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Assessment of daily dietary intake of Hg and some essential elements in diets of children from the Amazon region

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Daily dietary intake of Hg and some essential elements in diets of children from communities in the Jaú National Park, Amazon region, were assessed. Diet samples were analyzed for total Hg content using cold vapor atomic absorption spectrometry and Ca, Fe, K, Na, Se and Zn contents by instrumental neutron activation analysis. The weekly tolerable provisional intake for Hg in the communities studied varied from 13 to 57 μg of Hg per kg of body weight, exceeding the limit of 5 $\mu g \cdot kg^{-1}$ set by the WHO. Comparison of the daily dietary intake values to the new Dietary Reference Intakes (4–8 years), showed prevalence of inadequacy.

Introduction

Mercury and methylmercury (MeHg) contamination in humans and fish and its distribution in water and soil is an environmental problem of increasing concern in the Amazon region. Research groups have evaluated the Hg pollution, contamination sources, and its effects to human health in that area.[1–4]

Hg affects both ecosystems and human health.[5,6] Current investigations have shown that there are also natural sources of Hg contamination in the Amazon ecosystem.[7,8] The high levels in the food chain and its absorption by humans is universally recognized as a potential health hazard. Once Hg is released into rivers, lakes, and other aquatic environments, bacteria are able to transform it into an organic form, highly toxic MeHg, and it moves up the food chain to fish and eventually then to humans. Fish and fish products are usually the dominant source of MeHg in the diet and even the consumption of small amounts of contaminated fish can markedly affect the intake of MeHg in human beings.

Furthermore, Hg and other toxic elements such as Pb and Cd, even in extremely small amounts, can cause poisoning in humans and can be hazardous to the environment. Toxic elements strongly affect some essential element metabolisms, because they compete with these elements with binders in the biological system.[3] Such competition and the combination with binders can have adverse effects in the disposition and homeostasis of essential elements.

Recent studies indicate that the nutritional status of the individual and the dietetic interaction can increase the toxicity of Hg.[9,10] Nutritional status is not only determined by the quality and quantity of food consumed. Nutrition and pollution are interconnected: pollutants in the environment may deteriorate the nutritional status of populations. Poor nutritional status may increase health risks posed by pollutants. The purpose of this study was to assess the daily dietary intake of Hg and some traces of essential elements in diets of preschool children from communities in the Jaú National Park (JNP), in the Amazon region. Hg was determined by means of cold vapor atomic absorption spectrometry (CV/AAS) and the contents of some essential elements (Ca, Fe, K, Na, Se and Zn) were quantified in these samples by instrumental neutron activation analysis (INAA).

Study area

JNP is not only the largest national park in Brazil but also the world's largest rainforest park as well. It is located among Novo Airão and Barcelos counties, 220 km from Manaus, state of Amazonas. JNP protects the total basin of a black-water river (Jaú River), which presents the possibility of natural contamination by Hg. This park represents an important sample of the Amazon ecosystems (Fig. 1).

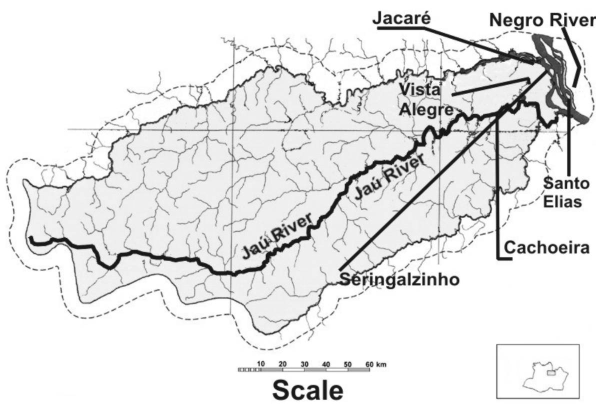

Fig. 1. Jaú National Park and the communities studied

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The inhabitants of JNP, as well as from other parts of the Amazon, are settled at the riverbanks. The proximity of water and forest resources assures the necessary conditions for the subsistence of family groups. Due to the importance of fish in the diet of Amazon populations living along the rivers and floodplain areas, JNP becomes an important area to study the dynamics of Hg and MeHg in the environment, as well as to formulate ways of monitoring and to help those nearby river inhabitants to support themselves appropriately.[11]

Experimental

Sampling location

Diet samples were collected in the following communities of JNP: Cachoeira, Jacaré, Santo Elias, Seringalzinho and Vista Alegre. In order to show the distinction existing between two different ecosystems within the Amazon region, the diets from Itapiranga and Novo Airão counties were also introduced for the sake of comparison. These counties are located near but outside the limits of JNP.

