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Color vision impairment with low-level methylmercury exposure of an Amazonian population – Brazil

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ABSTRACT

Land exploitation that follows deforestation and mining can result in soil erosion and the release of mercury to the waters of rivers in the Amazon Basin. Inorganic mercury is methylated by bacteria that are present in the environment and it serves as a source of human contamination through fish consumption in the form of methylmercury. Long-term exposure to low-level methylmercury in the riverside populations can lead to nervous system alterations, some of which are visual impairments such as loss of luminance contrast sensitivity, restricted visual fields and color vision defects. The present study sought to examine color vision in a group of adults living in the central Brazilian Amazon who were exposed to low-levels of methylmercury. Total Hg concentrations were measured from hair collected at the time of the testing. The D15d and FM100 color vision arrangement tests were applied in a population of 36 (22 males) and 42 (25 males), respectively. Controls were healthy volunteers from the cities of São Paulo for the D15d and Belém for the FM100. There was a statistically significant difference in performance between those who were exposed and controls for both tests ($p < 0.01$ and $p < 0.0001$, respectively, Mann-Whitney *U* test), meaning that adults living in this region of the Amazon made more mistakes on both tests when compared to controls. A linear regression was performed using Hg concentrations and test scores. Hg concentrations accounted for 7% and 2% of color D15d and FM100 arrangement test errors, respectively. Although other studies have previously found color vision impairment in the Amazon, they tested inhabitants on the east side of the Amazon, while this study was conducted in the central Amazon region and it is the first study in a population with no direct contact with the Hg source of contamination. These results suggest that long-term exposure to low-level methylmercury in riverside populations is more widely spread in the Amazon Basin than previously reported. This information is needed to implement public health policies that will ensure a safer environment for the Amazonian population.

1. Introduction

Mercury (Hg) is found everywhere in the environment due to constant off gassing of Hg from the earth's crust (Crinnion, 2000). The accumulation of Hg at the soil surface constitutes a natural reservoir, but gold mining, deforestation and subsequent land exploitation for agricultural purposes can result in soil erosion and hence exportation of Hg accumulated in these soils to the waters of the rivers in the Amazon Basin (Bastos et al., 2006; Lacerda and Marins, 1997).

Many investigations have reported an association between Hg exposure and visual function (Cavalleri et al., 1995; Ventura et al., 2005). Visual field constriction, contrast sensitivity losses, and color vision impairment have been found for both environmental exposure to the organic form (methylmercury) (Korogi et al., 2018)

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by fish eating and occupational exposure inthe inorganic form (Hg vapour) through inhalation (Barboni et al., 2009; Feitosa-Santana et al., 2007).

The Amazonian human population in South America has been continuously monitored for Hg exposure due to a long-term exposure to the metal. Gold-mining activity in the region is a danger for the inhabitants for two reasons. Hg is used to separate gold from the rocks, and when the gold-mercury amalgam is heated to obtain gold, the Hg is vaporized causing significant environmental pollution. Gold-mining activity also releasestons of Hg in the Amazonian river basins (Bastos et al., 2006; Dos Anjos et al., 2016). In the river, the metal is transformed by bacteria to methylmercury and is inserted in the food chain until it reaches humans. Many research groups have observed that Hg concentrations in hair are higher as there is higher intake by fishes in feeding habits (Bastos et al., 2006). Further, the Amazonian soil seems to be an important source of Hg, since even in populations that eat fish from rivers free from gold-mining activity, it was found that Hg exposure is higher than in non-fish eating populations (Barbosa et al., 2001).

The evaluation of neurological functions in riverside communities in some river basins has been used to investigate the effects of long-term and low Hg concentrations (Lebel et al., 1996, 1998). Many reports have shown there is impairment of visual, somatosensory, and cognitive functions. Color vision is one visual function that was observed to be altered in conditions of exposure to an inorganic form of Hg; i.e., Hg vapour (Cavalleri et al., 1995; Ventura et al., 2005), but there have been few investigations in Amazonian populations with high intake of fish feeding.

