ENVIRONMENTAL FACTORS AND BRAIN TUMOUR RISK IN YOUNG PEOPLE

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A mis padres

In appreciation

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Abstract

Risk factors and clinical characteristics of brain tumours (BT) in young people have been little explored, though BTs are one of the most frequent tumour types in children and adolescents. The purpose of this doctoral thesis was to study 1) the clinical characteristics and symptoms of BTs in young people, based on the international MOBI-Kids case-control study; 2) what is known about risk factors for BTs in young people based on a systematic review (SR) of the literature; 3) the risk of BT in patients 10-25 years old of young people in relation to chemicals present in drinking water and 4) to heavy metals.

The analyses of clinical characteristics revealed that the vast majority of tumours were neuroepithelial (mostly gliomas), followed by embryonal tumours. Overall, the most frequent symptoms were headache, followed by focal neurological signs and symptoms, nausea/ vomiting and visual signs and symptoms, being a 4% of the cases asymptomatic. The average time of diagnosis tended to be short (median 1.42 months), though this varied according to tumour type, age and type of symptom.

I found many studies that showed an association between environmental factors (including tobacco smoke, pesticides and diet, among other exposures) and BT risk in the SR. Because of methodological limitations however, the evidence about the role of these factors in the aetiology of this disease is still uncertain.

Our analyses in relation to water chemicals showed ORs below 1 for exposures to THMs, finding the strongest decreases in risk for

exposure in the first 2 years of life. For nitrate, we found ORs above 1 with a suggestion of an exposure-related increased risk of neuroepithelial BTs with residential nitrate levels in tap water, especially in exposures for early in life. Our analyses of heavy metals suggested reduced risk related to chromium and increased risk related to selenium that needs further exploration.

Overall, this thesis served to improve the knowledge concerning 1) the clinical characteristics of BT in young people, useful to both clinical practice and aetiological research; 2) causes of this disease; 3) and added evidence related to the role of heavy metals and ubiquitous chemicals in water and risk of BTs in young people. Further research needs on the aetiology and prevention of BTs in young people are discussed.

Resumen

Los factores de riesgo y las características clínicas de los tumores cerebrales (TCs) en los jóvenes han sido poco explorados, a pesar de que los tumores cerebrales (TC) son uno de los tipos de tumores más frecuentes en los niños y jóvenes. El propósito de esta tesis doctoral es el estudio de 1) de las características clínicas y los síntomas de los TC en los jóvenes, basados en el estudio internacional de casos y controles MOBI-Kids; 2) conocer qué se ha estudiado sobre los factores de riesgo de los TCs en jóvenes con una revisión sistemática de la literatura; 3) el riesgo de TC en pacientes jóvenes de entre 10-25 años de edad en relación a exposición de productos químicos presentes en el agua potable y 4) con los metales pesados.

Los análisis de las características clínicas revelaron que la gran mayoría de los tumores eran neuroepiteliales (principalmente gliomas), seguidos de tumores embrionarios. En general, los síntomas más frecuentes fueron dolor de cabeza, seguido de signos y síntomas neurológicos focales, náuseas/ vómitos y problemas en la visión, siendo un 4% de los casos asintomáticos. El tiempo promedio de diagnóstico tendió a ser corto (mediana 1.42 meses), aunque esto varió según el tipo de tumor, la edad y el tipo de síntoma.

Encontré muchos estudios que hallaron asociación entre los factores ambientales (incluido el humo del tabaco, los pesticidas y la dieta, entre otras exposiciones) y el riesgo de TC en la revisión sistemática. Sin embargo, debido a limitaciones metodológicas, la

evidencia sobre el papel de estos factores en la etiología de esta enfermedad aún es incierta.

Nuestros análisis en relación con los productos químicos del agua mostraron unos OR por debajo de 1 para exposiciones a THMs, sobre todo para exposiciones en los dos primeros años de vida. Para nitrato, encontramos OR por encima de 1 con una sugerencia de un mayor riesgo relacionado con la exposición de BT neuroepiteliales con niveles residenciales de nitrato en el agua del grifo. Nuestros análisis de metales pesados sugirieron un menor riesgo relacionado con el cromo y un mayor riesgo relacionado con el selenio que deben explorarse con más detalle.

En general, esta tesis sirvió para mejorar el conocimiento sobre 1) las características clínicas de la TC en los jóvenes, útiles tanto para la práctica clínica como para la investigación etiológica; 2) causas de esta enfermedad; 3) el papel de los metales pesados y los químicos presentes en el agua. Se ha identificado la necesidad realizar más investigaciones sobre la etiología y la prevención de las TC en los jóvenes.

Resum

Els factors de risc i les característiques clíniques dels tumors cerebrals (TC) en els joves han estat poc explorats, malgrat que els tumors cerebrals (TC) són un dels tipus de tumors més freqüents en nens i joves. El propòsit d'aquesta tesi doctoral és l'estudi de: 1) les característiques clíniques i els símptomes dels TC en els joves, basats en l'estudi internacional de casos i controls MOBI-Kids; 2) conèixer què s'ha estudiat sobre els factors de risc dels TCs en joves amb una revisió sistemàtica de la literatura; 3) el risc de TC en pacients joves d'entre 10-25 anys d'edat en relació a exposició de productes químics presents en l'aigua potable i 4) amb els metalls pesats.

Les anàlisis de les característiques clíniques van revelar que la gran majoria dels tumors eren neuroepitelials (principalment gliomes), seguits de tumors embrionaris. En general, els símptomes més freqüents són mal de cap, seguit de signes i símptomes focals neurològics, nàusees / vòmits i problemes a la visió, amb un 4% dels casos asimptomàtics. La mitjana de temps de diagnòstic tendia a ser curt (mediana 1.42 mesos), encara que això varia segons el tipus de tumor, l'edat i el tipus de símptoma.

Vaig trobar molts estudis que han associat l'associació entre els factors ambientals (inclòs el fum del tabac, els plaguicides i la dieta, entre altres exposicions) i el risc de TC en la revisió sistemàtica. No obstant això, a causa de limitacions metodològiques, l'evidència sobre el paper d'aquests factors en l'etiologia de la malaltia és incerta.

Els nostres anàlisis en relació amb els productes químics de l'aigua mostren ORs per sota d'1 per exposicions a THMs, sobretot per exposicions en els dos primers anys de vida. Per nitrat, trobem ORs per sobre d'1 amb un suggeriment d'un major risc amb l'exposició de TC neuroepitelials amb nivells residencials de nitrat en l'aigua. Les nostres anàlisis de metalls pesants van mostrar un efecte protector amb el crom i un major risc relacionat amb el seleni que se han d'explorar amb més detall.

En general, aquesta tesi va servir per millorar el coneixement sobre 1) les característiques clíniques dels TC en els joves, útils tant per a la pràctica clínica com per a la investigació etiològica; 2) les causes d'aquesta malaltia; i 3) el paper dels metalls pesats i els productes químics presents a l'aigua. S'ha identificat la necessitat de realitzar més investigacions sobre l'etiologia i la prevenció de les TC en els joves.

Preface

I completed a University degree in Environmental Sciences at the University of Huelva (Spain). I then did a Master's in 'Medical research: clinical and experimental' at the School of Medicine of the University of Seville (Spain) in 2013-2014. The Master's thesis focused on levels of heavy metals in toenails and risk of prostate cancer in adults, as part of the MCC-Spain study (http://www.mccspain.org/). I started working at CREAL (now ISGlobal) in 2010 as a Research Technician in the international MOBI-Kids study, as the field work coordinator and interviewer for the Andalusian node of the MOBI-Kids study in Spain. I also collaborated as fieldwork coordinator, interviewer and laboratory technician for the node of Huelva in the MCC-Spain study until September of 2015, date when I moved physically to Barcelona to work in the MOBI-Kids study as a PhD student.

The work in this thesis was conducted between 2015 and 2019 in the Radiation Programme of ISGlobal (previously CREAL), supervised by Prof. Elisabeth Cardis and co-supervised by Prof. Juan Alguacil (University of Huelva) and Dr. Gemma Castaño Vinyals (ISGlobal).

According to the rules of the PhD in Biomedicine of the Universitat Pompeu Fabra (UPF), this thesis contains one systematic review and three original papers, in which I explored the environmental risk factors and clinical characteristics of BTs in young people. Based on my experience as field work coordinator and interviewer

in Andalusia for the MOBI-Kids study, we decided to first include a descriptive article on the clinical characteristics of BT cases in young people, based on the data on 899 cases aged 10-24 in the 14 country study (Paper II), in order to fill a gap: while some articles exist for BTs in adults, very little has been published for BTs in young people.

As we found no recent and systematic literature review of the evidence for the role of environmental risk factors for BTs in children and young adults, I conducted a complete systematic review (SR) on this topic (Paper I). A preliminary review of the literature in the framework of the SR, identified a number of environmental risk factors for which evidence was insufficient. As information had been collected within MOBI-Kids substudies, I therefore decided to explore some of the risk factors in my thesis: exposure to tap water chemicals (Paper III) and to heavy metals (Paper IV). For Paper III, during the period 2015-2018, I collected information on water sources, treatment and historical measurements of various chemicals by municipality in Spain and coordinated the collection of this information in the other five countries which participated in the water substudy, with the help of the participating investigators and field-work coordinators. I then centralised and cleaned the data, estimated the levels for each of the residences in which the study subjects had lived and combined this information with water consumption habits from the MOBI-Kids questionnaire to estimate individual levels of exposure.

For Paper IV, the analysis of BT risk from heavy metals was conducted only in the Spanish node of the MOBI-Kids study, where toenails were collected from participants. In 2015, I went to Huelva to the laboratory where the metal concentrations of toenails were determined to learn the process of multielementary analysis by ICP-MS.

Finally, in order to obtain the International Mention and to take advantage of the BT clinical expertise of Prof. Michael Kundi (the Austrian MOBI-Kids Principal Investigator), I did a 4 months stay, in two separate periods in Vienna, one in 2017 and the other in 2018, at the Medical University of Vienna.

Abbreviations

Al	Aluminium
As	Arsenic
Be	Beryllium
BT	Brain Tumour
CBT	Childhood Brain Tumour
Cd	Cadmium
CI	Confidence Interval
CNS	Central Nervous System
Co	Cobalt
Cr	Chromium
Cu	Copper
DBPs	Disinfection By-Products
ELF	Extremely Low Frequency
Fe	Iron
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
Mn	Manganese
Mo	Molybdenum
Ni	Nickel
OR	Odds Ratio
Pb	Lead
Pt	Platinum
RF	Radiofrequency
Se	Selenium
SR	Systematic Review
TC	Tumores Cerebrales (Brain Tumours In Spanish)
THMs	Trihalomethanes
Tl	Thallium
U	Uranium
V	Vanadium
Zn	Zinc

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General Introduction

1. GENERAL INTRODUCTION

1.1. Epidemiology of brain tumours in young people

1.1.1. Incidence

Brain tumours (BTs) are the second most common tumour type in young people in Europe, third overall in high income countries around the world, after leukaemia and thyroid cancer, and the sixth in low income countries (IARC- Cancer Today, 2018). In 2018, around 23,982 new BTs in children (0-14 years old) were diagnosed in the world, and about 19,379 among adolescents and young adults (10-24 years old) (IARC- Cancer Today, 2018). The estimated age-standardized annual rate for brain and central nervous system (CNS) tumours in high income areas was 2.4 per 100,000 persons among people under 24 years of age compared to 0.5 in low income areas (IARC- Cancer Today, 2018). The worldwide incidence in children below the age of 15 is slightly higher in boys (1.3 per 100,000 persons/ year in 2018) than in girls (1.1 per 100,000 persons/ years in 2018). The incidence of brain and Central Nervous System (CNS) tumours varies also by race and ethnicity. For example, based on the SEER Cancer Statistics Review, in USA (2012-2016), the highest incidence of BTs was observed in white race compared other races among all ages (for example, annual rates of 3.0 in ages 10-14 per 100,000, compared to 2.8 for all races), followed by black race (2.1 per 100,000) (Howlader N et al., 2019).

Tumour morphology type varies with age. In older ages, the most frequent subtypes are gliomas located in the supratentorial region, specifically, in the frontal, temporal and parietal lobes. However, in younger ages, the most frequent tumours are gliomas (Stewart and Wild, 2014) and embryonal tumours, located mainly in the cerebellum and brain stem (Hemminki and Li, 2002). For the inherited genetic factors, a higher incidence of malignant BT were found in ancestries of European populations in previous publications (Ostrom et al., 2019), however, studies in migrant showed no difference in BT incidence between the offspring of migrants and those of the local population (Hemminki and Li, 2002).

It is not fully understood if the increasing incidence of BTs observed in the last decades in high income countries is only due to availability of improved diagnostic tools, more complete registration, or if then also is a real increased incidence, due, for example, to the increase of environmental exposures (McKinney, 2005; Patel et al., 2019).

1.1.2. Tumour characteristics and classification

The classification of central nervous system (CNS) tumours has changed over time. Originally, it was based on histology alone, not considering classifying tumours according to microscopic similarities (cells of origin and differentiation states) (Louis et al., 2016). The World Health Organization (WHO) Classification of Tumours of the Central Nervous System version 2000 and 2007

considered histological features. Since 2007, it has also been included the consideration of genetic alterations.

Nowadays, in order to code the tumour with a specific diagnosis ICD code (International Classification of Diseases), it initiates with the main histological characteristics associated to the tumour. Then, it is considered other specific clinical, pathological and molecular characteristics of the tumour type. When there is insufficient information about the genetic parameters and/ or alterations to classify the tumour, it is assigned a code that correspond with "NOS" (Not Otherwise Specified) (Louis et al., 2016). Tumour types are also classified according to the biological behaviour of the neoplasm (tumour grade) since 1979. Tumour grading supports the decision for tumour treatment, therapy and is related to prognosis. The tumour grade is classified into four categories. Grade I tumours are generally tumours with slow proliferation and high probability of cure. Grade II tumours are those that usually are infiltrative and often recur, and some of them often tend to progress into higher grades of malignancy. Patients with grade II tumours normally have a survival time of more than 5 years. Grade III tumours have clear histological evidence of malignancy and have a worse prognosis. Grade IV tumours are those that are malignant, with rapid cell division and are often fatal (International Agency for Research on Cancer, 2016).

Though the widespread use of imaging techniques have contributed to earlier diagnosis of CNS tumours, diagnostic latencies are still of

several months to over a year at least in adults (Dang-Tan and Franco, 2007; Dobrovoljac et al., 2002; Dorner et al., 2007; Hayashi et al., 2010; Klitbo et al., 2011; Mehta et al., 2002; Molineus et al., 2013; Reulecke et al., 2008; Veneroni et al., 2013; Wilne et al., 2012).

Symptoms preceding BT diagnosis occur not only in BT, they are often common in other diseases. High prevalence of headache, focal neurological signs and symptoms, nausea/ vomiting and visual signs and symptoms are among the symptoms described in BT cases, as reported in a previous meta-analyses (The Childhood Brain Tumor Consortium, 1991; Udaka and Packer, 2018; Wilne et al., 2007) and in individual studies (Dorner et al., 2007; Fukuoka et al., 2014; Hayashi et al., 2010; Molineus et al., 2013; Reulecke et al., 2008; Wilne et al., 2012).

The fact that some symptoms are also frequent in other diseases, like vomiting, which can be attributed to gastrointestinal problems, when in fact is provoked by the intracranial pressure due to the growing of the tumour in the brain, can possibly delay tumour diagnosis (Teo and Myseros, 2014). For teenage and adolescent cases, symptoms related to behaviour changes can be confused with normal behavioural changes at puberty (Madhusoodanan et al., 2015), especially, when these symptoms occurred isolated.

Long-term neurological and psychological sequelae has been suggested to result from delayed diagnosis (Reimers et al., 2003). However, the literature shows that time of diagnosis is between 1 to 2 months after the first symptom (Dang-Tan and

Franco, 2007; Dobrovoljac et al., 2002; Dorner et al., 2007; Hayashi et al., 2010; Klitbo et al., 2011; Mehta et al., 2002; Molineus et al., 2013; Reulecke et al., 2008; Veneroni et al., 2013; Wilne et al., 2012). Nevertheless, though most BT symptoms are also common in other diseases, which can delay the diagnosis, it is believed that more knowledge about symptoms of BT in the paediatric, adolescent and young adult populations could raise awareness among the medical community (Azizi et al., 2017; Wilne et al., 2010). Also, clinical differences seems to exist at least between some types of BT in children with respect to adults (Jiang et al., 2019).

1.2. Aetiology of brain tumours in young people

Though BT is one of the most frequent tumour types in young people, little is known about its causes. The only established risk factors are ionising radiation and genetic syndromes. A number of environmental factors have been studied in previous publications, however the literature at present do not supports a causal association between BT risk and these exposures.

The early peak of BT in childhood suggests an important inherited component (Berger et al., 2011), with cancer initiating events occurring before conception, during foetal life or in early infancy (Greenop et al., 2013), so the routes of exposure may be diverse.

Parents can be exposed to occupational environmental agents in their daily lives and that could affect the germ line and/or bio-accumulate in the bodies. In consequence, children can also be

exposed, especially during pregnancy (Martins et al., 2014) or after birth through breastfeeding.

1.2.1. Host factors

There are some risk factors related to the individual that might play a role in the susceptibility to develop a BT.

a) Role of the placenta and blood-brain barrier in protection of the brain against environmental factors

The placenta is an organ formed during pregnancy as the interface between the foetus and its mother. This organ not only provides the regulation of the nutrients, but also plays an important role in nutrition, respiration, excretion and protection (Caruso et al., 2012).

The human placenta is 2 to 3 cm of thickness, and it is composed of foetal material (coming from the blastocyst) and maternal material (from the endometrium) (Caruso et al., 2012). The main processes of exchange between the mother and the foetus though the placenta are based on diffusion, transporter-mediated mechanisms and endocytosis/ exocytosis (Burton Graham J. and Fowden Abigail L., 2015). Diffusion of small molecules occurs rapidly; however, diffusion rate it is influenced by the rate of blood flow across the membrane. Transporter proteins allow exchanging molecules across the membrane by different concentration gradient between both sides of barrier. In the process of endocytosis, molecules are captured by invaginations forming at the cell surface of the

placenta, then the vesicles traverse the cell by the process of exocitosis to get into the cytoplasm (Burton Graham J. and Fowden Abigail L., 2015).

The placenta acts as a literal protective barrier from external environmental toxicants, for the correct development of the foetus. An alteration, or reduced thickness, of this barrier can permit the entry inside the placenta or through the umbilical cord of some external molecules.

Another natural barrier, which protects the brain from external toxicants, is the blood-brain barrier (BBB). We refer to the central nervous system (CNS) vessels composed by endothelial cells that regulate the circulation of cells, molecules and ions through blood and the CNS. This barrier protects the CNS from chemicals (including toxins and drugs), pathogens, inflammation and lesions in order to ensure a correct neuronal function (Daneman and Prat, 2015).

The BBB can lose part of its protective properties due to inflammation, brain trauma, neuronal disorders, or in presence of other diseases. The ion regulation of the BBB can be altered and let molecules enter into the CNS, provoking neuronal dysfunction or degeneration (Daneman and Prat, 2015).

Although the BBB is developed before birth, some toxicants, such as some metals or drugs, may bind with proteins and cross the BBB and enter into brain and the cerebro-spinal fluid, thus affecting normal brain development (Harry and Tilson, 2010) and

damaging it. Some environmental toxicants are known for their neurotoxicological effects, for example lead and mercury (Goyer, 1990).

b) Genomic factors

Some inherited genetic conditions are known to be related with BTs, such as neurofibromatosis type 1 and 2, Li-Fraumeni syndrome, Turner syndrome, Turcot syndrome and tuberous sclerosis, among others. However, these syndromes explain only 1% of the incidence (McKinney, 2004; World Health Organization et al., 2014).

Gene mutations, copy number variations, structural rearrangements, and deregulation of the transcriptome contribute to the developmental biology of BT (Dubuc et al., 2010). Due to improvements of histological classification, tumour morphology classifications are being updated every several years. Nevertheless, few publications about genetics and BT risk in children have been conducted.

A recent review showed that some variants in genes for xenobiotic detoxification, or related to inflammation processes, DNA repair, cell cycle pathway, or folate metabolism, have been associated with childhood brain tumour (CBT) in three studies (Ostrom et al., 2019).

Mutations in the TP53 gene have also been related to BT risk, especially for gliomas. Metabolic processes such as detoxification,

oxidation, DNA repair and immune functions are associated with variations in the structure of genes and hence may be associated with BTs. Nevertheless, the role of environmental exposures on these genes and pathways are not sufficiently studied (McKinney, 2004).

