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Trace elements in drinking water and the incidence of attention-deficit hyperactivity disorder

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ABSTRACT

Background: Trace elements have been suggested to have neurotoxic effects and increase the risk of neurodevelopmental disorders, but studies of a potential role of trace elements in relation to Attention-Deficit/Hyperactivity Disorder (ADHD) are very limited. The objective of this study was to conduct an exploratory analysis investigating the associations between 17 geogenic trace elements (Ba, Co, Eu, I, Li, Mo, Rb, Re, Rh, Sb, Sc, Se, Si, Sr, Ti, U and Y) found in Danish drinking water and the risk of developing ADHD.
Methods: In this cohort study, 284,309 individuals, born 1994–2007, were followed for incidence of ADHD from the age of five until the end of study, December 31, 2016. We conducted survival analyses, using Poisson regression to estimate incidence rate ratios (IRRs) with 95 % confidence intervals (CI) in three different confounder adjustment scenarios.
Results: In a model including adjustments for age, sex, calendar year, parental socio-economic status, neighborhood level socio-economic status and parental psychiatric illness, we found that six of the 17 trace elements (Sr, Rb, Rh, Ti, Sb and Re) were associated with an increased risk of ADHD, whereas two (Ba and I) were inversely associated with ADHD. However, when including region as a covariate in the model, most trace elements were no longer associated with ADHD or the association changed direction. Four trace elements (I, Li, Rb, and Y) remained significantly associated with ADHD but in an inverse direction and for three of these (I, Li and Y), we found significant interactions with region in their association with ADHD.
Conclusion: The trace elements under investigation, at levels found in Danish drinking water, do not seem to contribute to the development of ADHD and our findings highlight the importance of examining consistency of associations across geographic areas.

1. Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is one of the most prevalent neurodevelopmental disorders, and it is estimated that 5–7 % of children and adolescents and 2.5 % of adults are affected by ADHD worldwide [1,2]. ADHD is characterized by symptoms of inattention, hyperactivity and impulsivity as well as some degree of difficulties in social interaction [3]. The aetiology of ADHD is still largely unknown but ADHD is considered a multifactorial disorder and to a) involve both genetic and environmental risk factors b) have an early onset and c) be the result of some disruption to brain development [4–6]. The developing and immature brain is considered particularly vulnerable to environmental toxins and more susceptible to cell injury. Consequently, disruptions caused by environmental insults are more likely to occur during early stages of brain development, than during adulthood [6–12].

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Trace elements are naturally occurring in the environment. They are found in drinking water in very small concentrations and locally with a high degree of variation. In humans, drinking water is one of the important sources of exposure to a wide range of trace elements and such exposure, even at low concentrations, may affect brain development [9]. Only a limited number of studies have investigated the association between trace elements in drinking water and ADHD but a recent cohort study by Schullehner et al. found that exposure to manganese from Danish drinking water was associated with later development of ADHD, specifically the inattentive subtype of ADHD [12]. The overall aim in our study was to conduct an exploratory analysis to investigate the associations between a range of geogenic trace elements and the risk of ADHD, using exposure data from two drinking water sampling campaigns, conducted in Denmark. To our knowledge, these associations have not previously been studied in a large epidemiological cohort study.

2. Materials and methods

2.1. Study population

The study population (N = 284,309) included all individuals born in Denmark between January 1, 1994 and December 31, 2007, who were living at a residential address in an area covered by the drinking water sampling campaigns (see below, 45 % of all residential addresses) on the 5^{th} birthday, and whose parents were both born in Denmark. Individuals, who had at least one residence outside of Denmark between birth and the 5^{th} birthday and individuals, who belonged to a household supplied by private wells, were not included in the study population.

The study population was identified from the Danish Civil Registration System (CRS), which was established in Denmark on April 2, 1968 [13] and includes all people, who had a residence in Denmark on this date and onwards. All residents in Denmark have a unique personal identification number, which is assigned at birth or by immigration. The personal identification number is the key that enables exact individual-level linkage between all the Danish registers. It is stored in the CRS together with information on sex, date and place of birth, continuously updated information of vital status (e.g. date of death or date of exit from Denmark) and the personal identification number of the parents. The CRS also holds full information about current and former residential addresses in Denmark as well as dates of all residential changes in Denmark.

2.2. Exposure window

The Danish Register on Official Standard Addresses and Coordinates has information on all residential addresses and their assigned geographical coordinates, and we linked this register with the residential addresses of our cohort in order to obtain geographical coordinates. The geographical coordinates were used to link the addresses to the correct water supply area and concentrations of trace elements. For each person in the study population, we defined the level of exposure of the geogenic trace elements in the water supply area at the person's residential address at the 5^{th} birthday as a proxy of the intake of the specific elements from drinking water during the first five years of life.