The present study examined color vision in a group of fish-eating adults living in the Brazilian Amazon at the Madeira River who were exposed to low levels of methylmercury (below 50 μg/g). The study took place in the area of Puruzinho Lake, Amazonas State, Brazil (Fig. 1), where it was found that the Hg biomagnification rate was higher than the expected value for its latitude due to the food web of its ichthyofauna (Azevedo-Silva et al., 2016). This part of the Brazilian Amazon was an important gold-mining area during the decades of 1980 and 1990. According to Bastos et al. (2006), despite the reduction of Hg deposition to the Madeira River from gold mining, Hg concentrations in fish and humans were similar to those measured during the gold rush. We aimed to compare the color vision of subjects with and without a history of methylmercury exposure, and to evaluate the existence of an association between methylmercury exposure and color vision.

2. Methods

2.1. Subjects

We compared performance on two color vision tests; i.e., the Lanthony desaturated test (D15d) and the Farnsworth-Munsell test (FM100), of subjects living in a methylmercury-exposed region and controls (a non-methylmercury-exposed region). The subjects living in a Hg-exposed area were recruited from fishermen communities near Puruzinho lake, Rondônia, Brazil (Fig. 1), while the control subjects were recruitedin the urban cities of Belém and São Paulo, Brazil. For the D15d test, the study population comprised 36 subjects (22 males) aged from 18 to 64 years (mean = 32.25 ± 11.67 years), and the control group comprised 37 healthy volunteers (18 males) aged from 18 to 62 years (mean = 32.3 ± 11.9 years). For the FM100 hue test, the study population comprised 42 subjects (25 males) aged from 17 to 54 years (mean =29.8 ± 11.1 years), and the control group was

Fig. 1. Map indicating locality of sampling and color vision test application in the Puruzinho riverine population.

composed of 157 subjects (85 males) aged from 17 to 92 years (mean = 33.2 ± 15.9 years). Since the control group was much bigger than the exposed group for the FM100 hue test, a statistical test was run to ensure there was no difference between these groups regarding age (Mann-Whitney test, W = 1998.5, *p*-value = 0.6329). Methylmercury-exposed groups overlapped by 21 volunteers who took both the D15-d and the FM100 tests. It was not possible to always test the same subjects on both tests because of their availability to complete all the tests. Subjects were invited to partake in the study as volunteers. Therefore, some subjects were evaluated with only one test in this study, and some subjects were evaluated with both tests (n = 21, see Table 1). Controls for both tests were selected from an urban population without any known exposure to Hg, including dietary exposure, matched to the Hg-exposed group by sex and age. Dietary exposure was defined by a fish consumption of less than twice a week.

Table 1
Data for all subjects in the exposed groups.

n	Gender	Hg μg/g (ppm)	D-15d	FM100
1	M			96
2	F	10.35	69.29	
3	M		61.82	
4	F	25.54	79.64	
5	M	15	77.76	
6	M	4.52	58.64	
7	M	20.72	74.32	
8	M	40.69	62.23	56
9	F			104
10	M	20.61	65.77	116
11	M			124
12	M			144
13	F			148
14	M	12.71	68.41	96
15	F			108
16	M			120
18	M			76
19	M			80
20	M			
21	M			76
22	M			64
23	M	17.87	56.41	
24	M			44
25	F			112
26	F		62.23	152
27	F		60.54	
28	F	18.2	70.17	160
29	F	24.74	81.76	
30	F	20.83	65.68	
31	F	21.66	84.7	112
32	F			184
33	F			68
34	F	17.4	61.82	
35	F		56.41	140
36	F			88
37	F		59.95	172
38	M		61.82	112
39	F	14.2	68.24	164
40	M		61.82	64
41	F			116
42	M	28.87	61.51	
43	M		61.82	128
44	M	18.58	62.18	104
45	M		61.51	
46	M			104
47	M	21.84	62.23	
48	M	21.84	61.82	56
49	M			76
50	M		56.41	40
51	M	26.64	72.42	116
52	F	17.8	73.15	108
53	M	2.28	62.23	
54	F	4.47	67.42	
55	M			152
56	M			72
57	M			164
58	F			92
59	F			84

Clinical history of both Hg-exposed subjects and controls was collected before the testing to ensure no history of other heavy metals or chemicals exposure, alcoholism, smoking, and systemic diseases that could have affected the visual system. Visual acuity was measured with the Snellen optotypes, and best visual acuity of 20/30 or better was the inclusion criterion for both groups of participants. Informed consent was obtained from all subjects. The testing procedures complied with the tenets of the Declaration of Helsinki and were approved by the Ethics Committees of the Institute of Psychology and the University Hospital of the University of Sao Paulo.

