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Estimation of human daily boron exposure in a boron-rich area

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Although, the safe limits of human daily boron (B) exposure are not absolutely clear, there is a growing interest in B and its effects on human health. The aim of the present study was to estimate daily B exposure in 66 males in Turkey living in a B-rich area using water containing at least 2 mg/l boron, with an average age of 38·55 (SE 1·66) years and an average number of years of residence in the B-rich area of 35·89 (SE 1·73). Another group of males (*n* 57), living in the city centres of Balıkesir and Ankara, were taken as controls; the average age and number of years of residence for this group were 29·44 (SE 1·43) and 10·26 (SE 1·83) years, respectively. As it is assumed that the B level in urine reflects daily B exposure, the amount of urinary B of both the study and control groups was analysed by using an inductively coupled plasma optical emission spectrometry (ICP-OES) technique. The average daily B exposure value was calculated as 6·77 (SE 0·47) mg in the study group and 1·26 (SE 0·1) mg in the controls. The results of this study are expected to contribute to creating a reference value for a safe daily B exposure.

Boron: Daily exposure: Risk assessment

Recent studies conducted both *in vitro* and epidemiologically have indicated that boron (B) exposure may make significant contributions to human health[1–3]. Humans are naturally exposed to B via food and drinking water[4]. B is absorbed almost completely by the gastrointestinal and respiratory system if exposure occurs, and is mostly present in body tissues and fluids as boric acid, $B(OH)_3$, and in lesser amounts as $B(OH)_4^-$ anion[5]. Studies indicate that B homeostasis is basically maintained by urinary excretion[6].

B was defined as a trace element in 1980[7]. In spite of the lack of epidemiological data, the WHO reported that boron may provide a physiological benefit for human health[8]. Safe and/or beneficial B levels have not been determined for humans, though animal studies have defined deleterious and beneficial B levels for animals[9].

In a study by Penland[10], it was outlined that B improves brain function and psychomotor response. It increases steroid hormone concentrations and has antioxidant properties in postmenopausal women[9,11]. There is also evidence that B plays a role in healthy bones and joints[12,13]. Additionally, in recent years there have also been some promising results concerning the treatment of certain malignancies[14,15].

Toxicological effects have also been reported in animals at higher doses, e.g in mice, rats and rabbits skeletal malformations and cardiovascular defects have been observed[16,17]. Although not confirmed, it was claimed that B had a deleterious effect in humans, the target organ being the testis. Atrophy and eventual degeneration of the organ in several animal species at high doses have been shown[18,19]. Initially, reproduction toxicology studies were based upon data obtained from animal studies and adapted to human risk evaluations[20]. It was reported in several field studies that B does not affect human reproductive systems or the sexual capabilities of people exposed to daily high levels[21–25]. Although these aforementioned field studies were performed in B-rich areas, the average daily B exposure in the field was not determined at that time. In order to define the average daily B level of exposure in this area further, this elaboration study was designed.

The purpose of this study is to establish the daily B exposure for a group of subjects who have been exposed to B for extended periods at relatively high levels with no reported specific health problems. The results may be useful in determining a reference value for the safe upper limit of B exposure.

Abbreviations: B, boron; ICP-OES, inductively coupled plasma optical emission spectrometry.

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Materials and methods

Geographic locations and sampling

The city of Balıkesir is in northwestern Turkey, bordering the Aegean and Marmara Seas. It is located about 180 m above the sea level, with a flat and wide topography, and has been known as a B-rich area since 1815. The studied B mining region and the nearby residential area are about 50 km from the city centre of Balıkesir, namely Osmanca and İskele villages. Colemanite, a calcium borate, and ulexite, a sodium calcium borate, are the main minerals at this location.

