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Affiliations

Environ Res 2021 Sep;200:111392.
doi: 10.1016/j.envres.2021.111392. Epub 2021 Jun 1.

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Affiliations

Affiliations

[1] ISGlobal, Barcelona, Spain; Universitat Pompeu Fabra (UPF), Barcelona, Spain; Consortium for Biomedical Research in Epidemiology & Public Health (CIBERESP), Madrid, Spain. Electronic address: angela.zumel@isglobal.org.

[2] ISGlobal, Barcelona, Spain; Universitat Pompeu Fabra (UPF), Barcelona, Spain; Consortium for Biomedical Research in Epidemiology & Public Health (CIBERESP), Madrid, Spain; IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain. Electronic address: gemma.castano@isglobal.org.

[3] Consortium for Biomedical Research in Epidemiology & Public Health (CIBERESP), Madrid, Spain; Centro de Investigación en Recursos Naturales, Salud y Medio Ambiente (RENSMA), Universidad de Huelva, Huelva, Spain. Electronic address: juan.alguacil@dbasp.uhu.es.

[4] ISGlobal, Barcelona, Spain; Universitat Pompeu Fabra (UPF), Barcelona, Spain; Consortium for Biomedical Research in Epidemiology & Public Health (CIBERESP), Madrid, Spain; IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain. Electronic address: cristina.villanueva@isglobal.org.

[5] Cancer Epidemiology Unit, Department of Medical Sciences, University of Turin and CPO-Piemonte, Turin, Italy. Electronic address: milena.maule@unito.it.

[6] ISGlobal, Barcelona, Spain; Universitat Pompeu Fabra (UPF), Barcelona, Spain; Consortium for Biomedical Research in Epidemiology & Public Health (CIBERESP), Madrid, Spain. Electronic address: esther.gracia@isglobal.org.

[7] School of Epidemiology and Public Health, University of Ottawa Faculty of Medicine, Ontario, Ottawa Hospital Research Institute, Ottawa, Canada. Electronic address: fmomoli@uottawa.ca.

[8] School of Epidemiology and Public Health, University of Ottawa Faculty of Medicine, Ontario, Ottawa Hospital Research Institute, Ottawa, Canada; McLaughlin Centre for Population Health Risk Assessment, University of Ottawa Faculty of Medicine, Ottawa, Ontario, Canada; Risk Science International, Ottawa, Ontario, Canada. Electronic address: dkrewski@uottawa.ca.

[9] University of Ottawa, Ottawa, Canada. Electronic address: cmohipp@cheo.on.ca.

[10] Department of Hygiene, Epidemiology and Medical Statistics, Medical School, National and Kapodistrian University of Athens, Athens, Greece; Hellenic Society for Social Pediatrics & Health Promotion, Athens, Greece. Electronic address: epetrid@med.uoa.gr.

[11] Hellenic Society for Social Pediatrics & Health Promotion, Athens, Greece. Electronic address: evibouka@hotmail.com.

[12] Cancer Epidemiology Unit, Department of Medical Sciences, University of Turin and CPO-Piemonte, Turin, Italy. Electronic address: franco.merletti@unito.it.

[13] CPO-Piemonte, AOU Città della Salute e della Scienza, Turin, Italy. Electronic address: enrica.migliore@cpo.it.

[14] Environmental and Occupational Epidemiology Branch, Cancer Risk Factors and Lifestyle Epidemiology Unit, Institute for Cancer Research Prevention and Clinical Network-ISPRO, Florence, Italy. Electronic address: s.piro@ispro.toscana.it.

[15] Department of Preventive Medicine, Dankook University College of Medicine, 119 Dandae-ro, Cheonan, Chungnam, Korea. Electronic address: minaha00@gmail.com.

[16] Centre for Public Health Research, Massey University, Wallace St, Mount Cook, Wellington, New Zealand. Electronic address: A.Mannetje@massey.ac.nz.

[17] Centre for Public Health Research, Massey University, Wallace St, Mount Cook, Wellington, New Zealand. Electronic address: A.J.Eng@massey.ac.nz.

[18] Consortium for Biomedical Research in Epidemiology & Public Health (CIBERESP), Madrid, Spain; Epidemiology Section, Public Health Division, Department of Health of Madrid, Spain. Electronic address: nuria.aragones@salud.madrid.org.

[19] ISGlobal, Barcelona, Spain; Universitat Pompeu Fabra (UPF), Barcelona, Spain; IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain. Electronic address: elisabeth.cardis@isglobal.org.

Abstract

Brain tumours (BTs) are one of the most frequent tumour types in young people. We explored the association between tap water, exposure to trihalomethanes (THM) and nitrate and neuroepithelial BT risk in young people. Analysis of tap water consumption were based on 321 cases and 919 appendicitis controls (10-24 years old) from 6 of the 14 participating countries in the international MOBI-Kids case-control study (2010-2016). Available historical residential tap water concentrations of THMs and nitrate, available from 3 countries for 86 cases and 352 controls and 85 cases and 343 for nitrate, respectively, were modelled and combined with the study subjects' personal consumption patterns to estimate ingestion and residential exposure levels in the study population (both pre- and postnatal). The mean age of participants was 16.6 years old and 56% were male. The highest levels and widest ranges for THMs were found in Spain (residential and

ingested) and Italy and in Korea for nitrate. There was no association between BT and the amount of tap water consumed and the showering/bathing frequency. Odds Ratios (ORs) for BT in relation to both pre- and postnatal residential and ingestion levels of THMs were systematically below 1 (OR = 0.37 (0.08-1.73)) for postnatal average residential THMs higher than 66 μg/L. For nitrate, all ORs were above 1 (OR = 1.80 (0.91-3.55)) for postnatal average residential nitrate levels higher than 8.5 mg/L, with a suggestion of a trend of increased risk of neuroepithelial BTs with increasing residential nitrate levels in tap water, which appeared stronger in early in life. This, to our knowledge, is the first study on this topic in young people. Further research is required to clarify the observed associations.

Keywords: Adolescents; Brain tumours; Children; Disinfection by-products; Nitrate; THM; Water; Young adults.

Highlights

• Brain tumours are one of the most frequent tumour types in young people.

• Trihalomethanes and nitrate are possible carcinogens.

• To our knowledge, this is the first study focusing on this topic in young people.

• We found a possible increased risk for nitrate, and no association for trihalomethanes.

1. Introduction

Brain tumours (BT) are the third most frequent tumour type in young people in high income countries, and the second worldwide (Siegel et al., 2019). Little is known about risk factors for BT in young people, apart from genetics and exposure to ionizing radiation. Some environmental exposures, including drinking water carcinogens, have been examined as potential risk factors for BT (Zumel-Marne et al., 2019).

