PREPARATION OF STANDARD HAIR MATERIAL AND DEVELOPMENT OF ANALYTICAL METHODOLOGY

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Abstract

The concept of the use of human scalp hair as a first level indicator of exposure to inorganic pollutants has been established by us earlier. Efforts towards the preparation of a hair reference material are described. The analytical approaches for the determination of total mercury by cold vapour AAS and INAA and of methylmercury by extraction combined with gas chromatography coupled to an ECD are summarized with results on some of the samples analyzed, including the stability of values over a period of time of storage.

1. INTRODUCTION

The potential of hair as an indicator of environmental exposure was discussed at the first Advisory Group Meeting in 1976, when we had suggested a possible approach [1]. This approach was substantiated by our study of countrywide student population and general population of metropolitan city of Bombay [2] , which clearly established the role of human scalp hair as an effective first level monitor in a multi-level scheme of monitoring environmental exposure to inorganic pollutants. It is in this context that we offered to provide an adequate material of hair, subjected to minimal chemical treatment as dictated by the social customs and the ready availability of relatively large quantities. The characterization of the hair sample with respect to trace elements in general, and mercury in particular, requires the cross validation for which different analytical techniques need to be developed and applied. The programme of work carried out since the second meeting of CRP had the following objectives:

(a) To prepare a large quantity of hair material for despatch to Vienna towards a reference material;

(b) to measure total and methylmercury in a variety of hair samples; and

(c) to measure mercury levels in a restricted population, whose staple diet happens to be fish.

2. STANDARD MATERIAL OF HAIR

The first lot of 35 kgs of hair purchased from a temple at Tirupati did not give a sufficient amount of clean hair (< 10 kg) after processing at the Bhabha Atomic Research

Centre, which followed the preliminary processing at Tirupati. Accordingly, additional 25 kg hair was procured and, after the preliminary processing of physical cleaning, washing with acetone-water and finally with acetone at Tirupati, this was mixed with the other major portion. The total sample was then cut into small pieces, between 5 to 10 mm lengths, and then subjected to the standard washing procedure (acetone, followed by three times with deionized distilled water, and finally again with analytical grade acetone), followed by air drying). All these operations were carried out in a "clean" room of the laboratory using non-metallic containers, which were initially cleaned in 1% nitric acid. It is to be noted that a more careful examination of the thoroughly cleaned sample gave a ratio of weight of uncleaned (as procured) to final processed hair at nearly 6:1 instead of 2.5:1 as reported earlier.

Several aliquots of these samples were analyzed for both total mercury and methylmercury, the total mercury determination using both cold vapour atomic absorption and instrumental neutron activation analysis. The other elements determined were selenium, zinc, cadmium and cobalt using Cold Vapour Atomic Absorption Spectrometry (CVAAS), Instrumental Neutron Activation Analysis (INAA), Graphite Furnace Atomic Absorption Spectrometry (GFAAS) and Square Wave Cathodic Stipping Voltammetry (SWCSV). Ten kg of this final material was packaged in two containers, each containing about 5 kg, sealed in polyethylene bags, radiation sterilized to 50 KGy at ISOMED of Board of Radiation & Isotope Technology, and was shipped by air to Vienna in May 1993.

We are glad to note that the homogenization process has been successfully completed as also the spiking and that the samples are already out for intercomparison experiments.

3. SAMPLES ANALYZED

The samples that have been analyzed during this period are: 1) aliquots of hair samples drawn from the bulk of the material; 2) hair samples collected from other rural population groups whose staple diet is fish; 3)the old fish homogenate of the IAEA; and 4) the new homogenized hair samples 085 and 086. One vial of sample containing 085 which was received in February 1994 for possible use at the Agency's Workshop on Environmental and Industrial Applications of Nuclear Analytical Techniques was analyzed for both total mercury and methylmercury. The two samples, 085 and 086 sent for intercomparison exercises were received only in May 1994 and as such, these could not be analyzed for the required constituents. These results will be reported at a later time.