To screen for congenital color vision deficiencies, the D15 test (Farnsworth, 1943) was applied, with quantitative and qualitative analyses of the color cap arrangement used to verify the color vision status. Subjects diagnosed with a congenital color vision deficiency were not included.

2.2. Equipment and procedures

2.2.1. Total-Hg determination

A sample of hair cut from the occipital area near the scalp was collected at the time of the color vision tests from the study population. The total Hg concentration (hair-Hg) was determined in individual hair samples according to routine laboratory procedures at the Wolfgang Christian Pfeiffer Environmental Biogeochemical Laboratory (BIOGEOQ) at the Federal University of Rondônia (UNIR). Briefly, after mineralization in acid-oxidant medium, total Hg determination was performed by cold vapour atomic absorption spectrometry on a Perkin-Elmer (Ueberlingen, Germany) FIMS-400 instrument (Bastos et al., 1998). For quality control, all analytical runs included material certified by the International Atomic Energy Agency (IAEA-085 and IAEA-086). Recovery rates were above 80% and the detection limit was below 0.03 mg/kg. The total Hg concentration in the hair reflects the history of Hg exposure within the last two months (Jo et al., 2015; Nuttall, 2006). No quantification of Hg in the hair was done for control populations.

2.2.2. D15d test

The D15d is an arrangement color vision test (Luneau, Prunay-le-Gillon, France), consisting of 16 desaturated color caps (1.2 cm in diameter). Each is contained in a circular black plastic support. The Munsell System defines their chromatic characteristics: varying only in hue, the color caps have the same lightness (Value = 5) and saturation (Chroma = 4).

For testing, the caps were placed on a desktop covered with a black cloth, under natural daylight illumination. Since subjects, especially elderly individuals, could have difficulty arranging these desaturated caps, we followed the guidelines for applying the D15d test: it was repeated up to three times, and the best result was considered in order to avoid false positives and to separate the color vision outcomes from a practice effect (Lanthony, 1986).

Errors in the D15d tests were quantified in terms of the Total Color Distance Score (TCDS; Lanthony, 1986; Vingrys and King-Smith, 1988). The minimum TCDS value is equal to 56.4 and occurs when all the caps are in consecutive order (Geller, 2001). Values higher than this imply a decrease in color discrimination ability. To find the TCDS, we considered the position of each cap chromaticity in CIE1976 color space. After hue ordering, the distances between consecutive ordered caps in the color space was summed and the integration of all the distances was the TCDS. To calculate the TCDS,

we used the table of color distance scores for quantitative scoring of the Lanthony desaturated color vision test (Geller, 2001).

2.2.3. FM100 hue test

The FM100 Hue Test in a computerized version consisted of 85 circular stimuli (1° of visual angle, mean luminance of 41.75 cd/m^2) of different hues and the same saturation (30%). Four sets of caps were shown separately in the same order of the conventional test. The subject was presented with the correct sequence of the stimuli for each set. They were then disarranged, and the subject was instructed to reorder the original hue sequence. Following the guidelines for applying this test, we had to ensure that the subject had learned the test in order to avoid false positives and to separate the color vision outcomes from a practice effect, and only after being sure he had learned the test was it then performed for the study. Errors of arrangement were measured for each position and the Total Error Score (TES) was measured to determine the test performance (Bento-Torres et al., 2016). To calculate the TES, first we considered that the caps had values from 1 to 85. After the hue ordering of a subject, we calculated the partial error score (PES) for each cap (Eq. (1)), which represented the sum of the absolute difference between the cap value in position i and in the neighbor positions $i+1$ and i-1. The correct ordering resulted in a PES value of 2 for each cap.

$$PES = |n_i - n_{i-1}| + |n_i - n_{i+1}| \quad (1)$$

TES is the integration of all 85 PES minus 170. The perfect ordering results in a TES of 0.