The study subjects were selected among residents who were born in these villages where the water supply for drinking and daily use is rich in B. Previous studies have shown that the fountain and tap water in this area contain 2 mg/l or higher levels of B[15,21]. The levels of B exposure of these subjects were compared with those of controls selected among the residents of Balıkesir and Ankara city centres. From both city centres, water samples were analysed prior to selection of the control regions; the B concentrations in tap water from the Balıkesir and Ankara control areas were found to be $<0{\cdot}20$ mg/l. Ankara, the capital of Turkey, is about 550 km away from the region; levels here reflect the contribution from the whole country. On the other hand, a certain region in Balıkesir was used as the control group because it did not have a B mining area. These two selected control groups were used to reflect the areas without B both in a nearby region and in the whole country.

The study group was selected through the records of the İskele Community Health Center; this population is labelled as group I. In the region, there were 583 males older than 20 years; approximately 10 % of this population was targeted. For preparation of the sample list, every eleventh person listed in the İskele Community Health Center's records was chosen. Some of the candidates were disqualified for reasons such as (1) being unable to provide samples in the required period; and (2) having irregular creatinine clearance values, outside the range of 80–125 ml/min. In such cases, new subjects were added using the same sampling technique. As a result, 66 males with the required qualifications constituted group I.

Group II consisted of 57 males from Balıkesir and Ankara as controls. The subjects were randomly selected among male healthy students and workers. The exclusion criteria were the same as mentioned above for group I. Some of the data regarding the characteristics of subjects in both groups are given in Table 1.

Method used to determine the level of daily boron exposure

Urine sampling for determination of daily boron exposure. Studies on B metabolism have clearly demonstrated that the B level in urine reflects the level of exposure[6,11,26]. In contrast to urine values, the concentrations of B in plasma are very low, at μg/l levels, and not very stable; therefore, 24-hour urine samples are commonly used to estimate B exposure[27].

For estimation of the daily B exposure, 24-hour urine samples were collected from individuals who had healthy kidney filtering functions represented by creatinine clearance levels within the range of 80–125 ml/min. Blood and urine creatinine values were used to calculate the creatinine clearance levels. It has been reported that the B excreted in the urine represented 85 % of the daily B exposure[6]. Taking this as an underlying consideration, the daily B exposure level was computed from the urine B values by multiplying these results by 100/85. The urine B level was calculated by the inductively coupled plasma optical emission spectrometry (ICP-OES) method.

For B exposure and health assessment, each subject was interviewed, and standardised questionnaires (prepared before visits) were filled out. The interviews were based on asking whether the subjects has any health problems and, in the case of any reported problem, the details of the disease as well as any treatment (surgery, medications, etc.) were recorded.

The daily B exposure level was based on the results of urine analysis and normalising these values from assumed value of 85 % to 100 % by computation.

Table 1. Results of descriptive statistics for all characteristics in group I and group II

Variable	Group	*n*	Mean	SEM	Minimum	Maximum
Age	I	66	38·55[a]	1·66	20	79
	II	57	29·44[b]	1·43	20	52
Residency period (years)	I	66	35·89[a]	1·73	10	79
	II	57	10·26[b]	1·83	1	46
Height (m)	I	66	1·72[a]	0·009	1·58	1·91
	II	57	1·76[a]	0·009	1·59	1·92
Weight (kg)	I	66	73·98[a]	1·64	55·00	110
	II	57	74·60[a]	1·56	53·00	123
BMI (kg/m^2)	I	66	24·68[a]	0·66	23·4	39·44
	II	57	21·64[a]	1·08	21·0	35·17
Creatinine clearance (ml/min)	I	66	106·99[a]	2·36	66·20	147·7
	II	57	106·40[a]	2·86	73·91	180·2
Urine volume (24 h, ml)	I	66	1363[a]	63·8	650	2750
	II	57	1350[a]	63·1	500	2500
B (mg/d)	I	66	6·768[a]	0·473	1·766	22·81
	II	57	1·256[b]	0·104	0·212	2·901
B (mg/kg)	I	66	0·093[a]	0·006	0·017	0·285
	II	57	0·017[b]	0·001	0·003	0·045

Mean values within a column with unlike superscript letters were significantly different ($P<0{\cdot}05$).

