# Journal Pre-proof

Association of maternal urinary fluoride concentrations during pregnancy with size at birth and the potential mediation effect by maternal thyroid hormones: The Swedish NICE birth cohort

Mariza Kampouri, Klara Gustin, Mia Stråvik, Malin Barman, Michael Levi, Vasiliki Daraki, Bo Jacobsson, Anna Sandin, Ann-Sofie Sandberg, Agnes E. Wold, Marie Vahter, Maria Kippler

Please cite this article as: Kampouri, M., Gustin, K., Stråvik, M., Barman, M., Levi, M., Daraki, V., Jacobsson, B., Sandin, A., Sandberg, A.-S., Wold, A.E., Vahter, M., Kippler, M., Association of maternal urinary fluoride concentrations during pregnancy with size at birth and the potential mediation effect by maternal thyroid hormones: The Swedish NICE birth cohort, *Environmental Research* (2022), doi: https://doi.org/10.1016/j.envres.2022.114129.

**CRediT authorship contribution statement**

**Mariza Kampouri:** Data analysis, Data visualization, Investigation, Writing – original draft, Writing – review & editing. **Klara Gustin:** Writing – review & editing. **Mia Stråvik:** Data visualization, Writing – review & editing. **Malin Barman:** Writing – review & editing. **Michael Levi:** Writing – review & editing. **Vasiliki Daraki:** Writing – review & editing. **Bo Jacobsson:** Writing – review & editing. **Anna Sandin**: Writing – review & editing. **Ann-Sofie Sandberg:** Writing – review & editing. **Agnes E. Wold:** Writing – review & editing. **Marie Vahter**: Conceptualization, Supervision, Investigation, Methodology, Writing – review & editing. **Maria Kippler:** Conceptualization, Supervision, Investigation, Methodology, Funding acquisition, Project administration, Writing – review & editing.

1 **Association of maternal urinary fluoride concentrations during pregnancy with size at birth and the**

2 **potential mediation effect by maternal thyroid hormones: the Swedish NICE birth cohort**

3 Mariza Kampouri[1], Klara Gustin[1], Mia Stråvik[2], Malin Barman[1,2], Michael Levi[1], Vasiliki Daraki[3], Bo

4 Jacobsson[4,5], Anna Sandin[6], Ann-Sofie Sandberg[2], Agnes E. Wold[7], Marie Vahter[1], Maria Kippler[1,#]

5

6 [1]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

7 [2]Food and Nutrition Science, Department of Biology and Biological Engineering, Chalmers University

8 of Technology, Gothenburg, Sweden

9 [3]Department of Endocrinology, University Hospital of Heraklion, Heraklion, Greece

10 [4]Department of Obstetrics and Gynecology, Sahlgrenska Academy, University of Gothenburg,

11 Gothenburg, Sweden

12 [5]Institute of Clinical Sciences, Department of Obstetrics and Gynecology, Sahlgrenska University

13 Hospital, Gothenburg, Sweden

14 [6]Department of Clinical Science, Pediatrics, Sunderby Research Unit, Umeå University, Sweden

15 [7]Institute of Biomedicine, Department of Infectious Diseases, Sahlgrenska Academy, University of

16 Gothenburg, Gothenburg, Sweden

17

18 [#]**Corresponding author**: Associate Professor, Ph.D., Maria Kippler, Institute of Environmental

19 Medicine, Karolinska Institutet, Box 210, SE-171 77, Stockholm, Sweden. Telephone: +46 730303131.

20 Telefax: +46 8 33 69 81. Email: maria.kippler@ki.se

21 **Declaration of conflicts of interest:** The authors declare they have no actual or potential competing

22 financial interests.

23   **Abstract**

24   **Background** Observational studies have indicated that elevated maternal fluoride exposure during

25   pregnancy may impair child neurodevelopment but a potential impact on birth outcomes is

26   understudied.

27   **Objectives** To evaluate the impact of gestational fluoride exposure on birth outcomes (birth size and

28   gestational age at birth) and to assess the potential mediating role of maternal thyroid hormones.

29   **Methods** We studied 583 mother-child dyads in the NICE cohort in northern Sweden. Maternal

30   fluoride exposure was assessed by measuring urinary concentrations at late pregnancy (median: 29th

31   gestational week) using an ion selective electrode. Plasma levels of free and total thyroxine (fT4, tT4)

32   and triiodothyronine (fT3, tT3), and thyroid stimulating hormone (TSH) were measured with

33   electrochemiluminescence immunoassays. The infant's weight, length, head circumference, and

34   gestational age at birth were extracted from hospital records.

35   **Results** Median urinary fluoride concentration was 0.71 mg/L (5th-95th percentile 0.31-1.9 mg/L;

36   specific gravity adjusted). In multivariable-adjusted regression models, every 1 mg/L increase of

37   maternal urinary fluoride was associated with a mean increase in birth weight by 84 g (95%CI: 30,

38   138), length by 0.41 cm (95%CI: 0.18, 0.65), head circumference by 0.3 cm (95%CI: 0.1, 0.4), and with

39   increased odds of being born large for gestational age (OR=1.39, 95%CI: 1.03, 1.89). Every 1 mg/L

40   increase of maternal urinary fluoride was also associated with a mean increase of the plasma fT3:fT4

41   ratio (B=0.007, 95%CI: 0.000, 0.014), but not with the hormones or TSH. In mediation analyses, the

42   maternal fT3:fT4 ratio did not explain the urinary fluoride-birth size relationships.

43   **Discussion** Gestational urinary fluoride concentrations were associated with increased size at birth

44   and even with increased odds of being born large for gestational age. The fluoride-related associations

45   with increased size at birth were not explained by changes in maternal thyroid hormone levels.

46   *Key words:* urinary fluoride, birth weight, birth length, birth head circumference, large for gestational

47   age, gestational age at birth, thyroid hormones

48  **1. Introduction**

49  Fluoride has clear benefits for dental health, but is otherwise not known to be required for any body

50  function (Featherstone, 1999). Exposure to fluoride occurs via fluoride-enriched dental care products,

51  via naturally high levels of fluoride in the drinking water in some geographical areas, or, in other

52  regions, via fluoride supplementation of drinking water, salt, or milk (O Mullane et al., 2016). The

53  content of fluoride in food is usually low, except for tea and marine fish, and foods prepared with

54  fluoride-rich water (European Food Safety Authority, 2013). It has been estimated that 80% or more

55  of ingested fluoride is absorbed passively in the gastrointestinal tract, half of which is fairly rapidly

56  excreted via the urine, while half is retained, mainly in bones and teeth (Buzalaf and Whitford, 2011).

57  High-level fluoride exposure may alter the structure of the mineralized tissues causing dental fluorosis,

58  a condition affecting tooth enamel, or even skeletal fluorosis, a metabolic bone disease (Chachra et

59  al., 2008). The World Health Organization recommends a maximum fluoride concentration in drinking

60  water of 1.5 mg/L to prevent fluorosis (World Health Organization, 2011).

61  Fluoride crosses the placenta (Malhotra et al., 1993; Montherrat-Carret et al., 1996; Sastry et al.,

62  2010), and exposure to elevated fluoride concentrations during pregnancy has recently been

63  associated with lower child intelligence in Canada (Green et al., 2019) and Mexico (Bashash et al.,

64  2017) and an increased risk of attention-deficit-hyperactivity-disorder symptoms in Mexico (Bashash

65  et al., 2018). However, studies regarding the effects of prenatal fluoride exposure on infant size or

66  gestational age at birth are limited and inconclusive (Aghaei et al., 2015; Goodman et al., 2021;

67  Mohanty et al., 2011). To our knowledge, there is only one prospective study on the impact of fluoride

68  exposure on size at birth, conducted in the MIREC cohort in Canada, which found no significant

69  association (Goodman et al., 2021). The underlying mechanism of early-life fluoride toxicity is unclear.

70  The U.S. National Research Council has suggested that fluoride may affect the endocrine system, and

71  in particular, the function of the thyroid gland (Carton and Park, 2006). Exposure to fluoride appears

72  to suppress thyroid hormones (Carton and Park, 2006), and observational studies in school-aged

73    children and adults have found associations between elevated fluoride exposure via drinking water

74    and increased odds of being hypothyroid (Kheradpisheh et al., 2018; Peckham et al., 2015; Singh et

75    al., 2014) or increased levels of thyroid stimulating hormone (TSH) among iodine-deficient individuals

76    (Malin et al., 2018). Thyroid hormones are strong determinants of fetal growth and development

77    (Forhead and Fowden, 2014), however, the impact of fluoride exposure on thyroid hormone levels in

78    pregnant women has so far not been evaluated.

79    In the present study, we aimed to evaluate the impact of maternal fluoride exposure, assessed

80    through urinary concentrations, on infant size (weight, length, head circumference) and gestational

81    age at birth in a birth cohort in northern Sweden, where natural fluoride concentrations in the drinking

82    water vary greatly due to geological properties (Aggeborn and Öhman, 2021; Aneblom, 2005). We,

83    also, aimed to assess if the fluoride exposure was associated with thyroid hormone levels in pregnancy

84    and if so, whether the latter mediates any identified association between maternal fluoride exposure

85    and the birth outcomes. A secondary aim was to identify dietary sources, other than drinking water,

86    of fluoride exposure in this population.

87    **2. Methods**

88    2.1. Study cohort

89    The birth cohort NICE (Nutritional impact on the Immunological maturation during Childhood in

90    relation to the Environment) consists of families recruited in 2015-2018, in the southern and eastern

91    parts of Region Norrbotten, in northern Sweden (Barman et al., 2018). Information concerning the

92    study was provided to the families at their first prenatal visit to the local maternity clinic in gestational

93    week 10-12. Those accepting to participate signed informed consent at the routine ultrasound visit

94    (gestational week 18-19). The inclusion criteria for participation in the NICE cohort included residing

95    in Norrbotten region, planning to give birth at the Sunderby Hospital, and being able to communicate

96    in written and spoken Swedish.

97    In total, 655 pregnancies were enrolled in the study (Figure 1). After excluding families already

98    participating with a previous birth, participant consent withdrawal, twin pregnancies, miscarriages,

99    and stillbirths, 629 mother-infant pairs remained, from whom, urine volume to measure fluoride

100   concentrations was available from 591 pregnant women (participants with available exposure data).