Epigenetic modifications regulate chromatin states of genes and of chromosomal regions to modulate gene expression levels. Somatic mutations in epigenetic regulator genes or histone genes in brain cancers have been identified (Maury and Hashizume, 2017). However, the impact of the genetic alterations occurring in BTs related to the 'chromatin machinery', and their interactions with other known oncogenes, tumour suppressor genes, or susceptibility genes needs to be further explored.

Telomere length has been also related with glioma. An increased risk for glioma was found in a recent review publication (Ostrom et al., 2019) for longer relative leukocyte telomere length. Longer telomeres length have been found in glioma tumour samples compared with other cancers types (Ostrom et al., 2019).

c) Medical conditions

Some specific medical conditions have been also related to BTs. For example, history of allergies or atopic skin conditions have been related with a reduced risk of BT in adults (Chen et al., 2011; Ostrom et al., 2019; Turner et al., 2013; World Health Organization et al., 2014), also in children (Johnson et al., 2014).

Other studies examining asthma or repeated infections found inconsistent associations with BT risk (Lupatsch et al., 2018; Turner et al., 2013).

Exposure to some drugs prescribed for other conditions, like statins (that can penetrate the BBB) or non-steroidal anti-inflammatory drugs, suggest a possible reduced risk of glioma (Greenland, 2017; Ostrom et al., 2019) in adults, though no or little evidence is available for childhood BTs (Seliger et al., 2016).

Infections from some particular viruses have been related with some human cancer types, including BT, for example John Cunningham (JC) virus (Zheng et al., 2009). This virus was introduced in humans through contaminated polio vaccines. Additionally, the polyomavirus SV40 has been found in human BTs biopsies samples, but the role in the aetiology of BTs is unclear, especially in children (World Health Organization et al., 2014).

d) Age

There are some differences in BTs incidence by age. For all CNS tumours, incidence rates are higher in children under 10 years of age and in young adults above the age of 25, with a decreased incidence in the range 10 to 20 years old (McKinney, 2005). An online analysis using the online Cancer Today database for 2018 (IARC- Cancer Today, 2018) showed that the incidence of BT in the

range of age from 0 to 14 years old was 8,290 cases per 100,000 persons in 2018 in high income countries compared to 2,013/100,000 in low income countries. For ages from 10 to 24 years old, the incidence was 6,605/100,000 in high income vs. 995/100,000 in low income countries (IARC- Cancer Today, 2018).

As previously mentioned in the introduction, there are also differences in tumour histology by age. Gliomas are generally frequent in all ages, but other specific types like pilocytic astrocytoma are more common in children and ependymomas in all ages. Meningiomas are generally more frequent in adults. Embryonal tumours and germ cell tumours are also much more common at early ages than in adults (Ostrom et al., 2019).

e) Sex

Incidence of BT is generally more frequent in males than females (IARC- Cancer Today, 2018), independently of the geographical area and the income level of the country. However, there are some differences by tumour type, with malignant tumours being more frequent in men than women, and the opposite for non-malignant tumours, particularly meningioma (INTERPHONE Study Group, 2010; Ostrom et al., 2019).

The different sex ratio between BT types has raised the issue of the possible role of hormones in BTs. Biological rationale for a role of estrogenic endocrine disruption in neurological disorders is available in the scientific literature (Preciados et al., 2016). Indeed, some studies reported by a review showed a suggestion of

a relationship with exposure to exogenous hormones intake (contraceptives, homone replacement therapies, fertility treatments drugs or cyproterone acetate use) with BTs in adults, finding no particular association for gliomas, but association with meningioma risk in some of them (Quach et al., 2017; Wigertz et al., 2006). Nevertheless, this association would not be relevant in children (except for drug exposures), though it could influence the risk of tumours in adolescents and young adults, even if the incidence of meningioma in this age range is very low.

f) Race/ ethnicity

Incidence of BTs varies with race/ ethnicity. Higher incidence of neuroepithelial tumours have been observed in European ancestry non- Hispanic white and meningiomas in more frequent in non-Hispanic black (Ostrom et al., 2019).

Incidence of BTs, particularly meningioma, appears to be higher in Jews than non-Jews in general (Inskip et al., 1995) and it also differs by origin, within Israel (Barchana and Liphshitz, 2013).

In the US, the frequency of different types of BTs has been found to vary by ethnic origin and race. Indeed, a higher frequency of meningiomas and pituitary adenomas has been observed among non-Hispanic black compared to other groups (Ostrom et al., 2019). In children and adolescents, a higher incidence rate of neuronal and mixed neuronal- glial tumours was observed among whites (less than 14 years old) (Gittleman et al., 2017) and a higher incidence of

germ cell tumours among Asian and Pacific Islander adolescents (below the age of 20) compared to other ethnicities (Gittleman et al., 2019). In the SEER Cancer Statistics Review of US population, showing age-specific rates, of 2012-2016, with higher incidence rates for white races than black races in young people populations (0 to 24 years old) compared to other (Howlader N et al., 2019).

1.2.2. Head injury

The idea that head injury may increase the risk of meningioma is not new (Walsh et al., 1969) and has been reported in both men and women in several earlier case-control studies (Preston-Martin et al., 1989, 1983, 1980). A cohort linkage study in Denmark found an increased incidence of BTs following head trauma, which, however, was considerably reduced when excluding tumours diagnosed in the first year: Standardised Incidence Ratio (SIR) 1.0 for glioma (CI = 0.8-1.2) and 1.2 for meningioma (CI = 0.8-1.7), suggesting previous findings may have been related to differential recall between cases and controls and to the chance discovery of asymptomatic tumours during imaging for trauma (Inskip et al., 1998). The hypothesis of differential recall bias has also been discussed by McKinney (McKinney, 2004)

A large scale multinational study of BTs also found an increased risk of BTs in adults following head trauma requiring hospitalisation (and hence unlikely to be subject to recall bias) in men; the increase was highest for meningioma (Preston-Martin et al., 1998).

In a small case-control study in Canada, a significantly elevated risk of BTs was also reported for head or neck injuries which required medical attention (Howe et al., 1989).

No more recent articles on the topic have been found though information on trauma has been collected in a number of recent large scale studies (Cardis et al., 2006; Sadetzki et al., 2014).

1.2.3. Environmental risk factors

Possible associations between specific environmental exposures and BT risk in young people are less studied than in adult populations. Exposures to heavy metals, pesticides, tap/ well water chemicals, air pollution, diet, environmental tobacco smoke or parental occupation have all been suggested to play a role in CBT.

Mothers may be exposed to genotoxic substances like heavy metals, pesticides and some organic solvents, and enter into contact with the child during pregnancy or breastfeeding. Children may also be exposed directly to toxicants that their parents bring from their jobs (clothes, skin, shoes, etc.) and that contaminate the home environment (Harry and Tilson, 2010). Moreover, children can be indirectly affected by paternal mutagenic or epigenetic changes in the sperm due to preconception environmental exposures, or directly affected by chemical transfer from the mother during conception or pregnancy (Rosso et al., 2008).

In this section of this thesis, ionizing radiation, as one of the most well established environmental risk factor for BT, is very brief

described, together with other specific environmental exposures particularly explored in this thesis: some water chemicals and heavy metals.

a) Ionising Radiation

Exposure to ionizing radiation, especially medical radiation to the head or neck in children, has long been associated with BT risk, in particular with risk of meningioma, but also to malignant tumours such as glioma (IARC, 2012, 2000; UNSCEAR, 2014, 2011; US NRC, 2006).

A number of recent publications on cohorts of paediatric patients having undergone at least one CT scan also indicate an increased risk of BTs in relation to low to moderate doses of radiation (Journy et al., 2015; Krille et al., 2015; Mathews et al., 2013; Meulepas et al., 2019; Pearce et al., 2012) although the exact magnitude of the risk is subject to discussion.

b) Water toxicants

Drinking water can contain various chemicals originated from the passage of surface water through contaminated areas, from infiltration of pollutants and chemicals to wells or aquifers - which drain to deeper areas – as well as from the by-products of chemicals used to disinfect the water. Particular chemicals of concern include pesticides, nitrate and trihalomethanes (a water disinfection by-product).

Nitrate come mostly from agricultural activity, wastewater and oxidation of nitrogenous waste products in human and animal excreta. Nitrate can be reduced to nitrite, and both are considered possibly carcinogenic in humans (Group 2A) since 2010 (IARC-Monographs Vol. 1-121, 2018). Children can be exposed through tap water and also through diet (used mainly as meat conservative). Two articles have been published on the possible association between exposure to nitrate through diet and CBT showing non-significantly increased risk for exposures during childhood (Lubin et al., 2000; Sarasua and Savitz, 1994). Three publications on exposures to nitrate or nitrite through tap water consumption showed increased risk of BT (Mueller et al., 2004, 2001; Weng et al., 2011).

Other important chemicals present in drinking water are the disinfection by-products (DBPs). These chemicals are mainly produced in the process of disinfection of the water, in the process that is carried out to kill bacteria and other pathogens for the prevention of illnesses after contact with tap water. The addition of some disinfectants like chlorine, chloramines, ozone, UV, and others to drinking water that contain natural organic matter, produce the DBPs. DBPs come also from other chemicals like pesticides, estrogens, parabens, bisphenol A, among other (Thompson et al., 2015). More than 700 DBPs have been identified, but still there are a lot of them that are unknown and consequently their effects on human health is unknown, if any. Trihalomethanes (THMs) and

haloacetic acids are the most commonly studied DBPs and are present in higher concentrations in drinking water compared to other DBPs (World Health Organization, 2017). Nevertheless, there is no any publication about BT risk in children and exposure to THMs in drinking water.

c) Heavy metals

Heavy metals are naturally in the Earth crust. Either originated by natural erosion of the soil or by industrial emissions, humans can get in contact with metals through air, soil and water. Metals can bioaccumulate in animals (aquatic and terrestrial) and plants that are subsequently ingested by humans. Diet is the main route of long-term human exposure to metals. Other exposures, for instance from private groundwater consumption, can occur, although they are isolated cases. Some metals have the ability to cross the placenta and the BBB (Goyer, 1990; Harry and Tilson, 2010), and their neurotoxic effects are proven (Carpenter, 2001), as is the case of mercury or lead.

Several recent studies have investigated the effects of occupational exposures to metals and risk of BTs in adults. While two studies report a possible association for meningioma (Rajaraman et al., 2006; Sadetzki et al., 2016) – the first possibly related to a genetic predisposition – no association has been reported between metal exposure in adults and risk of glioma (Parent et al., 2017).

There are very few publications on exposure to metals and risk of BT in young people.

d) Other environmental exposures (including non-ionising radiation, smoking, etc)

Tobacco smoke has been classified as carcinogenic to humans (Group 1) IARC Monographs programme (IARC- Monographs Vol. 1-121, 2018). While there is some evidence for an association between tobacco smoke and risk of BT in adults (Lee et al., 1997), the possible effects of active or passive smoking during pregnancy and in early life on BT risk is unclear, despite that 24 studies (Barrington-Trimis et al., 2013; Brooks et al., 2004; Bunin et al., 1994a; Cordier et al., 2004, 2004, 1994; Filippini et al., 2002, 2000, 1994; Gold et al., 1979, 1993; Howe et al., 1989; Hu et al., 2000; Ji et al., 1997; John et al., 1991; McCredie et al., 1994a; Milne et al., 2013; Norman et al., 1996; Pavlovic et al., 2005; Plichart et al., 2008; Pogoda and Preston-Martin, 1997; Preston-Martin et al., 1982; Tettamanti et al., 2016; Vienneau et al., 2016) examined the possible association between cigarette smoking and environmental cigarette smoking, finding 10 of them with indications of increased CBT risk. It is known, however, that some tobacco smoke compounds can cross the BBB and the placenta (Norman et al., 1996).

Air pollution has been associated with respiratory and cardiovascular diseases, but also with neurodevelopmental

problems in children (Chiu et al., 2016; Forns et al., 2018; Sunyer and Dadvand, 2019). Living in urban areas with high traffic density implies being exposed to different chemicals from vehicle exhausts. Two articles explored this possible association finding significant increased risk for some particular chemicals (Danysh et al., 2016; Von Ehrenstein et al., 2015).

Pesticides come mainly from agriculture. Their effects on human health, especially on BTs risk in children, have been explored in several articles. Use of pesticides by parents or use in wood houses to prevent plagues was associated with BTs in offspring in 11 studies. They showed positive associations with some particular BT types with exposures during pregnancy (Greenop et al., 2013; McCredie et al., 1994a; Pogoda and Preston-Martin, 1997; Rosso et al., 2008). Exposure during childhood was mostly statistically not significant. Other 5 studies explored the association with living on a farm during childhood or pregnancy (Bunin et al., 1994a; Cordier et al., 1994; Efird et al., 2003; Holly et al., 1998; McCredie et al., 1994a), showing a suggestion of increased risk during childhood and reduced for pregnancy.

Both extremely low-frequency (ELF) and radiofrequency fields (RF) (non-ionizing radiation) have been postulated to be risk factors for BTs in adults and possibly in children (Schüz, 2011). The evidence is inconclusive though both ELF and RF have been classified as possibly carcinogenic to humans (Group 2B) (IARC- Monographs Vol. 1-121, 2018).

1.2.4. Socio- economic factors

Little information is available on the relation between CBT and socio-economic status. Only two epidemiological studies have been published. The first, a case-control study, showed a strong increased risk for BT in children whose fathers had less than fourteen years of formal schooling and for children whose mothers were housewives or workers in comparison to children of clerks (Pavlovic et al., 2005). The second, a cohort study, observed an increased risk of astrocytomas for medium income families in comparison with high income families and a non-significantly reduced risk for ependymoma and choroid plexus tumours in children from low family income homes (Kollerud et al., 2015). Thus the evidence to date is poor and inconsistent.

Rationale

2. RATIONALE

BTs is one of the most frequent tumour types in young people with potential for long term sequelae in quality of life and little is known about their risk factors. Any valid information about its risk factors can prove useful to design strategies for its prevention. Some exposures during pregnancy and early life contribute to the risk, and hence, the information contained in this thesis could contribute with a better understanding of the aetiology and primary prevention of BTs in children.

Apart from genetics factors and ionizing radiation exposure which are establish risk factors for BT, studies have suggested that daily exposures to other factors like environmental tobacco smoke, air pollution, chemicals in food, chemicals in tap water, among other, can have a role in the aetiology of BTs. Few publications have explored the role of exposure to tap water toxicants though they have been related with other cancer types. The inconclusive results of these studies, due to small sample size mainly, made really interesting the possibility to explore this exposure in the much larger multinational MOBI-Kids study.

To my knowledge, only one study explored heavy metals body concentrations in children in relation with BTs risk, though some metals have been demonstrated as possibly neurotoxic. Further studies in a large sample sizes are needed to explore their role in the aetiology of BTs.

BTs if a mixed group of different tumour types, with widely different histologies and characteristics. The need to better

understand the clinical characteristics of different types of tumours with regards to morphology, symptoms, locations, grades and other characteristics is essential for a faster diagnosis, and consequently, preventing a case fatality or long-term neurological or psychological sequelae. The better knowledge of symptoms related to BTs is very important, especially since these symptoms are generally quite common in other diseases (headaches, visual problems, vomiting, etc.) and that fact could mask the real problem.

This thesis aimed to contribute to the understanding of the aetiology of BTs in young people and to a better understanding of the clinical characteristics of these tumour types.

Objectives

3. OBJECTIVES

The purpose of this doctoral thesis is to explore risk factors and clinical characteristics of BT, both malignant and benign, in young people.

To accomplish this objective, I conducted the work in two phases:

First phase:

- description of clinical characteristics and symptoms of BTs in young people, based on data on 899 BTs cases aged 10-25 years old from 14 countries, who participated in the international MOBI-Kids case-control study; and
- a Systematic Review (SR) of the literature on risk of BTs in young people in relation to environmental factors.

Second phase, based on the results and research gaps identified in the SR, I studied the risk of BT (overall and more specifically neuroepithelial tumours) in relation to:

- exposure to chemicals present in drinking water (THMs and nitrate); and
- heavy metals in toenails.

Methods

4. METHODS- The MOBI-Kids study

The MOBI-Kids study is a multinational prospective case-control study of BTs in young people (10-25 years old), coordinated by ISGlobal (former CREAL) and funded in particular by the European Community's Seventh Framework Programme under grant agreements number 226873 - the MOBI-Kids project - and 603794 – the GERoNiMO project. Additional funds for the coordination of MOBI-Kids were obtained from the Spanish Ministry of Science and Innovation (MINECO), while complementary funds for the conduct of MOBI-Kids in Spain were obtained from the Spanish Health Research Fund (FIS) of the National Institute for Health Carlos III. Additional grants were obtained in each of the participating countries. 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. The study was conducted in 14 countries (Australia, Austria, Canada, France, Germany, Greece, India Israel, Italy, New Zealand, Spain, Netherlands, Japan and Korea) using a common protocol. Details of the methods have been published (Sadetzki et al., 2014).

The primary objective of MOBI-Kids was to study the effects of childhood and adolescent exposure to EMF from mobile communications technologies on BT risk. The secondary objective was to study the relation between risk of BTs and a number of potential host and environmental risk factors.

Cases were defined as patients with diagnosis of first primary BT (encephalus or cranial nerves, and not located in the midline). To increase participation rates in this young population, two controls were selected from the lists of general surgery services of the hospitals in the case catchment areas on the basis of age, sex, region of residence and having an operation for appendicitis (a relatively frequent condition in the study target population, not related to socio-economic status and not thought to be related to the main risk factors under study). All participants, 899 cases and 1922 controls, were diagnosed during the period of the study (2010-2016, with the exact years depending on the country).

Ethics approvals were obtained from all necessary national, local and in some countries hospitals, ethics review boards as appropriate.

Among the cases identified, 899 were eligible and accepted (or the parents accepted, depending on the age of the subject) to participate in the study by signing an informed consent form and responding to a questionnaire administered in person to the subject and/or parent(s) by interviewers trained for the study.

Among the controls who were contacted, a total of 1922 were eligible and agreed (or their parents agreed) to participate.

In all participating countries, a clinical questionnaire was completed by the interviewers with help of neurosurgeons and/or pathologists. Entries into the clinical questionnaire were based on the available clinical records that included imaging, histopathology, surgery, symptoms and other clinical reports.

Six of the participating countries (Canada, Greece, Italy, Korea, New Zealand and Spain) participated in the water sub-study and collected information on personal water consumption habits. For this thesis, we coordinated the collection of the necessary information on residential tap water concentrations of THMs and nitrate in all six countries, and collected the information herself for Spain. The resulting information was validated and brought together in a common database and combined with questionnaire response about water consumption to estimate the ingestion and residential (both pre and post-natal) levels of various water chemicals for all participating subjects and evaluate their possible relation with risk of BTs (details are provided in paper III).

In Spain, participants who accepted provided toenails samples either during the interview or later (and sent by post). The toenails were analysed in 453 samples and concentrations of different metals were estimated.

Results

5. RESULTS

The results of this thesis are presented in four articles: a systematic review and three original research articles:

- **Paper I**: *Environmental factors and risk of brain tumours in young people - A systematic review*.

- **Paper II**: *Clinical presentation of brain tumours in young people (10-24 years old): Results from the international MOBI-Kids study*.

- **Paper III**: *Exposure to trihalomethanes and nitrate from drinking water and risk of brain tumours in young people: Results from the international MOBI-Kids study*.

- **Paper IV**: *Levels of heavy metals in toenail and brain tumours risk in young people: Results from the international MOBI-Kids study*.

5.1. Paper I

Zumel-Marne A, Castano-Vinyals G, Kundi M, Alguacil J, Cardis E. Environmental Factors and the Risk of Brain Tumours in Young People: A Systematic Review. Neuroepidemiology. 2019 Nov 1;53(3–4):121–41. DOI: 10.1159/000500601

5.2. Paper II

Zumel-Marne A, Kundi M, Castaño-Vinyals G, Alguacil J, Petridou ET, Georgakis MK, et al. Clinical presentation of young people (10–24 years old) with brain tumors: results from the international MOBI-Kids study. J Neurooncol. 2020 Apr 1;147(2):427–40. DOI: 10.1007/s11060-020-03437-4

5.3. Paper III

Angela Zumel-Marne, Esther Gracia-Lavedan, Gemma Castaño-Vinyals, Cristina M Villanueva, Juan Alguacil, Nuria Aragones, Franco Merletti, Andrea 'tMannetje, Amanda Eng, Eleni Petrou, Evi Bouka, Mina Ha, Charmaine Mohipp, Franco Momoli, Elisabeth Cardis.

Exposure to drinking water trihalomethanes and nitrate and the risk of brain tumours in young people: Results from MOBI-Kids study.

In preparation

Exposure to drinking water trihalomethanes and nitrate and the risk of brain tumours in young people

MOBI-Kids study. Participating countries: Canada, Greece, Italy, New Zealand, Spain and Korea.

