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DIETARY EVALUATION OF TOXIC ELEMENTS THROUGH INTEGRATED DIET

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Several potentially toxic trace elements, namely, Hg, Pb, Cd, As, Sb, Br and Se have been measured by INAA in combination with AAS techniques in the integrated diet representative of the inhabitants of Gujranwala, a highly industrialized city of Pakistan. The dietary intake values for these elements have been estimated from the prevailing concentration level in the summer and winter season diets, which reveals that present intake data are well within the reported WHO values and can be considered to be safe. Possible sources of food contamination by the toxic elements and their adverse impacts on human health are briefly discussed.

Introduction

Rapid establishment of diversified industries to provide various amenities of life are responsible for global indiscriminate pollution of biosphere and subsequent contamination of human food chain resources with harmful chemicals and toxic heavy metals. Being biologically undegradable and incompatible even at trace level with normal biochemical functions in human body, exposure of vital organs such as brain, nervous system, kidneys, liver, intestinal tract, lungs, etc., to these metals are likely to induce physiological disorder in a number of ways.[1,3] In order to get minimal adverse impact, it is important to measure and continuously monitor their levels in various environmental samples with particular emphasis on various food items. Food due to the introduction of mechanized farming, ever increasing use of chemicals, sprays, preservatives, food processing, canning etc., are likely to be further contaminated with the trace toxic elements. Gravity of this situation has attracted attention of national institutes in many countries and international organizations towards adopting measures for their effective control. Prior to any attempt on their control, it is necessary to define the safe levels in individual as well as integrated human foods and to estimate the dietary intake values. These objectives can be achieved by analyzing the trace elements in individual food items or determining them in an integrated diet prepared from basic food items. This approach though time consuming, is helpful in identifying the extent of toxic elemental

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contamination in individual food samples. However, later approach is suitable for obtaining necessary trace element data in the shortest possible time. Consequently, a study was undertaken to prepare integrated diets of various cities of Pakistan and to determine the dietary status of toxic trace elements. Previously, an integrated diet representative of a non-industrialized city, Islamabad was reported.[4] Similar IAEA co-sponsored studies were undertaken to determine the levels of nutrients and other trace elements in the total diets of USA, Canada, Sweden, Spain, Italy, Turkey, Iran, China, Thailand and Sudan.[5] This work describes the concentration level of such elements in the integrated diet of a highly industrialized city of Pakistan, Gujranwala.

Experimental

Preparation of the integrated diet

A comprehensive survey was conducted to assess general dietary pattern of the residents of Gujranwala in summer and winter seasons which revealed that the basic food items of daily use comprise mainly rice, wheat, pulses, vegetables, fruits, mutton, beef, poultry, dairy products, etc. (Table 1). Appropriate quantities of these items were collected from various farms and groceries across the city. The samples were washed with normal water, packed in polyethylene bags and brought for further processing to NAA laboratory at PINSTECH. In order to avoid chances of contamination from exposure of food sample to laboratory environment, all the food items were handled in a laminar flow box (Kotterman R. model 8580). Samples with high water content such as fruits, vegetables, dairy products, meat etc., were sequentially freeze-dried (BETA–A Christ) for 48 to 72 hours. All the samples were pulverized separately in a grinder with PTFE coated blades to minimize Fe, Cr and Ni contamination.

Based on the market basket survey and taking into consideration, as far as possible, nutritional habits, appeal and weekly requirements, four parallel average integrated diets (Table 2) representative of Gujranwala residents were prepared by blending 37 dried basic food items presented in Table 1. The prepared diets were filled in precleaned screw capped polyethylene bottles and placed in a freezer unless and until used for analysis.

