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Daily mercury intake in fish-eating populations in the Brazilian Amazon

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Although high levels of fish consumption and bioindicators of mercury exposure have been reported for traditional populations in the Amazon, little is known about their actual daily intake of Hg. Using an ecosystem approach, calculate daily mercury intake in adult fish-eaters, examine the relations between mercury intake and bioindicators of exposure and the factors that influence these relations. A cross-sectional dietary survey on fish and fruit consumption frequency was carried out with 256 persons from six villages of the Tapajós River. Fish portion per meal was determined. Mercury concentration was determined for 1123 local fish specimens. Daily mercury intake (μg/kg/day) was determined for men and women from each village using the average fish-mercury concentration for the fish caught in their fishing zone, the average quantity of fish per meal, fish-species frequency consumption and participants' body weight. Fish-mercury averaged 0.33 μg/g ± 0.33. Daily mercury intake varied between 0 and 11.8 μg/kg/day (mean 0.92 μg/kg/day ± 0.89) and varied by gender and village. Mean blood- and hair-mercury were 58.7 ± 36.1 μg/l and 17.9 ± 11.5 μg/g, respectively. There was a strong and positive relation between blood-mercury and daily mercury intake, with an inverse relation for fruit intake and schooling; significant variations were observed with immigrant status, and among villages. Hair-mercury was directly associated with daily mercury intake and inversely related to schooling and fruit consumption. Fruit consumption modified the relation between daily mercury intake and blood-mercury levels: for the same daily mercury intake, persons eating more fruit had lower blood-mercury concentrations (ANCOVA Interaction term: $F = 10.9$, $P < 0.0001$). The median difference of the ratio of blood-mercury to daily mercury intake between low and high fruit consumers was 26, representing a 26.3% reduction. These findings reveal high levels of daily mercury intake. Rigorous studies for developing risk-based reference doses in the Amazon should be undertaken to orient viable risk-management strategies to reduce exposure, while maintaining fish diet.

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Introduction

Although gold mining activities were considered the unique source of mercury pollution into the Amazon for many years (Malm, 1998), pioneering studies in the Tapajós River Basin in Brazil have shown that the mercury content in the water column is influenced by the amount of particulate matter, independently from upstream gold mining activities (Roulet et al., 1998a), and regional variations of mercury concentrations and burdens in soil compartments cannot be explained by the presence of gold mining centers in the region (Roulet et al., 1998b). In fact, removal of forest cover and destruction of root systems subsequent to deforestation and "slash and burn" agricultural practices deplete the soil, releasing naturally occurring mercury into the water systems (Roulet et al., 1999). Roulet et al. (2000), who examined mercury contamination in lacustrine sediments following deforestation, suggest that the environmental changes initiated between 1950 and 1970, which coincide with the first colonizing projects in the Amazon, probably correspond to the beginning of mercury leaching into the rivers. The resulting mercury pollution of the aquatic environment is taken up by the halieutic resources (Farella et al., 2001; Roulet et al., 2001). These important pathways of exposure, later confirmed for other watersheds of the Amazonian Basin (Fostier et al., 2000; Lechler et al., 2000; Fadini and Jardim, 2001; Wasserman et al., 2003; Lacerda et al., 2004; Almeida et al., 2005; Bastos et al., 2006; Farella et al., 2006, 2007; Mainville et al., 2006), constitute a continuous source of mercury exposure for the large number of traditional and immigrant communities of the region that rely on fish consumption as a dietary mainstay.

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This paper is dedicated to the memory of our dear and always CARUSO Project fellow, Dr. Marc Roulet, who made the fascinating discovery of deforestation and soil erosion as the major source of mercury into the riparian Tapajós region, and opened a new era of mercury biogeochemistry research in the Amazon. To this outstanding scientist, all our respect and acknowledgments.

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Despite many years of research and biomonitoring, adult traditional populations in the Amazon continue to be chronically exposed to high levels of mercury through their fish diet (Bastos et al., 2006, Pinheiro et al., 2006). In these populations, there is strong evidence of early nervous system dysfunction and, to a less extent, immunologic and cardiovascular effects among both adults and children (Lebel et al., 1996, 1998; Grandjean et al., 1999; Amorim et al., 2000; Dolbec et al., 2000; Harada et al., 2001; Cordier et al., 2002; Mergler, 2002; Yokoo et al., 2003; Silbergeld et al., 2005; Fillion et al., 2006).

During the last decade, a large number of exposure assessments of fish-eating populations have been conducted in different countries of the Amazon (Barbosa et al., 1997; Lebel et al., 1997; Cordier et al., 1998; Dolbec et al., 2001; Santos et al., 2003; Passos et al., 2003a; Gonçalves and Gonçalves, 2004; Webb et al., 2004; Dórea et al., 2005; Bastos et al., 2006; Pinheiro et al., 2006). However, little attention has been given to the importance of mercury intake in these populations, although a limited number of studies have tried to estimate the rates of mercury intake (Boischio and Henshel, 1996, 2000; Bidone et al., 1997; Hacon et al., 1997; Kehrig et al., 1998; Fréry et al., 2001). Daily oral exposures are used in many countries as the basis for risk assessment (Clarkson and Magos, 2006). In the Amazon, mercury daily dose is particularly difficult to estimate due to the diversity of fish and ecosystems, as well as seasonal changes in fish availability. Furthermore, studies in this region have shown that fruit consumption influences the relation between fish consumption and mercury concentrations both in blood and hair (Passos et al., 2003b, 2004, 2007a, b), but we do not know how it affects the relationship between mercury intake and exposure.

To determine oral doses, one requires not only information on human eating habits and bioindicators of exposure, but also a considerably large database on mercury concentrations in fish from different local ecosystems. As part of the Caruso Project (CARUSO, 2007), which has been studying mercury sources, its pathways through the ecosystem, human exposure and effects, there is extensive data on mercury concentrations in a large number of local fish species. The aims of the present study were as follows: (i) to estimate mercury intake in communities along the Tapajós River of the Brazilian Amazon; (ii) to analyze the relations between mercury intake and bioindicators of exposure; (iii) to examine the factors that influence the relation between mercury intake and bioindicators of exposure.