For 24-hour urine sample collection, 5·0 l plastic containers all of the same kind and colour were used. All the plastic containers were selected in Ankara, away from the mining site. These containers were checked randomly for any blank values regarding any possible B contamination; no related problems were observed. All the containers were kept in plastic bags before and after sampling. The subjects had been educated prior to sample collection regarding any contamination from hands and clothes; samples were taken accordingly. Although a large fraction of the subjects were not mine workers, samplings took place during weekends so that the possibility of contamination was minimised. The subjects were asked to collect their 24-hour urine in the containers starting after their second urination of the day; the first urination of the second day was included. The subjects were asked to provide one 24-hour urine sample. However, in the case of any time disorder in sample collection, the subject was excluded from the analysis. From each container, a portion of 5·0 ml was taken for urine creatinine levels; these were sent to Balıkesir Güven Tıp and Ankara A-Clinic Medical Center Laboratories as determined by the locations of the study groups. Two separate locations for creatinine clearance determination were used; these were near the sample collection sites. Therefore, rapid elimination of subjects was possible in cases where the results were negative.

The remainder of the urine sample was used for B determination using ICP-OES in Ankara, Middle East Technical University. For sampling, holidays and weekends were suggested in order not to disrupt the normal daily working activities of the subjects. For determination of the blood creatinine level, 2·0 ml blood samples were obtained from the left arm of the participants.

As a prerequisite to being involved in this study the subjects should have normal physiological renal functions, as determined by creatinine clearance values within the range of 80–125 ml/min. Ethical permission for this research was granted by Türkiye Ministry of Health, Balıkesir Provincial Health Director (December 30, 2004 and numbered 21 761/21 588). Verbal consent from all participants was obtained before sampling.

Determination of boron in urine. A 5·0 ml sample of urine was mixed with 5·0 ml of concentrated HNO_3; the resulting mixture was dissolved and digested using a microwave oven (Milestone Ethos PLUS microwave dissolution system, Shelton, CT, USA). The mixtures were brought from ambient temperature to 100°C in 3·0 min; held at 100°C for 5·0 min; brought to 150°C in 3·0 min; held at 150°C for 5·0 min; brought to 180°C in 3·0 min; and held at 180°C for 5·0 min. Finally, the oven was ventilated for 5·0 min for cooling. After cooling, all the samples were spiked with indium as internal standard to produce a final concentration of 10·0 mg/l after dilution to 25·0 ml using distilled water. An inductively coupled plasma optical emission spectrometer (ICP-OES; Leeman Model DRE, Leeman Labs Inc., Hudson, NH, USA) was used to measure the B concentration at a wavelength of 249·733 nm. Indium, the internal standard, was measured at 230·606 nm.

Creatinine clearance studies

Blood samples were analysed for creatinine levels in two different laboratories which are under the control of the BIO-RAD External Quality Assurance Services and the College of American Pathologists Surveys programs. A 2·0 ml sample of blood was centrifuged, and the serum fraction was collected. Samples of 1·0 ml of serum and 1·0 ml of urine were tested for creatinine content using a Roche-Cobas Integra 400 Plus instrument (Cat. No. 20764345, System-ID:07 6434 5; Roche Diagnostics Turkey/Swiss). Creatinine clearances were calculated according to blood and urine creatinine levels as well as the total amount in 24-hour urine.

Statistical analysis

Sample size was determined using the following equation:

$$n = \frac{NZ^2\sigma^2}{d^2(N-1)+Z^2\sigma^2},$$

where n is the sample size, N is the population size ($Z = 1{\cdot}96$), σ^2 is the assumed population variance, and d^2 is the error value[28].

One-way ANOVA was used to compare the groups. Pearson correlation and regression analysis were chosen for linear estimation in both groups. For calculations, MINITAB (for Windows; version 14, Minitab Inc., USA) statistical package programs were used.

Results

Subjects

In this study, none of the subjects in group I had reported any health problems which may be linked to high B exposure. No subjects from either group reported use of any mineral supplements in their diet. The ages, number of years of residence and other relevant data are given in Table 1. Although there was a significant difference between the average age and number of years of residence for both groups ($F_{1,115}$ 4·47, $P<0{\cdot}05$ and $F_{1,115}$ 34·64, $P<0{\cdot}01$), for height, body weight, BMI and creatinine clearance levels there was no statistically significant difference between the study and control groups.