101   We had available data on birth weight for 581 infants, on birth length for 548 infants, on head

102   circumference at birth for 562 infants, and on gestational age at birth for 583 infants. Cases which

103   lacked covariate data were excluded (season of sampling: 0.5%, maternal education: 1%, maternal

104   smoking: 0.3%, maternal BMI: 2.5%, gestational age at birth: 0.2%). This left 556 mother-child dyads

105   in the analyses of birth weight, 538 in the analyses of birth length and head circumference, and 558 in

106   the analyses of gestational age at birth (corresponding to 91% - 94% of the participants with available

107   exposure data).

108   This study was conducted in accordance with the ethical standards of the Helsinki declaration, and it

109   has been approved by the Regional Ethical Review Board in Umeå, Sweden. Written and verbal

110   information had been given to the participating parents before they provided written consent.

111   2.2. Sample collection

112   Blood and spot urine samples were collected at a visit to the local maternity health clinics in

113   gestational week 29 on average (SD: 1.5; range: 24-36) (Barman et al., 2018; Gustin et al., 2021).

114   Maternal urine samples were also collected at 4 months postpartum (n=486). Blood samples were

115   collected in 10-mL EDTA tubes (Becton Dickinson, Plymouth, UK). Urine samples were collected in

116   routine collection cups and transferred to 24-mL polyethylene bottles. The samples were stored at 4°C

117   until transported to the laboratory at the Sunderby hospital on the same or following workday. Blood

118   samples were centrifuged for 5 min at 2,400 rpm (Hettich Rotina 420, Hettich Lab Technology,

119   Tuttlingen, Germany), after which the plasma fraction was aliquoted in polypropylene tubes (Micronic,

120   Nordic Biolabs AB, Sweden) and stored at −80°C.

121    2.3. Exposure assessment

122    Fluoride exposure was based on urine concentration, a recognized marker of ongoing exposure (Till

123    et al., 2018). Although the excretion of ingested fluoride in urine is rapid (half-life in the circulation

124    about 5 h), the frequent intake of drinking water, water-based beverages, and food prepared with

125    water leads to fairly constant exposure, and thus, relatively constant urinary concentrations (Ekstrand

126    and Ehrnebo, 1983; Idowu et al., 2019; Whitford, 1994). Urinary fluoride concentrations were

127    measured at Karolinska Institutet, Stockholm, using a fluoride specific electrode (Orion 9609BNWP

128    and Orion Star A214 pH/ISE meter; Thermo Fisher Scientific, Waltham, MA USA) according to the user

129    manual. Aliquots of 0.5mL urine were diluted 1:1 with a total ionic strength adjustment buffer (Tisab

130    II, Thermo Fisher Scientific) and then vortexed. The external calibration curve ranged between 0.07

131    and 11 mg/L, and was prepared daily through serial dilution from a 100 mg/L fluoride standard

132    solution (Thermo Fisher Scientific) in deionized water with adjustment buffer (50% Tisab II). Urinary

133    fluoride concentrations were determined by comparing the mV readings with the mV of the standard

134    curve. The limit of detection was 0.05 mg/L [mean blank value (n=14) plus two times the standard

135    deviation] (Thomas et al., 2016). Only two urine samples had fluoride concentrations below the

136    detection limit and their concentrations were kept as measured (Helsel, 1990). To monitor stability

137    across each run, a standard solution (0.90-2.2 mg/L) was measured every second hour, showing an

138    average drift of 3±2%. The accuracy was monitored by measuring two reference materials, fluoride in

139    water (Product number: QC3162; Sigma-Aldrich, USA; certified fluoride value: 0.420 mg/L) and

140    Seronorm Trace Elements Urine L-2 (LOT: 1011645; Sero AS, Norway; recommended analytical

141    fluoride value: 4 mg/L), before the first and after the last urine sample in each run. The obtained mean

142    fluoride concentration was 0.48±0.04 mg/L (n=28, recovery 114%) and 3.8±0.2 mg/L (n=28, recovery

143    94%), respectively. In addition, an in-house control (a urine sample) was also measured before and

144    after each run (n=28 runs; mean±SD fluoride concentration 0.70±0.04 mg/L), showing an inter-day

145    coefficient of variation (CV%) of 6%.

146   To compensate for the variation in urine dilution, the fluoride concentration in each urine sample was

147   adjusted to the mean specific gravity (mean=1.017), measured using a digital refractometer

148   (EUROMEX RD712, Clinical Refractometer, Holland). The following formula was applied: adjusted

149   urinary fluoride concentration = unadjusted urinary fluoride concentration × (specific gravity$_{mean}$ −

150   1)/(specific gravity$_{sample}$ − 1), as reported previously (Nermell et al., 2008).

151   2.4. Dietary intake assessment

152   A semi-quantitative Food Frequency Questionnaire (FFQ; "Meal-Q") was e-mailed to the pregnant

153   women around gestational week 34 to collect information on their diet (Christensen et al., 2014;

154   Christensen et al., 2013; Nermell et al., 2008; Stråvik et al., 2019). The FFQ included 102–174 questions

155   on the intake frequency (times per day or week) and the quantity of the consumed food items during

156   the previous month. The exact number of questions in Meal-Q depends on the responses to specific

157   questions and the follow-up questions for different items. Information about portion size was

158   presented in pictures with portions of carbohydrates (pasta, rice, and potato), protein (meat, fish, and

159   vegetarian), and vegetables. The intake, in grams per day, was estimated based on both the frequency

160   of the intake and the reported portion size. Beverage consumption was recorded as the frequency of

161   drinking a predefined volume of each beverage. Intake of the specific food items (e.g., game meat)

162   was grouped to create the "total intake" of the food groups (e.g., total meat intake).

163   2.5. Birth outcomes' assessment

164   Information regarding birth weight (g), birth length (cm), head circumference at birth (cm), and

165   gestational age at birth (days; for IVF fetuses gestational age was determined based on the second

166   trimester ultrasound) was obtained from the hospital records at the Sunderby hospital. The ponderal

167   index was calculated as weight (grams) * 100 / length (cm)$^3$. Small for gestational age and large for

168   gestational age at birth were classified based on two different cut-off points and the mean birth weight

169   derived from ultrasound-based growth curves (Maršál et al., 1996). The cut-off points to classify

170    infants in the small-for-gestational-age category were weight below 2 SDs or weight below the 10th

171    percentile for the gestational age according to sex-specific growth curves derived from ultrasound

172    measurements in a Scandinavian population (Maršál et al., 1996). The respective cut-off points for

173    large-for-gestational-age classification were weights above 2 SDs from the mean birth weight and

174    weights above the 90th percentile for corresponding gestational age (Maršál et al., 1996). Preterm

175    delivery was defined as a birth delivered prior to gestational week 37.

176    2.6. Assessment of thyroid hormones and TSH

177    Maternal thyroid hormones were previously measured and the data for the cohort have been

178    reported in detail elsewhere (Gustin et al., 2021). In brief, the plasma concentrations of thyroid-

179    stimulating hormone (TSH) were analyzed at an accredited laboratory (Department of Clinical

180    Chemistry at the University Hospital of Malmö, Sweden) using a single-step sandwich method with

181    electrochemiluminescence immunoassays (ECLIA; Roche Cobas, Roche Diagnostics, Solna, Sweden),

182    while free and total thyroxine (fT4, tT4) and free and total triiodothyronine (fT3, tT3) were analyzed

183    via automated ECLIA (Roche Cobas, Roche Diagnostics, Solna, Sweden), consisting of a two-step

184    immunometric-competitive technique followed by chemiluminescent emission measurement. The

185    ratios of fT4 to tT4, fT3 to tT3, and fT3 to fT4 were calculated. As population and trimester-specific

186    reference ranges for maternal hormone concentrations are lacking, we used an upper TSH reference-

187    limit of 4.0 mIU/L as an indication of potential hypothyroidism (Alexander et al., 2017). Seven per cent

188    of the participating mothers had a diagnosis related to thyroid dysfunction (n=41). Mothers with a

189    thyroid dysfunction diagnosis were excluded from all the analyses regarding associations with thyroid

190    hormones.

191    2.7. Covariate data collection

192    Information on maternal age (years), parity (number of previous deliveries), education (elementary

193    school, high school, university), pre-pregnancy smoking (never, sometimes, daily), pre-pregnancy

194     alcohol consumption (never, sometimes, or daily), previously diagnosed maternal thyroid dysfunction

195     (yes/no), and infant sex (male/female) was obtained from the hospital records. Data on maternal

196     weight and length were obtained at the maternity clinic in the first trimester and maternal body mass

197     index (BMI) was calculated as weight (kg) divided by squared height ($m^2$). Pre-pregnancy maternal

198     smoking was used (ever, never) instead of smoking in pregnancy, since the latter was available only

199     for a sub-set of the women (n=447). Alcohol consumption during pregnancy was obtained from the

200     hospital records and no pregnant woman consumed any alcohol during pregnancy in accordance with

201     recommendations. Information on the source of drinking water at residency (municipality/private

202     well) was obtained through a questionnaire concerning environmental factors (Barman et al., 2018).

203     Season of sampling was defined as spring (March, April, May); summer (June, July, August); fall

204     (September, October, November), and winter (December, January, February). As iodine is essential

205     for thyroid hormone production (Werner et al., 2005), we also measured the maternal urinary iodine

206     concentration (UIC), a valid biomarker of recent iodine intake on a population level (World Health

207     Organization, 2013), in the same spot urine sample as fluoride.  The measurements of UIC have been

208     described in detail elsewhere (Gustin et al., 2021). The UICs were adjusted for specific gravity to

209     compensate for variation in urine dilution. We used the population cut-off UIC values suggested by

210     the World Health Organization for classification of iodine intake during pregnancy to divide the

211     participants into two categories (UIC <150 µg/L and UIC ≥150 µg/L) (World Health Organization, 2013).

212     2.8. Statistical analyses

213     Statistical analyses were performed using Stata/IC 13.0 (StataCorp, TX, USA) and R© version 3.6.2 (R

214     Foundation for Statistical Computing, Vienna, Austria). A *P-value* of less than 0.05 was considered as

215     statistically significant but robustness and consistency of the results were also considered in the

216     interpretation of the obtained results.

