Determination of Various Nutrients and Toxic Elements in Different Brazilian Regional Diets By Neutron Activation Analysis

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(Received November 1996/May 1997)

Summary

The concentration of 19 elements (As, Br, Ca, Cd, Cl, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Rb, Sb, Sc, Se and Zn) was evaluated in some diets taken from different regions of Brazil by Instrumental and Radiochemical Neutron Activation Analysis. Several populations with different socio-economic living conditions and inhabiting in different regions of Brazil were studied in order to estimate and to detect the variability of the mineral and toxic element content among Brazilian populational groups. The data obtained showed a significant difference between the contents of these elements in the diets from the regions studied. The general conclusions from the data obtained in this study were: 1) regarding the daily amounts of essential elements (Ca, Cl, Co, Fe, Mn, Na, K, Fe, Se and Zn), the Santa Catarina 2 diet showed the closest values when compared to the recommended values of RDA (Recommended Dietary Allowance) and/or WHO (World Health Organization). The Santa Catarina 1(low income groups) showed the lowest when compared to the same values. 2) The intake of toxic elements (As, Br, Cd, Hg, Sb) among the diets does not seem to be a major problem when compared to PTWI (Provisional Tolerable Weekly Intake, WHO), except for Hg intakes in regions near gold mining activities, like Manaus and Mato Grosso, where the values found were near the upper limit set by WHO.

Keywords: Neutron activation analysis, toxic elements, mineral contents, daily intake.

Introduction

From a public health point of view, it is important to assure the general population that the intake of all nutrients, including the essential elements, is adequate in the average, normal daily diet. At the same time, the ideal diet should not contain more than the allowed levels of toxic heavy metals(1). There exists a need to develop reliable analytical methods for monitoring diets and foods for as many major, minor and trace elements as possible, whether they are of nutritional or toxicological importance. For the minor elements like Ca, Cl, K, Mg, Na, P and S, which occur in foods at rather high concentrations, there is no methodologic problem for their determination. However, with trace elements the situation is quite different and, from an analytical point of view, they can be

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classified according to Iyengar (2) into the following three subgroups: (a)Those that are easy to determine routinely by various analytical techniques (e.g. Fe and Zn); (b) Those that are not always easy to assay, particularly at low concentrations (e.g. As, Se and Sn), and (c) those that require high levels of analytical expertise (e.g. Cd, Cr, Hg, Mn, Mo, Ni, Pb).

Methods of multielement determination, such as neutron activation analysis(NAA) combined with high resolution gamma ray-spectrometry applying Ge detectors and electronic data evaluation are therefore of particular interest in the area of toxicology and nutrition (3-6), since NAA is a very accurate and precise technique. Neutron activation with radiochemical separation (RNAA) combines characteristics of accuracy and precision with high sensitivity.

During the last years various Brazilian food items such as bread and milk (7), rice (8) and drinking water (9) have been analyzed for their elemental contents by INAA. By means of radiochemical separation various essential and toxic elements have been determined in biological materials (10). In the first part of the present work the elements As, Cd, Cr, Cu, Hg, Sb and Se were determined in diets from different regions of Brazil, by means of radiochemical separation (11).

Continuing to apply nuclear methods and due to lack of data on minor, trace and toxic elemental levels in Brazilian foodstuffs and diets, the same regional diets were analyzed in order to determine the concentration of fifteen elements (Br, Ca, Cl, Co, Cr, Fe, K, Mg, Mn, Na, Rb, Sb, Sc, Se and Zn) by instrumental neutron activation analysis and, consequently, to assess their daily dietary intake in these diets. The procedure developed was applied to the analyses of Total Diet (NBS-SRM 1548) and Citrus Leaves (NBS-SRM 1572) Standard Reference Materials for checking the accuracy and precision of the method.

Diets were prepared by market basket survey or dietary recall data techniques by the Food and Experimental Nutrition Department of the Faculty of Pharmaceutical Science, University of São Paulo.

The purpose of the present work was to evaluate whether the intake of elements among various population groups was safe and adequate. To this end, intake data for essential elements were compared with RDA/WHO values. For evaluating the intake of toxic elements the Joint Expert Committee of the WHO/FAO has recommended the use of PTWI, which was used in the present work.

Materials and Methods

Preparation of diets

The diets represent very different geographical regions in Brazil. Manaus is located in the North, Santa Catarina in the South and Mato Grosso in the central region of Brazil.