Methods

Study Design and Population

We conducted a cross-sectional study including six riparian communities situated in the Tapajós region, one of the most important tributaries of the Amazon River. Two of these communities are located on small tributaries of the Tapajós (villages of Açaituba and Santo Antonio), whereas the communities of São Luis do Tapajós and Nova Canaã are located on the east and west banks of the main channel of southern Tapajós, respectively. The villages of Vista Alegre and Mussum are located on the west bank of the lower Tapajós, near the small town of Aveiro (Figure 1).

In this area of the Tapajós, the only source of methyl mercury exposure for these remote riparian communities is the consumption of mercury-containing fish. The study population is composed of riparian villagers, most of whom are involved in subsistence fishing, agriculture and, to a lesser extent, cattle-raising. These traditional communities originated from the miscegenation of old indigenous populations with European colonizers and, to a less extent, with African slaves during the 18th and 19th centuries (Lima, 1992; Murrieta, 2001). Fish are obtained from the river and fluvial lakes close to the villages, without significant crossover between communities which are relatively far away from each other. Most villages can only be reached by boat.

Recruitment was performed during a house-to-house survey, which also served to estimate the sociodemographic characteristics of the total population. The study was explained at each household and persons were invited to participate on a voluntary basis. In addition, community meetings were held in each village to further explain the study. A total of 256 villagers, 15 years and older (121 women and 135 men), making up 38.2% of the underlying population of 670 adults, participated in the present study, which was carried out during the descending water season of 2003 (June and July).

All human and fish data were collected at the same period. Two interview-administered questionnaires were used. One targeted sociodemographic information (age, educational level, place of birth, length of time in the region, subsistence activities, as well as specific questions about working in gold mining and exposure to mercury through burning amalgam or other contaminants). The second was a food frequency questionnaire (described below). Hair and blood samples were taken and fish sampling was carried out.

The research protocol was approved by the Institutional Review Board of the Federal University of Rio de Janeiro (Brazil) and of the University of Quebec in Montreal (Canada). The study was explained individually, and persons agreeing to participate signed an informed consent form that was read to them.

Assessment of Fish-mercury Concentrations

Fish, from local village fishing sites, were captured with gill nets, and a piece of muscle tissue devoid of skin and bones was taken and frozen individually for future mercury analysis. The analyzed species included the following: Aracu (*Schizodon sp.*), Pescada (*Plagioscion squamosissimus*),

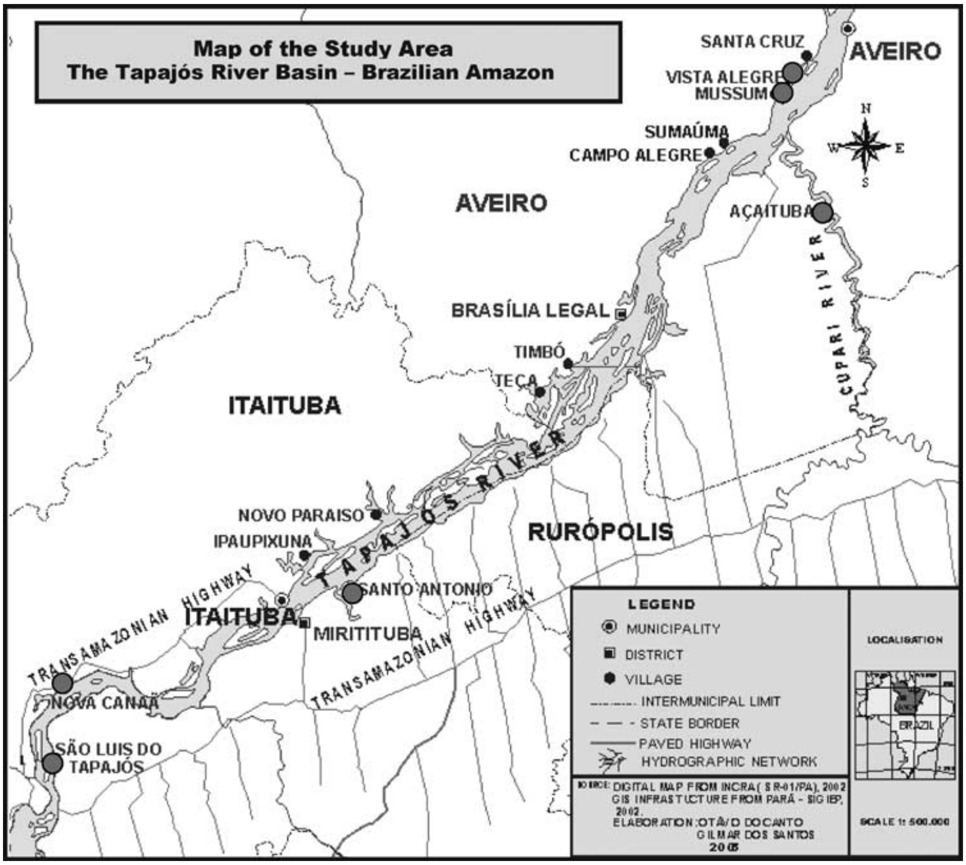

Figure 1. Map of the study area, the Tapajós River Valley. Participating communities are identified by a large dot.

Tucunaré (*Cichla monoculus* and *Cichla temensis*), Caratinga (*Geophagus proximus*), Pacu (*Mylossoma sp.*), Jaraqui (*Semaprochilodus insignis*), Piranha (*Pygocentrus sp.*), Branquinha (*Curimata inordata*), Acari (*Liposarcus pardallis*), Charuto (*Hemiodus unimaculatus*), Mandi (*Auchenipterus nuchalis*), Peixe-Cachorro (*Raphiodus vulpinus*), Surubim (*Pseudoplatystoma sp.*), Traira (*Hoplias malabaricus*), Sarda (*Pellona castelnaeana*), Curimata (*Prochilodus nigricans*), Tambaqui (*Colossoma macropomum*), Matrinxã (*Brycon cephalus*), and Curvina (*Pachypops furcraeus*). A total of 1339 fish were captured and analyzed. Here, we use the average mercury concentrations for the fish species that were effectively eaten in each community, comprising 1123 fish specimens. The species included here constitute 99.8% of the total fish diet as recorded in the dietary survey described below.

