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Neurotoxic Effects of Low-Level Methylmercury Contamination in the Amazonian Basin

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Many studies have demonstrated mercury contamination in the Amazonian ecosystem, particularly in fish, a dietary mainstay of populations in this region. The present study focused on potential health effects of this low-level methylmercury exposure. The study was carried out in a village on the Tapajós River, a tributary of the Amazon, on 91 adults inhabitants (15–81 years), whose hair mercury levels were inferior to 50 µ/g. Performance on a neurofunctional test battery and clinical manifestations of nervous system dysfunction were examined in relation to hair mercury concentrations. Near visual contrast sensitivity and manual dexterity, adjusted for age, decreased significantly with hair mercury levels ($P < 0.05$), while there was a tendency for muscular fatigue to increase and muscular strength to decrease in women. For the most part, clinical examinations were normal, however, hair mercury levels were signficantly higher ($P < 0.05$) for persons who presented disorganized movements on an alternating movement task and for persons with restricted visual fields. These results suggest dose-dependent nervous system alterations at hair mercury levels below 50 µg/g, previously considered a threshold for clinical effects. The profile of dysfunction in this adult population is consistent with the current knowledge on methylmercury poisoning. The long-term implications of these findings are unknown and need to be addressed. © 1998 Acadamic Press

Key Words: **methylmercury; low levels; human; hair; neurotoxicity.**

INTRODUCTION

The tragic events in Minamata and Niigata, Japan, where large populations suffered methylmercury poisoning from consumption of contaminated fish, dramatically brought to light the dangers of mercury incorporation into the food chain and its risk to human health (WHO, 1976, 1990). When released into aquatic environments, mercury can be methylated by bacteria present in the ecosystem. Methylmercury then becomes bioavailable to aquatic organisms and is biomagnified through every step of the food chain up to humans whose primary route of exposure is through fish consumption (WHO, 1989).

Since the late eighties, numerous studies in the Amazon Basin have confirmed the presence of mercury in various environmental compartments, particularly fish (Barbosa *et al.*, 1995; Lacerda *et al.*, 1991; Malm *et al.*, 1990;. Martinelli *et al.*, 1988; Palheta and Taylor, 1995; Pfeiffer *et al.*, 1989, 1991, 1993). Environmental studies in this region suggest that there may be two important sources for mercury contamination. The most well-known is gold mining activities (Martinelli *et al.*, 1988; Malm *et al.*, 1990; Lacerda and Salomons, 1991; Pfeiffer *et al.*, 1991, 1993). Gold-mining operations are responsible for the emission of an estimated 130 tons of mercury per year into the local environment (Lacerda and Salomons, 1991; Pfeiffer and Lacerda, 1988). In his detailed account of the Amazonian gold rush, Cleary (1990) describes the socioeconomic context, as well as the living and working conditions of the gold miners (*garimpeiros*). He underlines the fact that most *garimpeiros* are aware that mercury is dangerous, but have no notion of how toxic it is to themselves and the ecosystem. Cases of inorganic mercury

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poisoning, resulting from exposure to vapors given off during the burning of the gold mercury amalgam, have been described among gold miners (Branches *et al*., 1993; Aks *et al*., 1995).

More recent studies, carried out a few hundred kilometers downstream from the gold-mining activities, indicate that the soil in the Amazonian Basin is loaded with natural mercury. Slash and burn agricultural practices, coupled to important precipitation, lead to the lixivation of bare soil to the aquatic environment, rendering mercury bioavailable to aquatic organisms (Roulet *et al*., 1997, 1998). Furthermore, the Amazon Basin is subject to seasonal fluctuations of water levels, which can reach a difference of 6 m between the rainy season (mid-November to mid-May) and the dry season (mid-May to mid-November). During the rainy season, numerous fish species and invertebrates migrate to the flooded areas (Goulding, 1980). These sites are particularly favorable for mercury methylation due to important quantities of suspended organic matter, high temperature, acidic pH, and redox potential (Guimarães *et al*., 1995)

Studies on the mercury content of fish in the Amazonian river system have shown that levels often surpass 0.5 μg/g fresh weight (Barbosa *et al*., 1995; Boischio *et al*., 1995; Lacerda and Salomons, 1991; Lebel *et al*, 1997; Malm *et al*., 1995; Pfeiffer *et al*., 1991). This level is considered in many countries as a standard not to be exceeded for safe fish consumption. Hair mercury levels, assessed in human populations living in various areas of the Brazilian Amazon, are in the range of a few to 300 μg/g, with median exposure values in the order of 2 to ± 20 μg/g (Akagi *et al*., 1995; Barbosa *et al*., 1995; Boischio *et al*., 1995, 1996; Grandjean *et al*., 1993; Lebel *et al*., 1996, 1997; Nakanishi, 1992; Malm *et al*., 1995). Lebel *et al*. (1997), demonstrated that hair mercury levels varied seasonally, corresponding to changes in fish diet. Peak hair levels were observed during the rainy season when diet was composed primarily of piscivorous fish, while the lowest hair levels were observed in the dry season, when diet contained mainly herbivorous fish.

One of the predominant questions in recent years has been to determine at what level methyl mercury starts to affect exposed populations. The history of methylmercury exposure and health effects is recent and most of our knowledge in the recognition of signs of intoxication comes from the work done subsequent to the catastrophes in Japan and Iraq, where mercury hair levels were in the range of hundreds of micrograms per gram and exposure duration was relatively short, ranging from 2 to 3 months to a few years (Bakir *et al*., 1973; Inskip *et al*., 1985; WHO 1976, 1990). It was on the basis of these events that a working committee of the World Health Organization proposed exposure guidelines for maximum mercury hair concentration limits, founded on the risk of appearance of the first clinical signs of poisoning in adults (paraesthesia), which were observed between 50 and 125 μg/g (WHO, 1976). This recommendation was maintained by the International Program on Chemical Safety in 1990 (WHO, 1990) after re-evaluation of the data used in the 1976 report and on the basis of the absence of frank clinical neurological findings in studies carried out among Canadian aboriginal populations (McKeown-Eyssen and Ruedy 1983a,b; Spitzer *et al*., 1988; Wheatley, 1979). In these latter studies, methylmercury levels were lower than those encountered in the above-mentioned disasters, but the duration of exposure probably extended up to an entire lifetime.

