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Fish consumption and bioindicators of inorganic mercury exposure

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Abstract

Background: The direct and close relationship between fish consumption and blood and hair mercury (Hg) levels is well known, but the influence of fish consumption on inorganic mercury in blood (B-IHg) and in urine (U-Hg) is unclear.
Objective: Examine the relationship between fish consumption, total, inorganic and organic blood Hg levels and urinary Hg concentration.
Methods: A cross-sectional study was carried out on 171 persons from 7 riparian communities on the Tapajós River (Brazilian Amazon), with no history of inorganic Hg exposure from occupation or dental amalgams. During the rising water season in 2004, participants responded to a dietary survey, based on a seven-day recall of fish and fruit consumption frequency, and socio-demographic information was recorded. Blood and urine samples were collected. Total, organic and inorganic Hg in blood as well as U-Hg were determined by Atomic Absorption Spectrometry.
Results: On average, participants consumed 7.4 fish meals/week and 8.8 fruits/week. Blood total Hg averaged 38.6±21.7 μg/L, and the average percentage of B-IHg was 13.8%. Average organic Hg (MeHg) was 33.6±19.4 μg/L, B-IHg was 5.0±2.6 μg/L, while average U-Hg was 7.5±6.9 μg/L, with 19.9% of participants presenting U-Hg levels above 10 μg/L. B-IHg was highly significantly related to the number of meals of carnivorous fish, but no relation was observed with non-carnivorous fish; it was negatively related to fruit consumption, increased with age, was higher among those who were born in the Tapajós region, and varied with community. U-Hg was also significantly related to carnivorous but not non-carnivorous fish consumption, showed a tendency towards a negative relation with fruit consumption, was higher among men compared to women and higher among those born in the region. U-Hg was strongly related to I-Hg, blood methyl Hg (B-MeHg) and blood total Hg (B-THg). The Odds Ratio (OR) for U-Hg above 10 μg/L for those who ate >4 carnivorous fish meals/week was 4.00 [1.83–9.20].
Conclusion: This study adds further evidence to a positive relation between fish consumption and IHg in both blood and urine, which may result from absorption of IHg from fish or from demethylation of MeHg. The findings support the importance of assessing IHg exposure in fish-eating communities. Further studies should examine the potential toxicity of IHg in heavy fish consumers.

Keywords: Fish consumption; Inorganic mercury exposure; Demethylation; Amazon

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1. Introduction

It is well known that both freshwater and sea fish consumption are directly and closely related to increases of blood and hair mercury (Hg) concentrations (Clarkson, 2002; UNEP, 2002), whereas the influence of fish consumption on urinary mercury levels (U-Hg) is unclear. Recent studies of fish consumers in Europe and North America have revealed significant associations between fish consumption and U-Hg levels (Carta et al., 2002; Apostoli et al., 2002; Levy et al., 2004; Johnsson et al., 2005), and most of these authors suggest that demethylation of methyl mercury (MeHg) in the body may be responsible for these U-Hg levels. This hypothesis is supported by several human and animal studies, as well as recent MeHg toxicokinetic modeling (Bjorkman et al., 1995; Vahter et al., 1995, 2000; Carrier et al., 2001a,b; Young et al., 2001).

In the Amazon, Hg exposure through fish consumption has been the subject of much concern over the last two decades. A large number of exposure assessments of fish-eating populations have been conducted in many parts of the huge Amazon region, using blood and/or hair as biomarkers to study communities environmentally exposed solely through their fish diet (Pinheiro et al., 2006; Dórea et al., 2005; Gonçalves and Gonçalves, 2004; Webb et al., 2004; Passos et al., 2003; Santos et al., 2003; Dolbec et al., 2001; Boischio and Henshel, 2000; Cordier et al., 1998; Lebel et al., 1997). These have consistently shown elevated blood and/or hair Hg concentrations, strongly related to fish consumption. Recent studies indicate that fruit consumption may modulate