Sampling and sample preparation

Diets were collected in the houses by a 24-hour duplicate portion method, using plastic bags identified with the name of each child. About 5 to 10 individual preschool children's diet (12 to 60 months old) were collected from each community and mixed making a diet pool for each community sampled. As the study was carried out with a low income population, the diet samples collected were exchanged for small market baskets containing rice, beans, cooking oil, sugar and coffee distributed without previous notice to avoid families changing their daily diet. In all counties, the mayors and community leaders were contacted. In general, diets are composed of fish, manioc flour, coffee, rice, bread and some specific fruits and vegetables from Amazon region in different proportions and rarely meat (game meat).

After collection, the diet samples were frozen and transported to the Nutrition Laboratory of INPA (Amazon National Research Institute). The meals were separately weighed, mixed and homogenized in a domestic blender. All diets collected in the same community were mixed together. After this, the diets were dried in a ventilated oven at 60 °C until constant weight and again mixed and homogenized.[12] For chemical analysis the diet samples were sent to the Neutron Activation Analysis Laboratory (LAN/IPEN).

Methodology for total Hg determination – CV/AAS

Approximately 0.5 to 1.0 g of samples or reference materials were digested by a mixture of conc. HNO_3 (low Hg content) and conc. H_2SO_4, both from Merck, in Teflon vials. The vials were closed and left at room temperature overnight. The following day, the vials were put into a digestion block at 90 °C and left there for 3 hours. The vials were cooled to room temperature, 500 μl of 10% $K_2Cr_2O_7$ solution (w/v) was added and then diluted with Milli-Q water into a 50-ml volumetric flask. Total Hg determination was carried out by using FIMS (flow injection mercury system) from Perkin Elmer.

The precision and accuracy of total Hg determination were verified by analysis of reference materials: Fish Tissue (IAEA-407), Orchard Leaves (SRM 1571) and Typical Diet (SRM 1548a).

The limit of detection (LD) obtained in the present work for Hg analysis was determined from the calibration curve, by means of a regression curve in a 95% confidence level.[13] A value of 1 $ng \cdot ml^{-1}$ for the detection limit was found, which is in accordance with values from the literature for Hg determination.[14]

Determination of some essential elements by INAA

Aiming at multielemental analysis, approximately 200 mg of diet (duplicate samples) and about 150 mg of certified reference materials were accurately weighed and sealed in pre-cleaned double polyethylene bags for irradiation. Single and multi-element synthetic standards were prepared by pipetting convenient aliquots of standard solutions (SPEX CERTIPREP) onto small sheets of Whatman No. 41 filter paper. Diet samples, certified reference materials and synthetic standards were irradiated for 8 hours, under a thermal neutron flux of 10^{12} $n \cdot cm^{-2} \cdot s^{-1}$ in the IEA-R1 nuclear research reactor at IPEN.[15] The elements determined by INAA were Ca, Fe, K, Na, Se and Zn.

The precision and accuracy of the method were verified by measuring the certified reference materials Orchard Leaves (SRM 1541), Bovine Liver (SRM 1577b) and Peach Leaves (SRM 1547).

Statistical analysis

A one-factor ANOVA with a 5% Tukey multiple range test was used to evaluate the differences in mean Hg content in diets of the five communities from JNP. In order to verify distribution normality of Hg in the samples, a Shapiro-Wilk test was undertaken. After this procedure, *F*-test was applied, at the significance level of 5%, for homogeneity verification of the variances. All statistical analyses were performed using the statistical *R* programs.[16]

Results and discussion

The precision and accuracy of the methodology for total Hg in children diets were assessed by analysis of reference materials. The results are presented in Table 1. Relative standard deviations varied from 2.6% to 5.0% and the relative errors, from 2.7% to 3.9% showing the precision and accuracy of the analytical method, respectively. For Typical Diet (SRM 1548a), the relative standard deviation was 25% due to the low level of concentration. This material only presents an information value for Hg.

Hg in diets

Total Hg results for the diets from different communities located at JNP are presented in Table 2. The diets of Itapiranga and Novo Airão counties, located in the Amazon region but not at JNP, were also analyzed in order to compare the results obtained for total Hg.