Diet of Manaus

This diet was prepared from the data of Shrimpton (12) and obtained by market basket survey. The results of a household expenditure survey conducted in 1.200 families of Manaus city during a period of 1 year were taken into account. This search included the foods bought monthly by these families, based 100 families each month, during 12 months, using methods already known (13).

The Manaus diet is related to low-income groups (up to about US$ 200.00 per family, which corresponds to twice the minimum income) that live in the northern region of Brazil. This diet was prepared from foods produced and consumed in this region and the foods were obtained in the local supermarkets and fairs and prepared according to habits of this region. After preparation, the diet was placed in stainless steel trays and dried in a ventilated oven at 60 °C, for 12 hours or until totally dried. After that the diet was pulverized and homogenized in a stainless steel knife mill and kept at 4 °C before analysis.

Diets from Santa Catarina

These diets were prepared by market basket survey. The Santa Catarina 1 diet is related to low-income groups, while Santa Catarina 2 is related to an average of groups of several incomes, obtained by market basket survey from data of Secretaria da Agricultura of Santa Catarina State. The foods were obtained in the local supermarkets and fairs and prepared according to habits of the region.

Thereafter, the preparation procedure of the diets was as previously described item.

Diet of Mato Grosso

The regional urban diet of Mato Grosso State, located in the central region of Brazil (RUDMT) was made up based on the ENDEF data (14). Food items were pur-

chased from local shops in Cuiabá city, Mato Grosso, Brazil. The description and amounts of these foods items were given in a previous work (15). Thereafter, the preparation procedure of the diet was as previously described in item.

Figure 1 shows the basic composition of the diets analysed in this work.

Determination of macronutrient and fiber

The composition of the diets was determined according to the methods described by the Association of Official Analytical Chemists (16).

The fiber content was determined according to the method described by Asp et al. (17).

Preparation of standards

A multielemental comparison standard for NAA was prepared by weighing out and mixing together appropriate amounts of spec-pure elemental oxide/salts dissolved in nitric medium. Aliquots of 50 µL were dried on Whatman number 42 filter paper and subsequentely used as primary standard. After drying, the standards were introduced into a precleaned 2 cm^2 polyethylene bag which was sealed using a soldering iron.

Irradiation

About 200 mg of the diet samples (weighed in precleaned polyethylene bags), the synthetic standards and the reference material (NBS Total Diet used for quality assurance of the analytical method) were sealed, separately, in precleaned vials, placed into aluminium containers, and irradiated in the IEA-R1 research reactor. Short and long irradiations were implemented depending on the half-life of the radionuclides produced.

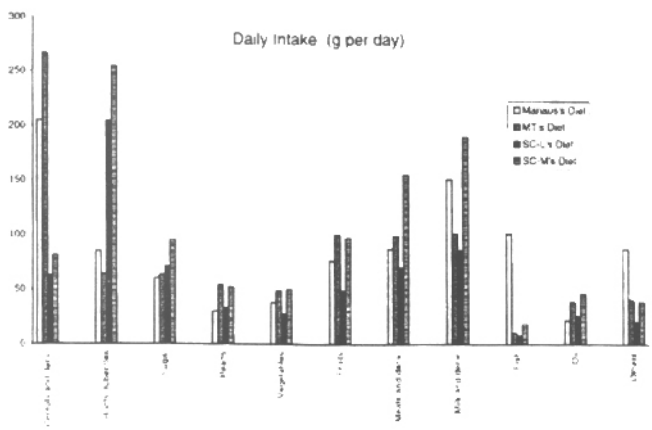

Figure 1. Food composition of the diets studied in the present work.

Short irradiation

This procedure was used for determination of the elements Mg, Mn, Cl, Na and K . In this case, the samples and standards were irradiated for 2 minutes, under a thermal flux of 10^{11} $n.cm^{-2}.s^{-1}$, and the activities were measured after different cooling times.

Long irradiation

The samples and standards were irradiated for 8 h, under a thermal neutron flux of 10^{12} $n.cm^{-2}.s^{-1}$, and after appropriate cooling periods, the following elements were determined: Br, Ca, Co, Cr, Fe, Na, Rb, Sb, Sc, Se and Zn.

Determination of minor and trace elements by INAA

The instrumental neutron activation analysis (INAA) technique was used in this work to determine quantitatively the elements Br, Ca, Cl, Co, Fe, K, Mg, Mn, Na, Rb and Zn in various Brazilian diets.

