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Methylmercury pollution in the Amazon, Brazil

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Abstract

In order to evaluate the extent of environmental mercury pollution due to goldmining activities in the Amazon, concentrations of total mercury and methylmercury were determined for human hair and fish samples from five fishing villages located mainly in the Tapajos river basin. Abnormally high levels of mercury were found in human hair from Jacareacanga and Brasilia Legal located near the main goldmining area as well as from Tres Bocas facing the lake in Amapa state, whereas the levels were much lower in Ponta de Pedras, a long way downstream. The values of male samples were about twice as high as females even in the same family. The predominant form of mercury in hair samples was methylmercury (average 90%), while high hair mercury observed in goldminers and workers in goldshops was mostly in the inorganic form. Longitudinal analyses of long hairs from people in fishing villages showed fairly constant and continuous exposure to methylmercury at least over the last few years with seasonal variations. Fish contained relatively high levels of mercury (up to 3.82 ppm) mostly in the form of methylmercury and most of the fish from upstream and some from downstream exceeded the allowable level of 0.5 ppm in Brazil.

Keywords: Methylmercury pollution; Total mercury; Gold mining; Amazon; Human hair samples, mercury pollution; Fish, mercury pollution

1. Introduction

Goldmining activities are quite widespread in the Amazonian region and the potential contamination by mercury used for collecting alluvial gold has become a matter of great concern.

Brazilian research groups have estimated that total mercury losses during the goldmining process in the entire Amazon river basin are of the order of 100 tons per year, of which 45% is released into river systems and 55% into the atmosphere [1,2].

The conversion of mercury in its various forms into methylmercury in the aquatic ecosystems, mainly in the bottom sediments has long been recognized as a critical process in aquatic environmental mercury contamination. It seems possible that the generation of methylmercury may similarly occur from inorganic mercury after en-

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tering the river systems and mercury levels in fish can become a health hazard through biomagnification. In fact, relatively high levels of total mercury in fish and human hair samples from the main tributaries of the Amazon river basin have already been reported by several research groups [3–5].

However, we are still unable to predict to what extent fish and people are exposed to methylmercury produced from inorganic mercury in the river systems, since information is limited to total mercury which includes contamination from both inorganic mercury and methylmercury.

The environmental mercury pollution in the Amazon originating from metallic mercury is quite different from that experienced in the past in Japan or Iraq, which originated from methylmercury. In goldmining areas, occupational poisoning due to inorganic mercury can occur among goldminers, whereas the people living downstream from goldmining might be exposed to both inorganic mercury and methylmercury. Therefore, the speciation of mercury is essential in surveying the chemical behavior of mercury in the ecosystems.

Recently, we have developed simplified and sensitive methods for the determination of nanogram amounts of total mercury and methylmercury in various biological and non-biological samples, including natural waters. These analytical techniques are suitable for multiple analyses of mercury in epidemiological studies, being able to analyze small amounts of mercury in various kinds of samples, quickly and precisely.

The work reported here was undertaken to evaluate the contamination levels of both total mercury and methylmercury in human hair as well as fish samples collected in fishing villages mainly from Tapajos river basin in the Amazon using these advanced analytical techniques.

2. Materials and methods

2.1. Study area

An enormous amount of mercury has been released mainly in the last 20 years in the Amazon area, and the Tapajos river basin is considered the oldest and most productive goldmining area. In order to evaluate the actual extent of methylmercury pollution in local populations, human hair samples were collected from inhabitants of various fishing villages at different distances from the goldmining area and analysed for total mercury and methylmercury. Human hair and fish samples analyzed in this study came from the areas as shown in Fig. 1.

2.2. Hair sampling

In total, 136 human head hair samples were collected from inhabitants of five fishing villages where people consume locally caught fish: 48 from Jacareacanga with a population of about 3000, about 600 km upstream from Itaituba; 11 from Rainha with a population of about 100, 20 km upstream from Itaituba; 56 (37 in March, 19 in July) from Brasilia Legal with a population of about 1000, 100 km downstream from Itaituba as the main gold commerce center in Tapajos river; 10 from Ponta de Pedras with a population of about 150, 350 km downstream from Itaituba; and 11 from Tres Bocas facing a lake where another goldmining area is located in Amapa state.

Most of hair samples were collected by author FJPB and some in Ponta de Pedras by author MH. At the same time, clinical examinations were also performed for nearly all individuals sampled.

The number of subjects together with the median and range of age in each village are summarized in Table 1. Hair samples from Rainha, Brasilia Legal, Ponta de Pedras and Tres Bocas were collected in March 1992 and those from Jacareacanga were collected in May 1992. Hair samples in Brasilia Legal were taken again in July 1992.