Disinfection of drinking water is a common practice in worldwide to inactivate microbial contaminants and prevent waterborne communicable diseases. Disinfectants such as chlorine added to raw water containing organic matter result in the formation of disinfection by-products (DBPs). Trihalomethanes (THMs) are one of the most common DBPs (World Health Organization, 2017).

Chlorinated drinking water has been associated mostly with bladder cancer in adults (IARC-Monographs Volume 84, 2004). Exposure to THMs occurs through inhalation, dermal contact and ingestion, and there is consistent evidence showing an association between long-term exposure and bladder cancer (Villanueva et al., 2007). There is no published study about BT risk and exposure to water DBPs in young people. However, two previous studies have evaluated exposure to water DBPs and BT risk in adults with mixed results (Cantor et al., 1999; Koivusalo et al., 1997).

Nitrate is another prevalent contaminant in drinking water. There is consistent evidence showing an association between long-term exposure to nitrate in drinking water and risk of colorectal cancer (Espejo-Herrera et al., 2016), and a suggestion of an association with other cancer types, like bladder and breast cancer (Ward et al., 2018). Nitrate can undergo endogenous nitrosation to form nitrite (World Health Organization, 2017) and both nitrate and nitrite are classified as probably carcinogenic to humans (Group 2 A) since 2010 (IARC- Monographs Vol. 1–121, 2018). However, the previous studies that explored the possible association with BT have yielded inconclusive results (Mueller et al, 2001, 2004, 2001; Weng et al., 2011).

A systematic review of BT risk factors in young people (Zumel-Marne et al., 2019) highlighted the lack of adequate evidence concerning the potential role of water disinfection by-products and of nitrate on risk of BT. Given the widespread exposure to these agents in the environment of children and pregnant mothers, we set-up the current study, focusing on assessing the role of exposure (pre- and postnatal) to THMs and nitrate in tap water on BT risk within the International MOBI-Kids study.

2. Methodology

2.1. Study population

MOBI-Kids is an international case-control study that recruited cases with a first primary benign or malignant BT, diagnosed between the ages of 10 and 24 years. For the main analyses in this article, we focused on neuroepithelial tumours as this includes the vast majority of cases (76%); the second largest group was embryonal tumour with a likely different aetiology. We did not collect midline tumours since MOBI-Kids was designed to assess risk of BTs in relation to radiofrequency radiation (RF) from mobile phone communication devices and the central part of the brain receives little RF exposure. Within neuroepithelial tumours, most of them were gliomas (83%) (Zumel-Marne et al., 2020). The corresponding ICD-O codes for the tumours (International Classification

of Disease for Oncology) were: 9380/3; 9381/3; 9382/3; 9383/1; 9384/1; 9391/3; 9392/3; 9393/3; 9394/1; 9400/3; 9401/3; 9411/3; 9412/1; 9413/0; 9420/3; 9421/1; 9421/3; 9424/3; 9425/3; 9430/3; 9440/3; 9441/3; 9442/3; 9450/3; 9451/3; 9492/0; 9505/1; 9505/3; 9506/1; 9509/1). Controls were selected among patients with a diagnosis of appendicitis in any of the hospitals in the catchment area of the case to which they were matched. Controls were matched to cases by age (±1 year from 10 to 17 and ±2 years from 18 to 24), sex and region. Subjects were recruited between 2010 and 2016. Information on tap water consumption and frequency of showers/baths was collected as part of a sub-study in 6 MOBI-Kids countries (Canada, Greece, Italy, New Zealand, Spain, and Korea). However, we could only include 3 of the 6 countries in the analyses of THM and nitrate: in Canada and New Zealand no participant fulfilled the criterion of having valid estimations of THMs and nitrates for at least 70% of their lifetime; in Greece all participants were served from the same water company, and thus there was insufficient variability of exposure. Ethics approvals for conducting the study were obtained from all appropriate national and regional review boards. All participants signed an informed consent form. Details about the study protocol and exclusion criteria have been reported previously (Sadetzki et al., 2014).

2.2. Individual information

Within MOBI-Kids, a face-to-face epidemiological questionnaire was administered to the study subjects, and/or their parents, by trained interviewers. The primary focus of the questionnaire was the use of mobile phones and exposure to other sources of electromagnetic fields (EMF). Additional questions were included to explore other potential risk factors for BT in young people, including questions about tap water use in the six countries mentioned above. Water questions were asked in relation to the study subjects' use of tap water during the year of diagnosis (defined from now on as "postnatal period") and that of their mothers during pregnancy (prenatal period). Questions were asked about frequency of drinking tap water at home (0, 1–2, and 3 or more glasses per day) and frequency of showering/bathing (1–3; 4 to 7; 8 or more times per week). The MOBI-Kids questionnaire also included a detailed residential history of the study subjects from birth until diagnosis. We assumed that the residence of the mother during pregnancy was the same as that at birth of the participant.

2.3. Exposure data

A survey was sent to the city authorities, treatment plants and national referents (depending on the country and municipality) to collect historical information on tap water characteristics of the municipalities of residence of the study subjects. This included: geographical area served (for the

water companies), water source (% surface, ground, other, annual values and changes over time); treatment (chlorination or disinfectants used), routine monitoring measurements (annual or monthly average levels of trihalomethanes and nitrate for as many years as available). Some countries provided nitrate levels as nitrate-nitrogen (Canada, New Zealand and Korea) and we converted these values into nitrate levels by multiplying them by 4.43 (Konieczynski and Wesolowski, 2007).

2.4. Estimation of historical data

THMs (in μg/L) and nitrate (in mg/L) levels either were provided by year or by month; in the latter case, annual THMs and nitrate levels were averaged over all months of the year. Levels below the limit of detection were assigned half the limit of detection. Total THMs levels were calculated by summing chloroform, bromoform, dichlorobromomethane and chlorodibromomethane levels, as done in previous studies (Font-Ribera et al., 2018). In Korea, THMs levels did not include bromoform because they were barely detected and this information was not provided. The information obtained about levels did not always cover the lifetime of all study subjects (1985–2016). For years with missing information, we estimated THMs and nitrate levels using the average levels of the available information, for each municipality/area as long as treatment and source of water was the same. If the treatment or source varied, we could not assume that the levels of nitrate and THM were similar to available years and hence levels for those years were kept as missing. We assumed that levels of THMs were equal to zero when no chlorination was used.