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Abstract

Introduction: Brain tumours (BT), and particularly neuroepithelial BTs - are one of the most frequent tumour types in young people and few risk factors have been established. Trihalomethanes (THMs) and nitrate are possible carcinogens found in drinking water. We explored the association between exposure to these agents and BT risk in young people.

Methods: We used information from participants in the 6 countries (Canada, Greece, Italy, Korea, New Zealand and Spain) of the international MOBI-Kids case-control study (2010-2015) which participated in the water sub-study. The analyses included 321 cases and 919 appendicitis controls with tap water use information. Historical data on disinfection mode and chemical concentrations was sought from water companies and/or municipality for the municipality of residences of the study subjects. Historical residential tap water concentrations of THMs and nitrate were modelled and combined with the study subjects' residential history and personal consumption patterns to estimate cumulative ingestion and residential exposure levels in the study population (both pre and post-natal). Water levels of THMs and nitrate could be assigned to only 86 cases and 352 controls due to difficulties in obtaining historical information in many municipalities. Conditional logistic regression was used, matched on sex, age and country, and adjusted for parental education.

Results: The highest levels and widest ranges were found in Spain for THMs (residential and ingested) and Italy and Spain for nitrate. No statistically significant difference was seen between cases and controls in tap water use for drinking or showering/bathing. Odds Ratios (ORs) for BT in relation to both pre- and postnatal residential and ingestion levels of THMs were systematically below 1; the strongest

decreases in risk were found for exposure in the first 2 year of life. For nitrate, all ORs were above 1, with a suggestion of an exposure-related increased risk of neuroepithelial BTs in association with residential nitrate levels in tap water both for pre- and post-natal exposures, which appeared slightly stronger when considering only exposures early in life.

Discussion: Though our study is the largest to date and the only focusing on BT in young people, exposure in most countries was very low, with very little variability between subjects. Our results do suggest a reduction of neuroepithelial tumour risk in young people in relation to levels of THMs in tap water, and an increased risk in relation to tap water levels of nitrate. Low response rates in controls and lack of information on other sources of exposure to these chemicals, may have affected our results, however. Further research is required to clarify the observed associations.

Key terms: brain tumours, water, disinfection by-products, nitrate, children, young adults, adolescents.

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 high and middle-income countries 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 chlorinated 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 in bladder cancer (Villanueva et al., 2007) among other cancer types. There is no published study about BT risk and exposure to water chemicals in young people. Two papers have addressed risk of BT in adults. In one, from the USA (Iowa) non-statistically significant associations where found between chlorinated surface water and BT risk in males who lived for over 40 years in residences with chlorinated surface water sources (Odds Ratio (OR)=2.5; 95% Confidence Interval (CI) 1.2-5.0; 13 cases and 81 controls) (Cantor et al., 1999). Analyses by lifetime average THMs exposure resulted in ORs below 1 for exposures levels lower than 32.5µg/L and above 1 for higher levels. A histological cohort study in Finland based on 621,431 persons adult (including 917 BTs) was conducted to assess the relation between historical exposure to drinking water mutagenicity and cancer risk (Koivusalo et al.,

1997). They found no relative risk (RR) for BT cases that lived in towns that used chlorinated surface water compared with those cases that did not (RR=0.99 (0.83-1.19)). 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 bladder cancer (Espejo-Herrera et al., 2015), colorectal cancer (Espejo-Herrera et al., 2016), and other cancer types. Nitrate can be reduced into nitrite (World Health Organization, 2017) and both nitrate and nitrite are classified as probably carcinogenic to humans (Group 2A) since 2010 (IARC- Monographs Vol. 1-121, 2018). A previous study that explored prenatal and early postnatal exposure to residential nitrite levels in water found a statistically significant association with BT risk for subjects with levels higher than 5mg/L (OR= 5.2 (1.2–23.3)) compared to non-exposed, based on small numbers (6 exposed cases and 8 exposed controls) (Mueller et al., 2004). Another publication from the same group showed a significantly increased BT risk for children exposed to nitrate before conception (OR= 8.8 (2.1–46)) in 13 exposed cases and 3 exposed controls for any nitrate level measured (Mueller et al., 2001). Another study, which explored the exposure to nitrate-nitrogen in 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 (1.07-1.84)) compared to lower levels (in 190 cases and 229 controls) (Weng et al., 2011). The systematic review of BT risk factors in young people we conducted (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.

Methodology

Population included

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 25 years. The vast majority of tumours were gliomas and other neuroepithelial tumours (75%), followed by embryonal tumours (14%) (Zumel-Marne et al, submitted). Controls were selected among patients operated for appendicitis in a hospital located in the catchment area of the cancer cases. Controls were matched to cases by age, sex and region. Subjects were recruited between 2010 and 2016, depending on the country. 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). 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 can be found in the MOBI-Kids methods paper (Sadetzki et al., 2014).

Individual information

Within MOBI-Kids, an epidemiological questionnaire was administered to the study subjects, and/or their parents, in a face-to-face interview by trained interviewers. The primary focus of the questionnaire was the use of mobile phones and exposure to other sources of electric and magnetic fields (EMF). Additional questions were included about 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 per week (1 to 3; 4 to 7; 8 or more). The MOBI-Kids questionnaire also included a detailed residential history of the study subjects from obtained birth until diagnosis. We assumed that the residence during pregnancy was the same as that at birth.

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 nitrates in the municipality where subjects lived (see below).

Exposure data

A survey was sent to the city authorities of the municipalities of residence of the study subjects as well as to the water companies, treatment plants and national referents (depending on the country and municipality) covering these municipalities to collect general historical information on tap water characteristics. 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 nitrogen-nitrate (Canada, New Zealand and Korea) and we converted these values into nitrate levels by multiplying them by 4.43 (Konieczynski and Wesolowski, 2007).

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 up the levels of chloroform, bromoform,

dichlorobromomethane and chlorodibromomethane, as done in previous studies (Font-Ribera et al., 2018). In Korea, THMs levels did not include bromoform because they were close to zero. The information obtained about levels did not always cover the entire lifetime of the study subjects (1985-2016). For years with missing information, if treatment and source of water was the same, we estimated the THMs and nitrate levels using the average levels of the available information, for each municipality/area. If the treatment or source varied, we could not assume that the levels of nitrates 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.

Individual exposure estimation

Residential postnatal exposure level was calculated by averaging the annual levels of THMs and nitrate from birth until 5 years before diagnosis (to allow for a minimum induction time between exposure and diagnosis of a potentially exposure related tumour), using only non-missing values. We also calculated the cumulative postnatal exposure by summing annual levels from birth up to 5 years before diagnosis, assigning a value 0 when exposure levels were missing (scenario 1). Prenatal exposure was estimated as the average levels of THMs and nitrate over all municipalities of residence in the year of the child's birth.

Ingestion THMs levels were calculated for each study subject, both for the pre- and postnatal periods, by combining the residential levels and the number of glasses consumed per day. Specifically, we multiplied the subject's average THMs level (μg/L) by the reported daily litres of tap water consumed – assuming that one glass of tap water was equivalent to 0.20L, 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 and nitrate.

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; Nolan et al., 2002; Wang et al., 2016), and no information was asked about the subject's consumption of water from these sources.

Statistical analysis

Descriptive analyses were conducted by age (in three groups: 10-14, 15-19 and 20-25), sex, country and parental education ('less than High school'; 'medium level tech/prof. school'; 'university' and 'other/don't know') obtained as the maximum of mother and father's education.

For the risk analyses, we used conditional logistic regression to estimate the odds ratio (OR) and 95% confidence interval (CI) of BT associated with exposure to residential THMs and nitrate, and ingestion of THMs, both for the pre- and postnatal period. Strata were created by age (1 year from 10 to 17 and 2 years from 18 to 25), sex and country. Analyses were systematically adjusted for parental education level.

For the analysis of postnatal residential and ingestion exposures, we included only the study subjects for whom information on THMs (86 cases and 352 controls) and nitrate (85 and 343 controls) was available for more than 70 percent of their life (Supplementary Table 1) 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 main analysis because no case fulfilled this criterion. We also excluded Greece because there of lack of variability in exposure (all participant

residences were supplied by the same source of water, and hence had the same annual residential average levels).

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

The main analyses focused on neuroepithelial tumours only (given the likely different aetiologies of tumours in the 10-24 years age-range).

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 the first 5 years of life. We could not take into account breastfeeding during this early period; previous publications suggest breastfeeding reduces nitrate exposure (Dusdieker, 1996; Fossen Johnson, 2019) while for THMs the results are inconsistent (Batterman et al., 2002). We also performed sensitivity analyses based on different scenarios to infer residential exposure level when data were missing: assigning the median residential level for the municipality for the missing periods (scenario 2) and the maximum residential level for those periods (scenario 3) – compared to assigning 0 (scenario 1) which was the basis for the main analyses. All statistical analyses were performed in STATA 14.0.

Results

A total of 491 cases and 1,002 controls were included in MOBI-Kids in the 6 countries which participated in the water contaminants sub-study. Among cases, 74% of the tumours were neuroepithelial (n=363). Stratification by age, sex and country reduced

the number of subjects in informative strata (i.e. with at least one case and one control) to 361 neuroepithelial tumour cases and 993 matched controls. Of these, 321 cases and 919 controls completed the personal residential history and tap water use questionnaires and mothers from 297 of the cases and 766 of the controls completed the maternal water use questionnaire. It was possible to collect information on water levels that cover more than 70% of the lifetime person-years of each subject for only 86 cases and 352 controls (Supplementary Table 1).

Table 1 shows the characteristics of the study subjects for neuroepithelial tumours, as well as for all BTs regardless of morphology. The mean attained age among participants included in the neuroepithelial analyses was 16.7 years (standard deviation, SD=4.3 years).

Table 1. Characteristics of the study population.

	Neuroepithelial		All BT tumours	
	Case N(%) N=361	Control N(%) N=993	Case N(%) N=491	Control N(%) N=1002
Age, mean(SD)	16.6 (4.3)	16.8 (4.3)	16.8 (4.4)	16.8 (4.3)
Age categories, years				
10-14	146 (40.4)	386 (38.9)	193 (39.3)	393 (39.2)
15-19	117 (32.4)	336 (33.8)	158 (32.2)	338 (33.7)
20-25	98 (27.1)	271 (27.3)	140 (28.5)	271 (27.0)
Sex				
Male	206 (57.1)	567 (57.1)	285 (58.0)	571 (57.0)
Country*				
Canada	17 (4.7)	21 (2.1)	23 (4.7)	24 (2.4)
Greece	39 (10.8)	86 (8.7)	54 (11.0)	87 (8.7)
Italy	123 (34.1)	342 (34.4)	160 (32.6)	342 (34.1)
New Zealand	13 (3.6)	25 (2.5)	16 (3.3)	29 (2.9)
Spain	145 (40.2)	421 (42.4)	208 (42.4)	422 (42.1)
Korea	24 (6.6)	98 (9.9)	30 (6.1)	98 (9.8)
Parental education *				
≤High school	130 (36)	300 (30.2)	178 (36.3)	303 (30.2)
Medium level tech/prof. school	99 (27.4)	244 (24.6)	129 (26.3)	244 (24.4)
University	94 (26)	274 (27.6)	133 (27.1)	278 (27.7)
Other and don't know	38 (10.5)	175 (17.6)	51 (10.4)	177 (17.7)

*Statistically significant heterogeneity by country (p=0.024) and parental education (p=0.001).

There was a higher proportion (39%) of participants aged 10 to 14 years old (Table 1) than 15-19 or 20-24. The sex ratio (male/female) was 1.33. Italy and Spain contributed with 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 unknown level of parental education was higher among controls than cases (Table 1). The distribution of tap water consumption habits among controls is shown, according to the main characteristics of the population, in Table 2. The proportion of study subjects consuming tap water differed by country and parental education level. Tap water consumption was highest in New Zealand (83%), Greece (80%) and Canada (72%) and lowest 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 pre- and postnatal periods). A high percentage of tap water consumption was seen for the pre-natal period among those with other/unknown parental educational level (75%), while the lowest was in the medium education level category (43%). Statistically significant differences in the frequency of showers/baths of study subjects were found also found by country, age, sex and, in the post-natal period, by parents' education level. Ninety-two percent of subjects reported showering or bathing more than 4 times/week New Zealand compared to 69% in Italy. Men tended to shower or bathe more frequently than women (83% vs. 72%) and older subjects more frequently than younger ones (87% and 70% respectively in the 20-24 and 10-14 years categories).

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

	Prenatal				Postnatal			
	Drinking tap water		Showering/ bathing		Drinking tap water		Showering/ bathing	
	Yes N (%)	p-value[1]	≥ 4 times/ week N (%)	p-value	Yes N(%)	p-value	≥ 4 times/ week N (%)	p-value
Age categories						0.179		<0.001
10-14	NA		NA		210 (58.8)		249 (69.7)	
15-19	NA		NA		165 (53.6)		249 (80.8)	
20-24	NA		NA		137 (54.2)		220 (86.6)	
Sex						0.790		<0.001
Male	NA		NA		291 (56.2)		430 (83.0)	
Female	NA		NA		221 (55.3)		288 (71.8)	
Country		<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)		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)		262 (66.0)		344 (86.6)	
Korea	29 (33.0)		74 (84.1)		25 (26.0)		72 (73.5)	
Parental education		<0.001		0.097		0.151		0.014
≤High school or less	141 (55.7)		211 (79.9)		142 (51.4)		208 (77.8)	
Medium level	92 (43.4)		168 (76.3)		124 (53.9)		168 (73.4)	
University	141 (58.5)		209 (85.0)		157 (61.0)		215 (83.3)	
Other, unknown	27 (75.0)		31 (86.1)		89 (57.8)		127 (82.5)	

[1]p-value from Chi-squared test for homogeneity. NA=Not applicable. Percentages are calculated based on the total of each category. 'No'=0 tap glasses of water; 'Yes'=1 or more glasses of tap water per day.

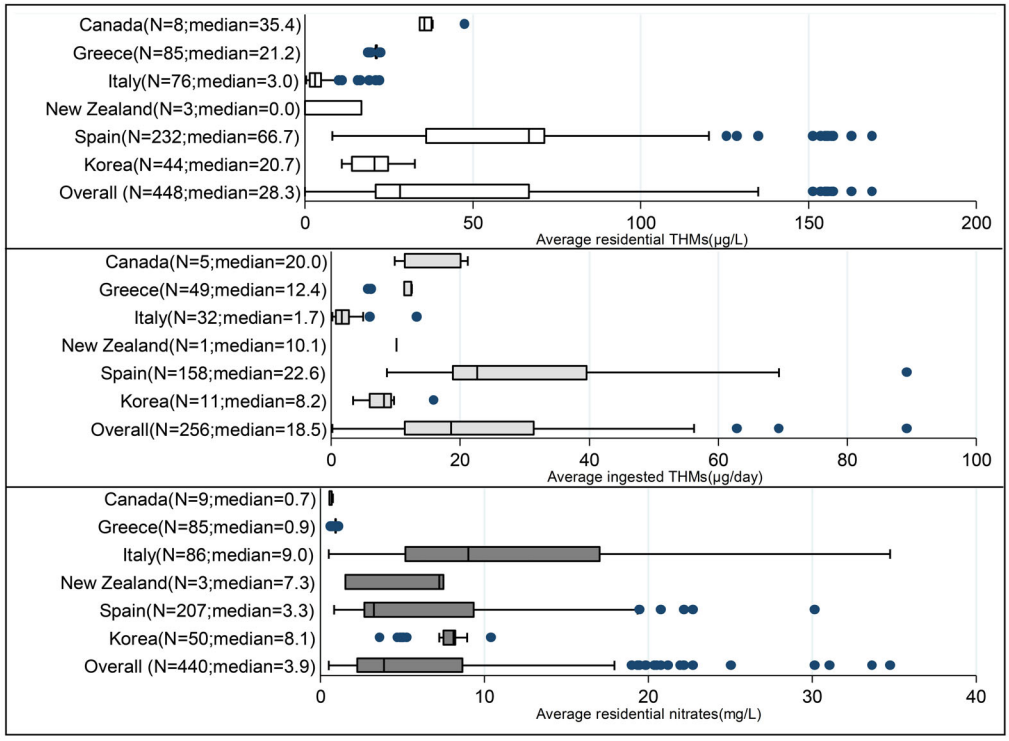
Those in the highest parental education category and those whose parental education level was “other/ unknown” had the highest frequency of showering/bathing over 4 times a week (83 and 82% respectively).

We found little differences in levels of THMs or nitrate by characteristics of the study population. Nitrate levels were higher in the older age group, and in participants with medium level of parental education (data not shown). Among subjects in the lowest category of residential THMs levels (less than 25.1 μg/L), the majority (61%) reported no tap water consumption, and 65% reported an average of 4 or more showers/ baths per week. Among those in the middle category of residential THMs 66% reported drinking at least 3 glasses of tap water per day (20% reported no tap water consumption), while among subjects in the highest tertile of residential THMs, there were similar percentages who consumed no tap water (41%) and 3 glasses or more per day (39%). Showering/bathing at least 4 times a week was similar in the middle and highest levels of residential THMs (87 and 88% percent respectively) (Supplementary Table 2).

For nitrates, the vast majority of subjects in the lowest tertile consumed 3 or more glasses of tap water daily (69%) and showered/bathed 4 or more times/week (88%). In the middle and highest tertiles of nitrate levels, similar distributions were found, with 55% reporting no tap water consumption, 34% and 33%, respectively, reporting drinking 3 glasses or more weekly and 74% and 70% respectively showering/bathing 4 or more times a week.

Figure 1 shows the average residential THMs, ingested THMs and residential nitrate levels in controls, by country and overall, for the postnatal period. Median residential THMs and nitrate levels were, respectively, 28.3μg/L, and 3.9mg/L. The highest average levels of residential THMs was seen in Spain and Canada and the lowest in Italy and New Zealand; a similar pattern was seen for ingested THMs.

Figure 1: Boxplot of average postnatal residential and ingested levels of trihalomethanes (THMs) and residential nitrate levels by country in participating controls (postnatal period).



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.

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.

We found no association between neuroepithelial BT risk and frequency of drinking tap water or of showering/ bathing, either during the pre- or the postnatal periods (Table 3). Participants lived, on average, in 2.1 municipalities with a mean duration of 11.2 years in each of them (data not shown).

Table 4 shows the ORs for neuroepithelial BT by tertile of residential and ingested THMs levels during the pre- and postnatal exposure periods. We found reduced ORs in all categories of exposure (both pre- and postnatal) for residential and ingestion exposures. There was no indication of a trend y exposure level, except for prenatal THMs ingestion levels, where the OR for ingestion level above the median was 0.33 (95% CI 0.16-0.70), based on 11 exposed cases. In the analysis by country, ORs were generally below 1, statistically significantly so for ingested THMs above the median in Spain for the prenatal period (Supplementary Table 3).

For nitrate (Table 5), ORs for neuroepithelial BT were systematically elevated in the 2nd and 3rd tertiles of pre- and postnatal exposure compared to the first tertiles, with a suggestion of a possible exposure related increasing trend (p=0.1 and 0.09, respectively, for pre- and postnatal average residential levels). Analyses by cumulative residential level show a significantly increased OR in the second tertile of exposure, statistically compatible, however, with the non-significant increase in the 3rd tertile.

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[1]	(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*

[1]Conditional logistic regression models, stratified on sex, age and country, and adjusted by parental education for all countries.

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 post-natal periods.

RESIDENTIAL THMs, (µg/L):	Cases (N)	Controls (N)	OR[1] (95% CI)
Prenatal			
Average residential THMs			
≤25.1	36	91	1.00
25.1-66.5	16	97	0.36 (0.14-0.93)
≥66.5	27	111	0.68 (0.28-1.69)
p trend			*0.99*
Postnatal			
Average residential THMs			
≤25.1	32	78	1.00
25.1-66.5	26	98	0.39 (0.09-1.77)
≥66.5	26	111	0.37 (0.08-1.73)
p trend			*0.46*
Lifetime cumulative residential THMs			
≤238.6	34	83	1.00
238.6-674.0	27	91	0.92 (0.30-2.85)
≥674.0	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 (≤19.7 µg/day)	24	69	0.93 (0.54-1.61)
Above median (>19.7 µg/day)	11	83	0.33 (0.16-0.70)
p trend			*0.01*
Postnatal			
Non-exposed	138	358	1.00
Below median (≤19.7 µg/day)	26	91	0.72 (0.43-1.21)
Above median (>19.7 µg/day)	22	97	0.71 (0.40-1.28)
p trend			*0.16*

[1]Conditional logistic regression models, matched by sex, age and country, and adjusted by parental education.

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.