217     Correlations between maternal urinary fluoride concentrations and maternal dietary data (g/day of

218     each food item or food group) were explored using unsupervised hierarchical cluster analysis and

9

219  visualized in a heat map using R version 3.6.2. In the heat map, the inserted dietary variables were

220  automatically clustered based on the correlations between them and were placed accordingly

221  (correlated variables placed close to each other in connected clusters). The direction of the

222  correlations between urinary fluoride concentrations and dietary data is coded by color (red indicates

223  positive correlations and blue indicates negative correlations) and the magnitude is visualized by color

224  shade (darker shades indicate higher magnitude). Correlations between maternal urinary fluoride

225  concentrations and dietary data were also stratified by the season of sampling.

226  Associations between maternal urinary fluoride concentrations, birth outcomes (weight, length, head

227  circumference, and gestational age), and potential covariates were initially explored using Spearman

228  rank test, Mann-Whitney U-test, or Kruskal-Wallis-test (combined with Dunn's test for post-hoc

229  pairwise comparisons), depending on the type of data. A directed acyclic graph (Textor et al., 2011)

230  was constructed based on previous knowledge and the observed associations in the data, using

231  DAGitty version 3.0, to identify potential confounders (Figure S1). Infant's sex (male, female) and

232  gestational age at birth (days) were selected as a priori adjustment factors. Thereafter, the analyses

233  of maternal urinary fluoride and birth outcomes were adjusted for maternal age (years), parity

234  (primiparous and multiparous), maternal BMI in early pregnancy (kg/m$^2$) or height (cm; in the analyses

235  of birth length), maternal education (no university degree, university degree), maternal smoking

236  status before pregnancy (ever, never), and season of sampling (spring, fall, winter, summer). Linearity

237  of the associations between the maternal urinary fluoride concentrations with birth-size outcomes

238  and gestational age at birth was examined using generalized additive models (Figure 2) and was

239  evaluated both visually and based on the *P*-gain (>0.1), defined as the difference in normalized

240  deviance between the generalized additive model and the linear model. As we did not observe any

241  indication of non-linearity in the models (Figure 3), linear regression models were performed to

242  explore the multivariable-adjusted associations of maternal urinary fluoride concentrations with birth

243  weight, length, head circumference, and gestational age. The associations of maternal urinary fluoride

244  and the categorical outcomes (small or large for gestational age and preterm delivery) were assessed

10

245 with multivariable-adjusted logistic regression models. Since previous studies have indicated that the

246 infant's sex may modify the association of fluoride exposure with other developmental outcomes

247 (Green et al., 2019; Liu et al., 2019), we added a multiplicative interaction term in the main models

248 and stratified the models by infant sex. Sensitivity analyses were conducted excluding women with

249 reported diagnosed thyroid dysfunction (n=41), infants born preterm (n=28), or families having a

250 private well at their residence. We also repeated the main models excluding maternal urinary fluoride

251 concentrations above the 99th percentile (n=6; corresponding to 3.9mg/L) to evaluate whether the

252 associations were impacted by extreme fluoride concentrations, and with additional adjustment for

253 the time of urine sample collection.

254 Pregnant women with diagnoses related to thyroid dysfunction (n=41) were excluded from all the

255 analyses concerning thyroid hormone concentrations, as done in previous relevant analyses (Gustin

256 et al., 2021; Gustin et al, submitted). Associations of maternal urinary fluoride concentrations with

257 maternal thyroid function biomarkers (TSH, tT4, fT4, tT3, fT3, fT4:tT4, fT3:tT3, fT3:fT4) were initially

258 explored with Spearman's rank correlation test. A separate directed acyclic graph was constructed to

259 select confounders for the models of maternal urinary fluoride concentrations with maternal thyroid

260 hormones and TSH (Figure S2; minimal suggested adjustment set: maternal age, parity, maternal BMI

261 in early pregnancy, maternal education, maternal smoking before pregnancy, and season of sampling).

262 TSH concentrations were $\log_2$-transformed to improve the distribution (right skewed). We used

263 generalized additive models to examine the linearity of the associations between maternal urinary

264 fluoride concentrations and thyroid hormones or TSH (Figure S3). The generalized additive models did

265 not suggest deviance from linearity. Therefore, the associations were evaluated using multivariable-

266 adjusted linear regression models. As an association between urinary fluoride concentrations and

267 altered thyroid hormones has previously been identified in iodine deficient individuals in a Canadian

268 study (Malin et al., 2018), we examined the role of maternal UIC in the associations between maternal

269 urinary fluoride concentrations and thyroid hormones' levels or TSH by inserting a multiplicative term

270 in the models (exposure * UIC categories [150 µg/L and ≥150 µg/L]) and stratifying the analyses by

11

271 these categories. Mediation analysis ("*paramed*" – Stata module for causal mediation analysis using

272 parametric regression models) was performed with parametric regression models between maternal

273 urinary fluoride concentrations, fT3:tT3, and birth size variables, to calculate a model for the mediator

274 conditional on exposure and covariates, and a model for the outcome conditional on exposure, the

275 mediator, and covariates (Valeri and VanderWeele, 2013).

276 **3. Results**

277 3.1. Participants' background characteristics and urinary fluoride concentrations

278 The background characteristics of the 558 mother-infant pairs included in the study are presented in

279 Table 1. Five per cent of the pregnant women delivered their children prior to gestational week 37

280 (preterm births). Fifty-five percent of the infants were girls. The mean birth length was 50.0 cm

281 (SD=2.1) for girls and 50.7 cm (SD=2.4) for boys, the mean head circumference was 34.6 cm (SD=1.4)

282 for girls and 35.3 cm (SD=1.6) for boys, and the mean birth weight was 3530 g (SD=503) for girls and

283 3640 g (SD=621) for boys. Seven percent of the participating girls (n=26) and 10% of the participating

284 boys were born small for gestational age (n=22), while 12% of the girls (n=38) and 16% of the boys

285 (n=39) were born large for gestational age ($10^{th}$ and $90^{th}$ percentile cut-offs, respectively).

286 The distribution characteristics of urinary fluoride concentrations at gestational week 29 and at $4^{th}$

287 month postpartum are presented in Table 2. The median urinary fluoride concentration, adjusted to

288 the mean specific gravity of 1.017, at gestational week 29 was 0.70 mg/L ($5^{th}$-$95^{th}$ percentile: 0.31-1.9),

289 with a maximum value of 6.4 mg/L. Concentrations were higher in women with a private well at their

290 residence (median=0.94 mg/L; n=34; P=0.013) than in women with access to municipality water

291 (median=0.70 mg/L; n=388). Women with a university degree had slightly higher urinary fluoride

292 concentrations (median=0.73 mg/L; n=389) than those without a university degree (median=0.68;

293 n=169; *P* <0.021). Also, urinary fluoride concentrations differed by the season of sampling ($chi^2$ =9.2;

294 *P*=0.024); in post-hoc pairwise comparisons the women's urinary fluoride concentrations were higher

295    in fall (median=0.78 mg/L; *P*=0.002) and winter (median=0.73 mg/L; *P* <0.05) than in spring

296    (median=0.66 mg/L), and higher in fall (median=0.78 mg/L) than in summer (median=0.69 mg/L; *P*

297    <0.05). The mothers' urinary fluoride concentrations at gestational week 29 were moderately

298    correlated with their urinary fluoride concentrations at 4 months postpartum (rho=0.5, *P*-value

299    <0.001).

300    The unsupervised clustered dietary analyses (Figure 2) showed that maternal urinary fluoride

301    concentrations were positively correlated with tea intake (rho=0.19, *P* <0.001). The correlation was

302    most evident in winter samples (rho=0.24, *P*=0.005), followed by the correlation in fall samples

303    (rho=0.19, *P*=0.037) and then summer samples (rho=0.16, *P*=0.049; spring: rho=0.12, *P*=0.119).

304    Moreover, the correlation of urinary fluoride concentrations was stronger with black tea (rho=0.17, *P*

305    <0.000) than with green tea (rho=0.11, *P*=0.013). Urinary fluoride concentrations were also weakly

306    positively correlated with fruit intake (rho=0.12, *P*=0.004), which clustered together with water and

307    water-based beverages.

308    3.2. Maternal urinary fluoride concentrations and birth outcomes

309    In the bivariate correlations, maternal urinary fluoride concentrations during pregnancy were

310    positively correlated, although weakly, with birth weight (rho=0.11*; P* <0.05) and birth length

311    (rho=0.13*; P* <0.05), while there was no correlation with head circumference (rho=0.07*; P*=0.08) or

312    gestational age at birth (rho=0.03; *P*=0.48).

313    In multivariable-adjusted linear regression models (Table 3), each 1 mg/L increase in maternal urinary

314    fluoride concentration was associated with a mean increase of 84 g in birth weight (95%CI: 30, 138;

315    *P*=0.003), of 0.41 cm in birth length (95%CI: 0.18, 0.65; *P*=0.001), and of 0.3 cm in head circumference

316    (95%CI: 0.1, 0.4; *P*=0.001). There was no association with gestational age at birth or the ponderal index

317    (birth weight x 100 / length$^3$). We did not observe any indication of an interaction between maternal

318    urinary fluoride concentrations and infant sex in relation to birth size or gestational age at birth (Table

319    3). Stratification by infant sex showed stronger associations of maternal urinary fluoride

320    concentrations with birth size measures in girls than in boys, however, the confidence intervals were

321    wide and overlapping (Table S1). In multivariable-adjusted logistic regression models (Table 4), each

322    1 mg/L increase in maternal urinary fluoride concentration was associated with 39% increase in the

323    odds (95%CI: 1.03, 1.89; $P$=0.034) of being born large for gestational age. No association was evident

324    between maternal urinary fluoride concentration and the odds of being born small for gestational age,

325    or of a preterm delivery.

326    In sensitivity analysis, the estimates were very similar to the respective estimates reported in the main

327    models when we excluded preterm deliveries (n=27), women with a reported diagnosed thyroid

328    dysfunction (n=41), or women with access to drinking water from a private well (n=34). Also, the

329    estimates were not markedly impacted when extreme urinary fluoride concentrations were excluded

330    from the analyses (>99th percentile; n=6) or when the models were additionally adjusted for the timing

331    of urine sample collection (n=488 to 520 depending on the outcome).