Recent studies conducted in The Seychelles on a population of children exposed during pregnancy to low levels methylmercury have been inconclusive in identifying a relation between maternal hair mercury concentrations and deleterious effects on the development of their offspring. Neuropsychological and neurobehavioral test batteries were administered at 6.5, 19, and 29 months of age. New results are expected for the cohort at older ages (Myers, 1995)

In the Amazon Basin, exposure probably started 20 to 35 years ago with the development of gold-mining activities (*circa* 1975) and slash and burn agricultural practices (*circa* 1960) (Roulet *et al*., 1997, 1998; Farella *et al*., 1996). Fish is the dietary mainstay, making up an average of 50% of all meals (Lebel *et al*., 1997). In a small, preliminary study of 29 outwardly healthy individuals from the Amazonian Basin, whose hair mercury levels were inferior to 50 μg/g, (median: 12.7 μg/g), results suggested that visual and motor functions, assessed with a sensitive neurofunctional test battery, decreased with increasing hair mercury levels (Lebel *et al*., 1996). The present study sought to further examine neurofunctional and clinical manifestations of nervous system dysfunction in relation to hair mercury concentrations at levels below 50 μg/g, in a larger population living in the Brazilian Amazon.

METHODS

Population

The study targeted persons living in a small village, Brasília Legal, situated on the bank of a tributary of the Amazon River: the Rio Tapajós

(3°59′00″S, 55°30′00″W). The village is approximately 250 km downstream from the most extensive gold-mining fields in Brazil and in the midst of extensively deforested river banks. The villagers are not exposed to mercury vapors. Brasília Legal is accessible only by water, a 12- to 18-h boat trip from Santarém, a city of several hundred thousand.

On our arrival in the village, a meeting was called by the local community health nursing aides in order to explain the study objectives to the villagers and invite them to participate. Further recruitment was carried out during a house-to-house survey, which likewise served to determine the total population and age distribution in the village. A total of 98 persons, representing 40.5% of the adult population ($\geq$15 years old) of 242 inhabitants, responded positively to our invitation.

The study took place at the Community Health Post, where hair samples were taken, a questionnaire including socio-demographic information, smoking and drinking habits, and medical and work history was given by interview; a neurofunctional test battery was administered; and a clinical examination was performed on a random subsample. The entire procedure took approximately 2 h.

Hair Sampling and Analysis

Hair mercury was selected as the bioindicator of exposure since it is highly specific for methylmercury and it provides the opportunity to reconstruct exposure patterns over time. Once incorporated into hair at the root, mercury remains stable, while hair continues to grow at approximately 1 cm per month (Clarkson *et al.*, 1988; Clarkson, 1997; Suzuki, 1988).

Hair strands from the root were taken from the occipital region and then placed in plastic bags, with the root end stapled. Analyses for mercury determination were conducted in the laboratories of the Environmental Research Chair of the University of Québec in Montréal, using cold vapor atomic fluorescence spectrophotometry (CVAF). Strands were cut in 1-cm segments and each segment (up to 24) was analyzed for total mercury, according to the procedure described by Bloom and Fitzgerald (1988). Inorganic mercury determination was done on the first segment using the methods described by Farant *et al.* (1981) and adapted for CVAF (Bloom and Fitzgerald, 1988). Analytical quality was ensured by including a Health Canada sample of powdered hair in the series. Methylmercury was calculated as the difference between total mercury and inorganic mercury for the first centimeter of hair.

The results of hair analyses, published elsewhere (Lebel *et al.*, 1997), revealed that there were significant seasonal variations in hair mercury levels. Thus, in the present study, four measures of hair mercury concentration were used: mean total hair mercury (HHgT) (averaged over all of the centimeters from each individual); total mercury in the first centimeter (HHg1); total mercury in the highest value obtained over all of the centimeters analyzed: peak total hair mercury (HHgP); and methylmercury in the first centimeter (MeHg1).

Neurofunctional Test Battery

Motor and visual functions were assessed using a battery of sensitive neurofunctional tests that could be applied under standard conditions, in a situation with no electricity. Instructions were given orally to the participants in Portuguese by trained Brazilian students. Special attention was given to ensure that the participants fully understood the instructions. The tests were administered in closed and quiet rooms. Neither participants nor test administrators had knowledge of participants' hair mercury levels.

Motor functions. Maximum grip strength was assessed for both hands with a dynamometer (Lafayette Instruments, Model 78010) over two trials. Mean values for the two trials were calculated for each hand, classified as dominant and nondominant, according to the subject's opinion. Participants were asked to maintain maximum grip strength for as long as possible and fatigue was estimated as the time (in seconds) taken to fall to one-half of the maximum value.

The Santa Ana test (Helsinki version) served to determine manual dexterity. Following a training period, there were two trials for each hand. The number of pegs successfully turned 180° in 30 s for the two trials were summed for the dominant and nondominant hand.

Visual functions. All tests were carried out monocularly. Due to the absence of electricity, tests of visual acuity, chromatic discrimination, and near visual contrast sensitivity were administered outdoors, in a shaded area and luminance was determined with a luxmeter (Opticon Model Kallight 400). Far and near visual acuity were determined at 6 m and 40 cm, respectively, with Snellen and National Optic Charts for illiterate persons. Resolution at 7.5 m for far visual acuity and 40 cm for near visual acuity was considered good visual acuity.