The NIST Total Diet Standard (SRM-1548) and Citrus Leaves (SRM-1572) were used for checking the accuracy and precision of the method.

Radiochemical separation

In the case of the toxic and some of the essential elements which cannot be determined by INAA, or are very difficult to determine, due to matrix effects and/or spectral interferences, RNAA was used for analysis in these diet samples. The results were completed by RUDMT analysis. The following elements were determined : As, Cd, Cr, Cu, Hg, Sb and Se. The details were presented in a previous work (11).

Measuring systems

Gamma ray measurements of irradiated samples and standards were carried out by using either of two measuring systems:

1.Gamma-X EG&G ORTEC detector coupled to a multichannel EG&G Ortec model 7450 and associated electronics (IBM/PC microcomputer, Monydata model NyDA 200 plus). The resolution of the system is 1.14

keV for the 121.97 keV gamma peak of ^{57}Co and 2.5 keV for the 1332.49 keV gamma ray peak of ^{60}Co.

2. GEM 20190 EG&G ORTEC POP TOP detector coupled to an EG&G ORTEC ACE 4K and associated electronics plus an IBM/PC microcomputer .The resolution of the system is 1.14 keV for the 121.97 keV and 1.94 keV for the 1332.49 keV gamma ray peak of ^{57}Co and ^{60}Co, respectively.

Spectrum analysis was performed by means of the VISPECT2 Software, developed by Dr D. Piccot, from Saclay, France.

Results and Discussion

According to the values presented in Table 1, except for to S. Catarina 1 diet, all other diets showed energy values close to the recommendations of the RDA for females (2090 kcal). However, the energy values were lower than recommended values for males (3150 kcal) for people from 18 to 30 years old (18). The protein level was adequate concerning the requirements of the population studied. Nevertheless, due to the energy deficit , part of the protein might be used as a source of energy, decreasing its use in tissue formation.

Table 1. Values of intake of macronutrients and dietary fiber in the diets studied in the present work

Diets	Energy (Kcal)	Protein (g)	Lipids (g)	Carbohydrate (g)	Fiber (g)
Manaus	1906	86.8	41.7	286	19.7
S.Catarina 1	1475	47.2	32.3	249	16.4
S.Catarina 2	2449	83.9	67.8	376	14.3
Mato Grosso	2100	57.0	51.0	354	17.0

The fat level was quite different among the diets studied, with the higher values being obtained for the diets related to higher income, as had been expected. The fiber content of the diets was close to the values recommended, ranging from 14.3 to 19.7 g (Table 1).

The accuracy and precision of the instrumental neu-

Table 2. Results obtained for multielemental determination in the Citrus Leaves and Total Diet Reference Materials by Instrumental Neutron Activation Analysis

	Citrus Leaves (NBS- SRM 1572)				Total Diet (NBS -SRM 1548)			
Element (mg/kg)	Found 1S.D.	Certified Values (19)	R.S.D. (%)	Relative Error (%)	Found 1S.D.	Certified Values(19)	R.S.D. (%)	R.E. (%)
Ca*	29.55 ± 0.47	31.50 ±1.00	1.6	6.1	1.70 ± 0.09	1.740 ± 0.07	5.5	2.0
Br	8.40 ± 0.04	(8.2)	0.5	-	-	-	-	-
Co**	21 ± 1	(20)	4.8	-	-	-	-	-
Cr	0.81 ± 0.02	0.8 ± 0.2	2.5	1.3	0.14 ± 0.03	-	2.1	-
Fe	90 ± 2	90 ±10	2.2	0	33 ± 2	32.6 ± 3.6	6.1	1.2
Rb	4.8 ± 0.3	4.84 ± 0.06	6.3	0	4.9 ± 0.3	(4.8)	6.1	-
Sc	10.4 ± 0.2	(10)	1.9	4.0	-	-	-	-
Se	-	-	-	-	0.21 ± 0.04	0.245 ± 0.005	19.1	14.3
Zn	28.3 ± 0.3	29 ± 2	1.1	2.4	33.6 ± 0.4	30.8 ± 1.1	1.2	9.1
Na***	0.156 ± 0.016	0.16 ± 0.02	10.3	5.9	5.90 ± 0.06	6.25 ± 0.26	1.1	5.5
K ***	19.40 ± 1.26	18.20 ± 0.60	6.5	6.6	6.08 ± 0.50	6.06 ± 0.28	8.2	0.4
Mn	23.7 ± 0.2	23 ± 2	0.8	3.0	5.2 ± 0.2	5.2 ± 0.4	3.8	0
Mg***	6.40 ± 0.25	5.80 ± 0.30	3.9	10.3	-	0.556 ± 0.027	-	-
Cl***	0.425 ± 0.012	(0.414)	2.8		8.54 ± 0.18	8.70 ± 0.40	2.2	1.8

