

DETERMINATION OF TOTAL MERCURY AND METHYLMERCURY IN HUMAN HEAD HAIR BY RADIOCHEMICAL METHODS OF ANALYSIS

IPEN-DOC-

5431

M.B.A. Vasconcellos¹, M. Saiki¹, G. Paletti¹, R.G. Baruzzi², D.A. Rodrigues², J. Cuten²

¹ Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, IPEN-CNEN/SP - Caixa Postal 11049, CEP 05422-970, São Paulo/SP, BRASIL

² Unidade de Saúde e Meio Ambiente, Departamento de Medicina Preventiva, Escola Paulista de Medicina - Rua Pedro de Toledo n° 675, CEP 04039, São Paulo/SP, BRASIL

Total mercury has been determined by instrumental neutron activation analysis in the hair of several Indian tribes living in the Xingu Park, in the Amazonic region of Brazil. Methylmercury and also total mercury have been determined in part of the samples by cold vapour atomic absorption spectroscopy, at the Nuclear Chemistry Department, Jozef Stefan Institute, Ljubljana, Slovenia.

All the tribes analysed presented mercury levels in hair very much higher than the control population. The arithmetic and geometric means found for total mercury ranged from about 10 to 20 ppm and the controls presented values of about 1 ppm for the corresponding means.

The results obtained for methylmercury have shown that most part of the element is present in the hair of the Indians as the organic form.

The Indian populations studied living in the Xingu Park can thus be considered as being at risk as regards contamination by mercury.

With the aim of applying neutron activation analysis for the determination of methylmercury in hair, experiments were made of irradiation at the IEA-R1 nuclear research reactor of cysteine and also thyoacetamide impregnated filter paper on which a methylmercury solution was pipetted. The results obtained have shown that all the mercury was lost from the cysteine-impregnated paper and about 90 % of the mercury remained on the paper impregnated with thyoacetamide.

1. INTRODUCTION

As described in the last Progress Report, three main Brazilian populational groups were the object of our study:

1. Control group, of 38 subjects with no suspicion of contamination by mercury (friends, colleagues and students from the University of São Paulo).
2. Group of 28 people living near the Billings Dam, which is located in one of the most heavily industrialized parts of the country, where there is possibility of pollution by chloralkali and other industries. This group consumes fish caught at the Dam, without much control from public health

authorities.

3. Indian tribes living at the Xingu Park, located in the Amazonic region, where the gold extraction activities have risen much concern due to the use of mercury in the process.

The results obtained for analysis of mercury in hair of these groups by instrumental neutron activation analysis have shown that the Indians, from three tribes (Suiá, Uaurá and Panará) presented very high amounts of mercury, as compared to the control group. The averages obtained for these tribes were from about 9 to 18 times higher than the controls.

Samples of hair from the Suiá tribe were also sent to Jozef Stefan Institute in Ljubljana, Slovenia, for analysis of methylmercury.

The results obtained have shown that most part of the mercury in the hair of the Indians is present as methylmercury (70 to 100% MeHg, with average of 89%).

The populational group living near the Billings Dam, on the contrary, showed amounts of mercury in hair lower in average than the controls.

Considering the overall results obtained, it was concluded that the Indian tribes living in the Xingu Park could constitute a group at risk as regards contamination by mercury and methylmercury. This was quite surprising, according to the group of physicians from the São Paulo School of Medicine who are collecting the hair samples, since the region of the Park was up to now considered as being free from contamination by mercury, because it is far from the sites of intensive gold exploration in the Amazonic region.

Lacerda and Pfeiffer^[1] have carried out a study on mercury contamination arising from gold mining in the Amazon environment and have shown that the mercury concentrations in Amazonian fishes are, in various sites, nearly five times the maximum permissible ones for human consumption.

All this data confirm the importance of carrying out this kind of study in Brazil, due to the risk of contamination that the populational groups consuming high amounts of fish caught in the Amazonic rivers are suffering.

2. METHODS

2.1 COLLECTION AND WASHING OF HAIR SAMPLES

In this phase of the study, hair samples were collected from the following groups of Indians, living in the Xingu Park:

1. Coicuro Tribe (4th Group) - 46 samples collected.
2. Matipu Tribe (5th Group) - 11 samples collected.
3. Pavuru Tribe (6th Group) - 44 samples collected.
4. Juruna Tribe (7th Group) - 49 samples collected.

