Drinking water disinfection by-products during pregnancy and child neuropsychological development in the INMA Spanish cohort study

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ABSTRACT

1   *Background*. Disinfection by-products (DBPs) constitute a complex mixture of

2   prevalent chemicals in drinking water and there is evidence of neurotoxicity for some

3   of them.

4   *Objectives*. We evaluated the association between estimates of DBP exposure during

5   pregnancy and child neuropsychological outcomes at 1 and 4-5 years of age.

6   *Methods*. We conducted a population-based mother-child cohort study in Spain with

7   recruitment at first trimester of gestation (INMA Project, 2003-2008).

8   Neuropsychological development was measured at 1 year of age using the Bayley

9   Scales of Infant Development and at 4-5 years with the McCarthy Scales of Children's

10   Abilities. Modeled tap water concentrations of trihalomethanes (THM) were combined

11   with personal ingestion, showering and bathing habits to estimate exposure as

12   ingestion uptake, all route (showering, bathing, ingestion) uptake (μg/day) and crude

13   levels (μg/l) in the residence. Chloroform, brominated THMs (bromodichloromethane,

14   dibromochloromethane, bromoform) and total THMs (chloroforom and bromianted

15   THMs) were analysed separately. Nine haloacetic acids levels were available in one

16   of the areas. Linear regression was used to estimate associations in 1855 subjects

17   adjusting for covariables.

18   *Results*. The median concentration of total THMs, chloroform, brominated THMs, total

19   haloacetic acids, dichloroacetic acid, and trichloroacetic acid were, respectively

20   30.3μg/L, 9.4μg/L, 11.6μg/L, 10.5μg/L, 2.7μg/L, and 3.1μg/L. The associations

21   between THM exposure and neuropsychological outcomes were null, except for total

22   and brominated THM uptake though all routes and the general cognitive score at 4-5

23   years, with a decrease in -0.54 points (95%CI -1.03, -0.05) and -0.64 (95%CI -1.16, -

24   0.12), respectively, for doubling total and brominated THM uptake. A positive

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1 association found between dichloroacetic acid and the mental score at 1 year did not

2 persist at 4-5 years.

3 *Conclusions*. Minor associations observed between DBP exposure during gestation

4 and child neuropshychological development at 1 year disappeared at 4-5 years.

5 Although a suggestive association is identified for exposure to brominated THMs and

6 the cognitive score at 4-5 years, chance cannot be ruled out.

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9 KEYWORDS: children, disinfection by-products, haloacetic acids, neurodevelopment,

10 trihalomethanes, water

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1. INTRODUCTION

Disinfection by-products (DBPs) are widespread chemicals in drinking water produced as undesired side effects of disinfection process, which is necessary to remove pathogens and prevent waterborne infections. Trihalomethanes (THMs) and haloacetic acids (HAAs) are the two classes at highest concentrations when the disinfectant used is chlorine-based (Richardson et al. 2007). Some DBPs, such as THMs are volatile and skin permeable and human exposure occur through different routes (ingestion, inhalation, skin contact) in activities involving water contact such as showering, bathing, swimming in pools, and intake of water and water-based fluids (Villanueva et al. 2015). On the contrary, HAAs are not volatile or skin permeable and incorporation is mainly through the ingestion route. Given the ubiquity of DBPs, the multiple routes involved and the potential adverse outcomes, DBPs constitute an environmental exposure of concern.

Trihalomethanes are a class of DBPs including chloroform, bromodichloromethane, dibromochloromethane and bromoform. The sum of these four is known as total THMs (TTHMs) and is regulated in the US and EU, among other countries, with a maximum contaminant level (MCL) of 80μg/l and 100μg/l, respectively (Villanueva et al. 2014). Haloacetic acids are a family of DBPs including 9 chemicals: monochloro-, dichloro-, trichloro-, monobromo-, dibromo-, tribromo-, bromochloro, bromodichloro, and dibromochloroacetic acids. The sum of monochloro-, dichloro-, trichloro-, monobromo-, and dibromoacetic acid is regulated in the US with a MCL of 60μg/l (Villanueva et al. 2014). Other DBPs, up to more than 700, have been identified in drinking water, including haloacetonitriles, haloketones among others (Richardson et al. 2007). These

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occur at much lower concentrations, in the range of ng/l and are not regulated in drinking water (Villanueva et al., 2014).

Dichloroacetic acid is a HAA with extensive evidence of neurotoxicity in humans at high doses. Dichloroacete salts were used as a drug to treat several metabolic, cardiovascular and cerebrovascular disorders in the past (Stacpoole et al. 1998). Peripheral neuropathy (extremity weakness, decreased nerve conduction velocity, ataxia, tremors) produced by dichloroacetate has been reported in humans (Kaufmann et al. 2006; Spruijt et al. 2001; Stacpoole et al. 1979). Mechanisms suggested by experimental studies in animals involve degeneration of spinal cord nerve fibers, myelin changes and gliosis, observed in rat brain and *in vitro* studies (Felitsyn et al. 2007; Moser et al. 1999). Other haloacetic acids have been shown to produce neurotoxicity in experimental studies, including trichloroacetic acid (neuroembryopathic effect in rats exposed during organogenesis) (Singh 2006), dibromoacetic acid (neuromuscular toxicity in rats) (Moser et al. 2004), and monocloroacetic acid (neuronal cell death through oxidative stress *in vitro*) (Chen et al. 2013; Lu et al. 2015).

Chloroform used as a solvent has been classified as neurotoxic in humans (Grandjean & Landrigan, 2006) but there is no human evidence on neurotoxicity of chloroforom or other THMs in drinking water. Autistic like behaviors have been observed in male mice after gestational and postnatal exposure to chloroforom and bromoform in drinking water (Guariglia et al. 2011). However, no evidence of neurotoxicity or neurobehavioural effects on motor activity has been observed in other animal studies (Balster & Borzelleca 1982; Moser et al. 2007). Experimental evidence in animals from

other DBPs occurring at lower levels in drinking water also show effects in murine studies. Chloroacetonitrile crosses the placenta and fetal blood-brain barrier and induces oxidative stress and apoptotic neurodegeneration in fetal brain in mice (Ahmed et al. 2005). Dichloroacetonitrile induces oxidative stress and developmental apoptotic imbalance in mouse fetal brain (Esmat et al. 2012). Detrimental behavioral effects in mice exposed to chloral during the prenatal and early postnatal period but no association among adult animals have been shown (Kallman et al. 1984).

The developing brain and nervous system during gestation is particularly vulnerable to environmental insults, with potential long-term consequences (Grandjean & Landrigan 2014). Small molecules such as chloroform crosses the placenta and can reach the fetus in humans (Dowty et al. 1976), but there is no evidence on the transplacental transmission for other DBPs. Given the widespread character of DBP exposure and the existing evidence suggesting potential neurotoxicity, the evaluation of neurodevelopmental impacts of DBP exposure *in utero* is warranted. We specifically aim to evaluate the association between markers of DBP exposure during pregnancy and neuropsychological outcomes at 1 and 4-5 years of age in a population-based mother-child cohort study in Spain.