332    3.3. Maternal urinary fluoride concentrations and thyroid hormones

333    In bivariate analyses, maternal urinary fluoride concentrations were weakly correlated with tT3 levels

334    during pregnancy (rho=0.09; $P$ <0.05; Table S3). In the multivariable-adjusted linear regression models

335    (Table 5), we did not find any association with thyroid hormones or TSH but each 1 mg/L increase in

336    maternal urinary fluoride was associated with a mean increase in the fT3:fT4 ratio of 0.007 (95%CI:

337    0.000, 0.014; $P$=0.047), which corresponds to a 0.1SD increase of fT3:fT4 ratio. We did not observe

338    any significant interaction between maternal urinary fluoride concentrations and UICs in relation to

339    thyroid hormone levels (Table S4). Stratification of the models by maternal UICs (<150μg/L &

340    ≥150μg/L) indicated different direction of the associations of urinary fluoride concentrations with fT4,

341    tT4, and TSH in the two categories, but none of the models was statistically significant. The

342    associations of maternal urinary fluoride concentrations with tT3 and fT3 did not differ between

343    women in the two UIC categories. The association of urinary fluoride concentrations and fT3:fT4 ratio

14

344 was statistically significant in mothers with UIC below 150 µg/L, but not in mothers with

345 concentrations equal to or above 150 µg/L.

346 As maternal fT3:fT4 was the only thyroid-related biomarker associated with maternal urinary fluoride

347 concentrations (Table 5), and it was also associated with birth weight and length as shown elsewhere

348 (Gustin et al, submitted), the fT3:fT4 ratio was evaluated as a possible mediator of these associations.

349 The mediation analysis (Table 6) indicated that maternal fT3:fT4 ratio did not explain the observed

350 association of maternal urinary fluoride with infant birth weight and length [(weight - indirect effect:

351 B=3 g, 95%CI: -1, 11) and (length - indirect effect: B=0.02 cm, 95%CI: -0.00, 0.06), corresponding to

352 less than 5% of the total-effect estimate on weight and length].

353 **4. Discussion**

354 To our knowledge, this is the first prospective cohort study to report a positive association between

355 maternal urinary fluoride concentrations during pregnancy and size at birth, which was evident with

356 weight, length, and head circumference. We did not find any evidence of sex differences in the

357 associations. Maternal urinary fluoride concentrations were also associated with increased odds of

358 being born large for gestational age. In cross-sectional analyses, the urinary fluoride concentrations

359 were weakly positively associated with the plasma fT3:fT4 ratio, but not with any other thyroid

360 biomarker. The observed associations between maternal urinary fluoride concentrations and size at

361 birth were not explained by changes in the maternal thyroid hormone levels.

362 There is a limited number of studies evaluating the impact of fluoride exposure on child

363 anthropometry at birth and their results have been inconsistent. An ecological study conducted in

364 regions of the geographical fluoride belt in Iran reported positive correlations of fluoride

365 concentrations in drinking water in 35 villages [median (range): 1.6 mg/L (0.3, 3.5)] with weight and

366 length at birth (Aghaei et al., 2015; Derakhshani et al., 2014). Conversely, a cross-sectional study in a

367 region of India with elevated water fluoride concentrations [drinking water: median (range)=2.2 (0.9-

368  5.2) mg/L; water used for cooking and household chores: median (range)=5.8 (3.6-16) mg/L] reported

369  that concentrations of fluoride in maternal serum and in cord blood serum were inversely correlated

370  with birth weight and gestational age at birth (Mohanty et al., 2011). However, both studies merely

371  conducted correlational analysis and did not adjust for any potential confounders. A recent Canadian

372  study (MIREC cohort), conducted in regions applying water fluoridation, is the only prospective study

373  which has evaluated multivariable-adjusted associations of maternal fluoride exposure during

374  pregnancy with birth outcomes (Goodman et al., 2021). In accordance with our finding, they did not

375  find any association of maternal urinary fluoride with gestational age at birth. Also, they reported a

376  positive association of maternal urinary fluoride with birth weight in the crude models, which was

377  attenuated in the multivariable-adjusted models (Goodman et al., 2021). However, the exposure

378  levels were slightly lower than in our study [urinary fluoride concentration in the MIREC study: median

379  (range)=0.5 mg/L (0.05-3.3 mg/L) and in the NICE study: median (range)=0.70 mg/L (0.07-6.4 mg/L)]

380  and the magnitude of the association with birth weight was only slightly smaller than the respective

381  in our study (55 g increase in the MIREC study and 84 g increase in the NICE study, per 1 mg/L increase

382  in urinary fluoride). To note, the focus of the MIREC study was small for gestational age while the

383  association with large for gestational age was not evaluated (Goodman et al., 2021).

384  Our results suggest that maternal urinary fluoride concentrations during pregnancy were associated

385  with increased odds of being born large for gestational age. We controlled for multiple well-

386  established determinants of increased size at birth (i.e., maternal obesity, multiparity, advanced

387  gestational age, higher maternal education, male infant sex, and maternal non-smoking status),

388  however, we did not have information on the mothers' size at birth. When we used the "above 2SD"

389  cut-off of birth weight to define large for gestational age, the association did not reach significance,

390  probably due to the fewer cases which resulted in limited power. However, the estimate was similar

391  in size and direction (27% increase) to the estimate of the model based on the "above 10th percentile"

392  cut-off of birth weight. To our knowledge, no other study has identified such an association. Given the

393  potential impact of macrosomia on both the mother and the newborn (i.e., increased risk for

394 caesarean delivery, postpartum hemorrhage, vaginal lacerations, increased rate of admissions to the

395 neonatal intensive care unit) further studies are obviously warranted. The birth size in our population

396 is consistent with nationwide data from the Swedish population (mean birth weight=3 494 g and 14%

397 of the newborns with weight above 4 000 g at birth) (Socialstyrelsen, 2020). Moreover, we have

398 previously shown that pregnancy outcomes, including infant birth weight and gestational age at birth,

399 do not differ between our participants and nonparticipating pregnant women in Norrbotten County

400 (N = 4976) (Ögge et al., 2022). Although Sweden has a low obesity prevalence in the general population

401 compared to most Western countries (especially among pregnant women; ≈14%) (Socialstyrelsen,

402 2020), an increasing rate of large size at birth, with a potential link to later obesity, has been reported

403 (Cnattingius et al., 2012; Sundquist et al., 2004; Surkan et al., 2004).

404 The distribution of the maternal urinary fluoride concentrations (range: 0.07, 6.4 mg/L) indicates a

405 wide variation of fluoride exposure in the studied women residing in the southern and eastern parts

406 of Region Norrbotten in northern Sweden. This is supported by previously reported wide variations of

407 fluoride concentrations in drinking water from both public water supplies (several plants reporting >1

408 mg/L) (Aggeborn and Öhman, 2021) and private wells (several wells with >4 mg/L) (Aneblom, 2005) in

409 the study area. However, these reports covered fairly few water sources and the overlap of these data

410 with the water sources of the NICE families is not known. In total 8% of the study participants reported

411 having water from a private well at their residence. However, having access to municipality water

412 instead of a private well did not attenuate the associations with birth size in this study. Urinary fluoride

413 concentrations were not correlated with the food intake data, apart from a weak positive correlation

414 with tea, which may be the result of the additional water consumption and of the tea intake itself

415 (Waugh, 2019). Black, followed by green, tea is known to contain more fluoride than other food items

416 (Malinowska et al., 2008). Indeed, the correlation between urinary fluoride concentrations and

417 reported tea consumption was stronger for black tea than for green tea. Notably, the urinary fluoride

418 concentration was more strongly correlated with hot tea consumption in winter and fall than in

419 summer and spring, probably due to the seasonal variation in the consumption of tea with more

420   frequent intake during the cold seasons (ice tea consumption is not common in Sweden).

421   Furthermore, the median urinary fluoride concentration was higher in winter and fall than in summer

422   and spring, following a similar pattern as with tea intake in our population. It is noteworthy that in

423   addition to several general factors that have been shown to or are expected to affect fluoride

424   metabolism and urinary excretion (i.e., renal clearance rate, skeletal uptake rate, pH of the gastric

425   contents, amount and form of consumed fluoride, diet), urinary fluoride concentrations during

426   gestation may also be influenced by an increased release of fluoride into the circulation due to the

427   high bone turnover in pregnancy (Åkesson et al., 2004; Black et al., 2000; Gulson et al., 1998).

428   Several experimental findings have reported suppressed thyroid hormones in fluoride exposed

429   animals (Basha et al., 2011; Bobek et al., 1976; Jianjie et al., 2016; Zhan et al., 2006), and findings from

430   a Canadian observational study suggested that exposure to fluoride may reduce thyroid hormone

431   levels in individuals who have moderate-to-severe iodine deficiency (Malin et al., 2018). Contrary to

432   the findings of the aforementioned studies (Basha et al., 2011; Bobek et al., 1976; Jianjie et al., 2016;

433   Malin et al., 2018; Zhan et al., 2006), we did not find any clear associations between the urinary

434   fluoride concentrations and thyroid hormone levels, except for a weak positive association with

435   plasma fT3:fT4 ratio. Also, we did not find any interaction between the urinary concentrations of

436   fluoride and iodine in relation to the thyroid hormone levels, conversely to the reported findings of

437   the previous Canadian study, despite that the mothers in the present study had low iodine intake

438   during pregnancy (Gustin et al., 2021; Stråvik et al., 2021).  On the other hand,  a statistically significant

439   increase in the fT3:fT4 ratio in women with UICs below 150 µg/L that was not evident in women with

440   concentrations equal or above 150 µg/L was found, which may suggest a possible increased

441   vulnerability of women with low iodine intake. Nevertheless, maternal thyroid hormones did not

442   explain the identified associations of maternal urinary fluoride concentrations with infant size at birth

443   (weight and length). The mechanisms involved in an effect of gestational fluoride exposure on size at

444   birth are unknown. Maternal hyperglycemia may be implicated as fluoride is known to inhibit insulin

445   synthesis causing elevated blood glucose levels (Lin et al., 1976; Menoyo et al., 2008), and the delivery

446    of hyperglycemic blood to the fetus leads to excessive glucose storage as glycogen and fat (Kamana et

447    al., 2015; Yan and Yang, 2014). Secondly, an effect of fluoride on maternal lipid metabolism can be

448    hypothesized as several experimental animal studies have shown that fluoride treatment affects lipid

449    metabolism (Chiba et al., 2015; Miltonprabu and Thangapandiyan, 2015; Pereira et al., 2016; Sun et

450    al., 2014; Umarani et al., 2015; Yu et al., 2021). Alterations in maternal lipid profiles have been linked

451    to fetal macrosomia regardless of the glucose tolerance status of the mothers (Rao and Ping, 2021).