The results showed high levels of Hg, particularly in the communities of Santo Elias (803 μg·kg^{-1}) and Vista Alegre (645 μg·kg^{-1}). For the diets from other regions, Itapiranga (89±9 μg·kg^{-1}) and Novo Airão (71±2 μg·kg^{-1}), much lower levels of mercury were verified.

The results from a one-factor ANOVA with a 5% Tukey multiple range test showed that there is significant statistical difference between the mean Hg content in these communities ($p<0.05$). However, no significant difference was observed for Hg concentration between Cachoeira/Jacaré and Vista Alegre/Seringalzinho ($p>0.05$). All p-values for Shapiro-Wilk test were larger than 0.05, which means that the mercury concentration in each community has normal distribution: Cachoeira (p-value = 0.4391); Jacaré (p-value = 0.8089); Santo Elias (p-value = 0.9069); Seringalzinho (p-value = 0.9383); Vista Alegre (p-value = 0.7861).

After this procedure, F-test was applied, at the level of 5% of significance, for verification of the homogeneity of the variances. The value of p for this test was 0.458, so the hypothesis of homogeneous variances for Hg contents is accepted.

The symmetry of the data and the mean values of Hg concentration in the diets can be observed in Fig. 2. The midline of each boxplot represents the median. The concentrations are different, except for the two pairs Cachoeira and Jacaré, Vista Alegre and Seringalzinho. Santo Elias community presented a considerably larger value than the others. Figure 2 also shows that there are no conflicting values inside each community, or in other words no outliers were observed for the Hg content in these communities.

Figure 3 shows the confidence interval for the difference between the Hg average in the diets of the studied communities. Only two pairs presented the same confidence interval that include zero, which are Cachoeira and Jacaré and Vista Alegre and Seringalzinho. The Tukey test had already confirmed this conclusion, where the Hg content does not differ statistically.

Table 1. Results obtained for total Hg in the reference materials compared to the certified values, as well as relative standard deviation (RSD) and relative error (RE) for the results

Reference materials	Total Hg, μg·kg^{-1} (mean ± s.d.)	Certified value, μg·kg^{-1}	RSD, %	RE, %
Fish Tissue, IAEA-407 (n=3)	228 ± 6	222 ± 24	2.6	2.7
Orchard Leaves, SRM 1571 (n=3)	161 ± 8	155 ± 15	5.0	3.9
Typical Diet, SRM 1548a (n=4)	4 ± 1	(5)	25	–

s.d.: Standard deviation.

Table 2. Total Hg content, Hg daily intake, daily food consumption, and total Hg weekly intake for preschool children's diet from the Jaú National Park (dry weight)

Community (JNP)	Total Hg, μg·kg^{-1} (mean ± s.d.)	Hg daily intake, μg/day	Daily consumption, g	Weekly intake per kg body weight,** μg/kg	PTWI*** intake/kg body weight, μg/kg
Cachoeira (n=3)	380 ± 22	18	47	13	5
Jacaré (n=3)	337 ± 23	61	182	43	5
Santo Elias (n=3)	803 ± 78	81	101	57	5
Seringalzinho (n=3)	571 ± 63	76	134	54	5
Vista Alegre (n=3)	645 ± 39	66	103	46	5
Itapiranga*	89 ± 9	14	153	5	5
Novo Airão*	71 ± 2	7	98	9	5

* Communities located near JNP in the Amazon region.
** The body weight was considered in average as 10 kg for each child.
*** PTWI – provisional tolerable weekly intake (5 μg of Hg per kg of body weight) (WHO, 1996).[19]

