.4%)	12 (4.8%)	
Preterm			*0.293*			*0.357*
No	279 (94.3%)	305 (96.5%)		344 (94.5%)	240 (96.8%)	
Yes	13 (4.4%)	8 (2.5%)		15 (4.1%)	6 (2.4%)	
Missing	4 (1.3%)	3 (0.9%)		5 (1.4%)	2 (0.8%)	
Small for gestational age			*0.860*			*0.564*
No	261 (88.2%)	288 (91.1%)		327 (89.8%)	222 (89.5%)	
Yes	24 (8.1%)	24 (7.6%)		26 (7.1%)	22 (8.9%)	
Missing	11 (3.7%)	4 (1.3%)		11 (3.0%)	4 (1.6%)	
Breastfeeding weeks	28.7 (21.4)	29.15 (19.9)	*0.808*	29.3 (21.2)	28.5 (19.7)	*0.671*

p value obtained by chi-squared test for categorical variables and student t test for continuous variables

Supplementary Table S2. Levels of MUFcr -mg/g- (mean and 95% CI) during pregnancy by sample characteristics

		Bayley N=316	McCarthy N = 248
		MUFcr (mg/g) at pregnancy mean (95%CI)	**MUFcr (mg/g) at pregnancy mean (95%CI)**
Mother			
Maternal age			
<30		0.64 (0.56 , 0.72)	0.60 (0.52 , 0.69)
30-33		0.67 (0.61 , 0.74)	0.65 (0.58 , 0.72)
>30		0.64 (0.54 , 0.74)	0.66 (0.56 , 0.76)
	p-value	*0.754*	*0.621*
BMI			
Less than 18.5		0.67 (0.14 , 1.20)	0.66 (0.22 , 1.11)
18.5 - 25		0.66 (0.61 , 0.71)	0.64 (0.59 , 0.69)
25 - 30		0.62 (0.48 , 0.75)	0.58 (0.45 , 0.72)
More than 30		0.67 (0.45 , 0.89)	0.82 (0.54 , 1.10)
	p-value	*0.916*	*0.385*
Educational level			
Primary or without education		0.62 (0.49 , 0.75)	0.61 (0.45 , 0.76)
Secondary		0.65 (0.58 , 0.72)	0.63 (0.55 , 0.70)
University		0.67 (0.61 , 0.73)	0.65 (0.59 , 0.72)
	p-value	*0.720*	*0.771*
Social class			
No manual		0.67 (0.61 , 0.72)	0.67 (0.61 , 0.74)
Manual		0.64 (0.58 , 0.71)	0.58 (0.51 , 0.66)
	p-value	*0.579*	*0.062*
Index of deprivation (4 years)			
1 Very low		0.61 (0.44 , 0.78)	0.58 (0.43 , 0.73)
2 Low		0.66 (0.58 , 0.74)	0.65 (0.57 , 0.74)
3 Moderate		0.72 (0.65 , 0.79)	0.70 (0.62 , 0.79)
4 High		0.56 (0.46 , 0.66)	0.55 (0.44 , 0.66)
5 Very high		0.59 (0.38 , 0.80)	0.53 (0.27 , 0.79)
	p-value	*0.127*	*0.144*
Smoking in pregnancy			
No		0.66 (0.61 , 0.71)	0.63 (0.58 , 0.69)
Yes		0.64 (0.54 , 0.74)	0.67 (0.54 , 0.79)
	p-value	*0.789*	*0.626*
Zone			
Non-fluoridated zone		0.46 (0.42 , 0.50)	0.45 (0.40 , 0.49)
Fluoridated zone		0.84 (0.78 , 0.90)	0.82 (0.76 , 0.89)
	p-value	***<0.001***	***<0.001***
Type of drinking water			
Community fluoridated drinking water (CFDW)		0.91 (0.83 , 0.99)	0.89 (0.81 , 0.98)
Community non-fluoridated drinking water (CNFDW)		0.43 (0.39 , 0.47)	0.41 (0.36 , 0.45)
Bottled water		0.62 (0.56 , 0.69)	0.63 (0.55 , 0.71)
	p-value	***0.040***	*0.078*

Parity			
0		0.67 (0.61 , 0.73)	0.66 (0.59 , 0.72)
>0		0.63 (0.57 , 0.7)	0.61 (0.54 , 0.68)
	p-value	*0.434*	*0.379*
Child			
Order between brothers/sisters			
Not first		0.64 (0.57 , 0.7)	0.62 (0.54 , 0.69)
First		0.67 (0.61 , 0.72)	0.65 (0.59 , 0.72)
	p-value	*0.459*	*0.451*
Sex			
Female		0.66 (0.59 , 0.72)	0.62 (0.55 , 0.69)
Male		0.66 (0.59 , 0.72)	0.65 (0.59 , 0.72)
	p-value	*0.989*	*0.501*
Nursery 1 year			
No		0.62 (0.56 , 0.69)	0.59 (0.52 , 0.66)
Yes		0.68 (0.62 , 0.74)	0.68 (0.61 , 0.75)
	p-value	*0.194*	*0.073*
Preterm			
No		0.66 (0.61 , 0.70)	0.64 (0.59 , 0.69)
Yes		0.64 (0.27 , 1)	0.64 (0.27 , 1.01)
	p-value	*0.908*	*0.996*
Small for gestational age			
No		0.66 (0.61 , 0.70)	0.64 (0.59 , 0.69)
Yes		0.59 (0.43 , 0.75)	0.66 (0.48 , 0.84)
	p-value	*0.385*	*0.793*
Breastfeeding weeks			
0		0.59 (0.47 , 0.70)	0.58 (0.46 , 0.71)
>0-16		0.68 (0.55 , 0.81)	0.62 (0.5 , 0.74)
>16-24		0.69 (0.58 , 0.80)	0.68 (0.55 , 0.80)
>24		0.64 (0.58 , 0.69)	0.64 (0.57 , 0.70)
	p-value	*0.583*	*0.772*
MUFcr -mg/g- (mean and 95% CI)			
Whole pregnancy		0.66 (0.61;0.70)	0.64 (0.59;0.68)
Week 12 of pregnancy		0.57 (0.52;0.62)	0.55 (0.50;0.60)
Week 32 of pregnancy		0.74 (0.69;0.79)	0.73 (0.67;0.79)
p-value		**<.001**	**<.001**

T test or ANOVA (two or more than two categories)

Supplementary Table S3. Maternal levels during pregnancy of MUFcr, MUF and Iodine in urine; Mn and As in serum, and Hg and Pb in cord blood.

N=248 McCarthy	min	max	IQ	mean	sd
MUF (mg/L) at pregnancy	0.08	1.67	0.35	0.48	0.27
MUF (mg/L) at week 12	0.05	1.77	0.47	0.46	0.33
MUF (mg/L) at week 32	0.05	1.93	0.39	0.51	0.32
MUFcr (mg/g) at pregnancy	0.15	1.91	0.45	0.64	0.38
MUFcr (mg/g) at week 12	0.05	2.36	0.43	0.55	0.40
MUFcr (mg/g) at week 32	0.13	3.07	0.49	0.73	0.48
Iodine pregnancy * (µg/L) aj creatinine (g/L)	61.59	836.65	253.3	291.18	138.70
Iodine at week 12 (µg/L) aj creatinine (g/L)	36.15	1405.56	230.4	243.53	173.27
Iodine at week 32 (µg/L) aj creatinine (g/L)	54.40	1096.15	324.0	341.97	200.69
Manganese level at pregnancy (ng/mL)* aj creatinine (g/L)	0.03	18.87	0.16	0.36	1.48
Manganese level at week 12 (ng/mL) aj creatinine (g/L)	0.02	3.00	0.09	0.13	0.29
Manganese level at week 32 (ng/mL) aj creatinine (g/L)	0.02	37.69	0.16	0.58	2.97
Arsenic level at pregnancy (ng/mL)* aj creatinine (g/L)	4.59	1546.23	101.4	113.78	158.53
Arsenic level at week 12 (ng/mL) aj creatinine (g/L)	3.95	2504.28	94.3	117.47	244.47
Arsenic level at week 32 (ng/mL) aj creatinine (g/L)	3.34	1028.55	113.1	110.93	152.98
Mercury (mg/l)	1.41	38.00	9.3	9.69	5.56
Lead (µg/dL)	< LOQ	4.00	< LOQ	-	-

Supplementary Table S4. Characteristics of the fluoridated and non-fluoridated communities

