

EVALUATION OF MERCURY LEVELS IN HAIR OF CHILDREN RESIDENT IN ARTISANAL GOLD MINING AREA IN THE CITY OF CHAPADA DE NATIVIDADE-TOCANTINS

**Seyna Ueno R.Mendes¹, Marina B.A.Vasconcellos², Marília Gabriela M.Catharino²,
Gilson A.Quinaglia³ and Barbara Held³**

¹Coordenação de Medicina
Universidade Federal do Tocantins
Campus de Palmas
Quadra 109 Norte, Av.NS15 ALC NO 14
77001-090
Palmas,TO
seynaueno@gmail.com

²Centro do Reator de Pesquisa-CRPQ
Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000, São Paulo, SP
mbvascon@ipen.br

³Setor de Análises Toxicológicas
CETESB
Av. Professor Frederico Hermann Jr., 345
05459-900, São Paulo, SP
quinaglia@sp.gov.br

ABSTRACT

The objective of the work is to evaluate the mercury exposure of children living in an artisanal gold mining area and in a control area, without mining. A cross-sectional study was conducted with children living in the municipalities of Chapada de Natividade and Porto Nacional, in the State of Tocantins, where hair samples were collected for laboratorial analysis of mercury concentrations, comparing between municipalities, gender, monthly fish consumption, profession of parents and the presence of amalgam in dental restorations. The hair samples were analyzed by the methods of neutron activation analysis and also by means of the DMA (Direct Mercury Analyzer) equipment, in the last case being a partnership with CETESB. There was no evidence of mercury-related diseases in the studied children. The mean concentration of mercury in children in Chapada de Natividade was significantly higher than in Porto Nacional. Children exposed to artisanal gold mining areas have higher concentrations of mercury than children living in non- artisanal gold mining areas. There was no significant relationship between mercury concentrations and gender, monthly fish consumption, parental profession and amalgam presence in dental restorations. The results suggest that the children living in an area exposed to mining experience greater environmental exposure to mercury, regardless of their eating habits or gender.

1. INTRODUCTION

Mercury (Hg) can be found naturally in the environment or as a consequence of human activities. Elevated levels of mercury in humans, mainly as methylmercury (MeHg) are related to the consumption of fish and also other seafood, such as shrimp, crab, octopus and others [1].

Methylmercury (MeHg) is one of the main chemical forms of mercury and it is a well known neurotoxin. MeHg in the human body presents a relatively long half life, of about 70 to 80 days [1].

Historic episodes of mercury intoxication of great populational impact occurred in Japan, in the 1960's and in Iraq, in the 1970's, and had as consequences sensorial and movement alterations in the exposed people and also resulted in many deaths [2]. In Japan, these effects became known as Minamata disease.

The adverse effects of the acute exposition to methylmercury during pregnancy have been documented since the episodes of Japan in the city of Minamata and include neurological symptoms and delays in the development of children [3].

On the other hand, hair samples have been widely used in several studies that evaluate levels of environmental exposure to mercury via food intake in humans [4].

The Ministry of Environment of Japan considers that the mercury concentration in hair is an useful biological monitor, frequently utilized to study MeHg exposure, because it reflects the concentration in blood in the occasion in which the hair was formed [5].

Besides, hair samples provide a simple sampling procedure, which is non invasive and with good adherence of the participants. They also have the advantage of being very stable and can be stored at room temperature [4,5].

Mercury concentration in hair is generally about 250-300 higher than the concentration in blood. Also, since hair grows at a rate of about 1 cm per month, it is possible to evaluate the past exposure. Nevertheless, the concentration of mercury in hair can be enhanced as a result of adherence of mercury vapor or diminish as a result of hair treatments and can be influenced also by the site of sample collection [5].

In cases where there is no exposure to external inorganic mercury, almost all hair mercury is in the form of MeHg and so the level of exposure to MeHg by dietary intake can be evaluated by measuring total mercury. For the general population of Japan, the hair mercury concentration is in the range of 1-5 ppm and rarely exceeds 10 ppm [5].

