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Hair Mercury Speciation as a Function of Gender, Age, and Body Mass Index in Inhabitants of the Negro River Basin, Amazon, Brazil

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Abstract. Human mercury contamination can be monitored through hair analysis of mercury's inorganic and organic form as methylmercury (Me-Hg). Hair total mercury and Me-Hg were studied in a Negro River fish-eating population in relation to age, gender, and body mass index (BMI). This riverbank population eats fish at least twice a day and is exposed to high levels of Me-Hg. Total mercury ranged from 1.51 μg/g to 59.01 μg/g, with only 21% of the sampled population having Hg concentration of less than 10 μg/g hair. The mean percentage of Me-Hg was 71.3% (range 34% to 100%) of the total mercury in hair. No statistically significant differences were found in regard to age groups (children and adults) or BMI. However, women had significantly lower total mercury in hair than men, but the percentage of Me-Hg was not significantly different. Women in fertile age (15–40 years) had hair total mercury ranging from 1.65–32.63 μg/g, and 65% in this subgroup had hair mercury above 10 μg/g hair. The percentage of Me-Hg concentration in hair of this freshwater, fish–eating population is comparable to populations eating ocean fish from different parts of the world and does not seem to be affected by age, gender, and BMI.

Mercury is widespread in the environment and constitutes an important contaminant for populations that depend on fish for daily sustenance. A series of complex chemical transformations allows the three-oxidation states of mercury (Hg^0, Hg^{+1}, Hg^{+2}) to cycle in the environment. The methylation reaction is the most important step for mercury to enter the food chain and thereby contaminate humans. This step takes place in water sediment and soil, and aquatic organisms become the most important bioconcentrators (Clarkson 1993). Bioaccumulation of Me-Hg in fish depends on the trophic level (Dietz *et al.* 1996), and it is also influenced by fish age, as measured by body size. In the elemental state mercury (Hg^0) is poorly absorbed from the intestine. However, as methylmercury (Me-Hg), the main form in aquatic organisms, it is easily absorbed by the gastrointestinal tract of humans.

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In the Amazon ecosystem, mercury contamination can occur either as a result of an occupational hazard, such as gold mining and processing activities, or as a result of environmental exposure due to consumption of mercury-contaminated food items (Harada 1993; Akagi *et al.* 1995a; Barbosa *et al.* 1995). Occupational exposure in the Amazon is mainly due to inorganic mercury used in gold amalgamation at mining sites or refining activities. In this case, most of the mercury in hair is inorganic (Harada 1993; Akagi *et al.* 1995a). Riverine populations of the Amazon Basin depend heavily on fish consumption for their nutritional needs (Giugliano *et al.* 1978) and therefore are naturally at risk of heavy body loads of methylmercury (Akagi *et al.* 1995a; Barbosa *et al.* 1995, 1997, 1998; Lebel *et al.* 1998; Kehrig *et al.* 1998; Harada 1997). Fetuses and breast-fed infants in these fish-eating populations of the Amazon Basin are exposed to Me-Hg contamination via placental transfer and maternal breast milk (Boischio and Henshel 1996; Barbosa *et al.* 1998; Barbosa and Dorea 1998), increasing the risk for neurodevelopmental deficits. In the Brazilian Amazon several reports have found nervous system dysfunction associated with Me-Hg contamination (Lebel *et al.* 1996; 1998; Grandjean *et al.* 1999; Dolbec *et al.* 2000).

Body retention of mercury, like other metals, is dependent on dietary and physiological factors. In the exposed individuals mercury is taken up from the blood stream during hair formation and varies with the level of contamination of the fish source (Lebel *et al.* 1997). Regardless of the level of seafood consumption, mercury is preferentially taken up by hair as the organic form than the inorganic form (Phelps *et al.* 1980). Physiological factors, such as gender, age, and body mass index (BMI), can modulate metabolism of nutritive or toxic metals. In this regard, the extent that these specific biologic factors can modulate the forms of mercury accumulation in hair needs to be evaluated. In this research, the two forms of hair mercury, inorganic and organic (presumably Me-Hg) are evaluated in the freshwater fish–eating population of the Negro River with regard to gender, age, and BMI.

