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Methylmercury exposure affects motor performance of a riverine population of the Tapajós river, Brazilian Amazon

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Abstract Gold mining and deforestation in the Brazilian Amazon are increasing mercury pollution of the extensive water system, exposing riverine populations to organic mercury through fish-eating. The aim of the present study was to evaluate the effect of such exposure on motor performance. This cross-sectional study was carried out in May 1996, in a village located on the banks of the Tapajós river in the Amazonian Basin, Brazil. Information concerning sociodemographics, health, smoking habits, alcohol drinking, dietary habits and work history were collected using an interview-administered questionnaire. Mercury concentrations were measured by cold vapor atomic absorption in blood and hair of each participant, of whom those aged between 15 and 79 years were assessed for motor performance ($n = 84$). Psychomotor performance was evaluated using the Santa Ana manual dexterity test, the Grooved Pegboard Fine motor test and the fingertapping motor speed test. Motor strength was measured by dynamometry for grip and pinch strength. Following the exclusion of 16 persons for previous head injury, working with mercury in the gold-mining sites, or for diabetes, the relationship between performance and bioindicators of mercury was examined using multivariate statistical analyses, taking into account covariables. All participants in the study reported eating fish, which comprised 61.8% of the total meals eaten during the preceding week. The median hair total mercury concentration was $9 \mu\text{g/g}$. Organic mercury accounted for $94.4 \pm 1.9\%$ of the total mercury levels. Multivariate analysis of variance indicated that hair mercury was inversely associated with overall perfor-

mance on the psychomotor tests, while a tendency was observed with blood mercury. Semipartial regression analyses showed that hair total mercury accounted for 8% to 16% of the variance of psychomotor performance. Neither hair nor blood total mercury was associated with the results of the strength tests in women and men. Although dose-effect relationships were observed in this cross-sectional study, they may reflect higher exposure levels in the past. The findings of this study demonstrated neurobehavioral manifestations of subtle neurotoxic effects on motor functions, associated with low-level methylmercury exposure.

Keywords Methylmercury · Amazon · Hair · Neurotoxicity · Motor performance · Fish

Introduction

Mercury contamination in the Amazon Basin is far more pervasive than originally thought. In addition to the mercury released into the environment from gold mining activities, estimated at 130 tons annually during the past two decades (Pfeiffer et al. 1993), slash and burn agricultural practices along the riverbanks cause the release of natural mercury through soil erosion into the aquatic ecosystem (Roulet et al. 1999). Recent evidence shows that mercury is not carried over long distances, and that the high levels present in areas remote from gold-mining sites are associated with the lixiviation of soils exposed to intense weathering after slash and burn of their forest cover (Roulet et al. 1998). This adds a new dimension to the problem of mercury pollution in the Amazon, largely increasing the regions and populations affected by mercury contamination.

Once released into the extensive Amazonian hydrographic basin, elemental mercury becomes available for bacterial biotransformation into organic mercury, a form more toxic to living organisms because of its high affinity for sulfhydryl groups. Through the process of bioconcentration and bio-amplification along the food

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chain, methylmercury eventually reaches humans through fish consumption (WHO 1989). Studies of increasing numbers of fish-eating populations in the Amazon region indicate mean or median hair mercury levels of between 2 and 20 $\mu\text{g/g}$ (Akagi et al. 1995; Barbosa et al. 1998; Boischio and Cernichiari 1998; Boischio et al. 1995; Grandjean et al. 1993; Lebel et al. 1996; Lebel et al. 1997; Malm et al. 1990; Malm et al. 1995).

The long-term effects of exposure to methylmercury through the ingestion of highly contaminated fish, following the Minamata and Niigata disasters, are still being documented (Harada 1995; Kinjo et al. 1993; Ninomiya et al. 1995; Takeuchi et al. 1996). These disasters revealed that the nervous system is a primary target of organic mercury poisoning, involving disturbances of sensation in the extremities, ataxia, dysarthria, deafness, constriction of the visual field and muscular weakness (WHO 1990). Recent studies conducted in the Brazilian Amazon by Lebel et al. (1996, 1998) reported manifestations of methylmercury toxicity in the visual and motor systems in adult riparian populations.