The three first groups studied were: Suiá Tribe, Uaurá Tribe and Panará tribe, of which the results for total mercury (all three groups) and methylmercury (Suiá Tribe) were presented in the last report.

All the samples were collected and washed according to the procedure recommended by the IAEA^[2].

2.2 DETERMINATION OF TOTAL MERCURY IN HAIR SAMPLES OF THE INDIANS FROM THE XINGU PARK BY INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS

Irradiation and measurements

About 100 to 200 mg of the prepared hair samples and of the reference material Chinese Human hair, SHINR-HH, were weighed in polyethylene envelopes previously washed with diluted nitric acid and desionized water. For each set of five samples, one reference material was analysed.

Irradiations were carried out for a period of one hour, in a pneumatic station, under a thermal neutron flux of about 10^{12} n.cm⁻².s⁻¹.

The standards were prepared by pipetting about 1 µg of mercury, in the nitrate form, onto sheets of Whatman No. 40 filter paper, previously impregnated with a solution of thioacetamide, to prevent mercury losses by volatilization before and during irradiation, as recommended by Noguchi et al^[3].

After a decay period of about 70 hours, samples, reference materials and mercury standards were measured in a GMX 20195 ORTEC Ge detector, with a resolution of 1.9 keV in the 1332 keV peak of ⁶⁰Co. The detector is coupled to an ADCAM 918A Multichannel Buffer and associated electronics.

Spectrum analysis was performed by means of VISPECT2 software, developed by D. Piccot, from Saclay, France^[4].

For calculation of mercury concentrations, the 77 keV peak of ¹⁹⁷Hg (*t*_{1/2} = 64.1 h) was used.

2.3 DETERMINATION OF METHYLMERCURY IN HAIR SAMPLES OF THE INDIANS FROM THE XINGU PARK

From the second group of Indians analyzed (Uaurá Tribe), whose results for total mercury were presented in the last report (n = 18), ten samples were sent to the Nuclear Chemistry Department of the Jozef Stefan Institute (Ljubljana, Slovenia) for analysis of methylmercury, by cold vapour atomic absorption spectroscopy. Total mercury was also analyzed in these samples.

From the sixth group of Indians (Pavuru Tribe) of which 44 hair samples were collected, 20 were analysed at IPEN for total mercury and 24 were sent to Ljubljana for analysis of total mercury and methylmercury.

The hair samples of the seventh group (Juruna Tribe) are being analyzed for total mercury by INAA at IPEN.

2.4 DETERMINATION OF SELENIUM IN HAIR OF SOME OF THE POPULATIONAL GROUPS STUDIED

As stated in the first divulgation of the CRP on analysis of mercury in hair of selected human populations, selenium is, besides mercury, an element of interest to the Programme.

Apparently it protects animals against the toxic effects of methylmercury and alters the tissue distribution and excretion of methylmercury ratio in tissues. This fact is due to the high affinity of methylmercury cations to selenides and diselenides.

In view of the fact that high amounts of mercury were found in the hair of the Indians from the Xingu Park although no symptoms of mercury intoxication could be detected in these populations, it was decided to start in this Project some analysis of selenium in hair by instrumental neutron activation analysis.

The groups studied up to now were: control group, group of the Billings Dam and the first group of Indians (Suiá Tribe).

Irradiations and measurements

Hair samples already analyzed for total mercury and the hair reference material SHINR-HH were irradiated for 90 seconds at the IEA-R1 nuclear research reactor, under a thermal neutron flux of 4×10^{11} n.cm⁻².s⁻¹ together with selenium standards.

The selenium standards were prepared by pipetting about 120 µg of selenium in the nitrate form onto sheets of Whatman No. 40 filter paper.

After a decay time of about 30 seconds, samples, reference material and standard were measured for 90 seconds in the γ-ray spectrometer already described in Item 2.2.

The short-lived radioisotope ^{77m}Se, with a half-life of 17.5 s was used for the selenium calculations.