(*) g/g; (**)µg/kg; (***) mg/g; (-) not determined

tron activation method have been tested by repeated analyses of NBS Total Diet and Citrus Leaves reference materials. The results are given in Table 2 (19). The results obtained using INAA are , in general, in good agreement with literature and reference concentration values. Relative standard deviations and relative errors were below 10%, for both materials, except for the element selenium in Total Diet reference material.

Under the optimized conditions developed, 14 elements were analyzed in 4 different regional diets from Brazil by instrumental neutron activation analysis; the results are presented, on a dry weight basis, in Table 3. Another 7 elements were analyzed by radiochemical neutron activation analysis and have been presented in a previous work (11).

It can be observed that the individual concentration results for these elements showed a significant difference among the diets studied. Nevertheless, the basic composition of these diets, as shown in Figure 1, were some combination of rice and beans, vegetables, fruits, meat or/and fish, eggs and milk.

The Manaus diet was caracterized by low vegetable, low legume, low fruit, high bread, high cassava flour and high fish consumption. The eating habits of this region are quite different from other regions of Brazil like Mato Grosso and Santa Catarina, where there is a high consumption of rice and beans with small amount of protein of animal origin.

From the 19 elements determined in the diets seven are considered as essential trace elements for plant and animal life (Co, Cr, Cu, Fe, Mn, Se, Zn); 4 others (Ca, Cl, K, Na) are also essential for man but present in much higher concentrations than trace elements, while 5 (As, Br, Cd, Hg, Sb) are classified as toxic (2).

Table 4 shows the results obtained for daily dietary intake in various Brazilian Regional Diets, and also a comparison of these results with a standard diet providing 10 MJ (2390 kcal) as well as with the values for recommended daily intake of essential elements by Recommended Dietary Allowance (RDA) from the Food and Nutrition Board, National Academy of Sciences, USA (18) and the World Health Organization (20) and Provisional Tolerable Weekly Intake (PTWI, WHO) for toxic elements.

The West Central Region of Brazil (Mato Grosso) and Northern Region (Manaus) present serious environmental contamination problems resulting mainly from the presence of mercury due to gold mining activities near these regions. The results obtained in this work confirm this situation because, in both cases, the Hg values are close to the upper limit set by WHO (20).

It can be observed that the estimated intake of the toxic element Cd is lower, in all cases, than the PTWI value. The Cd values observed in this study are similar to those

Table 3. Results of minor and trace element determination in Brazilian Regional Diets by Instrumental Neutron Activation Analysis(dry weight)

