Environmental Research Section A **84,** 204–210 (2000)
doi:10.1006/enrs.2000.4115, available online at http://www.idealibrary.com on IDEAL®

Exposure to Mercury in Pregnant Women from Alta Floresta—Amazon Basin, Brazil

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Received July 1, 1999

The region of Alta Floresta in the South of the Amazon basin, close to the Teles Pires River, was one of the main prospecting gold areas in the Amazon Basin until the beginning of the 1990s. The economic growth was accompanied by a considerable increase in the population, due to a massive influx of migrants from the southern region of the country. Women had an important role during that process. They worked in the "garimpos" in different activities, such as cooking or managing, where they were exposed mainly to indoor elemental mercury during burning of amalgam. They also worked in gold dealers' shops, where a great amount of amalgam was burned daily. Fish consumption was an important dietary protein source and also a possible exposure pathway, due to the high Hg concentrations reported in carnivorous species. The present study evaluates the mercury uptake and consequent risks involved for pregnant women from Alta Floresta at the end of the gold rush period. The survey included women at different pregnancy stages and it was supported by clinical exams, followed by an interview with a specific questionnaire. Mercury hair concentrations ranging from 0.05 to 8.2 μg/g were found among women, with 13% of them showing concentrations above 2 μg/g. According to the results, mean differences in hair mercury concentration were statistically higher for pregnant women if they had worked in gold mining areas, they had consumed alcohol, their husbands had worked as "garimpeiros," they ate fish, and/or they had malaria before and/or during pregnancy. © 2000 Academic Press

Key Words: **mercury; pregnancy; hair; Amazon Basin.**

INTRODUCTION

Studies on an outbreak of methylmercury poisoning from the consumption of contaminated bread indicated that prenatal exposure offered the greatest hazard (WHO, 1990). Methylmercury in contaminated fish or other foods is effectively absorbed by the gastrointestinal tract, enters the bloodstream, and finds its way rapidly to other parts of the body, including the brain and fetus. Methylmercury can be transformed to inorganic mercury in the brain and remains there for a long time (ATSDR, 1994). Metallic mercury vapor is effectively absorbed by the lungs, entering the bloodstream and rapidly diffusing to other parts of the body. Kidney and brain are the most affected organs by exposure to both metallic and mercuric mercury. Also, inhaled metallic mercury can easily reach the fetus (U.S. EPA, 1996). Most studies of occupational exposure to elemental mercury vapor on reproductive function have failed to find evidence of adverse effects (Alcser *et al.*, 1989; Erfurth *et al.*, 1990; McGregor and Mason, 1991). A few studies have shown at least suggestive evidence that elemental mercury exposure may adversely affect reproductive function (WHO, 1991). In a study of women exposed occupationally to mercury vapor (Sikorski *et al.*, 1987), a correlation was observed between scalp hair mercury and reproductive

This paper was presented at Mercury as a Global Pollutant: 5th International Conference, Rio de Janeiro, Brazil, May 23–28, 1999.

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0013-9351/00 $35.00

failure and menstrual abnormalities. However, that study is limited because it does not present exposure data. No evident effect on fertility was observed in exposed males, but one study of wives of Hg vapor-exposed workers in chloralkali plants found an increased rate of spontaneous abortions (Cordier *et al.*, 1991). It is possible that the wives were exposed to mercury as a result of handling contaminated clothing.

Methylmercury exposure causes an increased risk of fetal brain damage if the maternal mercury concentration in scalp hair exceeds a level of 10–20 μg/g (WHO, 1990). This threshold has been associated with mild or equivocal deficits on developmental or intelligence tests in prenatally exposed children (Kjellstrom *et al.*, 1989). A child may experience delays in speaking and walking, changes in muscle tone and reflexes, and, at higher doses, a syndrome resembling cerebral palsy. Adults may experience paresthesia, tremors, ataxia, deafness, and constriction of visual fields (WHO, 1990).