Estimated daily boron exposure values

Table 1 indicates the significant differences between the daily B exposure levels ($F_{1,115}$ 37·83, $P<0{\cdot}01$ and $F_{1,115}$ 37·23, $P<0{\cdot}01$) in the study and control groups. In group I, the average daily B exposure level was 6·768 (SE 0·473) mg/d with an average weight of 73·98 (SE 1·64) kg. In controls, these values averaged 1·256 (SE 0·104) mg/d and 74·60 (SE 1·56) kg, respectively. In groups I and II, the daily level of exposure to B per kg of body weight was 0·093 (SE 0·006) and 0·017 (SE 0·001) mg, respectively.

Relationship between the estimated daily boron exposure and other traits

When each group was analysed independently, in group I no statistically significant correlation between the daily B exposure values (both mg/d and mg/kg) and other traits such as age, number of years of residence, height, weight and creatinine clearance was found, and in group II a significant correlation was only observed between the daily B exposure

and age, with no relationship to number of years of residence, height, weight or creatinine clearance.

The decrease of B exposure with increasing age in group II is a result of the regression analysis which can be expressed as the equation: daily boron exposure (mg/d) = 2·073–0·028 age.

Therefore, a unit increase in age results in a decrease of 0·028 mg/d (R^2 0·15 t − 3·051, P <0·01) in mean daily B exposure (Table 2). When the same calculations are considered for each kg of body weight, daily B exposure (mg/kg per d) and age regression analysis is calculated as: daily boron exposure mg/kg = 0·028 – 0·00419 age. According to statistical findings, as the age of a person increases, daily B exposure decreases by 0·000419 mg/year (R^2 0·16 t − 3·19, P <0·01).

Discussion

The nutritional requirement for B has not been firmly established, because of the lack of epidemiological data. A study conducted in the USA revealed that the average daily exposure to B is around 1 mg in that country[29]. In another study reporting the results from four countries for adult males over 19 years of age, the daily B exposure values were reported as 1·04–1·11, 1·61–1·79, 1·89–2·36 and 1·94–2·03 mg/d for the USA, Germany, Mexico and Kenya, respectively[30]. It might be suggested that the variations observed for the different countries probably reflect the differences in diet traditions.

In the present study, a daily B exposure level of 6·77 (SE 0·47) mg/d was found for males living in İskele and Osmanlı villages of Bigadiç County in the province of Balıkesir. Şaylı *et al.*[21] called these towns 'natural human boron laboratories' as they are situated on large colemanite and ulexite deposits. In group II (controls), the daily B exposure was found to be 1·26 (SE 0·10) mg/d, and per kg of body weight it was calculated as 0·017 (SE 0·001) mg/d. When these values are considered, it can be concluded that group I subjects have been exposed to B by a factor larger than 5 (6·77/1·26) compared with controls. Therefore, this clearly demonstrates that subjects living in a B-rich mining area for almost all their lives (considering the ages and number of years of residence) are under chronic long-term B exposure. As there were no reported health problems of subjects in group I, as well as no accumulations of death due to certain diseases or a specific cause which may be linked to high B exposure, these values may be considered as safe levels for humans. Nielsen[31] concluded that – despite the lack of biochemical data – 1–13 mg B/d might be considered as a safe level for both humans and animals.

The main sources of B are drinking water and consumption of locally grown agricultural products[15,21]. In fact, determination of B in agricultural products from this area gave relatively high concentrations[32]. Additionally, since B mines are on the surface and open, exposure through inhalation is also highly possible.

The relevant figure for the control group II in Turkey is reasonable compared with the values given in the aforementioned countries, lying somewhere in between the numbers in the USA and Germany. Since some of our subjects in control group II are from Ankara – the capital, where there is a high rate of population mobility from all other regions – it would not be unreasonable to suggest that the value found for this group, 1·256 (SE 0·104) mg/d, may be a representative figure for the whole country.