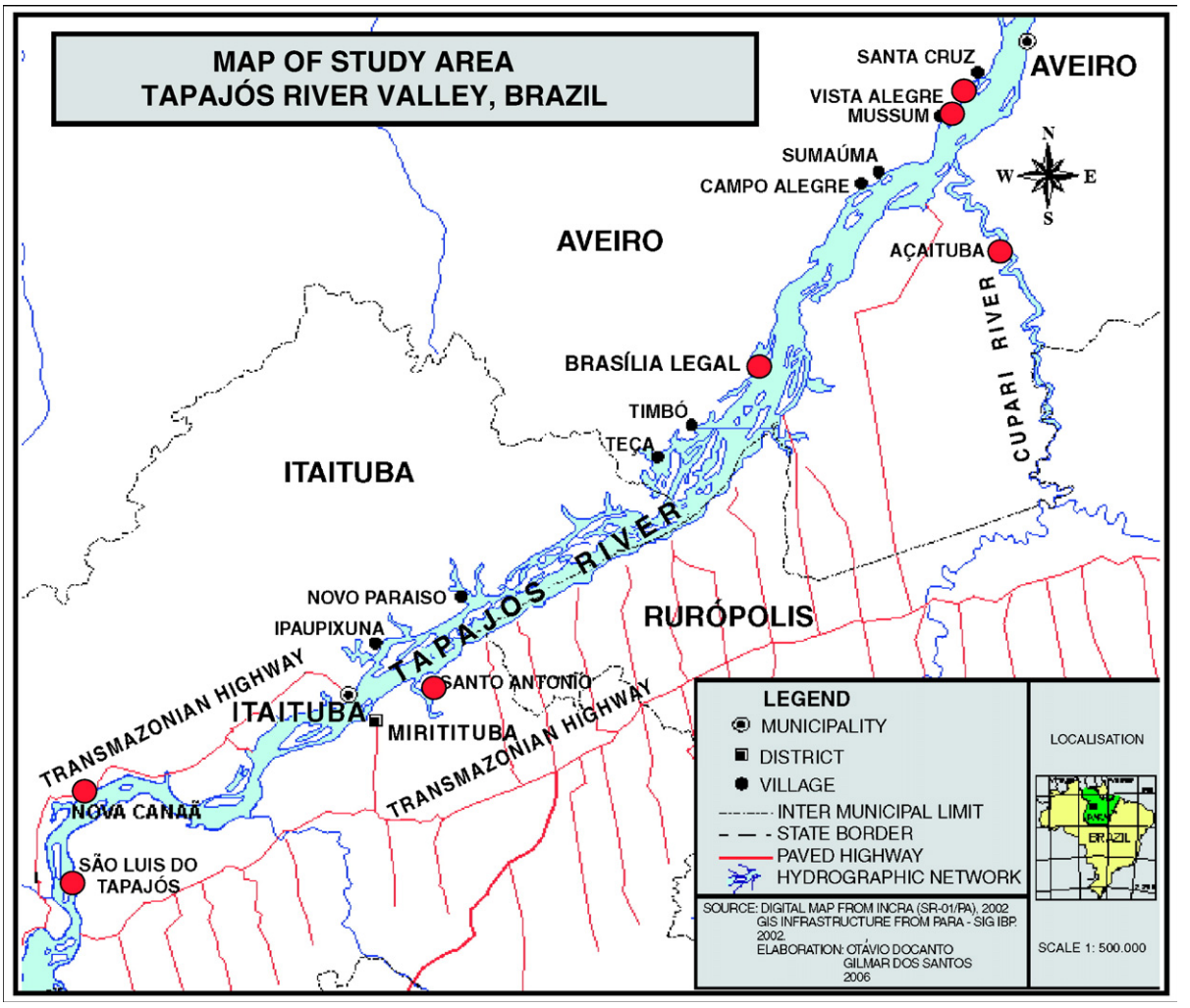

Fig. 1. Map of the study area. Participating communities are identified by a large red dot. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

this relation, with those who eat more fruit presenting lower hair Hg levels for the same amount of fish consumption (Passos et al., 2003, 2004).