There are three major human studies used for establishing developmental toxicity exposure benchmarks for MeHg. The U.S EPA has based their oral reference dose (RfD) of 1×10^{-4} mg/kg/day on the exposure of Iraqis to MeHg in bread prepared from contaminated seed grain (Cox *et al.*, 1989; Stern, 1993). Studies related to fish consumption in the Seychelles population suggest that there are no developmental effects associated with total Hg concentration in hair up to 26 μg/g (Swartout, 1999). However, the third study, concerning a population consuming whale meat and fish in the Faroes Islands, suggests that mental developmental effects may occur at Hg concentrations in hair as low as 10 μg/g or lower (Grandjean *et al.*, 1998). Other human studies suggest that neurological effects from MeHg exposure in adults may occur at hair Hg concentrations in the range 30–50 μg/g (U.S. EPA,1996).

Beginning in the 1980s, mercury contamination in the Amazon region became an important issue. During this period informal gold-mining activity has released about 2500 tons of Hg into the environment (Malm, 1998). This Hg has great potential to contaminate the environment and to enter the aquatic systems, where it is transformed into organomercuric compounds by sediment microbiota. Later it reaches the top of the food chain, with very high biomagnification factors. Exposure to methylmercury contamination through the consumption of contaminated fish can affect the fetuses of pregnant women.

In the past decades Amazonian communities have been exposed to Hg through different pathways and chemical species. Nowadays, the most relevant exposure in the Amazon basin is through fish consumption. Several areas of the Amazon region have been studied to identify the extent of contamination and the levels of human Hg exposure. The riverine communities are primarily exposed to MeHg through fish consumption because fish is the major food source. However, one must regard different exposure scenarios in the Amazon basin and the different social–cultural aspects (Hacon *et al.*, 1997).

The present study is concerned with the assessment of prenatal mercury exposure in the urban and rural areas of Alta Floresta. Different regression models were used to explain the results and their association and/or interactions between Hg in hair and variables such as occupational exposure, malaria, social habits, and fish ingestion rate, which may reflect exposure scenarios.

MATERIAL AND METHODS

Area and Population of Study

Alta Floresta county lies in the north of Mato Grosso State, located 800 km away from the city of Cuiabá, the capital of the state, near the Teles Pires River in the Southern part of the Brazilian Amazon Basin. Alta Floresta has a stable population of 46,000 inhabitants, with the great majority from the south of Brazil.

Gold mining in this area was the reason for the economic growth of the municipality until the early 1990s and during this period Alta Floresta was an important gold-trading center. Gold-mining activity in north Mato Grosso State has currently declined about 70%. However, the contaminated sites left by the "garimpeiros" are still a potential pathway for Hg exposure.

The colonization process in Alta Floresta was different from that of other areas of the Amazon Basin. It bagan as a private colony that was connected to a large-scale agricultural project. However, the "gold rush" transformed the stable social–economic project into a mobile economy. The southern migrant population, the farmers, had social and cultural habits that were distinct from those of the indigenous Amazonian population. The women arrived later, after the men had settled and established some sort of economic activity, which in most of the cases, was related to gold mining. After their arrival, most of the women were involved in child-rearing and, in general, did not have any economic activity. Then they began to help the men in the gold-mining activity by cooking or burning amalgam. In some cases they were involved in administrative activities related to gold marketing in the urban area or even in the "garimpo" areas.

The women of Alta Floresta were young, with 65% younger than 30 years. They belonged to a low social–economic status population, and had a low level of education. They had strong cultural habits, such as eating red meat and drinking a strong herb tea called "Chimarrão." The social profile of Alta Floresta women is related to urbanization of the region and regional origin. In Brazil, the role of women has changed, as evidenced by their increasing participation in the work force. In Alta Floresta the general social profile follows this standard, in contrast to the more traditional riverine population in the Amazon Basin.