2.5 EXPERIMENTS OF DETERMINATION OF METHYLMERCURY BY NEUTRON ACTIVATION ANALYSIS

Since no equipment, either of atomic absorption or gas chromatography is available at our Department for methylmercury analysis, it was decided to try a nuclear method for the analysis.

The method would be based on extraction of Me-HgCl in toluene followed by back extraction in filter paper impregnated with cysteine, as described by Horvat and Byrne^[5].

The paper containing MeHg in cysteine could then be irradiated together with mercury standards, in the same way as described for INAA of total mercury in hair (Item 2.2).

Since one of the problems in the irradiation of mercury compounds is the loss of the element by the effect of the radiation, it was decided to start the experiments by this last step.

2.5.1 PREPARATION OF THE METHYLMERCURY SOLUTION

The methylmercury solution to be irradiated was prepared by diluting to 1 : 50 a solution of methylmercury chloride, acquired from Johnson Matthey, and with a concentration of 1000 ppm.

2.5.2 PREPARATION OF THE CYSTEINE SOLUTION

As stated in the paper of Horvat and Byrne^[5], MeHg is extracted into aqueous cysteine solution only at neutral pH. So, a buffered solution with pH 7.00 was prepared by dissolving 21.20 mg of cysteine in a small amount of HCl 1 : 1 and adding a buffer of potassium diphosphate and sodium monophosphate.

2.5.3 IRRADIATION OF MeHg IN CYSTEINE-IMPREGNATED FILTER PAPER

An aliquot of 50 μL of the diluted MeHgCl solution, corresponding to about 1 μg of Hg was pipetted onto a sheet (2 x 2 cm) of Whatman No. 40 filter paper previously impregnated with the buffered cysteine solution (about 200 μg of cysteine were pipetted on each sheet of filter paper).

The filter paper was irradiated for 1 h under a thermal neutron flux of about $10^{12}\text{n.cm}^{-2}\text{s}^{-1}$ together with the Chinese Hair Standard, SHINR-HH-1.

2.5.4 IRRADIATION OF MeHg ON THIOACETAMIDE-IMPREGNATED FILTER PAPER

The MeHgCl solution was also pipetted on thioacetamide-impregnated filter paper, in the same way as for INAA analysis of total Hg and irradiated, together with the Chinese Hair Standard.

3 RESULTS

3.1 ANALYSIS OF REFERENCE MATERIALS

Analysis of a group of 10 samples of the Chinese Hair Reference Material, SHINR-HH-1, yielded an average of 2.15 ppm of Hg, with a relative standard deviation of 9.1 % and a relative error of 0.46 % as compared to the certified value of 2.16 ± 0.21 ppm.

As to the analysis of selenium in this reference material, the average obtained for six determinations was of 0.60 ppm Se, with a relative standard deviation of 21 % and a relative error of 3.4 % as compared to the certified value of 0.58 ± 0.05 ppm.

3.2 ANALYSIS OF HAIR SAMPLES

Table I presents the results obtained at the Jozef Stefan Institute for analysis of total mercury and methylmercury in hair of the Indians of the second group studied (Uaurá Tribe) which have been analyzed at IPEN for total mercury, as presented in the last Progress Report. The results of the first and third groups studied were also presented in the last Report.

In Table II are the results for total mercury in hair of the fourth group studied (Coicuro Tribe). The results were obtained at IPEN by INAA.

Table III presents the results for total mercury in hair of the fifth group of Indians (Matipu Tribe), also obtained by INAA.

In Table IV are the results for total Hg obtained by INAA of 20 samples of the sixth group of Indians (Pavuru Tribe). This group comprises 44 samples in the total, of which 24 were sent to the Jozef Stefan Institute (Ljubljana, Eslovenia) for analysis of total mercury and methylmercury by CVAAS.

Table V presents the results obtained for selenium by INAA in hair samples of 16 individuals from the control group. The very high results obtained for samples IQ3 and TFR9 were not considered for average calculations.

In Table VI are shown similar results for 15 individuals living near the Billings Dam, a highly industrialized region and whose results for total mercury have been previously presented. The very high result obtained for sample B23 was not considered for average calculations.

Table VII presents the concentrations of selenium obtained by INAA in the sample of the first group of Indians (Suiá Tribe) studied. The results of total mercury obtained by INAA for this group have been previously presented.