Chromatic discrimination was assessed with the Lanthony D-15 desaturated color panel, which requires participants to place 15 caps in order of chromatic similarity. Loss was estimated on a continuous scale using the Color Confusion Index (Bowman, 1982), a ratio based on the sum of the chromatic differences between juxtapositioned caps; a perfect score gives the value of one and scores greater than one indicate increasing deviation in cap order.

Near visual contrast sensitivity was determined with the Vistech 6000 test system. It is composed of three charts, each containing 45 circles (diameter, 1.3 cm) distributed in five rows of increasing sinusoidal grating frequency (1.5, 3, 6, 12, and 18 cycles/degree). In each row the contrast diminishes from left to right and the person is required to indicate whether the gratings are upright, to the left, or to the right. Participants indicated the direction of the gratings with their hand. The criteria for contrast threshold determination, at every frequency, were the same response on at least two of the three cards.

Clinical Examination

Since the physician was able to participate in the study for only a limited time, a randomization procedure was adopted for the choice of participants for the clinical neurological examination. Every third person who had attended was recalled, this procedure was continued until the physician had to leave. A total of 59 persons were thus selected and examined by a physician trained in recognition of methylmercury intoxication. The examination included the Branches Alternate Movement Task (BAMT), an original alternate hand movement task developed by Dr. Branches and illustrated in Fig. 1. Participants had the opportunity to practice the movement. Performance was classified as normal or disorganized. The disorganized classification included (1) inability to perform the alternate movement, and (2) inability to persist with the movement over time.

Statistical Analysis

Descriptive statistics are used to illustrate sociodemographic variables, mercury exposure, and the results of the test battery. Mean comparisons were carried out using the Student's t test (two-tailed). Stepwise regression models were used to examine the relation between potential confounding variables and the results from the neurofunctional tests. The relations between test results and mercury exposure were analyzed by multiple regression, with the previously identified confounders included in the model. For these multivariate analysis the PROC GLM procedure of the SAS statistical package was used. Results from the clinical neurological examination were dichotomized into normal and abnormal presentation and the distribution was examined with respect to socio-demographic variables using χ^2 tests. Mercury levels were compared between those with a normal and those with an abnormal presentation using Student's t test (two-tailed). Statistical significance levels were established at $P \leq 0.05$; tendency was noted for relations with $P \leq 0.10$. All analyses were performed with the SAS statistical package.

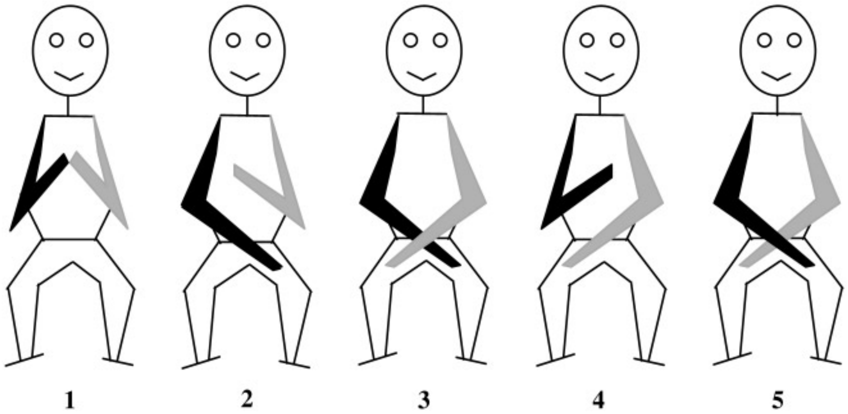

FIG. 1. The Branches Alternate Movement Task (BAMT). Initially, the participant has both hands in an upright position (1), then he or she has to touch the left knee with the right hand (2), followed by the crossing over of the left hand to touch the right knee (3). Right hand is then retrieved from the left knee (4) and crossed over the left arm to be redeposited on the left knee (5). The sequence (2 to 5) is then repeated alternately as fast as possible for a time period of approximately 30 s.

RESULTS

Population Characteristics

Using the data from the hair mercury measurements, four persons were excluded from the present analyses since at least one of the centimeters of hair mercury was above 50 μg/g. Three others were eliminated because one refused to give us a hair strand, one reported having suffered from cerebrovascular stroke, and one was not a resident of the village. The final group included 45 men and 46 women whose ages ranged between 15 and 81 years (mean: 32.9 years ± 15.2). The age distribution of the 91 participants is presented with the age distribution of the total population in Fig. 2.

Relevant socio-demographic data on age, educational level, sex, smoking, drinking habits, and history of malaria and parasitosis, as well as previous work in the *garimpos*, are summarized in Table 1. Educational level ranged between 0 and 12 years; 5 persons (5.4%) reported not having attended school, while 33 had completed more than 6 years in school (36.2%). For the current smokers, the number of cigarettes per day ranged from 1 to 4; 4% (3 persons) of the current drinkers reported drinking more than once or twice a week and only 4 persons reported previously using marijuana. One third (14 persons, 31.8%) of the men and 2 of the women had worked in the gold-mining region, where they could have been exposed to mercury vapors. It is in this area that most of the cases of malaria were contracted; of the 22 persons with a history of malaria, 12 (57.1%) had worked in the gold-mining region. A total of 16 persons reported history of parasitosis, 14 had suffered from gastrointestinal worms, and 2 from amoebas.