We used this information to estimate the annual average levels of chemicals in tap water for each year of life of the study subjects, based on the levels of THMs and nitrate in the municipality where subjects lived.

2.5. Individual exposure estimation

Average residential postnatal exposure level was calculated by averaging the annual levels of THMs and nitrate from birth until 5 years before diagnosis (of cancer for cases and appendicitis for controls) (to allow for a minimum induction time between exposure and diagnosis of a potentially exposure related tumour), using only non-missing values. We calculated the cumulative postnatal exposure by summing annual levels from birth up to 5 years before diagnosis. Prenatal exposure was estimated as the average levels of THMs and nitrate in the municipality of residence in the year of the child's birth.

Ingested THMs levels were calculated for each study subject, both for the pre- and postnatal periods, by combining the residential levels and the number of tap water glasses consumed per day

both by the subject and the mother. Specifically, for each subject, we multiplied the residential average THMs level (μg/L) by the reported daily litres of tap water consumed (L/day) – assuming that one glass was equivalent to 0.20 L, as done in previous studies (Villanueva et al., 2007) – thus obtaining the daily ingested amount of THMs (μg/day). We assumed that the amount of tap water consumption of participants did not vary over time. We also assumed that THMs levels were zero if participants did not drink tap water, as done in previous studies (Font-Ribera et al., 2010) or if they drank filtered tap water, since we assumed that filtering removes the majority of disinfection by-products (Carrasco-Turigas et al., 2013).

We could not estimate ingestion levels of nitrate, as nitrate can be found not only in tap water but also in bottled and well water (Alimohammadi et al., 2018; Espejo-Herrera et al., 2013; Nolan et al., 2002; Wang et al., 2016), and no information was asked about the subject's consumption of water from these sources.

2.6. Statistical analysis

Descriptive analyses were conducted by age (in three groups: 10–14, 15–19 and 20–24 years), sex, country and parental education (‘less or equal to high school’; ‘medium level: technical/professional school’; ‘university’ and ‘other/don't know’) obtained as the maximum of mother and father's education.

We used conditional logistic regression models to estimate the odds ratio (OR) and 95% confidence interval (CI) of BT associated with water uses (ingestion and showering/bathing), exposure to residential THMs and nitrate, and ingestion of THMs, both for the pre- and postnatal period. All models were adjusted for parental education level. Because the matching led to some cases having no controls, analyses were based on strata defined by age (within 1 year for ages 10 to 17 and 2 years for ages 18 to 25), sex and country rather than on the matched sets.

For the analysis of postnatal THMs and nitrate residential and THMs ingestion exposures, we also calculated the percentage of years with data available per subject. We used a cut-off of 70 percent to define subjects with valid estimation for the analysis on THMs (86 cases and 352 controls) and on nitrate (85 and 343 controls) (Supplementary Table 1) and to minimize exposure measurement error, as suggested in previous studies (Cantor et al., 1999; Espejo-Herrera et al., 2015). We excluded Canada and New Zealand from the THMs and nitrate analyses because no participants fulfilled this criterion. We also excluded Greece because of lack of variability in exposure (all participant residences were supplied by the same source of water, and hence all subjects had the

same annual residential average levels). We assessed potential confounding by maternal smoking during pregnancy or living on a farm (potentially related to nitrate exposure level) by including these variables in the model. A variable was judged to be a potential confounder if it modified the risk estimate by a factor of 10% of more.

For the main analyses, we used exposure as a categorical variable, defined as tertiles of exposure among controls. Level of ingestion of THMs was also grouped into three categories: non-exposed (for those that had 0 μg/day or did not report drinking tap water) and below and above the median of average THMs levels among controls.

We performed sensitivity analyses including all tumour types, including Canada, New Zealand and Greece, as well as exploring the possibility that children may be more vulnerable to THMs and/or nitrate induced BTs in the first years of life – for this, we restricted analyses to exposure in the first 2 and 5 years of life.

We also performed sensitivity analyses based on alternative scenarios to infer average residential exposure level when data were missing: assigning the median average residential level for the municipality for the missing periods (scenario 1); assigning 0 to missing periods (scenario 2), and the maximum average residential level for those periods (scenario 3).

As urban/rural residence may confound a possible association between THM and nitrate and risk of brain tumours, we conducted analyses restricted to subjects living in urban areas in Spain (where the degree of urbanisation of the municipalities of residence was at least 40%); it was not possible to conduct separate analyses of those who lived in rural areas (less than 3% of subjects in Spain). All statistical analyses were performed in STATA 14.0. Statistical significance was set at p-value <0.05.

3. Results

A total of 475 cases and 1002 controls were recruited in the 6 countries. Among cases, 76% of the tumours were neuroepithelial (n = 363). Of these, 321 cases and 918 matched controls had completed information on personal residential history and tap water use questionnaires, and mothers from 297 cases and 766 controls had completed the maternal water use questionnaire. Information on water levels covering more than 70% of the lifetime person-years of each subject was available in 3 countries and THMs and nitrate levels could be estimated for 86 cases and 352 controls and 85 and 343, respectively (Supplementary Table 1).

The sex ratio (male/female) was 1.33. Italy and Spain contributed the largest number of subjects. When considering education level of the parents, the largest proportion of subjects was in the category high school or less (36% of cases and 30% of controls); the proportion of subjects with other or unknown level of parental education was higher among controls than cases. The distribution of subjects by age, sex and parental education was similar when restricted to the three countries included in the analyses of THMs and nitrate (not show).

The distribution of tap water consumption habits among controls, according to the main characteristics of the population, is shown in Table 2. The proportion of study subjects consuming tap water differed by country and parental education level. Tap water consumption was higher in New Zealand (83%), Greece (80%) and Canada (72%) and lower in Italy (44%) and Korea (26%); similar differences were noted concerning mothers' tap water consumption during pregnancy. Among subjects with known parental education level, the highest tap water consumption was seen in the university level category (61% and 59% respectively in post- and prenatal periods). A high percentage of tap water consumption was seen for the prenatal period among those with other/unknown parental educational level (75%), while the lowest was in the medium education level category (43%). The same pattern was observed in the majority of the countries except in Greece and Korea (data not shown).

Statistically significant differences in the frequency of showers/baths of study subjects were also found by country, age, sex and parents' education level in the postnatal period (Table 2). Ninety-two percent of subjects reported showering or bathing more than 4 times/week in New Zealand compared to 69% in Italy. Male controls tended to shower or bathe more frequently than female controls (83% vs. 72%) and older controls more frequently than younger controls (87% and 70% respectively in the 20–24, and 10–14 years categories). Those in the highest parental education category and those whose parental education level was "other/unknown" had the highest frequency of showering/bathing, over 4 times a week (83 and 82% respectively). The same pattern was observed in all countries except in Greece and Korea.