Preparation of standard

Utilization of presently available wide range of reference materials[6] as standard is limited.[7] Therefore, a multi-element comparison basis and calibration solutions were prepared for NAA and AAS, respectively. The comparison basis was prepared by dissolving optimum quantity of specpure elemental salts in appropriate purified aqueous acids. 100 µl of the aliquot was dried on filter paper and used as standard after accounting

Table 1
Botanical name of common food items of the Gujrawala inhabitants

Sample, No.	Common name	Botanical name
1	Wheat	Triticum Vulgare
2	Rice	Oryza Sativa
3	Pulses	
(a)	Moong	Phaseolus Munga
(b)	Masoor	Lens Esculenta
(c)	Mash	Phaseolus Radiatus
(d)	Gram	Cicer Arietinum
4	Vegetables	–
(a)	Pumpkin	Cucurbita Pepo
(b)	Tinda	Citrulus Vulgaris
(c)	Zukini	Brassica Juncea
(d)	Arum	Zantedeschia
(e)	Potato	Solanum Tubirosum
(f)	Bitter gourd	Memordica
(g)	Brinjal	Solanum Melongena
(h)	Turnip	Brassica Rapa
(i)	Peas	Pisum Sativum
(j)	Carrot	Daucus Carota
(k)	Radish	Raphanus Sativus
(l)	Cabbage	Brassica Oleracca Var-capitala
(m)	Tomato	Lycoperscon Esculentum
(n)	Onion	Allium Cipa
(o)	Ginger	Zingiber Officinate
(p)	Garlic	Allium Sativum
(q)	Spinach	Spinacia Oleracea
(r)	Sweet Potato	Ipomoea Batatas
(s)	Mustard Leaves	Brassica Compestris
(t)	Corriander Leaves	Coriandrum Sativum
(u)	Green Chillies	Capsium Frutescins
5	Fruits	
(a)	Mango	Mangifera India
(b)	Pear	Pyrus Communis
(c)	Banana	Musa Sepientum
(d)	Plum	Prunus Domestica
(e)	Orange	Citrus Ovnantium
(f)	Kino	Citrus Ovnantium
(g)	Apple	Pyrus Malus
(h)	Guava	Psidium Guajava
6	Spices	
(a)	Red pepper	Capsium Frutescins
(b)	Corriander	Corriandrum Sativum
(c)	Termeric	Curcuma Domestica
(d)	Black pepper	Piper Nigrum
(e)	Clove	Eugenia Caryopyllata
(f)	Cummin seed (white)	Cuminum Cyminum
(g)	Caraway seed (black)	Carum Carvi
(h)	Ammomum	Ammomum Subulatum

Table 2
Weekly intake of basic food items of the adult popuplation of Gujranwala (g/person)

Sample, No.	Food items	Summer season		Winter season	
	Common name	Wet wt.	Dry wt.	Wet wt.	Dry wt.
1	Wheat	845	779	1024	962
2	Rice	731	674	733	676
3	Pulses				
(a)	Moong	41	37.8	36	33
(b)	Masoor	49	45.2	31	28
(c)	Mash	44	40.6	51	47
(d)	Gram	124	115	83	76
4	Sugar	284	256	377	338
5	Beef	47	12.7	31	8.5
6	Mutton	383	103	464	125
7	Chicken	87	23	202	54
8	Egg	181	49	287	78
9	Vegetables				
(a)	Pumpkin	373	25.5	–	–
(b)	Tinda	85	6.3	–	–
(c)	Zukini	114	9.4	–	–
(d)	Arum	34	7.9	–	–
(e)	Potato	69	24.4	324	115
(f)	Bitter gourd	173	18.7	–	–
(g)	Brinjal	149	23.9	124	17
(h)	Turnip	–	–	223	20.4
(i)	Peas	–	–	324	92
(j)	Carrot	–	–	409	50
(k)	Radish	–	–	291	22.2
(l)	Cabbage	–	–203	17	
(m)	Tomato	180	14.2	268	21.3
(n)	Onion	187	29.0	349	54
(o)	Ginger	13	0.2	39	0.6
(p)	Garlic	13	6.2	45	21
(q)	Spinach	–	–	209	27
(r)	Sweet potato	–	–	44	13
(s)	Mustard leaves	–	–	136	17.5
(t)	Corriander leaves	–	–6	0.7	
(u)	Green chillies	–	–	6	0.8
10	Fruits				
(a)	Mango	1640	21.8	–	–
(b)	Pear	125	1.3	–	–
(c)	Banana	700	202	560	162
(d)	Plum	164	1.4	–	–
(e)	Orange	–	–654	8.7	
(f)	Kino	–	–	509	6.7
(g)	Apple	–	–197	37	
(h)	Guava	–	–	110	22
11	Milk	2930	410	2816	394
12	Edible fats	496	486	496	486
13	Spices	15	15	15	15

for the contribution of the blank filter paper in the relative mode of NAA. Likewise, standard stock and blank solutions for AAS were prepared under indentical conditions and fresh working calibration solutions were always obtained from serial dilution of the stock solutions.