The basic principles of the experimental approaches followed for the determination of total mercury by CVAAS and INAA and of methylmercury by gas chromatography are briefly summarized below.

3.1. Total mercury: CVAAS

The hair sample is digested repeatedly with a mixture of nitric and sulphuric acid in a modified Bethge's apparatus. The last traces of organic matter are destroyed by fuming with perchloric acid. Mercury vapour is generated by stirring a sample aliquot with $SnCl_2$ solution which is then flushed with purified air into the absorption cell. A mercury vapour

lamp is used as the source of the incident beam. The concentration of mercury in the sample is worked out from a calibration graph.

3.2. Total mercury : INAA

The samples and standard solutions are sealed independently in quartz ampoules and irradiated at a flux of $10^{12} cm^{-2} \cdot s^{-1}$ in a pool reactor in which the temperature of water does not rise beyond 40°C; blank quartz ampoules are also included in the irradiation. Quantitation of radionuclides is based on two time-dependant measurements and, wherever possible, on more than one γ-ray energy. These measurements are carried out using a high purity germanium detector coupled to multi-channel pulse height analyzer.

3.3. Methylmercury

The hair sample is disintegrated in a solution of sodium hydroxide in presence of L-cysteine hydrochloride. MeHg is extracted from an aliquot of the solution into benzene and after the removal of emulsion, a small volume is injected into a gas chromatograph, which is equipped with a column of diethylene glycol succinate on Supelcoport, 100-200 mesh. MeHg is detected with an electron capture detector and its amount is determined by comparing the peak heights/areas with those of appropriate standards.

4. RESULTS

Some of the results are summarized in Tables I through IV. The analysis of samples from the West Coast of India from small towns and villages, who are basically fishermen, showed a large variation in the concentration of mercury. However, the maximum value was only marginally higher than that for normal population. The stability of values for total mercury and also methylmercury has been demonstrated by analysis of the same sample over a period of 1-2 years, as indicated in Tables I and II.

5. PLANS FOR FUTURE WORK

In future work we plan to look at other approaches to the determination of forms mercury, particularly methylmercury. More importantly, we also plan to analyse for selenium in some of the samples that we have already collected from specific population groups for determining mercury.

REFERENCES

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[2] GANGADHARAN, S., ARUNACHALAM, J., BHAT, K.R., YEGNASUBRAMANYAN, S., Neutron Activation Analysis in the Monitoring of Health-related Trace Element Pollutants, IAEA/TECDOC-330 (1985) 147-154.

TABLE I. TOTAL MERCURY (CVAAS) μg/gm

Hair Sample	30/07/92	17/09/93	04/0294
TPT 4	0.44	0.40	0.47
TPT 5	0.39	0.41	0.38
TPT 9	0.39	0.37	0.34
TPT 11	0.37	0.37	0.40

(Values reported on 04/02/94 are from the recent IAEA Workshop)

TABLE II. STABILITY OF METHYMERCURY IN HAIR SAMPLE

Sample	MeHg (μg/g ± SD)	
TPT 4	18/01/93	25/01/94
	0.40 ± 0.02	0.40 ± 0.02

TABLE III. MERCURY AND METHYLMERCURY

Sample	Concentration μg/g	
	Total Hg (INAA, CVAAS)	MeHg (GC - ECD)
Fish Homogenate (MA-A-2)	0.5 ± 0.03	0.35 ± 0.04
Fish Homogenate (305)	0.41 ± 0.03	0.31 ± 0.01
Hair (IAEA-085) (Received Feb. 1994)	19.5 ± 0.5	12.2 ± 0.2
Indian Population Hair	0.4 ± 0.03	0.35 ± 0.02

TABLE IV. TOTAL MERCURY IN HAIR

Sample	CVAAS	INAA	
		^{197}mHg	^{203}Hg
IAEA-085 (Bottle No. 3)	22.0 ± 0.4	26 ± 0.1	32
IAEA-086 (Bottle NO. 4)	3.3 ± 0.1	0.5	0.6

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