Overall, median residential THMs and nitrate levels were, respectively, 28.3 μg/L, and 3.9 mg/L. Levels of average residential and ingested THMs levels were the highest in Spain and Canada and the lowest in Italy and New Zealand when we stratified by country among controls for the postnatal period of exposure (Fig. 1). Most countries, except Spain, showed limited variability of exposure among controls. Controls in Italy, Korea and New Zealand had higher average levels of nitrate than those from other countries; variability of nitrate levels was very limited in Canada, Greece and

Korea. Considering that we calculated residential THM and nitrate exposure, we observed that, overall, participants lived, on average, in 2.1 municipalities, each one with a mean duration of 11.2 years (data not shown).

Few differences in residential levels of THMs or nitrate were observed by characteristics of the study population, except for residential nitrate levels that were higher in the older age group, and in participants with medium level of parental education (data not shown).

In the analyses of water uses reported by the participants in the 6 countries, we found no association between neuroepithelial BT risk and amount of drinking tap water or frequency of showering/bathing, either during the pre- or the postnatal periods (Table 3). Analyses by country showed similar results (data not shown).

ORs for neuroepithelial BT by tertile of residential and ingested THMs levels during the pre- and postnatal exposure periods are shown in Table 4, based on data from the subjects in the 3 countries for which exposure to these chemicals could be estimated. We found ORs below 1 in all categories of exposure (both pre- and postnatal) for residential and ingested THM exposures with no indication of a linear exposure-response trend. Results by country are shown in Supplementary Table 2.

For nitrate (Table 5), ORs for neuroepithelial BT were higher in the 2nd and 3rd tertiles of pre- and postnatal exposure compared to the first tertile, with a suggestive exposure-response trend for pre- and postnatal average residential levels (p □ 0.10). Cumulative residential levels showed a statistically significant increased OR in the second tertile of exposure, but exposure-response was not monotonic.

In Supplementary Table 3 we show the same analyses stratified by country.

We found some differences in tap water consumption between tertiles of THM and nitrate levels (Supplementary Table 4).

3.1. Sensitivity analyses

A sensitivity analysis was performed excluding those participants with no information on residential THM or nitrate levels from the reference group. We found similar results pre and postnatal period in a very small sample size. The analyses for exposure levels during the first 2 or 5 years of life, considered as a possible vulnerable period of life for brain development, showed significantly reduced ORs for residential THMs exposure (with no indication of an exposure-response trend), mainly driven by the results in Spain (Supplementary Tables 5 and 6). For nitrate,

we found an increased OR with a suggestion of exposure-response relationship for the average exposure during the first 5 years of life.

No major difference was observed in the results based on different scenarios for imputing exposure for the time periods where data were missing, and no trend was observed (Supplementary Table 7).

Analyses using all BT rather than only neuroepithelial for THMs and nitrate are shown in Supplementary Tables 8-10. While risk estimates differ slightly, results and interpretation are broadly consistent with those of the main analyses, apart from a much stronger dose-related decreased risk in relation to lifetime cumulative residential THMs in analyses of all BTs compared to neuroepithelial tumours only.

Neither maternal smoking during pregnancy nor living on a farm appeared to confound the association between BT risk and THM or nitrate, so analyses are not adjusted by these variables (not shown).

We explored possible differences in levels of THMs or nitrate by rural or urban areas. The majority of our participants lived urban areas, however, and an analysis adjusting for this variable yielded very similar results (data not shown). Further analyses restricted to subjects living in urban areas in Spain yielded very similar results (data not shown).

4. Discussion

We conducted, to our knowledge, the first study to explore the possible relation between neuroepithelial BT risk in young people and tap water consumption, assessing individual levels of THMs.

Tap water consumption habits – both ingestion and, to a lesser extent, frequency of bathing/showering - varied substantially by country and parental educational level. We found no association between risk of neuroepithelial BT and use of tap water either for drinking or showering/bathing during the pre- and postnatal periods.

All ORs for neuroepithelial BTs in relation to THMs exposure were below 1, for pre- and postnatal residential and ingestion exposures. Restricting analyses to exposures in the first two years of life suggest a stronger reduced OR in relation to residential THMs levels. These results are mainly attributable to Spain, the only country with subjects in the highest exposure category.

For nitrate, all ORs were above 1 with a suggestion of an increasing risk with increasing average residential nitrate level both for pre- and postnatal exposures. The trend was similar but slightly

stronger in analyses restricted to exposures in the first two years of life. There was substantial variability in levels between countries, with Italy, followed by Korea and New Zealand having the highest median levels. Italy and Spain contributed with the highest number of cases and controls and showed the greatest variability in exposure; thus the overall analyses are mainly driven by these two countries. In analyses restricted to Spain, increased ORs were seen both for prenatal and postnatal residential nitrate exposure for the higher exposure categories though there was no suggestion of an exposure-response.

While there are no previous reports on the risk of BT in young people related to THMs exposure, two studies investigated the effect of chlorinated water on BT risk in adults. In the first, conducted in Iowa (USA), researchers observed an increased BT risk in males who lived for over 40 years in residences with chlorinated surface water sources compared to those who did not (OR = 2.5; 95% (95% CI 1.2–5.0); 13 cases and 81 controls), but the age range and exposure duration differed substantially from that of our study (Cantor et al., 1999). Analyses by lifetime average THMs exposure resulted in ORs below 1 for exposure levels lower than 32.5 μg/L, and above 1 for higher levels. The other study, a historical cohort study conducted in Finland, based on 621,431 adult persons (including 917 BTs), found no association between BT risk and water mutagenicity level (Relative Risk (RR) = 1.00 (95% CI 0.85–1.18)) for water mutagenicity level of 3000 net rev/l – representative of average water mutagenicity in towns using chlorinated surface water –compared to no use of chlorinated surface water (Koivusalo et al., 1997). The other published article, based on a cohort of 917 adult BT cases from Finland (Koivusalo et al., 1997), found no association with exposure to chlorinated surface water. The exposure levels of the participants in our study are different from those of previously published articles in adults, where the highest category was 32.6 μg/L or more, a level which, in our study, is on the low side of our mid tertile of exposure level.