N (%) / mean (sd)	Bayley N=316	Non fluoridated zone N=153	Fluoridated zone N=160	*p value*	McCarthy N=248	Non fluoridated zone N=123	Fluoridated zone N=124	
Mother								
Maternal age	31.19 (3.40)	31.34 (3.54)	31.08 (3.20)	*0.499*	31.52 (3.40)	31.97 (3.48)	31.02 (3.21)	***0.026***
BMI	22.86 (3.31)	23.10 (3.55)	22.64 (3.08)	*0.229*	22.83 (3.39)	22.86 (3.50)	22.76 (3.25)	*0.810*
Educational level				*0.388*				*0.561*
Primary or without education	39 (12.34%)	23 (15.13%)	16 (10.00%)		33 (13.31%)	19 (15.45%)	14 (11.29%)	
Secondary	112 (35.44%)	53 (34.87%)	58 (36.25%)		89 (35.89%)	45 (36.59%)	44 (35.48%)	
University	164 (51.90%)	76 (50.00%)	86 (53.75%)		126 (50.81%)	59 (47.97%)	66 (53.23%)	
Missing	1 (0.32%)	1 (0.65%)						
Social class				*0.357*				*0.223*
No manual	177 (56.01%)	81 (52.94%)	94 (58.75%)		147 (59.27%)	68 (55.28%)	79 (63.71%)	
Manual	139 (43.99%)	72 (47.06%)	66 (41.25%)		101 (40.73%)	55 (44.72%)	45 (36.29%)	
Mothernal IQ	9.90 (2.78)	9.78 (2.93)	10.03 (2.59)	*0.537*	9.91 (2.73)	9.79 (2.92)	10.03 (2.51)	*0.507*
Smoking in pregnancy				*0.241*				*0.718*
No	252 (79.75%)	118 (77.63%)	132 (83.54%)		199 (80.24%)	97 (80.17%)	101 (82.79%)	
Yes	61 (19.30%)	34 (22.37%)	26 (16.46%)		45 (18.15%)	24 (19.83%)	21 (17.21%)	
Missing	3 (0.95%)	1 (0.65%)	2 (1.25%)		4 (1.61%)	2 (1.63%)	2 (1.61%)	
Living zone				***<0.001***				***<0.001***
Non-fluoridated zone	153 (48.42%)	153 (100.00%)	0 (0.00%)		123 (49.60%)	123 (100.00%)	0 (0.00%)	
Fluoridated zone	160 (50.63%)	0 (0.00%)	160 (100.00%)		124 (50.00%)	0 (0.00%)	124 (100.00%)	
Missing	3 (0.95%)				1 (0.40%)			
Drinks water				***<0.001***				***<0.001***
Community fluoridated drinking water (CFDW)	116 (36.71%)	0 (0.00%)	116 (73.42%)		88 (35.48%)	0 (0.00%)	88 (72.13%)	
Community non-fluoridated drinking water (CNFDW)	118 (37.34%)	116 (76.32%)	1 (0.63%)		95 (38.31%)	94 (77.69%)	1 (0.82%)	
Bottled water	77 (24.37%)	36 (23.68%)	41 (25.95%)		60 (24.19%)	27 (22.31%)	33 (27.05%)	
Missing	5 (1.58%)	1 (0.65%)	2 (1.25%)		5 (2.02%)	2 (1.63%)	2 (1.61%)	
Parity				*0.74*				*0.755*
0	186 (58.86%)	88 (57.52%)	96 (60.00%)		146 (58.87%)	71 (57.72%)	75 (60.48%)	
>0	130 (41.14%)	65 (42.48%)	64 (40.00%)		102 (41.13%)	52 (42.28%)	49 (39.52%)	

Supplementary Table S4. Characteristics of the fluoridated and non-fluoridated communities (cont.)

N (%) / mean (sd)	Bayley N=316	Non fluoridated zone N=153	Fluoridated zone N=160	p value	McCarthy N=248	Non fluoridated zone N=123	Fluoridated zone N=124	p value
Child								
Order between brothers/sisters				*0.83*				*0.958*
Not first	129 (40.82%)	64 (41.83%)	64 (40.00%)		100 (40.32%)	50 (40.65%)	49 (39.52%)	
First	187 (59.18%)	89 (58.17%)	96 (60.00%)		148 (59.68%)	73 (59.35%)	75 (60.48%)	
Missing								
Sex				*0.932*				*0.408*
Female	170 (53.80%)	83 (54.25%)	85 (53.12%)		123 (49.60%)	65 (52.85%)	58 (46.77%)	
Male	146 (46.20%)	70 (45.75%)	75 (46.88%)		125 (50.40%)	58 (47.15%)	66 (53.23%)	
Nursery 1 year				***0.012***				*0.105*
No	159 (50.32%)	90 (61.22%)	68 (45.95%)		123 (49.60%)	69 (57.50%)	53 (46.09%)	
Yes	139 (43.99%)	57 (38.78%)	80 (54.05%)		113 (45.56%)	51 (42.50%)	62 (53.91%)	
Missing	18 (5.70%)	6 (3.92%)	12 (7.50%)		12 (4.84%)	3 (2.44%)	9 (7.26%)	
Preterm				*1.00*				*1.00*
No	305 (96.52%)	147 (97.35%)	156 (97.50%)		240 (96.77%)	119 (97.54%)	120 (97.56%)	
Yes	8 (2.53%)	4 (2.65%)	4 (2.50%)		6 (2.42%)	3 (2.46%)	3 (2.44%)	
Missing	3 (0.95%)	2 (1.31%)	0 (0.00%)		2 (0.81%)	1 (0.81%)	1 (0.81%)	
Small for gestational age				*0.731*				*1.00*
No	288 (91.14%)	138 (91.39%)	148 (93.08%)		222 (89.52%)	110 (90.91%)	111 (90.98%)	
Yes	24 (7.59%)	13 (8.61%)	11 (6.92%)		22 (8.87%)	11 (9.09%)	11 (9.02%)	
Missing	4 (1.27%)	2 (1.31%)	1 (0.62%)		4 (1.61%)	2 (1.63%)	2 (1.61%)	
Breastfeeding weeks	29.15 (19.86)	30.26 (20.27)	27.77 (19.44)	*0.282*	28.53 (19.74)	30.79 (20.07)	26.17 (19.27)	*0.073*
MUF (mg/L) at pregnancy	0.51 (0.29)	0.36 (0.21)	0.65 (0.29)	***<0.001***	0.48 (0.27)	0.35 (0.20)	0.62 (0.26)	***<0.001***
MUFcr (mg/g) at pregnancy	0.66 (0.39)	0.46 (0.25)	0.84 (0.40)	***<0.001***	0.64 (0.38)	0.45 (0.26)	0.82 (0.39)	***<0.001***
Manganese level at pregnancy (ng/mL)* aj creatinine (g/L)	0.37 (1.37)	0.26 (0.55)	0.48 (1.85)	*0.147*	0.36 (1.48)	0.25 (0.54)	0.47 (2.02)	*0.258*
Arsenic level at pregnancy (ng/mL)* aj creatinine (g/L)	106.09 (144.68)	104.16 (162.08)	109.03 (127.58)	*0.768*	113.78 (158.53)	115.27 (178.68)	112.34 (137.09)	*0.85*
Mercury (µg/l) (blood)	9.23 (5.39)	8.63 (5.20)	9.78 (5.56)	*0.076*	9.69 (5.56)	8.64 (4.66)	9.87 (4.63)	*0.053*
Iodine pregnancy * (µg/L) aj creatinine (g/L)	283.92 (146.81)	275.57 (140.33)	293.16 (153.63)	*0.291*	291.18 (138.70)	292.35 (137.14)	287.79 (139.09)	*0.796*
Pb (µg/dL) (blood) (LOQ = 2 µg/dL)				*0.931*				*0.600*
< 2	256 (81.0%)	124 (91.8%)	130 (92.8%)		201 (81.1%)	98 (90.7%)	102 (93.5%)	
≥2	21 (6.6%)	11 (8.1%)	10 (7.1%)		17 (6.8%)	10 (9.3)	7 (6.4%)	
Missing	39 (12.3%)	18 (11.7%)	20 (12.5%)		30 (12.1%)	15 (12.2%)	15 (12.1%)	

Supplementary Table 5. MUFcr (mg/g) during whole pregnancy in fluoridated and non-fluoridated zones by categories of sociodemographic characteristics at the 1 and 4 years old follow ups