While, on average, the major part of total Hg content in fish is in the form of MeHg, the proportion of this chemical species may range from 57% to 100% (Ikingura and Akagi, 2003; Maurice-Bourgoin et al., 2000; Kehrig et al., 1998; Holsbeek et al., 1997; Akagi et al., 1995). Moreover, in fish-eating humans the proportion of MeHg in blood and hair varies between 20% and 100% (Lebel et al., 1996; Lodenius and Malm, 1998; Maurice-Bourgoin et al., 2000; Pinheiro et al., 2000; Barbosa et al., 2001; Dolbec et al., 2001). Despite these variations, most authors gloss over the amount of inorganic Hg or use total Hg as a proxy for MeHg.

The objective of the present study was to examine blood inorganic Hg (B-IHg) and U-Hg and the factors that influence their concentration, among fish-eating communities of the Brazilian Amazon.

2. Methods

2.1. Study design and population

This cross-sectional study was conducted over the months of January and February 2004 (rainy season), among 7 riparian communities situated on the banks of the Tapajós River, a major tributary of the Amazon (Fig. 1). The communities were selected in order to represent the diversity created throughout the colonization process in this region, as some of them were established after colonization began in the early 1960's, whereas others were established up to 100 years previously.

In this area of the Amazon, it is difficult to apply a random sampling strategy. Boats are the only means of transport from one village to another and villagers are spread out over large areas, which require small crafts and/or several hours of walking inland to reach. In addition, in many villages there are evangelical groups whose members systemically refuse to participate. Thus, a convenience sample was used and recruitment was carried out through village meetings and door-to-door invitation. Inclusion criteria for the present study were ≥ 15 years of age, no dental amalgams, not pregnant and no history of exposure to inorganic Hg in gold-mining. Age and sex distributions were then compared to the underlying population, which had previously been determined through a house-to-house survey in each community. Of the 216 who agreed to participate, 171 adults (79.2%) met the criteria. The age distributions and participation rates with respect to the underlying population are presented in Table 1. Distributions were similar for most categories, except for those under 24 and over 65 years, whose rates of participation were relatively lower.

Approval was obtained from Ethics Committees of the Federal University of Rio de Janeiro (Brazil) and the University of Quebec in Montreal (Canada). The study was explained individually, and persons agreeing to participate signed an informed consent form. This study is part of a larger interdisciplinary and ecosystemic investigation examining Hg dynamics in the environment, human exposure and health effects in the Tapajós region (CARUSO, 2006).

2.2. Assessment of fish and fruit consumption

Because of important seasonal differences in the availability of fish species in this region (Lebel et al., 1997; Dolbec et al., 2001; Passos et al., 2001), a seven-day dietary recall questionnaire (7-DDR) was used to determine current fish and fruit consumption frequency. Development and validation studies of this instrument have shown that it is easily administered and constitutes a sensitive method to assess short-term food consumption (Hebert et al., 1997). Our previous studies concur with this assessment. Using this tool, we have found good concordance between seasonal bioavailability of fish species and reported consumption of those fish species (Lebel et al., 1997), as well as between fish consumption frequency and bioindicators of exposure (Dolbec et al., 2001).

A list was prepared which included most of the fish species present in the region. Participants indicated for each day the number of meals containing fish, as well as the name of the fish species that were consumed. Based on the dietary habits of the fish species and the trophic classification proposed by Ferreira et al. (1998), fish were then grouped into carnivorous and non-carnivorous species. As for fruits, the procedure was similar, but in this case, for each fruit species, the participant

Table 1
Age distribution and rates of participation in the study population

Age category	Total adult population	Study population	% participation
15–24	308	25	8.1
25–34	214	39	18.2
35–44	174	36	20.7
45–54	132	31	23.5
55–64	95	30	31.6
≥65	79	10	12.7
Total	1000	171	17.1

indicated the number of fruits that had been eaten each day over the preceding seven days, whether during a meal or not. Here, total fruit consumption over the previous 7 days is used. Fish and fruit species that were not in the initial list were also recorded.