B homeostasis is regulated by the urinary system and, regarding the B exposure studies based on urinary sampling, a range of 0·35–10·0 mg B/d is considered as a good functional level for the kidneys, and no correlation between B and calcium clearance has been reported[6].

Previous studies have reported that B is not accumulated in soft tissues but only a small fraction is found in bones[33]. In our study, no correlation has been found between daily B exposure and the number of years of residence in group I. Considering that the male subjects were all adults over the age of 20, it can be proposed that any accumulation of B in bones would be at a negligible level when compared with adolescent subjects. Therefore, the impression obtained from our results is that B accumulation in soft tissues is negligible for the levels reported here; this is in agreement with the results of Murray[33].

A correlation has been found between the daily exposure level, 1·256 (SE 0·104) mg/d, and age for the control group II as given in Table 2. This finding demonstrates that subjects living elsewhere other than B mining region are not under continuous and significant B exposure, and their B exposure decreases with age.

In conclusion, for the male subjects living in B-rich area, a daily exposure level of 6·768 (SE 0·473) mg/d, and per kg of body weight of 0·093 (SE 0·006) mg/d was calculated for İskele and Osmanlı villages in Turkey. In the controls, these figures were 1·256 (SE 0·104) and 0·017 (SE 0·001) mg/d. The results of this study are expected to contribute to creating a reference value for a safe daily B exposure.

Table 2. Pearson correlation coefficients among some traits for each group

	Group	Age	Residency period	B (mg/d)
Residency period (years)	I	0·852**		
	II	0·625**		
B (mg/d)	I	0·042	0·107	
	II	−0·380**	−0·170	
B (mg/kg)	I	0·036	0·088	0·949**
	II	−0·396**	−0·181	0·964**

**P<0·01.

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References

1. Barranco WT & Eckhert CD (2004) Boric acid inhibits human prostate cancer cell proliferation. *Cancer Lett* **216**, 21–29.