Motor functions are easily measured in field situations, using neurobehavioral tests that can be administered under standard conditions, without electricity. In the studies in the Brazilian Amazon, Lebel et al. (1996, 1998) showed that manual dexterity and women's grip strength decreased with increasing hair mercury. The aim of the present study was to confirm, in another village in the Brazilian Amazon, the relationship between methylmercury exposure and reduced motor performance, and to extend our understanding of the effects of methylmercury on motor functions by evaluating a wider range of neuromotor outcomes.

Materials and methods

Subjects

This cross-sectional study was carried out at the end of the rainy season, in May 1996, with the participation of the population of Cametá, a village situated on the banks of the Tapajós river (Brazilian Amazon), approximately 250 km downstream from the gold mining area (3°17'08" S, 55°06'03" W). A house-to-house survey revealed that this village had a total population of 413 people. Because of time and resource limitations, 100 persons of 12 years and older, were targeted for the present study. No ad hoc exclusion criteria were applied and participation was on a "first come" basis.

During an initial meeting with the population, carried out in the local schoolhouse, the general objectives of the study were presented and the inhabitants of the village invited to participate on a voluntary basis. A total of 98 individuals, aged between 12 and 79 years old responded favorably within the 2-week time constraint. Because of interindividual differences in motricity, associated with the age of onset of puberty, only those of 15 years and older ($n = 84$) were included in the present analyses of motor performance. This group represented 39.1% of the village population in this age range: 41.4% of the women and 36.4% of the men.

The study protocol was approved by the ethics committees of the University of Québec at Montréal and the Federal University of Pará, Brazil. All participants gave their informed consent prior to the study.

Questionnaire

Information concerning sociodemographics, smoking habits, alcohol drinking, medical and work history were collected by a questionnaire, administered during interview by a physician. Questions were also asked about dietary habits, including fish consumption, with an emphasis on the most frequently consumed fish species and fish meals.

Determination of blood and hair mercury levels

Blood collection and analysis

Blood samples were collected by a nurse by venipuncture into heparinized Vacutainers of 7 ml capacity (Becton-Dickinson, BD367735). Risk of contamination and coagulation were reduced by the use of sterile, sodium heparinized tubes. All blood samples were stored at 4 °C until analyzed. Total mercury in blood was determined by atomic absorption spectrophotometry at the laboratories of the "Fundação Esperança", a medical assistance foundation, with a laboratory located in Santarém, Brazil, supported by the European Economic Community. The detection limit for blood mercury analysis was 3.0 $\mu\text{g/l}$. The laboratory participated in the Inter-Laboratory Comparison Program for blood mercury analysis of the Québec Toxicology Centre, Canada; the results obtained for five blood samples had a variance coefficient (R^2) of 0.9996 (Bastos et al. 1998).

Hair collection and analysis

Hair samples were taken immediately prior to being tested during the 2-week period. Hair from the occipital area of the head was cut close to the scalp, placed in an identified plastic bag and stapled to prevent the shifting of the hair strand. The hair samples were analyzed at the Laboratory of Indian and Northern Populations Health, Health Canada, Ottawa. Analyses of total and inorganic mercury were performed according to the method described by Farant et al. (1981). The level of organic mercury was obtained from the difference between the amounts of total and inorganic mercury. For these analyses, hair samples were cut into 1 cm lengths, starting from the scalp. Mercury concentration was determined by cold vapor atomic absorption using a mercury UV monitor (model 1235, Laboratory Data Control, Riviera Beach, Fla.). Detection limit was 0.5 $\mu\text{g/g}$. All the samples analyzed had total hair mercury levels above this detection limit, but some contained inorganic mercury below 0.5 $\mu\text{g/g}$.

Total hair mercury levels were used in the statistical analyses since they were determined directly, independently of the estimation of inorganic mercury levels. Since all participants had at least 2 cm of hair, the average mercury concentration from the first 2 cm of hair were used here as a bioindicator of mercury exposure. Precision and accuracy of mercury determination were ensured by the use of internal hair standards, provided by the Hair Mercury Inter-Laboratory Comparison Program, Health Canada, Ottawa. In the period over which these analyses were performed, 50 of the 54 standard samples were within 1 standard deviation and the other four were within 2 standard deviations.

Evaluation of motor performance

General procedure

Testing was carried out at the village school and tests were administered by trained Brazilian students from the Federal University of Pará. Each test was administered throughout by the same tester, who had no knowledge of the participants' mercury levels. The evaluation was carried out in the same order for each participant. The preferred hand was designated as that which the participant considered to be the most frequently used.