2.3. Analysis

Since the data were not normally distributed, nonparametric tests (Mann-Whitney U test and Wilcoxon matched pair test) were applied, with the level of significance of $p < 0.05$ (Statistica 6.0, StatSoft, Tulsa, USA). In addition, linear regression analysis was performed to investigate a possible relation between color vision results and Hg hair concentrations.

3. Results

We found color vision impairment among subjects exposed to low levels (<50 μg/g) of methylmercury with both D15d and FM100 tests when comparing them to the control groups. The composition and overlapping of the exposed groups may be observed in the full data set (Table 1).

3.1. D15d test

We found that the methylmercury-exposed group had higher variability in the TCDS values than the controls. Twenty-nine out of 37 controls had no error in the test while only 4 out of 36 methylmercury-exposed subjects had perfect performance. Eleven of 36 methylmercury-exposed subjects had a TCDS higher than the 95th percentile of the control group. Fig. 2 shows the data distribution of the TCDS for both groups. We observed that the methylmercury-exposed subjects had a mean TCDS of 65.37 ± 7.29 and the non-exposed group had a mean TCDS of 58.25 ± 4.12, and the difference between the groups was statistically significant ($p < 0.01$, Mann-Whitney U-test).

3.2. FM100 hue test

Fig. 2 shows the data distribution of the TESs for both groups. The methylmercury-exposed group had TES values of 108.4 ± 37.2, and the control group had TES values of 62.1 ± 33. The difference was statistically significant ($p < 0.0001$, Mann-Whitney U-test). Thirteen of 42 methylmercury-exposed subjects had a TES higher than the 95th percentile of the control group.

3.3. Correlation between the total-Hg concentrations in the hair and visual outcomes

Linear regression was performed using the D15d color arrangement test scores for 22 subjects that had their Hg concentration measured from a hair sample at the time of the test. Transformations of the data were tested, with the log of the Hg