Mean (95%CI)	Bayley N =316	Non-Fluoridated zone N=153	Fluoridated zone N=160	McCarthy N=248	Non-Fluoridated zone N=123	Fluoridated zone N=124
Maternal social class						
No manual	0.67 (0.61 , 0.72)	0.46 (0.4 , 0.51)	0.85 (0.77 , 0.93)	0.67 (0.61 , 0.74)	0.46 (0.4 , 0.52)	0.86 (0.77 , 0.95)
Manual	0.64 (0.58 , 0.71)	0.46 (0.4 , 0.52)	0.83 (0.73 , 0.93)	0.58 (0.51 , 0.66)	0.43 (0.36 , 0.5)	0.76 (0.65 , 0.88)
p-value	*0.579*	*0.979*	*0.710*	*0.062*	*0.569*	*0.195*
Mixed social class						
No manual	0.65 (0.6 , 0.71)	0.46 (0.4 , 0.51)	0.84 (0.77 , 0.92)	0.65 (0.59 , 0.71)	0.45 (0.4 , 0.51)	0.84 (0.76 , 0.92)
Manual	0.66 (0.58 , 0.73)	0.47 (0.4 , 0.54)	0.83 (0.72 , 0.95)	0.61 (0.52 , 0.69)	0.44 (0.36 , 0.52)	0.8 (0.66 , 0.93)
p-value	*0.951*	*0.786*	*0.901*	*0.422*	*0.819*	*0.610*
I. priv (1st trim)						
1	0.57 (0.41 , 0.72)	0.37 (0.28 , 0.46)	0.71 (0.46 , 0.96)	0.53 (0.39 , 0.67)	0.4 (0.29 , 0.51)	0.63 (0.41 , 0.86)
2	0.65 (0.58 , 0.73)	0.45 (0.39 , 0.52)	0.85 (0.73 , 0.96)	0.65 (0.57 , 0.73)	0.46 (0.38 , 0.55)	0.78 (0.68 , 0.89)
3	0.72 (0.65 , 0.79)	0.5 (0.42 , 0.58)	0.87 (0.78 , 0.96)	0.71 (0.63 , 0.79)	0.46 (0.37 , 0.55)	0.88 (0.78 , 0.99)
4	0.58 (0.47 , 0.69)	0.47 (0.38 , 0.57)	0.84 (0.57 , 1.11)	0.56 (0.45 , 0.68)	0.46 (0.36 , 0.55)	0.93 (0.59 , 1.27)
5	0.59 (0.38 , 0.8)	0.31 (0.21 , 0.41)	0.75 (0.42 , 1.08)	0.52 (0.24 , 0.8)	0.28 (0.19 , 0.37)	0.73 (0.22 , 1.25)
p-value	*0.142*	*0.294*	*0.705*	*0.117*	*0.531*	*0.276*
I. priv (3st trim)						
1	0.57 (0.41 , 0.72)	0.37 (0.28 , 0.46)	0.71 (0.46 , 0.96)	0.53 (0.4 , 0.66)	0.4 (0.29 , 0.51)	0.62 (0.42 , 0.82)
2	0.65 (0.58 , 0.73)	0.45 (0.39 , 0.52)	0.85 (0.73 , 0.96)	0.65 (0.57 , 0.73)	0.46 (0.38 , 0.55)	0.79 (0.68 , 0.9)
3	0.72 (0.65 , 0.79)	0.5 (0.42 , 0.58)	0.87 (0.78 , 0.96)	0.7 (0.62 , 0.78)	0.46 (0.37 , 0.54)	0.88 (0.78 , 0.99)
4	0.57 (0.47 , 0.68)	0.47 (0.38 , 0.56)	0.84 (0.57 , 1.11)	0.57 (0.45 , 0.68)	0.46 (0.36 , 0.55)	0.93 (0.59 , 1.27)
5	0.59 (0.38 , 0.8)	0.31 (0.21 , 0.41)	0.75 (0.42 , 1.08)	0.52 (0.24 , 0.8)	0.28 (0.19 , 0.37)	0.73 (0.22 , 1.25)
p-value	*0.112*	*0.281*	*0.705*	*0.136*	*0.533*	*0.233*
I. priv (4 years)						
1	0.61 (0.44 , 0.78)	0.48 (0.22 , 0.74)	0.71 (0.46 , 0.96)	0.58 (0.43 , 0.73)	0.53 (0.21 , 0.85)	0.62 (0.44 , 0.8)

2		0.66 (0.58 , 0.74)	0.46 (0.39 , 0.53)	0.84 (0.73 , 0.96)	0.65 (0.57 , 0.74)	0.47 (0.38 , 0.56)	0.78 (0.67 , 0.89)
3		0.72 (0.65 , 0.79)	0.5 (0.42 , 0.58)	0.87 (0.78 , 0.96)	0.7 (0.62 , 0.79)	0.46 (0.37 , 0.54)	0.89 (0.79 , 1)
4		0.56 (0.46 , 0.66)	0.44 (0.36 , 0.52)	0.87 (0.62 , 1.13)	0.55 (0.44 , 0.66)	0.43 (0.35 , 0.51)	0.97 (0.65 , 1.29)
5		0.59 (0.38 , 0.8)	0.31 (0.21 , 0.41)	0.75 (0.42 , 1.08)	0.53 (0.27 , 0.79)	0.28 (0.19 , 0.37)	0.72 (0.28 , 1.15)
	p-value	*0.127*	*0.384*	*0.704*	*0.144*	*0.408*	*0.123*

Supplementary Table S6. Pearson correlation coefficient between F, Iodine and metals (pregnancy/week 12/week 32) and Bayley and McCarthy scores of the different domains and Indexes.

	Bayley N =316	**McCarthy N = 248**	**Bayley N = 316**			**McCarthy N = 248**		
Metals (urine adjusted by creatinine)	**MUFcr (mg/g)**	**MUFcr (mg/g)**	**Mental**	**Verbal**	**Performance**	**Numeric**	**Memory**	**General cognitive**
MUFcr (mg/g) at pregnancy	-	-	0.04	0.11	0.19*	0.20*	0.14*	0.18*
MUFcr (mg/g) level at week 12 of pregnancy	-	-	0.01	0.06	0.14*	0.15*	0.10	0.12
MUFcr (mg/g) level at week 32 of pregnancy	-	-	0.05	0.13*	0.17*	0.19*	0.14*	0.19*
Manganese level at pregnancy (µg/g)	0.08	0.10	-0.01	-0.05	-0.003	-0.02	-0.05	-0.03
Manganese level (µg/g) at week 12	*0.24**	*0.19**	*0.05*	*0.02*	*-0.004*	*0.02*	*-0.02*	*-0.01*
Manganese level (µg/g) at week 32	*0.09*	*0.10*	*-0.02*	*-0.05*	*-0.007*	*-0.03*	*-0.05*	*-0.03*
Arsenic level (µg/g) at pregnancy	0.09	0.08	0.04	0.02	0.05	0.08	0.08	0.06
Arsenic level (µg/g) at week 12	*0.13**	*0.14**	*0.00*	*0.05*	*0.02*	*0.01*	*0.09*	*0.05*
Arsenic level (µg/g) at week 32	*0.1*	*0.09*	*0.07*	*-0.04*	*0.09*	***0.16****	*0.02*	*0.06*
Mercury (µg/l) cord blood	0.10/0.07/0.10	0.07/0.06/0.05	0.01	0.02	0.08	**0.25***	**0.15***	0.10
Iodine levels (µg/g) at pregnancy	0.19*	0.12	0.02	0.01	0.02	0.00	0.02	0.02
Iodine levels (µg/g) at at week 12	*0.30**	*020**	*0.01*	*0.04*	*0.07*	*0.01*	*0.03*	*0.05*
Iodine levels at week 32 (µg/g)	*0.12**	*0.18**	*0.03*	*-0.03*	*-0.03*	*-0.01*	*-0.01*	*-0.02*

* p<0.05

Supplementary Table S7. Multiple lineal regression models for the association between MUFcr (mg/g) levels and cognitive domains scores (Bayley and McCarthy) during pregnancy stratified by sex

Boys	**Bayley N=146**	**McCarthy N=125**				
Beta (IC95%)	Mental	Verbal	Performance	Numeric	Memory	General cognitive
MUFcr (mg/g) at pregnancy	3.84 (-5.04 , 12.72)	**13.38 (2.81 , 23.95)***	**12.24 (2.87 , 21.61)***	**11.09 (1.79 , 20.4)***	**11.3 (1.90 , 20.7)***	**15.03 (5.3 , 24.75)****
MUFcr (mg/g) at week 12	2.96 (-5.09 , 11.01)	3.78 (-6.16 , 13.71)	9.11 (0.47 , 17.75)	5.03 (-3.65 , 13.7)	4.28 (-4.51 , 13.06)	7.14 (-2.06 , 16.33)
MUFcr (mg/g) at week 32	2.50 (-4.46 , 9.46)	**11.79 (4.22 , 19.36)****	**7.17 (0.24 , 14.09)***	**8.56 (1.81 , 15.31)***	**9.26 (2.47 , 16.05)****	**11.39 (4.33 , 18.44)****
Girls	**Bayley N=170**	**McCarthy N=123**				
Beta (IC95%)	Mental	Verbal	Performance	Numeric	Memory	General cognitive
MUFcr (mg/g) at pregnancy	0.75 (-6.92 , 8.43)	-1.31 (-9.35 , 6.74)	2.03 (-4.77 , 8.83)	3.03 (-3.96 , 10.03)	-2.12 (-9.32 , 5.09)	-0.02 (-7.16 , 7.12)
MUFcr (mg/g) at week 12	-1 (-8.07 , 6.07)	-0.91 (-8.78 , 6.96)	1.10 (-5.53 , 7.73)	2.92 (-3.95 , 9.78)	-1.40 (-8.46 , 5.67)	0.21 (-6.77 , 7.19)
MUFcr (mg/g) at week 32	1.7 (-4.30 , 7.71)	-0.93 (-7.01 , 5.15)	1.69 (-3.44 , 6.83)	1.55 (-3.74 , 6.85)	-1.72 (-7.17 , 3.72)	-0.16 (-5.55 , 5.23)

Adjusted by age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, maternal social class, IQ and smoking. * = $p < .05$, ** = $p < .01$ and *** = $p < .001$ in beta coefficients.