Apart from these samples, seven hair samples from goldminers, four samples from workers in goldshops and 13 samples from former goldminers (inpatients with mercury poisoning and other diseases) in Itaituba collected in November 1989 were made available for comparison.

All these hair samples were taken from near the hair roots and cut finely with surgical scissors just before analysis. Some of the long hair samples were cut into 15-mm-long pieces for a longitudinal analysis of hair mercury.

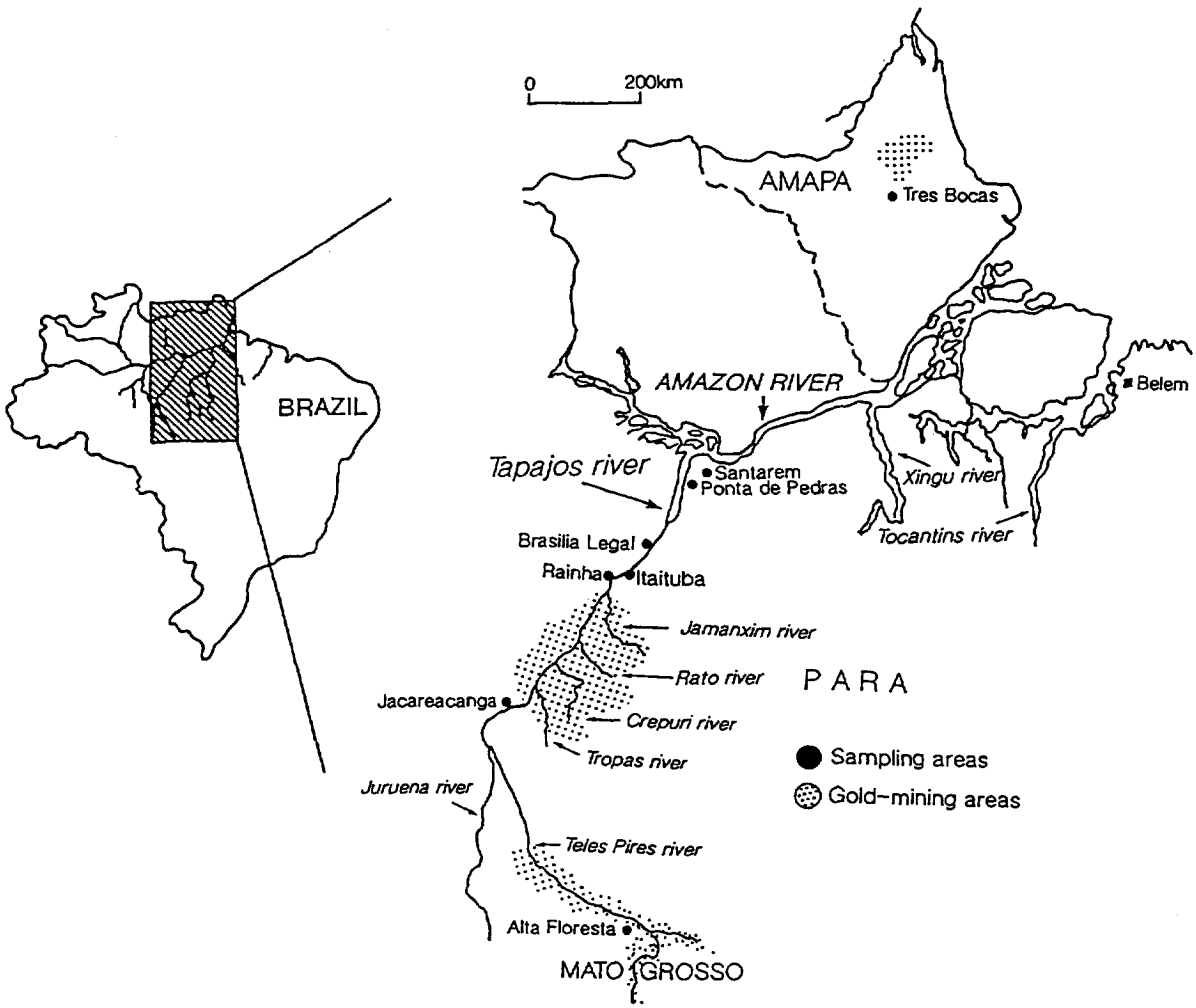

Fig. 1. Map of the study area.