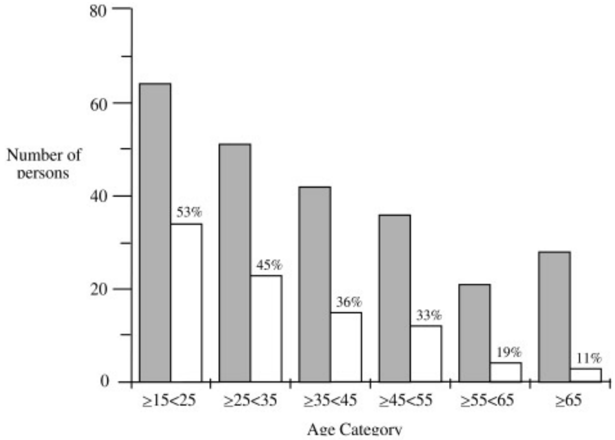

FIG. 2. Age distribution of the adult population of Brasília Legal (shaded bar; $n = 242$) and the study population (white bar; $n = 91$).

TABLE 1

Characteristics of the Study Participants

		n	Percentage
Sex:	Women	46	50.5
	Men	45	49.5
Age (years):	≥ 15 < 25	33	36.2
	≥ 25 < 35	23	25.3
	≥ 35 < 45	15	16.5
	≥ 45 < 55	11	12.1
	≥ 55 < 65	6	6.6
	≥ 65	3	3.3
Education (years):	0	5	5.4
	≥ 1 < 4	20	22.0
	≥ 4 < 7	39	42.9
	≥ 7 < 10	18	19.8
	≥ 10 < 13	9	9.9
Smoking habits:	Nonsmoker	48	52.7
	Exsmoker	20	22.0
	Smoker	23	25.3
Alcohol intake:	Nondrinker	36	39.6
	Drinker	55	60.4
Lived in gold-mining region:	Never	74	82.2
	Yes	16	17.8
History of malaria:	No	68	75.6
	Yes	22	24.4
History of parasitosis:	No	75	82.4
	Yes	16	17.6

Mercury Exposure

Figure 3 shows the percentile distribution for mean total hair mercury (HHgT), values for the first centimeter (HHg1), and peak levels (HHgP), as well as the level of methylmercury in the first centimeter (MeHg1). Methyl mercury constituted 72.8 to 93.6% of the total mercury in the first centimeter (mean: 89.6% ± 3.0). HHgT, HHgP, HHg1, and MeHg1

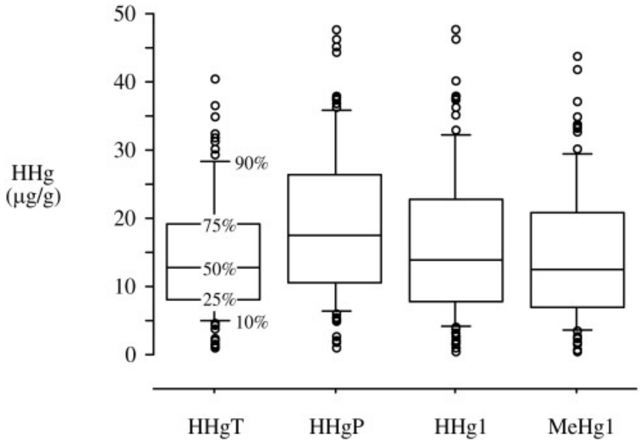

FIG. 3. Percentile distribution of hair mercury concentration in μg/g for the study participants ($n = 91$), (HHgT, mean total hair mercury; HHgP, peak hair mercury; HHg1, first centimeter total hair mercury; MeHg1, first centimeter hair methylmercury).

TABLE 2

Hair Mercury Levels in Fishermen, Nonfishermen, and Women (Mean ± SD)

	Fishermen ($n = 11$)	Other men ($n = 34$)	Women ($n = 46$)
Mean total hair mercury (μg/g)	23.9 ± 9.3*	14.3 ± 9.4	12.6 ± 7.0
Peak hair mercury (μg/g)	29.0 ± 10.3*	16.7 ± 11.4	18.6 ± 10.0
First cm total hair mercury (μg/g)	28.4 ± 10.4*	15.8 ± 11.0	14.1 ± 9.1
First cm hair methyl mercury (μg/g)	25.8 ± 9.5*	14.1 ± 10.0	12.7 ± 8.4

Note. ANOVA, Tukey's post hoc, $*P \leq 0.05$, differences between the fishermen and each of the two other groups.

were examined with respect to age, sex, work history in the gold-mining area, malaria, parasitosis, smoking, and drinking habits. Men had significantly higher levels of HHg than women (t test: $P \leq 0.05$) for all of the hair level measurements with the exception of HHgP. However, these differences could be attributed to the fishermen, who had significantly higher hair mercury levels as compared to the other men and the women (Table 2).

Neurofunctional Tests

Table 3 contains the results from the neurofunctional test battery. The values presented are averaged from left and right sides. There was no difference in performance between men and women for all of the tests with the exception of grip strength where men had significantly higher values than women and are presented separately. Possible confounders, such as sex, age, educational level, smoking, alcohol consumption, having spent time in the gold-mining region, malaria, or parasitosis were examined for each test using stepwise regression analysis. Performance on all of the neuro-outcomes decreased significantly with age. None of the other variables entered significantly into the model.

Luminance during the near visual contrast sensitivity testing ranged between 780 and 15,850 lux, mean: 4701 ± 3556. No relation was observed between the results of this test at each spatial frequency and luminance. During the color vision testing, luminance varied between 370 and 10,800 lux (mean: 3006 ± 2187) and no relation was observed with luminance.

The results of multiple regression analyses for the neurofunctional tests, with age and each of the four parameters of HHg included in the model, are given in Table 4. Overall, the intermediate and higher frequencies of near visual contrast sensitivity and manual dexterity, measured with the Santa Ana, vary with the level of hair mercury, however, the strongest relations are observed with peak mercury (HHgP). This was also the case for grip strength for women which decreased with increasing peak mercury level. Muscular fatigue in both men and women showed a tendency to increase with increasing mercury, with the higher correlations with peak and mean HHg ($P = 0.054$).