The exposure of children to mercury, as regard localization, can be divided in three categories: at home, at school and also at locations close to industries that are not adequately remediated and close to medical installations [6]. In all these locations, the primary exposure to elemental mercury is inhalation. Elevated levels of mercury vapor also were documented after spilling of amounts of mercury from broken thermometers, although effects in health in these cases are rarely related [6].

A great part of the mercury utilized in the process of extraction and amalgamation of the gold present in ores, in the artisanal gold mining activities, is thrown in rivers, and consequently deposited in the benthic sediments. By means of a series of chemical reactions, inorganic mercury is transformed in methylmercury, which can eventually become highly concentrated in carnivorous fish species, at the top of the aquatic food chain [7].

In one of the many studies conducted in the Amazonic region, it was shown that the human exposure to mercury by means of ingestion of fish caught in the region, constitute a potential risk for the occurrence of Minamata disease, which should recommend the maintenance of a program of epidemiological surveillance [7]. As a consequence, the consumption of fish that come from regions close to artisanal gold mining activities can give rise to a risk of exposure of adults and children to mercury in the Amazon.

Depending on the magnitude, duration and other characteristics of the exposure, the toxicological profile of mercury intoxication can be described as: acute or chronic, clinical or subclinical [4].

When the concentration of mercury vapors is high, mainly in confined working environments, acute intoxication of the exposed individuals can occur. The effects of inhalation of high mercury concentrations are present in the clinical form, causing respiratory problems, such as pneumonia and lung edema, and eventually can lead to death [4].

In the gold mining sites, where mercury is used for amalgamation of the precious metal, the individuals may present some neurobehavioral problems, arising from chronic exposure to mercury. The effects of this kind of exposure are generally subclinical and can be detected only by means of very specific tests [4].

The main symptoms observed in the human exposure to organomercury species, such as methylmercury (MeHg), are: tremors, dizziness, headache, cramps, weakness, numbing, depression, visual problems, dyspnea, cough, gastrointestinal inflammation, hair loss, nausea and vomit, eventually leading to death. Besides, the exposure to MeHg may be related to cardiotoxicity, with rise of blood pressure and alterations of cardiac rate. Also damage can be caused to fetal development, from neurological problems to brain malformation. The exposure to mercury inorganic species has been also related to nephrotoxicity. Some studies show evidence that the exposure to inorganic and organic mercury may also be associated to situations of immunotoxicity and autoimmune illnesses [4].

The exposure to MeHg by means of ingestion of contaminated seafood may result in irreversible toxic effects to the central nervous system. In Minamata, in Southwest Japan, it was described for the first time, in 1956, a syndrome caused by intoxication by methylmercury, discharged by residual waters from a chemical factory. This syndrome became known as Minamata disease and its typical symptoms are: sensory disturbances, ataxia, dysarthria, constriction of the visual field, hearing disorders and tremor [8].

The evolution of environmental contamination by mercury can be divided in five stages: 1 – environmental contamination by mercury vapor; 2 – contamination by soil and water, with further methylation, originating organic mercury compounds; 3 – concentration of organic mercury in the aquatic food chain; 4 – bioaccumulation in humans by seafood ingestion and 5 – appearance in some cases of symptoms of Minamata disease [8].

The efforts to reduce the exposure to methylmercury can bring high social and environmental benefits, since they can prevent the complications and long time consequences of the deficits in neurological development of children, since they are particularly vulnerable to the neurotoxic effects of metals [9].

So, it is of great relevance to determine the mercury concentrations in the hair of children living in a region of exposure to mercury due to artisanal gold mining activities, in the municipality of Chapada de Natividade, in the State of Tocantins-Brazil.

2. EXPERIMENTAL

2.1. Study Group and Sample Collection

A cross-sectional study was made, after approval of the project by the Ethics Committee in Research (CEP) of the Brazil Platform (CAAE 341122414.7.0000.5516), with children resident in two municipalities of the State of Tocantins. In the first one, Chapada de Natividade, artisanal gold mining (garimpo) activities are conducted, and in the second one, Porto Nacional, there are no such activities.