	Cases (N)	Controls (N)	OR (95% CI) [1]
Prenatal			
Average residential nitrate levels			
≤3.2	22	120	1.00
3.2-8.5	25	82	1.62 (0.74-3.53)
≥8.5	32	101	1.76 (0.91-3.41)
p trend			*0.10*
Postnatal			
Lifetime cumulative residential nitrate levels			
≤41.6	23	97	1.00
41.6-97.0	34	94	2.12 (1.02-4.40)
≥97.0	26	90	1.72 (0.82-3.63)
p trend			*0.18*
Average residential nitrate levels			
≤3.2	20	103	1.00
3.2-8.5	28	84	1.42 (0.66-3.09)
≥8.5	35	94	1.80 (0.91-3.55)
p trend			*0.09*

[1]Conditional logistic regression models, matched by sex, age and country, and adjusted by parental education.

In analyses by country (Supplementary Table 4), a statistically significantly elevated OR was seen in the highest tertile of residential nitrate level of exposure in Spain for the prenatal period (OR=2.30 (1.01-5.27); this OR was similar and statistically compatible to that seen in the 2nd tertile for both the pre- and postnatal periods. There was no evidence of an exposure response relationship. An increased OR was observed in the highest tertile in Korea, based on 3 cases and 2 controls, while no association was seen in Italy.

Sensitivity analyses:

In analyses by age at exposure, assessing possible vulnerable periods of life for brain development, we found significantly reduced OR for the middle and highest tertile of residential THMs exposure levels for the first 2 years of life (OR=0.07 in both exposure categories with no indication of an exposure related trend), entirely driven by the Spanish results as Spanish participants accounted for all cases and nearly all controls in the exposure categories (Supplementary Table 5). Results of analyses taking into account exposures in the first 5 years of life were broadly similar.

For nitrates, we found an increased OR both for the 2nd and 3rd (statistically significantly elevated only for the 3nd) compared to the 1st in analyses of exposure received in the first 2 years of life, with a suggestion of an exposure related trend (p=0.05). In analyses by country, we observed an increased OR both for the 2nd and 3rd tertiles (statistically significantly elevated only for the 2nd) in Spain, and reduced risks in both tertiles in Italy. There were too few exposed subjects in the other countries to estimate an OR. Results of analyses based on exposures received during the first 5 years of life (Supplementary Table 6), were broadly similar.

No major difference was observed in results of analyses based on different scenarios for imputing exposure for the time periods where data were missing, though there was some evidence for an increasing trend with increasing nitrate level both in scenario 2 (where the residential nitrate exposure was imputed to be the average of levels for the same municipality) and scenario 3 (where it was imputed to be the highest measured level for the same municipality) (Supplementary Table 7).

Analyses of risk using all brain tumours rather than only neuroepithelial tumours, as well as including Canada, Greece and New Zealand 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.

Discussion

The main strength of our study is that it is the first and largest study to explore the possible relation between neuroepithelial BT risk in young people and tap water consumption, assessing individual levels of THMs and nitrates exposure, using a homogeneous morphology of tumours, in a multinational study in six countries based on a common protocol, questionnaire and procedures.

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 post-natal periods.

All ORs for neuroepithelial BTs in relation to THMs exposure were below 1, for pre- and postnatal residential and ingestion exposures. An exposure-related decrease in ORs was seen for prenatal ingestion of THMs. Restricting analyses to exposures in the first

two years of life suggest a stronger reduced risk in relation to residential THMs levels, not related to exposure level, however. This was 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, followed by Spain showed the greatest variability in exposure and thus the overall analyses are mainly driven by these two countries. In analyses restricted to Spain, increased ORs were seen both for pre-natal and post-natal exposures in the higher exposure categories though there was no suggestion of an exposure- response.

It is difficult to compare our results to those in previously published papers, as no article to date has considered the risk of BT in young people related to THMs exposure. Two papers, however, investigated the effect of chlorinated water (and hence chlorinated disinfection by-products) on BT risk in adults. Our results, suggesting an exposure related decrease in risk of neuroepithelial tumours by levels of ingested THMs, are inconsistent with those of Cantor et al who found an increased risk of adult BTs in subjects who had resided for 40 years more in areas with chlorinated surface waters (Cantor et al., 1999), but the age range, exposure duration, and exposure characterisation differed substantially from that of our study. 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. It important to mention that the cut-off points of our exposure categories are different from those of previously

published article 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 medium exposure level.

For nitrate, increased ORs for adult BTs were observed in a small study (Mueller et al., 2001) conducted in US, Europe, Canada and Australia and in studies of nitrate-nitrogen exposure (Ho et al., 2011; Weng et al., 2011), giving support to our findings in children, adolescents and young people.

From the biological point of view, we know that an immune response of the central nervous system (CNS) activates when toxicants enter the body, providing protection to the brain against exposure to external chemicals. Nevertheless, excess inflammation caused by an immune reaction due to an infection or other processes can alter the blood-brain barrier (BBB) and let some toxicants enter the brain (Bondy and Campbell, 2018). In addition, considering the cerebral immaturity of the BBB of foetuses and newborns, as well as the rapid cellular division that occurs in uterus and early childhood, we can assume that children's brains may be more vulnerable to toxicants than adults. Moreover, some drinking water contaminants can disrupt mitochondrial function or increase the production of reactive oxygen species, increasing oxidative stress, and consequently, leading to abnormal brain function (Bondy and Campbell, 2018). Some water chemicals also have neurotoxic properties, including some metals and pesticides. 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). Another study suggested a possible association with neurodevelopment and DBPs in offspring whose mothers were exposed during pregnancy (Villanueva et al., 2018). Thus, it is possible that some water chemicals can cross the placenta. Therefore, from the biological point of view, exposures *in uterus* and in early life may be the most

relevant if an association between water chemicals and risk of BTs exist. Results of our analyses of prenatal exposures and of exposures during the first two years of life are consistent with this hypothesis.

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, at least 10 to 25 years before the interview. Random recall error may reduce the power of a study to find an effect, it is 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 brain tumour than tap water. Concerning post-natal 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. We also 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 city

halls 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.

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. We used average yearly levels for missing historical levels of THMs and nitrate assuming that levels did not vary if water treatment or source did not vary. These imputations have uncertainties.

Because of the amount of missing historical data, in order to minimise errors in our exposure estimates, we selected only participants for whom exposure measurements were available for more than 70% of residential history. Although risk estimates did not vary substantially, there was some evidence for an increasing trend with increasing nitrate level both in scenarios 2 and 3, where missing values were imputed based on the lifetime median or maximum levels of exposure for a particular subject.

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.

Exposure to THMs and particularly nitrate may come from other sources than tap water in the home. Questions on water consumption habits were very limited in the MOBI-Kids questionnaire, so the assessment of exposure from sources other than tap water

was not possible. Exposure to THMs through dermal contact or inhalation was not possible to estimate since we did not have information on duration of showering/ bathing as in Villanueva et al 2007. We could not explore the exposure to nitrate through other sources like diet or bottled water. We could also not retrieve data on nitrite levels, though association with BT risk in adults have been reported previously (Mueller et al., 2004, 2001).

Another limitation of the study is that we had a high percentage of controls who refused participate in our study, especially those with low parental education level (Turner et al., 2019). This could source of selection bias may affect our results if, for example subjects with higher education, as proxy for high income, used more bottle water, and consequently were less exposed to THMs.

The main strength of this study, however, is that it is the first article that explores the association between the risk of BTs in young people, focusing on a morphologically (and presumably aetiologically) homogenous group of tumours (neuroepithelial tumours) and individual exposure to THMs. It is also one of the few that explored nitrate exposure and BT risk in young people. Another important strength is the study's large sample size, including participants from 6 countries with different levels 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.

Conclusion

This is the first – and largest – study to explore the relationship between risk of BTs – in particular neuroepithelial tumours – in young people aged 10 to 24 years and tap water

consumption habits (drinking and showering/bathing), and, more specifically pre- and postnatal residential and ingestion levels of THMs and nitrate. Though THMs and nitrate are considered to be possibly and probably carcinogenic to humans, respectively, there is to date no evidence concerning their potential effects on the risk of BTs in young people.

Our results suggest a possible reduction in risk of neuroepithelial BTs in relation to THMs exposure both for pre- and postnatal residential and ingestion exposures, which appeared strongest in relation to exposures in the first 2 years of life. For nitrate, our results suggest an exposure-related increased risk of neuroepithelial BTs in association with residential nitrate levels in tap water both for pre- and post-natal exposures, which also appeared slightly stronger when considering only exposures early in life.

The statistical power of the study was hampered by the low levels of THMs and nitrate in most countries, and by uncertainties in exposure estimates. Further research is required to clarify these associations.

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Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Distribution of MOBI-Kids participants by country, availability of water questionnaire and of information on THMs and nitrate for at least for 70% of their life.

	Cases (N)	Controls (N)
Country	361	993
Canada	17	21
Greece	39	86
Italy	123	342
New Zealand	13	25
Spain	145	421
Korea	24	98
Participants with water questionnaire available		
Drinking tap water	321	918
Showering/ bathing	320	919
Mothers with water questionnaire available		
Drinking tap water	284	742
Showering/ bathing	297	766
Participants with THMs information[1]	86	352
Participants with nitrate information[1]	85	343

[1] For at least 70% of their life

Supplementary Table 2. Number of participants of frequency of drinking or showering habits by residential levels of THMs and nitrate.

	Drinking tap water (no. glasses)			**Showering/ bathing (times/week)**		
	0 (N(%))	**1-2 (N(%))**	**≥3 (N(%))**	**1-3 (N(%))**	**4-7 (N(%))**	**≥8 (N(%))**
THM levels (µg/L)						
≤25.1	91 (60.7)	13 (8.7)	46 (30.6)	52 (34.7)	84 (56.0)	14 (9.3)
25.1-66.5	25 (19.8)	18 (14.3)	83 (65.9)	17 (13.5)	94 (74.6)	15 (11.9)
≥66.5	58 (41.4)	29 (20.7)	53 (37.9)	17 (12.1)	104 (74.3)	19 (13.6)
Nitrate levels (mg/L)						
≤3.2	14 (11.5)	24 (19.7)	84 (68.9)	15 (12.3)	86 (70.5)	21 (17.2)
3.2-8.5	75 (54.4)	16 (11.6)	47 (34.1)	36 (26.1)	93 (67.4)	9 (6.5)
≥8.5	80 (54.4)	19 (12.9)	48 (32.7)	44 (30.0)	88 (60.0)	15 (10.0)

Supplementary Table 3. 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 post-natal periods, by country

Residential THMs levels:	Cases (N)	Controls (N)	OR (95% CI)
Prenatal			
Italy:			
≤25.1	21	52	-
25.1-66.5	0	0	-
≥66.5	0	0	-
Spain:			
≤25.1	7	29	1.00
25.1-66.5	16	89	0.51 (0.17-1.52)
≥66.5	27	111	0.88 (0.32-2.38)
Korea:			
≤25.1	11	23	-
25.1-66.5	0	11	-
≥66.5	0	0	-
Postnatal			
Italy:			
≤25.1	20	51	-
25.1-66.5	0	0	-
≥66.5	0	0	-
Spain:			
≤25.1	2	4	1.00
25.1-66.5	25	95	0.44 (0.07-2.72)
≥66.5	26	111	0.42 (0.07-2.56)
Korea:			
≤25.1	10	23	1.00
25.1-66.5	1	3	0.39 (0.01-9.93)
≥66.5	0	0	-
Ingest THMs levels:			
Prenatal			
Italy:			
Non-exposed	66	180	1.00
Below median (≤19.7 µg/day)	8	22	1.02 (0.42-2.48)
Above median (>19.7 µg/day)	0	0	-
Spain:			
Non-exposed	39	89	1.00
Below median (≤19.7 µg/day)	12	44	0.67 (0.31-1.45)
Above median (>19.7 µg/day)	11	83	0.31 (0.14-0.66)[2]
Korea:			
Non-exposed	11	31	1.00
Below median (≤19.7 µg/day)	4	3	4.14 (0.59-29.10)
Above median (>19.7 µg/day)	0	0	-
Postnatal			
Italy:			
Non-exposed	74	178	1.00
Below median (≤19.7 µg/day)	12	30	0.97 (0.44-2.15)
Above median (>19.7 µg/day)	0	0	-
Spain:			
Non-exposed	48	127	1.00
Below median (≤19.7 µg/day)	9	55	0.39 (0.18-0.88)
Above median (>19.7 µg/day)	22	97	0.62 (0.34-1.12)
Korea:			
Non-exposed	16	53	1.00
Below median (≤19.7 µg/day)	5	6	3.37 (0.64-17.60)
Above median (>19.7 µg/day)	0	0	-

[1]Conditional logistic regression models, matched by sex, age and country, and adjusted by parental education. [2]Only these results showed a p-trend <0.05.

Supplementary Table 4. Odds ratio (OR) and 95% confidence interval (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, by country.

Residential nitrate levels:	Cases (N)	Controls (N)	OR (95% CI)
Prenatal			
Italy:			
≤3.2	7	11	1.00
3.2-8.5	7	25	0.68 (0.16-2.90)
≥8.5	15	33	1.05 (0.30-3.66)
Spain:			
≤3.2	15	108	1.00
3.2-8.5	9	31	2.48 (0.90-6.85)
≥8.5	16	65	2.30 (1.01-5.27)
Korea:			
≤3.2	0	1	-
3.2-8.5	9	26	-
≥8.5	1	3	-
Postnatal			
Italy:			
≤3.2	5	7	1.00
3.2-8.5	7	23	0.59 (0.13-2.74)
≥8.5	17	33	1.01 (0.25-4.01)
Spain:			
≤3.2	15	96	1.00
3.2-8.5	12	31	2.47 (0.97-6.28)
≥8.5	15	59	1.89 (0.83-4.31)
Korea:			
≤3.2	0	0	-
3.2-8.5	9	30	1.00
≥8.5	3	2	7.37 (0.58-93.55)

[1]Conditional logistic regression models, matched by sex, age and country, and adjusted by parental education. Any country showed a p-trend <0.05.

Supplementary Table 5. Odds ratio (OR) and 95% confidence intervals (95% CI) for neuroepithelial brain tumours by tertiles of THMs nitrate residential tap water exposure during the first 2 years of life.

FIRST 2	Cases (N)	Controls (N)	OR (95% CI)[1]
Residential THMs levels			
All THM			
≤25.1	38	84	1.00
25.1-66.5	36	147	**0.07 (0.01-0.72)**
≥66.5	19	79	**0.07 (0.01-0.75)**
p trend			*0.29*
By country:			
Italy:			
≤25.1	24	56	-
25.1-66.5	0	0	-
≥66.5	0	0	-
Spain:			
≤25.1	2	1	1.00
25.1-66.5	36	142	0.08 (0.01-1.15)
≥66.5	19	79	0.08 (0.01-1.18)
Korea:			
≤25.1	12	27	-
25.1-66.5	0	5	-
≥66.5	0	0	-
Residential nitrates levels			
All nitrate			
≤3.2	23	115	1.00
3.2-8.5	34	93	1.71 (0.83-3.51)
≥8.5	37	96	**1.99 (1.02-3.86)**
p trend			*0.05*
By country:			
Italy:			
≤3.2	6	7	1.00
3.2-8.5	8	24	0.56 (0.27-2.50)
≥8.5	19	38	0.90 (0.23-3.50)
Spain:			
≤3.2	17	108	1.00
3.2-8.5	15	37	**2.80 (1.20-6.56)**
≥8.5	16	56	2.15 (0.96-4.80)
Korea:			
≤3.2	0	0	-
3.2-8.5	11	32	-
≥8.5	2	2	-

[1]Conditional logistic regression models, matched by sex, age and country, and adjusted by parental education.

Supplementary Table 6. Odds ratio (OR) and 95% confidence intervals (95% CI) for neuroepithelial brain tumours by tertiles of THMs nitrate residential tap water exposure during the first 5 years of life.

FIRST 5	Cases (N)	Controls (N)	OR (95% CI)[1]
Residential THMs levels			
All THM			
≤25.1	41	89	1.00
25.1-66.5	37	169	**0.11 (0.02-0.70)**
≥66.5	18	55	0.17 (0.03-1.10)
p trend			*0.94*
By country:			
Italy:			
≤25.1	27	63	-
25.1-66.5	0	0	-
≥66.5	0	0	-
Spain:			
≤25.1	2	2	1.00
25.1-66.5	37	163	0.16 (0.02-1.28)
≥66.5	18	55	0.24 (0.03-2.03)
Korea:			
≤25.1	12	12	-
25.1-66.5	6	0	-
≥66.5	0	0	-
Residential nitrates levels			
All nitrate			
≤3.2	23	117	1.00
3.2-8.5	35	96	1.76 (0.87-3.57)
≥8.5	40	98	**1.98 (1.03-3.01)**
p trend			***0.05***
By country:			
Italy:			
≤3.2	7	7	1.00
3.2-8.5	8	25	0.51 (0.12-2.02)
≥8.5	21	47	0.69 (0.19-2.51)
Spain:			
≤3.2	16	110	**1.00**
3.2-8.5	16	41	**2.83 (1.22-6.58)**
≥8.5	18	51	2.78 (0.76-4.57)
Korea:			
≤3.2	11	30	-
3.2-8.5	1	0	-
≥8.5	0	0	-

[1]Conditional logistic regression models, matched by sex, age and country, and adjusted by parental education.

Supplementary Table 7. Sensitivity analyses based on approaches to infer the missing (up to 30% data) on residential level - neuroepithelial tumours

	Cases (N)	Controls (N)	95% CI[1]
All THM			
Scenario 1 – Main analysis			
≤25.1	43	99	1.00
25.1-66.5	26	103	0.41 (0.80-2.09)
≥66.5	25	107	0.39 (0.08-2.01)
p trend			*0.52*
Scenario 2			
≤25.1	36	86	1.00
25.1-66.5	27	101	0.58 (0.14-2.47)
≥66.5	26	112	0.55 (0.12-2.43)
p trend			*0.59*
Scenario 3			
≤25.1	36	86	1.00
25.1-66.5	27	101	0.58 (0.14-2.47)
≥66.5	26	112	0.55 (0.12-2.43)
p trend			*0.59*
All nitrate			
Scenario 1-Main analysis			
≤3.2	29	131	1.00
3.2-8.5	33	86	1.60 (0.81-3.16)
≥8.5	32	92	1.53 (0.82-2.86)
p trend			*0.19*
Scenario 2			
≤3.2	20	103	1.00
3.2-8.5	29	86	1.45 (0.68-3.09)
≥8.5	35	94	1.80 (0.91-3.53)
p trend			*0.09*
Scenario 3			
≤3.2	20	103	1.00
3.2-8.5	29	84	1.50 (0.7-3.23)
≥8.5	35	96	1.75 (0.90-3.43)
p trend			*0.11*

Sensitivity analyses based on approaches to infer the missing (up to 30% data) on residential level was accomplished. The 3 approaches are: assigning 0 for the missing periods (scenario 1 – main analysis), assign the median level for the missing periods (scenario 2) and assigning the maximal residential level for those periods (scenario 3). [1]Conditional logistic regression models, matched by sex, age and country, and adjusted by parental education.

Supplementary Table 8. Odds ratio (OR) and 95% confidence intervals (95% CI) for all brain tumours and for all brain tumours in all countries associated with amount of tap water consumed and showering/bathing frequency, in pre- and postnatal exposure periods.

	All BTs[2]			All BTs in all countries		
	Cases (N)	Controls (N)	OR[1] (95% CI)	Cases (N)	Controls (N)	OR[1] (95% CI)
Prenatal						
Drinking tap water at home						
0 glasses per day	162	312	1.00	166	315	1.00
1-2 glasses per day	54	114	0.82 (0.55-1.22)	54	115	0.80 (0.54-1.20)
3 or more glasses per day	148	247	1.02 (0.76-1.38)	153	256	1.00 (0.74-1.35)
p trend			*0.26*			*0.29*
Showering/bathing						
1-3 per week	63	131	1.00	65	133	1.00
4-7 per week	261	468	1.09 (0.76-1.57)	268	475	1.09 (0.76-1.56)
8 or more per week	59	99	1.04 (0.64-1.69)	62	104	1.03 (0.64-1.65)
p trend			*0.20*			*0.21*
Postnatal						
Drinking tap water at home						
0 glasses per day	193	378	1.00	198	384	1.00
1-2 glasses per day	66	133	0.91 (0.62-1.27)	71	136	0.91 (0.64-1.29)
3 or more glasses per day	149	348	0.80 (0.61-1.05)	158	357	0.81 (0.62-1.06)
p trend			*0.27*			*0.34*
Showering/bathing						
1-3 per week	93	181	1.00	96	184	1.00
4-7 per week	256	550	0.89 (0.65-1.23)	267	560	0.90 (0.66-1.24)
8 or more per week	60	128	0.84 (0.54-1.32)	64	132	0.85 (0.55-1.31)
p trend			*0.88*			*0.96*

[1]Conditional logistic regression models, stratified on sex, age and country, and adjusted by parental education; [2]Only for countries included in the main analyses: Italy, Korea and Spain.