Total mercury concentrations in fish flesh were determined in the laboratory of the Environmental Research Chair-GEOTOP at the University of Quebec in Montreal, according to the method described by Pichet et al. (1999). A wet weight ranging from 100 to 200 mg was removed from each specimen for the acid digestion process. The digest was brought to a final volume of 30 ml with water, and then it was analyzed by measuring the atomic fluorescence (CVAFS) of the liberated mercury following its reduction by Sn (II). The system has a detection limit of 0.002 μg/g for 1 mg of sample. This laboratory participates annually in the Mercury Quality Assurance Program of the Canadian Agency for Food Inspection, as well as twice a year in the Interlaboratory Comparison Program of Health Canada. The accuracy of the method was verified using the TORT-2-certified standard (lobster hepatopancreas reference material from NRC). During the evaluation period, results averaged 284 ± 8 ng/g, which falls well within the certified value range of 272 ± 60 ng/g. For samples analyzed in duplicate, the average standard deviation was $4 \pm 3\%$.

Fish and Fruit Intake

In the Amazon, water levels vary by 6 m up to 20 m between the high- and low-water seasons causing important seasonal changes in fish habitat and behavior (Goulding et al., 1996; Bastos et al., 2007) and thus in the availability of different fish species for the riverside populations (Lebel et al., 1997; Dolbec et al., 2001; Passos et al., 2001). The rain cycle and local conditions likewise affect seasonal fruit availability (Passos et al., 2001). Because of these seasonal variations, an interview-administered 7-day recall food frequency questionnaire was used for fish and fruit consumption. For fish, 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 fish species that were consumed.

In addition, in each of the villages, the quantity of fish (g) eaten during a meal was determined in a subset of 72 villagers. The fish-meal, after cooking and before serving, was weighed using a Digital Scale Tanita™ (Model KD-200, 2 kg capacity, Min. grad 0 to 2000 g, $d = 2$ g). Data were analyzed separately for men and women and averaged for each village.

As for fruits, for each fruit species, the participant indicated the number of fruits that had been eaten each day over the preceding 7 days, whether during a meal or not. Fish and fruit species that were not in the initial list were also recorded.

Estimate of Mercury Intake

Estimated mercury intake was determined for men and women from each village using the average fish-mercury concentration for the fish caught in their fishing zone, and the average gender-specific quantity of fish eaten at each meal. In the case of fish species for which there were no mercury measurements (95 fish meals (5.3% of total fish meals)), we used the average regional mercury concentration of the fish's feeding habit (carnivorous or noncarnivorous). The product of fish species-specific mercury (μg/g) and the weight of the average meal (g) was then multiplied by the number of reported meals of the specific species over the last 7 days. These were then summed to provide the total intake over the past week. Total daily intake per body weight was then determined by dividing the weekly intake by 7 days and the person's weight, according to the formula bellow:

$$\begin{aligned}\text{Weekly Hg intake} = {} & \text{Fish quantity per meal } (g)\\ & *(\text{Fish species}_1\text{-Hg-concentration } (\mu\text{g/g})\\ & *\text{Number of fish meals of species}_1)\\ & +(\text{Fish species}_2\text{-Hg-concentration } (\mu\text{g/g})\\ & *\text{Number of fish meals of species}_2)\\ & +\ldots+(\text{Fish species}_\text{i}\text{-Hg-concentration } (\mu\text{g/g})\\ & *\text{Number of fish meals of species}_\text{i})\\ & = \mu\text{g Hg/week}\end{aligned}$$

$$\begin{aligned}&\text{Daily Hg intake per body weight} = \text{Weekly Hg intake } (\mu\text{g})\\ &/\text{body weight}/7\text{ days (kg)} = \mu\text{g/kg/day}\end{aligned}$$

Sampling and Analyses of Biomarkers of Exposure

Total mercury concentrations in whole-blood or scalp hair are the indicator media of choice in adults for methyl mercury exposure from fish consumption (Cernichiari et al., 1995; Clarkson and Magos, 2006; Mergler et al., 2007).

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° until analyzed. Total and inorganic mercury in blood were determined by Atomic Absorption Spectrometry at the laboratory of the Quebec Toxicology Center of the Quebec Public Health Institute (CTQ-INSPQ), Canada, according to the method described by Ebbestadt et al. (1975). The detection limit for blood-mercury analysis was 0.2 μg/l, and analytical quality control was ensured by the use of internal reference samples for blood analysis provided by the Interlaboratory Comparison Program conducted by the CTQ-INSPQ. The CTQ is accredited ISO 17025 and analytical performance for mercury analysis in the Interlaboratory Comparison Program for Metals in Biological Media was 36/36 for precision and 6/6 for reproducibility.

Hair strands from the occipital region were cut at the root and then placed in plastic bags, with the root end stapled. The first centimeter of each sample was analyzed at the Radioisotopes Laboratory of the Federal University of Rio de Janeiro (Brazil), by Atomic Absorption Spectrometry with an AA 1475 Varian and a cold vapor generator accessory VGA-76 Varian. Mineralization of samples was carried out with mixtures of acids (HCl, HNO_3, and H_2SO_4) and oxidants ($KMnO_4$, $K_2S_2O_7$, and H_2O_2), with techniques developed and adapted to the flow injection system vapor generator accessory (Malm et al., 1989). This laboratory participates regularly in interlaboratory comparison programs for total and inorganic mercury analysis (Gill et al., 2002), and analytical quality control was ensured by the use of standard reference materials (Human Hair 085 and 086) provided by the International Atomic Energy Agency (IAEA).

Statistical Analyses

Fish-mercury levels, fruit and fish intake, mercury daily intake, mercury exposures, and sociodemographics were characterized using descriptive statistics. Intergroup comparisons were made using parametric or nonparametric techniques, depending on data distribution. The relations between sociodemographic variables and daily mercury intake were examined using multivariate regression models. Where possible, continuous variables were used (age, years of schooling, number of fish meals, number of fruits). Smoking and alcohol consumption status, and villages were included as categorical variables. The influence of fruit consumption frequency on the relations between daily mercury intake and bioindicators of exposure was examined using analysis of covariance. For these analyses, all pregnant women were excluded. 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.).