Psychomotor tests

Santa Ana (Helsinki version)

The Santa Ana manual dexterity test (Hänninen and Lindstrom 1979) was used to evaluate motor coordination. The task consisted of lifting up square pegs with circular tops, turning them 180 degrees and replacing them in their holes as rapidly as possible. The number of pegs successively turned was recorded. Two trials of 30 s were done with each hand. The mean of the two trials is presented here.

Grooved pegboard

The Grooved Pegboard test (model 32025, Lafayette Instruments) was used to assess manual dexterity and fine motor movement. The participant was required to place 25 pins into holes, as quickly as possible. The pins were key-shaped and had to be rotated to fit into the holes. The test was performed first with the preferred and then with the non-preferred hand. The time required by the participant to complete this test was recorded. Mean time of two trials constituted the score for each hand. If persons did not complete the test within 5 min, the scores were not considered for the present analyses.

Fingertapping

The manual version of the Fingertapping test evaluates motor speed and coordination (Fleishman 1954). To accomplish this test, the participant taps with the index finger for 10 s, as quickly as possible, on a key connected to a counter. The participant performs first with the preferred and then with the non-preferred hand. For each hand, the number of taps recorded on the counter was noted. Three trials were given and the mean score was calculated for each hand.

Strength tests

Grip strength

The hand dynamometer (model 78010, Lafayette Instruments), adjusted to the size of the participant's hand, was used to evaluate motor strength (kilograms). The participant was asked to squeeze the hand dynamometer as hard as possible. One trial was given for the preferred hand and one for the non-preferred hand.

Pinch strength

The "Pinch gauge" manufactured by B&L Engineering and distributed by Lafayette Instruments (model 78005) was used to assess the fine strength of the hand and the fingers. The participants were asked to squeeze the handle of the gauge with the thumb, the index and the middle fingers (tripod pinch). The strength (kilograms) was measured three times per hand and the scores averaged for each hand.

Statistical analysis

Descriptive statistical analyses were used to describe the study population, mercury exposure and the results of motor performance. Multivariate analysis of variance (Manova), using Wilks' lambda, was used to examine the relationship between mercury level and overall performance on the psychomotor and strength tests. For these analyses, continuous independent variables were separated into two categories and the cut-off points were set near the median (age: 27 years; education level: 4 years; mercury: 10 µg/g). When the relationship with mercury was found to be statistically significant in the Manova, multiple regression models were

constructed to verify the contribution of mercury to the variation in performance on each motor task. The covariates were entered into a stepwise regression model and those $P \leq 0.1$ were retained. Because mercury concentrations and age were log-normally distributed, a log normal transformation was used in the multiple regression analysis. Alcohol consumption, smoking habits, gender and reported arthritis were treated as dummy variables in these models. Influential points, determined by the analysis of studentized residuals, were excluded from the regression analyses. Semipartial regression analyses, which determine the part of variance of the dependent variable not estimated by the other independent variables (Cohen and Cohen 1975), were carried out to examine the relationship between mercury concentration and motor performance when the latter was adjusted for relevant covariates. A P value of less than 0.05 was considered to be threshold for significance. Statistical analyses were performed using the SAS version 6.12 statistical package and Statview 4.5 (SAS Institute).

Results

Of the 84 participants, aged 15–79 years, 16 were excluded from the present analyses for the following reasons: head accident ($n = 6$), worked with mercury in gold-mining sites ($n = 9$) and self-reported diabetes ($n = 1$). The final group, which included 41 women and 27 men, is described in Table 1.

The majority of the participants practice subsistence fishing (60.3%), and all reported eating fish. In the diet survey, which covered 7 days in May, at the end of the rainy season, fish was included in an average of $61.8 \pm 24.6\%$ of the total meals eaten, ranging from none to 100%. Analysis of the types of fish eaten during this week showed that herbivorous fish made up 79.9% of the total fish meals, piscivorous fish, 18.2% and others, 2.1%. One person did not fill out the questionnaire on fish-eating habits.