2.3. Sampling and analyses of bioindicators

Urine samples were collected into polypropylene bottles (Nalgene 125 mL # 2104-0004) and then transferred to screw cap tubes with conical base (RPK PPGWB 15 mL, SARSTEDT™) for transport purposes. Samples were kept frozen until analyzed. U-Hg was determined by Cold Vapour Atomic Absorption Spectrometry. Following an acidic mineralization of the Hg present in urine at 50 °C, the resulting solution was diluted and analyzed on the FIMS 100 module from Perkin Elmer (M-568). Urinary Hg concentrations were adjusted for urine density.

Blood samples were collected by a nurse by venipuncture into 6 mL heparinized Becton Dickinson Vacutainer® (BD7863). All blood samples were kept frozen at −20 °C until analyzed. Total and inorganic Hg in blood were determined by Atomic Absorption Spectrometry according to the method described by Ebbestadt et al. (1975), with a detection limit of 0.2 μg/L. B-MeHg was estimated as the difference between T-Hg and B-IHg.

All urine and blood analyses were conducted at the laboratory of the Quebec Toxicology Center of the Quebec Public Health Institute (CTQ-INSPQ), Canada. Analytical quality control was ensured by routine checks of accuracy and precision, using reference materials from CTQ-INSPQ Inter-Laboratory Comparison Program. The CTQ is accredited ISO 17025 and analytical performance for Hg analysis in the Inter-Laboratory Comparison Program for Metals in Biological Media was 36/36 for precision and 6/6 for reproducibility.

2.4. Statistical analyses

Descriptive statistics were used to characterize the study population, fish consumption, as well as Hg exposure. Inter-group comparisons were performed using parametric or nonparametric techniques, depending on data distribution. The relation between fish consumption, fruit intake, socio-demographic variables and the bioindicators of Hg exposure were examined using linear multiple regression models. Where possible, continuous variables were used (age, years of education, number of fish meals and number of fruits). Alcohol consumption, smoking and immigrant status were included as categorical variables. The relation between the bioindicators of exposure was examined using correlational statistics (Spearman's rho), and the risk for having U-Hg levels above 10 μg/L was analysed using logistic regression analyses. Results were defined as statistically significant for a value of $P \leq 0.05$. Analyses were performed using Statview for Windows Version 5.0.1 and Jump 5.0.1a (SAS Institute Inc.).

3. Results

Participants' age ranged from 15 to 77 years (mean 40.2 years±15.1) with the distribution presented in Table 1. Other socio-demographic characteristics are shown in Table 2. Formal education was low, varying between 0 and 11 years (mean: 4.0 years±2.7). Seventy-one percent (71%) of the participants were originally from the Tapajós region, and 72.5% lived on the Tapajós River banks, while the others lived on one of its tributaries.

In this rainy period, 99.5% of the study population consumed at least one meal with fish over the preceding seven days. On average, participants consumed 7.4 fish meals/week, ranging from 0 to 24 meals/week; carnivorous fish made up an average of 65.2% of the fish diet, ranging from 0 to 100%.

