

DETERMINATION OF TOTAL AND METHYLMERCURY COMPOUNDS IN THE IAEA HUMAN HAIR INTERCOMPARISON SAMPLES - EXPERIENCE OF THE IAEA-MEL

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1. INTRODUCTION

The IAEA-MEL, Marine Environmental Studies Laboratory (MESL) in Monaco has developed considerable expertise in Quality Assurance/Quality Control for non-radioactive contaminants (trace elements and trace organics). It provides AQCS services to the Member States through the number of activities, such as, organization of intercomparison exercises, production and distribution of reference materials and provision of training courses for trace elements, and trace organic contaminant determinations. Most of its activities are funded through the UNEP's Regional Seas Programme. In recent years, MESL has also performed a lot of research on organometals in the marine environment (organomercury and organotin compounds), particularly on method development and testing. As a result, two reference materials were given a recommended value for methylmercury: IAEA-350, Tuna Fish Homogenate [1] and IAEA-356, Polluted Marine Sediment [2]. Future intercomparison samples for trace elements will also include methylmercury as one of the important parameter to be measured. In 1994, two such intercomparison samples will be prepared and distributed: a mussel tissue and a sea plant homogenate.

The programme of this CRP is focused on the analyses of human hair samples. There are only two human hair samples certified for total mercury, and no RMs for methylmercury compounds is available. One of the main objectives of the this CRP is to produce, through the IAEA AQCS Programme, a human hair intercomparison material for quality assurance requirements in population monitoring programmes for total and methylmercury exposure.

Through the reporting period, MESL has introduced a new method for simultaneous determination of total and methylmercury in biological samples. As the laboratory has close collaboration with the CRP's Reference Laboratory in Ljubljana, Slovenia, it has also been actively involved in the quality assurance component of this CRP. This report represents a summary of the results for total and methylmercury in two intercomparison samples, IAEA-085 and IAEA-086 using newly developed method.

2. EXPERIMENTAL

2.1. Determination of methylmercury compounds

The method consists of alkaline digestion (KOH/methanol) followed by aqueous phase ethylation, room temperature precollection on Tenax (or Carbotrap) separation of ethylated mercury species on the GC column (a wide bore capillary column Supelco SPB™, 30 m long, operated isothermally at 58°C). Separated mercury species were then transformed into elemental Hg by heating at 900°C and swept into atomic fluorescence detector (CV

AFS). The method is schematically presented in Figure 1 and described in details in other publications [3,4].

A comparative method based on the modified Westoo extraction procedure and GC-ECD detection was also used. Precise description is given in the publication [5]. Briefly after acidification of the alkaline digested sample, methylmercury bromide was extracted into toluene. In the clean-up step, methylmercury was adsorbed onto the paper impregnated with cysteine. After the cysteine paper was washed with fresh portions of toluene and dried, methylmercury bromide was back extracted into toluene after acidification. Finally, methylmercury was then measured by gas chromatography equipped with electron capture detector (ECD).

2.2. Determination of total mercury

Approximately 0.1 to 0.25 g of the sample was digested with 4 mL HNO₃ (Merck, Selectipur, 70%) and 0.5 mL of HCl (Merck, Suprapur, 36%) in closed Teflon vials (25 ml, Savillex Corp., USA) at 90°C for 6 to 12 hours. Before samples were diluted to the final volume (50 mL), 1 mL of BrCl oxidation solution was added. Samples were left for few hours before 0.1 mL of 10% hydroxyl amine hydrochloride solution was added to remove the excess of BrCl oxidizing solution. Samples were then diluted to the final volume with deionized water (MilliQ). An aliquot of the sample was introduced to the SnCl₂ reduction, aeration, amalgamation process. Finally, mercury was detected by CV AAS (Perkin-Elmer 1100, MHS-20) and/or CV AFS (double amalgamation technique coupled with a Brooks Rand, Ltd. AFS detector).

2.3. Data quality control

To control the accuracy of the measurements, a CRM, NIES No. 5, Human Hair sample was initially used. It was analyzed in duplicates in each batch of the analyses. Results obtained for total mercury compared well with certified value. Methylmercury has not been certified in this material. However, data obtained compared well with the results of previously methodological intercomparison study [6,7]. Unfortunately, during the reporting period, this sample was no longer unavailable. Therefore, a new intercomparison Human Hair sample from NIES No. 13 was used. Results for total and methylmercury in this sample are very similar to NIES No. 5. Results compared well with the preliminary results obtained from NIES (personal communication).

The accuracy of the results for both total and methylmercury determinations was also checked by analyzing a RM IAEA-350, Tuna Fish Homogenate. Results are given together with Tables of the results.