Results

Table 1 shows mean mercury concentrations for the fish species captured and effectively eaten in each community. Mercury concentrations averaged 0.33 μg/g for the 1123 fish samples, of which the 702 non-carnivorous specimens presented a mean concentration of 0.11 μg/g, whereas the 421 carnivorous specimens averaged 0.52 μg of Hg per g of fish tissue. No significant intercommunity variations in fish-mercury levels were observed (Kruskal–Wallis, $P = 0.5$).

Table 2 presents the participants' sociodemographic characteristics. The overall level of education was low (mean: 3.6 ± 2.6 schooling years), ranging from 0 to 11 years, and the age range was 15 to 89 years (mean 35.3 years ± 15.9). Eighty-one percent of the participants were originally from the Tapajós region and 73.4% lived on the Tapajós River banks, while the others lived on one of its tributaries.

In this season, 92.5% of the participants consumed at least one meal with fish over the preceding 7 days. On average, villagers had consumed 7 fish meals over the last week, with carnivorous fish constituting an average of 45% of the fish diet (ranging from 0 to 100%). The gender-differentiated rates of fish ingestion as well as anthropometric data for each community are summarized in Table 3. In all, participants consumed an average of 141 g of fish per meal, with a significant difference between women (mean: 124 g ± 65.9) and men (mean: 189.7 g ± 105.5) (Mann–Whitney U, $P = 0.0014$). Although no significant intercommunity differences were observed (Kruskal-Wallis, $P = 0.1$), when villagers

Table 2. Socio-demographic characterization of the sampled population

	Women		Men	
Characteristics	N	%	N	%
Age categories				
15 to 24 years	33	27.9	39	29.4
25 to 34 years	30	24.6	29	21.3
35 to 44 years	24	19.7	32	23.5
45 to 54 years	13	10.7	16	11.8
55 to 64 years	14	11.5	11	8.1
≥65 years	7	5.7	8	5.9
Current alcohol consumers	29	23.9	67	49.6
Current smokers	27	22.3	50	37.0
Schooling				
No formal education	13	10.7	23	17.0
Elementary school (1 to 8 years)	100	82.6	107	79.3
High school and more (≥9 years)	8	6.6	5	3.7
Village location				
On the Tapajós River banks	93	76.9	91	67.4
On an affluent	28	23.1	44	32.6

Table 1. Mean Hg concentrations (μg/g) in fish species captured and eaten in the participating communities

		Mean ± SD					
Fish species	Feeding habits	SLT*	NC*	SA*	VA*	MU*	AÇ*
Pescada (*Plagioscion sp.*)	Carnivorous	0.65 ± 0.33	0.57 ± 0.23	0.60 ± 0.28	0.45 ± 0.17	0.45 ± 0.17	0.39 ± 0.23
Aracu (*Schizodon sp.*)	Noncarnivorous	0.20 ± 0.14	0.17 ± 0.11	0.13 ± 0.11	0.10 ± 0.06	0.10 ± 0.06	0.14 ± 0.10
Caratinga (*Geophagus sp*)	Noncarnivorous	0.18 ± 0.12	0.18 ± 0.11	0.14 ± 0.06	0.07 ± 0.03	0.07 ± 0.03	0.11 ± 0.02
Tucunare (*Cichla sp.*)	Carnivorous	2.3 ± —	0.60 ± 0.55	0.39 ± 0.15	0.52 ± 0.36	0.52 ± 0.36	0.19 ± 0.13
Sarda (*Pellona sp*)	Carnivorous	0.43 ± 0.11	0.61 ± 0.22	0.66 ± —	0.77 ± 0.24	0.77 ± 0.24	0.66 ± 0.30
Piranha (*Serrasalmus sp.*)	Carnivorous	0.40 ± 0.26	0.57 ± 0.40	0.40 ± 0.21	0.40 ± 0.19	0.40 ± 0.19	0.31 ± 0.21
Branquinha (*Curimata sp.*)	Noncarnivorous	0.19 ± 0.06	0.17 ± 0.09	0.10 ± 0.03	0.06 ± 0.02	0.06 ± 0.02	0.07 ± 0.05
Charuto (*Hemiodus sp.*)	Noncarnivorous	0.09 ± 0.08	0.08 ± 0.06	0.14 ± 0.10	0.05 ± 0.05	0.05 ± 0.05	0.07 ± 0.03
Mandi (*Auchenipterus sp.*)	Carnivorous	0.46 ± 0.38	0.33 ± 0.15	0.41 ± 0.19	0.69 ± —	0.69 ± —	0.20 ± 0.09
Jaraqui (*Semaprochilodus sp.*)	Noncarnivorous	0.06 ± 0.02	0.09 ± 0.05	—	—	—	0.05 ± 0.002
Surubim (*Pseudoplatystoma sp.*)	Carnivorous	0.28 ± 0.16	0.27 ± 0.12	—	—	—	0.31 ± 0.12
Peixe-cachorro (*Raphiodus sp*)	Carnivorous	0.63 ± 0.52	0.58 ± 0.25	0.91 ± 1.2	—	—	0.42 ± 0.13
Matrinxã (*Brycon sp.*)	Noncarnivorous	0.1 ± —	—	—	—	—	—
Pacu (*Mylossoma sp.*)	Noncarnivorous	0.02 ± —	0.2 ± —	—	—	—	0.2 ± —
Tambaqui (*Colossoma sp.*)	Noncarnivorous	—	—	—	—	—	0.04 ± —
Curvina (*Pachypops sp.*)	Noncarnivorous	—	0.1 ± —	—	—	—	—
Traira (*Hoplias sp.*)	Carnivorous	—	0.3 ± —	—	—	—	0.4 ± —
Curimata (*Prochilodus sp.*)	Noncarnivorous	—	—	—	—	—	0.1
Sardinha (*Triportheus sp.*)	Noncarnivorous	—	0.02 ± 0.1	—	—	—	0.03 ± 0.2
Aruanã (*Osteoglossum bicirrhosum*)	Carnivorous	0.2 ± —	—	0.8 ± —	—	—	0.3 ± —
Acari (*Liposarcus pardallis*)	Noncarnivorous	—	0.1 ± —	0.1 ± —	—	—	0.04 ± —
Mapará (*Hypophthalmus sp.*)	Noncarnivorous	0.1 ± —	0.2 ± —	—	0.3 ± —	0.3 ± —	—
Cujuba (*Pseudodoras sp.*)	Carnivorous	—	—	—	—	—	0.2 ± —
Jacundá (*Crenicichla sp.*)	Carnivorous	—	0.5 ± —			—	—

*SLT: São Luis do Tapajós, NC: Nova Canaã; SA: Santo Antonio; VA: Vista Alegre; MU: Mussum; AÇ: Açaituba.