Fig. 2 presents the distributions of B-IHg and U-Hg. Blood total Hg averaged 38.6±21.7 μg/L (median: 34.6 μg/L, ranging from 4.2 to 114 μg/L). The average percentage of B-IHg was 13.8%, ranging from 7.4% to 23.4%. Average B-IHg was 5.0±2.6 μg/L (median: 4.7 μg/L, ranging from 0.4 to 14.8 μg/L). B-MeHg and B-IHg were highly correlated ($r=0.84$; $p<0.0001$). The percentage of B-IHg was not correlated to B-IHg levels ($p>0.1$), but was inversely related to B-THg ($r=-0.1$; $p<0.0001$). Average U-Hg was 7.5±6.9 μg/L (median: 5.6 μg/L, ranging from 0.2 to 36.1 μg/L), with

Table 2
Socio-demographic characteristics of the study population

	Women		Men	
	n	%	*n*	%
Schooling				
No formal education	7	7.4	16	21.1
Elementary school (1 to 8 years)	72	75.8	51	67.1
High school and more (≥9 years)	16	16.8	9	11.4
Born in the region (State of Pará)	72	75.8	51	67.1
Village location				
On the Tapajós River	72	75.8	52	68.4
On an affluent	23	24.2	24	31.6
Consumes alcohol	36	37.9	39	51.3
Current smokers	21	22.1	40	52.6

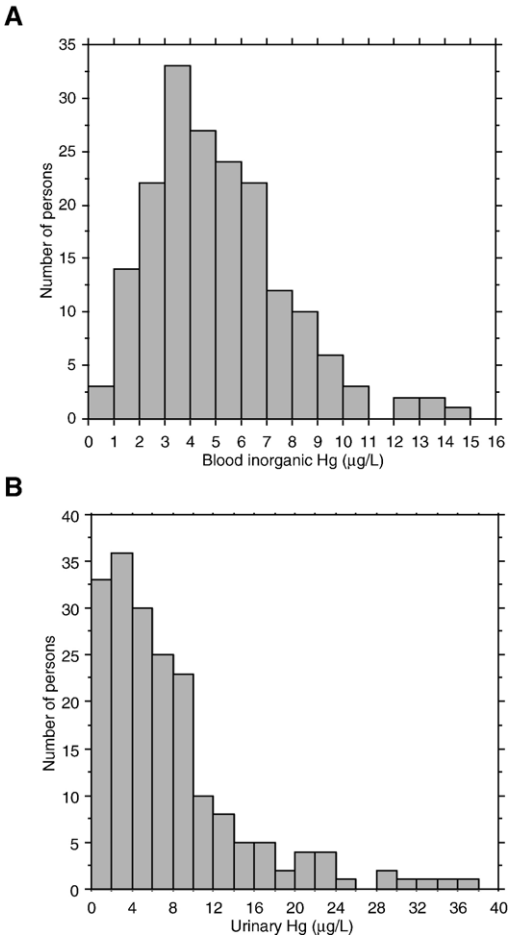

Fig. 2. Distribution of blood inorganic Hg (A) and urinary Hg (B) in the study population.

19.9% of participants presenting U-Hg levels above 10 μg/L, the upper limit for unexposed populations (WHO, 1991).

Table 3
Results of regression analyses for blood bioindicators of Hg exposure

	B-IHg (R^2) of model: 0.36		B-MeHg (R^2) of model: 0.52		B-THg (R^2) of model: 0.50	
	β coeff	p	β coeff	p	β coeff	p
Carnivorous fish	0.37	<0.001	2.25	<0.001	2.62	<0.001
Non-carnivorous	0.10	ns	0.93	0.04	1.03	0.04
Fruit consumption	−0.03	0.05	−0.18	0.04	−0.20	0.04
Age	0.03	0.05	0.05	ns	0.08	ns
Gender	0.05	ns	1.84	ns	1.79	ns
Immigrant	−0.91	<0.01	−7.77	<0.001	8.67	<0.001
Community		0.05		<0.001		<0.001

ns: non-significant.

