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CCQM-P39

As, Hg, Pb, Se and Methylmercury in Tuna Fish

FINAL REPORT

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NOTICE:

All the participants in CCQM-P39 gave their consent to the publication of this Final Report in the *Metrologia Technical Supplement*.

Contents

Abstract

CCQM-P39 was an activity of the Inorganic Analysis Working Group (IAWG) of CCQM and was organised by the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) of the European Commission (EC). In CCQM-P39 the amount contents of As, Hg, Pb, Se and Methylmercury (CH_3Hg) in tuna fish muscle were the measurands under investigation. Besides the National Metrology Institutes (NMIs) who are IAWG members, non-IAWG members, expert laboratories for mercury and methylmercury measurements, were also invited to participate in this pilot study. Finally, results were reported by 13 NMIs and 8 expert laboratories.

The results of the IAWG members for arsenic and selenium fall within a range of ± 10% with respect to the mixture model median (MM-median). For lead, the spread was within 2% and for mercury within 4% deviation from the MM-median. Also, for methlymercury the majority of the participating NMIs reported results within 4% deviation from the MM-median. Including the reported results from the invited expert laboratories, the spread of results increased for arsenic, lead, selenium and methlymercury up to 20-40%. Except for mercury, for this analyte the spread of all the reported results of the IAWG members and the invited expert laboratories was within 4%.

The methods applied were isotope dilution mass spectrometry (IDMS) using sector field or quadrupole inductively coupled plasma-mass spectrometry (ICP-MS), external calibration using time-of flight MS, atomic absorption spectrometry (AAS) or atomic fluorescence spectrometry (AFS). Instrumental neutron activation analysis (INAA) and k_0-neutron activation analysis (k_0-NAA) were also used as analytical techniques. Each of the 5 analytes under investigation was measured with at least 3 of these analytical techniques.

IAEA reported 2 results for arsenic, lead and selenium. They were measured with ICP-MS and AAS, respectively.

This report presents the participants' results in CCQM-P39 for all analytes under investigation. In Annex 1, the results with the MM-median are presented and sorted according to analytical technique applied. In Annex 2, the different approaches for methlymercury measurements are discussed in more detail. In Annex 3, the questionnaire data are presented. Annex 4 compiles all the CCQM-P39 information documents.

At the CCQM meeting in April 2004, it was decided to proceed this study to a key comparison and a pilot study for the same analytes in a salmon material, CCQM-K43 & CCQM-P39.1.

Introduction

The CCQM-P39 pilot study was agreed to in previous CCQM-IAWG meetings (Paris and Ottawa 2002). At the beginning of 2003, this pilot study was proposed and accepted by the CCQM-IAWG. The purpose of CCQM-P39 was to demonstrate the measurement capability of determining the amount content of metals in a real life fish sample. Organomercury was included for the first time in a pilot study in view of its toxicity. Furthermore, included in CCQM-P39 was arsenic, being a toxic mononuclidic element that cannot be determined by IDMS, further broadening the scope and degree of difficulty of measurements addressed by the CCQM-IAWG. It was agreed on that well known expert laboratories in mercury and methlymercury measurements would be invited to join this study. Some of these expert laboratories also took the opportunity to report results for arsenic, lead and selenium, even if they might be less experienced in this kind of analysis.

The same tuna fish material used in CCQM-P39 was measured by 235 laboratories in IMEP-20. Amongst those were 23 National Reference Laboratories (NRLs), 38 food control laboratories as nominated by their NRLs and 61 laboratories nominated by their National Accreditation Bodies (NABs). The IMEP-20 participants' report is accessible via the IMEP web-site [1]

Rationale for this comparison

In order to protect public health it is essential to keep contaminants at levels which are toxicologically acceptable. Thus surveillance measures are taken regarding the presence of contaminants in foodstuff, including fish.

Mercury is a potential environmental toxin. The main source of human intake of mercury contaminants originates from methylmercury in fish and fish products. Methylmercury is particularly interesting due to its high toxicity compared to inorganic mercury and its high proportion among organomercury species in the environment. Mercury species may induce alterations in the normal development of the brain of infants and may induce neurological changes in adults.

Lead may induce reduced cognitive development in children and increased blood pressure and cardiovascular diseases in adults.