The children were selected from the cadaster of the public service of basic attention (SUS), in their municipalities, and were invited to participate during visits at their homes. Their parents or other responsible adults were informed about the purposes and methodology of the study and were asked to give their formal authorization, by signing a Consent Term. Before collection of the hair samples, the clinical, sociodemographic and nutritional habits data of the children were collected and registered in a proper form, by the family and community physician responsible for the study.

The children included in the study had the following characteristics: age range from 5 to 6 years; no history of working in the artisanal gold mining; no infectious diseases in the scalp; both genders; no use of chemical products in the hair (such as dyes or smoothing products); presence of enough hair for sampling; parental consent.

About 500 mg of the children's hair samples were collected in the occipital region of the head, close to the scalp, using a titanium scissors, previously cleaned with ethanol. In the case of children with long hair, only the 10 cm closest to the scalp were utilized. Each sample collected was put in a polyethylene envelope, which was tightly closed, identified with a code and stored at room temperature, before being sent to the neutron activation analysis laboratory –LAN of IPEN-CNEN/SP, for preparation and analysis.

2.2. Sample Preparation.

The sample preparation was carried out according to the protocol of the International Atomic Energy Agency [10].

The collected hair samples were cut in small fragments, smaller than 2 mm, with the use of titanium scissors, which were previously cleaned with ethanol. The fragments were placed over a sheet of water proof paper, on a laboratory bench cleaned with ethanol, in a clean room. The hair samples were then transferred to Beaker flasks and washed, in order to

remove the exogenous contamination, which could be present at the surface of the hair strands, such as dust and particulate matter that are present in the environment.

The sequence of washing of the hair samples was:

- Washing with PA acetone, during 10 minutes, under shaking;
- Washing with distilled water, for three times, during 10 minutes for each washing;
- Final washing with acetone, with further filtering of the samples through CAAL number 1541 filter, placed over an Erlenmeyer flask.

The filter papers containing the washed hair samples were placed in Petri dishes, in order to dry the hair samples, at room temperature, for 24 hours. After drying, the samples were stored in polyethylene flasks, previously washed with 10 % HNO₃, being then ready for analysis.

At first it was tried to analyze the samples by neutron activation analysis, by irradiating at the IEA-R1 nuclear research reactor of IPEN-CNEN/SP, but due to the low concentrations of mercury in hair of the children, most of the results were below the detection limit.

Due to this fact, the mercury analysis in the hair samples were carried out at the Department of Toxicological Analysis of CETESB, the Company for Environmental Control of the State of São Paulo, by utilizing the DMA 80 equipment (Direct Mercury Analyzer), whose detection limit is of 0.01 nanograms of total mercury.

The analyses were performed according to USEPA – SW86, Method 7473 “Mercury in solids and solutions by thermal decomposition, amalgamation and atomic absorption spectrophotometry” and to the Application Book of Milestone Company [11].

This method was developed for the analysis of total mercury (organic and inorganic) in solid samples and samples in solution. The integration of sample preparation by thermal decomposition, followed by detection by atomic absorption, reduces the analysis time to less than 5 minutes. The samples were analyzed in duplicate and the uncertainty for hair analysis was of 20 %.

For quality control, the Human Hair – IAEA 086 Reference Material, from the International Atomic Energy Agency, was analyzed, together with the samples. This material has a recommended value for total Mercury of 0.573 µg g⁻¹, with a confidence interval at 95% of 0.534 to 0.612 µg g⁻¹.

3. RESULTS

Table 1 presents the results obtained for Hg concentrations in hair of the children resident in the municipality of Chapada de Natividade, where there are artisanal gold mining activities.

Table 1: Individual results of Hg concentrations in hair samples of children from the municipality of Chapada de Natividade - TO