Table 1
Number and median age of subjects sampled in each village

Village (sampling dates)	Sex	*N*	Age		
			Median	Range	Mean
Rainha	Male	2	—	28–61	44.5
(March, 1992)	Female	9	21.0	14–65	32.6
Brasilia Legal	Male	10	28.0	22–78	37.1
(March, 1992)	Female	27	20.0	4–78	27.1
Ponta de Pedras	Male	6	27.0	14–46	28.5
(March, 1992)	Female	4	33.5	7–63	34.3
Jacareacanga	Male	18	32.5	2–86	32.4
(May, 1992)	Female	30	18.0	4–62	23.5
Brasilia Legal	Male	9	49.0	25–74	49.7
(July, 1992)	Female	10	28.5	17–60	35.0
Tres Bocas	Male	7	32.0	22–56	35.1
(March, 1992)	Female	4	37.5	28–67	42.5

2.3. Fish sampling

Fish samples analyzed were obtained from Teles Pires river, Rato river, and the main channel of Tapajos river near Itaituba, Brasilia Legal and Santarem, respectively. Most of the fish samples were taken by the present research group at Federal University of Rio de Janeiro and were stored in a freezer until analysis for mercury. According to previous sampling and analyses by the University, these fish are representative of the upper Tapajos river basin. Also from previous experience in the area, 83% of the fish considered here are piscivorous (fish-eating).

2.4. Analytical procedures for mercury

In the present work, the determination of total mercury and methylmercury were made with sensitive and reliable methods recently developed in our laboratory [6].

Total mercury analysis in hair and fish. The procedure for total mercury in hair and fish is shown in Fig. 2. A known amount of sample (1–10 mg of hair and 0.5 g or less of fish) was placed in a 50-ml volumetric flask, to which 1 ml of water, 2 ml of nitric/perchloric acid (1:1) and 5 ml of sulfuric acid were added and heated at 230–250°C on a hotplate for 20 min. After cooling, the digested sample was made up to 50 ml with mercury-free water. An aliquot of the sample solution was introduced into an automated circulating air flow system with the addition of 10% stannous chloride solution. After air circulation for 30 s, the Hg in circulating air was measured by cold vapor atomic absorption spectrometry. This procedure is simple and can analyze accurately at least 100 samples a day. The detection limit is around 0.5 ng Hg.

Methyl mercury analysis in hair. As shown in Fig. 3, 10–20 mg of hair sample was placed in a 10-ml test tube with a screw cap, to which two drops of ethanol, 5 ml of 2 N hydrochloric acid and then a small amount of cotton were added to prevent the hair sample from floating. The test tube was capped tightly, and heated at 100°C in a water bath for 5 min. The sample solution was then stirred well using a voltex mixer. After cooling, 1 ml of the 2 N HCl extract was transferred to another 10-ml test tube with a screw cap

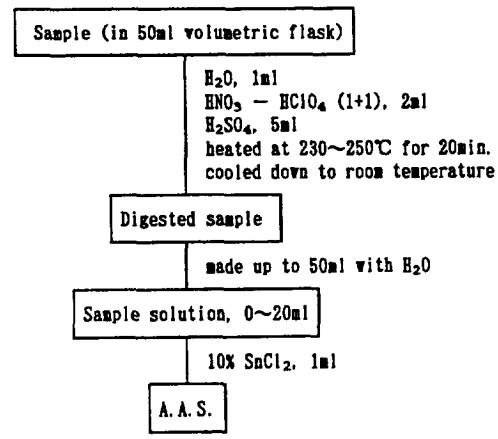

Fig. 2. Analytical procedure for total mercury in biological samples.

and extracted with 4 ml of benzene. Methylmercury in the final benzene extract was measured by ECD-gas chromatography.

Methyl mercury analysis in fish. The analyses of methylmercury in fish flesh were made with the combined method of dithizone extraction and ECD-gas chromatography. This method is based on the fact that methylmercury dithizonate in the final sample solution in benzene is converted into

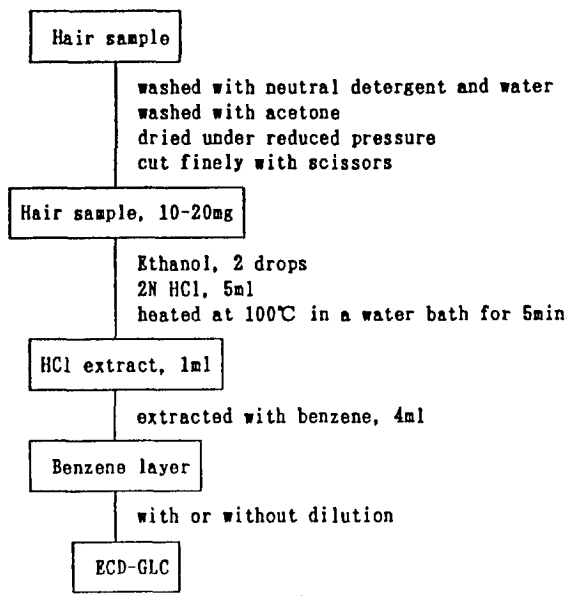

Fig. 3. Analytical procedure for methylmercury in human hair.

its chloride form as soon as it is subjected to conventional ECD-gas chromatography. Therefore, a special column with about 0.2 g of NaCl crystals added on the top of column (Hg-20A, GL Science Ltd.) was used throughout the methylmercury measurement in this study.