Further analyses of the data with respect to age revealed that some relations were stronger among the younger persons (≤35 years). Figure 4 shows the age-adjusted values of the Santa Ana test for those persons of 35 years or less and for those over 35 with respect to peak HHg. While HHgP explains 10% of the variance for the younger group ($F = 5.98$; $P \leq 0.02$), for the older group, the response does not vary with HHgP.

Figure 5 illustrates the near visual contrast sensitivity profiles for the younger and older groups with HHgP values above 20 μg/g compared to those with lower levels. For the younger group, differences (t test) are observed at 3 cpd ($P = 0.08$), 6 cpd ($P = 0.08$), 12 cpd ($P = 0.04$), and 18 cpd ($P = 0.04$). Near visual acuity measurements with the National Optic Chart showed that all of the young group with the exception of one had visual acuity of at least 20/30; the person with poorer visual acuity used the administrator's reading glasses to carry out the test. No differences were observed between the profiles for the higher and lower exposure groups for persons over 35 years.

TABLE 3

Results of the Neurofunctional Test Battery

		n	Mean ± SD	Range
Color vision (Color CCI)		87[a]	1.48 ± 0.44	1.00–3.22
Contrast sensitivity	1.5 cps	87[b]	25.8 ± 8.4	5.0–52.5
	3.0 cps		46.6 ± 21.0	9.5–127.5
	6.0 cps		51.3 ± 25.2	8.0–125.0
	12.0 cps		51.0 ± 30.5	0.0–125.0
	18.0 cps		23.1 ± 14.7	0.0–65.0
Grip strength (kg)	Men	45	37.8 ± 7.8	24.0–56.5
	Women	46	24.3 ± 4.7*	14.0–35.5
Fatigue (s)		91	51.2 ± 27.0	9–175
Manual dexterity (number)		91	36.4 ± 6.5	18.5–51.5

[a] Two Withdrawals of participants with congenital color deficit detected by the test, and two refusals.

[b] Four persons refused to perform the test.

*Differences between women and men (Student t test; $P < 0.001$).

TABLE 4

Results of the Neurofunctional Tests with Age and Mean Total Hair Mercury (HHgT), Peak Hair Mercury (HHgP), First cm Total Hair Mercury (HHg1), First cm Hair Methylmercury (MeHg1) in the Regression Model

			HHgT		HHgP		HHg1		MeHg1	
Test		(n)	β	t	β	t	β	t	β	t
Color vision (CCI)		87	− 0.007	− 1.32	− 0.005	− 1.39	− 0.005	− 1.40	− 0.006	− 1.36
Contrast sensitivity	1.5 cps	87	0.06	0.14	0.04	− 0.04	0.05	0.14	0.05	0.14
	3.0 cps		− 0.26	− 1.11	− 0.29	− 1.48	− 0.22	− 1.11	− 0.25	− 1.16
	6.0 cps		− 0.42	− 1.55	− 0.44*	− 2.05*	− 0.36	− 1.63	− 0.39	− 1.63
	12.0 cps		− 0.61*	− 1.97*	− 0.56*	− 2.31*	− 0.54*	− 2.16*	− 0.60*	− 2.20*
	18.0 cps		− 0.25†	− 1.77†	− 0.23*	− 2.00*	− 0.22†	− 1.91†	− 0.25†	− 1.93†
Grip strength (kg)	Men	45	0.06	0.58	0.05	0.60	0.03	0.29	0.03	0.31
	Women	46	− 0.17†	− 1.90†	− 0.11†	− 1.66†	− 0.09	− 1.30	− 0.10	− 1.30
Fatigue (s)		91	− 0.51†	− 1.94†	− 0.41†	− 1.94†	− 0.38†	− 1.77†	− 0.44†	− 1.86†
Manual dexterity (number)		91	− 0.10	− 1.55	− 0.11*	− 2.10*	− 0.08	− 1.43	− 0.08	− 1.45

Note. Beta coefficient (β) and t value for the mercury level are presented.
†$P \leq 0.10$; *$P \leq 0.05$.

Clinical Exam

Table 5 presents the results of the different elements of the clinical examination conducted on 59 persons randomly selected from the study participants. For the most part, clinical examinations were normal; however, high prevalences were observed for persons presenting disorganized movement for the BAMT (53.4%), restricted visual fields (47.5%), and patellar and bicepital hypereflexia (44.8% and 25.9%).

A χ^2 analysis was used to determine the distribution of these outcomes with respect to age (≤ 35 years, >35 years), educational level (≤ 6 years, >6 years) smoking, alcohol consumption, having spent time in the gold-mining region, malaria, and