Irradiation and radioassay

Prior to the sampling, the irradiation vials made of silica were leached with hydroflouric acid to remove surface trace elements followed by washing with purified nitric acid and bidistilled deionized water. About 250 mg of the diet sample, the synthetic standard and the reference materials, NBS Bovine Liver SRM-1577a used for quality assurance of the analytical method were sealed separately in precleaned vials and placed into aluminium containers, cold welded and irradiated in the reactor. This is a 5 MW swimming pool type research reactor (PARR–I) fueled with 90–93% enriched uranium alloy having a thermal flux of $2 \cdot 10^{13}$ n $\cdot$ cm$^{-2} \cdot$ s^{-1}. Following an appropriate cooling period, the samples were transferred into preweighed polyethylene capsules and reweighed.

The radioassay of the samples was carried out in a computerized γ-ray spectrometry system consisting of a high purity germanium detector (Intertechnique, resolution 1.9 keV with respect to 1332.5 keV full energy peak and peak to compton ratio of 1:50). CANBERRA spectroscopy amplifier (model 2010), 8k series 85 multichannel analyzer and VAX A/780 computer.

Optimum INAA experimental conditions based on earlier experience[7,8] were employed for the measurement of trace elements under consideration. After 6 hours irradiation and a cooling period of 3 days, As and Br were analyzed in the diet samples. The same samples were further cooled for 2 weeks and Hg and Se were determined.

Elemental analyses by AAS

For the analyses of Cd and Pb, diet samples weighing 0.1 g along with 5 ml of purified nitric acid were taken into 100 ml flasks fitted with 30 cm long air condensers and heated at 80 °C for 30 minutes. After cooling 2 ml of perchloric acid (70%) was added and then heated again at 250 °C with occassional shaking till white fumes evolved. The clear solution was brought to aqueous medium before analysis by AAS.[9]

Results

In order to check the blending procedure, 4 parallel diet composites were prepared and the homogeneity was monitored via analysis of Mn and K contents. Coefficient of variation (V_c, %) for Mn and K ranged from 2.6 to 3.1, indicating the diet was fairly homogeneous. Thereafter, concentration of Hg, Pb, Cd, As, Se, Sb and Br were measured with INAA and AAS techniques. The measured value reflects the prevailing concentration level of the toxic elements in the integrated diet samples for the residents of Gujranwala during summer (Table 3) and winter (Table 4) seasons. Based on the

Table 3
Trace element concentrations in summer season integrated diet. Concentration expressed on dry weight basis in ng/g; four parallel analyses of diet.

Element	No. 1	No. 2	No. 3	No. 4	Mean	Variation coefficient* (V_c), %
Hg	6.1	5.4	6.2	7.6	6.3	14.6
Cd	173	164	197	190	181	8.4
Pb	384	401	376	386	387	2.7
As	510	560	487	549	526	6.4
Sb	2.6	2.6	3.1	2.9	2.8	8.7
Br, µg/g	9.8	10.8	10.6	10.0	10.3	4.6
Se, µg/g	0.22	0.27	0.21	0.24	0.23	11.2

*The coefficient of variation reflects the homogeneity of blending of food items.

Table 4
Trace element concentrations in winter season integrated diet. Concentration expressed on dry weight basis in ng/g, four parallel analyses of diet

Element	No. 1	No. 2	No. 3	No. 4	Mean	Variation coefficient (V_c) %
Hg	6.7	5.9	5.3	6.2	6.0	10.0
Cd	125	118	137	107	122	8.9
Pb	160	200	190	240	197	14.4
As	606	593	639	564	600	4.5
Sb	2.8	2.5	3.0	3.1	2.9	8.0
Br*	8.8	8.5	7.9	8.1	8.3	4.2
Se*	0.1	0.23	0.21	0.25	0.22	8.6

*The coefficient of variation reflects the homogeneity of blending of food items.