Materials and Methods

During the month of March 1998 we surveyed six communities along the Negro River (see map, Figure 1) as part of an ongoing monitoring

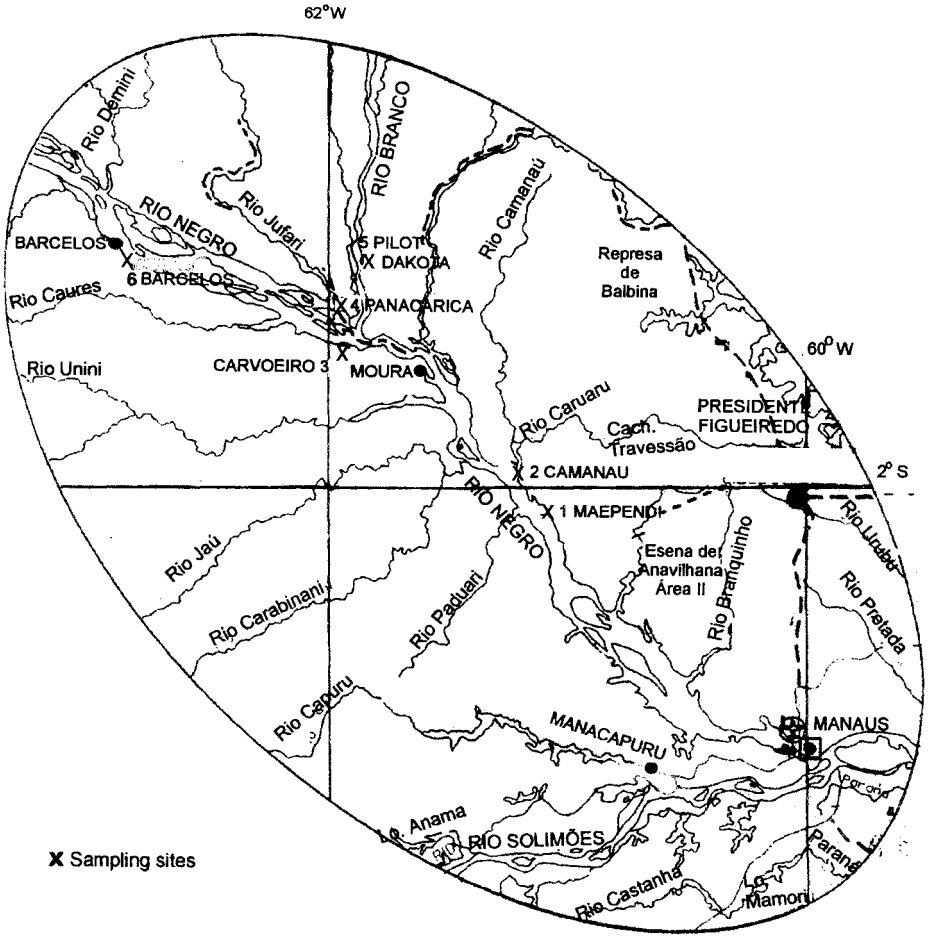

Fig. 1. Map of Amazon Basin showing sampling sites

project for mercury contamination of the Amazon Basin. All individuals sampled had the purpose of the study explained to them and gave verbal consent. After consent, hair of 149 individuals (men, women, and children) was sampled from the occipital area, cut close to the scalp with stainless steel scissors, bundled together with cotton thread, placed in an envelope, properly identified, and taken to the Laboratory of Environmental Chemistry of the University of Brasilia for analysis. Details of sample collection were given in a previous publication (Barbosa *et al.* 1995). The individuals were all members of families living along the shores of the Negro River and its tributaries. None of them declared to have worked in gold mining activities, and all subsisted on small agricultural activities and fishing. A simple questionnaire was applied asking how many fish meals were consumed daily. In a subsample of 50 adults, weight and height were measured and a questionnaire was filled with information on fish eating habits.

