Estimation of uncertainty of a reference material for proficiency testing for the determination of

total mercury in fish *in nature*

Estimation of uncertainty of a reference material for proficiency testing for the determination of total mercury in fish *in nature*

L V Santana[1], J E S Sarkis [1], J C Ulrich [1] and M A Hortellani[1]

[1] IPEN/CNEN Institute of Nuclear and Energy Research, São Paulo, Brazil

E-mail: santana-luciana@ig.com.br

Abstract: We provide an uncertainty estimates for homogeneity and stability studies of reference material used in proficiency test for determination of total mercury in fish fresh muscle tissue. Stability was estimated by linear regression and homogeneity by ANOVA. The results indicate that the reference material is both homogeneous and chemically stable over the short term. Total mercury concentration of the muscle tissue, with expanded uncertainty, was $0.294 \pm 0.089 \ \mu g \ g^{-1}$.

1. Introduction

Determination of property values of reference material requires the systematic assessment of uncertainties associated with studies of homogeneity and stability. In general these values are obtained using the Guide to expression of Uncertainty in Measurement (GUM) [1].

Characterization of a reference material is defined as the complete set of measurements and associated uncertainties needed to set the values of its properties, as opposed to assigning property values for batch material or for individual units. Several different approaches exist to characterize material from which the measurement methods are traceable to the references specified in the project planning. The main approaches are as follows [1, 2]:

1- Characterization by an independent method;

2- Characterization by multiple methods and/or different laboratories.

Homogeneity analysis is necessary to demonstrate that the material does not vary significantly in consistency and chemical content. Such analysis is performed between the sample containers and within each container to assess whether there is an excess of variability that might affect the specific property being evaluated [2, 3].

Stability analysis is carried out to verify transport conditions. Such analysis is typically performed by exposing reference samples to temperatures over short periods of time (3-6 months) that simulate a realistic range of transport conditions. The material is then evaluated for levels of degradation that may affect the state values of the evaluated property. The Stability study is considered ideal for proficiency testing samples from biological matrices [2, 3, 4].

In this paper we calculate short term uncertainties associated with homogeneity and stability study in order to obtain reference values for proficiency testing material for determination of total mercury content of fish fresh muscle tissue.

2. Methodology

2.1. Preparation of the material

Analysis was performed on two fish (589 g and 660 g) acquired from a retailer within Itaituba city, near the Tapajós River in the Amazon region of Brazil. The fish were prepared by removing the head, tail, intestines and skin with a stainless steel knife. Muscle tissue was triturated and filleted on a Walita® domestic multiprocessor, after which it was homogenized in a Walita® domestic blender (with "fouet" type attachments) for 10 minutes, resulting in 1.100 g of sample material [5]. The material was then divided into 86 sachets of 15 g each. Of this total, 43 sachets were randomly assigned to be irradiated in a cyclotron (Cyclone Applications ion beam 30), the Radiation Technology Center of IPEN, with 10.0 ± 1.05 kGy using a cobalt 60 source (^{60}Co) to temperature of 24 ± 4 °C for homogeneity and stability analysis.

2.2 Determination of total mercury

A 0.5 g sample was digested with 1 mL of HNO_3, H_2SO_4 and 2 ml of 1 mL $HClO_4$ on plate at 110 °C for 30 minutes. After digestion, the solution was diluted with 20 mL of water for analysis [6].

Total mercury was evaluated by atomic absorption spectrometry with cold vapor generation and flow injection (FIA-CV-AAS) using an atomic absorption spectrophotometer Varian model AA-220 FS, according to manufacturer's specifications. The reducing agent was $SnCl_2$ 25% (w / v) in HCl 25% (v / v) [6].

The certified reference material DORM-2 ("Dogfish") provided by the National Research Council of Canada (NRCC) was used for the validation of the analytical methodology. The value of total mercury concentration in the certified sample (with expanded uncertainty) is 4.64 ± 0.26 µg g^{-1} [6].

2.3. Homogeneity and stability study

Homogeneity study was performed by randomly assigning ten sachets to temperature treatments of 23 ± 0.2 °C. Analysis of Variance (ANOVA) was used to evaluate heterogeneity between and within sachets, as described in the ISO Guide 35 [1, 3].

Stability analysis was performed by exposing 16 randomly selected sachets to 4 different temperatures ranges 5 °C, 23 °C, 40 °C and 60 °C for a period of 0, 7, 15, 30 and 45 days. Data was statistically evaluated by linear regression analysis [2, 3].

2.4 Estimation of uncertainty of the material

The uncertainty characterization u(char) was calculated in a spreadsheet according to the 'Guide to the expression of uncertainty of measurement' (GUM) [1-3]. Relative uncertainty of the homogeneity study was evaluated by the mean square analysis of variance (ANOVA-one way) according to Equation 1 [1]:

$$s_{bb} = s_A = \sqrt{\frac{MQ_{between} - MQ_{within}}{n_0}} \tag{1}$$

Where: $s_{bb} = s_A =$ uncertainty due homogeneity; $MQ_{between} =$ mean square between the sachets; $MQ_{within} =$ mean square within the sachets and $n_0 =$ number of sachets.