The outline of the standard procedure established for methylmercury in fish is shown in Fig. 4. A known amount of fish sample (usually 0.5 g or less) was digested with 20 ml of 1 N KOH in ethanol in a 50-ml screw-capped centrifuge tube at 100°C in a water bath for 1 h. The digested sample was slightly acidified with 20 ml 1 N HCl. After washing with 5 ml of *n*-hexane, the methylmercury in the sample was extracted with 10 ml of 0.05% dithizone in benzene. The benzene layer was then washed twice with 5 ml of 1 N NaOH to remove the excess dithizone in the benzene layer. An aliquot of the benzene layer (usually 5 ml) was transferred to a 10-ml test tube with cap and back-extracted with 2 ml of 5 ppm Na_2S in 0.1 N NaOH/ethanol (1:1). After centrifuging, 1 ml of the lower layer was transferred to another 10-ml test tube, to which 1 N HCl was added dropwise with N_2 gas bubbling through the solution until a blue color appeared; bubbling was continued for a further 3 min to eliminate the excess sulfide ions as H_2S gas. To the sample solution, 2 ml of Walpole's buffer (pH 3.0) was added and the mixture was re-extracted with 1 ml of 0.05% dithizone in benzene purified with an equal volume of 0.1 N NaOH just before use. The benzene layer was washed with 3 ml of 1 N NaOH, and subsequently with 3 ml of distilled water and acidified with a few drops of 1 N HCl followed by ECD-gas chromatographic measurement. This method is applicable to various types of biological materials and is also suitable for the analysis of materials containing methylmercury at background levels. The detection limit of this method is around 5 ng/g sample for a 0.5-g sample on a wet weight basis.

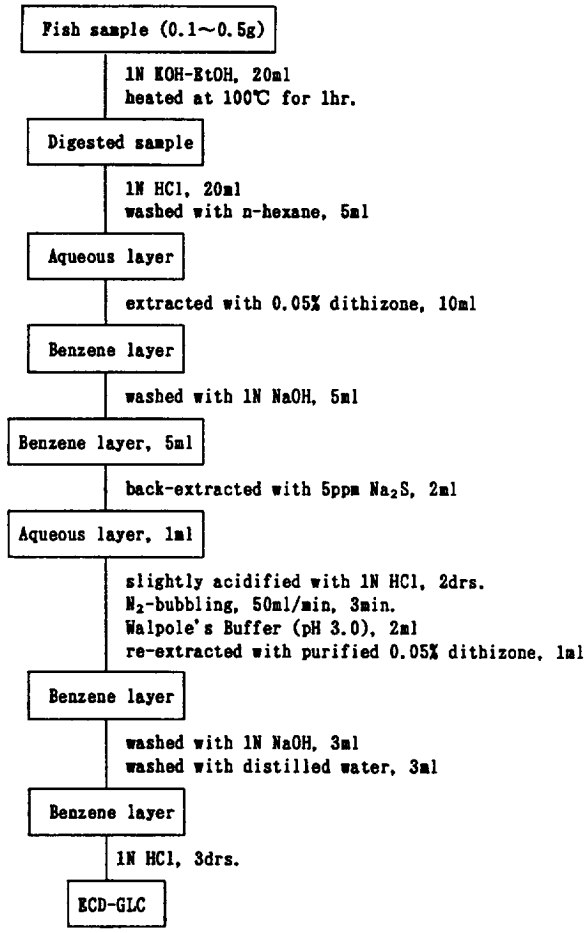

Fig. 4. Analytical procedure for methylmercury in fish.

3. Results and discussion

It is well recognized that people are mainly exposed to mercury through the consumption of fish, the main source of dietary methylmercury, and head hair has usually been chosen for monitoring its intake in persons at risk [7]. In the Amazon areas, however, special consideration must be given to the analysis of mercury in human hair if the data are to be compared meaningfully, because of the contribution of inorganic mercury originating from metallic mercury due to goldmining activities.