Mercury Determination

All glassware used in the analytical protocol was washed clean, rinsed with KOH and double-distilled water, respectively, and left to rest in 50% HNO_3 for 24 h. It was then rinsed again in double-distilled water and dried at 100°C for 12 h.

The hair samples were comminuted with stainless steel scissors, weighed, and digested before analysis. The method of Magos and Clarkson (1972) for mercury analysis of hair was employed as described previously (Barbosa *et al.* 1995). To a known weight of 10 mg of hair 1.0 ml of cysteine 8.3 mM and 2.0 ml of NaOH 11.3 M were added and heated together at 90°C for 15 min. Care was taken to avoid boiling. The digest was cooled in an ice bath and allowed to reach room temperature and then diluted with 7.0 ml of NaCl 171 mM. The weight of the digest was then registered.

The determination of total and inorganic mercury in hair was performed by cold vapor atomic absorption spectrometry (CV-AAS). The instrument used was a Mercury Monitor LDC Analytical, Model 1255 (LDC, Riviera Beach, FL) connected to a multimeter HP Model 3435 (Hewlet Packard, Palo Alto, CA) and a Neptune Dyna Pump (Magnetek Universal Electric, USA).

A 1.0-ml aliquot of digest was transferred to the reaction vessel and 1.0 ml of cysteine 8.3 mM, 20 ml NaCl 171 mM, 10 ml of H_2SO_4 8.0 M, 1.0 ml of $SnCl_2 \times 2H_2O/CdCl_2$, 20 ml of NaOH 13.3 M, respectively, were added. The reaction produces a vapor that passes through a water solution of tri-n-butyl-phosphate in ice next to the absorption chamber where the absorption reading takes place. After the C-Hg bonds are broken, Hg is reduced and measured as inorganic mercury. Total mercury is determined after breaking the C-Hg bonds in the presence of $CdCl_2$. Me-Hg is determined as the difference between total and inorganic mercury.

Precision and accuracy of mercury determinations was assured by the use of internal standards of hair, provided by the Hair Mercury–Interlaboratory Comparison Program (Ottawa, Ontario, Canada). As part of this intercalibration program that started in 1992, our results so far have been considered acceptable. Out of 27 samples analyzed, 25 (93%) gave results within 2 SD, and 18 (67%) within 1 SD of the corrected mean.

Summarized data (mean, SD, and ranges), Pearson correlation be-

Table 1. Mercury in hair of the riverine population of the Negro River, Amazon Basin

	Hg in Hair (μg/g)			
	Total	Inorganic	Me-Hg	Me-Hg (%)
Children (n = 73) < 15 years				
Mean	18.52	4.62	13.90	72.75
SD	10.04	3.56	8.66	15.52
Median	16.38	3.9	12.65	74.3
Range	0.51–45.89	0.55–22.34	0–44.53	34.0–97.1
Adults (n = 76) > 15 years				
Mean	21.40	5.9	15.25	70.89
SD	12.66	4.7	10.02	15.08
Median	17.8	4.71	12.58	71.05
Range	1.66–59.01	0–27.93	1.66–56.79	37.48–100

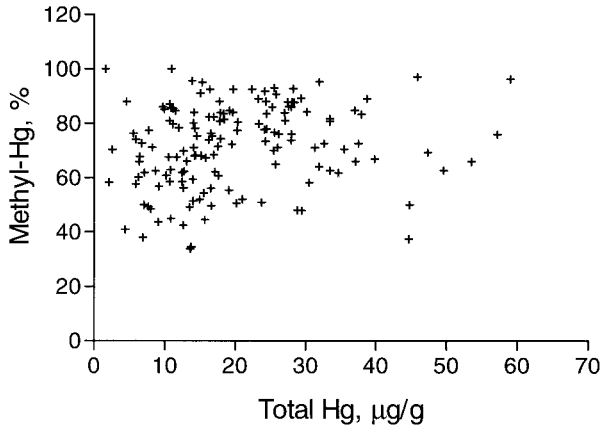

Fig. 2. Percentage of methylmercury in hair as a function total mercury in the Negro River population

tween variables, and analysis of variance, were performed using a SAS (SAS Institute, Cary, NC) computer program for PC. A *p* value of less than 0.05 was considered statistically significant.