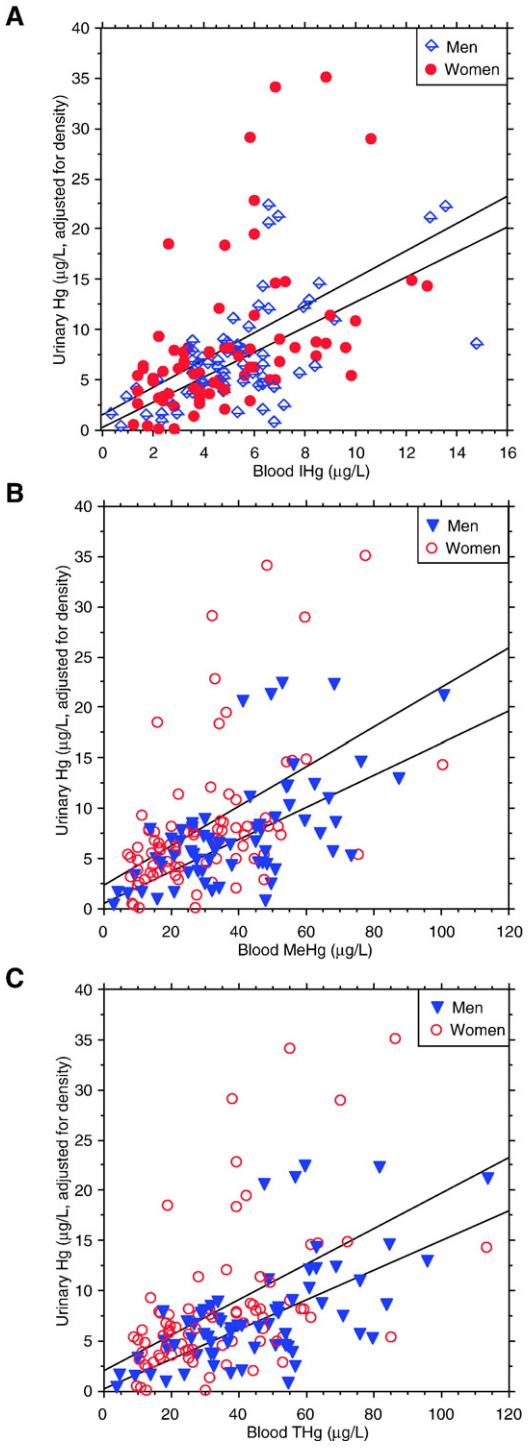

Fig. 3. Relations between density-adjusted U-Hg concentrations with respect to blood bioindicators of mercury exposure.

Table 3 presents the predictive factors for B-IHg, B-MeHg and B-THg. Carnivorous fish frequency consumption was highly significantly related to all three blood

bioindicators, while non-carnivorous fish consumption was related to B-MeHg and B-THg, but not B-IHg. Fruit consumption frequency was negatively related to all of these bioindicators. Age was only related to B-IHg. There were significant differences between communities and immigrant status; those who were not born in the region had significant lower levels of all of these bioindicators of exposure. Gender did not enter into any of the models, nor did smoking, alcohol or education (not shown here).

Urinary Hg was positively related to carnivorous fish consumption (β coeff: 0.39; $p<0.01$) and negatively to fruit consumption (β coeff: −0.07; $p=0.05$). Immigrants had lower U-Hg with respect to non-immigrants (β coeff: −1.76; $p=0.02$) and women had higher U-Hg compared to men (β coeff: 1.14; $p=0.03$). Since the blood bioindicators were highly inter-correlated, for the models of U-Hg each one was examined separately with potential covariables. Only gender entered significantly in all models. Fig. 3 (A, B, and C) shows the relations between density-adjusted U-Hg and B-IHg, B-MeHg and B-THg for men and women.