Both hair total mercury and blood total mercury were distributed logarithmically (Figs. 1 and 2). Men had

Table 1 Participants' sociodemographic characteristics

Characteristic	<i>n</i>	%		
Gender				
Women	41		60.3	
Men	27		39.7	
Alcohol consumption				
Non-drinker	25		36.8	
Spirits (cachaça)	13		19.1	
Beer	39		57.4	
Other spirits	27		39.7	
Smoking habits				
Smoker	31		45.6	
Non-smoker	27		39.7	
Ex-smoker	10		14.7	
Reported arthritis	29		42.6	
History of malaria	11		16.2	
History of parasitosis	26		38.2	
Born on the Tapajós river	54		79.4	
		Mean $\pm$ SD	Median	Range
Age (years) $n = 68$		31.8 $\pm$ 14.7	27.5	15–79
Education (years) $n = 67$		4.3 $\pm$ 2.3	4.0	0–11

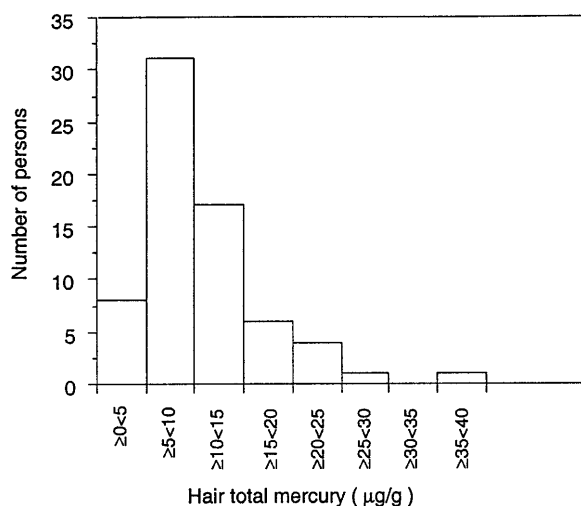


Fig. 1 Distribution of hair total mercury ($n = 68$)

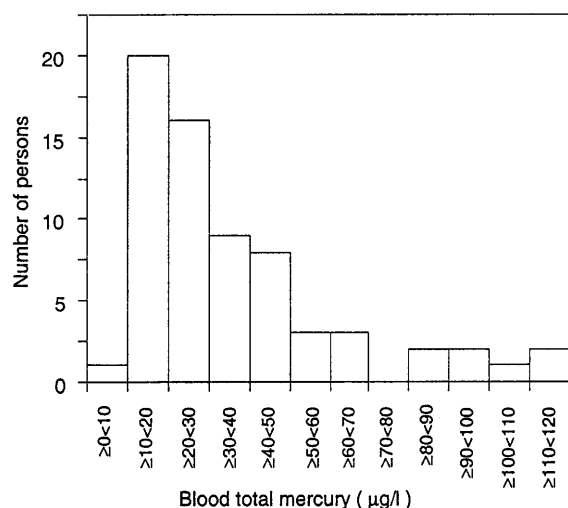


Fig. 2 Distribution of blood total mercury ($n = 67$)

significantly higher blood mercury levels compared with women, and a tendency in the same direction was present for hair mercury (Table 2). None of the other sociodemographic variables or reported symptoms or illnesses (reported arthritis, history of malaria or history

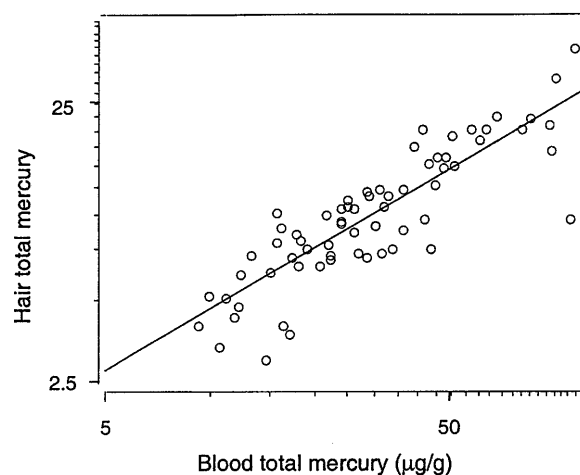


Fig. 3 Relationship between total mercury in hair and in blood (logarithmic scale)

of parasitosis) were related to hair mercury levels. In the hair, inorganic mercury accounted for $5.6 \pm 1.9\%$ of hair total mercury and organic mercury for $94.4 \pm 1.9\%$, varying from 89.9% to 97.6%. No differences were observed for these values between women and men. In this population, the correlation coefficient between the logs of total mercury level in blood and the first 1 cm of hair was 0.86 (Fig. 3). This coefficient was slightly less when the average of the two first 1 cm of hair were used for calculation ($r = 0.82$).