Sample Code	Hg concentration ($\mu\text{g g}^{-1}$)	Gender	Fish consumption
C1	0.205±0.0079	F	1x/month
C2	0.356±0.0020	F	4x/month
C3	0.108±0.0029	F	16x/month
C4	0.060±0.0046	M	8x/month
C5	0.690±0.0028	F	Rarely
C6	0.109±0.0024	M	12x/month
C7	0.520±0.0493	F	4x/month
C8	0.111±0.0024	F	3x/month
C9	0.436±0.0077	M	3x/month
C10	0.100±0.0020	M	2x/month
C11	0.313±0.0001	M	2x/month
C12	0.130±0.0039	F	2x/month
C13	0.103±0.0019	M	12x/month
C14	0.076±0.0042	F	1x/month
C15	0.098±0.0035	F	8x/month
C16	0.104±0.0024	F	1x/month
C17	0.243±0.0172	F	8x/month
C18	0.457±0.0013	F	8x/month
C19	0.133±0.0015	F	2x/month
C20	0.921±0.0484	F	4x/month
C21	0.237±0.0074	F	8x/month
C22	0.040±0.0024	F	4x/month
C23	0.117±0.0015	F	2x/semester
C24	0.230±0.0011	F	1x/month
C25	0.195±0.0044	F	2x/month
C26	0.649±0.0007	F	1x/month
C27	0.369±0.0006	F	2x/month
C28	0.171±0.0018	F	2x/month
C29	0.125±0.0005	F	1x/month
C30	0.070±0.0002	M	1x/month

The concentration range for the children of Chapada de Natividade was from 0.038 to 0.921 $\mu\text{g g}^{-1}$ Hg, and the arithmetic mean, geometric mean and median were, respectively, 0.249, 0.183 and 0.152 $\mu\text{g g}^{-1}$ Hg. None of the children presented values above 1.0 $\mu\text{g g}^{-1}$ Hg which is the limit set by the National Research Council of the USA [12].

In Table 2 are presented the results obtained for Hg concentrations in hair of the children resident in the municipality of Porto Nacional, where there are no artisanal gold mining activities.

Table 2: Individual results of Mercury concentration in hair of children living in the municipality of Porto Nacional - TO.

Sample Code	Concentration of Hg ($\mu\text{g g}^{-1}$)	Gender	Fish Consumption
P1	0.167±0.0032	F	4x/month
P2	0.079±0.0021	M	1x/month
P3	0.083±0.0059	F	2x/month
P4	0.175±0.0047	F	Rarely
P5	0.076±0.0011	F	12x/month
P6	0.222±0.0113	M	1x/month
P7	0.172±0.0029	F	8x/month
P8	0.132±0.0042	F	4x/month
P9	0.192±0.0011	F	1x/month
P10	0.195±0.0019	F	1x/month
P11	0.098±0.0002	F	Rarely
P12	0.375±0.0000	F	4x/month
P13	0.177±0.0012	F	4x/month
P14	0.141±0.0026	F	12x/month
P15	0.078±0.0022	F	8x/month

The concentration range for the children of Porto Nacional was from 0.076 to 0.375 $\mu\text{g g}^{-1}$ Hg, and the arithmetic mean, geometric mean and median were, respectively, 0.157, 0.142 and 0.168 $\mu\text{g g}^{-1}$ Hg. Also in this case, none of the children presented values above 1.0 $\mu\text{g g}^{-1}$ Hg which is the limit set by the National Research Council of the USA [12].

Table 3 presents the results obtained for the analysis of Hg in the IAEA 086 reference material, by the DMA method.

Table 3: Results of the determinations of mercury in the reference material IAEA-086 “Human Hair”, by the DMA method.

Reference Material	Hg concentration by DMA ($\mu\text{g g}^{-1}$)
	0.622
	0.615
“Human Hair” IAEA-086	0.614
	0.616
	0.606
	0.575
($\bar{x} \pm s$)	0.608 ± 0.017
Relative standard deviation (%)	2.8 %
Relative error (%)	6.1 %

The results obtained for the reference material IAEA-086 can be considered as satisfactory, as to the relative standard deviation and relative error obtained.

4. DISCUSSION

In the NAHNES (National Health and Nutrition Examination Survey) study [13], carried out in the United States, with children from 1 to 5 years and women of child-bearing age, the authors obtained a geometric mean for total mercury of $0.12 \mu\text{g g}^{-1}$ Hg for the children. The authors consider that the study constitutes a reference data base for the American children, as regards Hg concentration in this age range. This value is similar to the one obtained, of $0.142 \mu\text{g g}^{-1}$, for the non-exposed children of Porto Nacional.