2.3. Exposure to selected geogenic trace elements

The supply of drinking water in Denmark originates from groundwater and the majority of the population (approximately 98 %) are supplied by public waterworks, the rest from private wells. The number of active public waterworks in Denmark is approximately 2700 and during the period 2014–2016 the abstraction of groundwater for drinking water purpose was about 350 million m^3 per year [14]. In addition to the public waterworks there are approximately 50,000 small private wells (i.e. each serving less than 10 households) [15]. Consumption of bottled water is not very common in Denmark and per capita consumption is among the lowest in Europe [16], which means that the majority of water consumption for most individuals in Denmark originates from waterworks.

The measurements used in this study originate from two drinking water sampling campaigns conducted in 2013 [17] and 2016/2017 and analyzed using inductively coupled plasma mass spectrometry (ICP-MS) at the Geological Survey of Denmark and Greenland (GEUS) and Aarhus University in Denmark. The sampling campaign from 2013 has been described earlier [17–19]. The 2016/17 campaign followed a similar procedure. In short, drinking water samples were collected for 72 trace elements at 188 larger waterworks, evenly distributed within Denmark (Fig. 1). Altogether, these waterworks supply approximately half the Danish population with drinking water. The waterworks were linked to their supply areas (Fig. 1) [20]. The residential addresses in each of these water supply areas were established and this allowed for determining the exposure level for all residents within these areas [18]. The measurements in this study are an average of the values from the two campaigns unless there had been a change in the water treatment since 2013, in that case we used only the measurement from 2013.

The majority of the 72 trace elements included in the ICP-MS screening were under the detection limit (Table S1) at most of the waterworks. Therefore, we made a selection of trace elements to be included in this study based on the following criteria: First, we included elements for which more than 50 % of the measurements at the different waterworks were above the analytical detection limit and with high correlations between the samples obtained in 2013 and in 2016/17 at the same waterwork, indicating stable drinking water chemistry ($R^2 \geq 0.8$) (group a). Eight elements met these criteria: barium (Ba), iodine (I), lithium (Li), molybdenum (Mo), rubidium (Rb), rhodium (Rh), silicon (Si) and strontium (Sr). Second, we also included elements for which 10–50 % of the measurements at the different waterworks were above the analytical detection limit and with R^2 correlations between 2013 and 2016/17 ≥ 0.5 (group b). An additional eight elements met this second criterion: cobalt (Co), europium (Eu), rhenium (Re), antimony (Sb), scandium (Sc), titanium (Ti), uranium (U) and yttrium (Y). Finally, we included selenium (Se) (12.3 % of samples above the detection limit, $R^2 = 0.3$), as it is a known neurotoxic metal [21] and is considered a key chemical for large-scale health effects through drinking-water exposure by the WHO [22].

Trace elements concentrations were modeled as categorical quartiles and as continuous trend variables with the lowest quartile as the reference category and using exposure concentrations related to an individual's residential address at age 5 years as a proxy for exposure during the first five years of life. Concentrations below the detection limit were included in the lowest quartile and when more than 25 % of the study population was exposed to levels below the detection limit, the reference category was defined as below detection limit and the concentrations of the remaining study population classified in exposure tertiles (Table S2).

2.4. Assessment of ADHD

Information on clinical diagnoses of ADHD among cohort members was obtained from the Danish Psychiatric Central Research Register (DPCR) and from the Danish National Patient Register (DNPR) from which only contacts to departments of psychiatry, pediatrics, and neurology were included. The DPCR holds information about all inpatient admissions to Danish psychiatric hospital departments since 1969, and DNPR holds information on all inpatient admissions non-psychiatric hospital departments 1977, including departments of pediatrics. From 1995 and onwards, both DPCR and DNPR also include information on all contacts to outpatient departments and visits to emergency care units, psychiatric and non-psychiatric. Since 1994, clinical diagnoses in Denmark are based on the International Classification of Diseases, 10^{th} revision, Diagnostic Criteria for Research (ICD-10-DCR). For diagnoses