452    The main strengths of this study include the prospective design and the assessment of individual

453    fluoride exposure using urinary concentrations, which are considered to be the most suitable

454    biomarker for contemporary fluoride exposure at a population level (World Health Organization,

455    2014). However, the present study also has several limitations. Foremost, only one spot urine sample

456    collected in late pregnancy was used to assess urinary fluoride concentrations, which may have

457    resulted in exposure misclassification due to late-pregnancy physiology, although the concentrations

458    were adjusted for specific gravity to account for variation in urine dilution, and also due to participant

459    behavior prior to sampling (e.g., consumption of fluoride-rich water or food) as fluoride has a very

460    short half-life in the body. Still, we found that the mothers gestational urinary fluoride concentrations

461    were fairly well correlated with their urinary fluoride concentrations at 4 months postpartum; and

462    thus, most likely, they do reflect the ongoing fluoride exposure in the present population. Available

463    data on a thyroid dysfunction diagnosis and of thyroid hormone measurements during pregnancy gave

464    the opportunity to control for a possible impact of maternal thyroid disease on the reported findings,

465    and to examine whether a thyroid-disruption effect of fluoride would explain the observed

466    associations with birth size. However, thyroid hormones were, also, only measured once in late

467    pregnancy, and therefore, we cannot exclude a possible thyroid disruption in early pregnancy.

468    Moreover, the exposure and the mediator were measured in samples collected at the same timepoint;

469    while, in order to assume causal inference, the exposure should be measured prior to the mediator.

470    However, since the main source of fluoride exposure is drinking water, we expect that exposure is

471    fairly constant over time, which is supported by the correlation between the two separate sampling

472  time points presented above. Moreover, the power of the iodine-related analyses was fairly low, since

473  measurements of urinary iodine concentrations were not available for all study participants and they

474  are prone to short-term variations. We had no information on gestational diabetes, and we did not

475  measure maternal insulin levels or plasma lipids, which limited the interpretation of potential

476  underlying modes of action. Lastly, although we have adjusted our analyses for multiple potential

477  confounders and our associations were robust, we cannot exclude the possibility that residual

478  confounding has affected the reported findings.

479  **5. Conclusion**

480  This is the first prospective study suggesting that gestational fluoride exposure may increase size at

481  birth and even increase the risk of being born large for gestational age. We did not find any association

482  of maternal urinary fluoride concentrations with TSH or thyroid hormones in late pregnancy, except

483  for a weak positive association with the fT3:fT4 ratio, and maternal thyroid hormones did not explain

484  the observed associations between gestational urinary fluoride concentrations and infant size at birth.

485  Considering the ubiquity of fluoride in drinking water and that being born large for gestational age is

486  linked with perinatal complications, additional prospective cohort studies are warranted to confirm

487  our findings and to elucidate the mechanisms by which fluoride may affect intrauterine growth.

488  Furthermore, we believe that our findings highlight the need for further research on the possible

489  adverse effects of early-life fluoride exposure on child development and health.

**References**

500

501 Aggeborn, L., Öhman, M., 2021. The effects of fluoride in drinking water. Journal of Political Economy.

502       129**,** 465-491.

503 Aghaei, M., Derakhshani, R., Raoof, M., Dehghani, M., & Mahvi, A. H., 2015. Effect of fluoride in

504       drinking water on birth height and weight: an ecological study in Kerman Province, Zarand

505       County, Iran. Fluoride. 48**,** 160-168.

506 Åkesson, A., Vahter, M., Berglund, M., Eklöf, T., Bremme, K., & Bjellerup, P., 2004. Bone turnover from

507       early pregnancy to postweaning. Acta obstetricia et gynecologica Scandinavica. 83**,** 1049-

508       1055.

509 Alexander, E. K., Pearce, E. N., Brent, G. A., Brown, R. S., Chen, H., Dosiou, C., ... & Sullivan, S., 2017.

510       Guidelines of the American Thyroid Association for the diagnosis and management of thyroid

511       disease during pregnancy and the postpartum. Thyroid. 27**,** 315-389.

512 Aneblom, T., Thunholm, B., Rurling, S., & Gierup, J., 2005. Beskrivning till kartan över grundvattnet i

513       Norrbottens län [Description of the groundwater map in Norrbotten county]. SGU, Uppsala.

514 Barman, M., Murray, F., Bernardi, A. I., Broberg, K., Bölte, S., Hesselmar, B., ... & Sandin, A., 2018.

515       Nutritional impact on Immunological maturation during Childhood in relation to the

516       Environment (NICE): a prospective birth cohort in northern Sweden. BMJ open. 8**,** e022013.

517 Basha, P. M., Rai, P., & Begum, S., 2011. Fluoride Toxicity and Status of Serum Thyroid Hormones,

518       Brain Histopathology, and Learning Memory in Rats: A Multigenerational Assessment.

519       Biological Trace Element Research. 144**,** 1083-1094.

520 Bashash, M., Marchand, M., Hu, H., Till, C., Martinez-Mier, E. A., Sanchez, B. N., ... & Téllez-Rojo, M.

521       M., 2018. Prenatal fluoride exposure and attention deficit hyperactivity disorder (ADHD)

522       symptoms in children at 6–12 years of age in Mexico City. Environment International. 121**,**

523       658-666.

524    Bashash, M., Thomas, D., Hu, H., Angeles Martinez-Mier, E., Sanchez, B. N., Basu, N., ... & Hernández-

525        Avila, M., 2017. Prenatal fluoride exposure and cognitive outcomes in children at 4 and 6–12

526        years of age in Mexico. Environmental health perspectives. 125**,** 097017.

527    Black, A., Topping, J., Durham, B., Farquharson, R. G., & Fraser, W. D., 2000. A detailed assessment of

528        alterations in bone turnover, calcium homeostasis, and bone density in normal pregnancy.

529        Journal of Bone and Mineral Research. 15**,** 557-563.

530    Bobek, S., Kahl, S., & Ewy, Z., 1976. Effect of long-term fluoride administration on thyroid hormones

531        level blood in rats. Endocrinologia experimentalis. 10**,** 289-295.

532    Buzalaf, M. A. R., Whitford, G. M., 2011. Fluoride metabolism. Fluoride and the oral environment. 22**,**

533        20-36.

534    Carton, R. J., Park, A., 2006. Review of the 2006 United States National Research Council report:

535        fluoride in drinking water. Fluoride. 39**,** 163-72.

536    Chachra, D., Vieira, A. P., & Grynpas, M. D., 2008. Fluoride and mineralized tissues. Critical Reviews™

537        in Biomedical Engineering. 36.

538    Chiba, F. Y., Garbin, C. A., Mattera, M. S., Mota, M. S., Pereira, R. F., & Sumida, D. H., 2015. Chronic

539        treatment with a mild dose of Naf promotes dyslipidemia in rats. Fluoride. 48**,** 205.

540    Christensen, S. E., Möller, E., Bonn, S. E., Ploner, A., Bälter, O., Lissner, L., & Bälter, K., 2014. Relative

541        validity of micronutrient and fiber intake assessed with two new interactive meal-and Web-

542        based food frequency questionnaires. Journal of medical Internet research. 16**,** e59.

543    Christensen, S. E., Möller, E., Bonn, S. E., Ploner, A., Wright, A., Sjölander, A., ... & Bälter, K., 2013. Two

544        new meal-and web-based interactive food frequency questionnaires: validation of energy and

545        macronutrient intake. Journal of medical Internet research. 15**,** e2458.

546    Cnattingius, S., Villamor, E., Lagerros, Y. T., Wikström, A. K., & Granath, F., 2012. High birth weight and

547        obesity—a vicious circle across generations. International journal of obesity. 36**,** 1320-1324.

548    Derakhshani, R., Tavallaie, M., Raoof, M., Mohammadi, T. M., Abbasnejad, A., & Haghdoost, A. A.,

549        2014. Occurrence of fluoride in groundwater of Zarand region, Kerman province, Iran.

550        Fluoride. 47, 133-138.

551    Ekstrand, J., Ehrnebo, M., 1983. The Relationship Between Plasma Fluoride, Urinary Excretion Rate

552        and Urine Fluoride Concentration in Man. Journal of Occupational and Environmental

553        Medicine. 25.

554    European Food Safety Authority, 2013. Panel on Dietetic Products, Nutrition, and Allergies. Scientific

555        opinion on dietary reference values for fluoride. EFSA Journal. 11, 3332.

556    Featherstone, J. D., 1999. Prevention and reversal of dental caries: role of low level fluoride.

557        Community dentistry and oral epidemiology. 27, 31-40.

558    Forhead, A. J., Fowden, A. L., 2014. Thyroid hormones in fetal growth and prepartum maturation. J

559        Endocrinol. 221, R87-R103.

560    Goodman, C., Hall, M., Green, R., Hornung, R., Martinez-Mier, E. A., Lanphear, B., & Till, C., 2021.

561        Maternal fluoride exposure, fertility and birth outcomes: The MIREC cohort. Environmental

562        Advances. 100135.

563    Green, R., Lanphear, B., Hornung, R., Flora, D., Martinez-Mier, E. A., Neufeld, R., ... & Till, C., 2019.

564        Association between maternal fluoride exposure during pregnancy and IQ scores in offspring

565        in Canada. JAMA pediatrics. 173, 940-948.

566    Gulson, B. L., Mahaffey, K. R., Jameson, C. W., Mizon, K. J., Korsch, M. J., Cameron, M. A., & Eisman, J.

567        A., 1998. Mobilization of lead from the skeleton during the postnatal period is larger than

568        during pregnancy. Journal of Laboratory and Clinical Medicine. 131, 324-329.

569    Gustin, K Barman, M., Skröder, H., Jacobsson, B., Sandin, A., Sandberg, A. S., ... & Kippler, M., 2021.

570        Thyroid hormones in relation to toxic metal exposure in pregnancy, and potential interactions

571        with iodine and selenium. Environment International. 157, 106869.

572    Helsel, D. R., 1990. Less than obvious-statistical treatment of data below the detection limit.

573        Environmental science & technology. 24, 1766-1774.

574    Idowu, O. S., Azevedo, L. B., Valentine, R. A., Swan, J., Vasantavada, P. V., Maguire, A., & Zohoori, F.

575        V., 2019. The use of urinary fluoride excretion to facilitate monitoring fluoride intake: A

576        systematic scoping review. PloS one. 14, e0222260-e0222260.