Table 3. Mean values for the amounts of fish ingested during one meal, and weight information of study participants

	Mean fish consumption (g)		Mean weight (kg)	
Communities	Women	Men	Women	Men
São Luis do Tapajós	147.3±72.0	206.7±71.1	54.4±8.9	60.5±11.1
Nova Canaã	101.3±44.2	132.5±38.9	51.5±8.8	62.1±11.7
Santo Antonio	81.0±32.5	165.0±40.9	55.8±13.7	55.1±8.5
Vista Alegre	122.1±70.2	200.6±150.7	52.3±8.3	64.1±9.8
Mussum	122.1±70.2	200.6±150.7	50.9±8.1	62.6±7.5
Açaituba	80.3±25.1	133.1±30.2	48.5±7.6	60.2±7.4

were grouped by village location, persons living on the shores of the Tapajós ate larger portions (mean: 155.3±38 g of fish) compared to those living on its tributaries (mean: 121.5±36.3 g of fish) (Mann–Whitney *U*, $P<0.0001$). Persons born in the Tapajós region consumed significantly higher quantities of fish (151.1±40.8 g of fish) than immigrants from northeast Brazil (129.8±35.1 g of fish) (Mann–Whitney *U*, $P=0.003$). Multiple regression analysis confirmed the relation of fish ingestion rates with gender ($\beta=31.5$; $P<0.0001$) and village ($\beta=36.8$; $P<0.0001$), but showed no relation with schooling or immigrant status.

The overall mean body weight was 57±10.6 kg, which significantly differed between women and men (means: 52.9±9.5 and 60.8±10.1, respectively) (Unpaired *t*-test, $P<0.0001$). Although body weight did not significantly differ between communities (ANOVA, $P=0.7$) and did not vary with villages' location (Unpaired *t*-test, $P=0.2$), multiple regression analysis revealed a negative relation for body weight with fish consumption frequency ($\beta=-0.28$; $P=0.05$), a direct link with the quantity (g) of fish ingested ($\beta=0.11$; $P<0.0001$), and a weak but significant positive association with age ($\beta=0.08$; $P=0.04$).

Ninety-one percent of participants ate at least one fruit over the 7-day survey, with an average consumption of 8 fruits/week, ranging from 0 to 39 fruits/week. Fruit consumption was slightly but significantly different between women (mean: 7.1±7.5 fruits/week) and men (mean: 9.6±8.7 fruits/week) (Mann–Whitney *U*; $P=0.03$). Significant differences were also observed among communities (Kruskal–Wallis, $P<0.0001$), with villagers living in the southern Tapajós (São Luis do Tapajós, Nova Canaã, and Santo Antonio), presenting lower rates of consumption compared to villagers situated in communities close to the small town of Aveiro. The intake of fruit was also weakly but directly and significantly associated with years of schooling (Spearman = 0.15; $P=0.02$).

Estimated daily mercury intake varied between 0 and 11.8 μg/kg/day, with a mean of 0.92 and median of 0.59 μg/kg/day. There was one extreme value of 11.8 in the village of São Luis do Tapajós, which was excluded from the present analyses. Figure 2 shows the distribution of the daily intake for the entire population, without the above-mentioned outlier. Nonparametric comparisons showed no differences with respect to gender or smoking status, but a weak difference was observed between alcohol drinkers and nondrinkers, with the latter presenting lower rates of mercury intake (Mann–Whitney, $P=0.04$). Also, daily mercury intake was twice as high for persons born in the Tapajós region compared to immigrants (means: 0.97±0.92 and 0.48±0.61 μg/kg/day, respectively; Mann–Whitney, $P<0.0001$). Pronounced and significant differences among communities were observed, as shown in Figure 3 (Kruskal–Wallis, $P<0.0001$). Multivariate regression analyses showed that the daily mercury intake is gender-related, with men presenting higher intake rates than women ($\beta=0.1$; $P<0.05$); there was a tendency towards an inverse and weak association with age ($\beta=-0.01$, $P=0.07$), as well as strong variations between villages ($\beta=0.4$; $P<0.0001$).

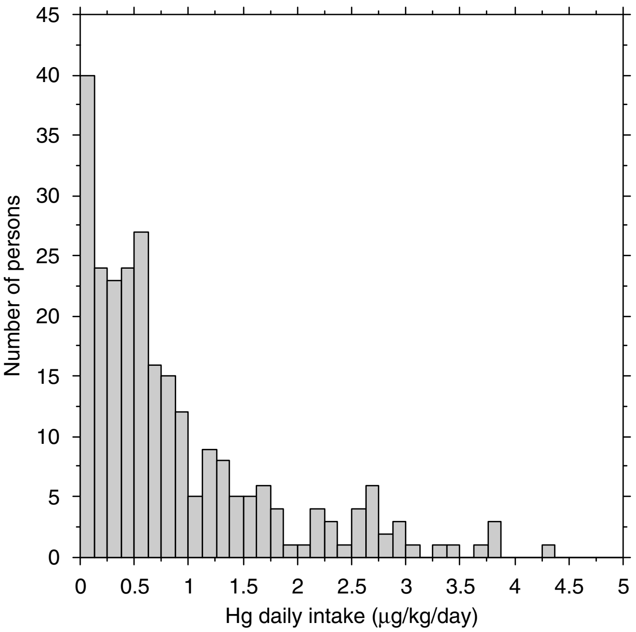

Figure 2. Overall distribution of the daily mercury intake for the entire population.

Mean blood total mercury for the whole cohort was 58.7±36.1 μg/l (median: 56.1 μg/l, ranging from 4.8 to 165.3 μg/l), of which 86.8% was in the form of methyl mercury, ranging from 75.2 to 94.3%. Inorganic mercury averaged 7.6±4.5 μg/l, with a maximum of 24 μg/l. Mean total hair-mercury concentration was 17.9±11.5 μg/g (median: 16.5, range 0.2 to 58.3 μg/g). Bioindicators of exposure differed significantly by gender and village, with men presenting higher concentrations than women (mean blood Hg: 65±40.9 and 55±35 μg/l; mean hair Hg: 19.3±11.9 and 16.3±11 μg/g, respectively), and São Luis do Tapajós and Santo Antonio representing the most and the least exposed communities, respectively (Kruskal-Wallis, $P<0.0001$).