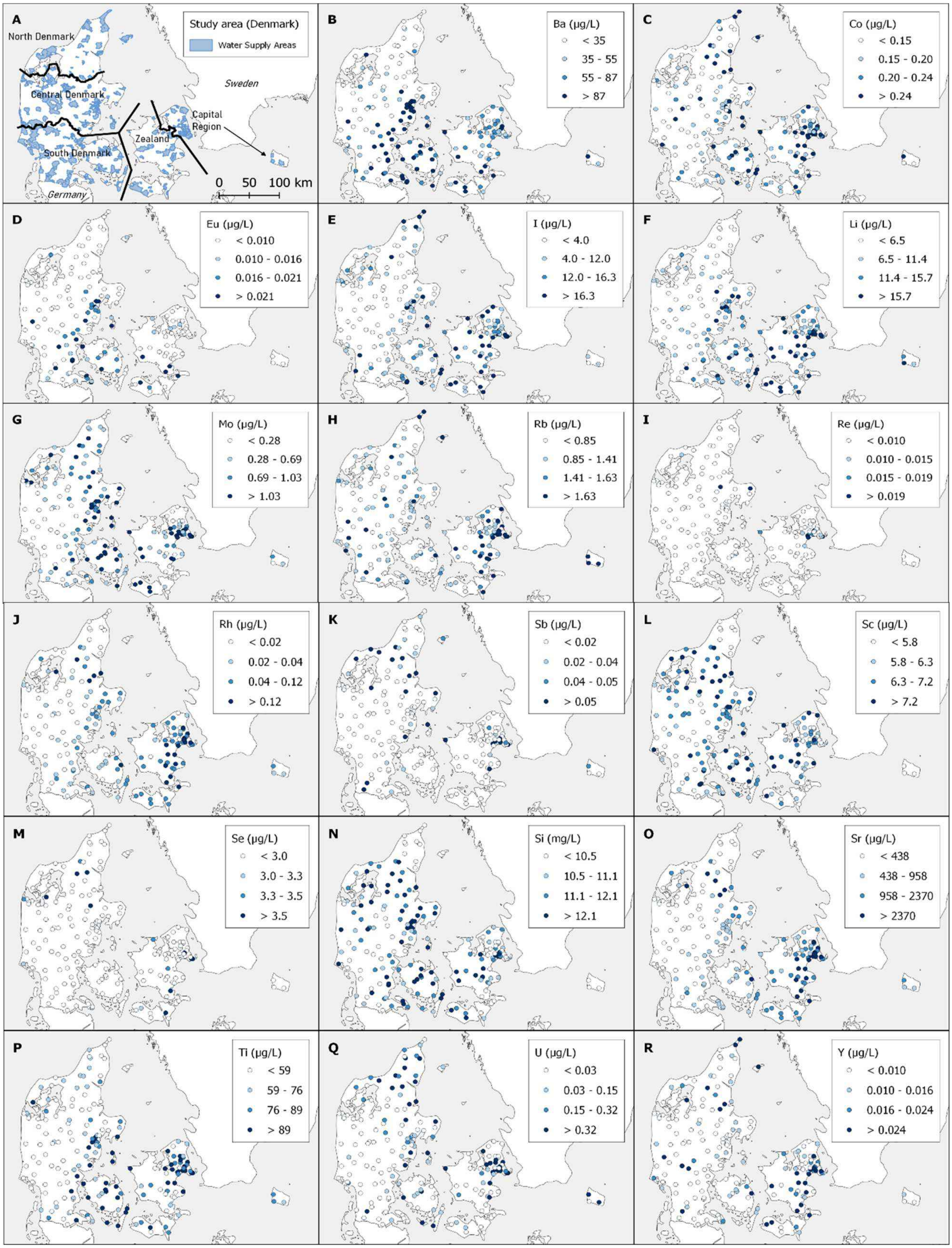

Fig. 1. Quartile distribution of trace elements and water supply areas in Denmark (sampled in 2013 and 2016/17). The results below the analytical detection limit are included in the lowest quartile.

of ADHD we used codes F90x (Hyperkinetic disorders) or F98.8 (Attention deficit disorder without hyperactivity). Diagnoses of ADHD are only registered in the DPCR after an assessment by a specialized child and adolescent psychiatrist and are considered to be of high validity [23]. In Denmark, public healthcare including mental health care is free of charge for the whole population, which enables a good national coverage of the health registries. Date of diagnosis of ADHD was defined as the first day with the diagnosis (inpatient or outpatient contact) and individuals, with an incident diagnosis of ADHD before their 5th birthday, were excluded from the cohort. The reason for excluding individuals with ADHD before five years of age was because follow-up started at the 5th birthday of each individual, and exposure must precede the outcome [24].

2.5. Covariates

Before conducting the analysis, we used the program DAGitty 3.0 [25] to construct a Directed Acyclic Graph (DAG) to identify potential confounders of the associations between trace elements exposure and the outcome, ADHD. Based on this DAG (Fig. S1), we decided to include age, calendar year, sex, parental socio-economic status (parental SES), neighborhood level socio-economic status (neighborhood level SES), parental psychiatric diagnoses and the administrative region (hereafter region, see Fig. 1a) as covariates. Information about parental SES, neighborhood level SES and region were accessed for the year the cohort member was born, whereas a parental psychiatric diagnosis must have been obtained before the birth of the child in order to be included.