Supplementary Table S8. Multiple lineal regression models for the association between MUFcr (mg/g) levels and cognitive domains scores (Bayley and McCarthy) during pregnancy adjusted by As urinary levels

		Bayley N = 316	**McCarthy N = 248**				
Beta (IC95%)		Mental	Verbal	Performance	Numeric	Memory	General cognitive
MUFcr (mg/g) at pregnancy	*All/Boys*	1.07 (-4.66 , 6.79)	**13.73 (3.72 , 23.74) †***	**5.86 (0.29 , 11.43) ɬ ***	**6.11 (0.5 , 11.71)***	**11.59 (2.54 , 20.65) †***	**15.27 (6.14 , 24.4) †***
	Girls		-1.55 (-9.38 , 6.28)	2.54 (-4.39, 9.46)		-1.78 (-8.87 , 5.3)	-0.26 (-7.4 , 6.89)
MUFcr (mg/g) at week 12	*All/Boys*	0.53 (-4.88 , 5.94)	0.35 (-5.77 , 6.46)	4.52 (-0.83 , 9.87)	4.48 (-0.95 , 9.91)	0.88 (-4.59 , 6.35)	2.77 (-2.79 , 8.32)
	Girls						
MUFcr (mg/g) at week 32	*All/Boys*	1.29 (-3.21 , 5.79)	**12.16 (4.85 , 19.47) †***	3.73 (-0.48 , 7.95)	3.51 (-0.65 , 7.67)	8.49 (1.97 , 15.01)	**11.17 (4.47 , 17.87) †***
	Girls		-0.54 (-6.54 , 5.46)			-1.52 (-6.88 , 3.83)	-0.15 (-5.65 , 5.36)

Adjusted by age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, maternal social class, IQ, smoking, cohort and As urinary levels.
† MUFcr and sex interaction statistically significant at the p <0.05 level. ɬ = interaction significant at the p <0.10 level. When this interaction is significant, first coefficient indicates the effect found in boys and the second in girls. When there is no interaction, the first and only coefficient is indicative of the effect detected for the whole sample. * = p < .05, ** = p < .01 and *** = p < .001 in beta coefficients.

Supplementary Table S9. Multiple lineal regression models for the association between MUFcr (mg/g) levels and cognitive domains scores (Bayley and McCarthy) during pregnancy adjusted by Mn urinary levels

		Bayley N = 316	**McCarthy N = 248**				
Beta (IC95%)		Mental	Verbal	Performance	Numeric	Memory	General cognitive
MUFcr (mg/g) at pregnancy	*All/Boys*	1.76 (-3.99 , 7.5)	**14.06 (4.08 , 24.04) †****	**6.29 (0.72 , 11.86)****	**6.51 (0.89 , 12.13)***	**11.79 (2.77 , 20.82) †***	**15.68 (6.6 , 24.76) †*****
	Girls		-1.09 (-8.96 , 6.78)			-1.44 (-8.56 , 5.68)	0.35 (-6.81 , 7.52)
MUFcr (mg/g) at week 12	*All/Boys*	1.20 (-4.08 , 6.48)	0.88 (-5.17 , 6.92)	4.23 (-1.04 , 9.51)	4.35 (-1.01 , 9.71)	1.17 (-4.24 , 6.59)	2.98 (-2.5 , 8.46)
	Girls						
MUFcr (mg/g) at week 32	*All/Boys*	1.69 (-2.8 , 6.18)	**11.91 (4.61 , 19.2) †****	4.11 (-0.07 , 8.29)	3.97 (-0.19 , 8.12)	**8.35 (1.84 , 14.85) †***	**11.26 (4.61 , 17.91) †***
	Girls		-0.57 (-6.58 , 5.44)			-1.5 (-6.85 , 3.86)	

Adjusted by age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, maternal social class, IQ, smoking, cohort and Mn urinary levels.
† MUFcr and sex interaction statistically significant at the p <0.10 level. When this interaction is significant, first coefficient indicates the effect found in boys and the second in girls. When there is no interaction, the first and only coefficient is indicative of the effect detected for the whole sample. * = p < .05, ** = p < .01 and *** = p < .001 in beta coefficients.

Supplementary Table S10. Multiple lineal regression models for the association between MUFcr (mg/g) levels and cognitive domains scores (Bayley and McCarthy) during pregnancy adjusted by Pb levels (unit)

		Bayley N = 316	**McCarthy N = 248**				
Beta (IC95%)		Mental	Verbal	Performance	Numeric	Memory	General cognitive
MUFcr (mg/g) at pregnancy	*All/Boys*	2.70 (-3.34 , 8.75)	10.21 (-1.2 , 21.63) **†**	4.44 (-1.48 , 10.36)	5.69 (-0.34 , 11.71)	0.97 (-5.26 , 7.2)	**12.43 (1.96 , 22.9) †**
	Girls		-1.85 (-9.77 , 6.08)				-1.01 (-8.28 , 6.25)
MUFcr (mg/g) at week 12	*All/Boys*	0.92 (-4.46 , 6.31)	-1.33 (-7.47 , 4.8)	4 (-1.43 , 9.43)	3.81 (-1.75 , 9.37)	-0.36 (-6.06 , 5.33)	1.36 (-4.3 , 7.02)
	Girls						
MUFcr (mg/g) at week 32	*All/Boys*	2.65 (-2.06 , 7.36)	**9.86 (1.77 , 17.95) † ***	2.27 (-2.14 , 6.68)	3.64 (-0.84 , 8.13)	1.23 (-3.39 , 5.85)	**8.61 (1.12 , 16.09) †**
	Girls		-0.4 (-6.38 , 5.57)				-0.4 (-5.92 , 5.13)

Adjusted by age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, maternal social class, IQ, smoking, cohort and Pb levels (<LOD/>LOD).
† MUFcr and sex interaction statistically significant at the p <0.05 level. **†** = interaction significant at the p <0.10 level. When this interaction is significant, first coefficient indicates the effect found in boys and the second girls. In the rest of the cases, the first and only coefficient is indicative of the effect detected for the whole sample. * = p < .05, ** = p < .01 and *** = p < .001 in beta coefficients.

Supplementary Table S11. Multiple lineal regression models for the association between MUFcr (mg/g) levels and cognitive domains scores (Bayley and McCarthy) during pregnancy adjusted by maternal urinary Iodine levels

		Bayley N = 316	**McCarthy N = 248**				
Beta (IC95%)		Mental	Verbal	Performance	Numeric	Memory	General cognitive
MUFcr (mg/g) at pregnancy	*All/Boys*	1.84 (-3.92 , 7.59)	**13.79 (3.71 , 23.87) † ****	**5.67 (0.11 , 11.23) ǂ ****	**6.25 (0.64 , 11.86)***	**11.68 (2.57 , 20.8) † ***	**15.16 (5.97 , 24.34) † ****
	Girls		-1.49 (-9.31 , 6.34)	2.02 (-4.87, 8.91)		-1.76 (-8.84 , 5.31)	-0.20 (-7.33 , 6.93)
MUFcr (mg/g) at week 12	*All/Boys*	1.09 (-4.21 , 6.39)	0.91 (-5.13 , 6.96)	4.33 (-0.86 , 9.52)	4.65 (-0.66 , 9.96)	1.69 (-3.75 , 7.14)	**3.18 (-2.33 , 8.69)***
	Girls						
MUFcr (mg/g) at week 32	*All/Boys*	1.71 (-2.89 , 6.31)	**12.47 (5.15 , 19.8) † *****	3.75 (-0.62 , 8.12)	**8.81 (2.01, 15.60) ǂ***	2.26 (-2.19 , 6.70) **†**	**11.62 (4.74 , 18.51) † ****
	Girls		-1.95 (-7.83 , 3.92)		1.15 (-4.30, 6.60	-2.21 (-7.67 , 3.25)	-1.12 (-6.64 , 4.41)

Adjusted by age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, maternal social class, IQ, smoking, cohort and urinary Iodine levels.
† MUFcr and sex interaction statistically significant at the p <0.05 level. ǂ = interaction significant at the p <0.10 level. When this interaction is significant, first coefficient indicates the effect found in boys and the second ir girls. When there is no interaction, the first and only coefficient is indicative of the effect detected for the whole sample. * = p < .05, ** = p < .01 and *** = p < .001 in beta coefficients.