2. METHODS

2.1. Study design and population

A mother-child cohort study was set up in 4 Spanish areas (Asturias, Gipuzkoa, Sabadell and Valencia) following a common protocol to constitute the INMA – INfancia y Medio Ambiente [Environment and Childhood] Project. For a detailed description of study areas see Supplemental Material. Study subjects were recruited at the first

trimester of gestation and followed until delivery. Eligibility criteria for enrollment were maternal age 16 years or older, singleton pregnancy, planning to deliver at the study hospitals, being able to communicate in either of the official languages, and not having followed an assisted reproduction program (Guxens et al. 2012). The study sample was representative of the target population in terms of prenatal care attendance in the public health system (used by more than 80% of the pregnant women). From 45% to 98% of the eligible pregnant women agreed to participate and enrollment periods ranged from November 2003 in Valencia to February 2008 in Gipuzkoa (Guxens et al. 2012). Recruited subjects at the first trimester of gestation were 494 in Asturias, 638 in Gipuzkoa, 657 in Sabadell and 827 in Valencia. Follow-up occurred at the third trimester of gestation, delivery, 1 year and 4-5 years of age. From the initial sample at recruitment, 475 children (96%) in Asturias, 599 (94%) in Gipuzkoa, 583 (89%) in Sabadell and 708 (86%) in Valencia were included at the 1 year follow up and mothers confirmed informed consent to participate for their children. The follow up at 4-5 years included 2028 children (453 in Asturias, 505 in Gipuzkoa, 514 in Sabadell and 556 in Valencia). The study protocol was approved by the Institutional Ethical Committees of the participating centers, and all included mothers gave written and voluntary consent in each phase of the study prior participation. See Figure S2 for more details on included and excluded subjects.

2.2. THM and HAA levels

Chlorine was the main disinfectant used for drinking water in all the study areas. Sampling locations were defined *a priori* to cover geographically the study areas (see Supplemental Material Figure S1). Water samples were collected from the tap with no filtration or other treatments that could affect THM or HAA concentration. Sample

collection in the different study areas was conducted by local study personnel, who was specifically trained to follow a standardized procedure (see Supplemental Material for details on Experimental THM and HAA analysis in tap water). The sampling strategy did not consider individual pregnancy periods but covered the period between the minimum and maximum conception dates of study subjects.

Trihalomethanes. Concentration of THMs was ascertained based on sampling campaigns and regulatory data from local authorities and water companies. Measurements were conducted at different time points: 2004–2008 (Asturias), 2006–2008 (Gipuzkoa), 2004–2006 (Sabadell), and 2004–2005 (Valencia). THMs were determined in 183 samples in Asturias (18 from our own sampling and 165 from regulatory measurements), 421 in Gipuzkoa (own sampling), 198 in Sabadell (148 own sampling, 50 regulatory), and 162 in Valencia (own sampling).

Haloacetic acids. Measurements of HAAs were available in one of the areas (Gipuzkoa), including 9 components: monochloro-, dichloro-, trichloro-, monobromo-, dibromo-, tribromo-, bromochloro, bromodichloro, and dibromochloroacetic acids. Only total, dichloro and trichloroacetic acid were further used to evaluate exposure since other HAAs occurred at low concentrations (Santa-Marina et al. 2010). A total of 264 measurements were conducted in 26 sampling points from 2007 to 2011.

2.3. THM and HAA modeling

Comparison of mean THM concentrations based on regulatory surveys and our own measurements did not show significant differences (*p*-value from *t*-test > 0.10), and data from both sources were used. Separate models for each area were conducted to predict total THM, chloroform, bromodichloromethane, dibromochloromethane, bromoform, total haloacetic acids, dichloroacetic acid and trichloroacetic acid to assign

a concentration to the distribution system of the municipality where women resided. For the modeling procedure, see Supplemental Material. Final models predicted average monthly levels from conception until delivery in each participant's residential water supply. Estimation of levels was not possible for all pregnancies followed to delivery because of missing concentration data in some municipalities, missing or incomplete address, or missing gestational age.

2.4. Exposure indices

Uptake factors (blood concentration) were used to convert exposure from different situations (ingestion, showering, bathing) to common units, to allow adding them and estimate total exposure at the residence. Uptake factors are available in the literature for THM only, and not for HAAs, thus precluding the study of HAA uptakes in the association of neuropsychological outcomes. The modeled residential THM level was multiplied by daily personal water use and uptake factors (see Supplemental Material Table S1), to derive an estimate of daily THM concentration in the bloodstream (Whitaker et al. 2003). Chloroform and brominated THM were analyzed separately because toxic properties differ among species, particularly brominated versus chlorinated species. A 90% reduction in ingestion was applied if a home filter was used (Egorov et al. 2003; Weinberg et al. 2006). Water consumption outside the home was mainly bottled in all areas (90% overall, range 79 to 96%) and was not considered. We averaged the 12- and 32-week tap water intakes to compute the ingested THM. Average THM uptake in the first, second, and third trimester and the whole pregnancy were calculated. Bathing and showering uptakes were added, and total household uptake was calculated by adding ingestion, showering, and bathing. More details on the exposure assessment is available in Villanueva et al. 2011.

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2 2.5. *Outcomes*

3 Child neuropsychological development was measured at ~14 months of age (median

4 14, range:13–15) using the Bayley Scales of Infant Development (BSID) (Bayley 1993)

5 by twelve experienced and trained psychologists in the presence of the mother at the

6 primary care center. The BSID consists of two scales, the mental and the psychomotor

7 scales. The mental scale consisted of 163 items that assessed age-appropriate

8 cognitive development in areas such as performance ability, memory, and first verbal

9 learning. The psychomotor scale consisted of 81 items assessing fine and gross

10 psychomotor development. All assessments were carried out according to a strict field

11 work protocol, and included inter-observer reliability tests estimated by intra-class

12 correlation tests (0.90 for the mental scale and 0.91 for the psychomotor scale).

13 Furthermore, Cronbach's Alpha Coefficient was used to determine the internal

14 consistency per each of the scales. Scale Alpha Coefficients were around 0.70 (good

15 to moderate). Raw scores were standardized for each child's age in days at the time

16 of test administration using a parametric method for the estimation of age-specific

17 reference intervals (Royston & Wright 1998). The parameters of the distribution are

18 modeled as a fractional polynomial function of age and estimated by maximum

19 likelihood. Standardized residuals were centered to a mean of 100 with a standard

20 deviation (SD) of 15 points. Participants for which neuropsychological tests were of

21 poor quality due to neurodevelopmental disabilities (Down syndrome and autistic traits)

22 or less-than-optimal cooperation of the child (due to tiredness, bad mood, or illness)

23 were flagged by the psychologists during evaluation and excluded from our analysis

24 (N=150), see Figure S2.

