

TITLE PAGE: LOW LEVEL METHYLMERCURY EXPOSURE AFFECTS NEUROPSYCHOLOGICAL FUNCTION IN ADULTS.

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List of Abbreviations: Hg – mercury; MeHg methylmercury, WHO – World Health Organization

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ABSTRACT

Background. The neurotoxic effects of methylmercury have been demonstrated in both human and animal studies. Both adult and fetal brains are susceptible to the effects of methylmercury toxicity. However, the specific effects of adult exposures have been less well-documented than those of children with pre-natal exposures. This is largely because few studies of methylmercury exposures in adults that have used sensitive neurological endpoints. The present study reports on the results of neuropsychological testing and hair mercury concentrations in adults (>17 yrs) living in fishing communities of Baixada Cuiabana (Mato Grosso) in the Pantanal region of Brazil.

Methods. A cross-sectional study was conducted in six villages on the Cuiaba River. Participants included 129 men and women older than 17 years of age. They were randomly selected in proportion to the age range and number of inhabitants in each village. Questionnaire information was collected on demographic variables, including education, occupation, and residence history. Mercury exposure was determined by analysis of hair using flameless atomic absorption spectrophotometry. The neurocognitive screening battery included the Wechsler Memory Scale, Digit Symbol, Concentrated Attention Test of the Toulouse-Pierron Factorial Battery, the Manual Ability Subtests of the Tests of Mechanical Ability, and the Profile of Mood States.

Results. Mercury exposure was associated with detectable alterations in performance on tests of fine motor speed and dexterity, and concentration. Some aspects of verbal learning and memory were also disrupted by mercury exposure. The magnitude of the effects increased with hair mercury concentration, consistent with a dose-dependent effect.

Conclusions. This study suggests that adults exposed to methylmercury may be at risk for abnormal mental decline. The functional domains disrupted in adults, namely attention, fine-motor function and verbal memory are consistent with those previously reported in children with prenatal exposures.

Key words: methylmercury; neurotoxicity; Amazonia; Brazil; fish

BACKGROUND

In recent years there has been a growing appreciation for acquired risk factors for neurologic disease and dysfunctions, including environmental exposures. In this investigation, we studied the effects of environmentally methyl mercury (MeHg) exposure on neurocognitive status of adults. This study is of critical importance as worldwide concerns have focused on mercury exposures. In many regions of the world mercury is used in small-scale gold mining and methyl mercury (MeHg) is a byproduct of these mining processes (1). In the US, adults as well as children are exposed to methyl mercury through the diet (2,3).

An intensive gold rush began in the early 1980's in the Pantanal and Amazon Regions of Brazil (4). Although declines in global prices for gold have led to decreased mining in recent years, previous mining activity continues to have consequences for the environment and its inhabitants. Small scale or informal gold mining, known in Portuguese as *garimpagem*, has significantly increased environmental mercury levels in water, sediments, and fish (5). In this process, metallic mercury (Hg^0) is used to recover particulate gold from alluvial deposits by crude amalgamation (6,7). During amalgamation and crude refining, significant amounts of Hg^0 are discharged into the surrounding water, soil and air (5,6,7,8). In aquatic systems inorganic mercury is converted to methylmercury (MeHg) by bacteria in sediments, bioaccumulated in fish and biomagnified through the aquatic food chain (3). Worldwide, the major source of human exposure to MeHg is through fish consumption (2,3,9).

The neurotoxic effects of MeHg especially during intrauterine development, have been demonstrated in both human and animal studies (3,10). MeHg exposure is

associated with a wide range of central nervous system dysfunction in both children and adults (11). Recent studies suggest an increased susceptibility of the developing nervous system to low level exposures, particularly during prenatal development (12,13,14, reviewed by 7). Neuropsychological data from most (but not all – ref 15) of these studies indicate that children exposed during prenatal development performed more poorly on several neuropsychological measures including concentration, fine motor speed, and verbal memory than non-exposed children. However the specific effects of environmental MeHg exposures in adults are less well-known.