Results

Hair mercury concentrations, including total and Me-Hg are shown in Table 1. Only 21% of this surveyed population had mercury levels in hair below 10 μg/g hair. In this Negro River population, fish is the main staple diet, and they indicated that they eat fish at least once a day (7.1%), but most (78.6%) consumed fish at least twice a day. Hair mercury distribution in this fish eating population was mostly below provisional standard level in hair established as 50 μg/g (Figure 2).

Summary of BMI and hair mercury as a function of gender among adults is shown in Table 2. The BMI between genders was not significantly different (p = 0.9053). However, women had a significantly (p = 0.02) lower concentration of total mercury, but the percentage of methylmercury was not significantly different (p = 0.7951) than that for men. Women in fertile age (15–40 years) had hair mercury ranging from 1.65 to 32.63 μg/g hair, and 65% of these women had hair mercury above 10 μg/g hair.

In this population with high fish consumption Me-Hg metabolism was studied through correlation analysis. Significant correlation (r = 0.1829; p = 0.0261) was found only between total mercury and Me-Hg (Figure 2). No statistically significant correlation was seen

Table 2. Body mass index (BMI) and mercury (total and Me-Hg) in hair of adult riverine population of Negro River (mean and SD)

		BMI	Total Hg (μg/g hair)	Methyl-Hg (%)
Female	31	22.41 (4.9)	18.32* (11.12)	72.94 (16.87)
Male	17	23.18 (3.0)	26.15 (13.73)	71.77 (15.12)

BMI = Weight/height^2.
* Significantly different (p < 0.02).

between total mercury and age (r = 0.1066; p = 0.2078); total mercury and BMI (r = −0.15335; p = 0.2981); Me-Hg and BMI (r = 0.05121; p = 0.7296), or Me-Hg and age (r = -0.0502; p = 0.5473).

Discussion

The hair mercury concentration, total and Me-Hg, we found in this sample from the Negro River population is in accordance with previous findings in other sites of the Amazon Basin. A comparison of studies reporting methylmercury in hair of different parts of the world is presented in Table 3. Most of the hair mercury is indeed Me-Hg, and our mean values were similar to other studies (Tables 1–3). Results of hair Me-Hg in Brazil were obtained from populations eating freshwater fish from the Amazon Basin. In this region fish intake can reach a daily per capita of 200 g, and mean total mercury intake has been estimated to vary from 40 μg to 200 μg (Barbosa *et al.* 1995). The Me-Hg percentage of the total hair mercury was comparable with previous studies in Brazil. However, it seems to vary among population studies worldwide. Regardless of frequency of fish consumption, or concentration of total mercury in hair, the percentage of Me-Hg is always high (Table 3) for people eating oceanic or freshwater fish.

Most of the studies in the Amazon reported methylmercury above 80% of the total hair mercury with comparable levels of fish consumption (Table 3). Regardless of levels of fish consumption, Harada *et al.* (1998) reported a relatively high percentage of methylmercury (84%). Such levels also seem to be independent of the total hair mercury concentration. Among the nonindigenous residents of the town of Balbina, also in the Amazon Basin (see map in Figure 1), where fish consumption is relatively lower Kehrig *et al.* (1998) reported a relatively lower hair total mercury (6.54 μg/g) with a high percentage of Me-Hg (95%).