577    Jianjie, C., Wenjuan, X., Jinling, C., Jie, S., Ruhui, J., & Meiyan, L., 2016. Fluoride caused thyroid

578        endocrine disruption in male zebrafish (Danio rerio). Aquatic Toxicology. 171, 48-58.

579    Kamana, K., Shakya, S., & Zhang, H., 2015. Gestational diabetes mellitus and macrosomia: a literature

580        review. Annals of Nutrition and Metabolism. 66, 14-20.

581    Kheradpisheh, Z., Mirzaei, M., Mahvi, A. H., Mokhtari, M., Azizi, R., Fallahzadeh, H., & Ehrampoush, M.

582        H., 2018. Impact of drinking water fluoride on human thyroid hormones: a case-control study.

583        Scientific reports. 8, 1-7.

584    Lin, B., Henderson, M. J., Levine, B. B., Nagy, B. R., & Nagy, E. M., 1976. Effects of iodoacetate and

585        fluoride on islet respiration and insulin biosynthesis. Hormone and Metabolic Research. 8,

586        353-358.

587    Liu, Y., Téllez-Rojo, M., Hu, H., Sánchez, B. N., Martinez-Mier, E., Basu, N., ... & Peterson, K. E., 2019.

588        Fluoride exposure and pubertal development in children living in Mexico City. Environmental

589        Health. 18, 1-8.

590    Malhotra, A., Tewari, A., Chawla, H. S., Gauba, K., & Dhall, K., 1993. Placental transfer of fluoride in

591        pregnant women consuming optimum fluoride in drinking water. Journal of the Indian Society

592        of Pedodontics and Preventive Dentistry. 11, 1-3.

593    Malin, A. J., Riddell, J., McCague, H., & Till, C., 2018. Fluoride exposure and thyroid function among

594        adults living in Canada: Effect modification by iodine status. Environment International. 121,

595        667-674.

596    Malinowska, E., Inkielewicz, I., Czarnowski, W., & Szefer, P., 2008. Assessment of fluoride

597        concentration and daily intake by human from tea and herbal infusions. Food and Chemical

598        Toxicology. 46, 1055-1061.

599  Maršál, K., Persson, P. H., Larsen, T., Lilja, H., Selbing, A., & Sultan, B. L., 1996. Intrauterine growth

600     curves based on ultrasonically estimated foetal weights. Acta paediatrica. 85, 843-848.

601  Menoyo, I., Puche, R. C., & Rigalli, A., 2008. Fluoride-induced resistance to insulin in the rat. Fluoride.

602     41, 260-9.

603  Miltonprabu, S., Thangapandiyan, S., 2015. Epigallocatechin gallate potentially attenuates Fluoride

604     induced oxidative stress mediated cardiotoxicity and dyslipidemia in rats. Journal of Trace

605     Elements in Medicine and Biology. 29, 321-335.

606  Mohanty, S., Bhongir, A. V., Mishra, A. K., & Rao, P., 2011. Association of higher maternal serum

607     fluoride with adverse fetal outcomes. International Journal of Medicine and Public Health. 1.

608  Montherrat-Carret, L., Perrat-Mabilon, B., Barbey, E., Bouloc, R., Boivin, G., Michelet, A., & Magloire,

609     H., 1996. Chemical and X-ray analysis of fluoride, phosphorus, and calcium in human foetal

610     blood and hard tissues. Archives of oral biology. 41, 1169-1178.

611  National Board of Health and Welfare, 2020. Statistics on Pregnancies, Deliveries, and Newborn

612     Infants 2020. https://www.socialstyrelsen.se/en/statistics-and-data/statistics/

613  Nermell, B., Lindberg, A. L., Rahman, M., Berglund, M., Persson, L. Å., El Arifeen, S., & Vahter, M., 2008.

614     Urinary arsenic concentration adjustment factors and malnutrition. Environmental research.

615     106, 212-218.

616  O Mullane, D., Baez, R. J., Jones, S., Lennon, M. A., Petersen, P. E., Rugg-Gunn, A. J., ... & Whitford, G.

617     M., 2016. Fluoride and oral health. Community dental health. 33, 69-99.

618  Ögge, L. E., Murray, F., Modzelewska, D., Lundqvist, R., Nilsson, S., Carré, H., ... & Barman, M., 2022.

619     Maternal characteristics and pregnancy outcomes in the NICE birth cohort: an assessment of

620     self-selection bias. The Journal of Maternal-Fetal & Neonatal Medicine. 1-9.

621  Peckham, S., Lowery, D., & Spencer, S., 2015. Are fluoride levels in drinking water associated with

622     hypothyroidism prevalence in England? A large observational study of GP practice data and

623     fluoride levels in drinking water. J Epidemiol Community Health. 69, 619-624.

624 Pereira, H. A. B. S., Dionizio, A. S., Fernandes, M. S., Araujo, T. T., Cestari, T. M., Buzalaf, C. P., ... &

625      Buzalaf, M. A. R., 2016. Fluoride Intensifies Hypercaloric Diet-Induced ER Oxidative Stress and

626      Alters Lipid Metabolism. PLOS ONE. 11, e0158121.

627 Rao, C., Ping, F., 2021. Second-trimester maternal lipid profiles rather than glucose levels predict the

628      occurrence of neonatal macrosomia regardless of glucose tolerance status: A matched cohort

629      study in Beijing. Journal of Diabetes and its Complications. 35, 107948.

630 Sastry, M. G Mohanty, S., & Rao, P., 2010. Role of placenta to combat fluorosis (in fetus) in endemic

631      fluorosis area.

632 Singh, N., Verma, K. G., Verma, P., Sidhu, G. K., & Sachdeva, S., 2014. A comparative study of fluoride

633      ingestion levels, serum thyroid hormone & TSH level derangements, dental fluorosis status

634      among school children from endemic and non-endemic fluorosis areas. Springerplus. 3, 1-5.

635 Stråvik, M., Gustin, K., Barman, M., Skröder, H., Sandin, A., Wold, A. E., ... & Vahter, M., 2021. Infant

636      iodine and selenium status in relation to maternal status and diet during pregnancy and

637      lactation. Frontiers in nutrition. 8.

638 Stråvik, M., Jonsson, K., Hartvigsson, O., Sandin, A., Wold, A. E., Sandberg, A. S., & Barman, M., 2019.

639      Food and nutrient intake during pregnancy in relation to maternal characteristics: Results

640      from the NICE Birth Cohort in Northern Sweden. Nutrients. 11, 1680.

641 Sun, L., Gao, Y., Zhang, W., Liu, H., & Sun, D., 2014. Effect of high fluoride and high fat on serum lipid

642      levels and oxidative stress in rabbits. Environmental Toxicology and Pharmacology. 38, 1000-

643      1006.

644 Sundquist, K., Qvist, J., Johansson, S. E., & Sundquist, J., 2004. Increasing trends of obesity in Sweden

645      between 1996/97 and 2000/01. International Journal of Obesity. 28, 254-261.

646 Surkan, P. J., Hsieh, C. C., Johansson, A. L., Dickman, P. W., & Cnattingius, S., 2004. Reasons for

647      Increasing Trends in Large for Gestational Age Births. Obstetrics & Gynecology. 104.

648 Textor, J., Hardt, J., & Knüppel, S., 2011. DAGitty: a graphical tool for analyzing causal diagrams.

649      Epidemiology. 22, 745.

650    Thomas, D. B., Basu, N., Martinez-Mier, E. A., Sánchez, B. N., Zhang, Z., Liu, Y., ... & Téllez-Rojo, M. M.,

651        2016. Urinary and plasma fluoride levels in pregnant women from Mexico City. Environmental

652        Research. 150, 489-495.

653    Till, C., Green, R., Grundy, J. G., Hornung, R., Neufeld, R., Martinez-Mier, E. A., ... & Lanphear, B., 2018.

654        Community Water Fluoridation and Urinary Fluoride Concentrations in a National Sample of

655        Pregnant Women in Canada. Environ Health Perspect. 126, 107001.

656    Umarani, V., Muvvala, S., Ramesh, A., Lakshmi, B. V. S., & Sravanthi, N., 2015. Rutin potentially

657        attenuates fluoride-induced oxidative stress-mediated cardiotoxicity, blood toxicity and

658        dyslipidemia in rats. Toxicology mechanisms and methods. 25, 143-149.

659    Valeri, L., VanderWeele, T. J., 2013. Mediation analysis allowing for exposure–mediator interactions

660        and causal interpretation: theoretical assumptions and implementation with SAS and SPSS

661        macros. Psychological methods. 18, 137.

662    Waugh, D. T., 2019. Fluoride exposure induces inhibition of sodium/iodide symporter (NIS)

663        contributing to impaired iodine absorption and iodine deficiency: molecular mechanisms of

664        inhibition and implications for public health. International journal of environmental research

665        and public health. 16, 1086.

666    Werner, S. C., Ingbar, S. H., Braverman, L. E., & Utiger, R. D. (Eds.). (2005). *Werner & Ingbar's the*

667        *thyroid: a fundamental and clinical text* (Vol. 549). Lippincott Williams & Wilkins.

668    Whitford, G. M., 1994. Intake and Metabolism of Fluoride. Advances in Dental Research. 8, 5-14.

669    World Health Organization, 2011. Guidelines for drinking-water quality, Edition, Fourth. World Health

670        Organization chronicle. 38, 104-108.

671    World Health Organization, 2013. Urinary iodine concentrations for determining iodine status in

672        populations. World Health Organization.

673    World Health Organization, 2014. Basic methods for assessing renal fluoride excretion in community

674        prevention programmes for oral health. World Health Organization.

675     Yan, J., Yang, H., 2014. Gestational diabetes mellitus, programing and epigenetics. J Matern Fetal

676          Neonatal Med. 27**,** 1266-9.

677     Yu, Y. M. Zhou, B. H., Yang, Y. L., Guo, C. X., Zhao, J., & Wang, H. W., 2021. Estrogen deficiency

678          aggravates fluoride-induced liver damage and lipid metabolism disorder in rats. Biological

679          Trace Element Research. 1-10.

680     Zhan, X. A., Li, J. X., Wang, M., & Xu, Z. R., 2006. Effects of fluoride on growth and thyroid function in

681          young pigs. Fluoride. 39**,** 95-100.