For nitrate, our results support the findings of previous publications (Mueller et al., 2001) conducted in US, Europe, Canada and Australia and of studies of nitrate-nitrogen exposure (Ho et al., 2011; Weng et al., 2011), which found suggestions of increased BT risk with exposure to nitrate in drinking water. Further larger scale studies with complete historical exposure information would be helpful to further explore this possible association. There is no evidence that risk of appendicitis (the diagnosis of our hospital controls) is related to either THM or nitrate. We found one article from Taiwan where the association between appendicitis among children and different levels of piped water supply was evaluated. This study observed that children who lived in low piped water

supply areas had a higher risk of appendicitis compared to those with high piped water supply. However, this relationship is appears to be related to differences in disinfection and hygiene (Li et al., 2018). In the high income countries in which MOBI-Kids was conducted, all tap water consumed is piped and correctly disinfected.

The biological plausibility of an association between THMs or nitrate exposure and risk of BTs is not clear. A study in rats suggested possible neurotoxicity of chlorinated compounds (Moser, 2004). In humans, another study suggested a possible association between exposure of mothers during pregnancy to DBPs and neurodevelopment in the offspring (Villanueva et al., 2018). Thus, there is some indication that some water chemicals might cross the placenta. Results of our analyses of prenatal exposures and of exposures during the first two years of life are not consistent with this hypothesis, and no increased risk of brain tumour was observed with exposure to THMs.

For nitrate exposures, Bilzer et al., (1989) suggested that exposure to nitroso-compounds can cause a base-mispairing in DNA and mutations in rats, and consequently, permanent cell proliferation (Bilzer et al., 1989; Chen et al., 2016). In humans, neural tube defects have been observed in progeny of women exposed to public water supply with high nitrate concentrations during pregnancy in a study conducted in California with mother of 538 cases and 539 non-malformed controls (Croen et al., 2001). We found a suggestive dose-exposure relationship with average nitrate levels and neuroepithelial brain tumours, pointing to the same direction of a possible effect on the neurological system. A previous childhood BT case-control study conducted in 7 countries explored prenatal and early postnatal exposure to residential nitrate and nitrite levels in tap water measured by dipstick found no clear association for nitrate and BT risk but a statistically significant association for nitrite levels higher than 5 mg/L (OR = 5.2 (95% CI 1.2–23.3)) compared to non-detected levels, albeit based on small numbers (Mueller et al., 2004). An earlier report on the US component of the study similarly showed a significantly increased BT risk for children with detectable nitrite levels in tap water compared to non-detectable (OR = 8.8 (95% CI 2.1–46)) based on 13 exposed cases and 3 exposed controls, while detectable nitrate levels were not associated with BT risk (OR = 0.6 (95% CI 0.3–1.1)) (Mueller et al., 2001). Another case-control study, which explored the association between nitrate-nitrogen in public water supplies and childhood malignant BTs and central nervous system tumours, found a statistically significant association for subjects who lived in towns with levels higher than 0.31 mg/L of nitrate-nitrogen in water (>1.37 mg/L nitrate) (OR = 1.40 (95% CI 1.07–1.84)) compared to lower levels (in 190 cases and 229 controls) (Weng et al., 2011).

Our study, like any other observational study, is subject to limitations. Recall error (random and systematic) is a definite possibility when mothers are asked about their water consumption habits during pregnancy, 10–24 years before the interview. Random recall error may reduce the power of a study to find an effect, if it exists, by biasing risk estimates towards the null. Systematic over and underestimate of risk is unlikely to change categorical ORs if non-differential between cases and controls. Differential recall of water consumption between mothers of cases and controls, while conceivable for the main exposures under study in MOBI-Kids, is less likely for water consumption as the number of questions was very limited and came at the end of a long questionnaire which covered many other topics, perhaps more obviously related to a possible risk of BT than tap water. Concerning postnatal exposures, questions focused only water consumption habits during the year of diagnosis, and any error, for the same reasons, is unlikely to be differential between cases and controls, though random error is certainly possible and exposure in the year of diagnosis may inadequately capture the exposure in the aetiologically relevant time period. We expect little error when reporting residential history since most participants had only 1 or 2 residences (73%). We have assumed that the residence during pregnancy was the same as the residence at birth, and this could lead to error when assigning the THMs and nitrate levels during the prenatal period; again, this is unlikely to be differential between cases and controls.

Assessment of residential exposure, however, relied not only on questionnaire data but also on data on disinfection history and methods and on historical measurement data for the particular chemicals of interest. Obtaining this information was a very complicated and tedious exercise, lasting over two years and requiring repeated contacts with local authorities and companies for over 300 municipalities. Despite our best efforts, data were missing for a large number of the MOBI-Kids subjects in the six participating countries. Missing data is unlikely to be related to case-control status, but it is related to time period, with earlier data being much more difficult to obtain than more recent data. As water companies and municipalities did not provide detailed information on DBPs as we requested, we had to use total THMs. Despite THMs not being the only and most carcinogenic compounds of the mixture of DBPs present in drinking water, we consider that they can be representative of water contaminants since the majority of the municipalities used chlorine as main disinfectant, and THM is one of the most frequent DBPs in these cases.

Many assumptions went into the modelling of historical exposure levels and interpolation between measurements in different years in order to assign yearly estimates of exposure to all the study subjects in the analysis. When we used average yearly levels for missing historical levels of THMs

and nitrate, we were assuming that levels did not vary (if water treatment or source did not vary), so these imputations have uncertainties. Additionally, we should consider that we selected only participants for whom exposure measurements were available for more than 70% of residential history, so older participants with longer exposures were less likely to be included.

Further, assignment of exposures based on municipality level data introduces another form of exposure measurement error, Berkson error, in which all individuals in a given municipality and time period are assigned the same level of exposure to THMs and nitrate. While Berkson error does not generally affect the magnitude of a linear dose-response, if it exists, it does cause greater uncertainty in individual assessment and reduce statistical power of a study. In categorical analyses, the impact of Berkson error on the magnitude of the risk estimates is, moreover, difficult to predict.

Furthermore, we have a limitation of sample size in the main analyses, since we had to exclude three of the original six countries as explained in methods.

Another limitation of the study is the response rate. We had a high percentage of controls who refused to participate in our study in general (46% of refusals in the 3 countries), especially those with low parental education level (Turner et al., 2019), a common situation in epidemiological studies. This may have affected our results if, for example subjects with higher education, as proxy for higher income, *may* used more bottled water, and consequently were less exposed to THMs. However, we did not have information on bottled water consumption.