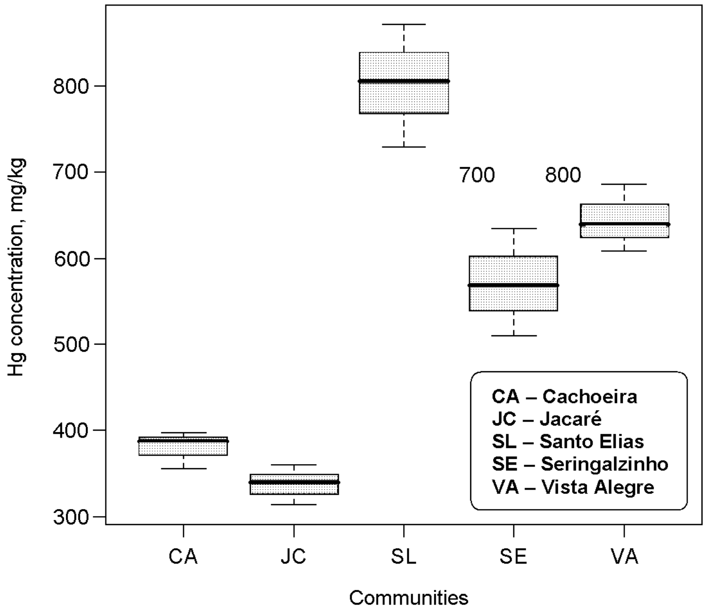

Fig. 2. Diet Hg percentil distribution of some Jaú National Park communities

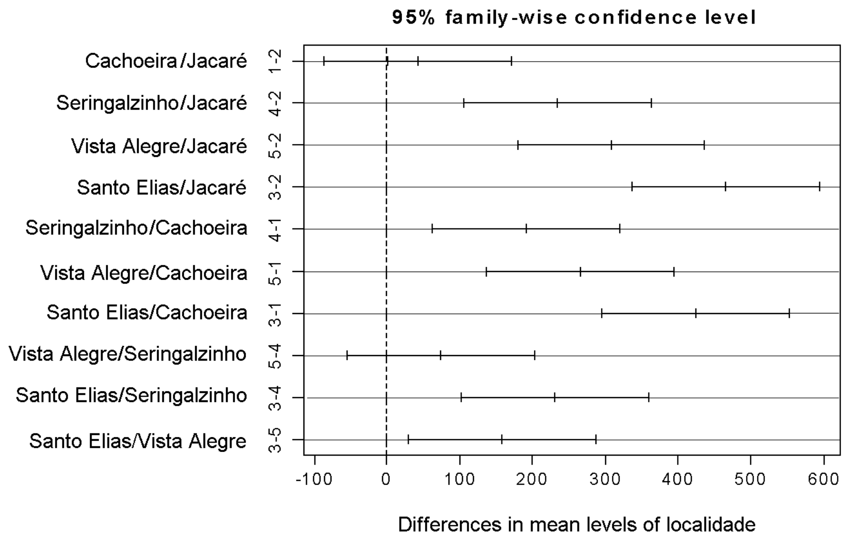

Fig. 3. Confidence interval for Hg average diet difference of the communities studied

Comparing the levels of Hg in diets from different regions of Brazil,[15] such as Santa Catarina 1 (9.0±0.5 μg·kg^{-1}), Santa Catarina 2 (22±3 μg·kg^{-1}), Mato Grosso (87±9 μg·kg^{-1}) and Manaus (87±4 μg·kg^{-1}), with the levels found in the present study, diets on JNP present much higher Hg values. It is important to emphasize that all diets contained fish in their composition, but only Mato Grosso and Manaus diets contained fish that came from the Amazon region and also a larger proportion of fish in the diet.

The World Health Organization,[17–19] through the Codex Alimentarius of FAO/WHO establishes, besides the necessities of some nutrients, the value of provisional tolerable weekly intake (PTWI) or the acceptable daily ingestion (ADI) values for the toxic or potentially toxic elements, such as Al, As, Cd, Hg, Sn and Pb. In the case of Hg, the PTWI value is 5 μg of Hg per kg of body weight weekly.[18] Adults and children are both susceptible to Hg toxicity, but the developing nervous system appears to be much more sensitive. Exposures to Hg during the prenatal period appear to interfere with the growth and migration of neurons, with the potential to cause irreversible damage to the central nervous system.[20]

Table 2 also presents the Hg daily intake for the diets analyzed in the present study. All communities of JNP showed values higher than PTWI. The community Itapiranga showed value close to the limit of 5 $\mu g \cdot kg^{-1}$ considering the average body weight as 10 kg for each child, while the community Novo Airão showed values higher than PTWI, but much lower than the values found for the communities of JNP.