In all models, we included age, sex and calendar year as covariates. Age and sex to control for individual confounding factors and calendar year to control for increasing incidence of ADHD diagnosis over time. From Statistics Denmark we obtained information about parental education (highest completed education in four categories, primary school, short education, medium long education and higher education) and parental income (gross income in quintiles) since parental SES has been associated with both choice of residential area [26] and ADHD [27]. Neighborhood level SES was included to control for effects of SES beyond the family and was measured as the proportion of individuals with low income and low education and the proportion of individuals outside the workforce in the municipality (the smallest administrative unit in Denmark). Parental psychiatric illness is associated with an increased risk of ADHD in offspring and can partly be considered a proxy of the genetic disposition for ADHD [28]. In this study, parents were classified as having a psychiatric illness, if they had a hospital contact resulting in a psychiatric diagnosis (ICD-8 codes: 290-315, ICD-10 codes F00-F99) before the child was born. Information about parental psychiatric diagnoses were obtained from the Danish Psychiatric Central Research Register (DPCR) and the Danish National Patient Register (DNPR). Studies have shown that the incidence of ADHD differ geographically across Denmark, possibly due to difference in access to diagnostic facilities and different diagnostic cultures in the hospitals [29,30]. In Denmark, all residents have free access to health care services, including help for psychiatric difficulties. Primary and secondary health care services are decentralized, and responsibility is divided between the five regions and the 98 municipalities. The municipalities have the responsibility for primary care, while the regions have the responsibility for secondary care (hospitals). Despite that health services are offered free of charge for all citizens some inequality exists since the number of hospitals and specialists are not equally distributed across the regions. As shown in Fig. 1, we also found very different geographical distribution patterns of the 17 trace elements, and these geographical differences point to the importance of considering potential confounding from geographical factors such as the region.

2.6. Statistical analysis

All individuals (n = 284,309) in the cohort were followed from their 5th birthday until the date of first diagnosis of ADHD, death, disappearance, emigration from Denmark or end of study (December 31, 2016) (whichever came first). Survival analyses were conducted using Poisson regression [31] in SAS version 9.4 (SAS Institute, Cary, N.C.) to estimate incidence rate ratios (IRRs). An IRR of 1.00 indicates no association between the trace element and ADHD. P-values and 95 % confidence intervals (CIs) were based on likelihood ratio tests [32] and the adjusted-score test [33] suggested that the regression models were not subject to over-dispersion.

Trace elements concentrations were analyzed as categorical quartiles and as continuous trend variables representing the lowest vs the highest quartile, using exposure concentrations related to the residential address at age 5 years as a proxy for exposure during the first five years of life.

We defined three models *a priori* to study the association between trace elements and ADHD. These were: model 1) adjustment for age, calendar year, and sex; model 2) adjustment for age, calendar year, sex, parental SES and neighborhood level SES and parental psychiatric illness and model 3) in which region was included in addition to the covariates included in model 2. In all models, we included age and calendar year as time-dependent variables, whereas other variables were treated as variables independent of time. When information on covariate data was not available, we performed complete-case analysis. To investigate the consistency of the effect of the trace elements across the 5 Danish regions, we examined whether region had interacting effects on the associations between trace elements and ADHD.

2.7. Ethics

The Danish Protection Agency and the Danish Health and Medicines Authority approved this study. By Danish law, ethical approval is not required for register-based studies, but all personal information from the registers was anonymized as required, when they are used for research purposes.

3. Results

The study population included 284,309 individuals born in Denmark between January 1, 1994 and December 31, 2007. During the study period (1999–2017), 9500 (3.34 %) of these were diagnosed with ADHD during 3,111, 871 person-years of risk. Individuals in the study population were followed for up to 18 years (10.9 ± 4.2 years). For 2183 individuals (0.7 %), follow-up was ended before the end of the study (i.e. censored), because they emigrated from Denmark (0.62 %), or disappeared or died (0.08 %). Characteristics of the study population are shown in Table 1.

3.1. Geogenic trace elements and ADHD

The geographical distribution of the 17 geogenic trace elements' concentrations in drinking water across Denmark in 2013 is shown in Fig. 1. Pairwise correlations between trace elements were mostly low to moderate, whereas a few were highly correlated, such as Rh and SR (Pearsons correlation coefficient (*r,* 0.99), Eu and Ba (*r,* 0.86) and Sc and Si (*r*, 0.83) (Table S3).

We constructed a DAG, to identify potential confounders of the associations between trace elements exposure and ADHD, and conducted analyses using 3 different adjustment models. Model 1 included base adjustments for age, sex and calendar year, while model 2 and 3 included adjustments for age, sex, calendar year, parental SES, neighborhood level SES and parental psychiatric illness. In addition model 3 included adjustment for region. Analyses were conducted with (model 3) and without region (model 2) in order to estimate the independent confounding effect of region.

In the first analyses (Model 1, Fig. 2) when trace elements were fitted as continuous trend variables scored from 0 to 1, we found that 9 trace elements (Co, Mo, Rb, Re, Rh, Sb, Sr, Ti and Y) were positively

Table 1
Characteristics of cases (N = 9500) in the overall cohort (N = 284,309).