Table 5
Validation of analytical methods used via analysis of US NBS Bovine Liver (SRM-1577a).
Concentration expressed in ng/g unless otherwise specified

Element	Our value	NBS value	Deviation, %
Hg	4.4 ± 0.5	4 ± 2	+10
Cd	411 ± 35	440 ± 60	–6.6
Pb	143 ± 10	135 ± 15	+2.9
As	51 ± 3	47 ± 6	+8.5
Sb	3.9 ± 0.5	(3)	+30.0
Se, μg/g	0.75 ± 0.06	0.71 ± 0.07	+5.6
Br, μg/g	9.4 ± 0.4	(9)	+4.4

Data in parentheses are non-certified values.

Table 6
Results of external quality assurance program for the execution of research contract organized by IAEA. Concentration expressed in ng/g unless otherwise specified

Element	Sample STD-1		Sample STD-2		Sample STD-3	
	Our value	IAEA	Our value	IAEA	Our value	IAEA
Hg	8 ± 2	6 ± 0.7	310 ± 30	280 ± 10	550 ± 60	470 ± 20
As, μg/g	0.43 ± 0.11	0.41 ± 0.05	7.0 ± 0.2	6.7 ± 0.6	2.8 ± 0.1	2.6 ± 0.1
Sb	–	–	80 ± 15	70 ± 30	–	5 ± 1
Br, μg/g	0.98 ± 0.09	(1)	950 ± 20	–	21.9 ± 1.5	–
Se, μg/g	0.48 ± 0.10	0.4 ± 0.1	2.8 ± 0.06	3.0 ± 0.2	1.9 ± 0.1	1.7 ± 0.3

Data in parentheses are non-certified values.

Table 7
Estimated weekly intake of toxic elements from the integrated diet of Gujranwala. Amount expressed on dry weight basis mg/person

Element	Estimated intake		Reported values[1,8,9,24]
	Summer season	Winter season	
Hg*	20.2	24.3	300
Cd*	622	493	400–500
Pb	1.3	0.77	3.0
As	1.7	2.4	2.8
Sb*	9.0	11.3	–
Se	0.74	0.88	1.54
Br	33.2	33.6	7

*Values in μg/person/week.

present concentration level in the integrated diet, dietary intake was estimated using Tables 2–4 and presented in Table 7 along with the literature values. The precision and accuracy of the method have been verified by replicate analyses (Table 5) of USA National Bureau of Standard, Bovine Liver (SRM-1577a). Further, our results on external quality assurance program of the International Atomic Energy Agency (IAEA) given in Table 6 along with the IAEA values show the reliability of analyses in three different matrices.

Discussion

Mercury

Although selenium interference in mercury analysis can be overcome by radiochemical NAA,[10] the instrumental method as adopted in earlier studies worked satisfactorily.[7,8,11,12] In INAA, contribution of the overlapping peak of ^{75}Se to the 279.2 keV full energy peak of ^{203}Hg was calculated and the necessary correction was applied. The precision of measurement was 11% mainly due to low counting statistics rather than any significant heterogeneity of the sample. The accuracy of Hg analysis by INAA is fairly good as, according to Table 5, our value deviates only +10% from the certified value.

Food contamination of mercury through agricultural practices are alkyl, alkoxyalkyl, aryl and inorganic mercury fungicides used for seed dressing. Compared to these, the amount of Hg entering the agricultural products as a result of various industrial activities is large.[13] Potential industrial mercury sources are chlorine production plants, dentistry, paints, electrical items, etc. Mercury measured in summer and winter season diets is similar. Since irrigation across Gujranwala mainly relies on river and canals, any significant mercury contribution from upstream during summer season would have been

Table 8
Comparison of dietary intake values in different countries (mg/person/week)

Element	Pakistan*	China[23]	Japan[23]	UK[24]	USA[24]	Canada[24]
Hg	0.016	0.078	0.1302	0.035–0.07	–	0.091
Cd	0.456	0.30	0.238	> 0.25	0.35–0.266	0.469
Pb	0.92	0.45	0.595	1.2	0.1–2.0	–
As	1.51	0.384	2.8	–	0.525–0.399	–
Br	27.6	5.4	–	–	–	–
Se	0.7	0.66	0.868	–	–	1.379

*The intake data for Pakistan reflects the median value of this study and earlier work[4].

reflected in the diet sample. Chlorine variation in the diets is insignificant. Therefore, possible Hg sources are mainly through local industrial activities.