682 **Table 1.** Characteristics of the mothers-infant pairs included in the study

| | n | Mean (±SD) or % | Median (range) |
|---|---|---|---|
| **Maternal characteristics** | | | |
| Age (years) | 558 | 30.6 (4.7) | 30 (19, 45) |
| Weight in early pregnancy (kg) | 558 | 71.0 (14.1) | 68.0 (43.4, 137.0) |
| Height (cm) | 558 | 167.0 (6.1) | 167 (149, 184) |
| BMI in early pregnancy (kg/m$^2$) | 558 | 25.4 (4.9) | 24.4 (16.6, 50.4) |
| Parity (%) | 558 | | |
|   Primiparous | 263 | 47 | - |
|   Multiparous | 295 | 53 | - |
| Maternal education (university; %) | 558 | | |
|   No | 169 | 30 | - |
|   Yes | 389 | 70 | - |
| Pre-pregnancy smoking status (%) | 558 | | |
|   Non-smoking | 523 | 94 | - |
|   Smoking | 35 | 6 | - |
| Drinking water source at residence | 422 | | |
|   Municipal | 388 | 92 | - |
|   Private well | 34 | 8 | - |
| Season of sampling | 558 | | |
|   Spring | 165 | 30 | - |
|   Summer | 146 | 26 | - |
|   Autumn | 115 | 21 | - |
|   Winter | 132 | 24 | - |
| Thyroid dysfunction (%) | 558 | | |
|   No | 517 | 93 | - |
|   Yes | 41 | 7 | - |
| **Infant characteristics** | | | |
| Gestational age at birth (weeks; %) | 558 | 39.9 (1.7) | 40.1 (29.0, 42.7) |
| Preterm delivery (<37 GWs) | 558 | | |
|   Full term delivery | 530 | 95 | - |
|   Preterm delivery | 28 | 5 | - |
| Sex (%) | 558 | | |
|   Female | 308 | 55 | - |
|   Male | 250 | 45 | - |
| Birth weight (g) | 556 | 3579 (561) | 3560 (1110, 5165) |
| Birth length (cm) | 538 | 50.30 (2.25) | 50 (42, 58) |
| Birth head circumference | 538 | 34.90 (1.60) | 35 (29.4, 39) |
| Ponderal index | 524 | 2.8 (0.2) | 2.8 (2.1, 3.6) |
| Small for gestational age (SGA; <10$^{th}$ percentile) | 556 | | |
|   No | 508 | 91 | - |
|   Yes | 48 | 9 | - |
| Small for gestational age (SGA; <2 SD) | 556 | | |
|   No | 547 | 98 | - |
|   Yes | 9 | 2 | - |
| Large for gestational age (LGA; >90$^{th}$ percentile) | 556 | | |
|   No | 479 | 86 | - |
|   Yes | 77 | 14 | - |
| Large for gestational age (LGA; >2 SD) | 556 | | |
|   No | 535 | 96 | - |

30

| | | | |
|---|---|---|---|
| Yes | 21 | 4 | - |

683 Abbreviations: BMI: body mass index; UFC: urinary fluoride concentration; GW: gestational week;
684 SG: specific gravity; SD: standard deviation

685 **Table 2.** Distribution characteristics of urinary fluoride concentrations at gestational week 29 and at
686 4 months postpartum

| | N | Mean (±SD) | Median (IQR) | Range | $5^{th}$ - $95^{th}$ percentile |
|---|---|---|---|---|---|
| UFC [a] (mg/L; GW 29) | 558 | 0.86 (0.67) | 0.71 (0.48) | 0.07 - 6.4 | 0.31 - 1.9 |
| UFC [a] (mg/L; 4 months postpartum) | 463 | 0.89 (0.54) | 0.77 (0.57) | 0.14 - 4.8 | 0.32 – 1.9 |

687 Abbreviations: UFC: urinary fluoride concentrations; GW: gestational week; SD: standard deviation;
688 IQR: interquartile range
689 [a] Urinary fluoride concentrations are adjusted for specific gravity

**Table 3.** Multivariable-adjusted linear regression models of maternal urinary fluoride concentrations at gestational week 29 with the birth outcomes (gestational age, weight, length, head circumference, and the ponderal index at birth)

| | | Maternal UFC at gestational week 29 [a] | | | | |
| | n | Crude models | | Adjusted models [b] | | Interaction by infant sex [c] |
| | | B (95% CI) | P -value | B (95% CI) | P -value | P-value |
| Birth weight (g) | 556 | 93 (24, 162) | 0.009 | **84 (30, 138)** | 0.003 | 0.894 |
| Birth length (cm) | 538 | 0.39 (0.11, 0.67) | 0.006 | **0.41 (0.18, 0.65)** | 0.001 | 0.183 |
| Head circumference at birth (cm) | 538 | 0.3 (0.1, 0.5) | 0.009 | **0.3 (0.1, 0.4)** | 0.001 | 0.309 |
| Gestational age at birth (days) | 558 | 0.2 (-1.3, 1.7) | 0.809 | 0.1 (-1.4, 1.7) | 0.865 | 0.230 |
| Ponderal index [d] | 524 | 0.00 (-0.03, 0.03) | 0.951 | 0.01 (-0.02, 0.04) | 0.638 | 0.444 |

Abbreviations: UFC: urinary fluoride concentration; CI: confidence interval

[a] Urinary fluoride concentrations are adjusted for specific gravity

[b] Estimates are adjusted for maternal age (continuous; years), parity (categorical; primiparous/multiparous), maternal body mass index in early pregnancy (BMI; continuous; kg/m$^2$) or maternal height in the analyses of length at birth (continuous; cm), maternal education (categorical; no university degree/university degree), maternal smoking status before pregnancy (categorical; ever/never), gestational age at birth (continuous; days; not included in the analyses of gestational age at birth), child sex (categorical; male/female), and season of urine sample collection (categorical; spring/summer/autumn/winter)

[c] P-for-interaction values are derived from the adjusted models with an addition of a multiplicative term (exposure * infant sex)

[d] Ponderal index = weight (grams) * 100 / [length (cm)]$^3$

701 **Table 4.** Multivariable-adjusted logistic regression models of maternal urinary fluoride
702 concentrations at gestational week 29 with the odds of a preterm delivery and of being born small or
703 large for gestational age

| | Maternal UFC (mg/L) at gestational week 29 [a] | | | |
|---|---|---|---|---|
| | *Controls, n* | Cases, n | OR (95% CI) [b] | *P-value* |
| SGA (< 10th percentile) | 508 | 48 | 0.71 (0.38, 1.33) | 0.287 |
| SGA (< 2SD) | 547 | 9 | 1.23 (0.55, 2.76) | 0.612 |
| LGA (> 90th percentile) | 479 | 77 | **1.39 (1.03, 1.89)** | 0.034 |
| LGA (> 2SD) | 535 | 21 | 1.27 (0.74, 2.19) | 0.387 |
| Preterm birth [c] | 530 | 28 | 0.80 (0.38, 1.66) | 0.543 |

704 Abbreviations: UFC: urinary fluoride concentration; SGA: small for gestational age; LGA: large for
705 gestational age
706 [a] Urinary fluoride concentrations are adjusted for specific gravity
707 [b] Odds ratios are adjusted for maternal age (continuous; years), parity (categorical;
708 primiparous/multiparous), maternal body mass index in early pregnancy (BMI; continuous; $kg/m^2$),
709 maternal education (categorical; no university degree/university degree), maternal smoking status
710 before pregnancy (categorical; ever/never), gestational age at birth (continuous; days; not included
711 in the analyses of preterm birth), child sex (categorical; male/female), and season of urine sample
712 collection (categorical; spring/summer/autumn/winter)
713 [c] Preterm birth was defined as a delivery prior to the 37th gestational week

714 **Table 5**. Descriptive statistics of maternal thyroid biomarkers and multivariable-adjusted linear regression models of maternal urinary fluoride
715 concentration with thyroid biomarkers at gestational week 29

| | | | | Maternal UFC (mg/L) at gestational week 29 [a] | |
|---|---|---|---|---|---|
| | $n$ | Mean (±SD) | Median (range) | B (95% CI) [b] | $P$ - value |
| Thyroid Stimulating Hormone (TSH; mIU/L, $\log_2$) | 516 | 1.8 (1.2) | 1.6 (0.07, 20.2) | 0.02 (-0.09, 0.12) | 0.741 |
| free Thyroxine (fT4; pmol/L) | 516 | 12.3 (1.6) | 12.3 (7.9, 17.5) | -0.13 (-0.34, 0.09) | 0.243 |
| free Triiodothyronine (fT3; pmol/L) | 516 | 4.1 (0.44) | 4.1 (2.9, 5.6) | 0.03 (-0.02, 0.09) | 0.224 |
| total Thyroxine (tT4; nmol/L) | 516 | 124 (19.6) | 122 (71.4, 193) | -0.03 (-2.63, 2.58) | 0.984 |
| total Triiodothyronine (tT3; nmol/L) | 516 | 2.6 (0.44) | 2.6 (1.5, 4.5) | 0.05 (-0.01, 0.10) | 0.083 |
| fT4:tT4 | 516 | 0.10 (0.01) | 0.10 (0.06, 0.14) | -0.001 (-0.003, 0.000) | 0.100 |
| fT3:tT3 | 516 | 1.60 (0.18) | 1.59 (1.12, 2.18) | -0.018 (-0.041, 0.004) | 0.115 |
| fT3:fT4 | 516 | 0.34 (0.06) | 0.33 (0.20, 0.55) | **0.007 (0.000, 0.014)** | 0.047 |

716 Abbreviations: UFC: urinary fluoride concentration; CI: confidence interval
717 [a] Urinary fluoride concentrations are adjusted for specific gravity
718 [b] Estimates are adjusted for maternal age (continuous; years), maternal body mass index (BMI; continuous; kg/m$^2$), maternal education
719 (categorical; no university degree/university degree), maternal smoking status before pregnancy (categorical; ever/never), parity (categorical;
720 primiparous/multiparous), and the season of urine samples collection (categorical; spring/summer/autumn/winter