	Number of cases with ADHD	Number of person-years at risk in total in the cohort	Incidence rate per 10,000 person-years
Year of birth			
1994–1997	3221	1362289.03	23.64
1998–2001	3265	943953.09	34.59
2002–2007	3014	805629.04	37.41
Sex			
Female	2934	1531643.22	19.16
Male	6566	1580227.94	41.55
Geographical region			
North Denmark	529	214090.64	24.71
Central Denmark	2359	719303.60	32.80
South Denmark	1429	600653.91	23.79
Capital Region	4271	1307249.93	32.67
Zealand	912	270573.07	33.71
Mother's level of education[‡]			
Primary school	3198	551584.49	57.98
Short education	4413	1519808.45	29.04
Medium long education	1510	747709.31	20.20
Long education	336	285066.00	11.79
Missing	<50		
Father's level of education[‡]			
Primary school	3188	569401.18	55.99
Short education	4868	1677409.71	29.02
Medium long education	813	456492.18	17.81
Long education	473	383114.33	12.35
Missing	158		
Mother's level of income[§]			
Below the 20th percentile	120	43530.54	27.57
20th to the 40th percentile	1334	278885.10	47.83
40th to the 60th percentile	4411	1104555.94	39.93
60th to the 80th percentile	2885	1205521.02	23.93
Above the 80th percentile	750	479370.62	15.65
Father's level of income[§]			
Below the 20th percentile	172	35827.85	48.01
20th to the 40th percentile	717	137451.48	52.16
40th to the 60th percentile	1563	317445.33	49.24
60th to the 80th percentile	3378	921479.39	36.66
Above the 80th percentile	3659	1698551.43	21.54
Maternal psychiatric illness			
Yes	756	108078.98	69.94
No	8744	3003792.17	29.1
Paternal psychiatric illness			
Yes	552	71626.06	77.07
No	8948	3040245.10	29.43
Neighborhood level of income in municipality			
Low income municipality	3217	1029351.40	31.25
Medium income municipality	3217	1054363.92	30.51
High income municipality	3066	1028155.83	29.82
Neighborhood level of education in municipality			
Low education municipality	2741	886843.05	30.91
Medium education municipality	3372	1054606.52	31.97
High education municipality	3387	1170421.59	28.94
Neighborhood level of unemployment in municipality			
High unemployment municipality	3656	1368031.23	26.72
Medium unemployment municipality	3083	971040.28	31.75
Low unemployment municipality	3656	1368031.23	26.72

Cohort consisted of 284,309 children born.1994–2007.
[‡]Highest finished education measured at the end of the year of the child's birth.
[§]Level of income at the year of the child's birth.

associated with ADHD with IRRs ranging from 1.07 to 1.31, whereas Ba (IRR: 0.90, 95 % CI: 0.86, 0.96) was inversely associated with ADHD, and the remaining trace elements were not associated with ADHD (Fig. 2). In analyses including trace elements modeled as quartiles, only Sb showed a significant dose-response relationship across quartiles (Table S4).

After additional adjustment for parental SES, neighborhood level SES and parental psychiatric illness (model 2, Fig. 2), we found that six (Rb, Re, Rh, Sb, Sr and Ti) of the 17 trace elements were associated with an increased risk of ADHD with IRRs ranging from 1.07 to 1.17, while two of the trace elements Ba (IRR: 0.89, 95 % CI: 0.84, 0.94) and I (IRR: 0.92, 95 % CI: 0.87, 0.98) showed an inverse association with ADHD. In the categorical analyses, none of the trace elements were associated with ADHD in a dose-dependent manner (Table S5).

Including region as a covariate in the final model (model 3, Fig. 2) changed many of the estimates, and associations were either strongly attenuated or reversed. After this adjustment, none of the trace elements were associated with an increased risk of ADHD, while four trace elements (Rb, Li, I and Y) were inversely associated with ADHD with IRRs ranging from 0.89 to 0.90. However, none of these trace elements showed a significant dose-response relationship in the categorical analyses (Table S6).

In a stratified analysis on the association between trace elements (modelled as continuous variables) and ADHD across regions and adjusted for parental SES, neighborhood level SES and parental psychiatric illness, we found that effects of Ba, Mo, Rb, Re, Sc, Se, Si and Sr were consistent across regions, while significant interactions with region were found for Co, Eu, I, Li, Rh, Sb, Ti, U and Y (Table 2). Only Rb was significantly (inversely) associated with ADHD in model 3, and with no interaction with region. However, in the stratified analysis of the association between Rb and ADHD estimates were not significant in any of the 5 regions.

4. Discussion

In this cohort study of 284,309 individuals, we performed an exploratory analysis of 17 geogenic trace elements naturally occurring in drinking water in Denmark, to investigate their potential contributions to the development of ADHD.