Mercury produces chronic as well as acute toxicity. Although known to man for over 2300 years, toxic effect was first noticed in Minamata accident in Japan in 1950. Mercury is known to be toxic in very low quantities[14] and even considered as "zero tolerance" element in food.[15] Prolonged exposure to low levels of mercury is likely to induce nervous disorder and myocardial necrosis,[16] whereas high dose may affect liver, kidney and brain tissues.[17] WHO maximum safe intake limit for mercury is 300 μg per person per week,[18] whereas estimated intake value for the Gujranwala diets ranged from 20 to 24 μg, which is only 8% of the WHO value. Although this diet is fairly safe from Hg intake point of view, it is 3 times higher than in the diet of Islamabad,[4] indicating mercury contamination of food mainly due to industrial activities across the city of Gujranwala. The comparison of our dietary intake value with those of other countries in Table 8 shows that our value of Hg is lower than those from USA, UK, Canada, Japan and China.

Lead

The measurement was carried out by AAS in electrothermal atomization mode. Precision of the measurement was 7% and the mean experimental value deviated only –3% from the NBS certified value, indicating good accuracy of the measurement.

Food contamination by lead is caused by various industrial uses over a long period of time and subsequent dispersion into the environment. Though used for glazing pottery by the Egyptians in ancient times, application in storage batteries, lead paint, cable, antiknock component of gasoline are mainly responsible for food contamination. Lead measured in the diets revealed that contents in summer season is higher by a factor of 2 than in winter season. A similar Pb distribution pattern was also observed in the summer and winter season diet of the residents of Islamabad.[4] The data suggest that local industrial effluents are not the only major contributor to lead contamination.

Lead poisoning may show up in the form of anemia, headache, dizziness, muscle weakness, renal damages, etc.[1] WHO suggested a maximum intake level per week of 3 mg/person,[18] whereas intake calculated for the Gujranwala diets ranged from 0.77 to 1.3 mg/person/week which is higher by a factor of about 2 than that of Islamabad.[4] The comparison of our dietary intake value with those of other countries in Table 8 reveals that our value of Pb is similar to that of UK but higher than those from China and Japan.

Cadmium

Although the radiochemical NAA method can be utilized for Cd analysis in biological and environmental samples,[19] the AAS method developed and applied earlier is simple,

rapid and fairly accurate.[8] In this study Cd was measured with good precision (8.5%) and fair accuracy. As can be seen in Table 5, our mean value deviates –6.6% from the NBS certified value.

Food contamination of cadmium may occur from fertilizers in the form of superphosphate and fungicides. Industrial contamination sources are metallurgical, nuclear, electrical, paint, plastic products, mining, etc. Measured value in the integrated diets revealed that the summer value is 1.5 times higher than that in the winter season, indicating the possibility of a non-local source of Cd contamination in the summer diet.

Cadmium is partially adsorbed and accumulated in the kidneys. Cadmium toxicity may result in vomiting, nausea, diarrhea and high amounts may cause renal damage, which in turn causes proteinuria, glycosuria, aciduria, etc.[1] Dietary assessment of the present diet indicates that this diet contributes up to 500 μg per week, which falls within the safe intake limit of WHO.[18] The comparison of our dietary value with those of other countries in Table 6 indicates that it is higher than those of China, Japan, UK and USA but similar to that of Canada.

Arsenic

INAA was used to determined As via the 559.1 keV full energy peak of ^{76}As. Partial overlapping of 554.3 keV peak of ^{82}Br, due to high count rate, with the peak of interest was overcome by Gaussian fitting of the doublet. The precision of the measurement was 5.9%, whereas deviation of the experimental value from the certified value was +8.5%, showing fair accuracy of the procedure adopted.

Food contamination of As in early days was mainly from food ingredients. Major sources of contamination include use of arsenic compounds as insecticides, herbicides, fungicides and food additives. Arsenic concentration in the Gujranwala diets does not vary in the two seasons, indicating that local industrial activities are mainly responsible for food contamination.

Arsenic was the first element to be noticed as toxic which, above tolerance limit, causes nausea, vomiting, diarrhea, burning of mouth and throat and extensive dosage may cause weakness, prostration, muscular aching, gastrointestinal disorder, etc.[1] The suggested WHO intake per week is 2.8 mg,[18] whereas the study revealed that the estimated intake level for the Gujranwala inhabitants ranged from 1.7 to 2.4 mg. The comparison of dietary intake values in different countries in Table 6 shows that our value for As is higher than those of China and USA, but lower than that of Japan.