Because of the logarithmic distribution of hair and blood mercury levels, the blood to hair ratios, calculated for each person, were averaged. Using this procedure, hair to blood ratio for mercury was 338 ± 99 , ranging from 81 to 624. This ratio differs between women (356 ± 108) and men (312 ± 78), although it is not statistically significant (Student's t -test: $P = 0.07$). Authors of other studies (WHO 1990) have used the slope of the regression line of the relationship between blood and hair mercury to determine the blood:hair ratio. Using the slope in the present study, we calculated that the ratio would be 1:203.

Table 3 presents the results from the motor performance tests for the preferred and non-preferred hands. Manova revealed that hair mercury level was

Table 2 Whole blood total mercury and hair total mercury concentrations for women and men ($n = 68$)

Biological indicator	n	Arithmetic mean	SD	Geometric mean	q1	Median	q3	M–W ^b <i>P</i>
Blood total mercury (µg/l)								
Women	40	33.0	27.6	11.1	25.5	22.1	33.1	< 0.05
Men	27	40.7	23.2	35.6	21.0	32.0	43.0	
Total	67 ^a	36.1	26.0	29.2	13.3	27.0	40.7	
Hair total mercury (µg/l)								
Women	41	9.9	5.6	8.7	5.1	8.0	10.9	0.08
Men	27	12.2	6.8	10.7	7.2	10.8	14.3	
Total	68	10.8	6.1	9.5	5.5	9.0	12.5	

^a One participant did not consent to give a blood sample

^b Mann-Whitney

Table 3 Results of performance for the psychomotor tasks and strength tests for the preferred and non-preferred hand

Test	<i>n</i>	Mean $\pm$ SD
Santa Ana (number)		
Preferred hand	67	20.3 $\pm$ 4.8
Non-preferred hand	68	19.1 $\pm$ 3.9
Fingertapping (number)		
Preferred hand	67	39.3 $\pm$ 7.9
Non-preferred hand	67	38.6 $\pm$ 7.2
Grooved Pegboard (s)		
Preferred hand	62	66.0 $\pm$ 9.0
Non-preferred hand	61	71.0 $\pm$ 10.7
Grip strength (kg)		
Women		
Preferred hand	39	24.3 $\pm$ 4.1 ^a
Non-preferred hand	40	21.7 $\pm$ 4.3 ^a
Men		
Preferred hand	26	36.8 $\pm$ 6.4 ^a
Non-preferred hand	27	33.6 $\pm$ 5.9 ^a
Pinch strength (kg)		
Women		
Preferred hand	40	6.6 $\pm$ 1.3 ^a
Non-preferred hand	40	6.0 $\pm$ 1.3 ^a
Men		
Preferred hand	26	8.6 $\pm$ 1.7 ^a
Non-preferred hand	27	8.0 $\pm$ 1.8 ^a

^a Differences between men and women (Mann-Whitney: $P < 0.0001$)

significantly associated with overall performance of the psychomotor tests. (Wilks' lambda value = 0.72; $F_{(6,53)} = 3.51$; $P < 0.01$). A tendency was also observed with blood total mercury (Wilks' lambda value = 0.82; $F_{(6,53)} = 1.88$; $P = 0.10$). The regression analyses presented here are for hair mercury, since that relationship was the stronger.

The results of the multiple regression analyses for the psychomotor tests are presented in Table 4. Although age entered significantly into the model only for the

Grooved Pegboard task and for the non-preferred hand of the Fingertapping test, it was included in all models of analysis. Men performed better on the Santa Ana and Fingertapping tests than women, however they took longer on the Grooved Pegboard test. The scores of the Santa Ana and of the Grooved Pegboard (decrease of time) improved with education level, which had no effect on the Fingertapping task. Performance on the Santa Ana was also affected by alcohol, smoking habits and self-reported arthritis. Mercury accounted for 8% to 16% of the variance of psychomotor tests (Table 4). Figure 4 shows the relationships between hair mercury levels and performance of these tests, with adjustment for covariates.

The results of the strength tests were very significantly different for men and women, so separate analyses were performed. Manova showed that overall, the strength tests were not influenced by hair mercury for both women (Wilks' lambda = 0.97; $F_{(4,33)} = 0.29$; $P = 0.89$) and men (Wilks' lambda = 0.69; $F_{(4,20)} = 2.21$; $P = 0.10$) or by blood mercury (for women: Wilks' lambda = 0.90; $F_{(4,33)} = 0.91$; $P = 0.47$); for men: Wilks' lambda = 0.78; $F_{(4,20)} = 1.37$; $P = 0.28$), therefore no further analyses were done for the strength tests.