In adults, methyl mercury exposure is thought to cause selective damage relatively restricted to the granule cells of the cerebellum and neurons in the visual cortex (11,16). In studies at Minamata, Japan, where the population consumed MeHg contaminated fish, high levels of prenatal exposure caused diffuse cerebral and cerebellar damage. In contrast, adults studied postmortem had more restricted, focal cerebral lesions (17). The most common clinical signs observed in adults in the Minamata episode were paresthesia, ataxia, gait disturbance, sensory disturbances, tremors, visual and hearing impairments (18). Formal neuropsychological assessments have not been reported. In 1971, a number of people in Iraq were exposed to mercury treated seed grain used mistakenly to make bread (19). The predominant symptom noted in adults was paresthesia, which typically emerged after a latent period of from 16 to 38 days (11). The most severely affected individuals presented with ataxia, blurred vision, slurred speech and hearing difficulties (10, 11). Standardized neuropsychological assessments were not conducted. Based upon these events the World Health Organization proposed that limiting exposure to a maximum hair mercury concentration of 50 to 125 µg/g would

prevent paresthesia (20). The International Program on Chemical Safety maintained this recommendation in its criteria document published in 1990 (9).

However, these recommendations are based upon very high exposure episodes that resulted in hair mercury concentrations in the range of hundreds of micrograms per gram (2). A critical contemporary issue in environmental health is the determination of the level at which methylmercury is toxic in persons with chronic low level exposure primarily by fish consumption (21). Recent studies of chronic low level MeHg exposures in adults suggest that the reference level of 50 µg/g in hair may not be protective.

Specifically, hair mercury concentrations below 50µg/g have been associated with neurobehavioral disturbances in aspects of visual (chromatic discrimination, contrast sensitivity and peripheral fields) and psychomotor functions (tremor, dexterity, grip strength, complex-movement sequences, hand-eye coordination, and rapid alternating movement) (6,22,23,24,25,26). Further studies are needed to delineate the potential neurocognitive effects associated with low level MeHg exposure for adults, in order to provide a rational basis for safety levels.

To this end, we evaluated the neurocognitive status of a riverine population in the Pantanal region of Brazil. These communities are dependent upon fish as a main source of food and protein (27,28). As a result of decades of small scale gold mining in this region, many aquatic systems are contaminated with mercury and many fish species contain relatively high levels of mercury (28, 29, 30, 31). The results presented in this paper are part of a larger regional project on “Mercury Contamination Risks Via Food Chain in Baixada Cuiabana, Pantanal – Brazil”. In this project, mercury levels have been measured in humans (blood, urine, hair), fish, water, and sediments. Levels of MeHg in

humans have been studied with respect to diet, fish consumption, socio-demographic information, self-reported symptoms and morbidity, and neurological and neurocognitive status (27). The substantial amount of data available on these exposures makes this a useful context for epidemiologic research.

METHODS

1. Population and Sampling:

The sample for this study was drawn from residents living in 6 fishing villages along the Cuiabá River, between the municipalities of Santo Antônio do Leverger and Barão de Melgaço in Baixada Cuiabana, Pantanal region – Brazil. This region is part of the Pantanal flood plain ecosystem in the southern region of Mato Grosso State. It is bordered by the tropical rain forest of the Amazon in the north and by the dry bush land savannah to the northeast. The studied area is approximately 250 km downstream from Poconé, an important gold mining area in Pantanal that was active in the early 1980s. According to data from the state of Mato Grosso, the annual gold production in this region in 1985 was 2.12 tons, which is estimated to involve the use of about 1 tons of mercury for amalgamation (5). Studies on exposures of gold miners and gold processors in Poconé demonstrate substantial exposures to mercury (29,32).

Based upon a census conducted along the Cuiabá River in 1995, the total population in the geographic region of interest was 1,387. The six largest villages were selected with a total of 1,159 inhabitants, representing 83% of the population. Using a maximum sampling error of 10% and a confidence interval of 95% , individuals were selected randomly and proportionally to the number of inhabitants in each village as well as proportionally by age groups (6 to 14, 15 to 49, and above 50 years of age). Persons

were excluded if they had lived in the study area for less than 6 months or worked in gold mining or in gold trading shops. For this study, 160 persons 17 years or older were selected from a total of 240. Neuropsychological data are reported in this study for these adult men and women. All participants gave informed consent to the study, which was approved by the institutional review board of the Universidade Federal de Mato Grosso.