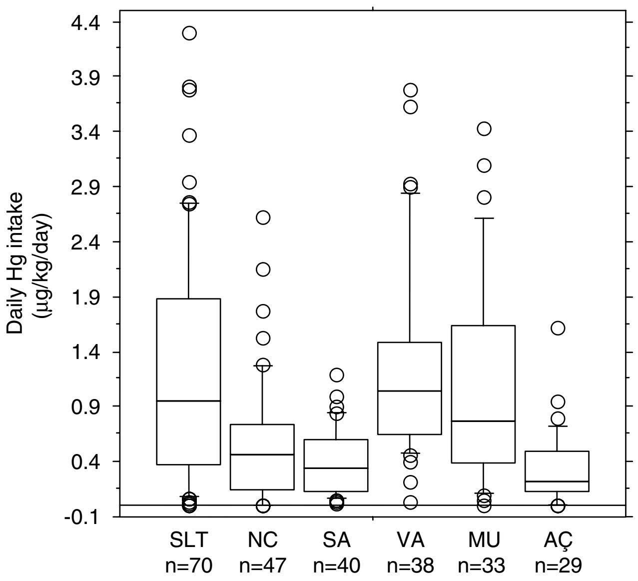

Figure 3. Percentile distributions of daily mercury intake for the six participating communities (SLT, São Luis do Tapajós; NC, Nova Canaã; SA, Santo Antonio; VA, Vista Alegre; MU, Mussum; AÇ, Açaituba).

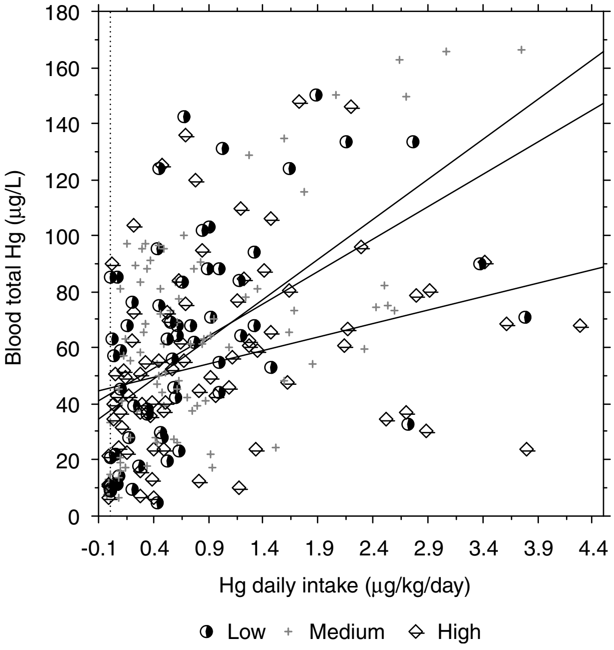

Figure 4. The influence of fruit intake on the relation between daily mercury intake and blood-mercury levels (low fruit consumption: ≤ 3 fruits/week, $n = 70$; medium fruit consumption: $> 3 \leq 10$ fruits/week, $n = 101$; high fruit consumption: > 10 fruits/week, $n = 85$).

Table 4. Beta (β) coefficients and significance levels for covariates explaining blood and hair total mercury concentrations in multiple stepwise regression models

	Bioindicators of exposure	
Covariates	Blood T-Hg (μg/l)	Hair T-Hg (μg/g)
Hg daily intake	19.7 ($P < 0.0001$)	3.4 ($P = 0.0001$)
Fruit intake	−0.56 ($P = 0.0357$)	−0.19 ($P = 0.0353$)
Gender	−3.6 ($P = 0.0880$)	−1.3 ($P = 0.05$)
Schooling	−2.8 ($P = 0.0028$)	−0.7 ($P = 0.0102$)
Smoking	−5.3 ($P = 0.0266$)	NS
Village location	−5.5 ($P = 0.0323$)	−3.5 ($P = 0.0002$)
Immigrant status	−6.9 ($P = 0.0104$)	−1.8 ($P = 0.0429$)
	Model $R^2 = 0.40$	Model $R^2 = 0.23$

NS, nonsignificant.

When total blood- and hair-mercury levels were examined as dependent variables in separate simple regression models, the daily mercury intake explained 28% and 9% of their variations, respectively. Table 4 shows the set of covariates explaining mercury variations in blood and hair. There was a strong and positive relation between daily mercury intake and blood total mercury levels, with an inverse relation for blood-mercury with fruit intake. We likewise observed a negative relation for blood-mercury with years of schooling, a tendency for differences between men and women, and significant variations with smoking and immigrant status, as well as between villages. Blood organic mercury concentration was also strongly related to the daily mercury intake ($\beta = 17.2$; $P < 0.0001$) and inversely associated with fruit consumption ($\beta = -0.5$; $P < 0.05$). It was inversely related to years of schooling ($\beta = -2.8$; $P = 0.001$), and varied with both immigrant status ($\beta = -5.7$; $P < 0.05$; Tapajós natives presenting higher levels than immigrants) and village ($\beta = 12.2$; $P = 0.002$). In turn, blood inorganic mercury was associated with daily mercury intake from fish ($\beta = 1.9$; $P < 0.0001$), and a tendency of inverse relation with fruit intake was observed ($\beta = -0.05$; $P < 0.09$). Hair-mercury was directly associated with daily mercury intake and inversely related to years of schooling. It was related to gender, with men presenting higher levels compared to women, and varied with villages as well as immigrant status. An inverse relation with fruit consumption was likewise observed.