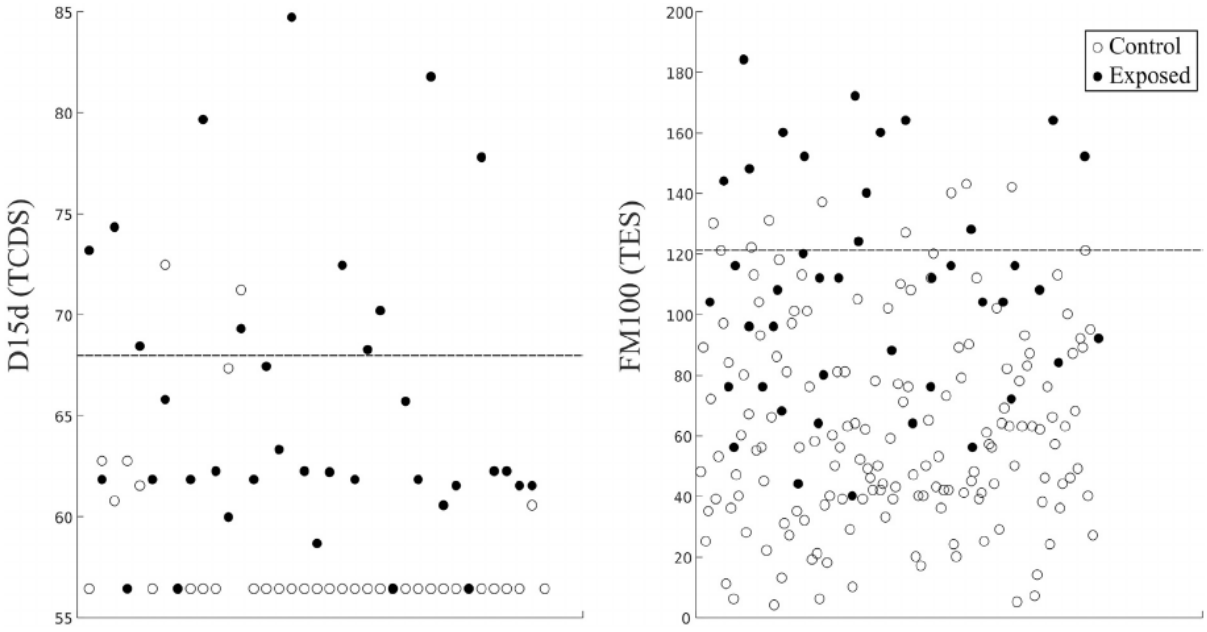

Fig. 2. TCDS (D15d, left panel) and TES (FM100, right panel) distribution of the control group (white circles) and methylmercury-exposed group (dark circles). For both tests, the methylmercury group had larger values and variability. Dashed lines represent the 95th percentile of the control group for each test. Methylmercury-exposed subjects had performances above the 95th percentile of the TCDS and TES (11/36 and 13/42, respectively) as estimated from the control group.

concentration yielding the best fit. The average Hg concentration for the exposed group was 18.4 ± 8.7 μg/g. The measured Hg concentration accounted for approximately 7% ($R = 0.27$, $R^2 = 0.073$) of the D15d score distribution variance (see Fig. 3).

Another linear regression was performed using the FM100 test scores for 12 subjects that had their Hg concentration measured from a hair sample at the time of the test. Transformations of the data were tested, but the raw data yielded the best fit. The average Hg concentration for the exposed group was 26.03 ± 15.9 μg/g. The measured Hg concentrations accounted for approximately 2% ($R = 0.14$, $R^2 = 0.019$) of the FM100 score distribution variance (see Fig. 4).

4. Discussion

We evaluated an Amazonian riverine population regarding their color vision performance on the D15d and the Farnsworth-Munsell FM100 Hue arrangement tests to assess the effects of chronic low-level methylmercury exposure on these measures. We found that this population was significantly different from a control group for both color vision tests. Although other studies have previously found color vision impairment in the Brazil Amazonian region (Lebel et al., 1996, 1998; Rodrigues et al., 2007), they examined subjects on the east side of the Amazonian region while this study was conducted in the central Amazon. This suggests that the issue of color perception impairment is not a local concern, but rather a widespread health hazard.

The present study showed alterations in color vision for subjects with Hg concentrations measured from hair samples below 50 μg/g, agreeing with previous findings from the literature (Lebel et al., 1998; Rodrigues et al., 2007). Despite this similarity, the subjects from the present study were not selected from a population in direct contact with the source of contamination; e.g. gold miners.

Previous works have reported Hg concentrations under 50 μg/g in the population of the whole Amazon Basin, which includes the Negro river (Barbosa et al., 2001; Kehrig et al., 1998), Tocantins river (Leino and Lodenius, 1995; Pinheiro et al., 2007, 2008; Pinheiro et al., 2000), Xingu river (Vasconcellos et al., 2000),

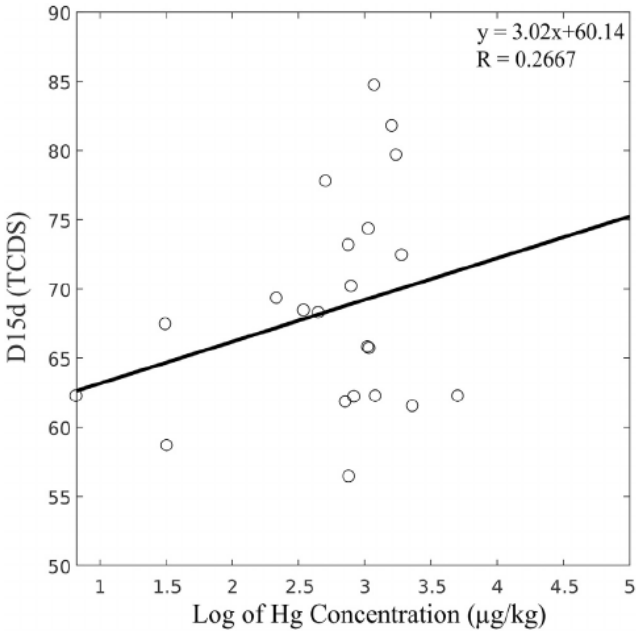

Fig. 3. Correlation measured by linear regression between Hg concentration and D15d test scores measured by hair samples.