Discussion

The findings of this study demonstrated that increased methylmercury exposure among people in this Amazonian village is associated with diminished psychomotor performance. This dose-effect relationship was observed at hair total mercury levels below 50 $\mu\text{g/g}$, the level presently associated with a 5% risk of neurological damage in adult populations (WHO 1990). These results were consistent with previous findings relating diminished motor functions to increased mercury among Amazonian fish-eating populations (Lebel et al. 1996; Lebel et al. 1998).

Table 4 Results of multiple regression analyses for the psychomotor tests. Partial regression coefficients (beta coefficients) associated to each variable entered in the models are presented

Psychomotor test	<i>n</i>	Beta coefficients							F value model	Adjusted R^2 model	Semi partial r^2 mercury
		Age ^a	Gender (men/women)	Education	Alcohol (yes/no)	Smoker (yes/no)	Arthritis (yes/no)	Hair total mercury ^a			
Santa Ana											
Preferred hand	66	-4.76	-1.86	0.62 ^b	0.76	-1.56	-2.82 ^c	-5.77 ^c	7.62 ^d	0.42	0.10
Non-preferred hand	67	2.14	-2.12 ^b	0.59 ^c	2.17	-1.95 ^b	-1.94 ^b	-6.42 ^d	6.42 ^d	0.37	0.16
Grooved Pegboard											
Preferred hand	61	24.61 ^d	-3.89 ^b	-1.08 ^b	—	—	—	11.56 ^c	10.31 ^d	0.38	0.12
Non-preferred hand	60	23.50 ^c	-7.57 ^c	-1.46 ^c	—	—	—	12.73 ^b	10.10 ^d	0.38	0.11
Fingertapping											
Preferred hand	67	-7.98	-5.75 ^c	—	—	—	—	-9.56 ^b	6.43 ^d	0.20	0.08
Non-preferred hand	67	-8.50 ^b	-7.50 ^d	—	—	—	—	-10.11 ^c	14.41 ^d	0.38	0.13

^a Logarithms of age and of mercury were used in the multiple regression models ^b $P < 0.05$; ^c $P < 0.01$; ^d $P < 0.001$