Supplementary Table S12. Multiple lineal regression models for the association between MUFcr (mg/g) levels and cognitive domains scores (Bayley and McCarthy) during pregnancy and adjusted by child's quality of the family context (Haezi-Etxadi Scale -HES-)

		Bayley N = 316			**McCarthy N = 248**		
Beta (IC95%)		Mental	Verbal	Performance	Numeric	Memory	General cognitive
MUFcr (mg/g) at pregnancy	*All/Boys*	0.82 (-5.15 , 6.79)	**11.96 (1.35 , 22.57) † ***	**11.35 (2.47 , 20.23) † ***	5.64 (-0.30 , 11.58)	**11.34 (1.76 , 20.93) † ***	**13.67 (4.31 , 23.02) † ****
	Girls		-2.69 (-11.12 , 5.74)	-0.61 (-7.66 , 6.45)		-4.8 (-12.42 , 2.81)	-2.87 (-10.31 , 4.57)
MUFcr (mg/g) at week 12	*All/Boys*	1.82 (-3.81 , 7.46)	0.16 (-6.47 , 6.80)	**10.9 (2.69 , 19.12) †****	4.06 (-1.81 , 9.93)	-0.15 (-6.17 , 5.87)	1.93 (-3.97 , 7.84)
	Girls			-1.63 (-8.86 , 5.61)			
MUFcr (mg/g) at week 32	*All/Boys*	-0.14 (-4.82 , 4.53)	**11.81 (3.77 , 19.84) † ****	2.28 (-1.95 , 6.52)	3.92 (-0.54 , 8.38)	**9.8 (2.49 , 17.1) † ****	**10.65 (3.49 , 17.8) † ****
	Girls		-1.72 (-7.87 , 4.42)			-3.07 (-8.66 , 2.52)	-1.70 (-7.17 , 3.78)

Adjusted by age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, maternal social class, IQ, smoking, cohort and child's quality of the family context (Haezi-Etxadi Scale -HES). † MUFcr and sex interaction statistically significant at the p <0.05 level. When this interaction is significant, first coefficient indicates the effect found in boys and the second in girls. When there is no interaction, the first and only coefficient is indicative of the effect detected for the whole sample. * = p < .05, ** = p < .01 and *** = p < .001 in beta coefficients.

Supplementary Table S13. Multiple lineal regression models for the association between MUFcr (mg/g) levels and cognitive domains scores (Bayley and McCarthy) during pregnancy adjusting for deprivation index

		Bayley N=316			**McCarthy N= 248**			
Beta (IC95%)		Mental	Verbal	Performance	Numeric	Memory	General cognitive	
MUFcr (mg/g) at pregnancy	*All/Boys*	1.39 (-4.44 , 7.23)	**12.65 (2.78 , 22.53) † ***	**6.62 (0.90 , 12.34)***	**6.39 (0.74 , 12.04)***	**10.78 (1.7 , 19.87) † ***	**15.15 (5.83 , 24.47) † ****
	Girls		-0.88 (-8.58 , 6.82)			-0.90 (-7.98 , 6.18)	0.8 (-6.46 , 8.06)
MUFcr (mg/g) at week 12	*All/Boys*	0.64 (-4.71 , 6)	0.72 (-5.20 , 6.63)	4.98 (-0.42 , 10.39)	4.45 (-0.91 , 9.82)	1.7 (-3.72 , 7.12)	3.4 (-2.20, 9)
	Girls						
MUFcr (mg/g) at week 32	*All/Boys*	1.36 (-3.17 , 5.89)	**11.99 (4.86 , 19.11) † ****	**4.36 (0.05 , 8.66)***	**4.38 (0.13 , 8.63)***	**9.02 (2.42 , 15.62) † ****	**11.89 (5.11 , 18.66) † *****
	Girls		-0.73 (-6.54 , 5.07)			-1.23 (-6.61 , 4.15)	0.25 (-5.27 , 5.77)

Adjusted by sex of the child, age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, MEDEA Index of deprivation, IQ and smoking.
† MUFcr and sex interaction statistically significant at the p <0.05 level. When there is no interaction, first coefficient indicates the effect found in boys and the second in girls. In the rest of the cases, the first and only coefficient is indicative of the effect detected for the whole sample. * = $p < .05$, ** = $p < .01$ and *** = $p < .001$ in beta coefficients.

Table S14. Multiple lineal regression models for the association between MUFcr (mg/g) levels and cognitive domains scores (Bayley and McCarthy) during pregnancy adjusted by As urinary levels and Pb blood levels

		Bayley N = 316			**McCarthy N = 248**		
Beta (IC95%)		Mental	Verbal	Performance	Numeric	Memory	General cognitive
MUFcr (mg/g) at pregnancy	*Boys*	2.5 (-3.61 , 8.61)	**10.29 (-1.19 , 21.76)ɬ**	4.42 (-1.53 , 10.38)	5.62 (-0.44 , 11.69)	1.00 (-5.27 , 7.26)	**12.43 (1.91 , 22.96)†***
	Girls		-1.8 (-9.76 , 6.16)				-0.94 (-8.26 , 6.38)
MUFcr (mg/g) at week 12		0.54 (-5 , 6.07)	-1.48 (-7.66 , 4.7)	4.44 (-1.06 , 9.94)	4.22 (-1.42 , 9.87)	-0.36 (-6.09 , 5.38)	1.55 (-4.11 , 7.22)
MUFcr (mg/g) at week 32	*Boys*	2.81 (-1.98 , 7.6)	**10.12 (1.93 , 18.31)†***	2.35 (-2.11 , 6.82)	3.05 (-1.38 , 7.48)	1.06 (-3.51 , 5.64)	**8.37 (0.78 , 15.95)ɬ***
	Girls		0.27 (-5.74 , 6.28)				0.09 (-5.48 , 5.65)

Adjusted by sex of the child, age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, maternal social class, IQ, smoking, cohort, As urinary levels, Pb levels (<LOD/>LOD). † Interaction between sex and MUFcr statistically significant at (p<0.05). **ɬ** = interaction significant at the *p* <0.10 level. No significative interaction between As and Pb was found.

Supplementary Table S15 Multiple lineal regression models for the association between MUFcr (mg/g) levels and cognitive domains scores (Bayley and McCarthy) during pregnancy adjusted by As and Mn urinary, Hg cord blood levels and Pb levels (<LOD/>LOD)

		Bayley N = 316			**McCarthy N = 248**		
Beta (IC95%)		Mental	Verbal	Performance	Numeric	Memory	General cognitive
MUFcr (mg/g) at pregnancy	*All/Boys*	2.89 (-3.36 , 9.14)	2.62 (-4.22 , 9.46)	5.34 (-0.64 , 11.33)	**6.26 (0.23 , 12.28) ***	1.59 (-4.75 , 7.94)	**12.42 (1.91 , 22.93) † ***
	Girls						0.10 (-7.30 , 7.50)
MUFcr (mg/g) at week 12	*All/Boys*	1.31 (-4.27 , 6.89)	-1.97 (-8.11 , 4.18)	4.57 (-0.95 , 10.09)	4.20 (-1.32 , 9.71)	-0.35 (-6.1 , 5.40)	5.93 (-2.90 , 14.77)
	Girls						
MUFcr (mg/g) at week 32	*All/Boys*	2.81 (-2.06 , 7.68)	**9.83 (1.58 , 18.08) ɬ ***	2.54 (-1.95 , 7.02)	3.23 (-1.16 , 7.61)	1.25 (-3.31 , 5.82)	3.14 (-1.47 , 7.76)
	Girls		0.67 (-5.42 , 6.75)				

Adjusted by sex of the child, age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, maternal social class, IQ, smoking, cohort and As and Mn urinary, Hg cord blood levels and Pb (<LOD/>LOD).

† MUFcr and sex interaction statistically significant at the *p* <0.05 level. **ɬ** = interaction significant at the *p* <0.10 level. When this interaction is significant, first coefficient indicates the effect found in boys and the second in girls. When there is no interaction, the first and only coefficient is indicative of the effect detected for the whole sample: * = *p* < .05, ** = *p* < .01 and *** = *p* < .001 in beta coefficients.

Supplementary Table S16 Multiple lineal regression model for the association between the zone (fluoridated/non-fluoridated) and cognitive domains scores (Bayley and McCarthy)

		Bayley N=316			**McCarthy N= 248**		
Beta (IC 95%)		Mental	Verbal	Performance	Numeric	Memory	General cognitive
Fluoridated zone	No	0 (Ref.)	0 (Ref.)	0 (Ref.)	0 (Ref.)	0 (Ref.)	0 (Ref.)
	Yes	3.01 (-1.35 , 7.38)	1.91 (-2.7 , 6.52)	-0.39 (-4.46 , 3.68)	**4.72 (0.67 , 8.76)***	1.81 (-2.35 , 5.97)	0.68 (-3.56 , 4.93)

Adjusted by sex of the child, age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, maternal social class, IQ and smoking.
* = $p < .05$.

Supplementary Table S17. Multiple lineal regression models for the association between MUFcr (mg/g) levels and cognitive domains scores (Bayley and McCarthy) during pregnancy, also including women with only one sample of urine (first or third trimester) available.