2. Mercury Exposure Assessment:

Hair mercury levels were utilized as a biomarker of chronic exposure, since hair analysis is generally used in assessing environmental exposures to MeHg (3,7,10,11,33). From each subject, a hair sample of approximately 2 cm was taken near the root just above the neck. Thus, hair mercury levels in this study represented relatively recent exposure, over the past 1-3 months (11). The hair was washed and stored in sealed plastic bags prior to measurement. Total mercury content in hair was determined by acid digestion and atomic absorption spectrophotometry, using standard methods, at the trace metal analysis laboratory of the Universidade Federal da Mato Grosso, which meets the QA/QC criteria of the Interlaboratory Comparison program coordinated by the Centre de Toxicologie du Quebec, Canada. Because exposures in this population were largely, if not exclusively, to MeHg in fish, a determination of total mercury in hair was likely to be between 80 – 95% methylmercury (3,14,23).

3. Neurobehavioral Tests:

The participants were administered neuropsychological measures recommended for assessing occupational neurotoxicity by the Health Secretariat of Sao Paulo State in Brazil (34), which are similar to the recommendations of the World Health Organization (35). These included the following measures:

1. The Orientation, Mental control, Logical Memory, Digit Span, Visual Reproduction and Paired Associate Learning tests from the Wechsler Memory Scale (Portuguese version) were administered and scored in standard fashion to assess attention, learning and memory (36).
2. The Digit Symbol Subtest of the Wechsler Adult Intelligence Scale (in Portuguese) was administered and scored according to standard procedures to assess sustained attention as well as clerical speed and accuracy (37).
3. The Manual Ability subtests of Leon Walter's Tests of Mechanical Ability (TAM, Testes de Aptidao Mecanica) were also administered. The subtests assess manual speed and dexterity and involved placing disks on spindles, bead threading and marking dots. The measures were administered and scored according to the TAM battery manual (38).
4. The Concentrated Attention Test of the Toulouse Pieron factorial battery was administered to assess graphomotor speed in the execution of simple visuospatial and attentional tasks. The measure was administered and scored according to the CEPA Battery manual. For data analysis, raw scores were used for total responses, total correct responses, total errors (errors of commission) and total omissions (errors of omission) (39).

In addition, the Profile of Mood States (POMS) was utilized as a general screening measure for assessing mood, anxiety and general distress. This test has been used in studies of occupational neurotoxicity in Brazil (34).

Four Brazilian psychologists, formally trained in the neurobehavioral procedures, administered and scored the measures. They were unaware of the participants' hair mercury concentrations (which were analyzed after separately and testing of the subjects).

4. Statistical Analyses

Descriptive statistics were used to characterize the studied population in terms of socio-demographic variables and hair mercury. Exposure was defined by hair mercury concentration. The relation between hair mercury and neurobehavioral function was first studied by correlation and partial correlations with three covariates in the analyses, age, sex and education level. Because there were no significant differences among the village populations, except for mercury exposures, the population was analyzed as a whole. All correlations were Pearson's correlations and two sided tests were applied throughout. The associations between exposure and outcome variables were then analyzed by multiple regression with adjustment for relevant covariates. In addition to age, sex and educational level, additional covariates in these analyses were: alcohol intake, occupation and residence time, all of which were examined as covariates using stepwise methods. The regression equation model with overall test F was considered statistically significant at $p \leq 0.05$. The regression model was also performed using a dichotomized stratification of exposure $<$ or $>$ $6 \mu\text{g/g}$, based on the WHO hair mercury reference level recommended for children and pregnant women exposed to MeHg since this is the only available international guideline for adult exposures (9). In addition, the results from each neurocognitive test were dichotomized by the median performance of the studied population in order to evaluate odds of poor performance. The relationships between

outcomes and exposure, stratified by the median of each test and by exposure, were analyzed.