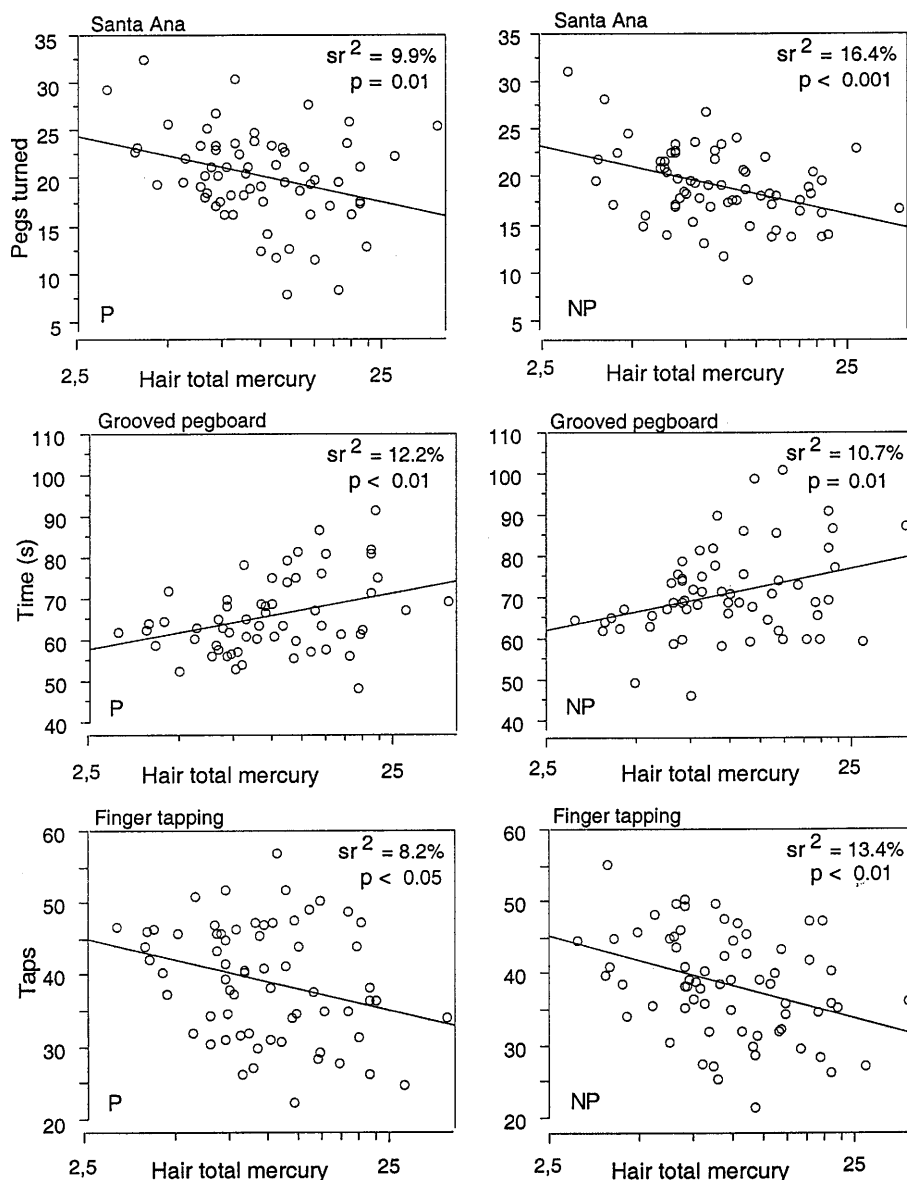


Fig. 4 Partial regression analyses of psychomotor testing. Adjusted score of each psychomotor task for the preferred hand (P) and the non-preferred hand (NP) in relation to the hair total mercury level adjusted for covariates. The adjusting covariates are those entered in the multiple regression analyses

Hair mercury levels measured in our study were similar to those reported in other studies along the Tapajós River (Akagi et al. 1995; Lebel et al. 1996; Lebel et al. 1997; Lebel et al. 1998; Malm et al. 1995) and in other regions of the Brazilian Amazon (Barbosa et al. 1995; Barbosa et al. 1998; Boischio and Cernichiari 1998; Boischio and Henshel 1996; Leino and Lodenius 1995; Malm et al. 1990). In the week preceding the study, fish was included in almost two thirds of the meals surveyed. In this remote village, fish is a dietary mainstay and is the major source of animal protein throughout the year. This is reflected by the high percentage of methylmercury in the hair samples. In other populations where fish is the major part of the diet, the

percentage of organic mercury has been shown to be over 80% of hair total mercury (WHO 1990); here, the range varied between 89% and 97%.

In this region, fish eating habits depend on the bio-availability of piscivorous and herbivorous fish (Lebel et al. 1997; Dolbec et al. submitted). Although mercury varies with the season and available species, people who consume more piscivorous fish have higher mercury levels than those who consume more herbivorous fish (Dolbec et al. submitted). Furthermore, mercury levels are related to the frequency of fish consumption (Dolbec et al. submitted). The other main elements in the diet include vegetables from the garden, manioc, beans, eggs and occasional poultry.

The village, like many others in the region, is isolated from areas where other sources of food, such as beef and pork, are available. These are traditional populations, who subsist on fish resources and what can be grown in their gardens or obtained from the surrounding forests.

There is minimal electricity only for a few hours daily and the lifestyle has not much changed during the past 50 years. Mercury contamination in this area of the Lower Tapajós River is attributed mainly to the intensification of deforestation and subsequent soil lixiviation (Roulet et al. 1998, 1999), which has been increasing rapidly over the past years (Nepstad et al. 1991). Although it is difficult to ascertain when the mercury pollution began, it has probably been prevalent for most of the lifetime of the majority of the persons studied here.

In the present study, the average mercury level in the first 2 cm of hair were used, since this provided a measure that would be similar for everyone, regardless of the length of hair. For methylmercury exposure, hair mercury is considered to be a better bio-indicator than blood mercury because it reflects body burden, since each segment represents the average blood concentration during the month that the segment was formed (Clarkson et al. 1988); blood mercury is more influenced by recent mercury intake and thus is more variable.

Motor function deficits have not been well established among adult populations exposed to low-level of methylmercury. An early study done by Valciukas and his coworkers (1986) reported no behavioral effects of methylmercury on a Mohawk Indian adult population. The authors attributed this to the fact that the levels of mercury measured in hair (total hair mercury mean = 0.69 µg/g) and in blood (total blood mercury mean = 2.45 µg/l) were too low to show any relationship. It should be noted, however, that the neurobehavioral tests used in the latter study measured more cognitive than motor functions.