Supplementary Table 9. Odds ratio (OR) and 95% confidence intervals (95% CI) for all brain tumours and for all brain tumours in all countries by tertiles of THMs residential exposure and ingestion from tap water during the pre- and post-natal periods.

	All BTs[2]			All BTs in all countries		
	Cases	Controls	OR[1] (95% CI)	Cases	Controls	OR[1] (95% CI)
Average RESIDENTIAL THMs, (µg/day)						
Prenatal						
≤25.1	52	97	1.00	107	179	1.00
25.1-66.5	31	106	0.38 (0.18-0.83)	32	107	0.41 (0.20-0.87)
≥66.5	33	111	0.46 (0.21-0.99)	33	111	0.48 (0.23-1.01)
p trend			0.07			<0.01
Postnatal						
≤25.1	43	92	1.00	97	173	1.00
25.1-66.5	46	114	0.35 (0.10-1.25)	46	114	0.37 (0.10-1.28)
≥66.5	33	117	0.25 (0.07-0.91)	33	117	0.26 (0.07-0.93)
p trend			0.15			<0.01
Lifetime cumulative residential THMs						
≤238.6	158	307	1.00	79	142	1.00
238.6-674.0	35	71	0.92 (0.57-1.48)	65	146	0.48 (0.20-1.17)
≥674.0	18	83	0.36 (0.19-0.66)	32	116	0.36 (0.13-1.02)
p trend			0.02			0.03
INGESTED THMs, (µg/day)						
Prenatal						
Non-exposed	96	184	1.00	162	308	1.00
Below median (<=19.7 µg/day)	267	560	0.90 (0.66-1.24)	56	114	0.90 (0.66-1.24)
Above median (>19.7 µg/day)	64	132	0.85 (0.55-1.31)	18	83	0.85 (0.55-1.31)
p trend			0.97			0.03
Postnatal						
Non-exposed	184	363	1.00	184	363	1.00
Below median (<=19.7 µg/day)	40	93	0.80 (0.52-1.25)	63	137	0.81 (0.52-1.25)
Above median (>19.7 µg/day)	29	100	0.60 (0.36-1.00)	29	100	0.60 (0.36-1.00)
p trend			0.03			0.03

[1]Conditional logistic regression models, matched by sex, age and country, and adjusted by parental education; [2]Only for countries included in the main analyses: Italy, Korea and Spain.

Supplementary Table 10. 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.

	All BTs[2]			All BTs in all countries		
	Cases	Controls	OR[1] (95% CI)	Cases	Controls	OR[1] (95% CI)
Prenatal:						
Average residential nitrate levels						
≤3.2	34	125	1.00	89	207	1.00
3.2-8.5	38	82	1.75 (0.92-3.29)	39	83	1.74 (0.93-3.26)
≥8.5	41	107	1.42 (0.80-2.51)	41	107	1.40 (0.79-2.47)
p trend			0.23			0.81
Postnatal:						
Lifetime cumulative residential nitrate levels						
≤41.6	40	111	1.00	94	191	1.00
41.6-97.0	41	104	1.44 (0.78-2.63)	41	105	1.32 (0.72-2.39)
≥97.0	39	102	1.29 (0.70-2.38)	39	102	1.24 (0.67-2.27)
p trend			0.97			0.20
Average residential nitrate levels						
≤3.2	31	114	1.00	84	194	1.00
3.2-8.5	45	95	1.56 (0.94-2.90)	46	96	1.60 (0.86-2.95)
≥8.5	44	108	1.39 (0.78-2.47)	44	108	1.38 (0.78-2.45)
p trend			0.20			0.92

[1]Conditional logistic regression models, matched by sex, age and country, and adjusted by parental education; [2]Only for countries included in the main analyses: Italy, Korea and Spain.

5.4. Paper IV

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Levels of heavy metals in toenails and brain tumours risk in young people: Results from MOBI-Kids study.

In preparation.

Levels of heavy metals in toenails and brain tumours risk in young people: Results from MOBI-Kids study

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Abstract

Introduction: Brain tumours (BT) are one of the most frequent tumour types in young people. The main objective of this study was to explore the association between cumulative heavy metals exposure (determined from levels measured in toenails) and risk of neuroepithelial BT in young people.

Methods: Toenails from 115 cases of neuroepithelial BT and 291 controls were collected from the Spanish participants of the international MOBI-Kids case-control study. Cases and controls were matched by sex, age and region. Levels of heavy metals in toenails were determined by plasma spectrometry techniques with mass detector (ICP-MS). Conditional logistic regression was used to test for an association between metals and BT risk. Analyses were adjusted for parental education, nails weight and laboratory batch.

Results: In crude analyses of the distribution of levels by case-control status, the median levels for the majority of metals tended to be lower in cases than controls, apart from selenium where levels were significantly higher in cases than controls. The results of the conditional logistic regression provided no consistent evidence of effect of any metal. There were suggestions, however, of an exposure-related increase for selenium, mainly in women and particularly among the youngest subjects, and reduced ORs for chromium overall, in both sexes and in the 15-19 and 20-24 years age group, with little evidence of an exposure related effect. There was also a suggestion of reduced ORs with arsenic exposure, overall, in both sexes and in all age-groups, with no evidence of an exposure-response relationship.

Discussion: Although the few publications on brain tumour risk in adults suggest a possible increased risk in relation to exposure to heavy metals and despite known associations between several metals and the risk of other cancer types, our results, related to exposures in the general environment of young people, did not support these findings. Levels of exposure in our population were generally low and our findings need confirmation in further studies.

Conclusion: In general, however, our study suggests that exposure to heavy metals in the general population in recent decades does not seem to be related with an increased risk of brain tumours in young people.

Keywords: heavy metals, brain tumours, children, young, lead.

Introduction

Little is known about factors that may influence in brain tumours (BT) risk despite it is one of the most frequent tumour type in young people (IARC- Cancer Today, 2018; Ostrom et al., 2016; Siegel et al., 2019). A number of environmental exposures, including heavy metals exposure, have been postulated as potential risk factors for BT (Zumel-Marne et al., 2019).

Heavy metals are naturally present in the Earth and humans exposure mainly results from natural erosion of the land surface and anthropogenic activities (mining, industrial effluents, among other) that facilitate the introduction of metals into the trophic chain. A particular example is consumption of fish that bioaccumulate metals (Zheng et al., 2019). The general population, including children, can also be exposed to metals from other sources, such as dental amalgams (Borchers et al., 2010; Jarup, 2003) or consumption of meat contaminated with metal compounds from vaccines. In areas with high environmental levels of metals in the soil and in houses with old white paints, young children can also ingest metals because of hand-to-mouth activities (Carpenter, 2001; García-Rico et al., 2019; Wilhelm et al., 1994).

Some metals are essential for physiologic functions, but, for many others, acute or chronic exposure can be harmful to health. The nervous system is particularly vulnerable to metals, as the brain can accumulate and integrate them in processes related to neuronal health and energy homeostasis. Some metals like aluminium (Al), arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr), lead (Pb), manganese (Mn), mercury (Hg), Nickel (Ni), Selenium (Se) and Zinc (Zn) are known to play a role in carcinogenic and/or neurotoxic diseases in humans (Caito and Aschner, 2015; IARC- Monographs Vol. 1-121, 2018), nevertheless their effects related with BT are not established. To our knowledge, only one study to date has investigated the relation between metals and BTs, reporting higher levels of Cd in samples of blood, urine, scalp hair and nails in children with BT than in controls ; the study was small, however, based on 4 BT cases from a selected population in Egypt (Sherief et al., 2015).

The main objective of this article is to explore the association of exposure to heavy metals in a general population-based case-control study conducted in Spain as part the international study MOBI-Kids and risk of brain tumours.

Methods

Study design

MOBI-Kids is an international case-control study, conducted in 14 countries with a common-core protocol and questionnaire (Sadetzki et al., 2014). Cases were patients with a first primary benign or malignant BT and controls between the ages of 10 and 24 years. Two controls were selected for each case, matched on age and sex, among patients operated for appendicitis in general surgery departments in the catchment areas of the cases. Recruitment of subjects was conducted between 2010 and 2015, with the exact recruitment period depending on the country; in Spain, subjects were recruited between 2011 and 2013.

Ethics approvals were obtained from all appropriate human review boards and all subjects (or their parents/guardians) who wished to participate signed an informed consent form. Patients with known genetic syndromes were excluded from the study. Participation included in particular responding to a questionnaire administered by a trained interviewer, during a face-to-face interview, and permission to access their medical history from clinical registries.

In the Spanish part of MOBI-Kids, a sub-study was conducted to evaluate the role of metals in BT risk. For this, at the time of interview, subjects who agreed to participate in MOBI-Kids were asked if they would agree to donate toe-nail clippings for the evaluation of their metal content. The nail clippings were either collected by the interviewer or, if the toenails were too short, sent by the participants or their parents by postal mail.

Study population

The Spanish part of MOBI-Kids was conducted in four autonomous communities: Andalusia, Catalonia, Madrid and Valencia. A total of 208 cases and 421 controls were eligible and participated in the study (this corresponds to 23% of all cases in the international MOBI-Kids study). The vast majority of cases (70%) had neuroepithelial brain tumours (NBT), with small numbers in other morphological categories. Because the

aetiology of tumours may differ by morphology (particularly at young ages where embryonal tumours was the second largest group of tumours), the main analyses was restricted to NBT.

Metal analysis

Samples were blinded and randomly distributed by case-control status to the laboratory. Determination of levels of heavy metals in toenails was carried out by the Department of Analytical Chemistry of the University of Huelva, using the recommendations established by the International Atomic Energy Agency (IAEA, 1985). Toenail samples were first cleaned twice using a 5% (weight/ volume) of Triton water solution, then, twice with Milli-Q water, and finally twice with acetone. Five minutes ultrasounds treatment was additionally applied. Each sample was digested in a mixture of HNO_3 and H_2O_2 of Ultra Trace Metals grade quality in a Teflon reactor for microwave-assisted attack. With the resulting extract, 1 mL of 0.5 ppm of Rh in water was added as internal standard (IS), and diluted to 5 ml with Milli-Q water and finally filtered through a 0.45 µm Polytetrafluoroethylene (PTFE) membrane filter before analysis. The multi-elemental analysis was carried out in a Thermo Scientific XSERIES2 plasma spectrometry techniques with mass detector (ICP-MS) equipped with a MicroMist nebulizer, Ni cones and Cetac ASX500 autosampler. The operating conditions were optimized with 2% aqueous solutions of lithium, cobalt, indium, uranium (1 ppb).

Daily validation was accomplished in the ICP-MS equipped with a collision cell using helium as collision gas. Human hair was used as reference material (NSC DC73347a). Every 20 samples analysed, a correction of the variability of measurement of the ICP-MS was accomplished. The effect of instrumental drift was further corrected by using rhodium (Rh) at a concentration of 100 µg/ L in all samples. In addition, samples whose internal standard response differed by ± 10% using the equipment software (PlasmaLab), were measured again to avoid specific problems to solvent possible obstructions in the micro-nebulizer, or due to alterations of the system sample introduction or autosampler.

Statistical analysis

Samples with values under the limit of detection were assigned half of that value. To avoid artefacts from outlier values, we excluded from the analyses all subjects with values above the 95th percentile. A sensitivity analysis was conducted including them.

The distribution of participants who provided or not toenail samples was evaluated by the main covariates (age, gender, geographical area, and parental education) using the Pearson Chi-square test for categorical variables and t-test for continuous variables. We compared levels of metals between cases and controls using nonparametric tests for two independent samples (U-Mann Whitney test).

Conditional logistic regression was used to estimate Odds Ratio (OR) and 95% Confidence Interval (CI) of BT risk associated with level of each heavy metal categorised in tertiles of its distribution among controls. The main analysis was stratified by age (1 year from 10 to 17 and 2 years from 17 to 25), sex and autonomous community and adjusted by parental education level, toenail weight and laboratory batch.

As controls tended to have higher level of parental education, we did a sensitivity analysis stratifying additionally by level of parental education (up to high school, secondary, university), to evaluate potential residual confounding by parental education. We also analysed the data using non-conditional logistic regression.

Statistical analyses were performed with STATA 14.0.

Results

Of the 208 cases of BT included in the study, 145 (70%) had neuroepithelial tumours and were matched to 421 controls. Toenail samples were obtained from 115 cases (79%) and 291 (69%) controls (Table 1). Among controls, but not cases, the proportion of subjects who gave toenail samples decreased with increasing age category. who gave samples were younger, on average, than those who did not. Participation rates were higher in Andalusia and Catalonia than in the rest of autonomous communities and the difference between case and control a participation rate was greatest in the Madrid and Valencian communities. Controls with the lowest level of parental education were less likely to provide toenail samples than their counterpart cases (Table 1). On average, the weight of the nail samples

was slightly higher in cases than controls (Table 2) and though samples from cases and control were randomly sent to the laboratory, an association was found between sample batch and case control status (Table 2). Weight of the samples was also associated with age (Spearman's rho=0.22; $p<0.01$) among controls (not shown).

Table 3 shows the median and interquartile range of the distribution of metal level (in ppb) in cases and controls for each metal considered. The median level of selenium was statistically significantly higher among cases than controls, while the median levels of arsenic, chromium, copper, lead, molybdenum and platinum were significantly higher among controls than cases. Median and IQ range are shown by sex in Supplemental Table 1. Selenium levels were higher in cases than controls in both males and females; no difference in levels of other metals were seen in females, while in males, levels of molybdenum, platinum and lead were lower among cases than controls.

Table 1. Distribution of cases and controls by socio-demographic characteristics and availability of toenails samples

	Cases			Controls		
Characteristics	**Provided nail samples**			**Provided nail samples**		
	No	**Yes**	**P - value[1]**	**No**	**Yes**	**p- value**
	N (%)	**N (%)**		**N (%)**	**N (%)**	
Number of subjects	30 (11)	115 (79)		130	291 (69)	
Age *(mean (SD))*	16.7 (4.3)	17.2 (4.5)	0.560	18 (4.1)	16.5 (4.2)	**0.001**
Sex						
Male	13 (16.9)	64 (83.1)		70 (53.8)	164 (70.1)	
Female	17 (25)	51 (75)	0.230	60 (46.2)	127 (67.9)	0.630
Age categories						
10-14	12 (22.6)	41 (77.4)		35 (26.9)	122 (77.7)	
15-19	9 (19.6)	37 (80.4)		48 (36.9)	96 (66.7)	
20-25	9 (19.6)	37 (80.4)	0.910	47 (36.2)	73 (60.8)	0.008
Region						
Andalusia	5 (11.9)	37 (88.1)		32 (24.6)	93 (74.4)	
Catalonia	4 (9.1)	40 (90.9)		19 (14.6)	119 (86.2)	
Madrid	10 (28.6)	25 (71.4)		39 (30)	58 (59.8)	
Valencia	11 (45.8)	13 (54.2)	**0.001**	40 (30.8)	21 (34.4)	**<0.001**
Parental education						
≤High school	12 (20)	48 (80)		45 (34.6)	91 (66.9)	
Medium level tech/prof. school	6 (19.4)	25 (80.6)		15 (11.5)	62 (80.5)	
University	5 (11.1)	40 (88.9)		12 (9.2)	96 (88.9)	
Don't know	7 (77.8)	2 (22.2)	**<0.001**	58 (44.6)	42 (42)	**<0.001**

[1] Chi-square homogeneity test.

Table 2. Distribution of nail weights and measurement batch by case/control status

	Cases	Controls	p- value[1]
	N (%)	N (%)	
Total number of subjects	115	291	
Nail weight (mg) – (mean (SD))	0.07 (0.03)	0.06 (0.03)	<0.001
Batch			
A	15 (13.0)	49 (16.8)	
B	16 (13.9)	44 (15.1)	
C	34 (29.6)	26 (8.9)	
D	13 (11.3)	48 (16.5)	
E	2 (1.7)	30 (10.3)	
F	23 (20.0)	77 (26.5)	
G	3 (2.6)	0 (0)	
H	9 (7.8)	17 (5.8)	<0.001

[1]Chi squared test of homogeneity for categorical variables, and T-test for continuous variables.

Table 3. Median (in ppb) and IQR of toenail metal levels in NBT cases and controls

	Cases			Controls			
Metals[2]	**N**	**Median**	**IQR**	**N**	**Median**	**IQR**	**p-value[1]**
Be	111	2	1 3	275	2	1 3	0.86
Al	112	46,108	26,825 68,312	274	45,544	29,067 70,068	0.30
V	111	64	33 117	275	64	38 117	0.44
Cr	**109**	**385**	**241 717**	**277**	**537**	**306 1,000**	**0.03**
Mn	111	643	330 1,057	275	679	377 1,176	0.19
Fe	110	34,033	18,133 49,273	276	29,553	17,456 54,373	0.90
Co	110	28	15 45	276	29	17 47	0.69
Ni	105	986	592 1,577	281	978	546 2,073	0.59
Cu	**111**	**4,847**	**4,076 6,041**	**275**	**5,617**	**4,193 7,190**	**0.01**
Zn	113	62,134	48,588 74,787	273	59,138	44,030 81,936	0.57
As	**109**	**108**	**80 160**	**277**	**124**	**94 185**	**0.05**
Se	**105**	**637**	**507 792**	**281**	**566**	**475 690**	**<0.01**
Mo	**111**	**15**	**12 22**	**275**	**18**	**13 27**	**<0.01**
Cd	109	11	7 17	277	13	8 24	0.07
Pt	**111**	**2**	**1 9**	**275**	**5**	**1 15**	**<0.01**
Tl	112	1	1 2	274	1	1 2	0.23
Pb	**110**	**708**	**358 1,287**	**276**	**919**	**502 1,579**	**<0.01**
U	104	9	6 14	282	9	6 13	0.19

[1] Mann-Whitney test; [2]Metals: Be=beryllium, Al=aluminium, V=vanadium, Cr=chromium, Mn=manganese, Fe=iron, Co=cobalt, Ni=nickel, Cu=cupper, Zn=zinc, As=arsenic, Se=selenium, Mo=molybdenum, Cd=cadmium, Pt=platinum, Tl=thallium, Pb=plumb, U=uranium

Table 4 shows the ORs and 95% CI for NBT by tertiles of metal levels for each of the metal analysed in the study, stratified on age, sex, autonomous community and adjusted for parental education, nail weight and date of heavy metals analysis. Significantly reduced ORs were seen in the middle and high tertiles of chromium with no indication of an exposure-response relationship. There were suggestions of an increasing trend in risk of NBT for selenium, and of a decreasing trend for arsenic, molybdenum and thallium.

In analyses in males and females separately (Supplementary Table 2) reduced ORs were seen for chromium in both sexes, though they were statistically significantly reduced only in males, with a suggestion of a trend of decreasing risk with increasing chromium levels. There was still a suggestion of an increasing trend with selenium levels, mainly in women, and of non-significantly reduced ORs for arsenic in both sexes, with no evidence on an exposure-response relationship.

Analyses by age group are shown in Supplementary Table 3. The increasing trend for selenium appears to be restricted to the youngest subjects, in the 10-14 years old category. A statistically significantly elevated OR was seen in the second tertile for iron among 15-19 year olds, and non-significantly increased ORs in the highest tertile among 15-19 and 20-24 year olds. For chromium and arsenic, reduced ORs were seen at all ages, statistically significant in the highest tertile for chromium except in the youngest age group, and only statistically significant in the highest tertile for in the oldest age group for Arsenic. As metal concentration levels tend to increase with attained age for most metals (except selenium and U) (not shown), comparison by age groups are difficult to make, with small numbers of subjects in each category, in particular for the higher tertiles in the lowest age groups.