RESULTS:

Of the 160 adults recruited for this study, 150 agreed to participate. The 10 refusals did not differ from the participants. The main reason given for refusal was lack of time to participate. Of the 150 participants, data on mercury exposure by hair mercury analysis was available on 129, and these subjects provide the data discussed here.. The number of persons completing each test varied, and the main determinant of failure to complete a test was advanced age (>60 years). No other single factor accounted for failure to complete the tests. The data reported in this study are for a group of 52 men and 77 women, with ages ranging from 17 to 81 years (mean: 35 ± 15). Other relevant socio-demographic data are summarized in Table 1. Most women (90%) reported being housewives; most men (67%) were involved in fishing. The hair mercury concentration in the 129 subjects ranged from 0.56 to 13.6 $\mu\text{g/g}$; the mean concentration was 4.2 ± 2.4 $\mu\text{g/g}$ and the median was 3.7 $\mu\text{g/g}$, as shown in Fig 1. Of these, fully 25% of the individuals had mercury concentration above the World Health Organization (WHO) reference level of 6 $\mu\text{g/g}$ (9). The general health of the population was good, with no obvious malnutrition or low body mass. In a separate study of diet and nutrition (Yokoo, in preparation), the composition of the diet was measured by self report over 3 days. In general the diet was somewhat – hypocaloric and low in carbohydrates, with normal lipid intake and high protein intake.

Neurobehavioral Tests

Table 2 shows correlations and partial correlations of neuropsychological scores related to hair mercury concentration. There were significant relationships between hair mercury and the Bead Threading, Digit Span, Digit Symbol, Paired Associated Learning Test and measures of Logical Memory. A significant relationship was also found between hair mercury concentration and errors of commission on the Toulouse-Pierron test. No relationship was observed between hair mercury and any measure of the POMS.

Partial correlations were adjusted by potential confounding variables, of sex, age, educational level, smoking, alcohol intake for each test using multiple regression analyses. These variables were selected because they are known to affect performance on these tests and because in these data they significantly affected the overall relation between mercury and test performance. No other variables entered significantly into the model. After adjustment only the Digit Symbol test results did not show significant correlations with hair mercury levels.

According to the β coefficients and 95% confidence intervals in the multiple regression analyses, increasing hair mercury concentration was associated with worse scores on threading beads (manual ability test), Digit Span (total score and number of digits accurately recalled in reverse order), Digit Symbol, Logical Memory and the Paired Associate Learning Test (easy and hard word pairs) and errors of commission on the Toulouse-Pierron Test. After adjusting for age, gender, and educational level, bead threading and errors of commission scores showed higher coefficients. Confidence intervals for Digit Symbol and learning difficult word pairs included zero (Table 3).

The multiple regression coefficients, using hair mercury dichotomized on the basis of the WHO reference criteria, are presented in Table 4. The Bead Threading, Digit Symbol and errors of commission predicted group membership (that is, persons with hair Hg levels above or below 6 µg/g) with higher coefficients than when Hg exposure was considered as a continuous variable. This finding was maintained when scores were adjusted for age, gender and education.

Table 5 presents neuropsychological test data dichotomized by a median split for each test. The associations between hair mercury and less than median performance on Bead Threading, Digit Span Backward, Digit Symbol, easy word pair learning, logical memory, and errors commissions were significant at p value ≤ 0.05 .

The relationship (OR crude and adjusted; 95% CI) between outcome stratified by median exposure or median test performance are reported in Table 6. The odds of obtaining a neuropsychological score below the median is higher for hair mercury concentrations above 6 µg/g for the Bead Threading (OR = 3.5), and Associate Learning (OR = 2.9) measures.

DISCUSSION

The results of this study indicate that adults exposed to methylmercury through fish consumption may have observable deficits in neurobehavioral performance measures, without detectable alterations in mood or affect. Since this is a cross sectional study, it is not possible to exclude the possibility that unmeasured exposures to methylmercury earlier in life (particularly *in utero*) may have contributed to the observed effects. However, this is not likely to wholly explain the results of this study of adults. First, length of residence in the region was not significantly associated with either hair

mercury level or outcome. Second, the major period of gold mining in this region began in the 1980s (29, 32), so that exposure to methyl mercury in fish, downstream from mining sites, was unlikely to have begun until some time after the mid 1980s, allowing for hydrologic dispersion of mercury and contamination of the aquatic food chain, at which point all of the persons in this cohort were already born.