Several of the environmental exposures that have been suggested as neurotoxic are trace elements including lead (Pb), mercury (Hg), arsenic (As), manganese [34], cadmium (Cd) and fluoride (F) [11]. However, the literature on potential neurotoxic effects of the 17 trace elements included in this study is sparse. The neurodevelopmental effect of Sb has not been widely investigated and the results are contradictory. One study did not find an association between prenatal exposure to Sb and

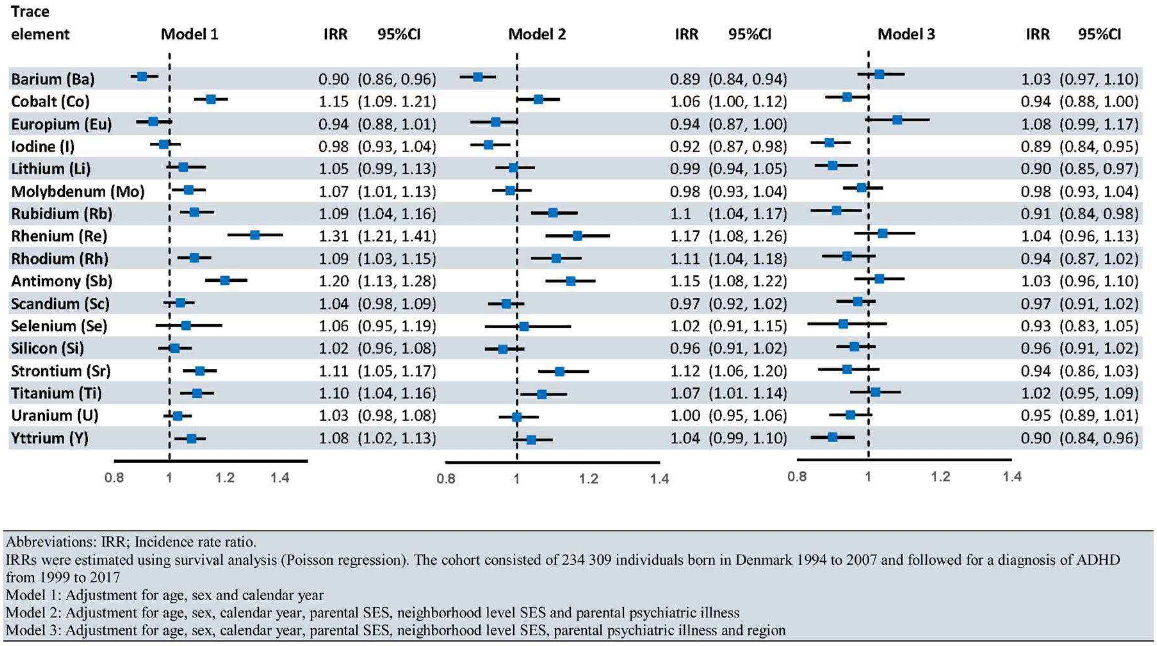

Fig. 2. Effects of trace elements on the risk of ADHD in different adjustment models.
Abbreviations: IRR; Incidence rate ratio.
IRRs were estimated using survival analysis (Poisson regression). The cohort consisted of 234 309 individuals born in Denmark 1994–2007 and followed for a diagnosis of ADHD from 1999 to 2017
Model 1: Adjustment for age, sex and calendar year
Model 2: Adjustment for age, sex, calendar year, parental SES, neighborhood level SES and parental psychiatric illness
Model 3: Adjustment for age, sex, calendar year, parental SES, neighborhood level SES, parental psychiatric illness and region

Table 2
Effects of trace elements (modelled as continuous variables) on the risk of ADHD stratified by region at birth (with interaction term) (Model 3).