In the European DEMOCOPHES project, with the participation of 17 countries, the geometric mean found, for children in the age range from 6 to 11 years old, was of $0.14 \mu\text{g g}^{-1}$. This mean concentration was also similar to the one obtained for the children of Porto Nacional, which was of $0.142 \mu\text{g g}^{-1}$ Hg (geometric mean). On the other hand, in the DEMOCOPHES project, it has to be pointed out that significant differences have been found between the countries, with a factor of 50 between the lowest (Hungary) and the highest (Portugal). Fish consumption and social status were found to be important and independent factors, determinant of mercury levels in hair [14].

A study carried out in a traditional riverine population of the Tapajós river, in the Amazonic region of Brazil, with 32 mothers and their 51 children, revealed very high concentrations of total Hg and MeHg. The ranges of total Hg and MeHg concentrations in hair were, respectively, of 1.0 to 51.0 and 0.5 to $41.4 \mu\text{g g}^{-1}$, with means of 12.4 and $10.2 \mu\text{g g}^{-1}$. The relative mean value of MeHg was 80 %. This population reported high fish consumption, from 4 to 14 times a week [15].

Due to the fact that children are more vulnerable to mercury toxicity, another study was made in the Amazonic region of Brazil, in the state of Rondônia, with children aged from 3 to 9 years old, in two localities: Demarcação (DA) and Gleba do Rio Preto (GRP). Besides Hg, selenium was also determined in the hair of the children, since it has been considered that it can delay mercury intoxication symptoms by forming an inert complex with Hg. High mean mercury levels were found in the children from both localities: $3.6 \mu\text{g g}^{-1}$ for DA and $6.2 \mu\text{g g}^{-1}$ for GRP [16].

4.1. Statistical Treatment

The results obtained for mercury in the hair of the children of the two municipalities were evaluated by means of statistical tests, considering $p < 0.05$ as the index of rejection of the null hypothesis by the Student t-test.

Figures 1 to 3 present, respectively, the mean and individual concentrations of mercury in the hair of children resident in the two municipalities studied and the concentrations of mercury related to fish consumption.

Figure 1, below, presents the mean concentrations of mercury in the hair of the children of Chapada de Natividade ($0.249 \mu\text{g g}^{-1}$) and Porto Nacional ($0.157 \mu\text{g g}^{-1}$).