The possibility of adverse effects in adults at lower levels of mercury exposure has been raised by other studies in Brazil of fish-consuming populations (6,23,24,25,26). Several of these studies have considered diet and nutrition as covariates (23,24). These populations are not undernourished or grossly deficient in nutrient intake. In general, health status is good to excellent, with the exception of risks of infection by endemic parasites and helminths (24). Alcohol consumption was not heavy in this population. These variables do not alter the observed associations between mercury exposure and neuropsychological outcomes. The strongest effects of mercury on persons in this study were detected on tests of fine-motor speed and dexterity (Bead Threading) and response inhibition (errors of commission or “false alarms” on the timed visual search and attention task). The size of the effects increased with hair mercury concentration, consistent with a dose-dependent effect. Mercury levels also affected performance on a measure of concentration and working memory (Digit Span Backward) and paragraph learning (Logical Memory), albeit to a lesser extent. It is plausible that disturbances in working memory, or mental tracking, interfered with the ability of the participants to link multiple pieces of information together for efficient paragraph learning. Decrements in performance on the visually guided fine motor speed and dexterity task (Bead threading) were also found, while gross motor functions (placing disks, marking dots) were

unaffected. These results are consistent with earlier studies (22, also reviewed by 40), which have suggested that mercury disrupts the cerebellum (a brain region associated with accurately aimed and smoothly executed coordinated movement) (41).

Errors of commission generally reflect disturbances in response inhibition or inhibitory control. Mercury exposure appears to be associated with increased responding, that is, both correct responses and commission errors. This is consistent with damage to cerebral inhibitory systems. Possible disturbances in select aspects of memory were also observed (working memory, new learning), which is consistent with previously reported findings (42). Adults chronically exposed to inorganic mercury in the workplace have demonstrated cognitive impairments, including deficits in attention and executive function, short-term memory, visuospatial ability, and motor function (16).

For most of the tests, the size of the effects - β coefficient - for the whole population is relatively small. However, when the population is stratified on the basis of the WHO standard, a relatively large effect of mercury exposure is observed, similar to findings for lead (43). Also, the risk of performing below the median on neuropsychological tests of fine motor speed, logical memory, and digit span (backward) is generally increased in persons with higher hair mercury. These stratified analyses were undertaken to examine odds of decrements in performance based upon an internationally recognized value. Testing associations between exposure and outcome as continuous variables provides more sensitive information on potential effects at low exposures, as demonstrated in the regression analyses, but these dichotomized analyses may be relevant to public health policy. Recent proposals for public health policy, such as advisory levels for fish consumption, have generally assumed that children are significantly more

sensitive than adults to the neurotoxic effects of methylmercury (2,9,20,44). However, this study suggests that adults may be as sensitive as children to methylmercury.

Although there are differences in the tests used, tests that evaluated similar domains or function showed decreased performance in adults (our study) and children (12,13,14) related to attention, fine-motor function and memory.

This is the first study of adults exposed to low levels of methylmercury, using neuropsychological tests comparable in sensitivity to the methods used in the Faeroes and Seychelles studies. Usually, motor function and visual field are the primary outcomes evaluated in cases of adult exposure. As in similar research on other environmental neurotoxins, it is important to distinguish individual risk from population risk. Decreases in mean scores may be too small to detect or to evaluate as to clinical significance for one individual, but they may be quite important when considered in terms of the impact on the population distribution of the affected function, as has been shown for lead (43). If this the case, then the public health impacts of methylmercury exposure at levels often encountered by adults in North America (2) may be inducing adverse effects on neurobehavioral performance. Further studies are necessary in order to confirm these findings and to understand mechanism by which methylmercury can affect central nervous system function in adults.

List of abbreviations used:

Hg – mercury; MeHg – methylmercury; WHO – World Health Organization, OR – odds ratio; CI – confidence interval; TAM – tests of mechanical ability, POMS – profile of mood states; CEPA – Centro de Psicologica Aplicada

Competing interests:

The corresponding author (Dr Silbergeld) declares, for all the authors, that there were no competing interests for any author in the conduct of the research, analyses of data, or writing of the paper. Support for this research and for Dr Yokoo's preparation of this paper were provided by the Federal Government of Brazil (CNPq). Support for Dr Silbergeld was provided by a Fogarty Center grant from the National Institutes of Health.