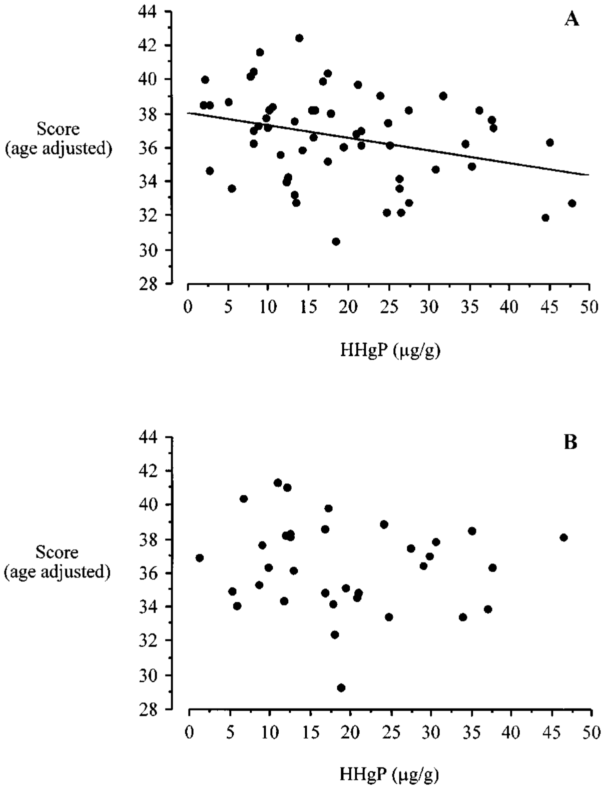

FIG. 4. Age-adjusted scores of the Santa Ana (Helsinki version) with respect to peak hair mercury concentration (HHgP) in µg/g. (A) Results for persons ≤ 35 years ($n = 57$; $F = 5.98$, $P \leq 0.02$; $R^2 = 0.10$). (B) results for persons >35 years ($n = 34$).

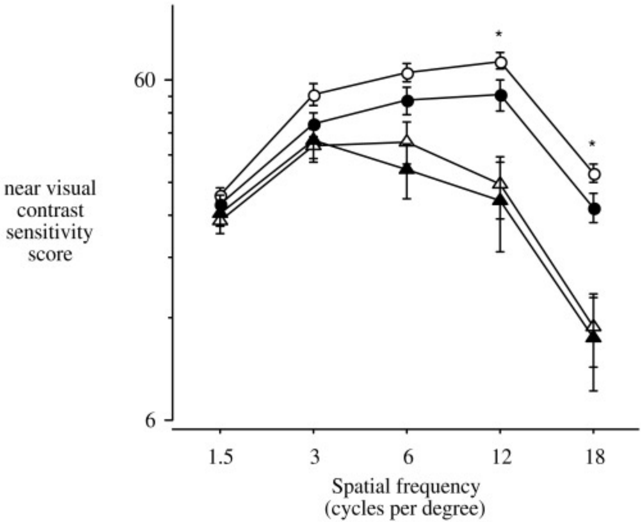

FIG. 5. Near visual contrast sensitivity profiles for the study participants with respect to peak hair mercury concentration (HHgP). Person ≤ 35 years with HHgP ≤ 20 µg/g (○, $n = 34$) and >20 µg/g (●, $n = 23$); persons >35 years with HHgP ≤ 20 µg/g (△, $n = 19$), and >20 µg/g (▲, $n = 11$). Standard error bars are indicated (*$P \leq 0.05$; students t test).

TABLE 5

Results of the Clinical Examination

Examination		Normal response	(*n*)	Abnormal response	(*n*)
Branches Alternate Movement Task (BAMT)[a]		Normal	27	Disorganized	31
Confrontation visual field		Normal	31	Reduced	28
Hyporeflexia:	Patellar	Absent	55	Lateral / bilateral	1 / 2
	Bicepital	Absent	58	Lateral / bilateral	0
	Glabellar	Absent	59	Bilateral	0
Hyperreflexia:	Patellar	Absent	32	Lateral / bilateral	3 / 23
	Bicepital	Absent	43	Lateral / bilateral	1 / 14
	Glabellar	Absent	58	Bilateral	1
Light touch:	Arms	Normal	51	Lateral / bilateral	2 / 4
	Legs	Normal	50	Lateral / bilateral	4 / 3
	Face	Normal	52	Bilateral	5
Pin prick:	Arms	Normal	52	Lateral / bilateral	2 / 3
	Legs	Normal	53	Lateral / bilateral	0 / 4
	Face	Normal	55	Bilateral	2
Pressure:	Arms	Normal	58	Lateral / bilateral	0 / 1
	Legs	Normal	58	Lateral / bilateral	0 / 1
Static equilibrium		Normal	51	Abnormal	8
Clonus of the feet		Present	57	Absent	2
Hands tremor		Absent	58	Present	1
Muscle strength		Normal	59	Increased / decreased	0 / 0
Muscular tonus		Normal	59	Abnormal	0
Calcanhar-Joelho		Normal	59	Abnormal	0
Index-nose movement		Normal	59	Abnormal	0
Ocular movement		Normal	59	Abnormal	0
Elocution		Normal	59	Abnormal	0
Audition		Normal	59	Abnormal	0
Walking		Normal	59	Abnormal	0

[a] One person refused to perform the BAMT.

parasitosis. Disorganized BAMT was significantly related to age: 10 (32.3%) of the 31 persons from the younger group displayed disorganized movements as compared to 21 (77.8%) of the 27 persons who composed the older group (77.8%) (χ^2: 12.4; $P < 0.001$). No significant differences were observed for disorganized BAMT and the other parameters. No relations were observed for visual field restriction and patellar and bicepital hypereflexia and any of the above variables.

Table 6 presents the results for normal and abnormal presentation for BAMT, visual fields, and

TABLE 6

Results of the Branches Alternate Movement Task (BAMT) and Visual Field and Patellar and Bicepital Reflexia in Relation to Mean Total Hair Mercury (HHgT), Peak Hair Mercury (HHgP), First cm Total Hair Mercury (HHg1), and First cm Hair Methylmercury (MeHg1)