The concentrations of total mercury and methylmercury detected in hair samples from goldminers (a–g) and workers in goldshops (h–k) together with 13 former goldminers among the inpatients at Itaituba hospital are shown in Fig. 5. Open circles and closed circles represent total mercury and methylmercury, respectively. Hair samples from goldminers (five males) and former goldmin-

ers (ten males) contained total mercury at median levels of 2.7 and 2.9 ppm, respectively, while much higher levels of total mercury were observed for workers in goldshops (four males); the median value was 11.6 ppm. In contrast to the large variation of total mercury levels, the results of methylmercury analysis showed fairly low and uniform levels ranging from 0.9–1.7 ppm in median values in these three groups. Thus, the average proportions (median) of methylmercury to total mercury were similar at 35.6–43.0% in goldminers and former goldminers, and lower (13.0%) in workers in goldshops. The levels of methylmercury in these groups are comparable to 2 ppm for the mean reference value for total mercury in human hair which can be regarded to be almost totally in the form of methylmercury [8].

These results indicate that goldminers and workers in goldshops have been exposed to substantial levels of inorganic mercury, probably mercury vapor, and as a consequence, the contribution of inorganic mercury to hair by direct uptake from air or from sweat is likely to be greater than that of the general population. Of these hair samples collected, one sample was underarm hair (asterisked in Fig. 5). This sample contained total mercury at 4.8 ppm but the methylmercury concentration was only 0.6 ppm, suggesting that a greater contribution of inorganic mercury in sweat is considered to be the predominant source of mercury in this hair.

The results for total mercury and methylmercury concentrations in hair samples from the inhabitants of five villages are presented in Table 2. Total mercury concentrations at various locations showed relatively high median levels ranging from 10 ppm to several 10s ppm with a large variation. It should be noted that the values of male samples are higher than females in all villages.

At Ponta de Pedras village relatively low levels were observed. The highest values were observed at Brasilia Legal (sampled in July) with a maximum total mercury of 151.2 ng/mg. At Jacareacanga (sampled in May) and Rainha village, around the same median values were found as Brasilia Legal, suggesting similarly high mercury

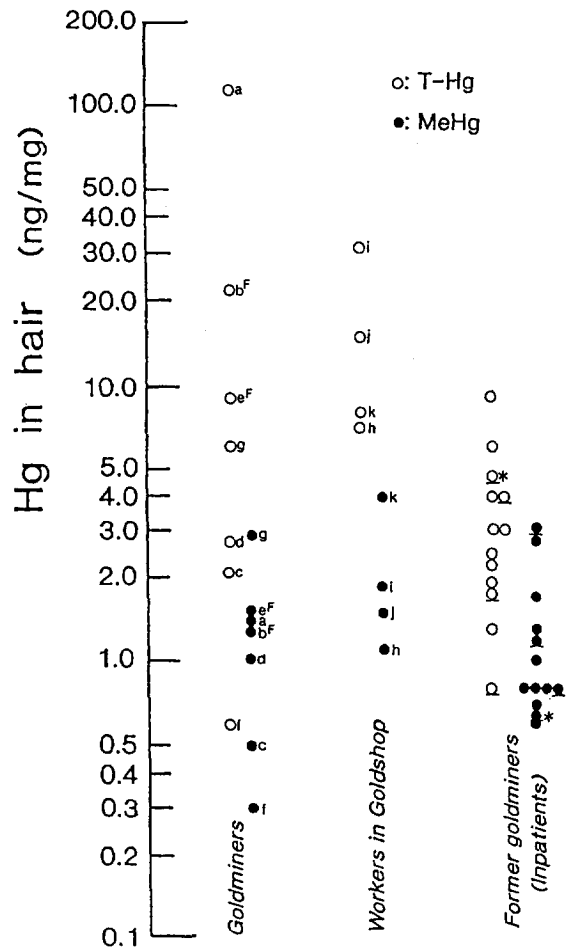

Fig. 5. Total mercury and methylmercury concentrations in hair from goldminers near Itaituba, workers in goldshops and former goldminers (inpatients) in Itaituba; F, Female goldminers; *, armpit hair was used for mercury analyses. ○,●, hair was sampled in November 1989; ○,● hair was sampled in March 1992.

exposures in the upstream Tapajos area when compared at the same sampling date (in March).