Trace Ele-ment	North Denmark IRR (95 % CI)	Central Denmark IRR (95 % CI)	South Denmark IRR (95 % CI)	Capital City IRR (95 % CI)	Zealand IRR (95 % CI)	P-value
Ba	1.35 (0.79, 2.17)	1.00 (0.91, 1.10)	1.20 (1.03, 1.41)	0.96 (0.85, 1.08)	1.14 (0.92, 1.41)	(p = 0.11195)
Co	0.99 (0.78, 1.25)	0.89 (0.79, 1.00)	0.73 (0.62, 0.87)	0.99 (0.90, 1.10)	1.22 (0.97, 1.54)	(p = 0.00307)
Eu	0.69 (0.20, 1.82)	0.95 (0.86, 1.06)	1.63 (1.40, 1.91)	1.02 (0.74, 1.38)	0.83 (0.63, 1.09)	(p=<.00001)
I	1.01 (0.83, 1.22)	0.83 (0.71, 0.96)	0.48 (0.40, 0.58)	0.99 (0.90, 1.08)	1.01 (0.85, 1.19)	(p=<.00001)
Li	1.05 (0.58, 1.83)	0.81 (0.72, 0.91)	0.73 (0.63, 0.86)	1.01 (0.91, 1.11)	1.35 (1.03, 1.78)	(p = 0.00013)
Mo	0.91 (0.66, 1.25)	0.95 (0.85, 1.05)	0.95 (0.83, 1.08)	0.98 (0.89, 1.07)	1.19 (1.00, 1.42)	(p = 0.24886)
Rb	0.85 (0.63, 1.14)	0.95 (0.80, 1.12)	0.88 (0.74, 1.05)	0.88 (0.78, 1.00)	1.01 (0.84, 1.22)	(p = 0.74389)
Re	0.85 (0.05, 4.38)	1.09 (0.87, 1.34)	1.42 (0.37, 3.73)	0.98 (0.89, 1.08)	1.26 (1.04, 1.51)	(p = 0.20449)
Rh	1.11 (0.82, 1.49)	0.76 (0.64, 0.90)	0.95 (0.75, 1.19)	1.06 (0.94, 1.19)	0.77 (0.60, 0.99)	(p = 0.00998)
Sb	1.01 (0.81, 1.24)	1.14 (0.99, 1.31)	1.78 (1.35, 2.32)	0.95 (0.87, 1.04)	1.07 (0.77, 1.47)	(p = 0.00080)
Sc	1.01 (0.83, 1.23)	0.98 (0.87, 1.10)	0.94 (0.83, 1.06)	0.96 (0.87, 1.05)	0.98 (0.83, 1.17)	(p = 0.96591)
Se	1.01 (0.69, 1.44)	1.03 (0.84, 1.25)	0.28 (0.01, 1.71)	0.92 (0.76, 1.12)	0.79 (0.59, 1.04)	(p = 0.42377)
Si	0.96 (0.77, 1.20)	0.86 (0.77, 0.97)	1.05 (0.92, 1.19)	0.95 (0.86, 1.04)	1.09 (0.93, 1.29)	(p = 0.10256)
Sr	1.05 (0.79, 1.38)	0.88 (0.70, 1.10)	0.86 (0.68, 1.09)	1.01 (0.89, 1.15)	0.73 (0.55, 0.98)	(p = 0.25796)
Ti	1.44 (0.98, 2.08)	1.03 (0.90, 1.16)	0.81 (0.70, 0.93)	1.09 (0.97, 1.22)	1.09 (0.91, 1.30)	(p = 0.00403)
U	1.07 (0.86, 1.35)	1.02 (0.88, 1.19)	0.47 (0.38, 0.59)	0.99 (0.91, 1.08)	1.00 (0.84, 1.19)	(p=<.00001)
Y	0.83 (0.62, 1.09)	0.75 (0.58, 0.95)	0.64 (0.52, 0.79)	0.97 (0.89, 1.07)	0.98 (0.83, 1.16)	(p = 0.00185)

Adjusted for age, sex and calendar year, parental SES, neighborhood level SES and parental psychiatric illness.

ADHD symptomatology at the age of four [35], while another study found that Sb was associated with susceptibility to ADHD and symptom severity in school-age children [36]. In a small study of 25 children with autism and 25 healthy controls, concentrations of Ba in hair and urine was found to be associated with a higher prevalence of autism in communities with high levels of Ba in drinking water [34], while another small case-control study found that Co deficiency was associated with ADHD [37] Li is known for its mood stabilizing effects and has been used as pharmacological treatment of several mental illnesses since the 1940−50 s, especially for bipolar disorder [38]. Like potassium and sodium, it is considered being essential for nerve conduction and a normal functioning of the nervous system. Observational data suggests that in patients with bipolar disorder, Li reduces the incidence of attempted and completed suicide [39,40], possibly through its capability of reducing aggression and impulsivity, which have been suggested to be associated with an increased risk of suicide in bipolar disorder [41]. Another study has found that long term exposure to Li in drinking water may be associated with a lower incidence of dementia [42] or inversely associated with depressive symptoms [43], while a recent study found that higher levels of Li in Danish drinking water were associated with increased risk of schizophrenia [44]. Rb has previously been used as pharmacological treatment for depression, bipolar disorder and schizophrenia but Rb was phased out and replaced by other pharmacological agents with higher efficacies [45]. A study by Meltzer et al. [46] suggested that Rb may increase alertness, activity and affect while another study found that Rb tended to increase the duration of mania but seemed to reduce the extremes of mood [47]. Deficiency of I has been suggested to cause cretinism, impaired development, cognitive disabilities [48] and the major impact of hypothyroidism caused by iodine deficiency is impaired neurodevelopment [49]. Insufficient Se levels may have harmful effects on brain function and may influence neuronal loss and Se deficiency may exacerbate symptoms of hypothyroidism, including substantial fatigue, goitre and cretinism [50]. Studies have shown that lower plasma Se concentrations in elderly people are associated with senility and increased cognitive decline [51] and low Se intake has been associated with an increased incidence of depression, anxiety, confusion and more hostile behavior [48,51–53]. Studies concerning the effect of Sr are very limited, but a small sample study by Yalçın et al. recently suggested that children with neurodevelopmental disorders had lower levels of Sr in both tooth dentin and blood [54].

We did not find clear evidence that any of the trace elements, included in this study, were associated with an increased risk of a diagnosis of ADHD at levels found in Danish drinking water. Regarding the trace elements with consistent effects across regions (Ba, Mo, Rb, Re, Sc, Si and Sr) it is unclear whether the estimates in Model 2 or Model 3 best describe the association with ADHD, as region may act as a confounder for some trace elements, though perhaps not for all. For this reason estimates resulting from both model 2 and 3 are presented. Adjusting for region significantly changed many of the estimated associations and may in some instances represent an over-adjustment. The stratified analyses may describe the true associations with ADHD across each of the five regions as these estimates are unaffected by potential interaction or confounding by region. However, in the stratified analyses most associations were heterogeneous across regions, with either significant interactions with region, with estimates in opposite directions or the association only significant in one or two of the five regions.