Exposure to THMs and particularly nitrate may come from sources other than tap water in the home. Questions on water use were very limited in the MOBI-Kids questionnaire, so we could not explore the exposure to nitrate from other sources like diet or bottled water, that could impact on endogenous nitration. Exposure to THMs through dermal contact or inhalation could not be estimated since we did not have information on duration of showering/bathing as in Villanueva et al., (2007). We could not take into account breastfeeding during this early period since information on breastfeeding was not collected in the MOBI-Kids study, though previous publications suggest breastfeeding reduces nitrate exposure (Dusdieker, 1996; Fossen Johnson, 2019) while for THMs the results are inconsistent (Batterman et al., 2002). We could not retrieve data on nitrite levels, so we could not assess the association with BT risk, as reported previously (Mueller et al, 2001, 2004). We assessed if there was any difference between levels of THM and nitrate by urban and rural areas, though most of our participants lived in urban areas, and results did not change. This was expected since the drinking water evaluated was tap water, and not well water, where there could have been more variations of levels of nitrate due to the nitrate infiltration into groundwater as a

consequence of agriculture. Another limitation is that since we excluded those tumours of the midbrain, our population could be not representative to the population.

One important strength is that our study includes participants from different countries with a wide range of levels of THMs and nitrate in water, recruited using the same protocol and answering a common questionnaire administered by trained interviewers. Not only did the study focus on a homogenous group of tumours but all cases with genetic diseases or conditions susceptible to BTs were excluded from the study population, thus reducing the potential for confounding and bias.

5. Conclusion

Our results exploring the exposure to THMs and nitrate in tap water consumption in what is to our knowledge the first study on this topic in young people show no increased risk of neuroepithelial BTs in relation to exposure to THMs, but suggest a possible exposure-related increased risk related to residential nitrate exposure, which appeared strongest for exposures early in life. Further research is required to clarify these associations.

Funding

Funding was obtained from the European Community's Seventh Framework Programme under grant agreements number 226873 and 603794, and from the Spanish Ministry of Science and Innovation (MINECO) and the Spanish Health Research Fund (FIS) of the National Institute for Health Carlos III. Italian participation was partially supported by a Ministry of Health grant (RF-2009-1546284). In Canada, Canadian participation in MOBI-Kids is supported by a university-industry partnership grant from the Canadian Institutes of Health Research (CIHR), reference number 110835, with the Canadian Wireless Telecommunications Association (CWTA) serving as the industrial partner. CWTA provides technical information on wireless telecommunications in Canada and facilitates access to billing records from Canadian network operators, but has no involvement in the design, conduct, analysis, or interpretation of the MOBI-KIDS study. D. Krewski is the Natural Sciences and Engineering Research Council of Canada Chair in Risk Science at the University of Ottawa. New Zealand participation was supported by Health Research Council and Cure Kids. MOBI-Kids Korea is supported by the ICT R&D program (2017-0-00961 and 2019-0-00102) of MSIT / IITP, Korea. Greek participation is partially supported by ELKE (Special Account for Research Grants of the National and Kapodistrian University of Athens) and

GGET (General Secretariat for Research and Technology). The funding sources had no role in: the study design; the collection, analysis, and interpretation of data; the writing of the report; and the decision to submit the article for publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to acknowledge all the research assistants and interviewers in the study centres, the staff of all participating hospitals, especially to all the surgeons, and, most of all the study participants and their families.

The coordinating center would like to thank Alex Albert, Francesc Badia, for database management and Chelsea Eastman Langer for her tireless efforts in coordinating the set-up and conduct of MOBI-Kids in all countries. We would also like to thank Gema Carretero, Eva Ferreras, Rebeca Sánchez, Marta Cervantes, Ma Ángeles Sierra, Irene Gavidia, Ma Antonia Muñoz and the other Spanish interviewers for their dedication; Patricia de Llobet for her support in different steps of the analysis and preparation of this paper. We are particularly grateful to all the municipalities, municipal water plants and local authorities that provided us information about disinfection methods and historical the levels of chemical in the water from their municipalities. ISGlobal also acknowledges support from the Spanish Ministry of Science, Innovation and Universities through the "Centro de Excelencia Severo Ochoa 2019–2023" Program (CEX 2018-000806-S), support from the Generalitat de Catalunya through the CERCA Program and support from the Secretariat of Universities and Research of the Department of Business and Knowledge of the Generalitat of Catalonia through AGAUR (the Catalan Agency for Management of University and Research Grants) (Project 2017 SGR 1487). The Italian study center would like to thank all water supply companies, local authorities and municipal water plants that provided information on water parameters and disinfection methods. We would also like to thank the Piedmont Regional Environmental Protection Agency (ARPA), the Environmental Protection Agency of the Tuscany Region (ARPAT), and in particular Stefano Mechinetti, the Tuscan Water Authority, and ATS Milano and Val Padana. We thank Laura Davico for data management. The Nationwide Registry