3. RESULTS AND DISCUSSION

3.1. Determination of methylmercury in IAEA-086 (spiked) human hair sample before grounding

Spiked human hair samples, cut in 2 - 4 mm segments, were received in four batches, marked as A, B, C, and D. Two types of experiments were performed.

- (a) Methylmercury was determined in each batch of the sample marked as A, B, C, and D. Analyses were performed in duplicates (sample weight varied from 20 to 50 mg) using an alkaline digestion, followed by aqueous phase ethylation, room temperature precollection on Carbotrap, isothermal GC and CV AFS detection. Comparative analyses by a modified Westoo procedure (extraction, GC-ECD) was also done.
- (b) The samples (without any pretreatment) were leached with toluene in order to check any possible release of methylmercury from the surface. Measurements were performed by GC-ECD.

Results are given in Table I. The overall methylmercury concentration was 26.34 ± 2.00 mg/kg (RSD = 7.6 %) (expressed as Hg). Relatively high RSD is probably related to the small sample weights. No significant difference of the results between the batches A, B, C, and D could be observed.

To control the accuracy of the measurements, a CRM NIES No. 13, Human Hair sample was analyzed together with the rest of the samples. The result obtained for methylmercury was 3.80 ± 0.18 mg/kg based on the analysis of four independent aliquots. Aliquots of the alkaline digested samples A(b) and C(c) were also processed using a modified Westoo procedure [6]. Two results presented in Table I compared well with other results.

3.1.1. Leaching with toluene

In order to check whether spiked methylmercury is well bound to the matrix, a leaching into toluene has been performed. Approximately 200 mg of each batch of the sample was put in a centrifuge Teflon vials and 5 mL of toluene was added. Each batch was done in duplicate. After two hours of shaking, an aliquot of 2 μ L was injected onto the GC column. No detectable amounts of methylmercury were measured (the absolute detection limit of measurements was 10 pg of methylmercury). Measurements were repeated after 24 hours of leaching. Even after this long period, there was no methylmercury detected. This indicated that methylmercury is mainly bound to the human hair structure.

3.2. Determination of methyl and total mercury after grounding and homogenization

3.2.1. Methylmercury

IAEA-085 (spiked) was received in nine different bottles. Methylmercury was isolated from the sample using three pre-separation procedures. Sample weights varied from 0.03 to 0.05 g.

- (1) Alkaline digestion (25% KOH in methanol) at 90°C for 12 hours
- (2) Distillation (aqueous phase, NaCl/sulphuric acid)
- (3) HCl leaching at room temperature (6M HCl, 12 hours)

For each pre-separation technique, the following detection system was used. An aliquot of the sample (alkaline digested, or distillate, or HCl leachate) was transferred into a bubbler for aqueous phase ethylation, room temperature pre-collection on Carbotrap (or Tenax), separation of ethylated mercury species on a GC column and determination by CV AFS.

The IAEA-086 was received in six bottles and the analyses were performed only with alkaline digestion method, followed by ethylation, GC, and CV AFS detection.

Each result in Tables II and III (IAEA-086, IAEA-085) is given as an average of two measurements. Alkaline digestion was done in duplicates. Evidently, results obtained by alkaline digestion and distillation are in very good agreement (Table II), while HCl leaching resulted in smaller results. Summary of the results is given in Table IV. It was assumed that not all of the methylmercury could be released by HCl leaching. In order to verify this conclusion, the residue after the leaching was thoroughly washed with water and submitted to distillation. Some of the sample was lost during this procedure. The methylmercury found in residues was around 0.5 mg/kg, which only partly covered the difference mentioned above. The question is, where is the missing methylmercury? Some of it has most probably been lost during washing of the sample and/or it was decomposed during leaching.

It is interesting to note that earlier method intercomparison study performed on human hair samples [5,6,7] have shown that HCl leaching coupled with ion-exchange separation compared well with other methods.

3.2.2. Total mercury

Spiked IAEA-085 sample was analyzed by both CV AAS and CV AFS. Results obtained by two different final detection systems are presented in Table II and summarized in Table IV. IAEA-086 sample was only analyzed by CV AAS. Evidently, a good agreement of the results have been obtained. Initially, when only HNO₃ acid was used for digestion, lower results (for 20 to 30 %) were obtained. This was mainly due to uncompleted digestion of the sample. Most probably, methylmercury was not completely decomposed to inorganic mercury and consequently could not be reduced with SnCl₂. It was found that addition of BrCl improved the results. Similar difficulties could also be expected in other laboratories. It is suggested to compare these results with another independent method, such as neutron activation analysis (preferably with a radiochemical separation).