A total of 34 persons (19.9%) had U-Hg levels above 10 μg/L, adjusted for urinary density. Logistic regression analyses for U-Hg levels above 10 μg/L, with respect to fish consumption, fruit consumption and socio-demographic co-variables showed that the only variable that entered significantly into the model was consumption of carnivorous fish. A total of 70 persons (40.9%) ate more than 4 carnivorous fish meals over the past 7 days. The Odds Ratio (OR) for having U-Hg above 10 μg/L for those who ate more than 4 carnivorous fish meals was 4.00 [1.83–9.20], with a Chi square $p<0.001$. Those with U-Hg levels above 10 μg/L had mean B-IHg concentrations of 7.71 μg/L± 2.67 (median: 6.81) while those with lower U-Hg concentrations had a mean B-IHg of 4.38 μg/L±2.18 (median: 4.00). Although the differences in IHg were highly significant (*t*-test; $p<0.001$), there was no difference between the 2 groups in the percentage of B-IHg in total B-Hg. (13.1% for those with higher U-Hg concentrations and 13.9% for those with lower levels).

4. Discussion

The present study shows high levels of IHg in a fish-eating population who have not been occupationally exposed to Hg vapours and without dental amalgams. For 19.9% of the participants, U-Hg concentrations surpass 10 μg/L, the upper limit for unexposed populations (WHO, 1991). IHg levels were directly associated with fish consumption and influenced by a number of socio-demographic factors.

Few studies have examined the association between fish consumption and IHg exposure in human populations. In Europe, an Italian polycentric study provided important insight on the relevance of assessing IHg exposure in fish consumers (Carta et al., 2002; Apostoli et al., 2002). These authors found a significant relation between the number of fish meals consumed weekly and U-Hg levels; they concluded that the organic compounds absorbed by usual sea fish consumption may be partially demethylated, thus increasing the IHg concentration in the kidney, and consequently its urinary excretion. More recently, two Swedish studies have corroborated these findings. Johnsson et al. (2005) reported a significant relationship between freshwater fish consumption and U-Hg levels, the latter being 15-fold higher in frequent fish consumers as compared to low consumers. The authors also suggested that demethylation of MeHg in the human body may explain such relations. A study examining the inter-individual variations of human biomarkers of Hg exposure, reported an average percentage of 6.8% for IHg in red blood cells, which increased with increasing consumption of fish, but not with increasing number of dental amalgam fillings (Berglund et al., 2005). A further study in Canada on U-Hg excretion in children (Levy et al., 2004) reported that, unexpectedly, children with higher levels of fish consumption excreted significantly elevated amounts of Hg in urine.

In the present study, the relation between fish intake and B-IHg and U-Hg concentrations was observed only with carnivorous fish consumption, suggesting that carnivorous fish may have higher levels of IHg or that when a bolus dose of MeHg is ingested from carnivorous fish, some may be converted to IHg. It is unlikely that this relation is due to a higher quantity of carnivorous fish consumption, since in these communities there is no difference between the preparation of carnivorous and non-carnivorous fish. Moreover, carnivorous fish can be large like Tucunaré (*Cichla* sp.) or small like Piranha (*Serrasalmus* sp.), the same being true for herbivorous fish which can be large like Tambaqui (*Collossoma macropomum*) or small like Pacu (*Mylossoma* sp.). Based on different studies, on average people eat approximately 163 grams of fish per day (Kehrig et al., 1998; Boischio and Henshel, 2000; Yokoo et al., 2001; Dórea et al., 2005). On the other hand, the conversion of MeHg into IHg in the body has long been proposed (Kozak and Forsberg, 1979). Demethylation of MeHg by microflora in the human gut is considered to be a key and probably a rate-determining process in the removal of MeHg, even though the microbes involved have not been identified nor have the biochemical mechanisms of cleavage of the carbon–mercury bond (Clarkson, 2002). This

biotransformation has recently been confirmed through the use of biologically- and physiologically-based toxicokinetic models, which describe the deposition kinetics of MeHg and its inorganic metabolites both in animals and humans (Carrier et al., 2001a,b; Young et al., 2001).