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The same children were interviewed/evaluated at 4-5 years of age (median: 4.5, range 4.4 – 5.6) by six trained psychologists using a standardized Spanish version of the McCarthy Scales of Children's Abilities (MSCA) (McCarthy 1996). The MSCA comprises 18 subtests that provide information on 5 scales (verbal, perceptual performance, memory, quantitative, and motor functions) and one general scale which is the sum of verbal, perceptual and quantitative scales, since memory scale overlaps with the other subscales' subtests. The verbal scale refers to cognitive tasks related to the processing of verbal information; the perceptual performance scale refers to cognitive tasks related to perceptual information processing, including manual performance; the memory scale considers short-term retention of information (verbal, visual, or numerical); the quantitative scale refers to numerical abilities; and the motor scale refers to fine (e.g., drawing) and gross (e.g., balance or accuracy) functions. In order to reduce multiple comparison problems, the present analysis selected the motor scale and general cognitive scale in which both of them contain all MSCA subtests. Raw scores were centered on a mean of 100 with an SD of 15. This was to obtain indexes in accordance with a local normative sample and to avoid the use of US norms provided in the manual. Testing was conducted according to a strict protocol, including neuropsychologist training, and for a small number of children, multiple neuropsychologist evaluations were performed with results reached by consensus. Alpha Coefficient for general cognitive scale was of 0.90 (excellent), MSCA motor subscale was of 0.64 each (good to moderate).

The standardization differs between the two scales, BSID and MSCA. BSID raw scores were regressed (fractional polynomials) on age and residuals of the regression become the outcome of interest due to age disparity between child cohort regions at

1   this developmental time, and due to their none-linear relation pattern observed. MSCA

2   age cohort differences were less important and a there was strong linear age-outcome

3   pattern that allowed us to center scores on a mean of 100 with and SD of 15 and further

4   include child age in the regression models, which is a simpler and preferred outcome

5   modeling.

6

7   *2.6. Covariables*

8   Parental and prenatal variables potentially influencing the evaluated outcomes were

9   considered. Maternal age, height, pre-pregnancy weight, education, marital status,

10   parity, country of birth (European, non-European) and paternal weight were collected

11   at enrolment (first trimester of gestation). Smoking and alcohol consumption during

12   pregnancy was recorded at third trimester of pregnancy. Date of the last menstrual

13   period was used as conception date. Maternal weight gain during pregnancy was

14   computed as the rate of weight gain during the second and third trimester in kg per

15   week (Rasmussen & Yaktine 2009), adjusted for gestational age at the last available

16   weight measure to correct possible heteroscedasticity and nonlinearity of the rate

17   (Dietz et al. 2006). Maternal social class was coded from the longest held job during

18   the pregnancy, using the 4-digit Spanish classification (CNO 1994), which is closely

19   related to the (ISCO 88). When the mother did not work during pregnancy (19.6% of

20   study subjects), the longest held job in the previous 10 years was used to define social

21   class. Among women who did not have a paid job in the previous 10 years (1.5%), the

22   occupation of the father was used. A proxy of maternal verbal intelligence quotient (IQ)

23   was measured through the Similarities subtest of the Wechsler Adult Intelligence-Third

24   Edition (WAIS-III) at the 4-5 year follow up. Use of bleach during pregnancy was

ascertained at week 32, and was explored in the models as cleaning has been identified as a source of THM exposure (Charisiadis et al. 2014).

2.7. *Statistical analyses*

A total of 1855 subjects were included in the BSID models at 1 year and 1453 in the MSCA models at 4-5 years (see Figure S2). Chloroform, brominated THMs, and total THM exposures were log transformed to normalize the distribution. We evaluated the association between the outcomes and log10 THM uptake and THM levels by linear regression adjusting for area, age at examination, sex, psychologist, quality of the test, parity and maternal age, social class, born in Europe, alcohol and smoking during pregnancy, height, pre-pregnancy body mass index, and weight gain during pregnancy. These were statistically significant covariates (p-value <0.05) or variables that modified ≥10% the β-coefficient either for BSID or MSCA outcomes. Missing values in some covariables yielded a slightly lower number of subjects included in the multivariate models. The effect of additionally adjusting for maternal IQ was estimated among the subset with available maternal IQ score (N=1351). To facilitate the interpretation of the associations as a change in the outcome for doubling exposure, coefficients from the regression models were multiplied by the logarithm of 2. Main analyses were conducted for average exposure in pregnancy. Alternative analyses were stratified by sex, given that previous evidence suggested an effect modification by sex (Guariglia et al. 2011), area, exposure by trimester of pregnancy, and social class. Sensitivity analyses were conducted by excluding children born preterm (4%) and those changing address during pregnancy (4%). Generalized additive models (GAMs) were used to evaluate the shape of the dose–response curves.

3. RESULTS

The characteristics of the study population are shown in Table 1. Half the population was female and 4% were born preterm. Median maternal age at delivery was 31 years, with a body mass index of 22.6, and a weight gain during pregnancy of 420 grams/week. Most of them were born in Europe (94%), were semi-skilled or unskilled workers (51%), and primiparous (57%). Smoking and alcohol consumption during pregnancy occurred among 31% and 9%, respectively.

The water use habits in the residence leading to DBP exposure are shown in Table 2. While showering and bathing habits were similar among areas, the type of drinking water consumed at home varied widely. Overall, bottled water consumption was the main source of drinking water at home, with an average of 62% ranging from 24% (Gipuzkoa) to 87% (Sabadell). In contrast, type of water consumed outside home was mostly bottled in all areas.

As shown in Figure 1, median level of total THMs in the residential tap water during pregnancy was 30.3 μg/L (percentile 25-percentile 75: 11.6 - 96.3 μg/L) in all areas combined. By area, the median ranged from 5.0 μg/L (Valencia) to 117.1 μg/L (Sabadell). Median chloroform level was 9.4 μg/L (p25-p75: 3.5-21.4 μg/L) in all areas combined. By area, the median value ranged from 0.3 μg/L (Valencia) to 29.4 μg/L (Asturias). Median brominated THM level was 11.6 μg/L (p25-p75: 7.4-86.9 μg/L) in all areas combined. By area, median ranged from 4.5 μg/L (Valencia) to 102.2 μg/L (Sabadell). Median total THM ingestion uptake was 0.001 μg/day (p25-p75: 0.0004-0.04 μg/day) in all areas combined. By area, median ranged from 0.000017 μg/day (Valencia) to 0.036 μg/day (Gipuzkoa). Median all route THM uptake was 0.40 μg/day

1 (p25-p75: 0.14-1.25 μg/day) in all areas combined. By area, median ranged from 0.11

2 μg/day (Valencia) to 1.57 μg/day (Sabadell). HAAs levels were available in Gipuzkoa,

3 with a median level of 2.7 μg/L (p25-p75: 1.8-4.0 μg/L) for dichloroacetic acid, 3.1 μg/L

4 (p25-p75: 2.4-4.8 μg/L) for trichloroacetic acid, and 10.5 μg/L (p25-p75: 8.0-13.7 μg/L)

5 for total haloacetic acids. Levels of HAAs and THMs were correlated (see table S2),

6 with highest correlations between trichloroacetic acid (TCAA) and chloroform

7 (Spearman rank correlation coefficient, r=0.85).