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TABLE I. METHYLMERCURY IN HUMAN HAIR INTERCOMPARISON SAMPLE BEFORE GROUNDING

Sample	Weight (mg)	Result (mg/kg) as Hg	
		Ethylation CV AFS*	Extraction GC-ECD
A (a)	0.019	25.46	
A (b)	0.50	27.56	26.9
A (c)	0.030	23.05	
B (a)	0.020	25.13	
B (b)	0.028	28.49	
B (c)	0.025	29.38	
C (a)	0.019	25.41	
C (b)	0.028	29.31	
C (c)	0.038	25.57	26.1
D (a)	0.019	26.52	
D (b)	0.025	24.01	
D (c)	0.022	26.23	
X ± SD		26.3 ± 2.0	

*Each result is expressed as the mean of two determinations.

TABLE II. RESULT FOR TOTAL AND METHYLMERCURY IN IAEA-085 (SPIKED)

Bottle No.	Moisture content (%)	Total - Hg mg/kg, dry weight		Methyl-Hg (as Hg) mg/kg, dry weight		
		CV AAS	CV AFS	Alkaline dig.	Distillation	HCl leaching
46A	8.9	25.44	26.61	24.46	24.87	23.78
		24.77	25.88	24.51		
46B	8.9	25.45	27.02	23.96	25.59	22.48
		27.48		27.02	24.67	
				26.94		
46C	9.1	26.65	26.11	25.42		23.13
		25.26		24.83		
47A	8.5	27.02	26.17	24.87	26.80	24.17
		26.32		28.62		
47B	8.3	23.92	27.23	24.56		
		26.15		22.96		
47C	7.9	26.71	24.24	26.39		
		29.08		27.26		
48A	8.0	26.07	27.07	24.57		
		26.60		21.88		
48B	8.5	24.47	25.19	26.48		
		27.42		27.80		
48C	8.4	24.44	26.83	26.20		
		26.90		23.79		
Mean ± SD	8.5 ± 0.4	26.11 ± 1.29	26.45 ± 1.15	25.06 ± 1.55	25.77 ± 1.06	23.39 ± 0.74
NIES No.13	8.8	4.72	4.32	3.84	3.75	
		4.27	4.53	3.64	3.74	
		4.63	4.54			
		4.27			3.80	
Mean ± SD		4.51 ± 0.22	4.46 ± 0.12	3.74 ± 0.14	3.76 ± 0.03	
Recomm. value*		4.10 - 4.30		3.90		
IAEA 350 Tuna Hom. Certified value	6.2		4.79 4.70 4.68 95% Conf. Int. (4.36 - 4.91)			

*NIES No. 13 is not as yet certified. Values recommended from NIES are given for comparison.

TABLE III. RESULTS FOR TOTAL AND METHYLMERCURY IN IAEA-086

Bottle No.	Moisture content (%)	Total-Hg mg/kg, dry weight	Methyl-Hg mg/kg, dry weight
		CV AAS	Alkaline digestion
086/1A	9.6	0.38	0.28
		0.39	0.28
086/1B	11.0	0.41	0.30
		0.40	0.27
086/1C	10.3	0.37	0.29
		0.36	0.31
086/2A	11.3	0.37	0.29
		0.37	0.31
086/2B	10.9	0.38	0.25
		0.41	0.28
086/2C	11.1	0.36	0.32
		0.38	0.30
Mean ± SD	10.3 ± 0.6	0.38 ± 0.02	0.29 ± 0.02
NIES No. 13	8.8	4.42	4.00
		4.51	4.12
		4.38	3.72
		4.53	
		4.36	
Mean ± SD		4.44 ± 0.080	3.95 ± 0.21
Recomm. value*		4.10 - 4.30	3.90

*NIES No. 13 is not as yet certified.

Values recommended from NIES are given for comparison.

TABLE IV. SUMMARY - TOTAL AND METHYLMERCURY IN IAEA-085 AND IAEA-086 HUMAN HAIR SAMPLES (CONCENTRATIONS ARE GIVEN IN mg Hg/kg, DRY WEIGHT)

	IAEA-085 (spiked)		IAEA - 086 (non-spiked)	
	Hg-total	MeHg	Hg-total	MeHg
After spiking Before grinding (2 - 4 mm segments)		26.3 ± 2.0 (A) n = 12 26.9, 26.1 (B) n = 12 25.0 ± 1.5 (A)** n = 4	---	---
After grinding and homogenization	26.1 ± 1.3 (CV AAS) n = 18 26.5 ± 1.2 (CV AFS) n = 11	25.1 ± 1.6 (A) n = 12 25.8 ± 1.1 (C) n = 5 23.4 ± 0.7 (D) n = 4 26.6 ± 1.8 (A)** n = 6	0.38 ± 0.02 (CV AAS)* n = 12 0.61 ± 0.41 (CV AFS) n = 6	0.29 ± 0.02 (A) n = 12
Intercomparison sample	24.41 ± 1.21 (CV AAS) n = 6 26.11 ± 1.60 (CV AFS)** n = 3	23.33 ± 0.91 (A) n = 6 22.17 ± 1.16 (F) n = 4 23.35 ± 1.33 (A)** n = 5	0.57 ± 0.02 (CV AFS) n = 6	0.29 ± 0.02 (A) n = 6

(A) alkaline digestion, ethylation, GC, CV AFS; (B) alkaline digestion, extraction, GC-EC; (C) distillation, ethylation, GC, CV AFS;

(D) HCl leaching, ethylation, GC, CV AAS; (F) microvolatilization, extraction, GC-ECD

* Low results due to matrix interference during reduction/amalgamation step.