METHODS

The sample frame was built from the medical records of the prenatal Health Community Center of Alta Floresta. This Community Center is the most important of the region (the only one with a permanent gynecologist), attending the whole region of north Mato Grosso State. According to these records, a total of 480 pregnant women were registered in this Community Center in 1995; this number included pregnant women from different municipalities of north Mato Grosso. During the sampling period (May 1995), about 130 pregnant women were expected for follow-up visits to the gynecologist at the Community Health Center. According to the medical records (gynecological information, obstetrician data, age, residential area), there was no significant difference between the expected group (130 pregnant women) and the group who came for prenatal follow-ups during the sampling period (110 pregnant women). The sample of 110 women was considered satisfactory. Individual interviews were performed with the available group, explaining the aims of the study, and a written consent was read to each of them. Only 75 agreed to provide hair sample and to answer the questionnaire. Once again, it was verified (taking into account the available data in the medical records) that such a difference in the sample size would not affect the sample profile. The reason presented during the interview for refusing to participate in the study was religious principles; it is well known some fundamentalist Christian churches do not allow their followers to cut their hair, and such churches became very popular in north Mato Grosso in recent years. Also, according to the Brazilian Constitution, groups or minorities cannot be forced to act against their beliefs.

Two nurses were trained to apply the questionnaire and to collect the hair. The coordinator of the project was responsible for the supervision of the study. The questionnaire asked for medical information such as obstetrical data and reproductive history, data on gynecological information, clinical data; and reported morbidity. Social–demographic information such as past living history, smoking habits, drug and alcohol ingestion, and fish-eating habits, age, and education was also surveyed. All women were clinically examined by an obstetrician, to determine their pregnancy stage and health status. In addition, a dentist determined the number of amalgam tooth fillings of each individual. After the interview, the nurses collected hair samples. Approximately 40 strands were selected in the occipital area of the head. The stands were tied with cotton thread, about 1 cm long, as close to the scalp as possible, cut with surgical scissors, and placed in a paper envelope. All of the women participating in this study were clinically confirmed to be pregnant by the gynecologist; therefore all hair samples corresponded to a pregnancy period.

Samples from five species of fish were collected from the Teles Pires River and the Cristalino River about 100 km from Alta Floresta, during the second and third weeks of May. The fish samples were collected by members of the research group and local fishermen. The species collected were the most representative for the local fish market, according to the fisheries and the fishermen, and the most consumed by the general population. A detailed study concerning this point has been published. (Hacon, 1996; Hacon *et al.*, 1997). For the present study, Hg concentrations were determined in the muscle of the five species of fish using CV AAS (Campos and Curtis, 1990). The weight and size of each individual species were measured. Although the sample size of each species was limited, the Hg concentration range was in agreement with that of other studies (Bidone *et al.*, 1997; Hacon *et al.*, 1997; Malm *et al.*, 1995) with larger sample sizes. Hair and fish samplings were performed in the same period (May 1995). The fish samples were frozen for later analysis.

The total Hg measured in fish and hair was determined by cold vapor atomic absorption spectrometry after acid digestion (Campos and Curtis, 1990). Analytical quality was ensured by the analysis of certified reference materials, a strict blank control (at least three blanks for a batch of 10 samples), duplicate analysis, and an internal reference sample. The data basing was carried out using Epi-Info (Version 6, 1997) and the statistical analyses were performed using SPSS (Version 8). The significance level used in this study was $P = 0.05$. All participants signed a written consent. This study was approved and supported by the Brazilian National Research Council and followed the ethical restrictions imposed by such council, which was the written consent.

RESULTS

General Characterization of the Pregnant Patients

The ages of the studied pregnant women ranged from 14 to 45 years. About 31% of them were younger than 20 years, 55.5% were between 20 and 30 years, 11% were between 30 and 40 years, and 2.5% were older than 40 years. The average number of children per family was three. About 93% of the women already had at least one child.

The education level of the group was low; 73% did not complete the first year of primary school and 20% were illiterate. About 56% of the women came from southern Brazil; the others came from different regions. The residential time ranged from 6 months to 10 years, 66.5% having lived in Alta Floresta for less than 5 years. Most of the women (85%) did not work outside the house. The others had general activities such as sellers in the local commerce; 17.5% reported having worked in gold-mining activities, as cooks or helping the husband burn amalgam. In relation to social habits, 30% of the group smoked, 85% usually drank coffee, and 28.5% drank alcohol during pregnancy. Only 2% of the women reported dying their hair during the pregnancy period.