for Childhood Hematological Malignancies and Solid Tumours (NARECHEM-ST) of Greece would like to specially acknowledge Dr. Phani Miskaki, Deputy Director of Planning & Development for Water Sector Projects at Athens Water Supply and Sewerage Company S.A, Greece, for providing information on tap water characteristics as well as the collaborating physicians Dr Maria Moschovi, Dr Apostolos Pourtsidis, Dr George Orfanides, Dr Konstantinos Oikonomou, Dr Panagiotis Nomikos, Dr Konstantinos Lampropoulos, Dr Helen Kosmidis, Dr Helen Dana, Dr Spyridon Sgouros, Dr Nikolaos Kelekis, Dr Mathilda Papathanasiou, Dr Stathis Boviatsis, Dr Spyridon Tzanis, Dr Panos Papanikolaou, Dr Vasiliki Xenaki, Dr Sophia Polychronopoulou, Dr Vassilios Papadakis, Dr Dimitrios Koliouskas, Dr Vaso Sidi, Dr Evgenia Papakonstantinou, Dr Spyros Voulgaris, Dr Athanasios Kyritsis, Dr George Alexiou, Dr Eftichia Stiakaki, Dr Antonios Vakis, Dr Katerina Manolitsi, Dr Basilios Zountsas, Dr Georgios Sfakianos, Dr Marios Themistocleous, Dr Ioannis Nikas, Dr Panagiotis Primikiris, Dr Vasilios Zerris, Dr Gerhard Friehs, Dr Alex Vyziotis, Dr Stergios Zacharoulis, Dr Emmanuel Hatzipantelis, Dr Savvas Papadopoulos, Dr Kalliopi Stefanaki, Dr Katerina Strantzia, Dr Eustratios Patsouris, Dr Triantafyllia Koletsa, Dr Charis Bourgioti, Dr Areti Gkantaifis, Dr Panagiotis Prassopoulos, Dr Konstantinos Nikas, Dr George Zografos, Dr Anastasios Machairas, Dr Vasilios Smirniotis, Dr Makis Kanavidis, Dr Konstantinos Simopoulos, Dr Grigorios Kouraklis, Dr John Tierris, Dr Antonios Kotsalis, Dr Antonia Papazoglou, Dr Efstratios Kouskos, Dr Konstantinos Tsimogiannis, Dr Konstantinos Papadopoulos, Dr Manolis Chrysos, Dr Sofia Xenaki, Dr Nikolaos Gatsoulis, nurses and hospital staff in MOBI-KIDS-Greece; special thanks are due young patients and parents for their collaboration. The Canadian team would like to thank our Investigators, Dr. John Spinelli and Dr. Paul Ritvo; our coordinators, Charmaine Mohipp, Fatima Kazoun, Tina Changoor, and Agnes Lai; Renee Sayegh for transcribing data; the municipal water supply contacts in Ottawa, Toronto, and Vancouver for data; and all the individuals that made recruitment possible: Dr. Michael Vassilyadi, Gail Macartney, CHEO SUPPORT team, Dr. Michael Taccone, Maria Andrews, Brendan Bakos, Ryan Woods, Cathy MacKay, Lisa Stellingwerff, Dr. Brian Toyata, Dr. Thomas J. Zwimpfer, Bonnie Giovannetti, Dr. O. Neely M. Panton; Dr. Danny Mendelsohn; Joleen Wright, Sandy Strandberg, Dr. Juliette Hukin, Colleen Fitzgerald, Jessica Davis, Dr. Eric M. Webber, Dr. Sonia Butterworth, Dr. Geoffrey Blair, Dr. James Murphy, Dr. Erik D. Skarsgard, Damian J Duffy, Roseanne Lim, Dr. Eric Bouffet, Dr. Mark Bernstein, and Dr. Nataliya Zhukova.

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Table 1. Characteristics of the study population.

	Neuroepithelial[a]		**All BT[a]**		**3 countries[b]**	
	Case N(%) N = 321	**Control N(%) N = 918**	**Case N(%) N = 430**	**Control N(%) N = 920**	**Case N(%) N = 292**	**Control N(%) N = 861**
Age (mean years (SD))	16.6 (4.3)	16.8 (4.3)	16.7 (4.4)	16.8 (4.3)	16.9 (4.3)	16.9 (4.2)
Age categories, years						
10–14	134 (41.7)	357 (38.9)	172 (40.0)	357 (38.8)	109 (37.3)	313 (36.4)
15–19	104 (32.4)	308 (33.6)	139 (32.3)	310 (33.7)	100 (34.2)	309 (35.9)
20–24	83 (25.9)	253 (27.6)	119 (27.7)	253 (27.5)	83 (28.4)	239 (27.8)
Sex						
Male	184 (57.3)	518 (56.4)	249 (57.9)	519 (56.4)	161 (55.1)	479 (55.6)
Country*						
Canada	17 (5.3)	18 (2.0)	20 (4.7)	20 (2.2)	–	–
Greece	25 (7.8)	61 (6.6)	35 (8.1)	61 (6.6)	–	–
Italy	114 (35.5)	322 (35.1)	148 (34.4)	322 (35.0)	114 (35.5)	322 (35.1)
New Zealand	13 (4.0)	24 (2.6)	16 (3.7)	24 (2.6)	–	–
Spain	128 (39.9)	397 (43.2)	183 (42.6)	397 (43.2)	128 (39.9)	397 (43.2)
Korea	24 (7.5)	96 (10.5)	28 (6.5)	96 (10.4)	24 (7.5)	96 (10.5)
Parental education *						
≤High school	115 (35.8)	276 (30.1)	155 (36)	277 (30.1)	108 (37.0)	255 (29.6)
Medium level	87 (27.1)	230 (25.1)	113 (26.3)	230 (25.0)	86 (29.5)	224 (26.0)
University	86 (26.8)	258 (28.1)	116 (27.0)	258 (28.0)	72 (24.7)	225 (26.1)
Other and don't know	33 (10.3)	154 (16.8)	46 (10.7)	155 (16.8)	26 (8.9)	157 (18.2)

Table 2. Distribution of tap water consumption and showering/bathing habits during the pre- and postnatal periods, by main characteristics among the controls matched to neuroepithelial BT cases.

	Prenatal				**Postnatal**			
	6 countries		**3 countries**		**6 countries**		**3 countries**	
	Drinking	**Showering/bathing**	**Drinking**	**Showering/bathing**	**Drinking**	**Showering/bathing**	**Drinking**	**Showering/bathing**
	Yes N (%)	≥4 times/week N (%)	Yes N (%)	≥4 times/week N (%)	Yes N(%)	≥4 times/week N (%)	Yes N (%)	≥4 times/week N (%)
Age categories					*0.179*	*<0.001*	*0.463*	*<0.001*

	Prenatal				Postnatal			
	6 countries		3 countries		6 countries		3 countries	
	Drinking	Showering/bathing	Drinking	Showering/bathing	Drinking	Showering/bathing	Drinking	Showering/bathing
(years)								
10–14	NA	NA	NA	NA	210 (58.8)	249 (69.7)	172 (48.2)	216 (60.5)
15–19	NA	NA	NA	NA	165 (53.6)	249 (80.8)	145 (47.1)	227 (73.7)
20–24	NA	NA	NA	NA	137 (54.2)	220 (86.6)	114 (45.1)	195 (76.8)
Sex					*0.790*	*<0.001*	*0.952*	*<0.001*
Male	NA	NA	NA	NA	291 (56.2)	430 (83.0)	237 (45.8)	375 (72.4)
Female	NA	NA	NA	NA	221 (55.3)	288 (71.8)	193 (48.3)	263 (65.6)
Country	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Canada	13 (81.2)	14 (87.5)			13 (72.2)	15 (83.3)		
Greece	47 (92.2)	44 (80.0)			49 (80.3)	43 (70.5)		
Italy	96 (34.5)	193 (66.6)	96 (34.5)	193 (66.6)	143 (44.4)	222 (69.2)	143 (44.4)	222 (69.2)
New Zealand	18 (81.8)	17 (77.2)			20 (83.3)	22 (91.7)		
Spain	198 (68.8)	277 (93.8)	198 (68.8)	277 (93.8)	262 (66.0)	344 (86.6)	262 (66.0)	344 (86.6)
Korea	29 (33.0)	74 (84.1)	29 (33.0)	74 (84.1)	25 (26.0)	72 (73.5)	25 (26.0)	72 (73.5)
Parental education	*<0.001*	*0.097*	*0.004*	*0.038*	*0.151*	*0.014*	*0.127*	*0.024*
≤High school	141 (55.7)	211 (79.9)	110 (43.5)	183 (69.3)	142 (51.4)	208 (77.8)	112(40.5)	179(66.9)
Medium level	92 (43.4)	168 (76.3)	81 (38.2)	155 (70.4)	124 (53.9)	168 (73.4)	112 (48.7)	158 (69.0)
University	141 (58.5)	209 (85.0)	111 (46.1)	180 (73.2)	157 (61.0)	215 (83.3)	127 (49.3)	184 (71.3)
Other, unknown	27 (75.0)	31 (86.1)	21 (58.3)	26 (72.2)	89 (57.8)	127 (82.5)	79(51.3)	117 (76.0)