In Table VIII, a summary is presented of all the results obtained in this period of the Project for total mercury in hair of individuals from three Indian tribes (4th, 5th, 6th group) and for total mercury and methylmercury in the Uaurá Tribe (2nd group).

Table IX presents a summary of the results obtained for the analysis of selenium by INAA, for the control group, the group of the Billings Dam and for the Suiá Tribe (first group) of the Xingu Park.

Table I - RESULTS OF THE ANALYSIS OF TOTAL MERCURY AND METHYLMERCURY IN HAIR SAMPLES FROM THE UAURÁ TRIBE (2nd GROUP), OBTAINED AT THE JOZEF STEFAN INSTITUTE (LJUBLJANA, SLOVENIA)

Sample Code Number	Total Hg (ppm)	Methyl-Hg (ppm)	% Methyl-Hg
210	-	7.55	-
418	-	12.9	-
537	11.5	9.22	80
705	13.9	11.5	83
727	10.1	7.70	76
937	21.7	11.6	53
5099	-	10.0	-
5192	12.1	11.0	90
5262	13.1	11.5	88
6029	10.1	9.21	91
	n = 7	n = 10	
	x = 13.2	x = 10.2	
	s = 4.0	s = 1.8	
	x _G = 12.8	x _G = 10.1	
	s _G = 1.3	s _G = 1.2	
	median = 12.1	median = 10.5	

Table II - RESULTS OF THE ANALYSIS OF TOTAL MERCURY BY INAA IN HAIR OF THE INDIANS FROM THE COICURO TRIBE (4th GROUP), FROM THE XINGU PARK

Sample Code Number	Total Mercury (ppm)
132	12.6
133	16.9
135	14.5
176	25.3
334	16.0
371	17.9
380	13.4
381	17.5
382	13.8
383	10.3
390	16.2
397	20.2
513	10.0
514	12.9
578	12.3
583	11.1
585	15.3
594	12.1
595	13.9
597	11.3
607	11.5
611	16.7
616	6.8
617	10.0
618	11.7
650	12.0
653	15.1
679	4.8
813	7.2
820	15.8
821	15.8
895	7.3
917	9.9
928	13.7
957	13.0
5018	10.2
5040	17.8
5051	11.1
5055	16.9
5057	13.3
5085	11.8
5127	16.7
5147	13.0
5148	10.0
5185	12.6
5200	8.8

n = 46
range = 4.8 - 25.3

\bar{x} = 13.2 s = 3.8
 \bar{x}_G = 12.7 s_G = 1.4

median = 13.0

Table III - RESULTS OF THE ANALYSIS OF TOTAL MERCURY BY INAA IN HAIR OF THE INDIANS FROM THE MATIPU TRIBE (5th GROUP), FROM THE XINGU PARK

Sample Code Number	Total Mercury (ppm)
3	8.3
286	11.5
316	13.0
389	9.2
633	7.3
634	1.7
653	15.1
682	12.2
692	10.4
5156	12.9
5198	14.7

$n = 11$ $\bar{x} = 10.6$ $s = 3.9$
 $\text{range} = 1.7 - 15.1$ $s_G = 9.4$ $s_G = 1.9$ $\text{median} = 11.5$

Table IV - RESULTS OF THE ANALYSIS OF TOTAL MERCURY BY INAA IN HAIR OF THE INDIANS FROM THE PAVURU TRIBE (6th GROUP), FROM THE XINGU PARK

Sample Code Number	Total Mercury (ppm)
305	18.1
425	18.7
435	13.0
438	24.4
440	18.6
444	22.7
447	28.2
448	17.6
449	17.5
452	8.1
458	12.2
463	13.0
466	13.0
480	21.6
524	27.7
883	21.8
5115	20.5
5278	18.9
5280	18.8
5353	57.3

$n = 20$ $\bar{x} = 20.6$ $s = 10.0$
 $\text{range} = 8.1 - 57.3$ $s_G = 19.0$ $s_G = 1.5$ $\text{median} = 18.8$