Signs and Symptoms Reported by the Pregnant Patients

Malaria and leishmania are endemic in the Amazon, but a high number of malaria cases occur in the garimpos areas. It was observed that 35% of 75 sampled women had at least one episode of malaria before the pregnancy. In the Amazon region there are cases of unsystematic malaria (Camargo *et al.*, 1999). The morbidity survey does not provide evidence of malaria and in this study there were no laboratory diagnoses. During the acute phase, malaria is easily diagnosed by clinical examination, especially in the Amazon region where physicians are skilled in recognizing this type of disease. As all pregnant women were subjected to such clinical examination and no malaria cases were detected, we believe that acute malaria was not underestimated. On the other hand, if we had some positive malaria cases and it was not detectable during the study, it may probably show an association between Hg and malaria, due to the strong association between gold-mining activity and malaria.

The most common symptoms and diseases during the pregnancy reported by the patients were swelling (34%), fever (31%), and malaria (10%). Some improvements in health and social conditions have been observed in the past 5 years in the region, but the reduction in morbidity in this period may also be due to the decrease in gold-mining activity and thus the bad health conditions related to this activity.

Obstetrical Data

The studied group went through clinical examination by the gynecologist of the Alta Floresta Municipal hospital. The group were at various stages of pregnancy: 31% were in the first trimester, 19% in the second trimester, and 31% in the third trimester. No statistical difference in hair Hg content was observed among these groups. Oral contraceptives had been taken by 60% of the group. About 13.5% reported to have had premature deliver, and 19% had abortion. Some medications were used during the pregnancy period, the most important in terms of effects for the pregnancy was the antimalarial, used by 15% of the interviewed pregnant women. Based on the present study and local medical registry, there was no increase in spontaneous abortions or stillbirths in pregnancies of women who had worked or whose husbands had worked in gold-mining activities.

Mercury Exposure

The exposure scenarios of Alta Floresta include different exposure pathways for adults and children in the population (Hacon *et al.*, 1997). In this study we assesser only pregnant women, due to the fact that they are a critical group for methylmercury exposure, through fish consumption. In some Amazon regions pregnant women have cultural habits of eating oily fish during pregnancy (Boishio and Barbosa, 1993). In Alta Floresta, we found the opposite. Fish consumption, even though it was low, followed the seasonal variability of the region. The group studied presented the general fish-eating habits of the region, consuming twice as many carnivorous fish (46%) as herbivorous fish (23%). About 31% of the group did not consume any fish at all. Of the 69% who consumed fish, 44% reported eating fish once a week and the others ate fish occasionally. For 25% of the group, the mean fish consumption rate was 8 g/day, and in 44% of the sample it was 20 g/day.

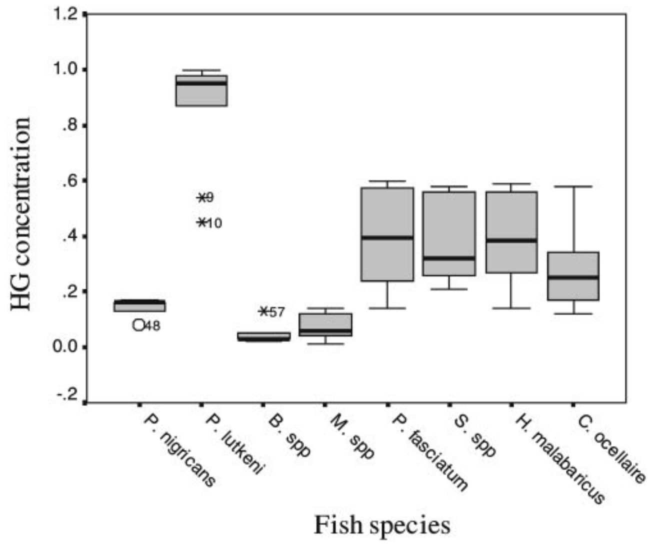

FIG. 1. Distribution of mercury concentration (mg/kg, ww) in fish by species consumed in Alta Floresta by the studied group.