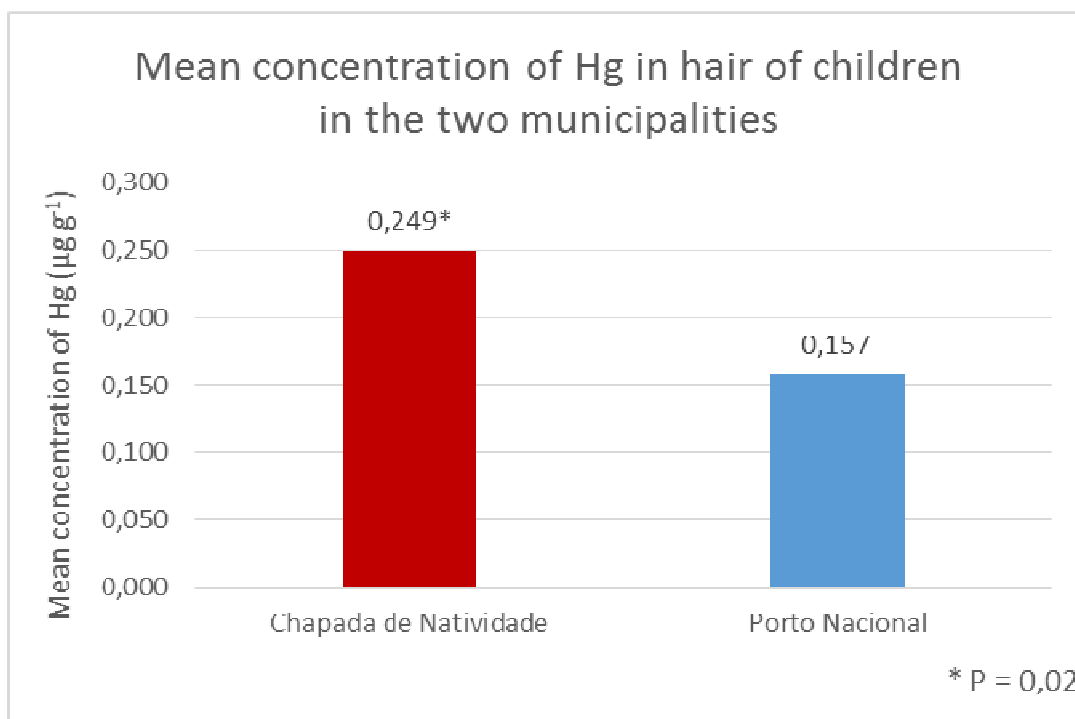


Figure 1: Mean concentrations ($\mu\text{g g}^{-1}$) in hair of children in the two municipalities studied $p = 0.02$ ($p < 0.05$)

Figure 2 below, presents the individual concentrations of mercury in the hair of the children of Chapada de Natividade and Porto Nacional.

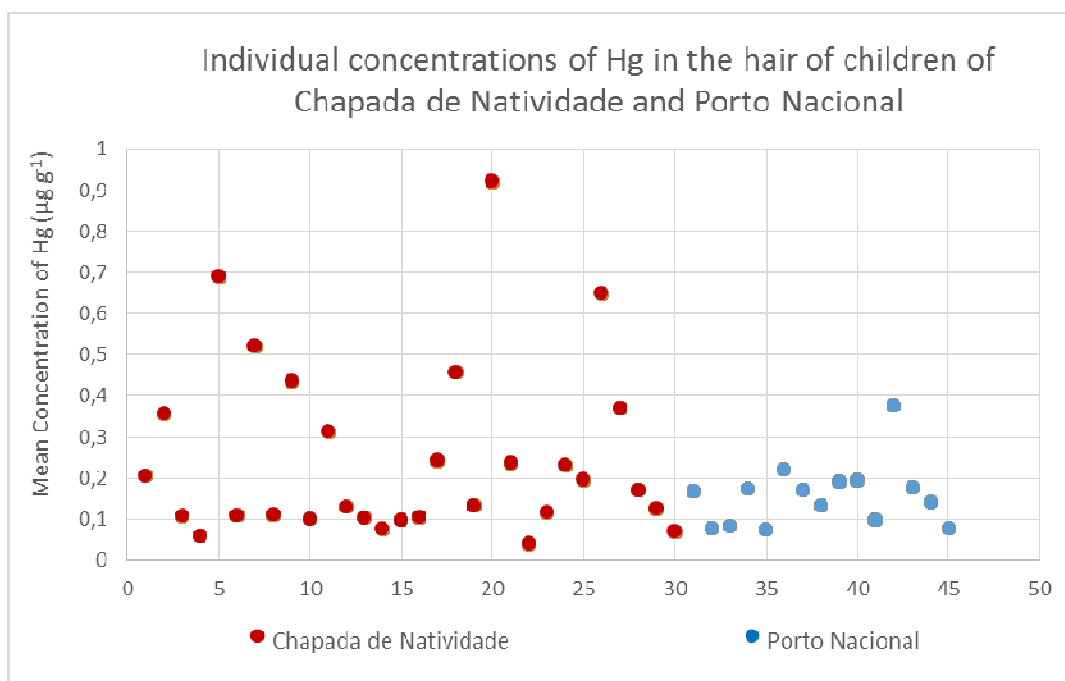


Figure 2: Individual concentrations of mercury in the hair of the children of Chapada de Natividade and Porto Nacional.