Bromine

Bromine was measured by INAA via the 554.3 and 776.5 keV full energy peaks and no interference was observed in the analysis. High counting statistics favored good precision of measurement (4.3%). The accuracy of the analysis was well within precision of the measurement (deviation +4.4%).

Food contamination from agricultural compounds includes methyl bromide and ethylene dibromide used as soil fumigants for controlling insects, weeds and as grain fumigants. Industrial use includes preparation of 1,2-dibromomethane added to gasoline containing tetraethyllead. This forms volatile lead bromide which ultimately escapes from automobile exhaust. Bromine is an essential ingredient in the emulsion of photographic films and it finds applications as hydraulic fluid, gauge and fire fighting fluid. The concentration of bromine in summer and winter season diets does not vary significantly, whereas it is 1.8 times higher than the concentration level of the Islamabad diet. This suggests that Br contamination is mainly through local activity.

Bromine intake at higher level is toxic. Since it is excreted quite slowly from the body, continuous exposure may lead to accumulation in body tissues which causes bromism with symptoms of mental disturbance, impaired memory and though process, drowsiness, etc. WHO maximum permissible intake limit is 1 mg/day/person.[20] The estimated intake is higher by a factor of 5 than the suggested WHO value.

Antimony

Antimony was measured via ^{122}Sb and ^{124}Sb radionuclides by short and long irradiations, respectively, and no interference was observed in the analysis. The precision of the measurement was around 13%, and our data deviate up to 30% from the NBS non-certified value. However, analyses of standards, namely, STD-1 and STD-2, under the IAEA external quality control measure show that our values deviate only 10 to 14% from the IAEA value (Table 6).

The concentration of Sb is similar in winter and summer season diets. The concentration level is also similar to the Islamabad diet, indicating uniform distribution of this element in our environment from where it has been introduced to food cycle.

Antimony is known to be highly toxic and affecting biological systems.[21] Since the chemistry of Sb and As are similar, it is likely that the toxicity of Sb is due to the same factors as that of As.[22] The estimated intake varied from 9 to 11.3 μg/person. This intake level is not different from the diet of the Islamabad area.

Selenium

Selenium was measured by INAA and no interference was observed in 264.7 keV full energy peak of ^{75}Se. Replicate analyses of SRM-1577a showed good precison (8%) and accuracy of the measurement as our value is in fairly good agreement with the certified value (deviation +5.6%).

Food contamination of this element may be caused by the use of Se fungicide. Other contamination sources include many plants which tend to absorb and accumulate large quantities of this element from soil. Industrial sources are glass manufacturing, paint, metallurgy, electronic industries, etc. The measured Se values in the summer and winter season integrated diets of Gujranwala are similar but higher than the values measured in the diets of Islamabad areas. On the basis of these considerations, it can be concluded that food contamination of Se may have occurred due to local industrial activities.

Although Se is an essential element, intake at higher levels produces toxic effects because the protein synthesis system incorporates Se instead of S, thereby removing the sulfhydryl group required for sustaining the oxidative process. Intake at higher levels may cause lassitude, depression, dermatitis, and gastrointestinal disorder.[1] The dietary intake level of Se from winter season diets ranged from 0.74 to 0.88 mg, which is nearly half the value reported for daily requirement[18] and can be considered to be safe and adequate. The comparison of dietary intake values in different countries in Table 6 reveals that our Se intake value is lower than that of Canada but similar to those of China and Japan.

Conclusion

INAA and AAS techniques have been utilized for the determination and study of base line levels of trace elements, namely, Hg, Pb, Cd, As, Sb, Br and Se in the integrated diets of the residents of Gujranwala, a highly industrialized city of Pakistan. The measured value has helped to assess the present dietary intake values for the residents of this city which, on comparison with earlier study,[4] shows that the intake level of Sb is similar. The intake values of Se are higher and those of As, Br, Pb and Hg are much higher in the Gujranwala diet than in the Islamabad diet. However, comparison with the suggested literature values reveals that the present intake data for all the elements studied except for Br are within safe limits.

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