VALIDATION OF THE INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS METHOD FOR MERCURY ANALYSIS IN HAIR SAMPLES USED FOR BIOMONITORING OF MERCURY ENVIRONMENTAL CONTAMINATION

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Abstract

VALIDATION OF THE INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS METHOD FOR MERCURY ANALYSIS IN HAIR SAMPLES USED FOR BIOMONITORING OF MERCURY ENVIRONMENTAL CONTAMINATION.

Environmental mercury contamination has lately become of widespread concern in Brazil owing to gold extraction activities in the Amazon region and also to industrial activities. It is estimated that tonnes of mercury are thrown in the rivers of the Amazon region annually or are evaporated into the open air owing to the amalgamation procedure employed in gold extraction. In the present work, as part of an IAEA Co-ordinated Research Programme (CRP), three main population groups were studied: a control group of individuals with no suspicion of contamination by mercury; a group of people living near the Billings Dam, located in one of the most heavily industrialized parts of the country, and several Indian tribes living in Xingu Park, an Indian reservation located in the Amazon region. An important part of the CRP was the validation of the analytical methodology for the analysis of total mercury in the hair samples of the population groups studied. Instrumental neutron activation analysis (INAA) was chosen for the analysis of mercury in these samples. It has the advantage of being non-destructive, which can be quite convenient when dealing with a large number of samples, which is the case in epidemiological studies. The experimental procedure consisted of irradiation of the hair samples in the IEA-R1 nuclear research reactor and measurement of the gamma radioactivity of ^{197}Hg. For the validation of the analytical methodology, the following reference materials (RMs) were analysed: IAEA MA-A-2/TM Fish Flesh Homogenate; SHINR-HH GBW-9101 Chinese Human Hair and the candidate RMs IAEA-085 and IAEA-086 Human Hair. The recently acquired RM BCR CRM-397 was also analysed. The accuracy and precision of the INAA method for the analysis of these RMs is discussed, as well as the applicability of the method to the analysis of mercury in the hair of the population groups studied.

1. INTRODUCTION

Contamination of the environment by mercury has become of general concern in Brazil in recent years owing to increasing industrial activities and mainly to gold extraction by amalgamation with mercury in the central and northern states of the country, mainly in the Amazon region [1, 2]. High levels of mercury in fish caught in the rivers of the region, as well as in human hair and urine, have been detected by different authors in the Tapajós, Madeira and Negro River Basins [3, 4].

The Radiochemistry Divison of Instituto de Pesquisas Energeticas e Nucleaires (IPEN/CNEN-SP) has recently been engaged in a similar kind of work, in the framework of an IAEA Co-ordinated Research Programme (CRP) entitled 'Environmental Exposure to Mercury in Selected Human Populations as Studied by Nuclear and Other Techniques' [5]. In this CRP, hair was chosen as the biological monitor owing to its ease of collection, stability during storage and the fact that it can trace the history of the contamination during the period of time in which it remains on the head [6].

In the case of the Brazilian project, the main focus of the study was an Indian reservation located in Xingu Park, in the Amazon region, where several Indian tribes have lived for many years. Xingu Park is at a considerable distance from the gold exploration sites of the Amazon region and initially it was supposedly free of mercury contamination. The collection and analysis of hair of individuals from ten of these tribes have shown mercury concentrations several orders of magnitude higher than those found in a control population.

For the analysis of total mercury in hair samples, instrumental neutron activation analysis (INAA) was the method of choice owing to its non-destructive character and adequate sensitivity for the levels of mercury found in hair. An important part of the project was also the validation of the analytical methodology for mercury determination, which was very much stimulated by the IAEA CRP.

For this validation, the following reference materials were analysed: IAEA MA-A-2/TM Fish Flesh Homogenate, SHINR-HH GBW-9101 Chinese Human Hair, and the candidate RMs IAEA-085 and IAEA-086 Human Hair. The recently acquired RM BCR CRM-397 was also analysed.

2. EXPERIMENTAL

2.1. Collection and washing of hair samples

The hair samples were collected and washed according to the protocol recommended by the IAEA [7]. The samples were cut using stainless steel scissors, from the occipital area of the head and as close as possible to the scalp in an amount corresponding to about 2 g.

The hair was then cut with the scissors into segments as short as possible and transferred to a glass vial for washing with acetone. The samples were covered completely with the solvent and stirred at frequent intervals for 10 min, and the solvent carefully decanted. After drying of the solvent at room temperature, the hair was homogenized and washed three times with distilled water. A final washing step with acetone was then carried out and the samples were left to dry in the open, at this point being ready for analysis.

2.2. Determination of total mercury in hair and in RMs by INAA

Owing to the need to analyse hundreds of hair samples for this study, it was considered to be more convenient to use INAA for total mercury determination and to use polyethylene envelopes for irradiation, which are cheaper and easier to handle than quartz ampoules. About 100–200 mg of the prepared hair samples and of the RM were weighed in polyethylene envelopes previously washed with diluted nitric acid and deionized water. Irradiation was carried out for a period of 1 h in a pneumatic station under a thermal neutron flux of about 10^{12} n·cm^{-2}·s^{-1} in the IEA-R1 research reactor.

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. [8].