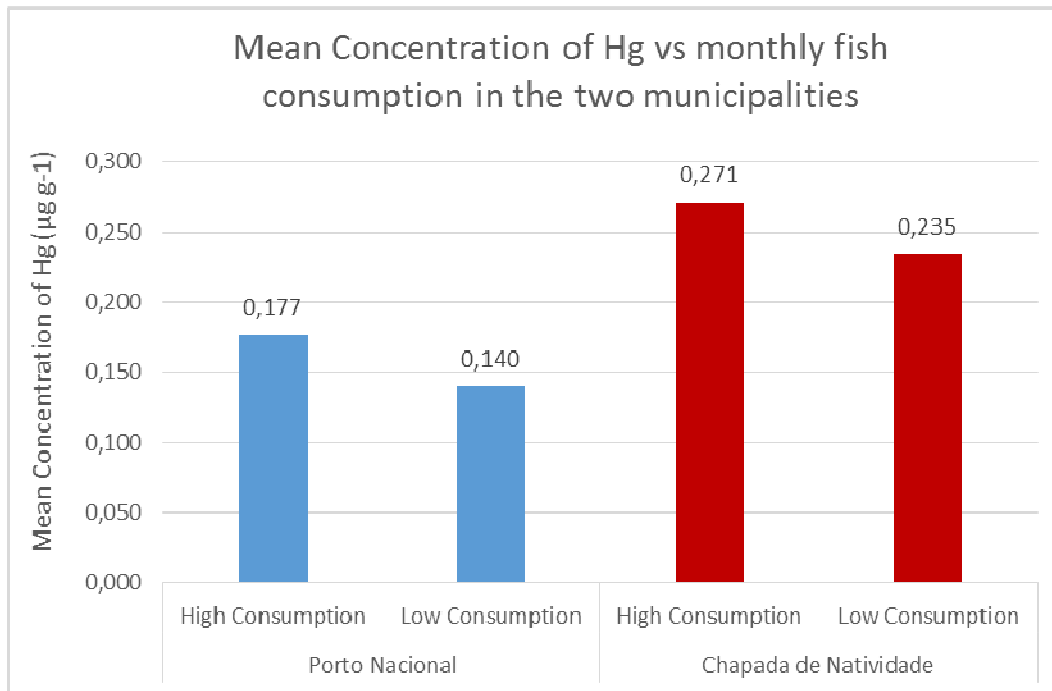


Figure 3: Mean concentration of Hg vs monthly fish consumption. $p = 0.30$ ($p > 0.05$).

According to Figure 1, it can be observed that the mean mercury concentration in the hair of the children of Chapada de Natividade was significantly higher ($p < 0.05$) than the concentrations of the children of Porto Nacional. As already commented, in Porto Nacional the value obtained for the geometric mean, of $0.142 \mu\text{g g}^{-1}$ Hg, was similar to the geometric mean obtained in the NHANES 1999-2000 study, in the US, which was of $0.12 \mu\text{g g}^{-1}$ Hg [13].

In Figure 2 the distribution of the individual concentrations of Hg in the samples can be observed. Some of the children in Chapada de Natividade presented values for Hg much higher than the mean, but no value exceeded the recommended by US-EPA ($1 \mu\text{g g}^{-1}$) and by WHO ($1.9 \mu\text{g g}^{-1}$).

It can be seen in Figure 3, that the children who consume fish frequently (equal or more than 4 times a month) present Hg concentrations higher, with mean concentration of $0.236 \mu\text{g g}^{-1}$ Hg, than the ones who do not consume or have a lower consumption of fish (less than 4 times a month), who present a mean concentration of $0.206 \mu\text{g g}^{-1}$. On the other hand, it was observed that there was no statistically significant difference as regards high vs low fish consumption, since $p = 0.30$ ($p > 0.05$).

A similar statistical treatment carried out for the results of both municipalities has shown that the mean Hg concentration for the feminine gender was of $0.232 \mu\text{g g}^{-1}$ and for the masculine gender it was of $0.166 \mu\text{g g}^{-1}$. The mean concentration of Hg in relation to the presence of

dental amalgam, which contains mercury, was $0.250 \mu\text{g g}^{-1}$ for the children that had amalgam fillings and of $0.210 \mu\text{g g}^{-1}$ for those without amalgam fillings. Both relations, (gender X Hg) and (amalgam X Hg), did not present statistically significant differences, with values of p equal to 0.12 and 0.28 ($p > 0.05$), respectively.

