



# REFERENCE SHEET

## REFERENCE MATERIAL

### IAEA-085

#### METHYLMERCURY, TOTAL MERCURY AND OTHER TRACE ELEMENTS IN HUMAN HAIR (METHYLMERCURY SPIKED)

Date of issue: May 2000<sup>⊕</sup>

#### Recommended Values (Based on dry weight)

Element	Recommended Value mg/kg	95% Confidence Interval mg/kg	N*
Hg	23.2	22.4 – 24.0	67
Fe	79.3	71.0 – 87.8	16
Zn	163	156 – 170	24
MeHg <sup>§</sup>	22.9	21.9 – 23.9	5

#### Information Values (Based on dry weight)

Element	Information Value mg/kg	95% Confidence Interval mg/kg	N*
Ca	929	847 – 1010	12
Cu	16.8	15.7 – 17.8	16
Mg	140	127 – 153	12
Mn	8.8	8.4 – 9.2	16
Sc	0.0092	0.0084 – 0.0100	9
Se	1.07	0.96 – 1.17	13

\* Number of accepted laboratory results which were used to calculate the recommended values and confidence intervals about the mean value.

§ As inorganic Hg

⊕ Revision of the original reference sheet dated November 1997

The reference values were established on the basis of results submitted by 74 participants from 68 institutes who participated in an international intercomparison exercise organized during 1994/1995. The details concerning the criteria for certification of recommended and information values may be found in the report (IAEA/AL/67) "Intercomparison Run IAEA-085/IAEA-086; Determination of Methylmercury, Total Mercury and Trace Elements in Human Hair". This report is in preparation and will be available upon request free of charge. A shorter version of it has been submitted for publication in an international journal [1].

### **Intended Use**

This material is intended as reference material primarily for the measurements of total mercury (Hg) and methylmercury (MeHg), but also for selected trace elements. The material can also be used for the assessment and control of a laboratory's analytical work, and for the validation of analytical methods used in a laboratory, i.e., in general quality assurance within a laboratory and for training purposes.

### **Origin and preparation of the material**

The material has been prepared from human hair. IAEA-085 represents hair with an elevated level of methylmercury.

Ten kg of human hair were collected and donated to the Agency for preparation of two reference materials. The material had been previously cut into uniform (1 cm) lengths, and cleaned with acetone and deionized water following the procedure developed by IAEA [2]. The material was then split into two portions, each approximately 5 kg, for the two reference materials, and was radiation sterilized at 50 kGy. Afterwards the material was labeled with methylmercury using an established procedure [3] to achieve an elevated level of methylmercury. During this process leaching of some trace elements might be possible. The hair was cryogenically homogenized using the stainless steel "CryoPalla" mill at the KFA-Juelich Specimen Bank facility [4]. The hair was subjected to consecutive millings, until approximately 70% was below 0.071 mm grain size. The material was then bottled, providing 750 5-g units. Sterilization of the material in the bottles was done at 12kGy using a <sup>60</sup>Co Source. Further details can be found in reference [5].

### **Homogeneity**

The material was analyzed to determine the degree of homogeneity. Aliquots from both the sub-batch of bulk homogenate and from the material after bottling and radiation sterilization were analyzed for these tests.

Several techniques were employed: instrumental neutron activation analysis (INAA) for total mercury and zinc, X-ray fluorescence for total Hg, cold vapor atomic absorption spectrometry (CV-AAS) for total Hg and gas chromatography with atomic fluorescence spectrometry detection (GC-AFS) for methylmercury. For the methods employing digestions, 50-100 mg sample sizes were used. The material did not have observable inhomogeneities greater than the measurement uncertainties (1-3% for CV-AAS, and 2-5% for GC-AFS). Sample sizes of 10 mg each were taken for the homogeneity tests using INAA. The observed relative standard deviation of the means within and between the sub-batches (14%) were on the same order or less than the measurement uncertainties (about 3.5-3.7%). The measurements for Zn gave similar results, with no significant differences observed greater than the measurement uncertainties.

The homogeneity results for total mercury by INAA and XRF on the material after final preparation were within 2% of the mean value, again comparable to measurement uncertainties. Thus, the material is considered homogeneous for Hg and Zn at the 10 mg sample size, and this can be considered comparable homogeneity for the other major biological trace elements. Further details can be found in reference [5].

### **Dry weight determination**

The recommended and information values are expressed on a dry weight basis. For this purpose, the volatile water content, at the time of analysis, should be determined in separate sub-samples. The recommended procedure is oven drying at 80°C for 2 hours or freeze-drying for 48 hours. These procedures have been shown to yield similar and stable results, which are in agreement with those obtained by microwave thermogravimetry analysis (within 0.5%).

### **Stability of the material**

Because the IAEA-085 material was a spiked, rather than a natural material, there was concern over possible loss of the spiked MeHg over time. Stability studies have been evaluated after two years of ambient storage and compared with material stored at liquid nitrogen temperatures, which have shown no discernible changes in the mercury, methylmercury or trace element concentrations in this material [1].

### **Establishing of reference values**

The recommended and information values represent overall mean values (excluding data that were detected and rejected as outliers) calculated on the basis of at least ten laboratory means. For the total mercury and methylmercury values, the results submitted by invited expert laboratories were used in addition to those from the intercomparison means. A confidence interval is given with each recommended value, which was calculated from the mean of the laboratories' means. The confidence interval for the information values was calculated in the same way, but these intervals should be interpreted with caution, because they did not fulfill all criteria to qualify as recommended values.

### **Instructions for use**

The recommended minimum sample size is 50 mg for organic (MeHg) analyses and 10 mg for inorganic analyses. Analysts are reminded to take care not to contaminate the rest of the material in the bottle. The direct introduction of metallic spatulas into the bottle should be avoided. It is recommended to store the material in a dark place, below 20°C (refrigeration is advised). From experience with similar materials, IAEA-085 is expected to remain stable for at least 5 years under these storage conditions.

### **Legal disclaimer**

The IAEA makes no warranties, expressed or implied, with respect to the data contained in this reference sheet and shall not be liable for any damage that may result from the use of such data.

## **References**

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- [2] Cortes-Toro, E., et al., The significance of hair material analysis as a mean for assessing internal body burdens of environmental pollutants: Results from an IAEA co-ordinated research programme, J. Radioanal. Nucl. Chem. **167** (1993) 413-421.
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- [5] Stone, S. F., et al., Production of hair intercomparison materials for the use in population monitoring programmes for mercury and methylmercury exposure, Fresenius J. Anal. Chem. **352** (1995) 184-187.

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