Authors' contributions:

Dr Yokoo carried out the field studies as part of her dissertation research for the PhD degree from the UERJ. She enrolled subjects, administered tests, collected hair samples, and participated in all steps of analysis and preparation of the ms. Prof Valente served as Dr Yokoo's thesis adviser, and guided her development of this project and her analyses of data. Dr Grattan advised Dr Yokoo in the appropriate analyses and interpretation of neurobehavioral test data. Dr Schmidt advised the UFMG research group on the selection of neurobehavioral tests for application in this field study. Ms Platt assisted Dr Grattan and Dr Yokoo in data analyses. Dr Silbergeld served as Dr Yokoo's research adviser during her fellowship in the US; she took final responsibility for the writing of this ms.

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Figure legend:

Figure 1. Distribution of hair mercury levels among participants (n = 129)

Table 1. Demographic characteristics of the study population.

Characteristic	N	Percentage
Gender		
Women	77	60.0
Men	52	40.0
Age (years)		
17-24	40	31.0
25-34	32	24.8
35-44	30	23.3
45-54	10	7.7
55-64	07	5.5
≥ 65	10	7.7
Education level		
Illiterate or write name	33	25.6
Basic primary	82	63.6
Uncomp.1 st grade	09	7.0
Comp. 1 st grade	03	2.2
Uncomp.2 nd grade	02	1.6
Smoking habits		
Nonsmoker	97	75.0
Smoker	32	25.0
Alcohol intake		
Nondrinker	103	80.0
Drinker	26	20.0
Occupation		
Fishing	11	9.3
Fishing + other	24	20.0
Agriculture	12	10.0
Housewife	69	40.0
Others	13	20.7
Residence time (years)		
<5	08	6.2
5 ≥ 20	37	28.7
20 ≥ 35	43	33.3
35 ≥ 50	23	17.8
≥ 50	18	14.0

Table 2. Pearson correlation $\text{\textcircled{R}}$ of adults' hair mercury concentration and neurobehavioral test results.

Test	N	R ^a	P value	R ^b	Pvalue
TAM					
Manual dexterity	107	- 0.02	0.79	- 0.007	0.94
Fine motor speed	113	- 0.26	0.006*	- 0.27	0.005*
WMS					
Information	129	- 0.08	0.35	- 0.10	0.28
Orientation	129	- 0.01	0.93	- 0.014	0.88
Mental Control	129	- 0.12	0.16	- 0.10	0.29
Logical memory	129	- 0.03	0.69	0.03	0.75
Log. Mem. first story	119	- 0.25	0.005*	-0.23	0.014*
Log.Mem.second story	121	- 0.15	0.10	- 0.14	0.13
Digit Span	128	-0.20	0.046*	-0.20	0.05*
Digit span forward	129	-0.13	0.15	-0.11	0.22
Digit span backward	129	- 0.20	0.05*	- 0.20	0.03*
Visual reproduction	129	- 0.10	0.29	- 0.06	0.56
Associative learning	129	- 0.15	0.08	- 0.15	0.12
Easy learning	129	- 0.20	0.03*	- 0.20	0.03*
Difficult learning	128	- 0.18	0.04*	- 0.15	0.10
Memory quotient	128	- 0.09	0.30	- 0.13	0.17
WAIS					
Digit symbol	122	- 0.242	0.007*	- 0.14	0.13
Toulouse – Pierron (CEPA)					
Total answers	116	- 0.08	0.41	- 0.01	0.30
Correct answers	116	- 0.07	0.45	- 0.01	0.30
Errors - commissions	116	0.22	0.016*	0.23	0.015*
Errors - omissions	116	- 0.16	0.08	- 0.12	0.20
POMS					
Tension	129	- 0.16	0.07	0.12	0.63
Anger	129	- 0.08	0.37	- 0.21	0.39
Vigor	129	0.04	0.64	0.07	0.76
Confusion	129	-0.02	0.83	- 0.12	0.61
Fatigue	129	- 0.04	0.65	- 0.14	0.56
Depression	129	- 0.01	0.96	- 0.15	0.53

^a bivariate correlations^b Partial correlations adjusted by age, sex and education level* p value ≤ 0.05

Table 3. Regression coefficients β of adults hair mercury concentration as a predictor of neurobehavioral test results.