Comparing results from different parts of the world there is a great

Table 3. Summary of studies comparing mean/median (and range) of total mercury and percentage of Me-Hg in hair

Reference	Country	n	Mercury In Hair		Sampling Observations
			Total (μg/g)	Methyl (%)	
This study	Brazil	163	20	71	Negro River
Akagi *et al.* (1995a)	Brazil	136	NG	> 90	Tapajós River
Akagi *et al.* (1995b)	Brazil	12	[3.1–36]	[85–96]	Tapajós River
Barbosa *et al.* (1995)	Brazil	55	34.2	87	Apiacás Reservation
Barbosa *et al.* (1997)	Brazil	142	17.2	79	Madeira River
Birke *et al.* (1972)	Sweden	6	47	80	
Brhun *et. al.* (1997)	Chile	33	[0.3–2.5]	[77–95]	8th Region
Buzina *et al.* (1995)	Yugoslavia	NG	6.4	86	Adriatic Sea
Cernichiari *et al.* (1995)	Seychelles	40	NG	80	
Chen *et al.* (1990)	Japan	49	0.8	0.2*	Initial values in China
			1.9	0.3*	After 1 year in Japan**
Dermelj *et al.* (1987)	Yugoslavia	26	4.3	79.4	
Egeland *et al.* (1999)	Alaska	16	1.3	< 2	
Feng *et al.* (1998)	China	64	1.69	25	
	Indonesia	55	3.1	25	
	Japan	243	4.6	77	
Foo *et al.* (1988)	Malaysia	150	6.1	52	Chinese
		44	5.2	55	Malay
		31	4.5	49	Indian
Harada *et al.* (1998)	Japan	191	[1.9–3.7]	[70–94]	
Ikingura and Akagi (1996)	Tanzania	29	[ND–5.4]	[ND–82]	Gold miners
Ishihara and Urushiyama (1994)	Japan	NG	10.4	84	0-month exposition
		NG	11.4	81	23-months exposition
Kehrig *et al.* (1997)	Brazil	112	NG	[56–86]	Several localities
Kehrig *et al.* (1998)	Brazil	20	6.5	95	
Kyle and Ghani (1982)	Papua	114	18	86	
		51	8.3	77	
		45	3.2	75	
Lee and Lee (1999)	Korea	315	1.7	61	Male and females
		104	1.1	48.8	Females
Lebel *et al.* (1996)	Brazil	29	14.0	86.1	Tapajós River
Lebel *et al.* (1998)	Brazil	91	12.5	89.6	Tapajós River
Malm *et. al.* (1995)	Brazil	121	[18–34]	85	Tapajós River
Sarmani *et al.* (1997)	Malaysia	10	1.7	59.7	
Soria *et al.* (1992)	Spain	50	2.8	58	Winter
		19	1.2	90	Summer
	Czeck				
Spevackova *et al.* (1997)	Rep.	32	0.5	[26–46]	
Smith *et al.* (1997)	USA	2,820	NG	0.24*	Fish-eaters
			NG	0.36*	Nonfish-eaters
Vural and Unlu (1996)	Turkey	12	NG	1.5*	Control
		35	NG	10.2*	Fish-eaters

NG = Not given; ND = Not detected.
* μg/g hair.
** Only 17 subjects.

variability in the percentage of Me-Hg. Foo *et al.* (1988) showed that low levels of total hair mercury (4.5–6.1 μg/g) among Chinese Malaysians and Indians had a relatively low percentage of Me-Hg (49—55%). Feng *et al.* (1998) found that the Japanese had a higher percentage of hair Me-Hg (77%) than Chinese and Malaysians (52% and 55%, respectively). Phelps *et al.* (1980) showed that in the U.S. population presumably with a relatively low fish consumption Me-Hg was greater than 90%. However, Brhun *et al.* (1997) compared fish-eaters (coastal Chile) with noneaters (inlanders) and found a relatively higher (95–95%) percentage of Me-Hg in the coastal inhabitants. Also, follow-up studies in hair mercury showed that after a 1-year increase in fish consumption there was increased mercury in hair (Chen 1990), but no change was observed after 23 months of occupational exposure (Ishihara and Urushiyama 1994). In this study the percentage of Me-Hg did not seem to be affected (Ishihara and Urushiyama 1994).

Mercury uptake and retention by fish is not well understood. Environmental factors may influence fish mercury metabolism, which has been reflected in differences in mercury content depending on fish trophic level. Herbivorous species have a lower content of mercury compared with piscivorous. Insomuch as fish is the main route of human mercury contamination, fish also is a good source of nutrients, such as selenium, known to counteract the toxic effects of mercury. Therefore, the content of selenium in the diet of the fish eating population varies considerably depending on the type of fish consumed (Dorea *et al.* 1998). The mercury to selenium molar ratio in fish may vary from 4.7 in piscivorous to 0.8 in herbivorous species (Dorea *et al.*

1998). Whether such differences in fish mercury and/or selenium concentrations may modulate uptake of mercury and its distribution in hair has not yet been elucidated. Suzuki *et al.* (1991) showed that in one case of mercury poisoning the hair concentrations of selenium coincided inversely with hair mercury concentration. More recently dietary selenium supplementation reduced mercury accumulation in pubic hair (Seppanen *et al.* 2000).

Marsh *et al.* (1995) reviewed the subject of body mercury load during pregnancy, indicating differences in clinical responses. Neurological effects were seen in children of Iraqi mothers with hair mercury concentrations as low as 20 ppm while in ocean fish–eaters of Peru, maternal hair levels of Me-Hg between 1.2 and 30 ppm showed no effect on infant neurological assessment. Marsh *et al.* (1995) attributed these differences to protective nutrients in the fish diet of Peruvian mothers that were absent in the grain diet of Iraqi mothers. One such nutrient, selenium, although present in cord blood in an average molar excess of 10-fold above Me-Hg offered no protection against mercury-associated decrease in neurologic optimality score (Steuerwald *et al.* 2000).

Gender differences in hair mercury varies greatly among reports. Lower levels in women's hair compared to men were reported by some (Shimomura *et al.* 1980; Airey 1983; Wakisaka *et al.* 1990) and the opposite by others (Grandjean *et al.* 1992). In the Amazon population, like Harada *et al.* (1998), we found a significantly low hair mercury in adult women. However Lebel *et al.* (1998) did not find gender differences in hair mercury. It is worth mentioning, however, that women of reproductive age (16–40 years) had a substantially lower concentration of mercury (organic and inorganic), but an equivalent percentage of Me-Hg. We showed in previous studies (Barbosa *et al.* 1998; Barbosa and Dorea 1998) that a decrease in hair mercury concentrations due to pregnancy and lactation. This in part could explain the gender differences we found.

In our surveyed population, women of reproductive age showed comparable hair mercury levels. It seems that the effects of pregnancy and lactation do not influence the mercury disposition of hair. In the subgroup of women of reproductive age, the percentage of Me-Hg does not seem to be altered. Human studies have shown that the blood to milk ratio of mercury varies over a wide range, thus indicating differences in blood to milk mercury transfer (Donangelo and Dorea 1998). Possible differences in milk mercury transfer due to different mercury species have not been extensively studied in humans. However, Oskarsson *et al.* (1996) suggested that inorganic mercury from amalgam fillings was the form transported to breast milk.

Dumont *et al.* (1998) noticed an increase in hair mercury with age in a population that was declining its mercury exposure. They speculated that food patterns might have played a role between generation lifestyles. In our population we did not noticed any age related difference, which agrees with others (Malm *et al.* 1995) working with Amazonian populations. Certain homogeneity of life style still persists in these investigated communities of the Negro River. For one thing, BMI was similar between genders. In this regard we did not find a significant difference either in total mercury or Me-Hg in hair. Also, there was no significant correlation between in either Me-Hg and BMI, or gender.

In conclusion, modulators of metal metabolism, such as gender, age, and BMI, are not significantly associated with the percentage of Me-Hg disposition of hair.

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