	HHgT (μg/g) mean (SD)		HHgP (μg/g) mean (SD)		HHg1 (μg/g) mean (SD)		MeHg1 (μg/g) mean (SD)	
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
BAMT	12.6	18.4*	16.5	24.4**	14.4	21.3*	13.0*	19.2*
	(5.7)	(10.3)	(7.1)	(12.3)	(7.5)	(13.1)	(6.8)	(12.0)
Visual field	13.6	18.2*	18.0	24.0*	16.0	20.3	16.0	20.3
	(7.9)	(9.3)	(10.5)	(10.5)	(10.7)	(11.5)	(10.7)	(11.5)
Patellar reflex	15.6	15.4	20.4	20.5	18.3	17.2	16.5	15.4
	(8.8)	(9.0)	(10.4)	(11.5)	(10.8)	(11.7)	(9.9)	(10.7)
Biceptial reflex	15.3	16.3)	19.7	22.6	17.3	19.1	15.6	17.1
	(8.9)	(8.7)	(10.6)	(11.5)	(10.8)	(12.3)	(9.9)	(11.3)

*$P \leq 0.05$; **$P \leq 0.01$.

patellar and bicepital reflexes. For the BAMT, there were significant differences for all hair mercury parameters, with the most significant differences observed with peak hair mercury level. For visual field, the differences were significant for mean hair mercury and peak mercury levels, but not with total or organic mercury from the first centimeter. There were no differences in any of the hair mercury parameters for patellar and bicepital hypereflexia.

Figure 6 presents the percentile distribution of peak hair mercury levels for those with normal and abnormal presentation for the BAMT and visual field for those below and above 35 years of age. The differences are significant for the younger group for the BAMT (*t* test: $P = 0.01$), but not for the older group. For visual field restriction, significant differences in HHgP are observed for the older group, but not in the younger group. It is noteworthy that in the younger group, the mean HHgP for those with both disorganized BAMT and reduced visual fields ($n = 5$) is 30.9 μg/g ± 13.6.

DISCUSSION

The findings of this study confirm our previous preliminary report of nervous system alterations at hair mercury levels below 50 μg/g in a riparian population of the Brazilian Amazon (Lebel *et al.*, 1996). There appears to be a dose-effect relationship for certain motor and visual functions, assessed quantitatively with a neurofunctional test battery and qualitatively by a clinical examination. For motor functions, manual dexterity, alternating hand coordination, and to a lesser extent, muscular fatigue were associated with hair mercury levels. Dose-dependent altered visual functions included near visual contrast sensitivity and restricted visual fields.

High levels of exposure to methylmercury, such as those experienced in Japan and Iraq, are known to produce important neuromotor disturbances including limb and gait unsteadiness, ataxia, abnormal reflexes, speech difficulties and muscular weakness (WHO, 1990). In a case-control study of possible neurological effects of low-level exposure to methylmercury among Cree Indians living in Northern Québec, bilateral incoordination was one of the two mandatory criteria for "case" classification (McKeown-Eyssen and Ruedy, 1983a,b). Although a positive association was observed between neurological abnormalities and methylmercury exposure, the authors concluded that they may not have been entirely attributable to methylmercury. The findings of the present study support the link between low-level, long-term exposure to methylmercury and deterioration of certain motor functions.

Neuromotor impairment has been observed in animals with long-term low-level exposure to methylmercury. Wild mice, captured in an industrially contaminated area of the Great Salt Lake, who appeared overtly normal, presented decreased swimming ability and ambulatory activities in an open field test; there was a direct correlation with hair mercury level which ranged between 0.3 and 22.0 μg/g (Burton *et al.*, 1977). Interestingly, the alternate hand movement task developed by Dr. Fernando Branches may call upon motion patterns similar to

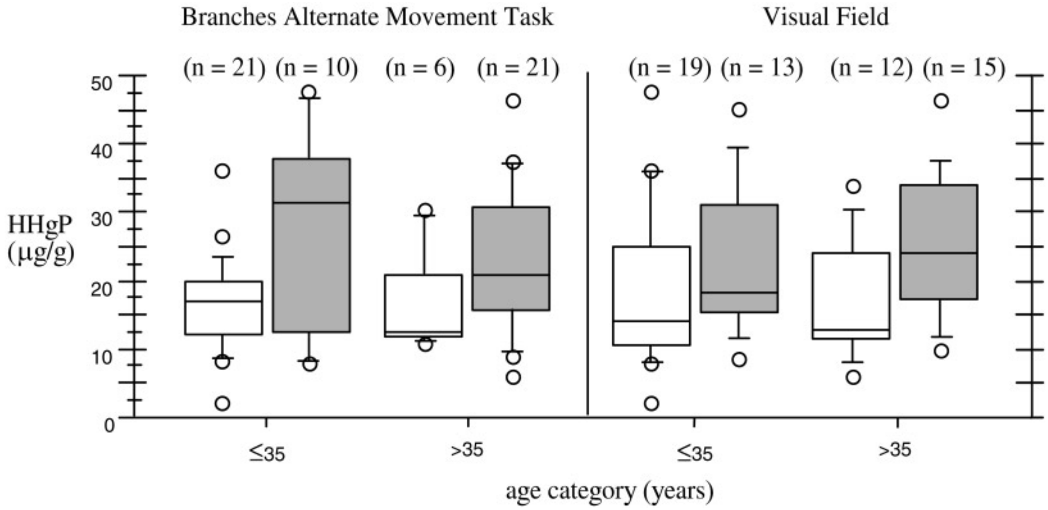

FIG. 6. Distribution of peak hair mercury (HHgP) in μg/g for study participants ≤ 35 years and > 35 years, for the Branches Alternate Movement task (normal (no shading), and disorganized (shading) and visual field (normal (no shading), and restricted (shading)).

those required for swimming since it involves repeated alternate arm movements. In monkeys, exposed to methylmercury chloride (50 μg/kg/day) from birth up to 7 years of age, and examined 6 years after cessation of exposure, fine motor performance, evaluated as the time required to retrieve raisins from recessed grids, was slower for methylmercury-treated animals in comparison to age-matched controls (Rice, 1989). In future studies, similar tasks should be included in test batteries for humans.