The high median values in Tres Bocas should be mentioned. They may be the result of acceleration of the methylation process and more methylmercury accumulation in fish in the lake with longer retention time compared with river systems, though further efforts are needed to examine the environmental mercury pollution including mercury contents in fish there. Much higher median values of both total and meth-

Table 2
Total mercury and methylmercury concentrations in hair from the inhabitants living along the Tapajos River and Lake Tres Bocas

Village (sampling date)	Sex	N	T-Hg			MeHg		
			Median	Range	Mean	Median	Range	Mean
Rainha	M	2	19.2	17.1–21.4	19.2	19.1	17.1–21.1	19.1
(March, 1992)	F	9	15.0	2.4–31.1	15.0	14.1	1.9–29.4	14.1
Brasilia Legal	M	10	25.5	5.4–46.9	24.7	23.4	4.8–42.6	23.3
(March, 1992)	F	27	9.9	3.5–32.2	12.5	8.6	0.9–32.2	10.7
Ponta de Pedras	M	6	11.6	9.7–12.6	11.4	10.8	9.1–12.0	10.8
(March, 1992)	F	4	8.1	6.2–10.8	8.3	6.8	4.7–10.8	7.3
Jacareacanga	M	18	20.0	4.8–46.1	22.7	19.9	3.9–49.9	20.8
(May, 1992)	F	30	13.6	1.5–32.9	13.0	11.7	1.1–32.7	11.9
Brasilia Legal	M	9	44.4	12.0–151.2	58.5	40.4	11.1–132.6	49.8
(July, 1992)	F	10	14.4	7.2–29.5	15.7	11.6	6.1–26.3	13.2
Tres Bocas[a]	M	7	34.5	8.4–53.8	31.4	33.6	6.1–50.3	29.7
(March, 1992)	F	4	19.1	15.4–35.1	22.2	17.7	13.5–32.1	20.2

[a]Tres Bocas is located near Lake Tres Bocas in Amapa (not Tapajos river basin).

ylmercury in July samples than those of March samples in Brasilia Legal could be explained by (1) higher intake of contaminated fish, (2) higher contents of methylmercury in fish in different seasons and/or (3) the direct consequence of more intense goldmining activity in the dry season.

Surprisingly, methylmercury analysis in hair showed that the values were very close to total mercury levels in almost all hair samples as shown in Table 2. The average proportions of methylmercury to total mercury were more than 90%, indicating that methylmercury was the predominant form of mercury in hair samples and that in these five villages people were mainly exposed to methylmercury. Malm et al. also observed similar levels of total mercury in human hair from Madeira river area (0.22–40.0, mean 9.2 ppm) [5]. In their studies, the total mercury levels in hair of people from Mato Grosso gold mining area, who did not consume any fish diet ranged from 0.04 to 6.3 ppm with an average value of 2.4 ppm, whereas people in Tapajos goldmining area, who consumed a fish diet had higher hair mercury concentrations ranging from 10.0 to 31.8 ppm with an average value of 18.7 ppm. They also confirmed with the control groups in Rio de Janeiro that people with a common fish diet had 5.4 ppm on average with a range from 1.5 to 13 ppm, whereas people with no regular fish diet had only 1.7 ppm on average, ranging from 0.9 to 3.1 ppm. Therefore, the abnormally high concentrations of mercury observed in the present study are considered to be the result of exposure to methylmercury through the consumption of contaminated fish.

In fact, measurements of hair mercury from fishermen and their families in Brasilia Legal showed much higher levels for the people with fishery occupations than the other family members (Table 3). As a consequence males presented higher levels of mercury than females, mostly in the form of methylmercury, probably due to their habit of eating proportionally more fish diet even in the same family. It is worthwhile mentioning that a single questionnaire was administered to all subjects for hair sampling in Brasilia Legal as a trial. The questionnaire contained eating habits including frequency of fish consumption, occupation, smoking and alcohol beverage, hair styling, dental therapy and some subjective complaints related to mercury poisoning.

No apparent symptoms which suggest typical Minamata Disease were observed by our clinical examinations in the study areas.

It is known that longitudinal mercury analysis along the strands of hair provides information on previous exposure levels in individuals. As hair grows at about 1.0–1.5 cm per month, complete regrowth is possible over several months or years depending on the length of the hair sample. Fig. 6

shows the results of such a longitudinal analysis of hair mercury. In the Amazon area, June–November is the dry season and the other half-year is the rainy season. From the results, individuals are exposed continuously to methylmercury at fairly constant levels at least over the last few years up to the present time in all locations with some seasonal variations, significantly so for those whose mercury levels are high.

It can be seen in Tres Bocas and Jacareacanga, in particular, that there is a tendency for increased levels of mercury in hair with time.

Sample No. B-15 in Brasilia Legal was an exception, showing a rapid decrease in hair mercury with the time. This woman (B-15) had dyed her hair 1 month before sampling and dyes it every month. This hair sample contained mercury almost all in inorganic form.