There are different explanations for our findings. Inconsistency plays an important role for some of the exposures, and using Sb as an example, this trace element was associated with a 1.15 (95 % CI: 1.08, 1.22) fold increased risk of ADHD in Model 2. However, we found an interaction with region ($p = 0.00080$) and for Sb to be causally associated with ADHD, consistency across regions would be expected. In addition, Sb was only associated with an increased risk of ADHD in one of the five regions (IRR: 1.78, 95 % CI: 1.35, 2.32) (South Denmark). Potential confounding by region is another important issue to consider in the interpretation of our data, and using Sr as another example, we found that Sr was associated with a 1.12 (95 % CI: 1.06, 1.20) fold increased risk of ADHD in Model 2. No interaction with region was found ($p = 0.26$), however in stratified analyses, the association was not significant in any of the 5 regions and when adjusting for region (Model 3), the effect was reduced from 1.12 to 0.94. In addition, results from model 2 showed that individuals being exposed to the highest quartile of Ba had a risk of 0.89 compared to individuals exposed to the lowest quartile of Ba. The effect of Ba was consistent across region ($p = 0.11$, Table 2), but the overall estimate of 0.89 (Fig. 2, model 2) was not within the range of the region-specific estimates (Table 2, range 0.96–1.35).

4.1. Strengths and limitations

The study was designed as a register-based cohort study, which offered a unique possibility to link exposure data of 17 trace elements with a large study population, which could be followed prospectively for up to 18 years with a minimum of losses to follow-up. In addition, information about the outcome was obtained from the Danish National Health registers using data on clinical diagnoses of ADHD set by a specialized child and adolescent psychiatrist and based on the International Classification of diseases (ICD-10). Studies have found high validity of ADHD-diagnoses in the register [23,55]. Also, exposure data was based on high precision sampling and analyses at the waterworks of the trace elements which was linked to the exact residential address of the individuals. Finally, information from the registers made it possible to adjust for parental SES, neighborhood level SES and parental psychiatric illness as potential confounders in our analyses and estimate interactions with region.

However, our study also has important limitations. For the exposure (concentrations of the different trace elements), we relied on measurements obtained in 2013 and 2016/2017, as an approximation of exposure from birth to age 5, assuming that levels of these trace elements have remained constant over the period of 1994–2016 and restricted the selection of trace elements in this study to those with moderate to high correlation between the samples taken in 2013 and 2016/17 [22]. In addition, we did not have information about concentration levels of trace elements in drinking water at the children's daycare institutions. However, since most children in Denmark attend daycare close to their residential address, the daycare institution will most likely obtain drinking water from the same waterwork as the child's residential address. We did neither have information of the chemical speciation of the elements, which for U and other, non-included elements (As and Cr), have proven important for understanding health effects [22]. Another important limitation is that some elements, like Ba, I and Li [19] are co-correlated in groundwater due to geologic origin and geochemical processes in the aquifers. Furthermore, they could be correlated to other, major elements that were not included in this study, e.g. Cl, Ca or Mg, which also may have some effect. Finally, exposure to trace elements does not only exist through drinking water, and therefore other unmeasured sources of exposure may also be important in addition to exposure from drinking water.

5. Conclusion

This exploratory cohort study is the first to investigate a large range of trace elements found in drinking water and their potential associations with ADHD. We did not find evidence that the exposure to higher concentrations in the Danish drinking water of any of these trace elements was associated with a higher incidence of ADHD, and our findings point towards the importance of examining consistency of associations across geographic regions and the potential confounding effects of regional-level factors, such as diagnostic differences, when studying health effects of low-dose trace element exposure in drinking water. However, caution has to be taken in interpreting the results as only a limited number of previous studies have investigated these associations, and as an association may be observed at higher exposure rates. Denmark is generally characterized by relatively low levels of pollution, thus the findings of this study do not exclude a potential contribution of the trace elements into adverse neurodevelopmental outcomes at other exposure levels. Hence, more studies are needed to support our findings.

Author statements

Malene Thygesen: Literature search, data preparation, data analyses, interpretation of data and writing of manuscript.

Jörg Schullehner: Data preparation, exposure assessment, study design, interpretation of data and revision of manuscript.

Birgitte Hansen: Study design, interpretation of data and revision of manuscript.

Denitza D. Voutchkova: Study design, interpretation of data and revision of manuscript.

Søren Munch Kristiansen: Study design, interpretation of data and revision of manuscript.

Torben Sigsgaard: Study design, interpretation of data and revision of manuscript.

Carsten Bøcker Pedersen: Conception of scientific idea, study design, input in statistical methods, interpretation of data and revision of manuscript.

Søren Dalsgaard: Conception of scientific idea, study design, interpretation of data, clinical expertise and revision of manuscript.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

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