Table V - RESULTS OF THE ANALYSIS OF SELENIUM BY INAA IN HAIR OF 16 INDIVIDUALS FROM THE CONTROL GROUP

Sample Code Number	Selenium Concentration (ppm)
C1	0.42
C3	0.47
C4	0.39
C5	0.34
C6	0.50
IQ1	0.43
IQ3	84.3
TFR1	0.42
TFR2	0.44
TFR3	0.44
TFR4	0.48
TFR5	0.36
TFR6	0.46
TFR7	0.40
TFR8	0.45
TFR9	7.43

$n = 16$ $\bar{x} = 0.43$ $s = 0.04$
 range = 0.34 - 84.3 $\bar{x}_G = 0.43$ $s_G = 1.11$ median = 0.43
 Obs.: For the calculation of averages and of the median the values for samples IQ3 and TFR9 were not considered

Table VI - RESULTS OF THE ANALYSIS OF SELENIUM BY INAA IN HAIR OF 15 INDIVIDUALS FROM THE GROUP OF THE BILLINGS DAM

Sample Code Number	Se Concentration (ppm)
B2	0.33
B3	0.26
B4	0.53
B5	0.43
B6	0.33
B7	0.27
B8	0.46
B9	0.39
B10	0.33
B11	0.52
B12	0.30
B13	0.41
B14	0.17
B15	0.64
B23	26.8

$n = 15$ $\bar{x} = 0.38$ $s = 0.12$
 range = 0.17 - 26.8 $\bar{x}_G = 0.36$ $s_G = 1.41$ median = 0.36

Table VII- RESULTS OF THE ANALYSIS OF SELENIUM BY INAA IN HAIR OF 15 INDIVIDUALS FROM THE FIRST GROUP (SUIÁ TRIBE) FROM THE XINGU PARK

Sample Code Number	Selenium Concentration (ppm)
1225	0.33
1226	< 0.28
1228	< 0.28
1230	0.57
1234	0.34
1241	0.37
1242	0.86
1244	< 0.28
1245	0.40
1247	< 0.28
1248	0.64
1250	0.39
1251	0.25
1253	0.84
1255	0.35
1269	0.50
1274	< 0.28
1277	< 0.28
1278	< 0.28
1280	0.53
1281	0.46
1285	0.32
1286	< 0.28
1293	0.41
1324	0.50
1341	0.51
1652	< 0.28

n = 27
range = <0.28 - 0.86

\bar{x} = 0.47
 \bar{x}_G = 0.45

s = 0.01
 s_G = 1.39

median = 0.43

Table VIII - SUMMARY OF THE RESULTS OBTAINED FOR TOTAL MERCURY AND METHYLMERCURY CONTENTS IN THE HAIR OF THE POPULATIONAL GROUPS STUDIED (in ppm)

POPULATIONAL GROUP	X	S	X _G	S _G	Median	Range
COICURO TRIBE (4 th Group) (*)	13.2	3.8	12.7	1.4	13.0	4.8 - 25.3
MATIPU TRIBE (5 th Group) (*)	10.6	3.9	9.4	1.9	11.5	1.7 - 15.1
PAVURU TRIBE (6 th Group) (*)	20.6	10.0	19.0	1.5	18.8	8.1 - 57.3
UAURÁ TRIBE (2 nd Group) (*)	13.2	4.0	12.8	1.3	12.1	11.5 - 21.7
UAURÁ TRIBE (2 nd Group) (**)	10.2	1.8	10.1	1.2	10.5	7.7 - 12.9

* Values for total mercury

** Values for methylmercury

Table IX - SUMMARY OF THE RESULTS OBTAINED FOR SELENIUM CONTENTS IN HAIR OF THREE OF THE POPULATIONAL GROUPS STUDIED (in ppm)

POPULATIONAL GROUP	X	S	X _G	S _G	Median	Range
CONTROL GROUP	0.43	0.04	0.43	1.11	0.43	0.34-84.3
BILLINGS DAM GROUP	0.38	0.12	0.36	1.41	0.36	0.17-26.8
SUIÁ TRIBE (1 ST GROUP)	0.47	0.01	0.45	1.39	0.43	<0.28-0.86

4 DISCUSSION

The results of the analysis of 10 samples of the Chinese Hair Reference Material, SHINR-HH-1, of 2.15 ppm of total Hg, showed good agreement with the certified value, of 2.16 ± 0.21 ppm. The relative standard deviation was of 9.1%.