Element	Manaus diet	S.Catarina 1 diet	S.Catarina 2 diet	Mato Grosso diet
Br (mg/kg)	17.2 ± 0.3 (7)	6.1 ± 0.2 (6)	9.3 ± 0.2 (8)	5.9 ± 0.1 (5)
Cl (g/kg)	12.04 ± 0.65 (5)	6.584 ± 0.334 (6)	5.296 ± 0.041 (2)	11.642 ± 0.538 (7)
Co (µg/kg)	79 ± 12 (6)	36 ± 3 (4)	28 ± 4 (7)	60 ± 10 (4)
Fe (mg/kg)	24 ± 2 (6)	17 ± 1 (8)	20 ± 1 (6)	27 ± 1 (6)
K (mg/kg)	4282 ± 317 (5)	3876 ± 386 (5)	4074 ± 140 (7)	3178 ± 158 (6)
Mg (mg/kg)	561 ± 30 (4)	427 ± 45 (3)	212 ± 16 (2)	416 ± 53 (2)
Mn (mg/kg)	5.9 ± 0.2 (5)	5.7 ± 0.1 (6)	5.3 ± 0.1 (4)	8.4 ± 0.3 (3)
Na (mg/kg)	7738 ± 361 (8)	4140 ±163 (8)	3514 ± 118 (6)	7868 ± 236 (6)
Rb (mg/kg)	13.3 ± 0.4 (6)	9.0 ± 0.4 (8)	13 ± 1 (6)	6.9 ± 0.2 (7)
Sc (µg/kg)	1.0 ± 0.2 (4)	1.8 ± 0.1 (8)	1.39 ± 0.04 (4)	1.8 ± 0.2 (5)
Se (µg/kg)	225 ± 19 (3)	147 ± 11 (4)	246 ± 10 (4)	130 ± 30 (2)
Zn (mg/kg)	17.8 ± 0.6 (6)	14.1 ± 0.5 (6)	17.0 ± 0.6 (7)	21.5 ± 0.8 (7)
As(µg/kg)*	37.2 ± 0.5 (2)	138 ± 5 (2)	259 ± 17 (2)	36 ± 2 (2)
Hg(µg/kg)*	87 ± 9 (2)	19 ± 3 (2)	22 ± 3 (2)	87 ± 4 (2)
Sb(µg/kg)*	7.4 ± 0.1 (2)	9.0 ± 0.5 (2)	10.7 ± 0.9 (2)	7.4 ± 0.5 (2)
Se(µg/kg)*	210 ± 16 (2)	147 ± 11 (2)	241 ± 12	130 ± 16 (2)
Cd(µg/kg)*	<73 (4)	21 ± 4 (4)	24 ± 5 (4)	<73 (4)
Cr(µg/kg)*	970 ± 50 (4)	350 ± 40 (4)	680 ± 10 (4)	960 ± 20 (4)
Cu(µg/kg)*	2420 ± 20 (4)	1870 ± 50 (4)	2100 ± 100 (4)	2500 ± 200 (4)
Ca (%)	0.094 ± 0.014 (3)	0.078 ± 0.006 (8)	0.088 ± 0.005 (6)	0.077 ± 0.034 (5)

( ) - number of determinations ; (*)- results obtained by RNAA (11).

Table 4. Comparison of the Daily Dietary Intake of the elements in various Brazilian Regional Diets with a standard diet providing 10 MJ (2390 kcal) and Recommendations(WHO,RDA).

	Diet of the region (kcal)									
Elements/ Daily Intake	Manaus (1906)	standard	S.Catar. 1 (1475)	standard	S.Catarina 2 (2449)	standard	M.Grosso (2100)	standard	WHO (20)	RDA (18)
Br (mg/d)	7.7	9.7	2.3	3.7	5.4	5.3	2.7	3.1	1(c)	
Ca (mg/d)	423	530.4	289	468	508	496	356	405	400-500	800-1200
Cl (mg/d)	5418	6794	2436	3947	3056	2982	5379	6122		750(d)
Co (µg/d)	35.6	44.6	13.3	21.6	16.2	15.8	27.7	31.5	2	3 (e)
Fe (mg/d)	10.8	13.5	6.3	10.2	11.5	11.2	12.5	14.2	10(male) 20(female)	10(f) 15(g)
K (mg/d)	1927	2416	1434	2324	2351	2294	1468	1671		2000(d)
Mg (mg/d)	252	316	158	256	122	119	192	219	300	350 (f) 280 (g)
Mn (mg/d)	2.7	3.4	2.1	3.4	3.1	3.0	3.9	4.4	2 -3	2.0 - 5.0
Na (mg/d)	3482	4366	1532	2482	2028	1979	3635	4137		500(d)
Rb (mg/d)	6.0	7.5	3.3	5.3	7.5	7.3	6.0	6.8		
Sc (µg/d)	0.45	0.56	0.7	1.1	0.8	0.78	0.64	0.73		
Se (µg/d)	101	126.6	54.4	88.1	142	138.6	60.0	68.3	70	55 (g) 70 (f)
Zn (mg/d)	8.0	10.0	5.2	8.4	9.8	9.6	9.9	11.3	10-15	15 (f) 12 (g)
As (b) (µg/d)	16.7	20.9	51.1	82.8	149	145	16.6	18.9	130(h)	
Hg (b) (µg/d)	39.2	49.2	7.0	11.3	12.7	12.4	40.2	45.8	43(h)	
Sb (b) (µg/d)	3.3	4.1	3.3	5.3	6.2	6.1	3.4	3.9		
Se (b) (µg/d)	94.5	118.5	54.4	88.1	142	138.6	60	68.3	70	55— 70
Cd (b) (µg/d)	<32.9	41.2	7.8	12.6	13.8	13.5	<33.7	<38.3	60(h)	
Cr (b) (µg/d)	437	548	130	211	392	382.6	444	505		200
Cu (b) (mg/d)	1.09	1.37	0.69	1.11	1.21	1.18	1.12	1.28	3.5	1.5 - 3.0