Alterations of visual function are one of the most frequently reported signs of methylmercury intoxication. In Japan and Iraq, visual field restriction was a predominant, but not invariable, symptom among intoxicated individuals (Iwata, 1980; WHO, 1990). In the present study, visual field restriction, assessed by confrontation testing, was linked to HHg levels, particularly peak and mean Hg values. In a report on the North West Québec native population, reduced visual fields, evaluated by perimetry, were observed for an individual with hair mercury level of 7 μg/g; no indication was given of possible previous higher exposures (Barbeau *et al.*, 1976). In Niigata cases of visual field constriction, measured by perimetry, were reported in individuals with HHg levels of 52 and 66 μg/g at the beginning of the outbreak (Iwata, 1980).

Diminished contrast sensitivity was also reported subsequent to the Niigata contamination episode (Mukuno *et al.*, 1981). In studies of monkeys with pre- and postnatal methylmercury exposure over a 4-year period, impaired visual spatial frequency function was observed with no obvious signs of intoxication; the loss was particularly important in the high spatial frequencies (Rice and Gilbert, 1982, 1990). The findings of the present study confirm our initial observations on depressed near visual contrast sensitivity profiles in the intermediate and high spatial frequencies, in the absence of near visual acuity loss. However, contrary to our previous findings, in this population, color vision did not vary with mercury hair levels.

The hair mercury levels observed here are similar to those measured previously among a smaller sample living in the same area (Lebel *et al.*, 1996). Different measures of hair mercury content (mean, peak, first centimeter and MeHg first centimeter), provided similar results with respect to neuro-outcomes, although to slightly differing degrees. Mercury exposure varies over time and hair analyses can provide an indicator of past exposure since hair grows at approximately 1 cm per month (Cernichiari *et al.*, 1995; Clarkson *et al.*, 1988; Clarkson, 1997). There are limits, however, to whichever measure is used to describe exposure. The first centimeter has the advantage of being present for all individuals, but it reflects very recent exposure. Mean hair level presents the advantage of providing integrated information over time, although the precision of the measure is limited by the person's hair length. The same is true for peak hair levels, which are likewise dependent on hair length.

Peak levels may be of particular physiological importance, and, in this study, among the various parameters of hair mercury, it is the most strongly related to neuro-outcomes. In the village, fish consumption and mercury hair levels vary sinusoidally with the seasons (Lebel *et al.*, 1997). Testing was carried out in March, during the rainy season, so that even among those with short hair, the peak would probably be close to the highest level during the year. Peak values of hair mercury as a predictor of neuro-outcomes should be examined in future studies.

This study was carried out in a remote village, where fish constitute an important source of protein. It is an integral part of the diet and consumption appears to be relatively constant from year to year, as evidenced by the stable levels of hair mercury over a 2-year period, with seasonal variations corresponding to changes in dietary practices from a more piscivorous fish diet to a more herbivorous fish diet (Lebel *et al.*, 1997). The mercury hair levels in this population probably reflect long-term exposure. Thus, the dose-effect relations are most likely related to cumulative exposure, rather than short-term exposure, but further studies should be done with these populations in order to understand the toxico-kinetics of mercury in this situation. Indeed, the hot, humid climate may be an important factor. Yamaguchi *et al.* (1984), who examined the effect of methylmercury toxicity in rats, reported that the onset of neurotoxicity was more rapid for those maintained in an environment of 33°C, compared to those at 22°C, or at 11°C. It is interesting to note that in this animal study, one of the sensitive measures of methylmercury toxicity was "hind-leg crossing."

All hair mercury parameters in this population were inferior to 50 μg/g which has to date been considered as the limit below which clinical signs are not apparent. The dose-effect relations observed below this level, for both visual and motor outcomes, may have several explanations. Previous studies on low-level methylmercury exposure did not use sensitive neurofunctional test batteries, but relied on neurological signs that were present in Japan and

Iraq. It is also possible that there is a factor in this population, which may be genetic or environmental, that makes them more susceptible to the effects of methylmercury than others. The neurofunctional and clinical tests used here should also be applied in studies of other populations with similar levels of mercury contamination.

One of the interesting findings in this study is that for those under 35, many relations between hair mercury and neuro-outcomes are stronger than for the older group. These differences in dose response may be due to the history of mercury exposure in this region and the lifetime period at which exposure to the contaminant began. Results from the environmental portion of our study suggest that mercury levels began increasing approximately 40 years ago with the implantation of population settlement and forest burning practices for agricultural activities, which contribute to the lixiviation of mercury from the soils to the river system (Roulet *et al.*, 1997, 1998; Farella *et al.*, 1996). At the end of the seventies, a new enrichment of mercury came from the booming gold-mining activities and also from increasing forest burning practices necessary for immigrant settlements in this region. If this is so, persons under 20 years of age may have substantial methylmercury exposure *in utero*, while those between 20 and 35 years were exposed during perinatal and infancy period. The older population would only have been exposed in adulthood. In animal studies on the sequelae of low-level methylmercury exposure, more severe effects have been observed for *in utero*, as compared to perinatal and adult exposure periods (Rice, 1989). The implication of this finding is critical for the population of Brasília Legal and for other populations within this region since the proportion of persons with exposure only in adulthood will diminish over time, while those with *in utero* exposure will increase.

In a situation where mercury levels are below the recognized threshold, and exposure is extended over a long period of time, the results of this study, coupled to those of our preliminary study (Lebel *et al.*, 1996), suggest that there may be subtle nervous system changes that can be assessed with sensitive quantitative neurofunctional tests and adequate neurological examination. In this particular study, it was difficult to administer an extensive neurobehavioral test battery due to environmental conditions, the absence of electricity, and geographical remoteness. More studies are clearly needed in this and other populations to elucidate the profile of impairment and to determine the long-term significance of these alterations.

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