8

9 The outcome variables are continuous scores and higher values mean better

10 performance in the neuropsychological tests. The distribution of the outcome variables

11 is shown in Figure 2. The median raw mental/cognitive score was 114 (percentile 25-

12 percentile 75: 109-119) points at 1 year and 135 (p25-p75: 117-162) points at 4-5 years

13 in all areas combined. The median raw motor score was 51 (p25-p75: 48-53) points at

14 1 year and 41 (p25-p75: 36-49) points at 4-5 years in all areas combined. The variables

15 used in the multivariate models were the centered to an average of 100 with standard

16 deviation of 15.

17

18 The associations between exposure to total THM, chloroform and brominated THMs

19 during pregnancy and neuropsychological outcomes at 1 year and 4-5 years are shown

20 in Table 3 (total THMs) and Table 4 (chloroform, brominated THMs). The associations

21 were generally null. However, residential uptake through all routes to total THMs (Table

22 3) and brominated THMs (Table 4) was associated with a slightly reduced cognitive

23 score at 4-5 years, with a decrease of -0.54 (95% confidence interval, CI, -1.03 to -

24 0.05) and -0.64 (-1.16 to -0.12) points, respectively for doubling total THM and Br-

25 THMs uptake. These associations were similar, although not statistically significant in

the analyses separately for boys and girls, and by trimesters of pregnancy (see Table S3). The GAM of the cognitive score at 4-5 years showed a slightly decreasing curve with the continuous all route brominated THM uptake, confirming a linear association (Figure 3). A positive association was found for the mental score at 1 year among girls (Table 3), driven by brominated THMs (Table 4). The GAMs showed flat curves (see Figure S3) and linear trends except for THM levels and the mental score at 1 year, where the curve showed non-linear associations (p-value for gain compared to the linear model 0.03).

Adjusting for maternal IQ led to similar associations, with wider confidence intervals compared to results in Table 3, with and effect of -0.47 (-0.96 to 0.03) for the cognitive score at 4-5 years associated with doubling all route THM uptake. Excluding preterm births and those that changed address during pregnancy led to similar associations compared to the main models in Table 3. The effect (95% CI) for the cognitive score at 4-5 years associated with doubling all route THM uptake was -0.61 (-1.11 to -0.11) after excluding preterm births and -0.62 (-1.12 to -0.12) after excluding those changing address, and the number of observations of the models were, respectively 1373 and 1384.

Analyses stratified by area showed mostly non- statistically significant associations around the null (see Table S4), similarly to the main combined analyses. A negative statistically significant association between all route THM uptake and the mental score at 1 year was observed in Asturias (effect=-1.75, -3.47 to -0.04), where the examination was done mostly after 1.5 years of age. A statistically significant positive association is observed in Sabadell for ingestion THM uptake and the cognitive score at 4-5 years

(Table S4). An effect modification by social class is observed in the association between the ingestion THM uptake and the motor score at 1 year (Table S5).

The associations between levels of haloacetic acids during pregnancy and neuropsychological outcomes are shown in Table 5. A statistically significant positive association driven by girls is observed between dichloroacetic acid and the mental score at 1 year, with an increase of 1.78 points (0.12 to 3.45) for doubling dichloroacetic acid levels, that does not persist at 4-5 years (effect=0.91, -0.81 to 2.62).

4. DISCUSSION

To our knowledge this is the first epidemiological study evaluating the association between markers of DBP exposure during pregnancy and neuropsychological outcomes in children. Average residential levels of total THMs, chloroform, brominated THMs, total haloacetic acids, dichloroacetic acid, and trichloroacetic acid in the study areas were, respectively 50.5, 12.7, 34.5, 11.3, 3.0, and 3.6μg/L. A high proportion of women consumed bottled water during pregnancy and showering-bathing was the most important contributor to residential THM uptake. The associations for THM exposure were generally null, except from a small negative association between all route total THM and Br-THM uptake and the general cognitive score at 4-5 years. There was an effect modification by sex for some associations, and a positive association was found among girls for the mental score at 1 year that did not persist at 4-5 years. Results were consistent among areas.

An overall interpretation of the associations evaluated for THM exposure indicates mostly lack of association between child neurodevelopment and exposure during

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pregnancy. A small but statistically significant association is suggested between total and brominated THMs and the cognitive score at 4-5 years, similar between boys and girls and for trimesters of exposure. A similar result is observed in the area with highest levels of brominated THMs (Sabadell), although the association is not statistically significant probably due to limited sample size. However, previous evidence is limited to sustain this association. Behavioral toxicity of brominated THM has been tested among adult mice, showing effects on operant behavior with development of tolerance (Balster & Borzelleca 1982), and no neuropathological effects have been observed in a second study evaluating bromodichloromethane in rats (Moser et al. 2007). Other brominated DBPs evaluated in rats include dibromoacetonitrile and dibromoacetic acid, showing no effects for dibromoacetonitrile (Moser et al. 2007), while dibromoacetic acid produced concentration-related neuromuscular toxicity and degeneration of spinal cord nerve fibers (Moser et al. 2004). However, none of these studies evaluated prenatal exposure. In contrast, chloroform has been classified as neurotoxic solvent in humans (Grandjean & Landrigan 2006), while we do not find any evidence of association in our study at the observed exposure levels. Our study is not comparable with the only, to our knowledge, evaluation of infant neuropsychological development in relation to water DBP exposure in humans, since they measured motor development at 6 months (Lin et al. 2011). In consequence, although consistent and suggestive, the association we observe should be cautiously interpreted and chance cannot be ruled out given the large number of tests performed and the small magnitude of the association. We cannot discard, however, that adverse effects of prenatal DBP exposure might appear later in childhood. The prospective nature of the INMA Project, with neuropsychological developmental measurements until 11 years of age will allow the estimation of such late eventual effects.

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2 Although dichloroacetic acid was the DBP with a higher prior related to

3 neuropsychological development, the study had limited power and narrow exposure

4 variability to test the effect of haloacetid acids, since data was only available in one of

5 the areas. An unexpected positive association is observed between dichloroacetic acid

6 levels and the mental score at 1 year driven by girls, that disappears at 4-5 years. In

7 contrast, a negative (although not statistically significant) association was observed for

8 trichloroacetic acid levels and the motor score at 1 year, that did not persist at 4-5

9 years either. The adverse effects previously observed for dichloroacetic acid have

10 been reported at doses of 5-150 mg/day (Stacpoole et al. 1998), whereas average

11 (maximum) ingested dose in the study area was 3.4 μg/day (13.8 μg/day). In

12 consequence, our results show that haloacetic acids at the levels observed here do

13 not involve adverse effects on child neuropsychological development.