The results of measurements of total mercury and methylmercury in fish from Tapajos basin are presented in Table 4. Total mercury in fish ranged from 0.08 to 3.82 ng/mg, and more than half (58%) of the fish samples analyzed had relatively high mercury concentrations exceeding the Brazilian allowable limit of 0.5 ng/mg. Extremely high mercury levels of 3.82 and 2.85 ng/mg in Piraiba (*Brachyplatystoma filamentosum*) were observed in Teles Pires river near Alta Floresta, far upstream in the Tapajos river basin, where the goldmining activities had been growing more and more in recent years. The average value in all fish samples from this area is 1.3 ng/mg. The predominant form of mercury was also found to be methylmercury in all samples analyzed, with on average 91.5%.

Relatively high values of fish mercury were also found in other locations near main gold mining areas, while far downstream in Tapajos river basin, near Santarem, levels were lower, with 0.08 and 0.39 ng/mg even in piscivorous fish. This suggests high and widespread contamination of this kind of fish in the upper Tapajos river basin. From these results, it seems that the regional differences in methylmercury levels in each local fish should be reflected in the levels of mercury in hair of people in the individual areas.

To confirm and elucidate the pathway of the methylmercury from fish to human hair, more epidemiological studies are needed on eating habits, and fish consumption, together with further surveys on the mercury pollution levels in various fish species.

Table 3
Hair mercury contents (ng/mg) of fishermen's families from Brasilia Legal in March 1992

No.	Relation	Age	Occupation	T-Hg	MeHg
38	Father	49	Fishery & agriculture	37.5	32.3
36	Mother	45	Housewife	17.0	13.9
01	Son	22	Fishery	41.9	37.5
39	Daughter	17	Housewife	14.7	12.2
03	Mother	45	Housewife	7.8	6.8
04	Daughter	16	—	5.2	4.9
05	Son	22	Fishery & agriculture	5.4	4.8
13	Father	47	Agriculture	48.3	47.6
44	Mother	51	Housewife	19.0	15.3
43	Son	25	Fishery & agriculture	44.4	40.4
40	Father	71	Fishery	56.7	46.7
47	Mother	60	Housewife	29.5	26.3
41	Son	29	Fishery	82.9	68.1
30	Father	60	Fishery	31.7	27.5
31	Mother	57	Housewife	16.5	14.9
32	Daughter	22	Servant	12.0	10.5

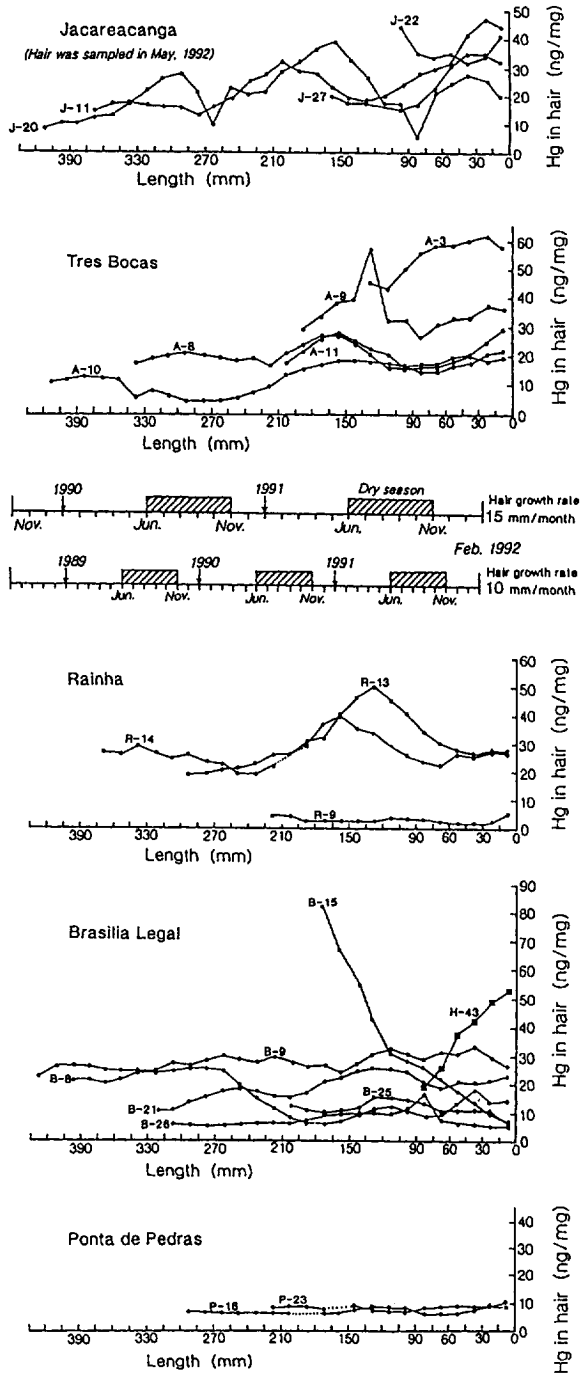

Fig. 6. Longitudinal mercury analyses along strands of hair. Hair was sampled in March 1992 except for one case (H-43) which was sampled in July 1992.