Apart from the demethylation of MeHg in the human body, the IHg fraction of fish Hg may provide another explanation of the relation between fish consumption and IHg concentrations. Despite the general assumption that MeHg constitutes the major part of the Hg content in fish, recent studies have shown substantial variations of the IHg fraction in fish flesh, both in the Amazon and elsewhere. For example, in a study on Hg speciation and accumulation in freshwater and anadromous fish from Bangladesh, Holsbeek et al. (1997) reported an accumulation mechanism reflected by increasing levels of IHg combined to low and constant MeHg levels, leading to a relative decrease of the MeHg fraction with age. According to the authors, this unexpected pattern had only been reported in cases of some marine species where it seemed to be linked to demethylation mechanisms or regional influences on fish Hg levels. An intermediate accumulation pattern with increasing concentrations of both MeHg and IHg fraction with age was found in one bottom dwelling species. In the Amazon, Maurice-Bourgoin et al. (2000) reported IHg fractions in fish varying from 2% to 27% of the total Hg in fish of the Madeira River. In a more recent investigation, analyses of total Hg and MeHg levels in fish from hydroelectric reservoirs in Tanzania show that approximately 0–44% of the total Hg in these fish was IHg (Ikingura and Akagi, 2003). There is thus reason to believe that IHg ingested from fish may contribute to IHg in the human body, which is reflected in the blood and urinary Hg levels. Although in humans IHg is less well absorbed by the gastro-intestinal tract than MeHg, at rates of about 7% and 95% respectively (Rahola et al., 1973; Aberg et al., 1969; cited in Berglund et al., 2005), the findings of the present study suggest that even this relatively low absorption rate of IHg can result in substantial blood and urinary IHg levels in heavy fish consumers.

Another interesting finding in the present study was that both B-IHg concentrations and the percentage of B-IHg were positively associated to the age of participants. This association might be explained by increasing rates of IHg accumulation with time, under a chronic exposure scenario. Indeed, physiologically-based toxicokinetic models which extrapolate experimental data to much longer periods of exposure, suggest that the concentrations of IHg can surpass those of MeHg overtime, thus potentially reflecting increased blood and urinary IHg levels (Young et al., 2001).

Even when fish consumption frequency was taken into account, villagers who were born in the Tapajós region presented significantly higher B-IHg and B-MeHg than immigrants from northeast Brazil. Those who were born in the Tapajós region have had a fish-based diet throughout their life, while those who were arrived later consumed much less fish prior to their arrival in the region. Over the last years there has been growing interest in evaluating the potential of perinatal and lifetime exposures to determine different MeHg pharmacokinetic patterns during adulthood, since chronic and low-level exposures to environmental pollutants often begin prenatally and then continue through the lifetime (Stern et al., 2001). In experimental conditions which sought to obtain basic information about blood and brain Hg levels in mice under conditions of chronic lifetime exposure, these authors observed that both brain and blood Hg levels rose significantly between 14 and 26 months, suggesting an interaction between dose and age. Lifetime exposure might, therefore, explain the higher blood Hg levels observed in persons born in the Tapajós region, when compared to persons who arrived in this region during adolescence or even adulthood.

In this study a convenience sample was used. Convenience samples have been shown to appropriately represent underlying populations in different settings (Kelly et al., 2002; Zelinski et al., 2001) and in the present study, 17.1% of the population of these villages surveyed well represented most age categories. Although this sampling strategy may have introduced some selection bias it would probably not affect the relation between fish consumption and blood or urinary inorganic Hg.

In conclusion, the present results support the need to measure and better understand the role of the different chemical forms of Hg from fish consumption, since Hg chemical species present different toxicological properties. Given the degree of environmental exposure in the present cohort, attention should be paid to the potential of toxicity of IHg, since inorganic metabolites may, in part, be responsible for some of the neurotoxic effects induced by MeHg (Charleston et al., 1994; Vahter et al., 1994, 1995), even if the high load of IHg in the kidneys may leave less IHg circulating in blood and available for transfer to the brain (Carrier et al., 2001b). Future studies should examine the potential toxicity of IHg in heavy fish consumers.

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