Diminished performance on the Santa Ana manual dexterity test has also been associated with increasing hair mercury levels in previous studies done on similar populations (Lebel et al. 1996, 1998). In the present study, we likewise evaluated finger dexterity by the Grooved Pegboard test, which is sensitive to mercury exposure. We selected the Grooved Pegboard test since it is similar to a laboratory task that was used by Rice (1989) on mercury-exposed non-human primates, who were trained to retrieve raisins from a recessed grid. Six years after cessation of exposure to methylmercury, the monkeys, who had been exposed to methylmercury chloride (50 µg/kg/day) from birth to 6.5–7 years of age took longer to retrieve raisins than did the control monkeys. The author of the latter study suggested that the somatosensory system may be implicated and affected by long-term exposure to methylmercury. Indeed, the findings from the animal and human studies may reflect a combination of fine motor and somatosensory effects.

Fingertapping, which reflects motor speed, also diminished with increasing hair mercury levels in the present study. In a case-control study of children with prenatal exposure to methylmercury in the Faeroe Islands, Grandjean and co-workers found poorer performance in the fingertapping test associated with mercury exposure in boys (Grandjean et al. 1998).

The different measures of motor performance were not independent of one another, so multivariate analyses confirmed that psychomotor performance was affected by methylmercury exposure. No relationship was observed in the present study on tests for grip or finger strength. Lebel et al. (1996, 1998) reported decreased handgrip strength with increasing hair mercury levels in women. Considering that grip strength can differ for the same individual between different trials (Young et al. 1989), the one time evaluation done in the present study may account for a non-representative state of the actual individual grip strength. This could explain the difference between the results of the present and previous studies, where the average of two trials for each participant was the outcome measure. However, it should be noted that the previous work showed a significant relationship between mercury levels and grip strength, only in women. It would be important, in future studies, to consider body mass index in examining the association between mercury exposure and strength.

In this study, the covariates that influenced motor performance were in accordance with previous reports in the literature. Anger and co-workers (1997) carried out a study the aim of which was to identify human factors that may have had similar effects to neurotoxic exposure on behavioral functions, and that would lead to erroneous conclusions on neurotoxicity. They demonstrated that education level, age, gender and cultural group affected performance of motor tests. In the present study, motor performance varied as expected, with age, education and gender; the group was homogeneous with regard to culture. Cigarette smoking and alcohol consumption, factors that may potentially modify or confound the relationship between chemical substances and neurotoxic effects (Stephens and Barker 1998), affected performance on the Santa Ana motor dexterity test, as did reported arthritis. There was a high frequency of reported arthritis in this population, that was inversely associated with manual dexterity. These types of ailments are important considerations in studies on remote populations.

Recruitment was done on a voluntary basis, which may have introduced a selection bias. Indeed, proportionally more young than old people participated. However, persons had no knowledge of their hair mercury levels, and subtle rather than overt effects were evaluated, which would minimize bias due to recall or the presence (or absence) of illness. Future researchers should do a house-to-house survey to determine the reasons for non-participation.

In the area studied, it was not possible to find a control group without exposure, since fish-eating is widespread. The dose-effect relationship showed diminishing psychomotor performance with increasing mercury concentrations, however, this may be related to previous rather than current mercury levels. On the other hand, it was not possible to distinguish long-term effects from short term consequences, since fish-eating is an on-going activity. Although there are limits inherent

in this cross-sectional study, the consistency between these findings and those of other human and animal studies strongly supports the dose relatedness of the response. Only 14 persons were born in regions other than the Tapajós, where fish is the mainstay; thus, it was not possible to determine the eventual contribution of in utero exposure. It is also possible that nutritional deficiencies or some genetic factor in this area could accentuate the effects of mercury, that would limit generalization to other populations.

The findings of this study demonstrated neurobehavioral manifestations of subtle neurotoxic effects on motor functions, associated with low-level methylmercury exposure. The changes represent early indicators of methylmercury neurotoxicity in the study population. These sub-clinical effects may be reversible and their early detection is important for the prevention of further nervous system deterioration. Alterations in behavioral functions can, in themselves, be harmful by affecting exposed people's capacity to function in their daily activities. Fish is the main source of protein for this population and has obvious nutritional advantages; thus, further studies should focus on the identification of the mercury content of different species, in order to reduce mercury exposure while maintaining an adequate diet.

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