Figure 1 shows the median, 5, 25, 75, and 95% percentiles for Hg concentration in the different species consumed by the studied group. The range of Hg concentration in carnivorous fish species was 0.3 to 1.0 mg/kg (wet weight). The weight variation of these species was from 0.3 to 3.0 kg, with an average weight of 2.8 kg. The highest fish Hg concentration was found in carnivorous fish, *Paulicea lutkeni* (1.0 mg/kg) (jau) and *Pseudoplatystoma fasciatum* (0.6 mg/kg) (pintado), which dominate the fish consumption period from June to November. In herbivorous species, *Myleus* spp. (Pacu), *Prochilodus nigricans* (Curimatã), and *Brycon* spp. (Matrinchã), the levels of Hg were very low, as expected, ranging from 0.01 to 0.17 mg/kg. The weight variation was from 0.1 to 3.5 kg, with an average weight of 2.5 kg. These results corroborate some other data from the Amazon region (Akagi *et al*., 1995; Malm *et al*., 1995; Hacon *et al*., 1997; Bidone *et al*., 1997).

In relation to water consumption, most of the people (84%) used water from wells without any type of primary treatment, and about only 4% used water from a supply system. The mean total Hg concentration in water consumed by the population was 3.0 ng/L. The exposure estimated from water consumption can be regarded as negligible (Hacon *et al*., 1997).

The Hg concentration in scalp hair ranged from 0.051 to 8.2 μg/g with a mean of 1.12 μg/g (SD 1.17); only 13% of the results showed concentrations above 2 μg/g). This represents a low contamination level from fish intake in Alta Floresta. To understand how the different variables may affect the relationship of Hg exposure and Hg in hair, mean tests and regression linear models were carried out using statistical analysis software.

Table 1 shows the results of the univariate analysis. For each selected independent variables two categories of answers were proposed. To compare the meaning of these answers (yes or no), a mean test was applied and the *P* values showed that the variables working in gold-mining activity, alcohol consumption, being the wife of a "garimpeiro," eating fish, having malaria previously, and having malaria during the pregnancy period, presented a statistical significance for the positive answer. For some others variables, including age, time of exposure, residential area, Hg concentration in water, education level, residential time, smoking, dying hair, and number of amalgam tooth fillings did not show any statistical significance in relation to Hg concentration in hair.

The data were analyzed by multiple linear regression models with variables selected from the univariate analysis as the independent variable and Hg in hair as the dependent variable; the results are showed in Table 2. The model explains the mercury concentration in hair in pregnant women with a determination coefficient of 0.44 (r^2). The results revealed that if they had malaria the Hg levels in hair increased significantly ($P = 0.009$). There were significant interactions between alcohol intake and previous malaria ($P = 0.008$), and husband occupation and previous malaria ($P = 0.01$).

DISCUSSION

The mercury baseline exposure scenario for the studied group was a low-level chronic exposure. The

TABLE 1

Means and *t* Test for Hair Mercury Concentration Considering Possible Predictor Variables in Pregnant Women, Alta Floresta, Brazil

Independent variable	Category	Hg mean (ppm)	*T* Test *P* value
Work in gold-mining activity	Did not work	0.96	
	Worked	1.86	0.01
Alcohol intake	Did not drink	0.88	
	Drank	1.53	0.03
Husband occupation	Others	0.83	
	"Garimpeiro"	1.5	0.02
Fish consumption	No consumption	0.62	
	Consumer	1.23	0.01
Malaria during pregnancy	Yes	1.0	
	No	2.90	0.00
Previous malaria	Yes	1.53	
	No	0.90	0.01

TABLE 2

Coefficients (β), 95% Confidence Interval and *P* Value of Regression Linear Analysis Model for Mercury Concentration in Hair in Pregnant Women, Alta Floresta, Brazil

Independent variables	β	95% CI	P value
Constant	0.519	(− 0.511; 1.549)	0.327
Working in gold mining activity	0.531	(− 0.087; 1.149)	0.097
Alcohol intake	0.130	(− 0.374; 0.633)	0.616
Husband occupation	0.007	(− 0.501; 0.515)	0.979
Fish consumption	0.410	(− 0.160; 0.979)	0.164
Malaria during pregnancy	1.144	(0.146; 2.142)	0.028
Previous to malaria	2.857	(0.774; 4.940)	0.009
Interaction (alcohol intake and previous malaria)	1.779	(0.517; 3.041)	0.008
Interaction (husband occupation and previous malaria)	− 2.385	(− 3.585; − 1.185)	0.001

Note. $r^2 = 0.44$.

way people are exposed to Hg depends on several factors, such as the levels of contamination in the environmental compartments; the existence of potential pathways for human exposure; the size, nature, and habits of the community; and the frequency and duration of exposure.