14

15 Our analysis was limited to prenatal exposure since data on postnatal exposure was

16 not available for all cohorts. We cannot rule out an effect of postnatal exposure, and

17 this deserves to be evaluated in future studies. Although we were careful to minimize

18 measurement error, exposure misclassification is a concern and we cannot rule out

19 that it could partly explain the null associations. The use of THMs and HAAs as

20 surrogates of other DBPs has limitations. Other DBPs occurring at lower levels may

21 have an effect on the neuropsychological development (Ahmed et al. 2005; Esmat et

22 al. 2012), and these are poorly correlated with THMs and HAAs (Villanueva et al.

23 2012). The different distribution of levels between areas is a concern in the analysis

24 combining all the areas, for the possible ecological comparisons. However, despite the

25 differences, there was an overlap in the distributions allowing the all-area combined

analyses. The area-stratified analyses showed some discordances that might suggest residual confounding. However, most of the associations were similar to the all-area combined analyses, providing reliability to these results. The study population had a higher educational level compared to the general population. To address this, all multivariate models were adjusted for social class, and we further tested the potential effect modification by social class. There was no evidence of effect modification in most of the tests, except for the association between the motor score at 4-5 years and ingestion THM uptake, suggesting an implausible positive effect of the exposure among the wealthiest.

The main strengths of this study include its population-based prospective design, large sample size, a large number of covariables available and a common protocol for exposure and outcome assessment across cohorts. Potential confounding was addressed in multivariable analyses adjusted for a wide range of socioeconomic and life-style factors. Residual confounding for other variables associated both with the exposure and the outcome is possible but unlikely. Finally, we include a population with a wide range of exposure, which allows the generalization of our results to other settings.

5. CONCLUSIONS

Overall, results does not show a consistent association between exposure to drinking water disinfection by-products during gestation and child neuropsychological development at 1 and 4-5 years. Associations observed at 1 year did not persist at 4-5 y ears. Although a suggestive association is identified for exposure to brominated THMs and the cognitive score at 4-5 years, chance cannot be ruled out.

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1   Table 1. Characteristics of the study population (n=1855).

2

Variable	Categories	N (%) / Median (p25-p75)
Area	Asturias	341 (18%)
	Gipuzkoa	454 (24%)
	Sabadell	475 (26%)
	Valencia	585 (32%)
Child		
Sex	Female	925 (50%)
	Male	930 (50%)
Preterm	No	1773 (96%)
	Yes	70 (4%)
	Missing	12
Age at Bayley test (months)		14.3 (12.6-15.4)
	≤ 14 months	706 (38%)
	> 14 months	1149 (62%)
Age at McCarthy test (years) [a]		4.5 (4.4-5.6)
	<5 years	1004 (69%)
	≥5 years	449 (31%)
Mother		
Age (years)		31 (28-33)
Height (cm)		163 (159-167)
Pre-pregnancy weight (Kg)		60 (55-67)
Body mass index (Kg/m^2)		22.6 (20.8-25.1)
Weight-gain during pregnancy (Kg/week)		0.42 (0.33-0.51)
Parity	0	1057 (57%)
	≥1	798 (43%)
Social class	I/II Managers/Technicians	405 (22%)
	III Skilled	501 (27%)
	IV/V Semi-skilled/unskilled	949 (51%)
Country of birth	Europe	1749 (94%)
	Outside Europe	106 (6%)
Smoking during pregnancy	No	1256 (69%)
	Yes	567 (31%)
	Missing	32
Alcohol during pregnancy	No	1642 (91%)
	Yes, ≥ 1 drink/week	165 (9%)
	missing	48

3   [a] N=1453

Table 2. Description of water use habits among the study subjects.

Variable	All (n=1855)	Asturias (n=341)	Gipuzkoa (n=454)	Sabadell (n=475)	Valencia (n=585)
Source of drinking water at home (%)					
Bottle	62	52	24	87	75
Tap filtered	7	5	11	6	6
Tap non filtered	26	36	64	3	12
Other	5	7	1	3	8
Missing (n)	32	14	12	6	0
Source of drinking water outside home (%)					
Bottle	90	90	79	96	93
Tap (non filtered)	9	9	21	4	6
Other	1	1	1	1	1
Missing (n)	40	18	13	8	1
Tap water ingestion (L/day)[a]					
percentile 50 (25-75)	1.2 (0.9-1.8)	1.2 (0.6-1.4)	1.2 (0.9-1.4)	1.4 (0.9-1.8)	1.2 (0.9-1.8)
n	474	117	283	13	61
Showering/bathing (%)					
Shower only	88	91	94	86	84
Bath only	2	3	2	1	2
Shower & bath	10	6	5	12	15
Shower frequency (times/week)					
percentile 50 (25-75)	7 (6-7)	7 (7-7)	7 (4-7)	7 (6-7)	7 (5-7)
n	1790	318	434	462	576
Shower duration (min)					
percentile 50 (25-75)	10 (7-15)	10 (7-15)	10 (5-15)	10 (7-15)	10 (10-15)
n	1789	318	434	462	575
Bath frequency (times/month)					
percentile 50 (25-75)	2 (1-3)	2 (1-6.5)	2 (1-3)	1 (1-2)	2 (1-3)
n	214	28	29	65	92
Bath duration (min)					
percentile 50 (25-75)	30 (20-30)	30 (17.5-37.5)	20 (15-30)	30 (20-30)	30 (20-30)
n	215	28	29	64	94

[a] Among those drinking unfiltered tap water

Table 3. Estimated change[a] in neuropsychological scales from a linear regression for doubling total trihalomethane exposure in average over pregnancy.