A research shows that adverse effects can be caused by the exposure to low concentration of Hg, and epidemiological studies suggest that subtle effects at the nervous system can be associated to previous exposures that were considered safe.[20] Besides, deficient diets can favor the harmful effects of Hg mainly because this element, as some others, can strongly affect some essential representative metabolism. Some studies suggest also that an appropriate ingestion of Se can have a protective effect against Hg and its poisonous compositions.[21]

Essential elements in the diets

The quality control for the INAA methodology was carried out by analyses of certified reference materials, whose results are in Table 3. The relative standard deviation ranged from 2.4% to 11% and the relative error from 0.8% to 12%, showing good precision and accuracy.

Table 4 shows the concentration values obtained for the diets from the five communities located at JNP. A high variation was observed in the concentration of the essential elements determined in the children diets: Ca (576 to 8275 $mg \cdot kg^{-1}$), Fe (26 to 68 $mg \cdot kg^{-1}$), K (3646 to 12000 $mg \cdot kg^{-1}$), Na (3369 to 14786 $mg \cdot kg^{-1}$), Se (0.21 to 0.29 $mg \cdot kg^{-1}$) and Zn (13 to 29 $mg \cdot kg^{-1}$).

From the concentration values obtained and considering the average daily food consumption for each diet (between 47 and 182 g, dry weight), it was possible to calculate the daily intake for each element (Table 5). Table 5 also shows estimated average requirements (EAR) and adequate intake (AI) values for children (4–8 years).[22,23]

Table 3. Results obtained (in $mg \cdot kg^{-1}$) for the certified reference materials Bovine Liver (SRM 1577b), Orchard Leaves (SRM 1571) and Peach Leaves (SRM 1547), average of five determinations (mean ± standard deviation)

Element	Bovine Liver,		RSD,	RE,	Orchard Leaves		RSD,	RE,	Peach Leaves		RSD,	RE,
	Found	Certified	%	%	Found	Certified	%	%	Found	Certified	%	%
Ca	–	116 ± 4	–	–	17590 ± 1586	20900 ± 300	9	16	15468 ± 1000	15600 ± 200	6	0.8
K, %	1.05 ± 0.03	0.990 ± 0.002	3	6	1.39 ± 0.06	1.47 ± 0.03	4	5	27216 ± 654	24300 ± 300	2.4	12
Fe	–	184 ± 15	–	–	–	300 ± 20	–	–	832 ± 40	870 ± 30	5	4.4
Na	2207 ± 158	2420 ± 60	7	9	88 ± 8	82 ± 6	9	7	–	24 ± 2	–	–
Se	0.69 ± 0.03	0.73 ± 0.06	4	4	0.09 ± 0.01	0.08 ± 0.01	11	10	0.130 ± 0.005	0.120 ± 0.009	3.8	8.3
Zn	111 ± 5	127 ± 16	4	12	28 ± 1	25 ± 3	4	12	18.3 ± 1.5	17.9 ± 0.4	8	2

–: Not determined.

Table 4. Results obtained for the diets (in dry weight) from communities of Jaú National Park, by NAA (in $mg \cdot kg^{-1}$) (mean ± standard deviation)

Element	Cachoeira	Jacaré	Santo Elias	Seringalzinho	Vista Alegre
Ca	4047 ± 250	8275 ± 564	576 ± 89	2388 ± 350	1141 ± 144
Fe	63 ± 2	62 ± 4	26 ± 2	41 ± 10	68 ± 3
K	5834 ± 428	4829 ± 747	5560 ± 378	3646 ± 892	12000± 809
Na	9905 ± 440	12631± 481	3369 ± 44	5940 ± 894	14786± 769
Se	0.25 ± 0.01	0.29 ± 0.01	0.27 ± 0.02	0.22 ± 0.03	0.21 ± 0.02
Zn	24 ± 2	29 ± 6	13 ± 1	15 ± 1	20.4 ± 0.9

Table 5. Values of daily intake of some essential elements in the diet samples and AI and EAR values, all expressed in mg/day, except for Se (in µg/day)

Element	Cachoeira	Jacaré	Santo Elias	Seringalzinho	Vista Alegre	EAR*	AI*
Ca	192	1505	117	320	58	–	800
Fe	3.0	11.2	7.0	6.7	2.6	4.1	–
K	277	974	1235	1642	559	–	3800
Na	470	2297	1521	664	339	–	1200
Se	12	54	22	33	28	23	30
Zn	1.1	5.3	2.1	2.2	1.3	4.0	5.0

* EAR (estimated average requirements) and AI (adequate intake) for children in preschool age (4–8 years).

Daily dietary intake values obtained for each element and their comparison to the AI and EAR values are discussed below.