The influence of fruit consumption on the relation between daily mercury intake from fish and blood total mercury levels was further examined through analysis of covariance, as illustrated in Figure 4. The regression lines are drawn for those with low fruit consumption (≤ 3 fruits/week; $n = 70$), medium fruit consumption ($> 3 \leq 10$ fruits/week; $n = 101$), and high fruit consumption (> 10 fruits/week, $n = 85$) in relation to blood-mercury concentrations. The intercepts of the three regression lines are similar, but their slopes are significantly different (ANCOVA Interaction term: $F = 10.9$, $P < 0.0001$), indicating that for the same daily mercury intake, persons eating fruit more frequently have lower blood-mercury concentrations. The difference of mean mercury concentrations between high and low fruit

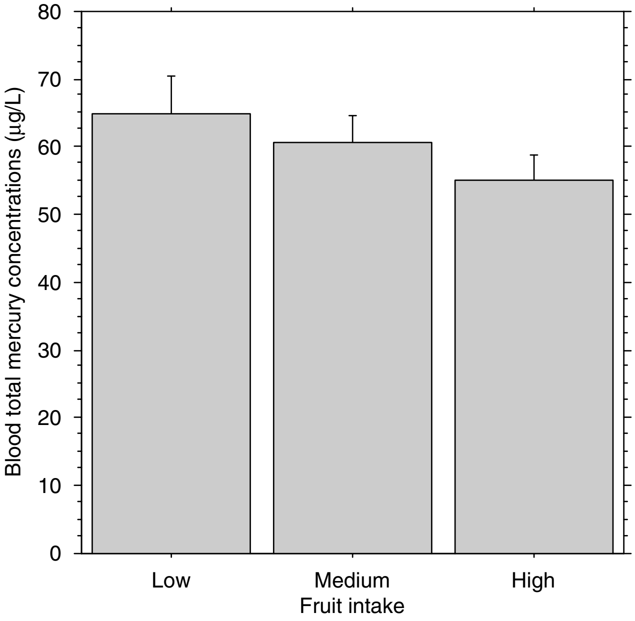

Figure 5. Mean blood total mercury concentrations according to the levels of fruit intake (low fruit consumption: ≤ 3 fruits/week, $n = 70$; medium fruit consumption: $> 3 \leq 10$ fruits/week, $n = 101$; high fruit consumption: > 10 fruits/week, $n = 85$).

consumers is 9.3 μg/l, corresponding to 14.4% reduction in mean blood-mercury (Figure 5). In addition, the median difference of the ratio of blood-mercury to daily mercury intake between low and high fruit consumers was 26, representing a reduction in the order of 26.3% in terms of the input of ingested mercury into the blood stream.

Discussion

This study shows high rates of daily mercury intake in riparian communities of the Amazonian Tapajós region, who rely heavily on fish consumption. The calculations, based on measured concentrations of mercury for local fish species consumed by the different communities and weighed meal quantities, provide a high level of precision. Bioindicators of exposure were highly correlated to daily mercury intake averaged over the past 7 days, with blood, an indicator of current exposure, showing a much stronger correlation than hair. Fruit consumption significantly modified these relations.

Regulatory guidelines to limit environmental mercury exposures have been developed by governmental agencies throughout the world (Adams and Schantz, 2006). Based primarily on the results of large longitudinal neurodevelopmental toxicity studies conducted in New Zealand, the Seychelles and the Faroe Islands, and using varying interpretations of the epidemiological evidence, recommended intake without appreciable risks currently range from 0.1 to 0.3 μg/kg/day (Clarkson and Magos, 2006; Innis et al., 2006; Wijngaarden et al., 2006). In comparison, daily mercury intake of the Amazonian population in the present study averaged 0.92 μg/kg/day, ranging from 0 to 4.3 μg/kg/day, with an outlier whose intake was 11.8 μg/kg/day. In this population, 86.3% of participants presented daily intake above 0.1 μg/kg/day and 72.4% above 0.3 μg/kg/day. Thus, the large majority of participants surpassed all recommended reference doses (*RfD*), which have been derived by various agencies. The *RfD* is "an estimate of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime" (US EPA, 1998). Even if for some authors, exceeding these guidelines by a small margin is meaningless in terms of actual risk because of so many uncertainties (Clarkson and Magos, 2006), given the wide range of variation of the daily intake in this Amazonian cohort, there is reason for concern about the potential for deleterious health effects that could be caused by high mercury intake. Indeed, almost 50% of the population surpassed the Benchmark dose (BMD) for blood-mercury (58 μg/l) that was used to calculate the US NAS/NRC reference dose (NRC, 2000), and over 50% surpassed the BMD for hair-mercury (14 μg/g) used to determine the World Health Organization reference dose (WHO, 2003).

There are important intervillage differences in mercury intake, varying from a median of 0.22 to 1.05 μg/kg/day. Daily mercury intake was much higher among persons born in the Tapajós region compared to immigrants, and for persons living on the shores of the Tapajós as opposed to those residing in the tributaries. This probably reflects the fact that those born on the Tapajós have a longer tradition of eating fish and those that live directly on the Tapajós eat fish more frequently and consume bigger portions. In addition, environmental mercury levels around the villages, differences in methylation rates (Guimarães, 2001; Roulet et al., 2001; Miranda et al., 2004), or simply changes in the availability of the different fish species present in the various ecosystems can also contribute to intervillage differences (Dolbec et al., 2001).

Men presented higher intakes compared to women, who consumed significantly lesser quantities of fish than men, even when considering their lower body weight. Men also showed higher mercury intake rates despite higher self-reported alcohol consumption as compared to women. Even though the inhibition of mercury absorption by dietary ethanol in dentists has recently been suggested (Martin and Naleway, 2004), to date only one study showed such evidence in a fish-eating population (Grandjean et al., 1992). The latter study of the Faroes birth cohort, which includes a large number of participants, reported that blood-mercury levels were slightly lower if the mother had occasionally ingested alcohol beverages. In the current study, multivariate analyses did not reveal any effect of alcohol consumption on mercury exposure.

In the present study, there were substantial intervillage differences in mean fish portion size per meal, ranging from 171 g in São Luis do Tapajós to 115 g in Açaituba. Pronounced variations have also been reported for different areas of the Amazon basin. For example, Boischio and Henshel (1996) reported fish portions of 200 g for the riverside population of the Madeira River Basin, while Hacon et al. (1997) presented an average fish ingestion rate of 110 g/day for fishermen of the Alta Floresta region. A daily consumption of about 110 g has likewise been reported for the villagers of the Balbina reservoir (Kehrig et al., 1998), whereas Fréry et al. (2001) showed that in periods of abundance the amount of fish consumption may reach over 600 g/day and that in times of scarcity these amounts may be relatively low. These latter authors report an average daily consumption surrounding 340 g for adult men of native Amerindian communities in French Guiana. Although there are variations in portion size, depending probably on the size and amount of fish captured and the availability of different fish species, throughout the Amazon, large amounts of fish are consumed daily.