Table 4. Odds ratio (OR) and 95% confidence interval (95% CI) for NBT in relation to tertiles of toenail metals levels – analysis stratified on sex, age and region and adjusted for parental education, nail weight and date of heavy metals analysis

Metal[1]	OR	95% CI	Metal	OR	95% CI
Be			**Zn**		
1st	1.00		1st	1.00	
2nd	0.80	0.40 1.61	2nd	0.73	0.29 1.82
3rd	1.17	0.55 2.51	3rd	0.76	0.24 2.34
Al			**As**		
1st	1.00		1st	1.00	
2nd	0.92	0.44 1.91	2nd	0.61	0.29 1.28
3rd	1.05	0.50 2.20	3rd	0.54	0.25 1.16
V			**Se**		
1st	1.00		1st	1.00	
2nd	0.77	0.38 1.57	2nd	1.25	0.52 2.98
3rd	0.71	0.33 1.55	3rd	1.47	0.52 4.18
Cr			**Mo**		
1st	1.00		1st	1.00	
2nd	**0.40**	**0.20 0.82**	2nd	0.70	0.34 1.44
3rd	**0.34**	**0.15 0.77**	3rd	0.55	0.21 1.48
Mn			**Cd**		
1st	1.00		1st	1.00	
2nd	1.00	0.48 2.07	2nd	1.16	0.55 2.42
3rd	0.86	0.39 1.88	3rd	1.16	0.47 2.87
Fe			**Pt**		
1st	1.00		1st	1.00	
2nd	2.02	0.93 4.38	2nd	0.92	0.30 2.77
3rd	1.45	0.64 3.25	3rd	0.40	0.08 2.14
Co			**Tl**		
1st	1.00		1st	1.00	
2nd	1.45	0.67 3.10	2nd	0.83	0.40 1.74
3rd	1.18	0.54 2.61	3rd	0.55	0.21 1.44
Ni			**Pb**		
1st	1.00		1st	1.00	
2nd	1.24	0.60 2.55	2nd	0.83	0.40 1.73
3rd	1.06	0.44 2.54	3rd	0.78	0.37 1.68
Cu			**U**		
1st	1.00		1st	1.00	
2nd	1.13	0.53 2.42	2nd	1.09	0.49 2.43
3rd	0.91	0.38 2.18	3rd	0.98	0.43 2.24

[1] Heavy metals: Be=beryllium, Al=aluminium, V=vanadium, Cr=chromium, Mn=manganese, Fe=iron, Co=cobalt, Ni=nickel, Cu=cupper, Zn=zinc, As=arsenic, Se=selenium, Mo=molybdenum, Cd=cadmium, Pt=platinum, Tl=thallium, Pb=plumb, U=uranium.

Sensitivity analyses

Analyses including all subjects, i.e. including those who had values above the 95th percentile of any metal, and using non-conditional logistic regression showed very similar results (not shown)

Stratifying analyses on parental education level as well as age, sex and autonomous community (Supplementary Table 4) reduced the number of informative cases and controls. For selenium, there was no apparent trend with exposure. Statistically significantly reduced ORs were still apparent for chromium in the middle and highest tertiles, with little suggestion of an exposure-response relationship. A suggestion of an increased risk of NBT with increasing level of exposure was seen for beryllium and uranium and of decreasing risk with increasing level of exposure for lead.

Discussion

The aim of this study was to explore the possible association between cumulative exposure to metal (assessed by levels of metals in toenails) and risk of NBT in young people. This is the largest study of BTs in young people with individual assessment of cumulative exposure to metals to date.

We found no consistent evidence of effect with any metal, though a suggestion of an exposure-related increase was seen for selenium, mainly in women and particularly among the youngest subjects and reduced ORs for chromium overall, in both sexes and in the 15-19 and 20-24 years age group, with little evidence of an exposure related effect. There was also a suggestion of reduced ORs with arsenic exposure, overall, in both sexes and in all age-groups but with no evidence an exposure-response relationship.

The selenium results are somewhat surprising given the general belief that selenium may prevent the risk of cancer. Recent studies and a systematic review (Vinceti et al., 2018), however, conclude that increased selenium intake does not reduced cancer risk and that more research is needed to evaluate how various chemical forms of selenium compounds might affect cancer risk in different populations.

Previous studies have suggested that exposure to certain metals, like lead, methylmercury, arsenic, manganese or cadmium, can have toxic actions on the brain and consequently,

neurodegenerative diseases or neurobehavioral (Carpenter, 2001; Rodríguez-Barranco et al., 2013).

Chromium has been shown to be as a potential risk factor for other cancer types (Núñez et al., 2016), but its penetrance intro cerebrospinal fluids is unclear (Harrison-Brown et al., 2019). Arsenic is a recognized neuro-toxicant (Rodríguez-Barranco et al., 2013) and human carcinogen based on data on bladder, lung and skin cancer. To our knowledge no specific analytical study on risk of brain tumours in young people has been conducted. Results of an ecological in Spain suggests increased brain cancer mortality in adults in relation to arsenic topsoil contents (López-Abente et al., 2018; Núñez et al., 2016) and a record linkage study in Sweden suggests an association between occupational arsenic exposure and risk of adult glioma (Navas-Acién et al., 2002). At least two studies in areas polluted with arsenic in drinking water found no excess of risk of brain cancer among children in Chile (Liaw J., et al 2008) or Nevada (Moore LE., 2002). The lack of an exposure response in our study suggests that the observed decreased risk in our study may be due to chance, though it merits further study.

The study by Sherief et al. (2015) reported higher levels of Cd in children with BT than in controls ($p<0.001$) (Sherief et al., 2015). However, the results were based in only 4 BT cases, who might have been exposed to much higher levels of CD than the subjects in our study, a population-based study conducted in the four most populated autonomous communities in Spain. In fact the mean toenail levels of Cd reported by Sherief were equivalent to 350 ppb among cases and 20 ppb among controls, much higher in cases than the 11 ppb observed in our study.

The mainly inverse associations found in our study are compatible with the results from a recent study that reviewed the available literature on trace element changes related to brain tumours (Cilliers et al., 2019) and concluded that altered trace element levels differ amongst different tumour types, as well as malignancy grades, which makes it almost impossible to compare results across most studies.

It is also possible that reporting bias might have limited the number of published papers showing no or reduced associations between metals and brain tumour risk.

Despite the important strengths of our studies – including the relatively large sample size for a study of rare tumours like brain tumours in young people and the availability of toenail samples for the determination of metal exposures, the study also suffers from some important limitations.

Metals metals can keep in toenails about 6 months to 1 year of exposures before sample collection, considering that toenails growth is less than 0.05-1.2 mm per week (Abdulrahman et al., 2012), and does not capture exposure during pregnancy or early life. This would introduce non-differential exposure misclassification, which would tend to bias risk estimates towards null associations, even if a risk exists.

Another important issue is that the levels of exposure to metals are low in our population, as we recruited subjects from the general population instead of selecting subjects from areas with important environmental pollution. However, as most the population lives in areas without relevant exposure to metals, we believe that our results are interesting for public health in general. Also, following Knudson's two hit theory, tumours diagnosed among younger people are more likely to carry a higher genetic component, and it could be that cumulated exposure to carcinogenic metals at low levels are less relevant than among populations of adults.

Although mercury is an important neurotoxic metal (Caffo et al., 2014; McCaulley, 2019), we did not measure mercury in our study for two reasons: because ICP-MS measures total mercury, and the evidence of neurotoxicity is mainly for methylmercury, and because of logistics: measuring mercury using ICP-MS requires in depth cleaning of the ICP system with a gold solution after each sample to avoid a "memory effect" and this was not feasible in our study.

Another issue that needs to be taken into account in interpreting results is the possible effect of selection bias. We observed that parental education levels in our controls was higher than in the cases. Lower participation rates among controls than among cases within population with lower educational level is common in case control studies (Castaño-Vinyals et al., 2015), as cases are more motivated to participate in medical studies. Exposure to metals measured in nails has been reported to be different by social status in adult cancer patients in Spain (Camargo et al., 2019), as has exposure to other environmental factors in the general population in Spain (Gasull et al., 2013).

A sensitivity analysis, stratifying on parental education level (in order to remove possible residual confounding compared to an analysis simply adjusted on this factor) reduced the informative number of cases and controls but provided little additional insight in the associations reported here.

Another important issue is that the toenails samples with lower weight tended to have higher levels than those with higher weight. This could be a concern as most samples were below the minimum weight needed to guarantee a linear calibration of the assay. Among controls, younger subjects tended to have higher levels of metals, and younger subjects tended to provide lower amount of toenail sample. Controls were on average half a year younger than cases, and their nails were 0.01 mg lighter than those from cases. However, we believe that the possible impact of a measurement bias is likely to be low in our study, as the analyses were matched by age, and adjusted by weight of the nail sample and batch number, and the analyses by age group – though underpowered - showed no substantial changes in the main results of this study.

As mentioned above, the present study has a number of important strengths. We have a high number of cases with a homogeneous diagnosis: neuroepithelial brain tumours (while most studies to date considered all brain tumours as a group), and despite the rarity of such tumours in young people we have been able to perform separate analyses by sex and age group. Also, cases and controls were recruited very closely in time, which is important to avoid biases due to differences in environmental exposures over time.

Conclusion

Overall, we found no association between levels of most metal in toenails and the risk of NBT in our study apart, from a suggestion of a reduced risk related to chromium and possibly arsenic and of an increased risk in relation to selenium level. These results need further confirmation. In general, however, our study shows that exposure to neurotoxic metals in the general population in recent decades does not seem to be related with an increased risk of brain tumours in young people.

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Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Median levels of heavy metals in toenails by sex

	MALES								FEMALES							
	Cases				Controls				Cases				Controls			
	N	Median (ppb)	IQR		N	Median (ppb)	IQR	p-value[1]	N	Median (ppb)	IQR		N	Median (ppb)	IQR	p-value[1]
Be	63	2	1	3	157	1	1	3	0.93	48	2	1	3	118	2	1	3	0.79
Al	64	45,546	26,180	69,078	152	42,382	26,940	64,398	0.46	48	46,802	28,800	66,037	122	48,678	32,129	77,864	0.49
V	63	65	37	109	153	60	37	112	0.51	48	58	32	140	122	70	40	126	0.72
Cr	60	356	207	678	159	535	276	843	0.08	49	405	331	752	118	537	350	1,080	0.14
Mn	62	644	344	966	152	615	334	1,118	0.31	49	608	321	1,250	123	735	452	1,269	0.44
Fe	61	32,981	19,618	48,277	154	25,495	16,222	52,126	0.78	49	37,091	15,633	58,443	122	33,470	18,813	55,011	0.96
Co	63	26	14	41	152	27	16	47	0.41	47	32	16	46	124	31	18	50	0.66
Ni	60	1,013	571	1,934	158	919	498	1,909	0.48	45	887	625	1,477	123	1,120	619	2,654	0.98
Cu	63	4,628	3,911	5,861	156	5,209	3,978	6,824	0.08	48	5,394	4,531	6,467	119	6,050	4,590	7,728	0.12
Zn	63	59,251	45,765	69,788	154	58,223	42,253	81,440	0.34	50	63,227	52,633	92,851	119	59,485	47,860	83,979	0.86
As	61	110	88	160	154	125	97	189	0.06	48	104	79	165	123	122	89	174	0.44
Se	60	639	507	791	158	566	472	691	**0.008**	45	629	507	795	123	588	477	690	**0.01**
Mo	63	14	12	19	152	17	12	23	**0.005**	48	17	13	29	123	20	15	29	0.52
Cd	62	10	7	16	157	13	8	22	0.07	47	12	8	20	120	13	9	24	0.49
Pt	62	1	1	6	157	6	1	14	**<0.001**	49	3	1	13	118	4	1	17	0.31
Tl	63	1	1	2	156	1	1	2	0.27	49	1	1	2	118	1	1	2	0.59
Pb	62	645	339	1,148	154	873	461	1,469	**0.012**	48	807	440	1,573	122	1,063	602	1,695	0.26
U	59	9	6	14	159	9	6	13	0.34	45	9	6	14	123	10	6	14	0.34

[1] Mann-Whitney test

Supplementary Table 2. Odds ratio (OR) and 95% confidence interval (95% CI) for NBT in relation to terciles of toenail metals levels by sex – analysis stratified on age and region and adjusted for parental education, nail weight and date of heavy metals analysis

	Male			Female				Male			Female		
	OR	**95% CI**		**OR**	**95% CI**			**OR**	**95% CI**		**OR**	**95% CI**	
Be							**Zn**						
1st	1.00			1.00			1st	1.00			1.00		
2nd	0.98	0.42	2.28	0.83	0.28	2.44	2nd	0.60	0.19	1.96	0.94	0.24	3.72
3rd	0.96	0.37	2.50	1.21	0.40	3.71	3rd	0.41	0.09	1.78	1.71	0.30	9.69
Al							**As**						
1st	1.00			1.00			1st	1.00			1.00		
2nd	0.93	0.40	2.15	1.47	0.41	5.37	2nd	0.46	0.18	1.19	0.51	0.15	1.73
3rd	1.20	0.48	3.02	1.19	0.36	3.97	3rd	0.41	0.15	1.09	0.51	0.15	1.71
V							**Se**						
1st	1.00			1.00			1st	1.00			1.00		
2nd	0.79	0.34	1.85	0.68	0.20	2.28	2nd	1.02	0.35	2.98	1.47	0.34	6.42
3rd	0.93	0.35	2.47	0.51	0.16	1.66	3rd	1.13	0.32	3.96	1.61	0.25	10.14
Cr							**Mo**						
1st	1.00			1.00			1st	1.00			1.00		
2nd	**0.41**	**0.17**	**1.00**	0.47	0.15	1.50	2nd	0.81	0.33	1.98	0.59	0.19	1.80
3rd	**0.17**	**0.06**	**0.54**	0.42	0.12	1.51	3rd	0.33	0.09	1.14	1.60	0.30	8.68
Mn							**Cd**						
1st	1.00			1.00			1st	1.00			1.00		
2nd	1.51	0.63	3.58	0.57	0.17	1.98	2nd	1.25	0.49	3.16	0.86	0.28	2.66
3rd	0.96	0.36	2.54	0.97	0.28	3.32	3rd	1.22	0.36	4.10	1.06	0.29	3.86
Fe							**Pt**						
1st	1.00			1.00			1st	1.00			1.00		
2nd	3.19	1.27	8.02	0.98	0.28	3.40	2nd	0.21	0.04	1.03	5.16	0.87	30.64
3rd	1.64	0.60	4.45	1.57	0.43	5.64	3rd	0.09	0.01	1.04	1.88	0.16	22.91
Co							**Tl**						
1st	1.00			1.00			1st	1.00			1.00		
2nd	1.30	0.53	3.22	1.57	0.44	5.57	2nd	0.95	0.38	2.41	0.69	0.23	2.11
3rd	0.98	0.38	2.50	2.06	0.55	7.69	3rd	0.47	0.14	1.55	0.68	0.15	3.06
Ni							**Pb**						
1st	1.00			1.00			1st	1.00			1.00		
2nd	1.52	0.63	3.64	1.15	0.35	3.82	2nd	1.37	0.59	3.20	0.28	0.07	1.10
3rd	1.36	0.44	4.17	0.64	0.16	2.53	3rd	0.52	0.19	1.37	0.80	0.23	2.76
Cu							**U**						
1st	1.00			1.00			1st	1.00			1.00		
2nd	0.72	0.27	1.94	1.56	0.49	5.01	2nd	0.85	0.33	2.16	1.05	0.26	4.25
3rd	0.70	0.24	2.06	0.87	0.23	3.36	3rd	0.96	0.35	2.63	1.69	0.43	6.69

Supplementary Table 3. Odds ratio (OR) and 95% confidence interval (95% CI) for NBT in relation to terciles of toenail metals[1] levels by age group – analysis stratified on sex and region and adjusted for parental education, nail weight and date of heavy metals analysis

	10-14 years old			15-19 years old			20-24 years old				10-14 years old			15-19 years old			20-24 years old		
	OR	**95% CI**		**OR**	**95% CI**		**OR**	**95% CI**			**OR**	**95% CI**		**OR**	**95% CI**		**OR**	**95% CI**	
Be										**Zn**									
1st	1.00			1.00			1.00			1st	1.00			1.00			1.00		
2nd	1.94	0.48	7.84	0.68	0.21	2.19	0.75	0.24	2.36	2nd	1.28	0.25	6.51	0.43	0.11	1.77	0.86	0.12	5.95
3rd	1.75	0.43	7.07	1.05	0.32	3.43	1.10	0.23	5.17	3rd	0.72	0.10	5.45	0.49	0.08	2.91	1.95	0.16	23.97
Al										**As**									
1st	1.00			1.00			1.00			1st	1.00			1.00			1.00		
2nd	1.31	0.37	4.66	0.66	0.18	2.35	1.88	0.51	6.84	2nd	0.38	0.08	1.81	0.53	0.16	1.76	0.42	0.09	1.87
3rd	0.64	0.15	2.74	1.52	0.39	5.90	2.21	0.61	8.00	3rd	0.49	0.12	1.96	0.83	0.27	2.58	**0.08**	**0.01**	**0.67**
V										**Se**									
1st	1.00			1.00			1.00			1st	1.00			1.00			1.00		
2nd	1.15	0.30	4.33	0.71	0.23	2.24	0.54	0.15	1.91	2nd	1.74	0.31	9.90	1.20	0.35	4.14	0.33	0.05	2.09
3rd	0.56	0.12	2.55	0.96	0.26	3.56	1.32	0.33	5.27	3rd	2.22	0.27	17.99	0.69	0.14	3.51	1.06	0.16	6.98
Cr										**Mo**									
1st	1.00			1.00			1.00			1st	1.00			1.00			1.00		
2nd	0.54	0.12	2.36	0.37	0.12	1.14	0.42	0.10	1.73	2nd	2.24	0.44	11.24	0.68	0.23	1.97	0.35	0.10	1.27
3rd	0.55	0.11	2.74	**0.29**	**0.08**	**0.99**	**0.04**	**0.00**	**0.44**	3rd	1.43	0.26	7.83	0.65	0.12	3.36	0.51	0.06	4.41
Mn										**Cd**									
1st	1.00			1.00			1.00			1st	1.00			1.00			1.00		
2nd	0.91	0.23	3.67	2.22	0.68	7.25	0.34	0.09	1.38	2nd	0.76	0.19	3.09	1.38	0.38	4.95	1.14	0.30	4.31
3rd	0.65	0.16	2.54	2.13	0.54	8.39	1.24	0.29	5.29	3rd	1.92	0.37	9.95	0.51	0.11	2.32	2.90	0.46	18.24

	10-14 years old			15-19 years old			20-24 years old				10-14 years old			15-19 years old			20-24 years old		
	OR	**95% CI**		**OR**	**95% CI**		**OR**	**95% CI**			**OR**	**95% CI**		**OR**	**95% CI**		**OR**	**95% CI**	
Fe										Pt									
1st	1.00			1.00			1.00			1st	-	-	-	1.00			1.00		
2nd	1.75	0.39	7.90	**3.62**	**1.05**	**12.44**	1.12	0.30	4.18	2nd	-	-	-	1.41	0.20	10.17	0.56	0.06	5.56
3rd	1.05	0.24	4.67	2.89	0.80	10.49	3.04	0.64	14.52	3rd	-	-	-	0.65	0.04	9.62	1.23	0.05	28.38
Co										Tl									
1st	1.00			1.00			1.00			1st	1.00			1.00			1.00		
2nd	2.00	0.40	9.91	1.29	0.41	4.03	0.84	0.22	3.25	2nd	0.90	0.17	4.73	0.89	0.27	2.98	0.75	0.23	2.45
3rd	1.79	0.32	10.01	0.96	0.28	3.24	2.31	0.57	9.38	3rd	0.32	0.03	3.32	1.06	0.26	4.28	0.23	0.03	1.59
Ni										Pb									
1st	1.00			1.00			1.00			1st	1.00			1.00			1.00		
2nd	2.81	0.67	11.83	1.57	0.51	4.85	0.73	0.21	2.61	2nd	1.47	0.40	5.42	0.23	0.06	0.87	2.03	0.55	7.52
3rd	2.01	0.42	9.64	0.88	0.22	3.46	1.47	0.23	9.33	3rd	0.67	0.16	2.89	0.48	0.15	1.58	1.32	0.33	5.27
Cu										U									
1st	1.00			1.00			1.00			1st	1.00			1.00			1.00		
2nd	0.60	0.15	2.42	1.21	0.36	4.11	1.16	0.26	5.15	2nd	0.82	0.20	3.28	2.95	0.64	13.70	0.24	0.05	1.25
3rd	0.27	0.06	1.33	1.02	0.24	4.31	1.41	0.27	7.45	3rd	0.87	0.20	3.72	5.71	1.09	29.81	0.55	0.11	2.69

[1]Heavy metals: Be=beryllium, Al=aluminium, V=vanadium, Cr=chromium, Mn=manganese, Fe=iron, Co=cobalt, Ni=nickel, Cu=cupper, Zn=zinc, As=arsenic, Se=selenium, Mo=molybdenum, Cd=cadmium, Pt=platinum, Tl=thallium, Pb=plumb, U=uranium.