During the hair samples collection, in the municipalities of Chapada de Natividade and Porto Nacional, no cases of children's work in the artisanal gold mining were detected. On the other hand, some families lived in houses that were built on the same land where the artisanal gold mining was carried out, mainly when the parents or other responsible adults worked as gold miners. There were some reports also that the children played close to the places where the gold – mercury amalgam was heated to eliminate mercury, with the consequence of the children inhaling mercury. Besides the risks of environmental exposure inside the residence, the families were also exposed to the mercury residues disposed in the surrounding soil, where domestic animals and also animals raised for feeding, such as chicken and swines are present. It was observed that the children whose parents or responsible adults were workers in artisanal gold mining activities had higher mercury concentrations ($0.294 \mu\text{g g}^{-1}$) than those whose parents worked in other professions ($0.205 \mu\text{g g}^{-1}$), but without statistical significance, which suggests that the environmental exposure to mercury can be extended to houses in which there are no gold extraction activities.

On the other hand, children can also be frequently exposed to mercury by accidental spilling of the metal, mainly by the inadequate use or when a proper cleaning is not made after the spilling. Broken thermometers are the main source of exposure, as it is reported in the usual calls to the centers of toxicological control. Nevertheless, these calls are diminishing and this reduction can reflect the decreasing availability of the mercury thermometers. Only two children participating in the present study had a history of accidents with mercury thermometers, one from Chapada de Natividade who presented mercury concentration $0.076 \mu\text{g g}^{-1}$, with an accident four years ago and another from Porto Nacional with an accident a month ago and with a concentration of Hg in hair of $0.078 \mu\text{g g}^{-1}$.

In a study carried out in three communities of the Brazilian Amazon, in which two of them were close to gold mining activities and the third was situated far from these activities, the authors pointed out that industries and gold mining extraction, besides other nutritional, natural or anthropic sources, could be responsible for the increase of mercury concentrations in the environment of the region and that children are more vulnerable to mercury exposure [17].

Molina-Villaba et al [18] made an evaluation of the potential contribution of gender, water consumption, site of residence and body mass index, as related to mercury and other metals concentrations in children from 6 to 9 years old, inhabiting for at least one year in a region close to industries and mining activities in Spain, in one of the most polluted estuaries in the world. The arithmetic and geometric means were, respectively, of 1.282 and $0.407 \mu\text{g g}^{-1}$ Hg in hair of the children. The authors concluded that the body burden of Hg and also Cd of the children living near industrial and mining areas can have an impact on health. The authors also point out that the children are more vulnerable to the neurotoxic effects of metals and so deserve special attention of biomonitoring programs [18].

Montuori et al [19] studied, also in Spain, the Hg speciation in the hair of pre-school children, of age 4, living in two areas, both exposed to different sources of Hg: a point source in Ribera

d Ebre (RE) and a diffuse source on the island of Menorca (MC). The total Hg and MeHg median values found for RE were much higher than the values found for MC. Children who consumed fish more than three times a week had higher methylmercury (MeHg) concentrations than those who consumed less than once a week. On the other hand, the differences in MeHg between the children from RE and MC remained statistically significant even after adjustment for fish consumption and other variables, which suggests that populations with similar fish consumption may receive different levels of exposure to mercury due to their proximity to mercury-related industrial activities.

5. CONCLUSIONS

The study demonstrated that the mean concentration of Hg in hair samples of the children living in an area exposed to artisanal gold mining activities, in the municipality of Chapada de Natividade - TO was significantly higher than in a non-exposed area, in the municipality of Porto Nacional - TO.

There was no statistically significant difference related to gender, monthly fish consumption of the parents, accidents with mercury thermometers or presence of amalgam fillings in teeth.

These results are in agreement with the ones obtained by Villaba [18] in Spain, who studied children resident in areas close to industrial and mining activities. The authors report that the body burden of Hg and also cadmium in these children can have an impact on health. The authors also point out that the children are more vulnerable to the neurotoxic effects of metals and so deserve special attention of biomonitoring programs.

Also Montuori et al [19], who studied mercury speciation in hair of pre-school children living near a chlor-alkali plant concluded that populations with similar fish consumption may receive different levels of exposure to mercury due to their proximity to mercury-related industrial activities.

The results of the present work thus suggest that children living in a region exposed to artisanal gold mining are environmentally exposed to mercury, regardless of their eating habits or gender.

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