In the present study, several factors were predictive of blood- and hair-mercury levels. As expected, the strongest associations were observed with the daily mercury intakes. The intake of fruit was associated with lower mercury concentrations in both bioindicators, corroborating recent findings, which indicate that individuals consuming fresh fruit more frequently have much lower blood- and hair-mercury concentrations for the same level of fish consumption, as compared to those who consume fruit less frequently (Passos et al., 2003b, 2004, 2007a, b). In an exploratory study, which covered a large variety of dietary information such as the intake of vegetables and many other foods, only fruit consumption was related to bioindicators of mercury exposure, when fish consumption frequency was considered (Passos et al., 2001, 2003b). In the present study, fruit consumption was the only element that modified the relation between mercury intake and bioindicators of exposure.

There has been much debate about how to address the risks of mercury exposure and the benefits of fish consumption with questions raised about the suitability of regulating human risk based either on controlling average daily intake, as recommended by the EPA, or excluding fish with high mercury levels from the diet as put forward by FDA (Stern et al., 2004; Clarkson and Magos, 2006). In the Amazon, because fish is a dietary mainstay and there is high mercury intake, there is a need for a suitable strategy that would reduce mercury exposure while maintaining fish consumption.

Some researchers have proposed that fish intake in the Amazon should be limited. Kehrig et al. (1998) suggested that pregnant women and women of childbearing age should consume less fish to limit their exposure to potential sources of methyl mercury, while Boischio and Henshel (2000) put forward that risk communication for this exposed population could be addressed using fish advisories for consumers, fishermen, and retailers, which would recommend an acceptable number of fish meals to be consumed according to species. Whereas both these studies used predictive models to provide important insights on the potential risks to human health, their recommendations are limited by their methodological approach. For example, despite some inaccuracies (Passos and Mergler, 2006), a recent study which reconstructs methyl mercury intakes in indigenous and riparian populations from biomarker data, indicates that notwithstanding the chronic exposure scenario in the Amazon, steady-state assumptions are not in order because of the strong seasonal fluctuations in methyl mercury exposure (Gosselin et al., 2006). These findings, coupled to the results of the present study which show regional ecosystem variations in mercury concentrations, as well as intercommunity differences in fish portions, and considering the socioeconomic conditions as well as the dietary and cultural background of these communities (Passos et al., 2001, 2003a; Mertens et al., 2005), suggest that a risk management strategy that focuses solely on limiting the number of fish meals might deprive this population of an important food source. In French Guyana, Fréry et al. (2001) encouraged close collaboration within local populations, public health authorities, and scientists to develop concrete recommendations and adopt measures to reduce the environmental sources of this high exposure. A community-based campaign carried out in a village on the Tapajós, which focused on "eating more fish that don't eat other fish" was successful in reducing mercury exposure by close to 40% while maintaining the same quantity of fish consumption (Mergler et al., 2001; Bahia et al., 2004; Lucotte et al., 2004; Mertens et al., 2005). Further interventions, focusing on agro-forestry practices that would limit deforestation and/or reduce soil erosion, would serve to reduce the source of mercury contamination in these ecosystems.

Some authors have recently minimized the importance of mercury exposure in the Amazonian region, suggesting that despite high concentrations of methyl mercury in fish, daily consumption of large amounts of this central and highly nutritious food over the course of a lifetime, poses no health hazards for Amazonians (Dórea, 2003, 2004; Dórea et al., 2005). There has been the suggestion that based on the general health status of the population, "normal" values higher than those proposed by WHO could be established in the Amazon (Santos et al., 2002). However, another Amazonian study concluded that differences in exposed and nonexposed populations in this region would argue against an Amazonian reference level, particularly as levels surpass those of WHO (Pinheiro et al., 2006). Despite attempts to establish reference values and background parameters as well as eliminate uncertainties about the reference dose in the Amazonian context, few studies have adopted a rigorous procedure for developing risk-based reference doses (Gaylor and Kodell, 2002; Rice, 2005). Dietary and genetic factors (Passos et al., 2003b, 2004, 2007a, b; Klautau-Guimarães et al., 2005) as well as other specificities of the Amazonian traditional populations, make it difficult to evaluate the suitability of using current reference doses for methyl mercury exposure in the Amazon. Despite this difficulty, allegations that daily consumption of mercury-contaminated fish in large amounts poses no health hazards, based on the absence of mercury-induced clinical effects and the existence of general health problems (Dórea, 2003, 2004), do not take into consideration the importance of changes in biological functions on the population level and their usefulness for risk assessment (Bellinger, 2004; Rice, 2005). Simplistic allegations may be misleading and do not contribute to the establishment of scientifically based guidelines able to effectively orient a viable risk management strategy in the Amazon.

The effects of fruit consumption with respect to the relation between mercury intake and biomarkers of exposure corroborate a relevant avenue that should be further explored as a potential additional short-term intervention strategy, during which time mercury levels in the environment should be brought under control through other intervention projects aiming to reduce the release of mercury from soils toward aquatic ecosystems, based on better farm land-use management strategies (Farella et al., 2006, 2007). The data from the present study suggest that reference doses for mercury in fish-eating populations where fruit is an important component of diet could be considerably different from those without daily access to fresh fruit. These findings further underline the importance of considering the influence of dietary factors in risk assessment.

Conclusion

The present results for riparian populations of the Tapajós River Basin indicate that a variety of factors determine both the rates of mercury daily intake and their relations with bioindicators of exposure. The findings support the need to conduct rigorous studies for developing risk-based reference doses adapted to the Amazonian context. In particular, future studies examining early neurotoxic and cardiovascular effects as well as immunologic outcomes associated with mercury exposure should attempt to determine benchmark doses, which could form the basis for the establishment of reference doses suitable to orient viable risk management strategies for these populations. Meanwhile, the high level of daily mercury intake points out the need for intervention projects, which should not only seek to reduce the degree of exposure in the human populations while maintaining fish consumption, but also re-establish the integrity of the ecosystems.

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