Outcome	Exposure	Effect[a] (95%CI)	N	Effect[a] (95%CI)	N	Effect[a] (95%CI)	N	interaction
		All		Boys		Girls		p-value
Mental/cognitive score								
1 year								
	Ingestion uptake, µg/day	-0.07 (-0.27, 0.13)	1855	0.03 (-0.25, 0.32)	930	-0.15 (-0.43, 0.13)	925	0.89
	All route uptake[b], µg/day	0.00 (-0.47, 0.46)	1823	-0.42 (-1.10, 0.25)	916	0.42 (-0.22, 1.07)	907	0.07
	Residential levels, µg/L	0.09 (-0.49, 0.66)	1855	-0.76 (-1.59, 0.07)	930	**0.88 (0.09, 1.68)**	925	**0.02**
4-5 years								
	Ingestion uptake, µg/day	0.17 (-0.03, 0.38)	1453	0.20 (-0.10, 0.50)	719	0.14 (-0.14, 0.42)	734	0.07
	All route uptake[b], µg/day	**-0.54 (-1.03, -0.05)**	1429	-0.52 (-1.25, 0.20)	708	-0.54 (-1.20, 0.12)	721	0.50
	Residential levels, µg/L	-0.35 (-0.96, 0.25)	1453	-0.59 (-1.50, 0.32)	719	-0.09 (-0.91, 0.73)	734	0.78
Motor score								
1 year	Ingestion uptake, µg/day	-0.07 (-0.26, 0.11)	1855	0.02 (-0.24, 0.28)	930	-0.14 (-0.42, 0.13)	925	0.52
	All route uptake[b], µg/day	-0.05 (-0.49, 0.38)	1823	-0.24 (-0.85, 0.37)	916	0.22 (-0.41, 0.85)	907	0.46
	Residential levels, µg/L	0.00 (-0.54, 0.53)	1855	-0.66 (-1.41, 0.08)	930	0.69 (-0.09, 1.48)	925	0.16
4-5 years								
	Ingestion uptake, µg/day	0.06 (-0.15, 0.27)	1453	-0.02 (-0.34, 0.30)	719	0.19 (-0.10, 0.48)	734	0.39
	All route uptake[b], µg/day	-0.16 (-0.66, 0.35)	1429	-0.30 (-1.08, 0.47)	708	0.02 (-0.65, 0.68)	721	0.10
	Residential levels, µg/L	0.01 (-0.62, 0.64)	1453	-0.60 (-1.55, 0.36)	719	0.65 (-0.18, 1.48)	734	**0.01**

[a] Additive change in the mean of the outcome when doubling the exposure, from a linear regression adjusted for area, age at examination, sex, psychologist, quality of the test, parity and maternal age, social class, born in Europe, alcohol and smoking during pregnancy, height, pre-pregnancy body mass index and weight gain during pregnancy.
[b] All route uptake is the total residential THM uptake through ingestion, showering and bathing.

Table 4. Estimated change[a] in neuropsychological scales from a linear regression for doubling exposure in average over pregnancy.

Outcome	Exposure metric	THM component	Effect[a] (95%CI)	N	Effect[a] (95%CI)	N	Effect[a] (95%CI)	N	Inter.
Mental/cognitive score			All		Boys		Girls		p-value
1 year	Ingestion uptake, µg/day	Chloroform	-0.06 (-0.25, 0.12)	1855	0.03 (-0.24, 0.30)	930	-0.14 (-0.41, 0.12)	925	0.62
		Brominated	-0.10 (-0.33, 0.14)	1855	0.03 (-0.30, 0.36)	930	-0.19 (-0.52, 0.13)	925	0.58
	All route uptake[b], µg/day	Chloroform	0.06 (-0.24, 0.37)	1823	-0.19 (-0.64, 0.25)	916	0.31 (-0.12, 0.73)	907	**0.01**
		Brominated	0.01 (-0.49, 0.51)	1823	-0.38 (-1.11, 0.34)	916	0.39 (-0.29, 1.08)	907	0.43
	Residential levels, µg/L	Chloroform	0.12 (-0.20, 0.44)	1855	-0.24 (-0.70, 0.22)	930	0.44 (-0.01, 0.89)	925	**<0.01**
		Brominated	0.13 (-0.46, 0.72)	1855	-0.64 (-1.49, 0.21)	930	**0.84 (0.02, 1.66)**	925	0.24
4-5 years	Ingestion uptake, µg/day	Chloroform	0.17 (-0.03, 0.36)	1453	0.22 (-0.07, 0.51)	719	0.11 (-0.16, 0.38)	734	**0.03**
		Brominated	0.18 (-0.05, 0.42)	1453	0.20 (-0.15, 0.55)	719	0.16 (-0.17, 0.49)	734	0.12
	All route uptake[b], µg/day	Chloroform	-0.26 (-0.58, 0.06)	1429	-0.34 (-0.82, 0.13)	708	-0.17 (-0.61, 0.26)	721	0.34
		Brominated	**-0.64 (-1.16, -0.12)**	1429	-0.62 (-1.39, 0.16)	708	-0.64 (-1.34, 0.06)	721	0.84
	Residential levels, µg/L	Chloroform	-0.16 (-0.50, 0.17)	1453	-0.33 (-0.83, 0.17)	719	0.01 (-0.44, 0.46)	734	0.49
		Brominated	-0.39 (-1.01, 0.24)	1453	-0.65 (-1.59, 0.28)	719	-0.09 (-0.93, 0.75)	734	0.87
Motor scale									
1 year	Ingestion uptake, µg/day	Chloroform	-0.07 (-0.25, 0.10)	1855	0.00 (-0.24, 0.24)	930	-0.12 (-0.38, 0.14)	925	0.70
		Brominated	-0.08 (-0.29, 0.14)	1855	0.01 (-0.29, 0.31)	930	-0.14 (-0.46, 0.18)	925	0.36
	All route uptake[b], µg/day	Chloroform	-0.05 (-0.34, 0.23)	1823	-0.27 (-0.67, 0.13)	916	0.20 (-0.22, 0.61)	907	0.10
		Brominated	-0.06 (-0.53, 0.41)	1823	-0.25 (-0.90, 0.40)	916	0.20 (-0.47, 0.87)	907	0.82
	Residential levels, µg/L	Chloroform	-0.02 (-0.32, 0.28)	1855	-0.37 (-0.79, 0.04)	930	0.34 (-0.09, 0.78)	925	0.04
		Brominated	-0.01 (-0.56, 0.55)	1855	-0.70 (-1.47, 0.06)	930	0.72 (-0.08, 1.53)	925	0.36
4-5 years	Ingestion uptake, µg/day	Chloroform	0.08 (-0.13, 0.28)	1453	0.02 (-0.28, 0.32)	719	0.18 (-0.09, 0.45)	734	0.45
		Brominated	0.05 (-0.20, 0.30)	1453	-0.04 (-0.41, 0.33)	719	0.19 (-0.14, 0.52)	734	0.26
	All route uptake[b], µg/day	Chloroform	0.01 (-0.32, 0.34)	1429	-0.22 (-0.72, 0.29)	708	0.23 (-0.21, 0.66)	721	0.09
		Brominated	-0.30 (-0.85, 0.24)	1429	-0.27 (-1.10, 0.55)	708	-0.29 (-1.00, 0.42)	721	0.17
	Residential levels, µg/L	Chloroform	0.07 (-0.28, 0.42)	1453	-0.29 (-0.82, 0.23)	719	0.42 (-0.04, 0.88)	734	**0.03**
		Brominated	-0.10 (-0.75, 0.55)	1453	-0.58 (-1.57, 0.41)	719	0.42 (-0.43, 1.28)	734	**0.02**

[a] Additive change in the mean of the outcome when doubling the exposure, from a linear regression adjusted for area, age at examination, sex, psychologist, quality of the test, parity and maternal age, social class, born in Europe, alcohol and smoking during pregnancy, height, pre-pregnancy body mass index and weight gain during pregnancy.

[b] All route uptake is the total residential THM uptake through ingestion, showering and bathing.

Table 5. Estimated change[a] in neuropsychologial scales from a linear regression for doubling residential haloacetic acid levels (µg/L) in average over pregnancy.