Until the mid-1990s the Alta Floresta population was exposed to Hg through different routes and sources of exposure and different concentrations. The gold trade in the urban area until 1995 was responsible for a Hg emission factor of 0.034 t/a. The indoor Hg in "gold shops" reached an average concentration of 40 μg/m^3 for a work day. The Hg concentrations in the urban atmosphere ranged from 0.01 to 5.7 μg/m^3. This variability depended on the amount of gold processed daily and the external environmental conditions (Hacon *et al*., 1995).

The population in this region is also exposed to Hg via fish intake. Fish from the region presented high Hg concentration, mainly carnivorous fish. But the fish consumption rate in this group was very low compared to that in some other Amazon areas (Hacon *et al*., 1997). Studies have estimated 200 g/day as the average fish consumption among the riverine communities in the Amazon region (Boishio *et al*., 1996; Rodrigues *et al*., 1994). However, in Alta Floresta the fish ingestion rate ranged from 5 to 110 g/day. This difference was mainly caused by the differences in eating habits, occupational activity, sex, and age. The local adult population had a low fish consumption of 56 g/week (Hacon *et al*., 1997). The variability of the fish consumption and the low rate of fish ingestion may be one factor that could explain the low level of Hg in hair in this group when compared to some other areas in the Amazon Basin.

This study confirmed the results of previous research carried out with the local adult population of Alta Floresta that reported low exposure (Hacon *et al*., 1997). These results may be associated with the maintenance of strong cultural food habits of the population: most of the group was from the south of Brazil and still eating mostly red meat. Although fish consumption was low for the whole group, it was possible to distinguish the difference in Hg concentration in hair among those who consumed fish (1.23 μg/g) and the ones who did not eat fish (0.62 μg/g), with twice the mercury concentration in hair for those who consumed fish.

Some variables related with gold-mining activity showed a relationship with Hg concentration in hair. To have had malaria before and during pregnancy were variables with significant statistical association with Hg in hair. The previous malaria cases were related to the period that the women worked at the "garimpo" areas, where the environmental degradation favors mosquito breeding due to the poor water drainage. During the pregnancy period some women were still indirectly exposed to elemental mercury since their husbands were still burning gold amalgam at home or even being directly exposed by working in the gold-mining areas.

The interactions of previous malaria with consumption of alcohol and husband occupation also showed statistical significance (Table 2). This may be explained by the fact that the women's gold-mining activities were directly related to the husband's occupational activity. Women used to go with their husbands when they went to work in garimpos, being exposed to malaria and other social effects related to this occupational activity, such as alcohol consumption. Alcohol consumption in garimpos areas was very high; this may be explained by the psycho-social effects of this activity.

The main variables that were included in the model that could better explain the determination of mercury concentration in hair were related to working in a gold-mining activity. This occupational activity could also increase exposure through fish consumption, because in the "garimpos" areas food availability was low, resulting in an increase in fish consumption, although in this study the variable fish consumption did not present a coefficient with statistical significance in the regression equation.

The variables working in gold-mining activity, having had malaria, and alcohol consumption can be regarded as risk factors for increased Hg exposure. Although it is unlikely that present mercury levels in hair represent a serious health risk for the pregnant women, the possibility of subtle developmental changes cannot be excluded for a small group that presented Hg levels in hair close to 8 μg/g.

ACKNOWLEDGMENTS

This study could not have taken place without the collaboration of the physicians, nurses, and administrative workers of Municipal Hospital of Alta Floresta and a dentist of the local public health center. Our fieldwork was greatly facilitated by the nurses of the local Public Health Center and by the fishermen of the region. We express our gratitude to the urban and rural population of Alta Floresta. We thank Hilton Silva for helpful English review. This work was financially supported by the Brazilian National Council Research and Development—CNPq. This study was conducted in accordance with the national and institutional guidelines for protection of human subjects and animal welfare.

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