Abbreviations: BT, brain tumour. [a]p-value from Chi-squared test for homogeneity. NA=Not applicable, this column refers only to the mother, during the prenatal period, hence age and sex the study subjects is not applicable. Percentages are calculated based on the total of each category. Drinking tap water: 'No' = 0 tap water glasses; 'Yes' = 1 or more tap water glasses per day. Showering/bathing: <4 times/week and ≥4 times/week. 3 countries are those included in the final analyses.

Table 3. Odds ratio (OR) and 95% confidence intervals (95% CI) of neuroepithelial brain tumours associated with amount of tap water consumed and showering/bathing frequency, in pre- and postnatal exposure periods.

	Cases (N)	Controls (N)	OR[a] (95% CI)
Prenatal			
Drinking tap water at home			
0 glasses per day	127	317	1.00
1–2 glasses per day	39	112	0.82 (0.52–1.29)
3 or more glasses per day	112	254	1.04 (0.74–1.45)
p trend			*0.80*
Showering/bathing			
1–3 per week	50	131	1.00
4–7 per week	199	477	1.07 (0.72–1.59)
8 or more per week	43	101	0.94 (0.55–1.61)
p trend			*0.85*
Postnatal			
Drinking tap water at home			
0 glasses per day	146	390	1.00
1–2 glasses per day	53	137	0.92 (0.62–1.37)
3 or more glasses per day	120	358	0.87 (0.64–1.18)
p trend			*0.36*
Showering/bathing			
1–3 per week	74	189	1.00
4–7 per week	196	564	0.91 (0.65–1.30)
8 or more per week	48	132	0.91 (0.56–1.48)
p trend			*0.68*

Abbreviations: OR, odds ratio; CI, confidence intervals. [a]Conditional logistic regression models, stratified on age, sex and country and adjusted by parental education. 6 countries included.

Table 4. Odds ratio (OR) and 95% confidence intervals (95% CI) for neuroepithelial brain tumours by tertiles of THMs residential exposure and ingestion from tap water during the pre- and postnatal periods.

	Cases (N)	Controls (N)	OR[a] (95% CI)
RESIDENTIAL THMs (µg/L):			
Prenatal			
Average residential THMs			
≤25.0	36	91	1.00
25.1–66.5	16	97	0.36 (0.14–0.93)
≥66.6	27	111	0.68 (0.28–1.69)
p trend			*0.99*
Postnatal			
Average residential THMs			

	Cases (N)	Controls (N)	OR[a] (95% CI)
≤25.0	32	78	1.00
25.1–66.5	26	98	0.39 (0.09–1.77)
≥66.6	26	111	0.37 (0.08–1.73)
p trend			*0.46*
Lifetime cumulative residential THMs			
≤238.6	34	83	1.00
238.7–674.0	27	91	0.92 (0.30–2.85)
≥674.1	23	113	0.82 (0.23–3.02)
p trend			*0.74*
INGESTED THMs (µg/day):			
Prenatal			
Non-exposed	119	304	1.00
Below median (≤19.2)	24	69	0.93 (0.54–1.61)
Above median (>19.3)	11	83	0.33 (0.16–0.70)
p trend			*0.12*
Postnatal			
Non-exposed	138	358	1.00
Below median (≤19.2)	26	91	0.72 (0.43–1.21)
Above median (>19.3)	22	97	0.71 (0.40–1.28)
p trend			*0.59*

Abbreviations: OR, odds ratio; CI, confidence intervals; THM, trihalomethanes.[a]Conditional logistic regression models, stratified on age, sex and country and adjusted by parental education. Data from Spain, Korea and Italy (the 3 final countries with information on water levels covering more than 70% of the lifetime person-years of each subject).

Table 5. Odds ratio (OR) and 95% confidence intervals (95% CI) for neuroepithelial brain tumours for tertiles of exposure to residential nitrate (mg/L) levels present in tap water during pre- and postnatal exposure periods.

RESIDENTIAL NITRATE (mg/L):	**Cases (N)**	**Controls (N)**	**OR [a] (95% CI)**
Prenatal			
Average residential nitrate			
≤3.26	22	120	1.00
3.27–8.47	25	82	1.62 (0.74–3.53)
≥8.48	32	101	1.76 (0.91–3.41)
p trend			*0.12*
Postnatal			
Average residential nitrate			
≤3.26	20	103	1.00
3.27–8.47	28	84	1.42 (0.66–3.09)
≥8.48	35	94	1.80 (0.91–3.55)
p trend			*0.10*
Lifetime cumulative residential nitrate (mg/day)			

RESIDENTIAL NITRATE (mg/L):	**Cases (N)**	**Controls (N)**	**OR [a] (95% CI)**
≤41.60	23	97	1.00
41.70–97.00	34	94	2.12 (1.02–4.40)
≥97.10	26	90	1.72 (0.82–3.63)
p trend			*0.38*

Abbreviations: OR, odds ratio; CI, confidence intervals. [a]Conditional logistic regression models, stratified on age, sex and country and adjusted by parental education. Data from Spain, Korea and Italy (the 3 final countries with information on water levels covering more than 70% of the lifetime person-years of each subject).

Fig. 1. Boxplot of average postnatal residential and ingested levels of trihalomethanes (THMs) and residential nitrate levels by country in participating controls (postnatal period). Abbreviations: THM, trihalomethanes. Only controls with more than 70 percent of lifetime years with known information on THMs or nitrate levels during their life were included in the figure. For average ingested THMs, participants without ingestion of tap water were not included in the figure. Number of controls by country is indicated in parenthesis. The pink boxplot called 'overall 3 countries' is the mean of Italy, Spain and Korea, the countries included in the main analyses.