Relative uncertainty of the stability analysis was calculated by multiplying the value of the standard error of the slope of the regression curve for the time period (in months) according to equation 2 [2, 3].

$$u_{sts} = s_{(b1)} . t \tag{2}$$

Assuming the uncertainties associated with homogeneity and stability are independent, the overall uncertainty associated with the property values of the reference material, with coverage factor (k = 2), was calculated as follows [2]:

$$u_{MR} = k\sqrt{u_{car}^2 + u_{bb}^2 + u_{sts}^2}$$

(3)

3. Results

The characterization of uncertainty u(char) gave a value of 0.033 µg^{-1} g (12%), obtained from a single measurement method (FIA CV-AAS). Error propagation was carried out by the Ishikawa model diagram, where each parameter was calculated by spreadsheet, developed in the laboratory for the determination of mercury in fish (test accredited in CGCRE / INMETRO second ABNT: ISO / IEC 17025: 2005). The results were submitted to Grubbs test for the presence of outliers. According to the ANOVA, the studied material was homogenous ($F_{calc}<F_{crit}$, Table 1). The Shapiro-Wilk test (p=0.05) indicated that the sample was drawn from a normal population.

Table 1-Average results ± standard deviation (SD), in µg g^{-1}, of homogeneity study at 23 ± 0.3 °C

Average ± SD	0.294 ± 0.009
RSD %	1.3
P-value	0.19
$W_{calc.}$	0.78
$F_{crít.}$	3.88
$F_{calc.}$	1.85
ubb, µg g^{-1}, (%)	0.015 (5 %)

Wcrit.(0.05;5)=0.762. F_{calc}: calculated; F_{crit}: critical, F value for α = 5 %; P-value>0.05; $u_{bb:}$ uncertainty of homogeneity; sachets n° 56, 14, 21, 42, 3, n=30.

Likewise, the sample showed a considerable degree of stability under the experimental conditions imposed on the samples (Table 2).

Table 2- Average results ± standard deviation (SD), in µg g^{-1}, obtained for stability study over 0, 7, 15, 30 and 45 days

	Freezer (5 ± 0.2 °C)	Room (23 ± 0.3 °C)	Warm house (40 ± 1.3 °C)	Warm house (60 ± 1.2 °C)
Average ± SD	0.313±0.015	0.325± 0.025	0.375±0.038	0.380±0.029
RSD %	1.5	2.5	3.8	2.9
b1	0.0006	-0.0073	0.0024	-0.0003
s(b1)	0.00502	0.01325	0.00018	0.0013
|b1| < t(0,95,n-2)x s(b1)	0.02	0.02	0.03	0.03
usts, µg g^{-1}, (%)	0.010 (1 %)	0.026 (26 %)	0.001 (01 %)	0.003(03 %)

$b_{1:}$ slope curve; s_b: standard deviation in b_1; u_{sts}: uncertainty of stability; t0, 95, n-2 x s(b1) value for factor t (Student's) for n-2 degrees of freedom to a level of confidence 95 %[2], n=48.

The statistical evaluation of the data obtained for the concentration of mercury in fish muscle tissue during the time intervals and temperature ranges showed no significant trend - | b_1 | is smaller than the value of the condition t0, 95-n 2 xs (b1) [2, 7]. Table 4 shows the value of total mercury concentration with an estimated expanded uncertainty for reference material according to equation 3:

$$u_{MR} = 2 \times \sqrt{(0.033)^2 + (0.015)^2 + (0.026)^2} = 0.089$$

Table 4- Estimation of the combined standard uncertainty in µg g^{-1}, for the element mercury

	[a]Average ± U (µg g^{-1})	U (%)
$U_{MR(Hg)}$	0.294 ± 0.089	30

[a]Mean obtained in the study of homogeneity, represented by the mean values of 30 replicates.

4. Conclusions

The material produced for proficiency tests showed high levels of homogeneity for Hg, both within and between sachets. The stability of the material was also robust in the short-term, demonstrating the efficacy of the irradiation process to transport the samples at room temperature. Proficiency testing was performed at 30 days and, for this reason, it was not deemed necessary to assess long-term stability.

The calculated uncertainties were compatible with the measurement technique used to estimate uncertainty of the material. Therefore, the material produced was considered fit for use in proficiency testing, providing an additional tool for control and quality assurance in the analysis of mercury content in fish.

References

[1] BIPM, IEC, IFCC, ISO, IUPAC, IUPAP, OIML 2008 *Guide to the expression of uncertainty in measurement*, 1st edn. International Organization for Standardization (ISO), Geneva

[2] ISO, International Organization of Standardization 2006 *Certification of reference materials— general and statistical principles*. ISO Guide 35. 3rd edn, Geneva

[3] van der Veen A M H, Linsinger T P, Pauwels J 2001 *Accredit. Qual*. Assur. **6** 290

[4] van der Veen A M H, Linsinger T P J, Lamberty A, Pauwels J 2001 *Accredit. Qual. Assur*. **6** 257

[5] Aquino J S, Silva J A, Prado J P, Cavalheiro J M O 2008 *Rev. Inst. Adolfo Lutz*, 67(**3**):190-195

[6] Azevedo J S, J Sarkis E S, Hortellani M A, Ladle R J 2012 *Water, Air, & Soil Pollution*. **223** 3911-3922

[7] van der Veen A M H, Pauwels J 2000 *Accredit. Qual. Assur*. **5** 327