Supplementary Table 4. ORs and 95% CI stratified by age, sex, region, and parental education

Metal[1]	OR	95% CI	Metal	OR	95% CI
Be			**Zn**		
1st	1.00		1st	1.00	
2nd	1.42	0.55 3.65	2nd	1.58	0.40 6.32
3rd	1.68	0.60 4.72	3rd	0.74	0.14 3.91
Al			**As**		
1st	1.00		1st	1.00	
2nd	0.68	0.26 1.77	2nd	0.38	0.14 1.04
3rd	0.84	0.30 2.32	3rd	0.65	0.27 1.55
V			**Se**		
1st	1.00		1st	1.00	
2nd	0.56	0.21 1.52	2nd	1.21	0.43 3.39
3rd	0.48	0.19 1.24	3rd	0.84	0.24 3.02
Cr			**Mo**		
1st	1.00		1st	1.00	
2nd	**0.33**	**0.13 0.88**	2nd	0.71	0.30 1.70
3rd	**0.23**	**0.08 0.73**	3rd	0.47	0.13 1.66
Mn			**Cd**		
1st	1.00		1st	1.00	
2nd	1.32	0.45 3.81	2nd	1.14	0.44 2.96
3rd	0.78	0.28 2.15	3rd	0.86	0.29 2.57
Fe			**Pt**		
1st	1.00		1st	1.00	
2nd	1.02	0.41 2.56	2nd	0.85	0.20 3.70
3rd	0.84	0.32 2.23	3rd	0.32	0.03 3.67
Co			**Tl**		
1st	1.00		1st	1.00	
2nd	1.35	0.50 3.67	2nd	1.40	0.57 3.44
3rd	1.44	0.56 3.70	3rd	0.74	0.24 2.26
Ni			**Pb**		
1st	1.00		1st	1.00	
2nd	1.49	0.61 3.60	2nd	0.63	0.26 1.54
3rd	0.63	0.22 1.82	3rd	0.37	0.13 1.04
Cu			**U**		
1st	1.00		1st	1.00	
2nd	0.91	0.38 2.16	2nd	1.31	0.47 3.66
3rd	0.79	0.25 2.50	3rd	1.59	0.56 4.50

[1]Heavy metals: Be=beryllium, Al=aluminium, V=vanadium, Cr=chromium, Mn=manganese, Fe=iron, Co=cobalt, Ni=nickel, Cu=cupper, Zn=zinc, As=arsenic, Se=selenium, Mo=molybdenum, Cd=cadmium, Pt=platinum, Tl=thallium, Pb=plumb, U=uranium.

Discussion

6. DISCUSSION

The aim of this chapter is to give an integrated and general interpretation of the four articles included in this thesis.

To achieve the main objective of this thesis, that was to explore risk factors and clinical characteristics of BTs in young people, two phases of work were done: firstly 1) a study of the symptoms and other clinical characteristics of 899 cases recruited in the MOBI-Kids study (Paper II) and 2) a SR of the literature on risk of BTs in young people in relation to environmental factors (Paper I); and in a second phase the exploration of 1) the association between tap water contaminants and BT risk with the cases and controls from six of the participating countries in MOBI-Kids study (Paper III) and 2) the association between heavy metals levels in toenails samples and BT risk with the cases and controls from the Spanish node of MOBI-Kids (Paper IV).

6.1. Main findings and contribution to current knowledge

6.1.1. Environmental determinants of BT in young people

After reading the majority of the reviews published so far, we can say that, to date, we did one of the most complete systematic reviews focused on potential environmental risk factors (excluding ionising radiation, already a well-established cause of BT) for BT among young people (Paper I). Since the SR, a number of papers

were published on specific exposures and do confirm the conclusions of the SR (Van Maele-Fabry et al., 2017), however in some aspect such as smoking, results were not consistent (Ostrom et al., 2019).

The main findings in the review are shown in Table 2 of Paper I and summarised below:

Environmental tobacco smoke: This is one of the best-established carcinogens for humans (IARC- Monographs Volume 100C, 2012). It is well established that there are hundreds of chemical and toxic compounds in environmental tobacco smoke (Paumgartten et al., 2017). Nevertheless, their role in the aetiology of BT risk is not clear. One of the results of the systematic review is that the majority of the 22 studies that were reviewed suggest a link between environmental tobacco smoke and BT, particularly for mother's exposure to passive smoking during pregnancy and child postnatal exposures. However, the results are not conclusive though the majority of the significant results were close the 1 in the confidence interval and based on small sample sizes.

Few publications found positive associations with the exposure to some air pollutants from traffic density. However, there are several studies that explored air pollution and its possible implications on neurological problems (such as attention deficit and hyperactivity disorder) in children (Chiu et al., 2016; Forns et al., 2018; Sunyer and Dadvand, 2019), finding positive results. These findings suggest that some chemicals in air pollution could be neurotoxic. It

is still for us to understand if these neurological effects could also be related to BT. Further studies should explore in depth the implications for BT risk in young people.

Living on a farm could be the origin of exposure to different agents, from pesticides to other chemicals and to biological agents. We found some studies that suggest that living on a farm during pregnancy may increase BT risk in young people, as may living on a farm during early childhood. In articles focusing on pesticide exposures, we found suggestions of associations between childhood BT risk and exposures during both pre- or postnatal periods, especially for parental handling of pesticides and for professional extermination at home or in the garden. However, these associations are difficult to interpret. Children spend a lot of time at home, and living on a farm may also entail contact with other chemicals, for example disinfectant products for cleaning the animal's living areas and viruses from cattle.

One concern these days is the ever-growing exposure of young people to communication devices which emit non-ionizing radiation. Few articles to date have been published about their possible association with BT risk. Electric and magnetic fields (EMF), both ELF (extremely low frequency) and RF (radiofrequency) have been classified by the IARC monograph as possibly carcinogenic to humans (IARC- Monographics Vol. 80, 2002; IARC-Monographics Vol 102, 2002). Few studies have studied the association of EMF and BT risk in children and their

power has been severely limited by the small proportion of long term and heavy users, hence it is not possible to draw conclusions about the presence or absence of a risk. Further, large-scale studies with much improved exposure assessment are needed to better evaluate the possible association of EMF and BTs in young people. This is in fact the main objective of the MOBI-Kids study and manuscripts are in preparation.

In recent decades, potential detrimental occupational exposures have been more strictly regulated than before. However, we found significant association with some parental occupations and CBT risk in exposures before child's birth, like factory workers, machine repair workers, painters, among others. Nevertheless, some non-statistically reduced risks were found for some particular job exposures including unspecified chemicals, hydrocarbons, electromagnetic fields, or metals workers among others, medicine and science in general, activities related to aerospace, agriculture and farming. For exposures during childhood, other publications found increased risk for parental exposures like father exposed to electrical repairing, ionizing radiation and agriculture, among other. Nevertheless, the results are difficult to interpret because the majority of the studies explored different occupations, which imply exposure to a high variety of biological, chemical and physical agents.

Tap water contains a very wide variety of water chemicals that have been related to other cancer type; nevertheless their possible role

in BT risk is unclear. In the SR of this thesis we found no study that explored the possible association of water disinfection by-products (DBPs, in particular THMs, in drinking water with young people populations and BTs risk, though DBPs are related to risk of other cancer types. In this thesis, we therefore explored the association of THMs within the MOBI-Kids study (Paper III). Contrary to our expectation, we found that exposure to THMs both during the pre- and postnatal periods reduced the risk of BT in young people (the association was statistically significant only for prenatal medium residential exposure levels and especially for prenatal ingested THMs above the median). This finding may be due to a number of methodological limitations including: assumed that the residence during pregnancy was the same as the residence at birth; we had a high percentage of controls that refused to participate in our study, especially those with low parental education level and consequently controls had higher parental education; among other methodological limitations on the exposure assessment since we had to make some assumptions for the years with missing levels.

Regarding other tap water chemicals, few articles related to water consumption found an increased risk of BTs in participants who consumed tap water with presence of nitrite or nitrate during pregnancy and early infancy. The results were not conclusive for nitrate since only two articles reviewed this exposure and found non-statistically significant association for BT risk in a small sample size study (Mueller et al., 2004; Weng et al., 2011). For nitrite, in Mueller's 2004 they found significant increased BT risk

in a bigger sample size, however the exposure assessment is not conclusive since it was based on punctual water samples. We therefore explored the association of nitrate within the MOBI-Kids study (Paper III). We found a suggestion of an exposure-related increased risk of neuroepithelial BTs in asociation with residential nitrate levels.

However, we could not take into account another important source of exposure to nitrate, which is diet, since nitrate compounds are quite commonly used as meat preservatives. Indeed, in the SR (Paper I), the majority of articles about diet suggested an association with BT risk and high meat consumption, being consistent with the classification of IARC (Monographs volume 114 of 2018) where the consumption of red meat is classified as 2A "probably carcinogenic to humans" (IARC-Monograph Vol. 114, 2002, p. 114). Moreover, we found articles that suggested reduced BT risk related to regular ingestion of other type of food like fruits, vegetables, grains and fish, or iron supplements. This could mean that ingestion of some other foods or supplements could reduce the detrimental effects of nitrate and be an effective approach to primary prevention of BT.

Regarding other environmental exposures possibly related to BT, we found only one article about metals exposure in the SR, with significantly higher levels of Cd in cases samples than in controls samples (Paper I). In this thesis, we explored the association between heavy metals in toenails (like As, Al, Be, Mn, Pb, Pt, Tl,

U, V, among other), known to be relevant in carcinogenic or neurotoxic processes (IARC- Monographs Vol. 1-121, 2018) (Caito and Aschner, 2015), and BT risk in young people within the MOBI-Kids study (Paper IV). The analyses were restricted to Spain (the unique country in which toenail samples were collected). We did not find substantial suggestions of any increased risk for any of the metals studied. Instead, we found some unexpected inverse associations with Cr. The literature could not support this association since Cr has been known as a potential risk factor for other cancer types (Núñez et al., 2017), but its penetrance intro cerebrospinal fluids is unclear (Harrison-Brown et al., 2019). An explanation for the lack of association in our study is that the levels of exposure to metals are low, as we recruited subjects from the general population instead of selected subjects from areas with an important environmental pollution. There were suggestions of an exposure-related increase for selenium, however this is not supported by literature (Vinceti et al., 2018), and we consider more research is needed to evaluate how selenium compounds affect in different populations. We have to consider that median levels of metals contained in toenails were a slightly higher in controls than in cases, as opposed to the results of Sherief's article (Sherief et al., 2015). Sherief et al results were based in only 4 BT cases, who might be exposed to much higher levels of metals than our population, that was recruited from the general population. We have to consider that parental education in our controls was higher than in cases since participants with lower parental education refused to participate more often. Nevertheless, we did a sensitivity

analysis matching by parental education, which provided no differences in the main results of our study.

6.1.2. Clinical characteristics of BT in young people

Given the paucity of information on symptoms and clinical characteristics of BTs in young people, we analysed the data from the clinical questionnaire in the MOBI-Kids study (Paper II). This paper is an important addition to the literature. It provides a comprehensive analysis of symptoms, time to diagnosis as well as distribution of tumour morphologies and topographies among young people, based on data from the largest study so far of BT cases in young people with clinical data, where we found that the vast majority of cases presented were neuroepithelial tumours, especially gliomas. The type of tumours vary across age groups and sex overall, however embryonal tumours (the second largest group of tumours) being much more frequent among males and at early ages and much rarer in adolescence and young adulthood, contrary to meningiomas and other non-neuroepithelial which were more frequent in females very rarely identified in this age range. The most frequent anatomical locations of the tumours were in the cerebellum, frontal and temporal lobes, though this varied across age groups. Brain stem and cerebellar tumours were more frequent at early age categories, however frontal lobe and cerebral meninges were more frequent at older ages. We also observed particular differences with morphology: gliomas were mostly located in the

frontal lobe and cerebellum, while other neuroepithelial tumours were mainly recorded in the temporal, frontal and cerebral ventricles. With respect to non-neuroepithelial, embryonal tumours were most frequently located in the cerebellum, while the majority of other non-neuroepithelial tumours were located in cranial nerves.

When we explored the timing between first symptom reported until diagnosis, a very important issue to ensure the best prognosis of this often very damaging tumour type in young people, we found that BT overall were diagnosed rapidly in this age range, between 1 to 2 months after reporting of first symptoms. However, longer latencies, of the order of 1 year after first symptoms were reported in 12% of the participants, with 6% diagnosed 1 year after and 6% two years. The average and maximum durations between symptoms and diagnosis varied by morphology and as well as by symptom. The most frequent earliest symptom was headaches in overall cases, especially for gliomas and embryonal tumours. Altered sensitivity was the symptom with the longest median time as earliest symptom in overall (median=2.67 months), being for embryonal tumours convulsions and seizures (5.33 months) and for meningioma cognitive, memory and behavioural changes (13.9 months). For the latest symptom, headache was the most frequent in overall cases, reported as well in gliomas, embryonal and non-neuroepithelial tumours. Visual problems were the symptom with the shortest median time as latest symptom in overall (0.47 months) and the longest median time was dizziness (1.10 months). The shortest median time for those cases whose latest symptom was visual sign

and symptoms or altered consciousness was 0.50 months. The longest median time for the latest symptom to diagnosis was for dizziness (median= 1.10 months). Overall, the most frequent symptoms reported were headaches, focal neurological signs and symptoms, nausea/vomiting and visual signs and symptoms. To be noted, 27 cases were asymptomatic. Differences were seen based on the tumour location, type and grade. Headaches were most frequently reported in cases with gliomas and for other neuroepithelial tumours, and for the later, also with convulsions and seizures. Headaches were also most frequently reported in non-neuroepithelial tumours except for meningiomas that were visual signs and symptoms. Differences by topography were particularly observed with headaches being more frequent in brain stem, cerebellum, parietal lobe, cerebral ventricles, overlapping lesion of the brain and for brain NOS. Nausea and vomiting was reported by the majority of cases tumours located in the cerebellum. Cases with tumours located in brain stem reported most frequently focal neurological signs and symptoms. Convulsions and seizures were reported for cases with tumours located in brain stem, cerebellum, cranial nerves and brain NOS; and dizziness for cases with brainstem or cerebellar tumour.

We also found a common sequence pattern of symptoms repeatedly: headaches, followed by nausea and vomiting, then followed by visual signs and symptoms Headaches and nausea/ vomiting was the unique similarity found occurring together for the majority of topographies and morphologies.

Table 6.1. Summary table of the main findings of this thesis.

Article	Participating countries	Participants involved	Exposure/ outcome	Overall results
1. Systematic review	EU, USA,…	70 articles analysed	Heavy metals	↑Cd concentrations in cases
			Tap water chemicals	↑and ↓risk for presence of nitrate or nitrite
			Diet	↑risk for meat consumption and ↓ for other (fruit, vegetables…)
			Tobacco smoke	↑risk for maternal passive smoking
			Air pollution	↑risk for some specific pollutants
			Lived on a farm	↑risk
			Pesticides	↑risk
			Non-ionizing radiation	NA
			Parental occupation	↑risk for some specific jobs
2. Clinical article	Australia, Austria, Canada, France, Germany, Greece, India, Israel, Italy, New Zealand, Spain, Netherlands, Japan and Korea	899 cases		More frequency of neuroepithelial tumours (especially gliomas)
				Higher frequency of locations: cerebellum, frontal and temporal lobes
				Median of 1 to 2 months for diagnosis after the first symptom
				12% of participants with longer lag time for diagnosis (more than 1 year after the first symptom)
				Higher frequency of headaches, nausea/vomiting, visual signs and symptoms and focal neurological sign and symptoms, with particular differences by grades, morphologies and topographies. Common pattern in symptoms
3. Water article	Canada, Israel, Italy, New Zealand, Spain		Residential THM	↓risk for medium THMs levels (25.1-66.5µg/L) in prenatal
			Ingestion THM	↓risk for levels above median (>19.7 µg/day) prenatal exp. levels
			Residential nitrate	Suggestion ↑risk for residential nitrate levels
4. Heavy metals article	Spain		Heavy metals in toenails	No statistical significant associations for metals, except ↓risk for exposure to Cr and suggestion ↑risk for Se.

6.2. Methodological considerations: strengths and limitations

After reviewing the literature on environmental risk factors and conducting specific analyses within the MOBI-Kids study, we can conclude that there is some evidence for a relationship between BT in children and young people and exposure to some specific environmental exposures. In this section we explain the strengths and limitation in our research.

The main strength of this thesis is that it constitutes a complete and coherent research piece on the epidemiology of BT in young people. We started with a systematic review (Paper I) of all studies published so far about BT risk in young people, with 70 articles analysed. This was complemented by an analysis of the clinical characteristics of BTs in young people based on the largest compilation to date of clinical data on primary BTs in young people (Paper II). This allowed us to identify the main types of tumours in young people – tumours with very different aetiologies. This informed the analyses for the MOBI-Kids risk papers – both included in this thesis as well as others conducted or in preparation by other MOBI-Kids collaborators. It also provided insights into combinations of symptoms for different types and locations tumours which may influence the rapid diagnosis of tumours in young people. In Paper III we explored for the first time in the literature, the association with THMs and BT risk in young people, also analysing risk of BT in relation to estimated residential levels of

nitrate in tap water based on the entire residential history of the subjects – thus improving substantially over previous paper where exposure assessment was very limited. Finally, we explored for the first time in the literature, the association between the levels of metals in human samples and risk of BT in young people (Paper IV). Another important strength of this thesis is that Papers II, III and IV are based on a large collection of subjects from different areas and countries, with data collected using a common protocol and questionnaires (Sadetzki et al., 2014).

Thus this thesis provides information on BTs based on a very large sample size and much refined exposure assessment, thus overcoming some of the limitations observed in the previous studies reviewed in Paper I.

As mentioned previously, the primary objective of MOBI-Kids was to assess the role of EMF – from mobile communicating devices and other sources in the work and general environment – and risk of BTs in young people. Because of this, we excluded a large proportion of tumours which were located in the mid-brain area where it was expected that exposure to RF from phone use would be very small. Thus, our clinical epidemiology paper lacks a substantial proportion of tumours in this age range and hence some of the conclusions should be interpreted with caution.

Further, the information on clinical characteristics in Paper I comes from medical records and answers concerning symptoms could have been influenced by the clinician seeing the patients and also may

have been differently reported by subjects (or parents) depending on their level of worry or stress. We have no gold standard to evaluate the adequacy of the symptom information in the medical records, unfortunately.

The secondary objectives of MOBI-Kids were to assess the possible effects of other possible risk factors on the risk of brain tumours in young people. For the water sub-study, which was conducted in only 6 of the MOBI-Kids country, however the list of questions on water consumption and use was unfortunately limited. While we could estimate life-time and in-uterus exposure levels both for THMs and nitrate, ingestion levels could only be estimated for THMs. Indeed, to study the effect of nitrate ingestion levels we missed information on consumption of water from sources other than tap water, in particular bottled water which contains varying levels of nitrates. Further, for both THMs and nitrate, we could not estimate either dermal or inhalation exposure because, though we had information on frequency of showering/ bathing, the questionnaire did not include questions about the average duration of showers and baths. The same applies to swimming, where we had only limited information on frequency and none about duration. For swimming, we would also have had to collect information on THMs and nitrate levels in each type of swimming pool in the different areas, as the source of water and treatment can greatly influence the presence of these chemicals. We also had no information on nitrate from the diet as no dietary questionnaire was included in the study.

Another issue that could influence the results and may explain the significantly negative trends found in relation to THM exposure may be selection bias. We tried to minimise this by recruiting hospitalized controls who we thought would be more likely to participate in the study as they would have more time immediately after their operation, but in practice, many of the potential controls were approached weeks or even months after their operation and hence had restarted their school, work and social activities and hence participation rates among controls were much lower than in cases (Turner et al., 2019). If the controls who participated were not representative of the population from which the cases came from, they may not be representative of the population in terms of water consumption and diet, possibly explaining some of the negative trends observed.

Another possible bias which could affect results in this thesis is recall error (both random and systematic) in Paper III, where participants had to report the number of tap water glasses they drank and their frequency of showering/bathing and using swimming pools. Interviewers usually did not explain to the participants why they asked these questions if the participants did not ask directly and there may have been misclassification in number of glasses of water drank (if subjects inadvertently counted all the water they drank including from bottles) and differential recall among cases (who are looking for a reason for their disease) and controls.

Recall bias is also possible in the majority of the articles analysed in the systematic review (Paper I), because exposure assessment was mostly based on personal interviews without measurements, and no analysis of possible exposure- response relationship was provided.

Another important limitation in Paper I was that the majority of the articles included were based on small samples sizes, with poor exposure characterisation, and hence the statistical power to detect an effect if it exists was low. Additionally, the comparison of studies of parental occupational exposures was made difficult by the inclusion of different occupations in the different papers.

We consider that some detection bias may could occurred, since in Paper III, levels of water chemicals were provided from different labs and in Paper IV metals were detected in different batches. We observed that there were significant differences in levels of metals by batch, so we included this variable in the analysis as adjusting variable.

We consider that possible information bias could occur in Paper III since we estimated historical exposures of THMs and nitrate for missing data. Another limitation is that for Paper IV we have to consider that we are exploring recent exposures, since metals can keep in toenails about 6 months to 1 year of exposures, considering that toenails growth is less than 0.05-1.2 mm per week (Abdulrahman et al., 2012).

Confounding is also a possibility in Papers III and IV as we had only limited information on other potentially confounding variables. We ran sensitivity analyses including covariates available in the study, including “living on a farm” (yes/no) and “smoking during pregnancy”, and found no major differences in results. While water chemicals could well be related to pesticide use and living on a farm hence residual confounding is possible, it is unlikely that parental smoking would be related to any of the exposures under study and thus would confound the results.