Test	β^*	95% CI		β^{**}	95% CI	
Fine motor speed	-3.40	-5.8	0 -1.00	-3.20	-5.40	-1.00
Digit span	-0.14	-0.29	-0.001	-0.15	-0.29	0.003
Digit span backward	-0.09	-0.18	-0.001	-0.09	-0.19	-0.009
Digit symbol	-1.21	-2.08	-0.33	-0.54	-1.2	0.16
Easy learning	-0.37	-0.70	-0.04	-0.34	-0.64	-0.04
Difficult learning	-0.21	-0.42	-0.001	-0.15	-0.34	0.03
Log. Memory first story	-0.29	-0.51	-0.09	-0.27	-0.49	-0.06
Errors commissions	1.39	0.26	2.5	1.45	0.28	2.6

* – not adjusted;

** – adjusted by age, gender and education level;

Table 4. Regression coefficients β of adults hair mercury concentration stratified on the base of the WHO* reference as a predictor of neurobehavioral test results in children.

Test	β^a	95% CI		β^b	95% CI	
Fine motor speed	-19.0	-31.0	-6.7	-17.7	-29.4	-6.3
Digit symbol	-7.82	-12.4	-3.2	-4.28	-7.9	-0.48
Errors commissions	9.0	3.1	14.9	9.0	2.9	15.1

a – not adjusted;

b – adjusted by age, gender and education level;

* 6 $\mu\text{g/g}$ (WHO reference)

Table 5. Mean of hair mercury concentration ($\mu\text{g/g}$) stratified by the median of each neuropsychological test.

Tests	Median	Hair Hg	P value*
Speed Fine motor	< 30	4.8	0.006
	\geq 30	3.6	
Digit symbol	< 20	4.8	0.03
	\geq 20	3.8	
Logic memory total	< 03	4.6	0.05
	\geq 03	3.7	
Digit span backward	< 02	4.7	0.007
	\geq 02	3.6	
Easy learning	< 14	4.7	0.02
	\geq 14	3.7	
Log. Mem. first story	< 02	4.9	0.01
	\geq 02	3.7	

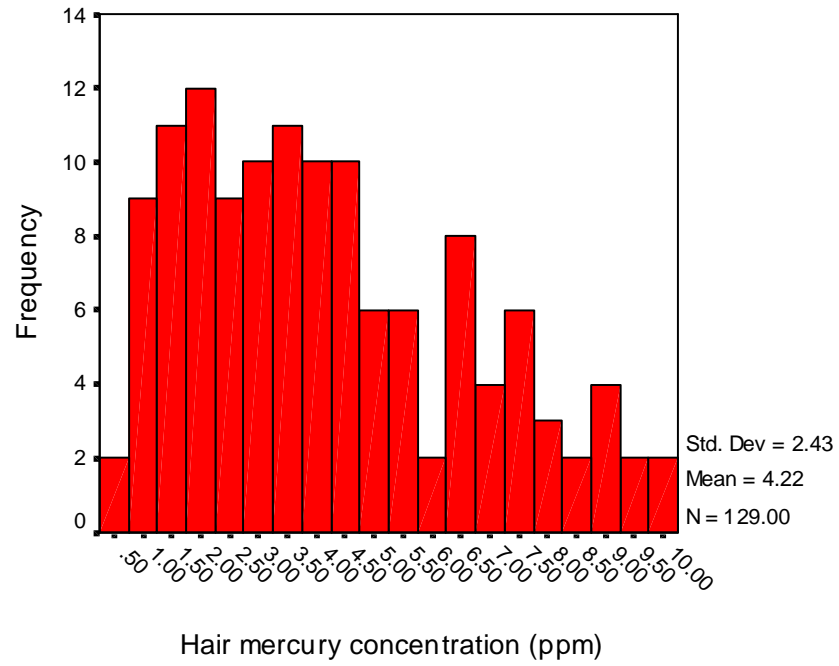
*T-test with p value \leq 0.05

Table 6. Association between hair mercury concentration stratified on the base of the WHO reference and neurocognitive tests stratified by the median of each test.

Test	OR	95% CI		OR*	95% CI	
Fine motor speed	3.3	1.3	8.0	3.5	1.2	9.3
Digit symbol	2.4	1.1	5.2	1.6	0.6	5.0
Log. Mem. first story	2.6	1.1	6.0	2.3	1.0	5.8
Digit span backward	2.6	1.1	5.6	2.3	1.1	6.0
Easy learning	2.9	1.3	6.5	2.9	1.1	7.3

*Adjusted by age, sex and educational level

Fig 1. Distribution of hair mercury concentration



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