After a decay period of about 70 h, samples, RMs 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 ^{60}Co. The detector is coupled to an ADCAM 918A multichannel buffer and associated electronics. Spectrum analysis was performed by means of the VISPECT2 software developed by D. Piccot from Saclay, France [9]. For the calculation of mercury concentrations, the 69 and 77 keV peaks of ^{197}Hg ($T_{1/2}$ = 64.1 h) were used.

3. RESULTS AND DISCUSSION

Table I presents the results obtained for the analysis of total mercury by INAA in the following RMs: IAEA MA-A-2/TM Fish Flesh Homogenate; SHINR-HH GBW-9101 Chinese Human Hair; BCR CRM-397; and in the candidate RMs IAEA-085 Human Hair, elevated level and IAEA-086 Human Hair, low level.

It can be observed that the relative errors obtained for the human hair RMs were very good: 1.3% for Chinese Human Hair and 2.8% for BCR CRM-397. The relative error for the RM of marine origin, i.e. Fish Flesh Homogenate, of 5.7% was not as good as for the hair RMs, but still acceptable at this concentration level. This could

TABLE I. ANALYSIS OF MERCURY IN RMs BY INAA

Reference material	ppm Hg (certificate)	ppm Hg (present work)[a]	Relative error (%)	Relative standard deviation (%)
IAEA MA-A-2/TM Fish Flesh Homogenate	0.47 ± 0.02	0.50 ± 0.06	5.7	9.4
SHINR-HH GBW-9101 Chinese Human Hair	2.16 ± 0.21	2.13 ± 0.05	1.3	7.8
IAEA-085 Human Hair	[b]	26.8 ± 1.1	[b]	8.7
IAEA-086 Human Hair	[b]	0.66 ± 0.04	[b]	9.5
BCR-CRM 397 Human Hair	12.3 ± 0.5	12.0 ± 0.9	2.8	7.2

[a] Confidence limits at the 95% level.
[b] Material in the process of certification.

be explained by the difference in matrix composition, since the materials of marine origin present high activities, owing to the high concentrations of elements such as sodium and bromine, and consequently present more complex gamma ray spectra, the hair matrix being cleaner for INAA.

The relative standard deviations present higher values than the relative errors and varied between about 7 and 9%, which can also be considered as being acceptable for analysis at the ppm level.

Table II presents some of the results obtained for the analysis of hair samples in the framework of the IAEA CRP entitled 'Assessment of Environmental Exposure to Mercury in Selected Human Populations as Studied by Nuclear and Other Techniques' [10]. It can be observed that the means varied from less than 1 ppm (group of residents at Billings Dam, São Paulo, Brazil) to more than 20 ppm (Indian Group 6), which corresponds approximately to the concentration range of the RMs analysed.

One important conclusion that arose from the CRP, apart from considerations about the validation of the methodology, was that all the Indian tribes analysed present concentrations of mercury in hair much higher than the controls, which could mean that they are at risk of mercury contamination. The most probable source of contamination is fish, which is the main source of protein for these populations, but

TABLE II. SUMMARY OF THE RESULTS OBTAINED FOR MERCURY CONTENTS IN THE HAIR OF THE BRAZILIAN POPULATION GROUPS STUDIED

Population group	$\bar{x}$	$\bar{s}$	Median	$\bar{x}_G$	Range
Controls	1.06	0.61	0.96	0.90	0.26–2.9
Billings Dam	0.88	0.68	0.74	0.71	0.30–3.0
Indian group 1	18.50	5.9	18.0	17.1	6.87–34.3
Indian group 2	12.0	4.0	10.7	11.4	6.54–21.6
Indian group 3	8.7	3.0	8.2	8.2	4.5–18.5
Indian group 4	13.2	3.8	13.0	12.7	4.8–25.3
Indian group 5	10.6	3.9	11.5	9.4	1.7–15.1
Indian group 6	20.6	10.0	18.8	19.0	8.1–57.3
Indian group 7	16.5	5.5	15.8	15.5	2.5–30.2
Indian group 8	17.2	6.0	16.2	16.3	2.10–31.7
Indian group 9	17.7	4.1	16.6	17.3	10.9–25.0
Indian group 10	8.1	9.0	2.8	4.7	1.5–33.1

$\bar{x}$ = arithmetic mean
$\bar{x}_G$ = geometric mean

further investigation has to be conducted in the region of Xingu Park and materials other than hair have to be analysed, such as fish, sediments, plants and aerosols.

The INAA method can be considered as adequate for the monitoring of total mercury in the hair of the populations that were object of the study as regards accuracy, precision and required sensitivity.

The procedure employed for INAA, with a relatively short irradiation time of 30 min to 1 h and using mercury standards pipetted on filter paper impregnated with thioacetamide to avoid loss of mercury during irradiation, was convenient to handle the number of samples involved in this kind of study. Up to the present time about 400 hair samples have been analysed and many more analyses will have to be done, since the study is being extended to other regions of Brazil where gold exploration occurs.

4. CONCLUSIONS

The analysis of several RMs, mainly of human hair matrices, has shown that the INAA method was very adequate in terms of accuracy, precision and sensitivity for monitoring mercury levels in the hair of several Brazilian population groups. The procedure adopted for INAA, using relatively short irradiation times and mercury

standards pipetted on filter paper impregnated with thioacetamide and encapsulated in polyethylene envelopes, allowed the determination of mercury in about 400 hair samples.

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