We observed that controls had higher levels for the majority of metals analysed. Parental education in our controls was higher than in cases (Turner et al., 2019). Lower participation rates among controls than among cases within populations with lower educational level is common in case- control studies (Castaño-Vinyals et al., 2015), as cases are more motivated to participate in medical studies. The exposure to metals measured in nails has been reported to be different by social status in cancer patients in Spain. As our control population had a higher level of parental education, we did a sensitivity analysis matching by parental education, which provided no differences in the main results of our study.

Additionally, we observed that toenails samples with lower weight tended to have higher levels than those with higher weight.

This could be a concern as most samples were below the minimum weight necessary to guarantee a lineal calibration of the assay. Among controls, younger subjects tended to have higher levels of metals, and younger subjects tended to provide lower amount of toenail sample. Controls were on average one year younger than cases, and their nails were 0.01mg lighter than those from cases. However, we believe that the possible impact of a possible measurement bias would be low in our study, as the stratified analyses by age showed no substantial changes in the main results of this study, and the analyses were matched by age, and adjusted by weight of the nail sample and batch number.

Another explanation for the lack of association in our study is that the levels of exposure to metals were low, as we recruited subjects from the general population instead of selected subjects from areas with an important environmental pollution. However, as most people live in areas without relevant exposure to metals, we believe that our results are of interest for public health. Also, analyses from paper III and IV are matched by geographic area, which tends to dilute differences in environmental exposures, resulting in lower power and null results.

Finally, an important limitation affecting the systematic review (Paper I) is publication bias, occurring either because of difficulties of publishing negative results or because of pressure on researchers to publish in high impact journals and hence to delay or avoid writing up negative results.

Hence the conclusion, though limited by small sample sizes and inadequate exposures assessment, may be affected by publication bias. Another limitation of studies to date is that they tended to study BTs as a group, though in young people BTs include a number of mophologies, possibly with different aetiologies, as we observed in Paper II.

6.3. Implications for public health

Apart from ionizing radiation exposure and genetics factors, there is a lack of understanding of the aetiology of BTs in young people, with no clearly identified risk factor for BT prevention, particularly since most studies have tended to study BTs as a group, though the frequency of BT types varies substantially with age and, for example, embryonal tumours are likely to have a very different aetiology than tumours diagnosed later in childhood, adolescence and young adulthood. Without a better knowledge of risk factors, primary prevention of these life and quality of life threatening tumours in young people is very difficult.

More articles than expected reviewed environmental exposures and BT risk in young people (70 articles), and they found a suspected increased risk for most of them. However, several limitations that were already mentioned have to be taken into account for future research.

However, these suspects have to be considered for public health since some exposures can be prevented specially for children, like mother's passive smokers. We encourage governments to make campaigns with more information on prevention of smoking close to pregnant women and children, especially to raise awareness in the population that partners of pregnant wives should avoid or quit smoking close to them during this period, always providing support to quit smoking and avoiding iatrogenic distress to people who want to quit from smoking and cannot. We also would like to emphasize the potential importance of using more ecological substances for society and the environment in general, for example highlighting the potential danger of use or exposure to pesticides during pregnancy or close to children.

Concerning the results of the literature review related to consumption of meat, further research is needed to determine whether nitrate consumption (in diet and in water is really related to BT risks in young people.

Though results in Paper III are not conclusive, we noticed considerable variability across areas and countries in levels of THMs and nitrate in drinking water, with some communities having levels very close to current maximum permitted levels. Because of the evidence of carcinogenicity of both of these exposures – even if the association with BTs is unclear – a review of permitted levels is important. Currently legal levels of chemical permitted for

consumption are established for the control of other diseases or cancer types, for example levels of nitrate in drinking water are regulated to prevent the methemoglobinemia in children (Ho et al., 2011, especially because there is no solid evidence for BTs risk. Therefore, we encourage promoting more prevention and research on the potential effect of these exposure on BT risk.

We recommend more studies assessing the exposure to metals and BTs risk, since general population have presence of metals in their body samples, as we observed in Paper IV, the real implication on BT aetiology is not clear.

This thesis also contributes to the literature on semiology of BTs confirming the findings of previous publications about symptoms associated with BTs, but with the advantage that in this article the sample size was larger (Paper II). We also contribute confirming the fact that there is a short period of time to BT diagnosis, so the current process of diagnosis of BTs in children, among the 14 countries explored, is generally quite fast. Nevertheless, 12% of the cases in our study with symptoms information were not diagnosed before at least 1 year after 1st symptoms. While this depends on tumour types and symptoms, we have suggested some combinations of symptoms of interest and recommended that more effort is put on studying the clinical epidemiology of BTs in young people in order to refine criteria for rapid diagnosis of some rarer tumour types with longer times between symptoms and diagnosis.

6.4. Future research

There are still gaps in the knowledge of risk factors and mechanisms of BT development. Hence, we present in this section some areas in which more research is required.

Literature and incidence rates indicate that the more common types of BT are more frequent in males than females and that the incidence of some types of tumours differs between people of different ethnic origins. Further research investigating these differences would help elucidate the mechanisms and risk factors underlying these differences. We encourage doing more research exploring why this male incidence is higher, probably due to some possible genetic or external hormonal intake exposures.

Much of the research published to date suffers from important limitations that should take into account for future research:

- Sample sizes for most of the studies reviewed in Paper I was often very limited, thus making any conclusion difficult to reach.

- Exposure assessment is an important issue for environmental risk factors. Most studies reviewed classified exposure in a very crude way (with different classifications across studies, complicating the review of evidence) thus limiting conclusions and reducing statistical power to detect an effect if there is one. Considering exposures over the

course of the lifetime – and the ability to estimate exposure in different time windows to assess particular sensitive time windows (such as during pregnancy and in early life) is essential to correctly identify a risk if there is one, and this requires complete residential history, sufficiently detailed questionnaires (for example for water consumption and diet) and complete historical data on exposure levels in these areas. The latter is not a simple task: it took two years in MOBI-Kids to obtain all the information and major efforts in contacting and convincing all relevant entities to obtain the data and still, some were missing.

- Tumour types in young people are very variable – with the most prevalent types in MOBI-Kids being neuroepithelial and embryonal tumours. These types most likely have different aetiologies and it is important in future studies to separate the analyses by tumour type in order to obtain more relevant information and reduce misclassification.

- This also applies to analyses of symptoms to allow the identification of groups of symptoms more strongly related to specific tumour types that could, if properly identified, reduce the time to diagnosis of specific rare types of tumours avoiding fatalities and long term consequences.

- We recommended that future research carefully collects and takes into account information on potential confounders and

effect modifiers. Very few studies in the systematic review (Paper I) had that information.

Conclusions

7. CONCLUSIONS

The main conclusions of this doctoral thesis are:

- Many articles published so far found positive associations related to the exposure to some environmental risk factors and BT risk in young people, though conclusions could not be reached due to small sample sizes, limited exposure assessment and other methodological issues.
- This thesis corroborates (and provides much more details) the findings of the small number of previous studies concerning the clinical characteristics of BTs in young people, especially those related to frequency on type of tumours, locations and symptoms.
- This thesis does not provide evidence of an increased risk of BTs from exposure to THMs.
- The suggestion of a possible increased BT risk for exposure to nitrates through drinking water found in other previous studies is supported by the results found in this thesis. Nevertheless, results have to be interpreted with caution because some bias could affect to the results.
- This thesis provided, for the first time, information on BT risk in young people in relation to a number of metals. Despite suggestions of a decreased risk in relation to exposure to Cr and As and an increased risk in relation to Se, our findings are weak, with little evidence of an exposure-response relationship.

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Annexes

9. ANNEXES

9.1. List of publications

- **Ángela Zumel-Marne**, Gemma Castano-Vinyals, Michael Kundi, Juan Alguacil, Elisabeth Cardis. Environmental Factors and the Risk of Brain Tumours in Young People: A Systematic Review. NED [Internet]. 2019 Jun 5 [cited 2019 Jun 6];1–21. Available from: https://www.karger.com/Article/FullText/500601
- **Ángela Zumel-Marne**, Michael Kundi, Gemma Castaño-Vinyals, Siegal Sadetzki, Andrea 't Mannetje, Hans-Peter Hutter, TobiasWeinmann, Franco Momoli, Brigitte Lacour, Eleni Petridou, Franco Merletti, Rajesh Dikshit, Roel Vermeulen, Mina Ha, Naohito Yamaguchi, Maria Morales Suárez-Varela, Charmaine Mohipp, Dan Krewski, John Spinelli, Paul Ritvo, Thomas Remen, Evdoxia Bouka, Papathoma Paraskevi, Rajini Nagrani, Milena Maule, Graziella Filippini, Noriko Kojimahara, Amanda Eng, Angela Thurston, Hyung-ryul Lim, Juan Alguacil, Elisabeth Cardis. *Clinical characteristics of brain tumours in young people: Results of the international Mobi-Kids study.* Submitted in Journal of the National Cancer Institute (JNCI).
- **Ángela Zumel-Marne**, Cristina Villanueva, Gemma Castano-Vinyals, Franco Merletti, Andrea 't Mannetje, Amanda Eng, Eleni Petrou, Evi Bouka, Mina Ha, Charmaine Mohipp, Franco Momoli, Juan Alguacil , Elisabeth Cardis. *Tap water chemicals and risk of brain tumour in young people.* In progress.
- **Ángela Zumel-Marne**, Gemma Castano-Vinyals, Maria Morales, Nuria Aragones, Juan Alguacil, Elisabeth Cardis. *Levels of heavy metals in toenails and risk of brain tumour in young people*. In progress.

9.2. Presentations in scientific congresses

- **Ángela Zumel-Marne**, Michael Kundi, Gemma Castaño-Vinyals, Siegal Sadetzki, Andrea 't Mannetje, Hans-Peter Hutter, TobiasWeinmann, Franco Momoli, Brigitte Lacour, Eleni Petridou, Franco Merletti, Rajesh Dikshit, Roel Vermeulen, Mina Ha, Naohito Yamaguchi, Maria Morales Suárez-Varela, Charmaine Mohipp, Dan Krewski, John Spinelli, Paul Ritvo, Thomas Remen, Evdoxia Bouka, Papathoma Paraskevi, Rajini Nagrani, Milena Maule, Graziella Filippini, Noriko Kojimahara, Amanda Eng, Angela Thurston, Hyung-ryul Lim, Juan Alguacil, Elisabeth Cardis. *Clinical characteristics of brain tumours in young people: Results of the international Mobi-Kids study*. Oral presentation. Brain tumor Epidemiology Consortium (BTEC) Annual Meeting, Copenhaguen, Denmark. June 19th- 21st, 2018.
- **Ángela Zumel-Marne**, Gemma Castaño-Vinyals, Juan Alguacil, Elisabeth Cardis. *"Brain tumors and environmental risk factors in young people- A literature review"*. Poster presentation. 28th Conference of the International Society of Environmental Epidemiology (ISEE). September 1st–4th, 2016, Rome, Italy. Publication: Research Triangle Park, NC: Environmental Health Perspectives; http://dx.doi.org/10.1289/ehp.isee2016.
- **Ángela Zumel**, Juan Alguacil, Laura Costas, Esther Gracia, Miguel Santibáñez, Nuria Aragonés, Beatriz Perez-Gomez, Tania Fernández-Villa, Javier Llorca, Víctor Moreno, Mikel Azpiri, Marcela Guevara, Silvia de Sanjose, José J. Jiménez-Moleón, Guillermo Fernández-Tardón, Rocío Capelo, Rosana Peiró, Rafael Marcos-Gragera, Jose María Huerta, Gemma Castaño, Marina Pollán, Ana M. García, Manolis Kogevinas. "*Occupational exposure to metals and risk of breast, chronic lymphocytic leukaemia, colorectal, prostate, and stomach cancer in the MCC-spain case control study*". Poster presentation. 25th Congress on Epidemiology in Occupational Health (EPICOH). September 5th-

8th 2016, Barcelona, Spain. Publication: Occup Environ Med 2016;73:Suppl1 A139-A140 doi:10.1136/oemed-2016- 03951.

- Antonio Ruiz, Rocio Capelo, Antonio Pereira, Miguel Angel Garcia, Marian Diaz-Santos, **Angela Zumel**, Rocio Jara, Alejandro Pascagoza, Jose Luis Fernandez de Liger, Tamara Garcia, Juan Alguacil. *"Occupational exposure to metals and respiratory symptoms among workers exposed to metals"*. Poster presentation. 25th Congress on Epidemiology in Occupational Health (EPICOH). Publication: Occup Environ Med 2016;73:Suppl 1 A216 doi:10.1136/oemed-2016-103951.598.
- Juan Alguacil, **Angela Zumel**, Rocío Capelo, Macarena González, Marian Diaz-Santos, Miguel Ángel García, Rocío Jara, Manuel Contreras, Amanda Gago, Tamara García. "*Impact of seafood consumption before sample donation on urinary and toenail metal levels in workers exposed to heavy metals"*. 25th Congress on Epidemiology in Occupational Health (EPICOH). September 5th-8th, 2016, Barcelona, Spain. Occup Environ Med 2016;73:Suppl 1 A47 doi:10.1136/oemed-2016-103951.127.
- Javier Vila, Michelle C Turner, Ana Espinosa, Esther Gracia, Gemma Castaño-Vinyals, Joseph D Bowman, Juan Alguacil, Vicente Martín, Pilar Amiano, Eva Ardanaz, Javier Llorca, Victor Moreno, **Angela Zumel**, Adonina Tardón, Rosana Peiró, Rafael Marcos-Gragera, Miguel Santibáñez, Elisabeth Cardis, Marina Pollán, Manolis Kogevinas. *Occupational exposure to extremely low frequency magnetic fields and risk of breast cancer in the mcc-spain study.* 25th Congress on Epidemiology in Occupational Health (EPICOH). September 5th-8th, 2016, Barcelona, Spain. Occup Environ Med 2016;73:Suppl 1 A19-A20 doi:10.1136/oemed-2016-103951.52.

9.3. Outreach activities

- Poster presentation titled *"Brain tumors in young people and environmental risk factors".* PhD symposium ISGlobal. November 6th, 2018, Barcelona, Spain.

- Poster presentation titled *"Brain tumors in young people and environmental risk factors"*. Jornada d'Investigadors Predoctorals Interdisciplinaria (JIPI). February 28th, 2017, Barcelona, Spain.
- Poster and flash talk presentation titled *"Brain tumors in young people and environmental risk factors"*.3rd ISGlobal PhD Symposium. November 28th, 2016. Barcelona, Spain.
- Poster presentation titled*"Brain tumors in young people and environmental risk factors"*. 2nd ISGlobal-CREAL PhD Symposium. November 11th, 2015. Barcelona, Spain.

9.4. Other activities

- Seminar. Oral presentation at the Water department of Medical University of Vienna "Water chemicals from tap water and brain tumour risk in young people". March 21st, 2019. Vienna, Austria.
- Oral presentation to students of Environmental Sciences bachelor at the PRBB. March 2nd, 2018. Barcelona, Spain.
- Organization of the weekly scientific seminars of ISGlobal since January to September 2017. The tasks were since contact with the speakers until presenting the day of the seminars and moderate the questions part. As weel to supervise all the administrative an technical aspects.
- Grant for the Encuentro para la Excelencia de la Investigación en Salud Pública (CIBERESP). September 20-22th, 2017. Menorca, Spain.
- Competition Rin4 (Research in 4 minutes). Oral presentation titled "Environmental factors and brain tumour risk in young people". Universitat Pompeu Fabra. April 19th, 2016. Barcelona.
- BioJunior 2016. I did a speech for visitors for the BioJunior at PRBB. April 15th, 2016. Barcelona, Spain.
- Open day PRBB. I did a poster presentation explaining the Radiation Programme of CREAL (now ISGlobal). October 3rd, 2015. Barcelona, Spain.

- 10th aniversay of CREAL. I did an speech about Radiation Programme to the visitors for the 10th aniversary of CREAL (now ISGlobal). November 8th, 2015. Barcelona, Spain.

9.5. International stay

Two stays at Department of Environmental Health at the Medical University Vienna, supervised by Prof. Michael Kundi, as part of the International Mention for this thesis: first one in October-November 2017; and the second one in February- March 2019.

9.6. Water questionnaire

mobi-kids
Study on Communication Technology, Environment and Brain Tumours in Young People

QUESTIONNAIRE ABOUT DRINKING WATER: SOURCE, TREATMENT AND QUALITY

The study is funded by the European Union (grant agreement FP7-ENV-2008-226873) and by local and national financial resources.

CREAL centre de recerca en epidemiologia ambiental

GENERAL INFORMATION

1. Organization or company that answers the questionnaire:……..

……

2. Address and phone number:……

……

3. Name and position of the person who answers this questionnaire:……

……

4. Date when questionnaire is answered: _____/_____/_____

5. Municipalities or towns supplied (all of them): ……

……

……

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……

6. In case of city councils, please write the name of the companies that supply the municipality: ……

……

……

……

……

SOURCE OF WATER

7. Indicate the source of drinking water currently supplied. If it is a mixture of sources, please indicate the approximate % of each source.

Since: _____/_____ (month / year)	**%**	
Superficial (river or reservoir)	⌊_____⌋	Name of the river or reservoir: .....
Groundwater (aquifers): wells, spring, etc.	⌊_____⌋	
Seawater (desalinated)	⌊_____⌋	
Other (specify):	⌊_____⌋	

8. If previously the source of the water was different from today, indicate the sources by periods:

	Since (year)_____ Until (year)_____	**Since (year)_____ Until (year)_____**	**Since (year)_____ Until (year)_____**	**Since (year)_____ Until (year)_____**
% superficial	⌊_____⌋	⌊_____⌋	⌊_____⌋	⌊_____⌋
% groundwater	⌊_____⌋	⌊_____⌋	⌊_____⌋	⌊_____⌋
% seawater	⌊_____⌋	⌊_____⌋	⌊_____⌋	⌊_____⌋
% other: (specify)	⌊_____⌋	⌊_____⌋	⌊_____⌋	⌊_____⌋
Name of the river or reservoir	.....	.....	.....	.....

TREATMENT

9. Do you chlorinate or disinfect the water? Yes ☐ No ☐

10. When did you start distributing chlorinated or disinfected water?* _____
* If you don´t know the year, please indicating an approximate range of years.

11. What agent did you use for disinfection? Select the appropriate ones in the following table:

	Yes	**No**	**If yes, indicate starting year:**
Chlorine gas (Cl_2)	☐	☐	⌊_⌋⌊_⌋⌊_⌋⌊_⌋
Hypochlorite (ClO^-)	☐	☐	⌊_⌋⌊_⌋⌊_⌋⌊_⌋
Chlorine dioxide (ClO_2)	☐	☐	⌊_⌋⌊_⌋⌊_⌋⌊_⌋
Chloramine (NH_2Cl)	☐	☐	⌊_⌋⌊_⌋⌊_⌋⌊_⌋
Ozone (O_3)	☐	☐	⌊_⌋⌊_⌋⌊_⌋⌊_⌋
Hydrogen peroxide (H_2O_2)	☐	☐	⌊_⌋⌊_⌋⌊_⌋⌊_⌋
Potassium permanganate ($KMnO_4$)	☐	☐	⌊_⌋⌊_⌋⌊_⌋⌊_⌋
Other (indicate):	☐	☐	⌊_⌋⌊_⌋⌊_⌋⌊_⌋

12. Have you always used the same disinfectant? Yes ☐ No ☐

13. If you have previously used other disinfectants, please indicate the period in which they were applied:

Since _____/_____ until _____/_____ Disinfectant: .....

Since _____/_____ until _____/_____ Disinfectant: .....

[if there are more, please indicate]

14. Do you currently apply any other water treatments?

No ☐

Yes ☐ Please indicate: .....

.....

15. When did you start using this treatment process? _____/_____ (month /year)

16. If you have previously applied a different treatment to the current one, please indicate previous treatment (including disinfectant used) and the period:

Since ____/_____ until ____/_____.....

.....

.....

Since ____/_____ until ____/_____.....

.....

.....

[if there are more, please indicate]

QUALITY

To complete information about levels of chemicals, we would be grateful if you could send a copy of all water analysis providing historical data for the period 1985 (or earliest year available, please specify) until 2015:

Supply zone
Date
Concentration (and units)
Sampling point (exit of treatment plant / network/ tap / if other, please specify)

About the following compounds:

TRIHALOMETHANES

Chloroform ($CHCl_3$)
Dichlorobromomethane ($CHCl_2Br$)
Chlorodibromomethane ($CHClBr_2$)
Bromoform ($CHBr_3$)
Total of trihalomethanes (THM)

NITRATE AND METALS

Nitrate
Arsenic
Selenium
Lead
Mercury
Chromium
Nickel
Cadmium
Zinc

THANK YOU VERY MUCH FOR ANSWERING THE QUESTIONNAIRE