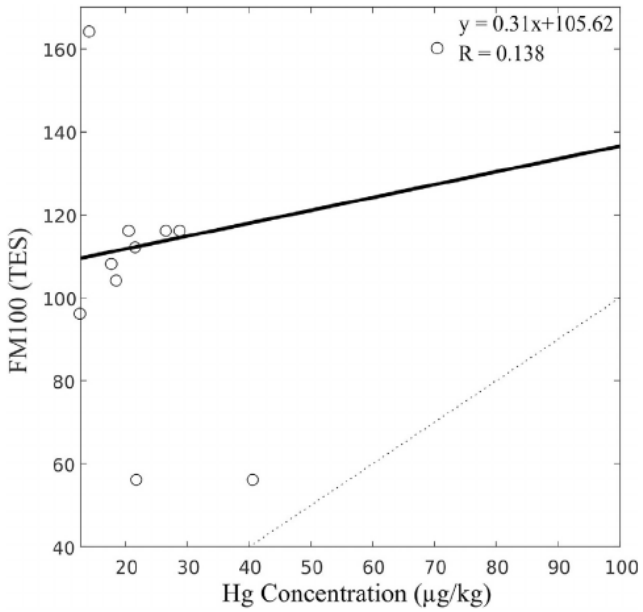

Fig. 4. Correlation measured by linear regression between Hg concentrations and FM100 test scores measured in hair samples.

Tapajós river (Malm et al., 1995), and Madeira river (Bastos et al., 2006). According to our results, these populations may show similar color vision impairments even though they fall within the safe concentration of Hg defined by the World Health Organization.

Linear regression performed for D15d and FM100 tests and Hg concentrations measured in hair explained little of the data variance ($R^2 = 0.073$ and $R^2 = 0.019$, respectively). Damage to color vision due to methylmercury consumption may be irreversible, as previously shown for Hg vapour intoxication (Feitosa-Santana et al., 2008). Therefore, color test scores may reflect harm caused in the past due to Hg peaks in developmental windows or chronic consumption of fish, which would not correlate directly to measures of Hg-concentrations at the time of sampling.

The present study has some limitations: (i) due to time limitations, we tested just one eye of each subject. As the Hg exposure is systemic, the possible damage would be similar for both eyes, thus which eye was chosen would not have an important role in our results. Moreover, even when there is asymmetry in the damage between eyes, it is usually non-significant; (ii) the control group was from an urban population. There is no previous description about the influence of social-economic condition on hue ordering tests, and we do not expect for any influence of it in the color vision test performance; (iii) no Hg quantification in the hair for the controls. There are several reports that found an association between fish consumption frequency and Hg concentrations in the hair in urban samples, suggesting that the fish consumption up to twice a week does not affect Hg concentration in the hair (Vieira et al., 2015; Abelsohn et al., 2011).

The color vision of the population in this region of the Amazon is impaired, although Hg concentrations were low (less than 41 μg/g). We cannot assert our findings as a causal association, but it expands the knowledge of visual function in riverside communities in the Brazilian Amazon, and health programs for riverside communities must take into account the broader temporal and spatial range of the Hg exposure issue. Although other studies have previously found color vision impairment in the Amazon Basin, they tested only individuals on the east side of the Amazon. The present study was conducted in a community with long-term exposure to low-levels of methylmercury in the central Amazon Basin, in a population with no direct contact with the source of

contamination; e.g. gold miners. Future investigations should include longitudinal approaches, other susceptible population (such as children or elderly) and control population from similar cultural-social-environmental background. The present study is needed in order to implement public health policies that will ensure a safer environment for the Amazonian population.

Con ict of interest

None.

Approval by the ethics committee for research with human subjects

This research was performed following the international regulations of ethics for research with human subjects, and so testing procedures complied with the tenets of the Declaration of Helsinki and were approved by the Ethics Committees of the Institute of Psychology and the University Hospital of the University of Sao Paulo.

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