** Samples re-analyzed in December 1994 by Dr. Lian Liang

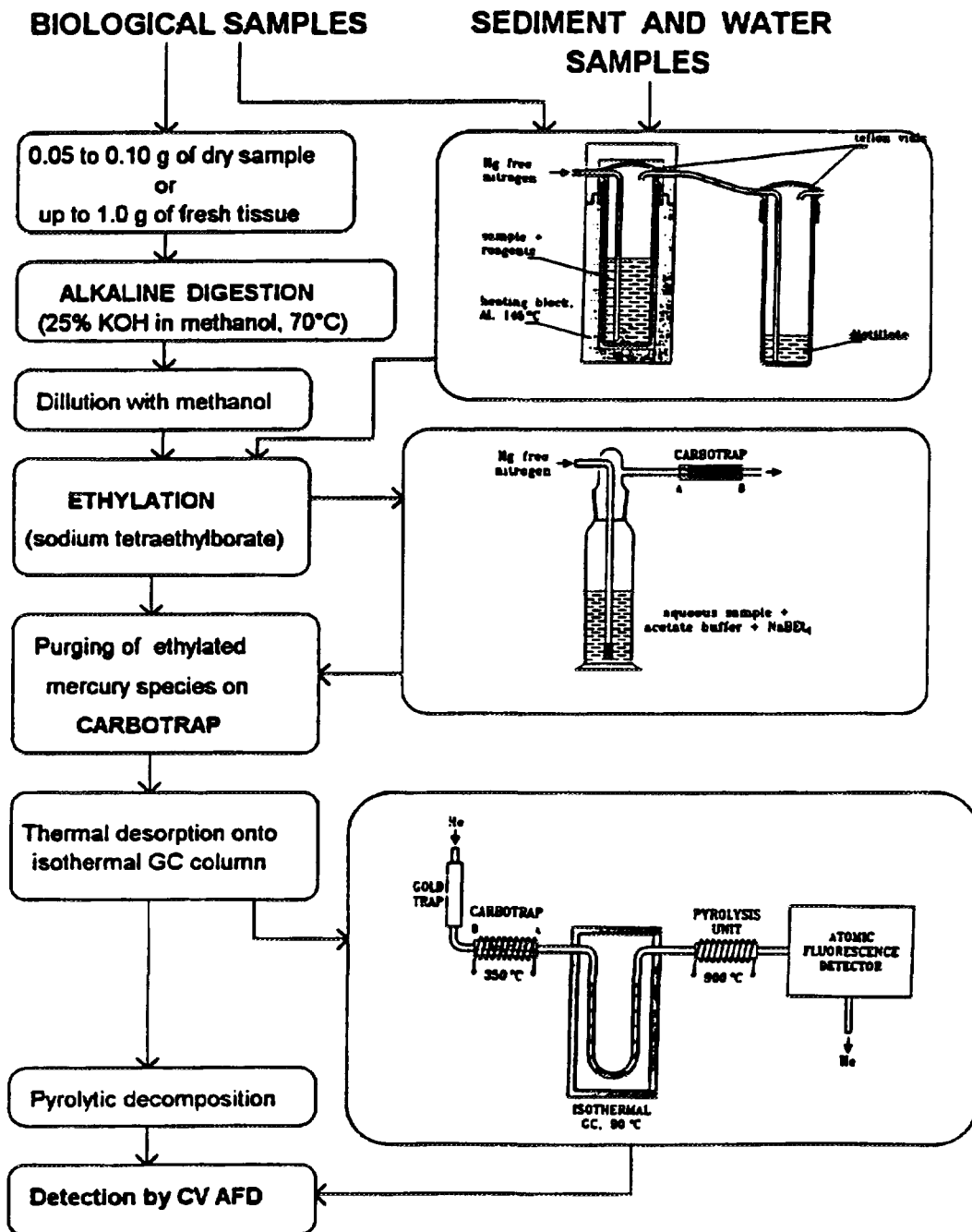


Figure 1. A schematic flow-chart for methylmercury determination by ethylation, room temperature preconcentration, GC separation and CV AFS detection.