(a) Recommended dietary allowance or estimated safe and adequate daily dietary intake
(b) Results obtained by RNAA (11)
(c) Br (WHO)= 1 mg Bromide ion per kilogram body weight per day.
(d) Minumum Requirements of Healthy Persons (RDA)(18).
(e) Recommended daily dietary allowances in terms of Vitamin B_{12}.
(f) RDA values for males (18)
(g) RDA values for females (18)
(h) Provisional Tolerable Daily Intake (PTDI) as calculated from the WHO/FAO Provisional Tolerable Weekly Intake for a person with a body weight of 60 kg.

reported by others authors from other countries (21-22).

The total daily dietary intake of arsenic depends, to a great extent, on the amount of seafood in the diet, but also on the group meat, fish and poultry (23). This could explain why the highest As levels were in Santa Catarina diets because this is a coastal region with large consumption of these kinds of foodstuffs (Table 4).

There is no official recommended intake of Co except in terms of Vitamin B_{12}. The estimated intake range obtained in this work (0.013-0.036 mg/day) is very similar to the value obtained for diets of Spain (24).

The Cr content of the diets was very high and we concluded that there was a contamination of the foods which must have occurred during the drying in stainless steel trays or even when the foods were transformed into powder by means of a knife mill and homogenized. Liu and collaborators (25) studied the contamination from the stainless steel blade during homogenization and verified that there was a slight contamination , particularly for the elements Cr, K, Sc and Se. The average contamination ranged from 0.7 to 3 %, except for the 27.3% in the worst case of Cr.

Generally, the results presented by S. Catarina diet, related to a medium-income group, showed total daily amounts of the essential elements (Ca, Cl, Co, Fe, Mn, Na, K, Se and Zn) nearest to the recommended values of the RDA and/or WHO. Values obtained from the S.Catarina 1 diet (related to low-income groups) were the lowest when compared to the recommended values.

Considering a standard diet that provides 2390 Kcal or 10MJ of energy, and a standard quality of the diet, the following conclusions about mineral elements were reached:

Ca: the results of daily intake have increased but they

have not reached the RDA values. The results would be adequate only with regard to WHO recommendation.

Cu: all the results have increased and the values are near the lowest limit established by the RDA; however it is still lower than the WHO recommended value.

Fe: the results of Fe daily intake have also increased but they are still below the RDA and WHO recommendations for women.

Mg: the results of Mg daily intake have increased, however the values were below the RDA and WHO recommendations, except for the Manaus diet.

Se: there has been an increase in the daily intake, mainly for Santa Catarina 1 and Mato Grosso diets, which reached the recommendations set by WHO and RDA.

Zn: all the results have increased and the values are near the lowest limit set by WHO.

In conclusion we could say that even if the individuals took in the recommended quantity of energy, maintaining the same food pattern they would have deficiencies in some of the micronutrients.

Concerning the toxic elements, given the recommended caloric intake we can see there could be a risk of exceeding the limits set by WHO(20), with regard to the PTWI values for Hg (Manaus and Mato Grosso diets) and for As (Santa Catarina diet).

Conclusion

Considering the mineral elements Ca, Cu, Fe, Mg, Se and Zn in Brazilian diets, we were able to verify that all the diets analyzed in the present work have not reached the values recommended by RDA and WHO. If we take a standard diet providing 10MJ, with the same food pattern, there is still a risk of deficiency, meaning there should be some kind of supplementation.

It can be concluded that in the diets analyzed in the present work there is no danger of exceeding the limits for toxic elements (As, Br, Cd, Sb), except for Hg in the Manaus and Mato Grosso diets and As in the Santa Catarina diet.

The results obtained are preliminary and the analyses will be continuied in a more extensive study using the methodology of duplicate portion for collecting the diets, for various groups in the Brazilian population where we can make a more accurate determination of the daily dietary intake.

Acknowledgement

The authors wish to thank the CNPq from Brazil for financial support.

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