Outcome	Exposure	Effect[a] (95%CI)	N	Effect[a] (95%CI)	N	Effect[a] (95%CI)	N	Interaction
		All		Boys		Girls		p-value
Mental/cognitive score								
1 year	Dichloroacetic acid	**1.78 (0.12, 3.45)**	454	0.44 (-1.95, 2.84)	215	**2.84 (0.48, 5.21)**	239	**0.07**
	Trichloroacetic acid	0.90 (-1.63, 3.43)	454	-0.31 (-3.91, 3.30)	215	2.07 (-1.54, 5.69)	239	0.27
	Total haloacetic acids	2.54 (-0.23, 5.32)	454	0.60 (-3.43, 4.63)	215	**3.96 (0.05, 7.87)**	239	0.10
4-5 years	Dichloroacetic acid	0.91 (-0.81, 2.62)	313	1.18 (-1.47, 3.84)	146	1.15 (-1.33, 3.63)	167	0.88
	Trichloroacetic acid	-0.33 (-3.31, 2.65)	313	1.26 (-3.44, 5.96)	146	-1.63 (-5.78, 2.53)	167	0.32
	Total haloacetic acids	0.58 (-2.33, 3.48)	313	1.26 (-3.23, 5.75)	146	0.45 (-3.77, 4.66)	167	0.68
Motor score								
1 year	Dichloroacetic acid	-0.57 (-2.43, 1.28)	454	1.04 (-1.63, 3.71)	215	-2.41 (-5.08, 0.26)	239	0.54
	Trichloroacetic acid	-2.68 (-5.48, 0.11)	454	-2.29 (-6.30, 1.71)	215	-2.83 (-6.89, 1.23)	239	0.92
	Total haloacetic acids	-2.04 (-5.12, 1.04)	454	-0.74 (-5.23, 3.74)	215	-3.53 (-7.94, 0.88)	239	0.81
4-5 years	Dichloroacetic acid	-0.75 (-2.55, 1.05)	313	-1.29 (-4.24, 1.67)	146	0.08 (-2.38, 2.55)	167	0.48
	Trichloroacetic acid	0.26 (-2.87, 3.39)	313	-0.68 (-5.92, 4.56)	146	1.26 (-2.86, 5.38)	167	0.65
	Total haloacetic acids	-1.58 (-4.63, 1.46)	313	-2.24 (-7.24, 2.75)	146	-0.65 (-4.83, 3.52)	167	0.70

[a] Additive change in the mean of the outcome when doubling the exposure, from a linear regression adjusted for area, age at examination, sex, psychologist, quality of the test, parity and maternal age, social class, born in Europe, alcohol and smoking during pregnancy, height, pre-pregnancy body mass index and weight gain during pregnancy.
[b] All route uptake is the total residential THM uptake through ingestion, showering and bathing.

Figure 1. Distribution of the modeled exposures evaluated among the study areas. Boxes are delimited by the 25th (left hinge) and 75th (right hinge) percentiles, the central vertical line represents the median value, the whiskers represent ±1.5 times the interquartile range, and the points outside the whiskers represent outliers.

All route uptake is the total residential THM uptake through ingestion, showering and bathing.