Calcium: There are no functional criteria for Ca status that reflect response to dietary intake in infants. Thus recommended intakes of Ca are based on an adequate intake (AI) that reflects the derived mean intake of infants fed principally with human milk. The values obtained are below 800 mg recommendation for healthy individuals at this age: C. Cachoeira 192 mg/day, C. Seringalzinho 320 mg/day, C. Santo Elias, 117 mg/day and C. Vista Alegre, 58 mg/day. Ca intake obtained for the population of the Jacaré community was higher than the recommendation (1505 mg/day), but the other communities showed intakes much lower than the recommended ones.[23] From the results, it can be concluded that 80% of the diets showed the prevalence of inadequacy. This can be explained by a low consumption of dairy products by these populations.

Iron: Comparing Fe daily intake obtained in the present work (from 2.6 to 11.2 mg/day) with the EAR values, according to the new DRIs for children (4 to 8 years), 40% of the diets showed the prevalence of inadequacy considering the EAR value (4.1 mg/day). The estimated average requirement (EAR) is the average daily nutrient intake level sufficient to meet the requirement of half healthy individuals in a particular life stage gender group. It can be observed that in the present study the EAR was not reached for Fe intake in the diets from JNP.[22]

Potassium: K requirements have been evaluated only in a few studies, but since this element is a necessary constituent of each body cell, an increase in lean body mass is a major determinant of K needs. For children from 1 to 10 years old 1500 to 2000 mg/day is required.[22] Due to an insufficient data from dose-response trials, an estimated average requirement (EAR) could not be established, and a recommended dietary allowance (RDA) could not be derived, thus recommended intakes of K are based on an adequate intake (AI = 3800 mg/day). The results obtained (from 277 to 1642 mg/day) shows that no community was within the recommended adequate intake, or 100% of the diets showed the prevalence of inadequacy.

A number of dietary factors, including dietary fiber and Na, can affect K balance. Some studies demonstrated that at dietary Na intakes greater than 6.9 g/day (300 mmol/day) there was a net loss of K-urinary excretion.[22]

Sodium: Although it is accepted that Na is essential for human life, there is no agreement for the daily minimum requirement. However, it has been estimated that the daily total intake of 120–400 mg should supply children needs and 500 mg for adults.[22] Results obtained are based on recommended intakes of K or Na on an adequate intake (AI = 1.2 g/day) for children (4–8 years). In the present study only the diets of the communities Jacaré and Santo Elias reached those new recommendations. In this case, 60% of the diets showed the prevalence of inadequacy.

Selenium: The daily intake value obtained for Se varied from 12 to 54 µg/day. According to the new DRIs for children (4–8 years old) 40% of the diets showed the prevalence of inadequacy comparing to the EAR value (23 µg/day). Se on animal tissue presents itself in different quantities as selenomethionine or selenocysteine, better known as selenoprotein. Some of the recognized functions of Se (by means of its association to proteins) include the protection against the oxidative stress, action regulation of the thyroid hormone, regulation of redox status of vitamin C and a protecting paper regarding intoxication with Hg.[22]

Zinc: From the results obtained for Zn intake (from 1.1 to 5.3 mg/day) and EAR (4 mg/day), 80% of the diets showed the prevalence of inadequacy. Digestion process produces the opportunity for Zn to bind to exogenous and endogenous constituents in the intestinal lumen, including peptides, amino acids, nucleic acids and other organic acids and inorganic anions such as phosphate.[22]

Conclusions

INAA technique provided concentration values for elements Ca, Fe, K, Na, Se and Zn important from a nutritional point of view.

The present study concluded, from the obtained results, that the diets of the children living in the JNP are, in general, deficient in the essential elements determined, evidencing the need for a more extensive nutritional study.

Regarding Hg, the weekly intake obtained for the diet samples was close or above PTWI value, contrary to the values for the diets of other Brazilian regions. This was due to the consumption of freshwater fish from Hg contaminated waters. This suggests that further studies are needed as well as clinical evaluation and/or preventive actions. Hg pollution offers a serious risk to health, because it can cause congenital problems, damage to the brain and to the liver that can cause death. Its effects are particularly serious on fetus and small children. The exposure assessment of the Brazilian population – including those with high fish consumption – is needed to provide a full picture of the MeHg and total Hg distribution and exposure both regionally and nationally.

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