		Bayley N=483	**McCarthy N=379**				
Beta (IC95%)		Mental	Verbal	Performance	Numeric	Memory	General cognitive
MUFcr (mg/g) at pregnancy	*All/Boys*	0.63 (-3.44 , 4.69)	**9.53 (2.75 , 16.32) †****	**4.3 (0.5 , 8.09) †****	**9.16 (2.82 , 15.5) †****	**7.3 (0.65 , 13.96)** †*	**11.66 (5.23 , 18.09)†*****
	Girls		-1.86 (-7.01 , 3.29)	0.89 (-3.76 , 5.53)	-1.00 (-3.81 , 3.81)	-2.22 (-7.27 , 2.83)	-1.14 (-6.01 , 3.74)
MUFcr (mg/g) at week 12	*All/Boys*	0.24 (-4.42 , 4.9)	1.89 (-3.33 , 7.11)	**4.72 (0.17 , 9.27)***	2.48 (-2.27 , 7.23)	2.5 (-2.43 , 7.43)	3.89 (-1.03 , 8.81)
	Girls						
MUFcr (mg/g) at week 32	*All/Boys*	1.16 (-2.67 , 4.99)	**10.64 (4.63 , 16.65) †****	**8.22 (2.68 , 13.76) †****	**8.68 (3.1 , 14.27) †****	**7.58 (1.82 , 13.34)** †**	**10.97 (5.4 , 16.53) †*****
	Girls		-2.49 (-7.34 , 2.37)	-0.12 (-4.59 , 4.35)	-0.13 (-4.64 , 4.38)	-2.76 (-7.41 , 1.89)	-2.15 (-6.64 , 2.34)

Adjusted by sex of the child, age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, maternal social class, IQ and smoking.
† MUFcr and sex interaction statistically significant at the p <0.05 level. When this interaction is significant, first coefficient indicates the effect found in boys and the second in girls. When there is no interaction, the first and only coefficient is indicative of the effect detected for the whole sample. * = $p < .05$, ** = $p < .01$ and *** = $p < .001$ in beta coefficients.

Supplementary Table 18. Multiple lineal regression models for the association between MUFcr (mg/g) levels and cognitive domains scores (Bayley and McCarthy) during adjusted by fluoridated and non-fluoridated zone

		Bayley N=316			McCarthy N= 248		
Beta (IC95%)		Mental	Verbal	Performance	Numeric	Memory	General cognitive
MUFcr (mg/g) at pregnancy	*Boys*	-0.52 (-7 , 5.95)	**13.35 (2.95 , 23.74) †***	**7.82 (1.58 , 14.07)***	4.08 (-2.21 , 10.36)	**10.96 (1.56 , 20.36) †***	**16.11 (6.64 , 25.59)†***
	Girls		-2.11 (-10.71 , 6.48)			-2.58 (-10.36 , 5.19)	0.69 (-7.15 , 8.52)
MUFcr (mg/g) at week 12		-1.00 (-6.66 , 4.65)	0.27 (-6.12 , 6.65)	5.50 (-0.07 , 11.07)	2.63 (-2.96 , 8.23)	1.01 (-4.74 , 6.77)	3.5 (-2.36 , 9.36)
MUFcr (mg/g) at week 32	*Boys*	0.33 (-4.52 , 5.19)	**11.71 (4.31 , 19.12) †***	**4.67 (0.08 , 9.26)***	2.53 (-2.06 , 7.13)	**8.71 (1.99 , 15.43) †***	**11.74 (4.94 , 18.53)†***
	Girls		-1.51 (-7.94 , 4.91)			-2.57 (-8.4 , 3.26)	-0.17 (-6.06 , 5.73)

Adjusted by sex of the child, age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, maternal social class, IQ , smoking and zone (fluoridated yes/no). † MUFcr and zone interaction statistically significant at the p <0.05 level. When this interaction is significant, first coefficient indicates the effect found in boys and the second in girls. When there is no interaction, the first and only coefficient is indicative of the effect detected for the whole sample. * = p < .05, ** = p < .01 and *** = p < .001 in beta coefficients.

Supplementary Table 19 Multiple lineal regression models for the association between MUFcr (mg/g) levels and cognitive domains scores (Bayley and McCarthy) during pregnancy excluding children with scores below 2SD

		Bayley N=309			**McCarthy N= 233**		
Beta (IC95%)		Mental	Verbal	Performance	Numeric	Memory	General cognitive
MUFcr (mg/g) at pregnancy	*All/Boys*	1.72 (-3.76 , 7.2)	**15.14 (6.14 , 24.15) ŧ****	**7.7 (2.25 , 13.16)****	**6.55 (0.84 , 12.26)***	**12.82 (3.96 , 21.67) ŧ****	**16 (7.42 , 24.57) ŧ*****
	Girls		-4.57 (-11.84 , 2.69)			-3.28 (-10.43 , 3.87)	-0.73 (-7.65 , 6.19)
MUFcr (mg/g) at week 12	*All/Boys*	1.05 (-3.95 , 6.06)	-0.18 (-5.67 , 5.32)	**6.15 (1.07 , 11.23)***	4.81 (-0.53 , 10.14)	1.32 (-4.01 , 6.66)	3.26 (-1.96 , 8.48)
	Girls						
MUFcr (mg/g) at week 32	*All/Boys*	1.45 (-2.84 , 5.74)	**14.05 (7.56 , 20.54) ŧ*****	**4.64 (0.55 , 8.74)***	4.2 (-0.07 , 8.47)	**10.66 (4.21 , 17.12) ŧ****	**12.77 (6.52 , 19.02) ŧ*****
	Girls		-3.18 (-8.52 , 2.16)			-2.89 (-8.2 , 2.42)	-1.09 (-6.23 , 4.05)

Adjusted by sex of the child, age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, maternal social class, IQ and smoking. **ŧ** MUFcr and sex interaction statistically significant at the p <0.05 level. **ŧ** = interaction significant at the p <0.10 level. When this interaction is significant, first coefficient indicates the effect found in boys and the second in girls. When there is no interaction, the first and only coefficient is indicative of the effect detected for the whole sample. * = p < .05, ** = p < .01 and *** = p < .001 in beta coefficients.

Supplemantary Table 20. Multiple lineal regression models for the association between MUFcr (mg/g) levels, stratified by fluoridated and non-fluoridated zone (MUFcr x Zone), and cognitive domains scores (Bayley and McCarthy) during pregnancy.

		Bayley N=316		**McCarthy N= 248**			
Beta (IC95%)	Fluoridated/Non-fluoridated Zone	Mental	Verbal	Performance	Numeric	Memory	General cognitive
MUFcr (mg/g): pregnancy	Both zones/ Non-fluoridated	-0.52 (-7 , 5.95)	**15.58 (3.71 , 27.45)†****	**7.82 (1.58 , 14.07)***	4.08 (-2.21 , 10.36)	2.71 (-3.77 , 9.18)	**15.46 (4.55 , 26.36)†****
	Fluoridated zone		-2.4 (-11.17 , 6.37)				1.96 (-6.09 , 10.02)
MUFcr (mg/g): week 12	Both zones	-1 (-6.66 , 4.65)	0.27 (-6.12 , 6.65)	5.5 (-0.07 , 11.07)	2.63 (-2.96 , 8.23)	1.01 (-4.74 , 6.77)	3.5 (-2.36 , 9.36)
MUFcr (mg/g): week 32	Both zones/ Non-fluoridated	0.33 (-4.52 , 5.19)	**16.11 (7.4 , 24.81)†****	**4.67 (0.08 , 9.26)***	2.53 (-2.06 , 7.13)	2.17 (-2.56 , 6.9)	**12.88 (4.82 , 20.94)†****
	Fluoridated zone		-2.3 (-8.6 , 3.99)				0.11 (-5.73 , 5.95)

Adjusted by sex of the child, age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, maternal social class, IQ , smoking and bottled zone (fluoridated yes/no). †*MUFcr and zone interaction statistically significant at the p <0.05 level. When there is no interaction, first coefficient indicates the effect found in non-fluoridated and the second fluoridated zones. When there is no interaction, the first and only coefficient is indicative of the effect detected for both zones. * = p < .05, ** = p < .01 and *** = p < .001.

Table 2. Multiple lineal regression models for the association between MUFcr (mg/g) levels and cognitive domains scores (Bayley and McCarthy) during pregnancy for boys

	Bayley N=146			**McCarthy N=125**		
Beta (IC95%)	Mental	Verbal	Performance	Numeric	Memory	General cognitive
Zona NO *fluorada*	*Bayley N=70*			*McCarthy N=58*		
MUFcr (mg/g) at pregnancy	-1.81 (-14.79 , 11.17)	**29.76 (14.09 , 45.43)**	**19.63 (5.89 , 33.38)**	12.08 (-0.38 , 24.54)	**15.56 (2.49 , 28.62)**	**27.71 (13.83 , 41.59)**
MUFcr (mg/g) at week 12	-3.64 (-15.76 , 8.48)	12.23 (-4.46 , 28.91)	16.13 (2.87 , 29.39)	2.88 (-9.35 , 15.1)	4.08 (-8.97 , 17.13)	**15.19 (0.57 , 29.81)**
MUFcr (mg/g) at week 32	0.24 (-8.57 , 9.04)	**20.67 (10.27 , 31.08)**	9.54 (-0.12 , 19.2)	**9.45 (1.21 , 17.7)**	**11.98 (3.39 , 20.57)**	**17.31 (7.75 , 26.88)**
Zona fluorada	*Bayley N=75*			*McCarthy N=66*		
MUFcr (mg/g) at pregnancy	2.63 (-13.08 , 18.34)	-1.13 (-16.19 , 13.93)	9.34 (-6.95 , 25.63)	3.45 (-13.07 , 19.97)	6.97 (-10.39 , 24.33)	5.06 (-11.46 , 21.58)
MUFcr (mg/g) at week 12	4.42 (-8.97 , 17.81)	0.51 (-12.32 , 13.34)	7.08 (-6.86 , 21.01)	3.58 (-10.47 , 17.63)	5.22 (-9.6 , 20.04)	4.54 (-9.53 , 18.6)
MUFcr (mg/g) at week 32	-0.61 (-14.71 , 13.48)	-1.86 (-13.77 , 10.05)	5.59 (-7.41 , 18.6)	1.23 (-11.87 , 14.33)	4.23 (-9.57 , 18.02)	2.43 (-10.7 , 15.55)

Adjusted by age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, maternal social class, IQ and smoking.