Ingestion THM uptake

Chloroform | BrTHM | Total THMs
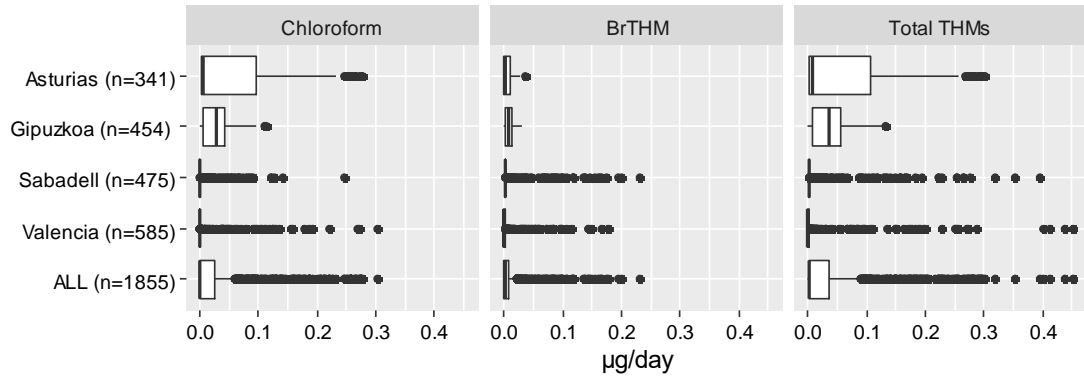
All route THM uptake
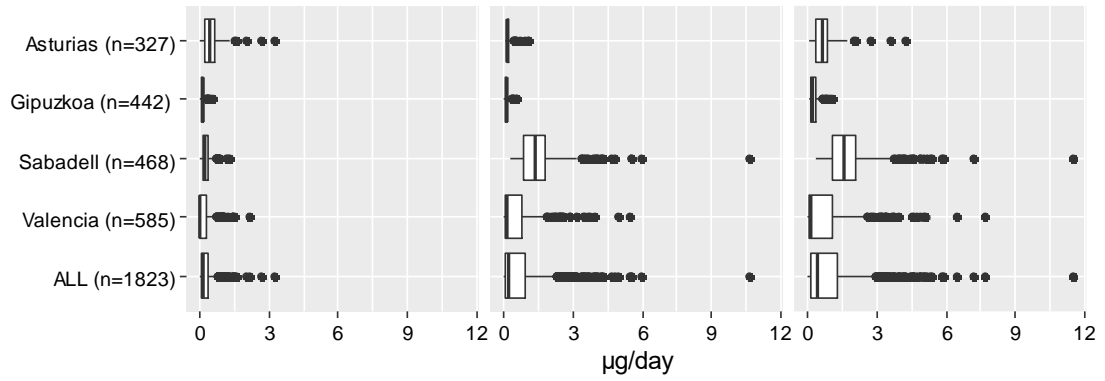
THM levels
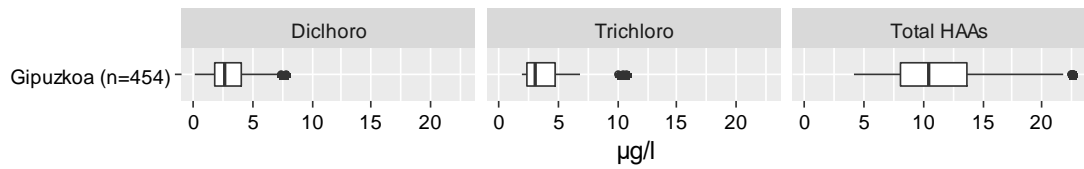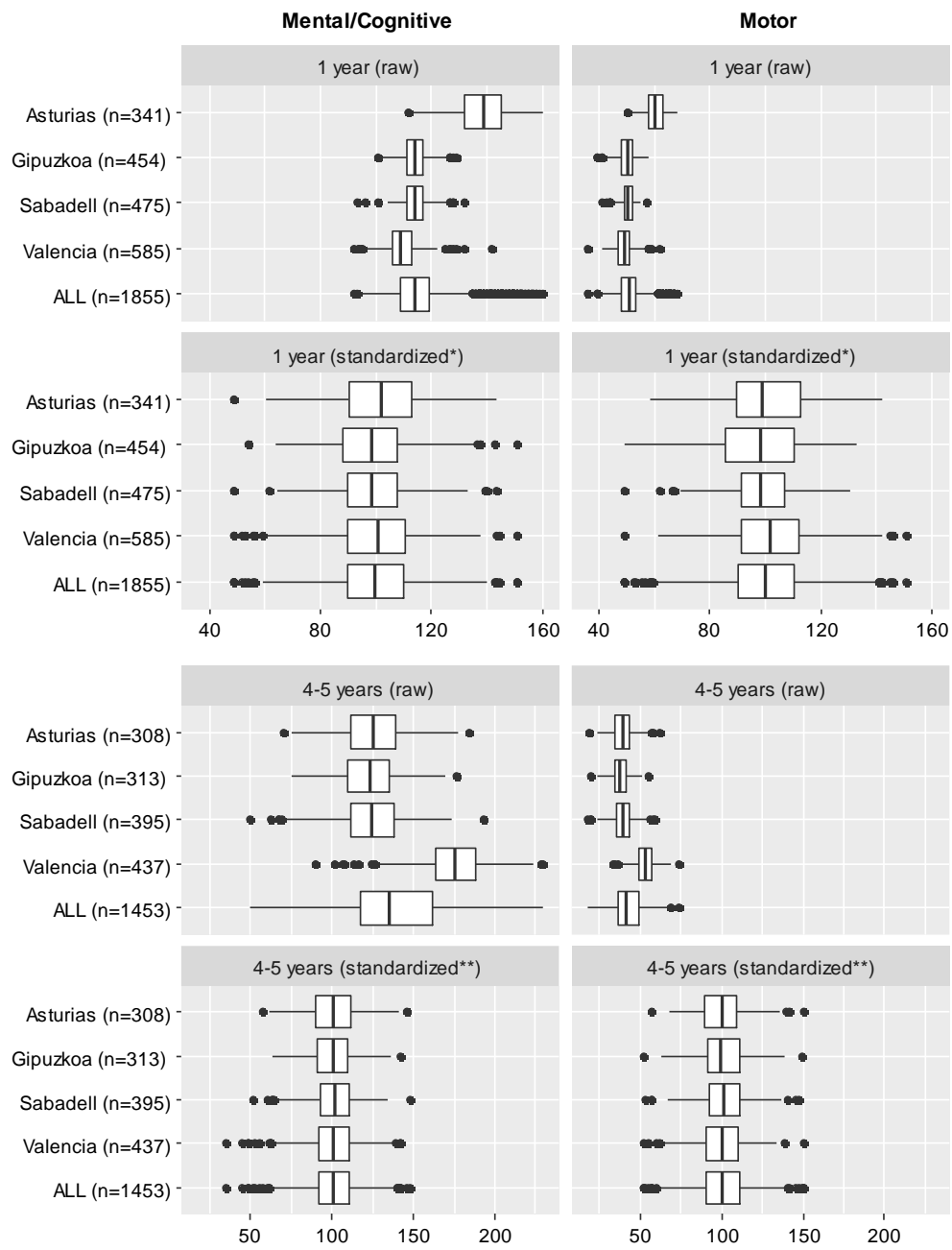
Haloacetic Acids
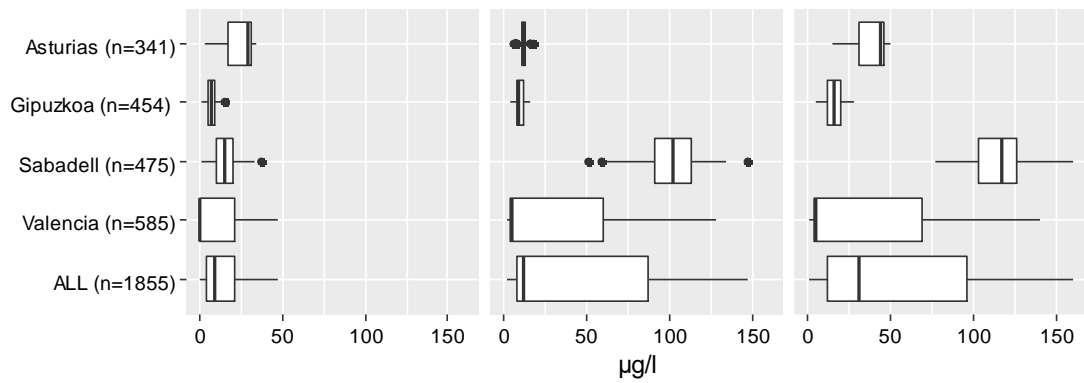
Diclhoro | Trichloro | Total HAAs

Figure 2. Distribution of the neuropsychological scores at 1 year (Bayley Scales of Infant Development) and 4-5 years (McCarthy Scales of Children's Abilities) in the study population.

Footnote: Only the standardized scores were used in the multivariate models to estimate the association with the exposure.

	Mental/Cognitive	Motor

1 year (raw)

- Asturias (n=341)
- Gipuzkoa (n=454)
- Sabadell (n=475)
- Valencia (n=585)
- ALL (n=1855)

1 year (standardized*)

- Asturias (n=341)
- Gipuzkoa (n=454)
- Sabadell (n=475)
- Valencia (n=585)
- ALL (n=1855)

40   80   120   160   40   80   120   160

4-5 years (raw)

- Asturias (n=308)
- Gipuzkoa (n=313)
- Sabadell (n=395)
- Valencia (n=437)
- ALL (n=1453)

4-5 years (standardized)**

- Asturias (n=308)
- Gipuzkoa (n=313)
- Sabadell (n=395)
- Valencia (n=437)
- ALL (n=1453)

50   100   150   200   50   100   150   200

* Age-adjusted and centered by cohort to a mean of 100 and standard deviation of 15 points.
** Centered by cohort to a mean of 100 and standard deviation of 15 points.

Figure 3. Exposure-response relationship between log-transformed brominated trihalomethane (THM) uptake through all routes and general cognitive score at 4-5 years. Smoothed spline with 3 degrees of freedom from general additive models adjusted for area, age at examination, sex, psychologist, quality of the test, parity and maternal age, social class, born in Europe, alcohol and smoking during pregnancy, height, pre-pregnancy body mass index and weight gain during pregnancy. P-value for gain compared to linear model: 0.09

[a] Additive change in the mean of the outcome when doubling the exposure.

[b] All route uptake is the total residential THM uptake through ingestion, showering and bathing.

Log brominated THM all route uptake‡