The analysis of total mercury in the hair of four Indian tribes (Coicuro, Matipu, Pavuru and Uaurá) showed that the arithmetic means, geometric means and medians obtained were very much higher than the corresponding values for the controls, presented in the last report (around 1 ppm).

In Table VIII it can be noted that the Coicuro, Matipu and Uaurá Tribe presented mean values from about 10 to 13 ppm and the Pavuru Tribe values of around 20 ppm.

The results obtained at the Jozef Stefan Institute for Me-Hg in the Uaurá Tribe showed that most part of the mercury contained in the hair of these Indians is presented as Me-Hg (about 80% in average).

Together with the values for Hg and MeHg presented in the last report, it can be concluded that all the Indian Tribes studied up now could be at risk as regards contamination by mercury.

At first sight it could be concluded that this contamination could arise from consumption of fish caught in the rivers of the Xingu Park, since these populations consume fish almost daily.

As stated in the Introduction, Lacerda and Pfeiffer^[1] have shown that the mercury concentrations in some Amazonian fishes are, in various rivers, nearly five times the maximum permissible ones for human consumption.

Anyway, a more detailed study of several compartments of the Xingu Park such as water, sediments, aerosols and other foodstuffs consumed by the Indians must be carried out.

As to the analysis of selenium in hair, no significant difference was found among the Control Group, the Group of the Billings Dam and the Suiá Tribe.

The means and medians presented were very similar, all around 0.4 ppm of selenium.

The irradiation experiments carried out for MeHgCl solution pipetted on filter paper impregnated with cysteine and thioacetamide showed that in the first case practically all the mercury was lost.

The paper impregnated with thioacetamide presented better results, as about 89.5% of the Hg pipetted as CH₃HgCl remained on the filter paper after irradiation.

5 PLANS FOR FUTURE WORK

The following scheme is devised for the next period of the Research Contract:

1. Collection of additional hair samples from Indian tribes living in the Xingu Park.
2. Preparation of the hair samples collected (cutting, washing, drying) according to the procedure recommended by the IAEA.
3. Analysis of reference materials.
4. Analysis of total mercury in the hair samples by instrumental neutron activation analysis.
5. Analysis of methylmercury in part of the hair samples by CVAAs, at the Jozef Stefan Institute (Ljubljana, Slovenia).
6. Experiments for analysis of methylmercury by neutron activation analysis:
 - Extraction of MeHg from the samples by hydrochloric acid, as in the method of May et al.^[6].
 - Extraction of MeHg in toluene, twice, as in the method of Horvat and Byrne^[5].
 - Re-extraction with cysteine solution.

This step can also be substituted by shaking of the toluene phase with cysteine paper. The paper could be irradiated directly for neutron activation analysis.

7. Analysis of selenium in the hair samples of Indians, via the short lived isotope ^{77m}Se (*t*_{1/2} = 17.5 s).

ACKNOWLEDGMENTS

The authors would like to thank CNPQ and FAPESP (Brazil) and the International Atomic Energy Agency for financial support.

Also thanks are due to Dr. A.R. Byrne, from the Nuclear Chemistry Department, Jozef Stefan Institute, Ljubljana, Slovenia, for analysis of total mercury and methylmercury by CVAAS.

REFERENCES

[1] L.D. LACERDA, W.C. PFEIFFER, Mercury from gold mining in the Amazon environment - an overview, *Química Nova*, **15** (2) (1992) 155.

[2] Reference Methods for Marine Pollution Studies, No. **46** (draft), October 1987.

[3] K. NOGUCHI, M. SHIMIZU, K. MORIKAWI, Activation analysis of mercury in head hair of dentists, *Radioisotopes*, **29** (1980) 221-5.

[4] D. PICCOT, Personal Communication.

[5] M. HORVAT, A.R. BYRNE, K. MAY, A modified method for the determination of methylmercury by gas chromatography, *Talanta*, **37** (1990) 207-12.

[6] K. MAY, M. STOEPLER, K. REISINGER, *Toxicol. Environ. Chem.*, **13** (1987) 153.