Supplementary Table 22. Multiple lineal regression models for the association between MUFcr (mg/g) levels and cognitive domains scores (Bayley and McCarthy) during pregnancy stratified by maternal social class; only for boys

	Bayley N=146			**McCarthy N=125**		
Beta (IC95%)	Mental	Verbal	Performance	Numeric	Memory	General cognitive
Maternal social class (manual)	*Bayley N=65*			*McCarthy N=50*		
MUFcr (mg/g) at pregnancy	5.52 (-10.69 , 21.73)	10.74 (-5.75 , 27.24)	10.81 (-4.42 , 26.03)	13.1 (-1.53 , 27.73)	**15.77 (1.1 , 30.44)**	13.63 (-2.43 , 29.69)
MUFcr (mg/g) at week 12	-0.37 (-14.17 , 13.42)	5.87 (-8.35 , 20.09)	9.06 (-3.86 , 21.98)	9.87 (-2.67 , 22.41)	**12.6 (0.06 , 25.15)**	9.11 (-4.76 , 22.97)
MUFcr (mg/g) at week 32	7.25 (-5.38 , 19.88)	7.71 (-4.88 , 20.3)	5.22 (-6.6 , 17.03)	7.22 (-4.19 , 18.64)	8.11 (-3.51 , 19.74)	8.45 (-3.97 , 20.87)
Maternal social class (non-manual	*Bayley N=81*			*McCarthy N=75*		
MUFcr (mg/g) at pregnancy	0.25 (-11.32 , 11.82)	**18.2 (3.74 , 32.66)**	**14.17 (1.01 , 27.34)**	9.69 (-3.17 , 22.54)	9.43 (-3.39 , 22.25)	**18.1 (4.91 , 31.29)**
MUFcr (mg/g) at week 12	3.19 (-8.47 , 14.85)	7.51 (-8.52 , 23.54)	12.45 (-1.6 , 26.5)	1.55 (-12.27 , 15.38)	-1.4 (-15.18 , 12.38)	9.88 (-4.76 , 24.52)
MUFcr (mg/g) at week 32	-1.55 (-10.42 , 7.32)	**14.47 (4.56 , 24.39)**	8.35 (-0.98 , 17.68)	8.78 (-0.06 , 17.62)	**9.84 (1.13 , 18.55)**	**13.33 (4.18 , 22.47)**

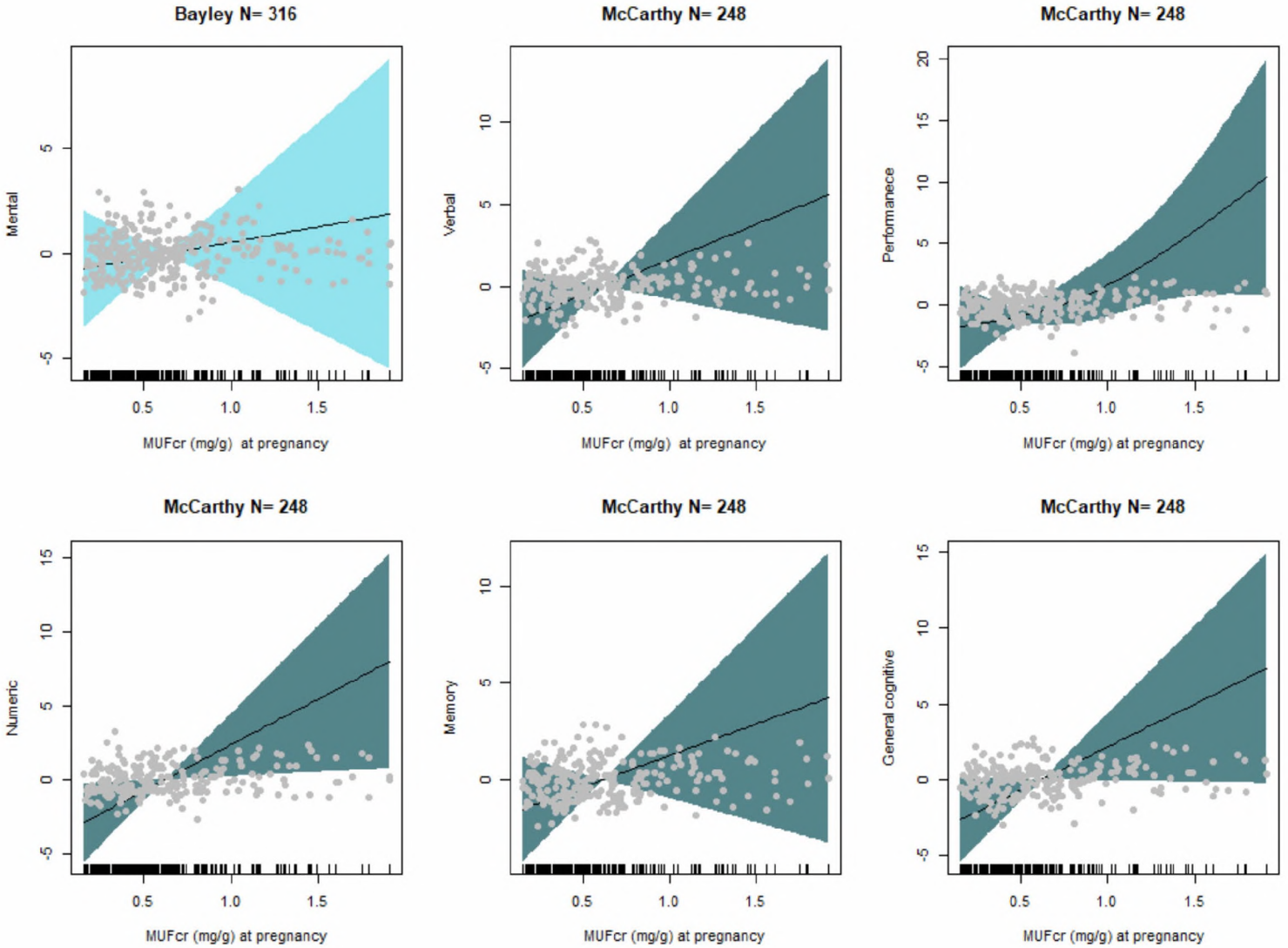
Adjusted by age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, IQ and smoking.

Supplementary Table 23. Multiple lineal regression models for the association between MUFcr (mg/g) levels and cognitive domains scores (Bayley and McCarthy) during pregnancy, stratified by quality of the family context; only for boys