These data together with the fact that human hair samples contain relatively high levels of mercury mostly in the methylated form suggest strongly that metallic mercury released into the river systems is transformed into methylmercury somewhere in the aquatic environment, probably in the bottom sediments, and ultimately accumulates in fish bodies by biomagnification in these ecosystems.

Further studies are needed to elucidate these phenomena, that is, the origin and the main pathways of methylmercury found in fish and human hair in this and in other Amazon river systems. Also, detailed epidemiological information such as eating habit, amounts of fish in their diet and species commonly consumed should be more thoroughly investigated for people living in these areas.

4. Conclusions

In order to evaluate the actual extent of methylmercury pollution, human hair samples and fish samples were collected from five fishing villages at different distances from a goldmining area.

1. The methylmercury contents of hair from the inhabitants of fishing villages located upstream on Tapajos river were higher than those downstream. A few people among a total of 136 hair samples examined had methylmercury levels exceeding 50 ppm (minimum of threshold value; 50–125 ppm for methylmercury poisoning as indicated in a recent IPCS publication [8]). It should be noted that quite a high median mercury level was present in the fishing village of Tres Bocas facing the lake.
2. The lowest median values for both total mercury and methylmercury were found in Ponta de Pedras village located downstream on Tapajos river, but even these levels were higher than for people without a specific mercury contamination (1–5 ppm in hair as total mercury in Brazil and other countries).
3. The longitudinal mercury analysis of the long hair samples indicated that the inhabitants of fishing villages in this study were exposed continuously to methylmercury at least over the last few years to the present time with fairly constant average values, and even a

Table 4
Total mercury and methylmercury concentrations in various fish species from Tapajos river basin

Origin	Name of fish	Weight (g)	T-Hg (ng/mg)	MeHg (ng/mg)	MeHg/T-Hg (%)
Teles Pires river	Daurada	2700	0.60	0.57	95.0
(near Alta Floresta)	Jau	23000	0.61	0.58	95.1
	Jau	24000	0.75	0.70	93.3
	Jau	25000	1.03	0.82	79.6
	Jau	32000	0.39	0.36	92.3
	Piraiba	22000	3.82	3.29	86.1
	Piraiba	40000	2.85	2.44	85.6
	Piranha	400	0.29	0.29	100.0
Rato river	Aruana	515	0.28	0.24	85.7
	Mandube	290	0.53	0.54	101.9
	Mandube	330	0.56	0.60	107.1
	Peixe-cachorro	100	1.60	1.60	100.0
	Traira	6000	0.95	0.83	84.4
	Jacare (alligator)[a]	11500	0.32	0.27	84.4
Tapajos river	Acara	160	0.17	0.15	88.2
(near Brasilia Legal)	Apapa	1040	0.60	0.52	86.7
	Pacu	1430	0.10	0.09	90.0
	Pescada	910	0.41	0.36	87.8
	Tucunare	570	1.16	1.12	96.6
Tapajos river	Apapa	450	0.54	0.46	85.2
(near Itaituba)	Filhote	4000	1.00	0.95	95.0
	Pescada	200	0.56	0.52	92.9
Tapajos river	Apapa	390	0.39	0.37	94.9
(near Santarem)	Pirarucu	20000	0.08	0.07	87.5

[a]Not a fish.

slightly increasing trend for some of them. In other words, no decreasing trend was observed with time. A significant seasonal variation in mercury levels was observed for the hair samples with high contents of mercury.

4. The methylmercury content of fish from most of the upstream area and some of the downstream area in Tapajos river exceeded the limits (< 0.5 ppm in Brazil) for Hg in edible fish tissues.
5. Thus, it could be concluded that high and widespread contamination with methylmercury in Tapajos river basin was strongly suggested by the present methylmercury concentrations determined by both human hair and fish samples.
6. Analysis of hair samples from goldminers and workers in goldshops clearly showed that they were contaminated from inorganic mercury as well as methylmercury. Thus, analysis solely for total mercury is not sufficient, and methylmercury should be determined in areas where contamination with both methylmercury and inorganic mercury occurs as in the Amazon area.

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