2. Cui Y, Winton MI, Zhang ZF, Rainey C, Marshall J, De Kernion JB & Eckhert CD (2004) Dietary boron intake and prostate cancer risk. *Oncol Rep* **11**, 887–892.
3. Yazbeck C, Kloppmann W, Cottier R, Sahuquillo J, Debotte G & Huel G (2005) Health impact evaluation of boron in drinking water: a geographical risk assessment in Northern France. *Environ Geochem Health* **27**, 419–427.
4. Rainey CJ, Nyquist LA, Christensen RE, Strong PL, Culver BD & Coughlin JR (1999) Daily boron intake from the American diet. *J Am Diet Assoc* **99**, 335–340.
5. Hunt CD (1998) Regulation of enzymatic activity: one possible role of dietary boron in animals and humans. *Biol Trace Elem Res* **66**, 205–225.
6. Sutherland B, Leslie R, Woodhouse PS & Janet CK (1999) Boron balance in humans. *J Trace Elem Exp Med* **12**, 271–284.
7. World Health Organization (1996) *Trace Elements in Human Nutrition and Health*. Geneva: WHO.
8. World Health Organization (1998) *Boron. World Health Organization, IPCS (Environmental Health Monograph) 204*. Geneva: WHO.
9. Devirian TA & Volpe SL (2003) The physiological effect of dietary boron. *Crit Rev Food Sci Nutr* **43**, 219–231.
10. Penland JG (1994) Dietary boron, brain function, and cognitive performance. *Environ Health Perspect* **102**, 65–72.
11. Hunt CD, Herbel JL & Nielsen FH (1997) Metabolic responses of postmenopausal women to supplemental dietary boron and aluminum during usual and low magnesium intake: boron, calcium, and magnesium absorption and retention and blood mineral concentrations. *Am J Clin Nutr* **65**, 3803–3813.
12. Chapin RE, Ku WW, Kenney MA, McCoy H, Gladen B, Wine RN, Wilson R & Elwell MR (1997) The effects of dietary boron on bone strength in rats. *Fundam Appl Toxicol* **35**, 205–215.
13. Newnham RE (1994) The role of boron in human nutrition. *J Appl Nutr* **46**, 81–85.
14. Barranco WT & Eckhert CD (2006) Cellular changes in boric acid-treated DU-145 prostate. *Br J Cancer* **94**, 884–890.
15. Korkmaz M, Uzgoren E, Bakırdere S, Aydın F & Ataman OY (2007) Effects of dietary boron on cervical cytopathology and on micronucleus frequency in exfoliated buccal cells. *Environ Toxicol* **22**, 17–25.
16. Heindel JJ, Price CJ, Field EA, Marr MC, Myers CB, Morrissey RE & Schwetz BA (1992) Developmental toxicity of boric acid in mice and rats. *Fundam Appl Toxicol* **18**, 266–277.
17. Price CJ, Marr MC, Myeos CB, Seely JC, Heindel JJ & Schwetz BA (1996) The developmental toxicity of boric acid in rabbits. *Fundam Appl Toxicol* **34**, 176–187.
18. Ku WW, Chapin RE, Wine RN & Gladen BC (1993) Testicular toxicity of boric acid (BA): relationship of dose to lesion development and recovery in the F344 rat. *Reprod Toxicol* **7**, 305–319.
19. Weir RJ & Fisher RS (1972) Toxicologic studies on borax and boric acid. *Toxicol Appl Pharmacol* **23**, 351–364.
20. Moore JA, with an Expert Scientific Committee (1997) An assessment of boric acid and borax using the IEHR evaluative process for assessing human development and reproductive toxicity of agents. *Reprod Toxicol* **11**, 123–160.
21. Şaylı BS, Tüccar E & Elhan AH (1998) An assessment of fertility in boron-exposed Turkish subpopulations. *Reprod Toxicol* **12**, 297–304.
22. Şaylı BS (1998) An assessment of fertility in boron-exposed Turkish subpopulations 2: evidence that boron has no effect on human reproduction. *Biol Trace Elem Res* **66**, 409–422.
23. Şaylı BS (2001) An assessment of fertility in boron-exposed Turkish subpopulations 3: evaluation of fertility among sibs and in 'borate families'. *Biol Trace Elem Res* **81**, 255–267.
24. Şaylı BS (2003) Low frequency of infertility among workers in a borate proccessing facility. *Biol Trace Elem Res* **93**, 19–29.
25. Tüccar E, Elhan AH, Yavuz Y & Şaylı BS (1998) Comparison of infertility rates in communities from boron-rich and boron-poor territories. *Biol Trace Elem Res* **66**, 401–407.
26. Beattie JH & Peace HS (1993) The influence of a low-boron diet and boron supplementation on bone, major mineral and sex steroid metabolism in postmenopausal women. *Br J Nutr* **69**, 871–884.
27. Pahl VM, Culver BD, Strong I, Murray JF & Vaziri DN (2001) The effect of pregnancy on renal clearance of boron in humans: a study based on normal dietary intake of boron. *Toxicol Sci* **60**, 252–256.
28. Yamane T (1967) *Elementary Sampling Theory*. New York: Prentice-Hall.
29. Meacham SL & Hunt CD (1998) Dietary boron intakes of selected populations in the United States. *Biol Trace Elem Res* **66**, 65–78.
30. Rainey CJ & Nyquist LA (1998) Multicountry estimation of dietary boron intake. *Biol Trace Elem Res* **66**, 79–86.
31. Nielsen FH (1998) The justification for providing dietary guidance for the nutritional intake of boron. *Biol Trace Elem Res* **66**, 319–330.
32. Şimşek A, Velioğlu SY, Coşkun LA & Şaylı BS (2003) Boron concentrations in selected foods from borate-producing regions in Turkey. *J Sci Food Agric* **83**, 586–592.
33. Murray FJ (1998) A comparative review of the pharmacokinetics of boric acid in rodents and humans. *Biol Trace Elem Res* **66**, 331–341.