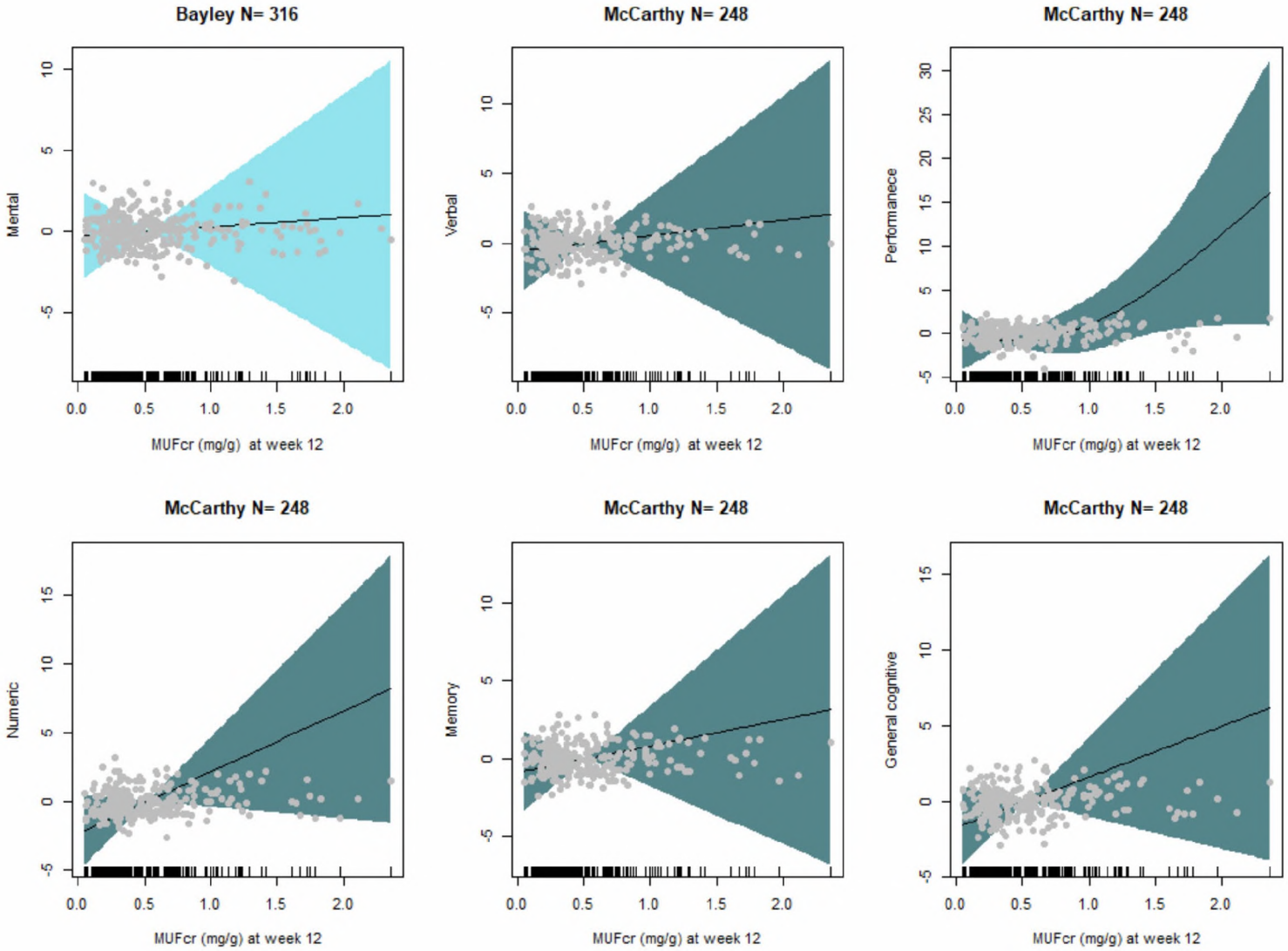
	Bayley N=146			**McCarthy N=125**		
Beta (IC95%)	Mental	Verbal	Performance	Numeric	Memory	General cognitive
≤ median HES	*Bayley N=56*			*McCarthy N=51*		
MUFcr (mg/g) at pregnancy	4.3 (-11.48 , 20.07)	**24.88 (4.89 , 44.87)**	**17.79 (0.82 , 34.76)**	**20.06 (3.51 , 36.62)**	16.09 (-2.2 , 34.38)	**23.96 (6.25 , 41.67)**
MUFcr (mg/g) at week 12	4.97 (-9.85 , 19.79)	12.2 (-9.7 , 34.1)	12.1 (-5.75 , 29.96)	5.85 (-12.48 , 24.17)	3.82 (-15.61 , 23.25)	12.32 (-7.29 , 31.93)
MUFcr (mg/g) at week 32	2.11 (-11.19 , 15.42)	**20.13 (5.93 , 34.33)**	**12.62 (0.16 , 25.08)**	**18.34 (7.05 , 29.62)**	**15.16 (2.35 , 27.98)**	**19.08 (6.5 , 31.67)**
> median HES	*Bayley N=56*			*McCarthy N=50*		
MUFcr (mg/g) at pregnancy	2.17 (-11.33 , 15.68)	2.88 (-12.39 , 18.14)	6.71 (-5.99 , 19.41)	10.63 (-3.12 , 24.38)	8.78 (-4.43 , 21.99)	7.05 (-6.08 , 20.17)
MUFcr (mg/g) at week 12	4.75 (-9.01 , 18.51)	-2.83 (-17.32 , 11.65)	10.22 (-1.51 , 21.95)	**13.26 (0.55 , 25.97)**	6.66 (-6 , 19.32)	5.94 (-6.56 , 18.44)
MUFcr (mg/g) at week 32	2.11 (-11.19 , 15.42)	5.25 (-6.36 , 16.87)	1.23 (-8.68 , 11.13)	3.86 (-6.99 , 14.71)	6 (-4.21 , 16.21)	4.42 (-5.71 , 14.56)

Adjusted by age of the child at the time of the test (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, IQ and smoking.
There were 34 and 24 missing values at each follow up.

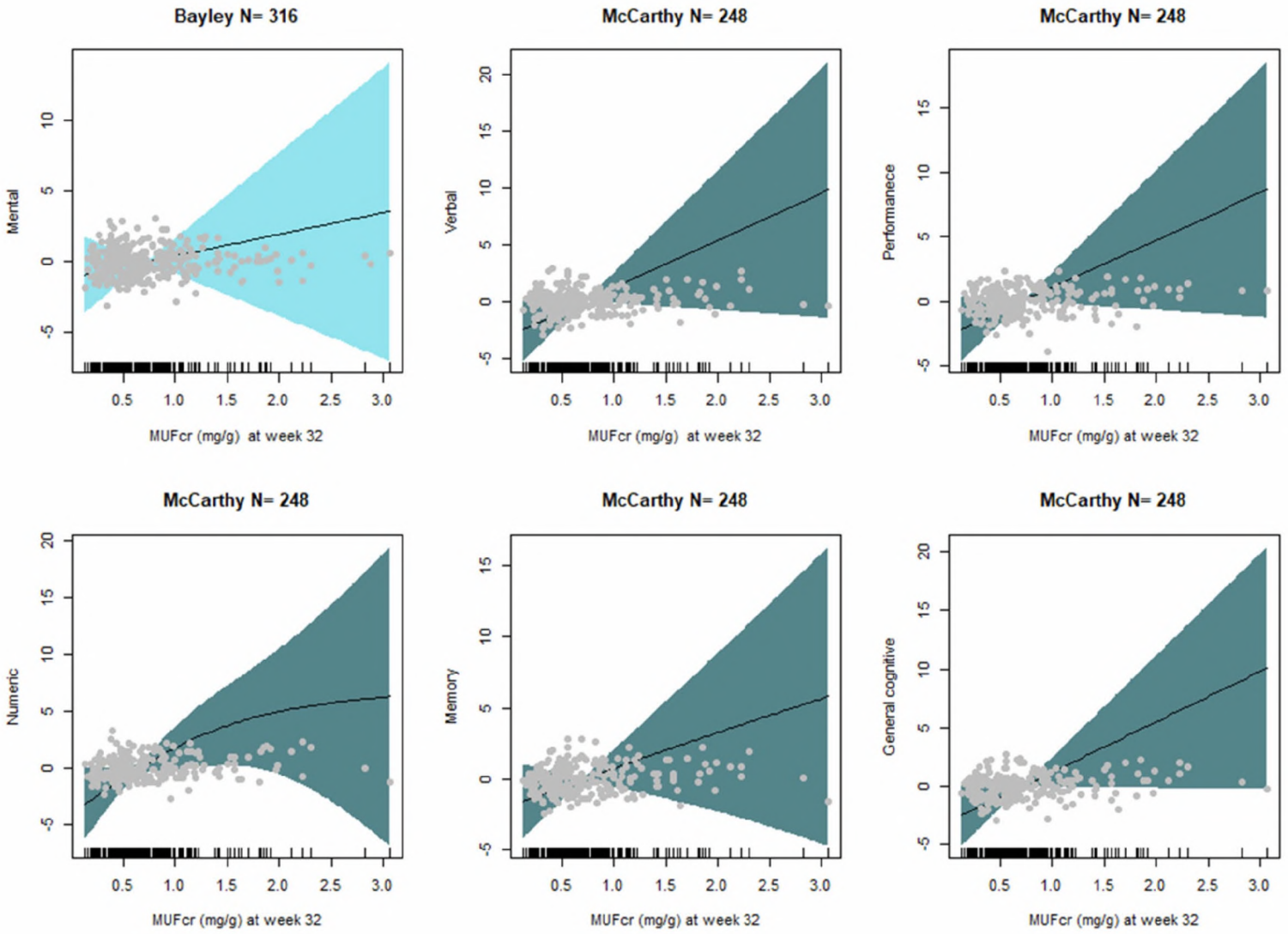
Supplementary Figure 1. GAM models depicting Bayley's scores in function of MYFcr (mg/g) levels by time of pregnancy.



Supplementary Figure 1 (cont.). GAM models depicting Bayley's scores in function of MYFcr (mg/g) levels by time of pregnancy.



Supplementary Figure 1 (cont.). GAM models depicting Bayley's scores in function of MUFcr (mg/g) levels by time of pregnancy.



Supplementary Figure 2. McCarthy's verbal, memory and General Cognitive Index scores: Sex-MUFcr interaction term plots.