721 **Table 6.** Mediation effects investigating whether maternal ratio of free triiodothyronine concentration to
722 total triiodothyronine concentrations mediate the association between maternal urinary fluoride
723 concentration and infant weight and length at birth

| Exposure | Outcome | Mediator | Total effect[α] | Direct effect[α] | Indirect effect[α] |
|---|---|---|---|---|---|
| | | | B (95%CI) | B (95%CI) | B (95%CI) |
| UFC[α] | Birth weight | fT3:fT4 | **56 (10, 98)** | **53 (9, 95)** | 3 (-1, 11) |
| UFC[α] | Birth length | fT3:fT4 | **0.32 (0.10, 0.54)** | **0.31 (0.10, 0.53)** | 0.02 (-0.00, 0.06) |

724 Abbreviations: UFC: urinary fluoride concentration; free triiodothyronine: fT3; total triiodothyronine: tT3
725 [a] Urinary fluoride concentrations are adjusted for specific gravity
726 [b] Estimates are adjusted for maternal age (continuous; years), maternal body mass index (BMI;
727 continuous; kg/m2) or maternal height in the analyses of birth length (continuous; cm), maternal
728 education (categorical; no university degree/university degree), maternal smoking status before
729 pregnancy (categorical; ever/never), parity (categorical; primiparous/multiparous), gestational age at
730 birth (continuous; days), child sex (categorical; male/female) and the season of urine samples collection
731 (categorical; spring/summer/autumn/winter)

732 **Figure captions**

733

734 **Figure 1.** Flowchart of the mother-child pairs included in the present study
735 Abbreviations: NICE: Nutritional Impact on the Immunological Maturation during Childhood in
736 relation to the Environment; UFC: urinary fluoride concentration; GA: gestational age; BW: birth
737 weight; BL: birth length; BHC: birth head circumference

738

739

740 **Figure 2.** Heat map of clustered bivariate correlations between maternal urinary fluoride
741 concentrations and maternal diet during pregnancy based on a Food Frequency Questionnaire. The
742 associations are based on Spearman rank correlation test; the direction of the correlations is
743 indicated by color (red indicates positive and blue indicates negative correlations); the magnitude
744 of the correlations is denoted by the colour shade (deeper shades depict stronger correlations and
745 lighter weaker) and statistical significance is presented by asterisks (∗P < 0.05; ∗∗P < 0.01;
746 ∗∗∗P < 0.001)

747

748

749 **Figure 3.** Generalized additive models (GAMs) of maternal urinary fluoride concentration (UFC) at
750 gestational week 29 (GW 29) adjusted for specific gravity with birth outcomes (gestational age,
751 weight, length, head circumference, and the ponderal index at birth); GAMs are adjusted for
752 maternal age (years), maternal parity (primiparous/multiparous), maternal body mass index (BMI;
753 kg/m$^2$) or maternal height in the analyses of child birth length (cm), maternal education (university
754 degree/no university degree), maternal smoking status before pregnancy (ever/never), gestational
755 age at birth (days; not included in the GAM of gestational age at birth), child sex (male/female), and
756 season of urine sample collection (spring/summer/autumn/winter).

**Fluoride in relation to diet**
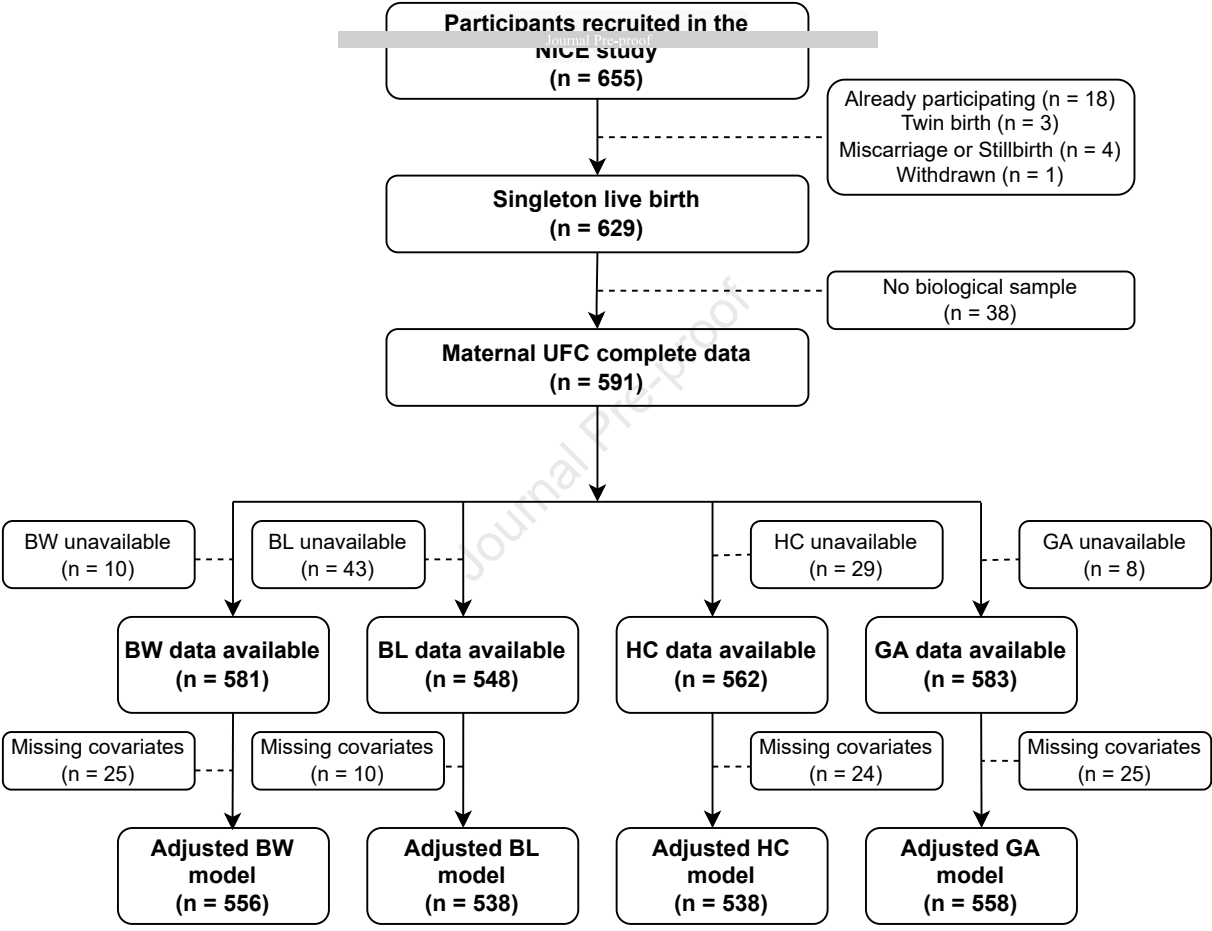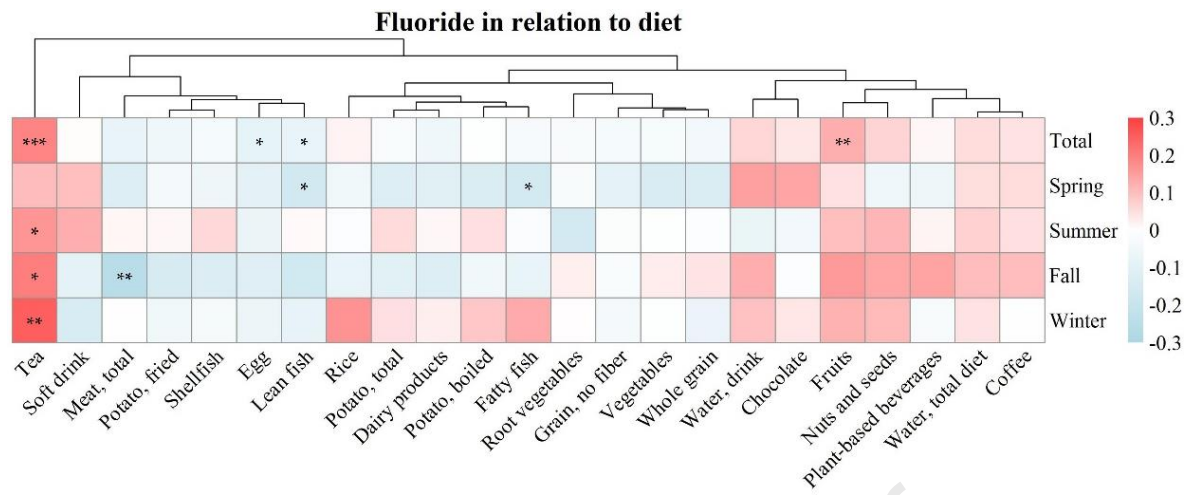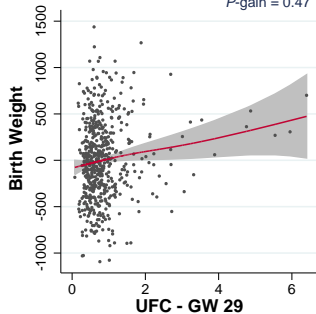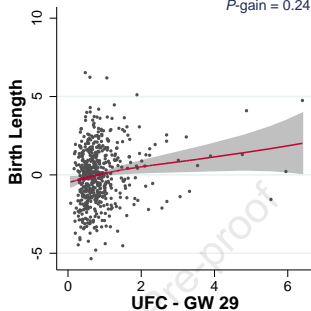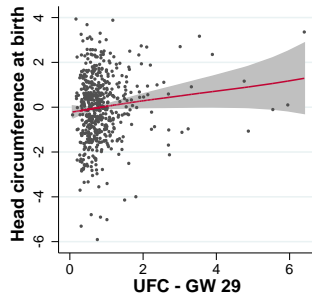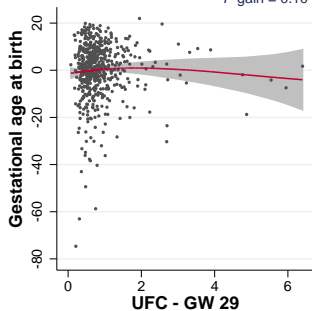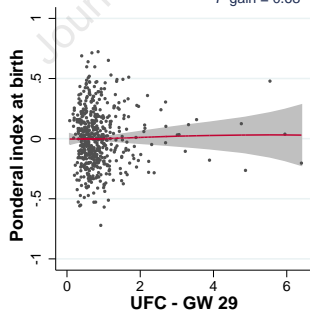
**Highlights**

- Gestational fluoride exposure was associated with increased infant size at birth.

- Fluoride exposure was linked with increased odds of large for gestational age.

- Maternal thyroid hormone levels did not explain the observed associations.

**Declaration of interests**

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: