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Methylmercury in Fish and Hair Samples from the Balbina Reservoir, Brazilian Amazon

Helena do Amaral Kehrig,*[,1] Olaf Malm,* Hirokatsu Akagi,† Jean R. D. Guimarães,* and João Paulo M. Torres*

**Laboratório de Radioisótopos Eduardo Penna Franca, Instituto de Biofísica Carlos Chagas Filho, Centro de Ciências da Saúde, UFRJ, 21949-900 Rio de Janeiro, Brazil; and †Department of Epidemiology, National Institute for Minamata Disease (NIMD) 4058-18, Hama, Minamata, Kumamoto 867, Japan*

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The present study aimed to evaluate methylmercury in fish and human hair samples from an important hydroelectrical reservoir, Balbina (Brazil, Amazon). It presents a quite intense fishing activity and there is no known goldmining activity in its watershed. Fish and human hair were analyzed with a new extraction technique and measured by GC-ECD. Analytical quality was checked through intercomparisons between two laboratories with local samples and certified standards from IAEA. Methylmercury in hair ranged from 2.0 to 21.6 $\mu g \cdot g^{-1}$ with a mean of 8.76 $\pm$ 5.20 $\mu g \cdot g^{-1}$ ($N = 20$), while the methylmercury percentages were above 90. Fish presented methylmercury levels ranging from 0.03 to 0.9 $\mu g \cdot g^{-1}$ wet wt with a mean of 0.24 $\pm$ 0.18 $\mu g \cdot g^{-1}$ wet wt ($N = 32$), which is below the limit established for food by Brazilian legislation (0.5 $\mu g \cdot g^{-1}$ wet wt) and methylmercury mean percentages were above 95%. The total mean daily methylmercury intake ranged from 11 to 55 μg for 70% of the sampled population from the village based on a daily consumption of about 110 g of fish with methylmercury concentrations in the range of 0.1 to 0.5 $\mu g \cdot g^{-1}$. This calculation is consistent with methylmercury concentrations in hair samples in the range of 2.6 to 13.1 $\mu g \cdot g^{-1}$. © 1998 Academic Press

Key Words: **Methylmercury; human hair; fish; Amazon; reservoirs.**

INTRODUCTION

In Brazil, the informal gold mining activity, locally known as "garimpos," is the main source of mercury released into environment. In the Brazilian Amazonian region, large amounts of metallic mercury (Hg°) are used to amalgamate the fine gold particles. The mercury is discharged into aquatic systems during gold prospecting and into the atmosphere when burning the amalgam. It is estimated that around 100 tons of Hg have been released annually, during around 20 years, of which 45% is discharged into river systems and 55% is discharged into the atmosphere (Pfeiffer and Lacerda, 1988).

Mercury is converted to methylmercury, the most toxic mercury compound, in the environment, by bacteria in sediment and aquatic plants (Jernelöv, 1973) and is then bioaccumulated in fish and biomagnified through the aquatic food chain. The major source of human exposure to methylmercury is the consumption of fish (WHO, 1990).

Methylmercury, and also metallic mercury, are well known neurotoxic agents (WHO, 1990). One of the main target organs for methylmercury is the central nervous system for both adults and fetus (Igata, 1986). The developing brain is much more vulnerable and sensitive to the effects of methylmercury (Choi, 1989). High prenatal exposure to methylmercury is known to cause severe derangement of the developing central nervous system with psychomotor retarding in fetus. Lactating females have an increased rate of clearance of methylmercury from blood, compared with non-lactating females (Greenwood *et al.*, 1978). This can be explained, at least partly, by excretion via breast milk, a route of maternal excretion of toxic elements (Oskarsson *et al.*, 1996).

The present study was aimed at evaluating the human exposure levels to methylmercury by analysis of hair collected from a group of people living in a village near one important reservoir constructed for hydroelectrical generation, Balbina reservoir. In this reservoir watershed, there is no goldmining

[1]To whom correspondence should be addressed. Fax: 55 21 280-8193. E-mail: kehrig@leao.biof.ufrj.br.

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activity (R. Miyai, personal communication). The main mercury source is expected to be the atmospheric deposition.

The other aim was to evaluate the level of methylmercury in the muscle of certain fish sampled in this reservoir, which is the most frequently consumed by the local population. The pattern of fish consumption was established and used to estimate the range of methylmercury ingestion.

MATERIALS AND METHODS

Study Area

This study was conducted at one important reservoir constructed for hydroelectrical generation, Balbina reservoir, in the Brazilian Amazonian region (Fig. 1). This hydroelectrical power plant is situated about 200 km northeast from Manaus city (Amazon). It was constructed from 1982 to 1987 and started

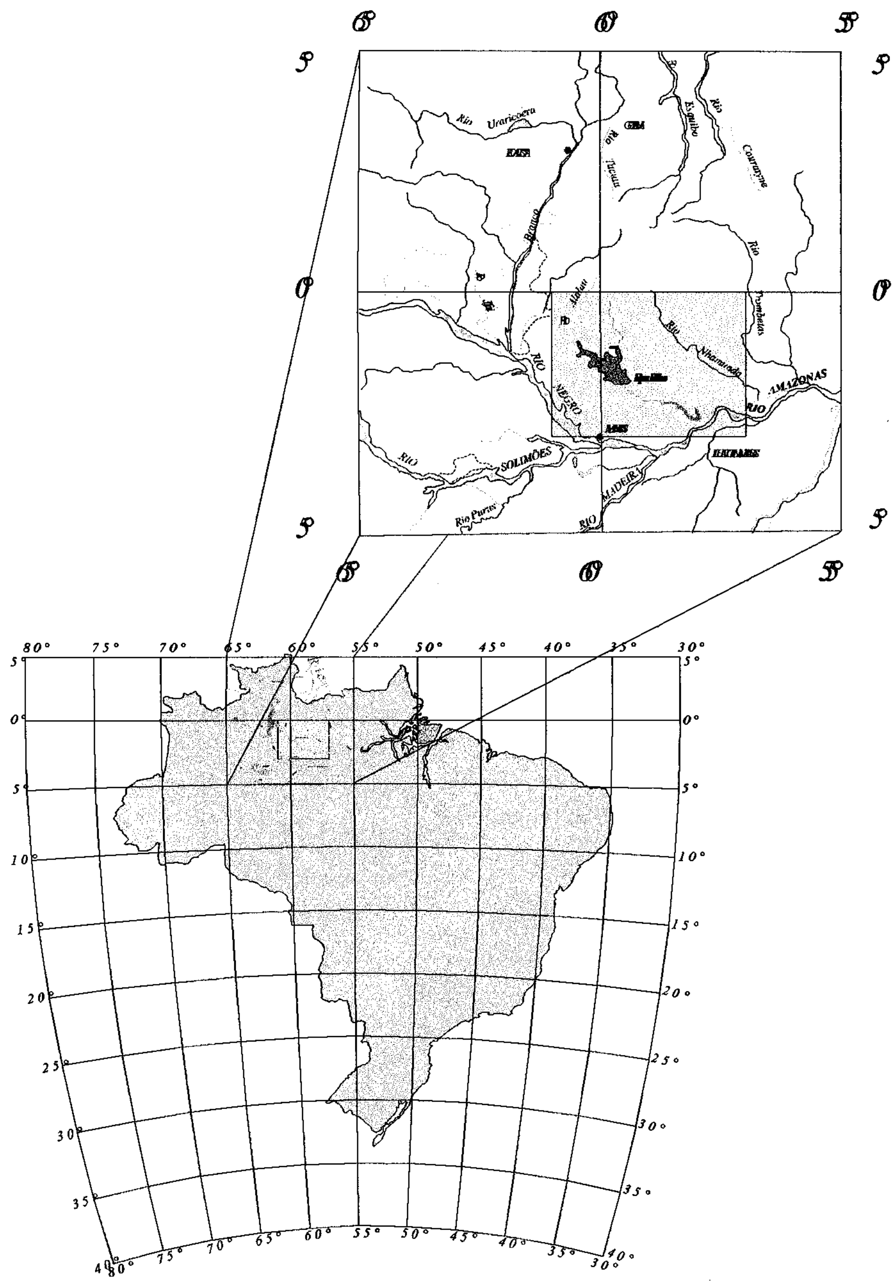

FIG. 1. Study area.

operating in 1989. The reservoir covers a total area of 2360 km^2 and the average flow rate is 577 $m^3 s$. The lake is formed from waters of Uatumã river and it contains more than 4000 islands. Most of the area was not deforested before the reservoir filling, and large areas of tropical rainforest were submerged. The water is poor in basic nutrients and is considered oligotrophic. Fishing activity is quite intense in the reservoir, producing about 20 tons of fish per month. Tucunaré (*Cichla spp.*), a carnivorous fish, is the predominant commercial specie caught in the reservoir.

Balbina Village, locally known as Waimiri-Atroari, which was built by the hydroelectrical company (Eletronorte), has a population of about 2000 inhabitants and is situated close to Balbina dam (2 km from the dam).

Sampling and Sample Preparation

A total of 43 fish samples, mainly on carnivorous (piscivorous) and omnivorous species, were collected in March 1996 at Balbina reservoir. Fish were caught with nets or directly acquired from local fishermen. Identification of the fish was done with the help of the fishermen. Weight and length were measured on every fish, and a piece of dorsolateral muscle tissue was sampled for analysis.

A total of 58 human hair samples were collected at the same time at Balbina village. They were cut in the occipital region from a representative group of people, selected on the basis of a questionnaire. Individuals were chosen from 14 families from whom we obtained also information on their nutritional habits, other possible mercury exposure sources, smoking, and alcohol ingestion.

Total mercury and methylmercury analysis were performed on fish and human hair samples at Laboratório de Radioisótopos (UFRJ) and at the National Institute for Minamata Disease (NIMD), Japan. Methylmercury analysis was performed on 32 fish samples and on 20 human hair samples from five families, the selection being based on previous total mercury data obtained at UFRJ laboratory.

As far as we know, it is the first time certified mercury and methylmercury analysis were done in this reservoir.

Analytical Methodology

Total mercury analyses were performed for both fish and human hair samples at UFRJ laboratory. Samples were acid digested and subjected to atomic absorption spectrometry with an AA 1475 Varian instrument, equipped with a cold vapor generator accessory (Varian VGA-76), with sodium borohydride as a reducing agent (Malm *et al.*, 1989). For methylmercury, we used an analytical procedure developed at the NIMD laboratory and adapted at UFRJ. Methylmercury in hair was first extracted with hydrochloric acid and then with benzene. The benzene layer was subjected to electron-capture detection gas chromatography (ECD-GC) at a Shimadzu GC 14B instrument (Akagi and Nishimura, 1991). The methylmercury analysis in muscle tissue of fish was made by digesting fish samples with an alcoholic potassium hydroxide solution followed by dithizone–benzene extraction and analysis by ECD-GC (Akagi and Nishimura, 1991).

Analytical methylmercury quality was certified through intercomparison exercises between the two laboratories using fish samples and certified standard samples from the International Atomic Energy Agency (IAEA).

An analytical intercalibration exercise for methylmercury analysis in human hair between the two laboratories cited above is described elsewhere (Kehrig *et al.*, 1997).

RESULTS

Comparison between the results of the analysis performed at the two laboratories for 32 fish samples collected at Balbina reservoir showed good agreement with a highly significant correlation ($r^2 = 0.99$); a paired t test showed that they are similar ($0.50 < P_{t_{32}} < 0.25$).

Precision and accuracy of the analytical method were demonstrated with a comparison exercise with human hair and tuna fish certified reference samples from IAEA. The results of analysis of these certified samples are shown in Table 1.

Mercury concentrations of carnivorous and omnivorous species ($N = 43$) ranged from 0.02 up to 1.1 $\mu g \cdot g^{-1}$ wet wt and the average was 0.26 ± 0.26 $\mu g \cdot g^{-1}$ wet. wt. Only six specimens (14%) had a mercury concentration above the maximum limit of 0.5 $\mu g \cdot g^{-1}$ wet wt established for food by the Brazilian legislation (Brasil, 1975).

We analyzed methylmercury in 32 fish samples, 17 of them being "Tucunaré" (*Cichla spp.*). This species make up half of the total fish catch and is the most important dietary item for the population living around the Balbina reservoir. "Tucunaré" is found in all parts of the reservoir. In Tucurui, an other reservoir in the Brazilian Amazon, Porvari (1995) found that "Tucunaré" had the same importance in fisheries and diet as in Balbina. The data on

TABLE 1

Methylmercury Concentration in Certified Standard Fish Sample and Human Hair Sample ($\mu g \cdot g^{-1}$)

Sample	Certified value		UFRJ value		
	Mean ± SD	Range	Mean ± SD	Range	(*N*)
IAEA 085 sample 486, human hair	21.6 ± 4.20	(20.1–23.0)	20.6 ± 1.8	(18.1–23.6)	12
IAEA 086 sample 376, human hair	0.29 ± 0.09	(0.25–0.33)	0.31 ± 0.04	(0.25–0.36)	10
IAEA 350 sample 256, tuna fish	3.65 ± 0.35	(3.32–4.01)	3.59 ± 0.41	(2.55–4.50)	27

methylmercury in fish samples and on the feeding habits for the individuals are summarized in Table 2. The average of methylmercury for all fish ($N = 32$) was $0.24 \pm 0.18\ \mu g \cdot g^{-1}$ wet wt.

The mean percentages of methylmercury to total mercury for all fish samples (carnivorous and omnivorous) were 96 ± 4% with a range of 82 to 100%, indicating that organic mercury was the predominant form of mercury in the fish muscle tissue.

The population of Balbina village consumes fish at least once a week. The average weekly consumption of fish ranges from one to seven times a week. The estimated average daily consumption of fish per capita was about 110 g for adults.

The mercury and methylmercury levels in human hair are good indicators of human exposure to methylmercury. The longitudinal analysis of mercury and methylmercury along hair strands provides information on previous methylmercury exposure (WHO, 1990).

The averages and ranges of the levels of mercury and methylmercury and percentages of methylmercury in human hair samples from Balbina village are summarized in Table 3. The total mercury concentration in the hair samples ($N = 53$) from Balbina did not correlate significantly (R (Spearman) = 0.031; $P > 0.50$) with the number of weekly fish meals among the population, which mainly consumed the carnivorous fish "Tucunaré."

Human hair methylmercury concentration was determined in hair from 20 individuals of five different families sampled at the village near Balbina reservoir. The methylmercury concentration of $8.76 \pm 5.20\ \mu g \cdot g^{-1}$ found for this group seems coherent with their moderately elevated carnivorous fish consumption The mean percentages of methylmercury to total mercury were about 95%, indicating that organic mercury was the predominant form of mercury to which people were exposed through the consumption of fish.

Special attention must be paid to the methylmercury concentrations found among females of childbearing age and females infants: one-half of them

TABLE 2

Methylmercury Concentration in Fish Muscle Tissue from Balbina Reservoir (March 1996)

Species	(*N*)	Feeding habits[a]	Mean MeHg ± SD ($\mu g \cdot g^{-1}$) wet wt	Range
Tucunaré Pinima (*Ciclha temensis*)	14	C	0.3 ± 0.2	0.06–0.7
Tucunaré Açu (*Ciclha acellaris*)	3	C	0.13 ± 0.003	0.12–0.13
Peixe Cachorro (*Acestrorynchus falcirostris*)	1	C	0.7	
Piranha (*Serrasalmus rhombeus*)	4	O	0.6 ± 0.4	0.05–0.9
Acará Tinga (*Geophagus surinamensis*)	10	O	0.06 ± 0.03	0.03–0.1
Total	32		0.24	0.03–0.9

[a]C, carnivorous; O, omnivorous.

TABLE 3

Concentration ($\mu g \cdot g^{-1}$) in Human Hair from Balbina Village [Mean±Standard Deviation (Range)]

Population		Total mercury	Methylmercury	% Methylmercury
Children	Female	7.7 ± 6.8 (1.3–22.0) (*N* = 16)	11.3 ± 7.6 (2.3–21.6) (*N* = 6)	93 (69–100) (*N* = 6)
	Male	5.3 ± 2.5 (2.5–11.4) (*N* = 12)	6.1 ± 2.6 (3.3–10.5) (*N* = 6)	95 (88–101) (*N* = 6)
Adults	Female	7.4 ± 4.6 (2.2–15.5) (*N* = 12)	8.5 ± 4.9 (2.0– 15.2) (*N* = 6)	97 (91–103) (*N* = 6)
	Male	5.5 ± 3.5 (1.2–12.2) (*N* = 13)	10.0 ± 2.1 (8.5–11.5) (*N* = 2)	96 (94–98) (*N* = 2)
Mean		6.54 ± 5.45 (*N* = 53)	8.76 ± 5.20 (*N* = 20)	95 ± 7 (*N* = 20)

presented methylmercury levels above 10 μg · g^{-1}. The results of longitudinal analysis of hair methylmercury showed no seasonal variation of methylmercury intake in women Balbina population.

The results of a longitudinal analysis of hair total mercury and methylmercury in a 17-year-old mother, who only consumed fish once a week, did not have any dental amalgam, and was exposed to methylmercury during pregnancy, presented hair mercury levels above 13 μg · g^{-1} and a percentage of methylmercury near 100%. Maternal hair methylmercury concentrations decreased slightly during pregnancy. After birth methylmercury concentrations increased slightly up to 25 μg · g^{-1}. Her baby, 12 months old, had a hair mercury concentration of 5.3 μg · g^{-1} and methylmercury being near 100%.

The results of a longitudinal analysis of hair methylmercury and percentage of methylmercury in a 24-month-old child, who stopped breastfeeding when he was 9 months, presented an increase in the methylmercury concentration and in the percentage of methylmercury from 12 to 28 μg · g^{-1} and from 80 to 100%.

To evaluate the daily methylmercury intake and methylmercury values obtained in hair by the sampled population, we used the interconversion between fish methylmercury, methylmercury intake and hair methylmercury based on a kinetic model described by Clarkson *et al*. (1988), using the following relationships:

$$\text{Methylmercury daily intake } (\mu g) = \text{fish } (110\ g) \times \text{fish methylmercury } (\mu g \cdot g^{-1}) \quad (1)$$

$$\text{Blood [methylmercury] } (ng \cdot mL^{-1}) = 0.95 \times \text{daily intake } (\mu g) \quad (2)$$

$$\text{Hair [methylmercury] } (\mu g \cdot g^{-1}) = 250 \times \text{blood[methylmercury] } (ng \cdot mL^{-1}) \quad (3)$$

Table 4 presents the percentages of occurrence hair and fish methylmercury levels observed in intervals in the samples of this study in comparison to the estimated methylmercury intake levels derived from hypothetical hair and fish methylmercury levels using the equations above. The total mean daily methylmercury intake is in the range of 11 to 55 μg for 70% of the sampled population from the Balbina Village. The mean methylmercury daily intake of approximately 35.2 μg was calculated from the observed mean fish methylmercury concentration of 22 samples (0.32 μg · g^{-1}) and the fish consumption was estimated as 110 g. The interconversion of the observed mean hair methylmercury (8.76 μg · g^{-1}) into methylmercury intake (using the equations above) also resulted in a estimated daily intake of approximately 36.8 μg methyl mercury during the whole year.

TABLE 4

Percentage of Occurrence of Methylmercury in Human Hair and Methylmercury Fish (Intervals) from Balbina Reservoir Compared to Methylmercury Intake as Estimated by Fish and Hair Methylmercury Observed Levels

Estimated results[a]			Observed results	
Fish methyl-mercury (μg · g^{-1})	Methyl-mercury intake (μg/day)	Hair methyl-mercury (μg · g^{-1})	Hair methyl-mercury (% of $N = 20$)	Fish methyl-mercury (% of $N = 22$)
< 0.1	< 11	< 2.6	10	13
0.1–0.5	11–55	2.6–13.1	70	64
0.5–0.9	55–88	13.1–23.5	20	18
> 0.9	> 88	> 23.5	0	5
0.32[b]	35.2[b]	8.76[b]		

[a] Interconversion of methylmercury concentration in hair from methylmercury intake based on 110 g of daily fish consumption during the whole year (Clarkson *et al*., 1988).

[b] Observed MeHg daily intake and mean fish and human hair MeHg concentration.

DISCUSSION

The variation in mercury and methylmercury levels in the fish of each trophic level is a result of a varied diet of the fish throughout the dry and raining season. Freshwater fish from areas considered to be unpolluted (without any anthropogenic mercury point source input) usually have levels lower than 0.2 μg Hg · g^{-1} wet wt (Meili, 1991). In Balbina reservoir, 13 fish samples analyzed (40%) presented methylmercury concentrations higher than 0.2 μg · g^{-1} wet wt.

"Tucunaré," which is on the top end of the aquatic food chain, is a good indicator of mercury in fish of Balbina reservoir. In Tucurui reservoir, situated close to a gold mining area, all carnivorous fish presented mercury concentrations above 0.5 μg · g^{-1} wet wt and the mean mercury concentration value was 1.3 μg · g^{-1} wet wt ($N = 121$), showing the contamination in this reservoir (Porvari, 1995). In the Tapajós river basin, Malm *et al*. (1997) observed a mean mercury value of 0.5 μg · g^{-1} wet wt ($N = 122$) in carnivorous fish. Mean mercury in piscivorous fish in Madeira river basin was 0.9 μg · g^{-1} wet wt ($N = 284$) (Malm *et al*., 1997).

It is noteworthy that for certain species such as the omnivorous Piranha (*Serrasalmus rhombeus*), a large range of methylmercury levels is found (Table 2). It probably occurs due to the fact that this species has quite diversified feeding habits. Despite considered omnivorous, "Piranha" is quite specific as carnivorous, feeding small pieces bitten from the prey, but also eats some fruits and seeds, mainly endosperm material (Val and Almeida Val, 1995). Three specimens of "Piranha" which had weights above 600 g, and one had of the highest methylmercury concentrations found in this study. Omnivorous species eat the most varied diet, which is reflected in varied flesh mercury and methylmercury concentrations. Boischio *et al*. (1995) observed the same fact for total mercury on fish sampled from the Madeira river basin (Brazilian Amazon).

This high methylmercury content in the fish muscle tissue can be considered consistent with preliminary data from Madeira river basin (98%) and Tapajós river basin (90%) (Malm *et al*., 1995) or with data from Minamata area fish with values often higher than 95% (Akagi and Nishimura, 1991).

One of the parameters, which may affect the mercury concentration in Balbina fish, is the age of the reservoir. Reports from Sweden and Canada suggested that the impoundment of rivers for the construction of hydroelectric reservoirs generally leads to an elevation of methylmercury in fish, even in the absence of any man-made point source of mercury (WHO, 1990). In new reservoirs, in which the methylation process is fast, the mercury concentrations in fish are highest and decrease as the reservoir ages (Bodaly and Hecky, 1979). Verta (1986) calculated that the half-life for the fish Hg concentration was about 15 years for Finnish reservoirs and only a small decrease can be expected to occur in reservoirs aged 20 years or more.

The hair-mercury data document an increased exposure related to fish diet. Some people of this village consume protein from a variety of sources such as beans, eggs, poultry, and meat. In other fishery villages of Amazon, however, the population normally consumes only fish and fish products. Hence, the mercury concentration in hair samples correlates positively with the number of weekly fish meals. One example of this fact was observed with the population of Tucuruí village (Leino and Lodenius, 1995).

In previous studies on one riverine village from the Tapajós river, a group of women of different ages, exposed continuously to methylmercury through a high carnivorous fish intake, presented a clear seasonal variation of methylmercury intake (Agaki *et al*., 1995; Kehrig *et al*., 1997).

The mother's breast milk was the main source of inorganic and organic mercury for the 24-month-old child, probably justifying the low (80%) percentages of methylmercury in the child's hair. However, in this child's near scalp hair, we observed a peak of mercury and methylmercury concentration and percentages of methylmercury of 100%, reflecting the onset of solid food intake, predominantly fish. The mother of this child was continuously exposed to methylmercury through fish consumption.

In a previous study on one tributary of Madeira river, a similar case was observed with a mother of one riverine family, during pregnancy, which was continuously exposed to methylmercury through fish consumption, presented high methylmercury concentrations in maternal hair samples. These concentrations decrease slightly during pregnancy. The longitudinal analysis of hair methylmercury and percentage of methylmercury in her baby showed high levels of methylmercury and occurred an increase in the percentage of methylmercury in the child's hair from 40 up to 80% (Kehrig *et al*., 1997).

The Joint FAO/WHO Expert Committee on Food Additives (1989) established a provisional tolerable weekly intake of methylmercury of 200 μg (or 30 μg methylmercury/day) for the general population but noted that pregnant women and nursing mothers are likely to be at greater risk of adverse eects from methyl mercury.

The total mean daily methylmercury intake for the Balbina Village is consistent with methylmercury concentrations in hair samples in the range of 2.6 to 13.1 $\mu g \cdot g^{-1}$, based on a daily consumption of about 110 g of fish with methylmercury concentrations in the range of 0.1 to 0.5 $\mu g \cdot g^{-1}$. This consistence was observed here due to data was treated in groups of people. Previously, correlations were tried individually between mercury in hair and number of weekly fish meals and then weak correlations were obtained.

The interconversion of the observed mean hair methylmercury into methylmercury intake (using the equations described by Clarkson *et al*., 1988) confirm that fish consumption is the main source of Hg exposure in this population.

This population had a mean methylmercury daily intake (35.2 μg), which is similar to the FAO/WHO recommended provisional tolerable daily intake. Based on these calculations, and considering that adverse effects on infant development have been associated with levels of exposure that result in few, if any, signs of maternal clinical illness or toxicity, it is recommended that pregnant women and women of child-bearing age consume less fish, to limit their exposure to potential sources of methylmercury.

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