To protect public health, maximum levels of mercury and lead in fish products are laid down in relevant regulations. The EC Directive 2001/22 describes the community methods for the sampling, the sample preparation and the analysis of mercury and lead in fish [2]. The EC Regulation (466/2001) endorses officially the threshold value of 1 mg kg^{-1} mercury and 0.2 mg kg^{-1} lead in tuna fish [3].

Selenium is an essential trace element for human beings. Seafood is an important source of selenium intake for people in some regions. Certain forms of cancer and cardiovascular diseases have also been associated with selenium deficiency. Selenium is also counted among the most important elements in terms of food-chain contamination. Selenium has the narrowest plateau between concentrations that show deficiency and toxic effects, respectively. Recently, the EC requested the Scientific Committee on Food (SCF) to review the upper level of daily intake of individual vitamins and minerals, amongst them selenium, and to provide the basis for the establishment of safety factors [4].

Rules for measurements of arsenic are set in the commission decision on implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results [5].

Participation in CCQM-P39

CCQM-P39 PARTICIPANT	COUNTRY
IAWG members	
BAM Bundesanstalt für Materialforschung und –prüfung	**Germany**
BNM-LNE Bureau National de Métrologie - Laboratoire National d' Essais	**France**
CENA Centre for Nuclear Energy and Agriculture	**Brazil**
IAEA International Atomic Energy Agency	**United Nations**
IMGC Institute of Metrology G. Colonnetti - University of Pavia	**Italy**
IRMM Institute for Reference Materials and Measurements	**European Commission**
IRMM-SCK Institute for Reference Materials and Measurements - Studiecentrum voor Kernenergie	**European Commission**
KRISS Korea Research Institute of Standards and Science	**Republic of Korea**
LGC Ltd. Laboratory of the Government Chemist	**United Kingdom**
NARL National Analytical Reference Laboratory	**Australia**
NIST National Institute of Standards and Technology	**United States of America**
NMIJ National Metrology Institute of Japan	**Japan**
NRC National Research Council Canada	**Canada**
SP Sveriges Provnings- och Forskningsinstitut	**Sweden**
Invited expert laboratories	
BKÁE-ÉT-Szent Istvan University Budapesti Közgazdaságtudományi és Államigazgatási Egyetem	**Hungary**
ENEA Ente per le Nuove Technologie, l' Energia e l' Ambiente	**Italy**
Frontier Geosciences Inc.	**United States of America**
JSI Jozef Stefan Institute	**Slovenia**
LCABIE – University of Pau Laboratory of analytical and bio-inorganic environmental chemistry	**France**
Trent University	**Canada**
Umeå University	**Sweden**
University of Oviedo	**Spain**

The tuna fish sample

The tuna fish CCQM-P39 sample is a freeze dried and ground tuna muscle powder bottled in amber glass vials each one containing about 4 g. Participants who measured all the 5 analytes received 15 g of this material. The others received 8 g of the tuna fish.

This tuna fish originates from the Mediterranean Sea close to Messina and was taken off the market due to its elevated amount content of Hg. The range of metal amount contents was just slightly exceeding the upper limits as stated in the EC Regulation (466/2001) [3]. Therefore, this tuna fish material was perfectly appropriate for the purpose of a CCQM pilot study. Participants in CCQM-P39 could demonstrate their measurement capabilities on this “real-life” sample.

Homogeneity characterisation

Within and between bottle homogeneity tests for Hg and Pb were carried out on 10 sub-samples of 10 bottles using solid sample Zeemann AAS (SS-ZAAS) and on 3 subsamples from 3 bottles applying IDMS. For As and Se, the homogeneity was assessed by analysing 3 sub-samples from 5 bottles applying k_0-NAA. Results from these measurements were evaluated accordingly and compared to the procedures established in ISO Guide 35:1989 for the certification of reference materials based on analysis of variance ANOVA [6, 7]. The homogeneity of all 5 analytes was found to be adequate for this pilot study.

Participant Coordination

IAWG members and expert laboratories were invited for participation in CCQM-P39 during February and March 2003. The information package contained the tuna fish samples together with a Certified Reference Material (CRM) BCR-464, certified for its MeHg content and was sent to all registered participants at the end of April 2003.

In June/July the IRMM-670 MeHg isotopic CRM spike solution enriched in ^{202}Hg was sent on dry ice to the participants that previously indicated on the registration sheet that they would measure the MeHg in the tuna fish. The participants were asked to report their results and questionnaire answers by means of input in templates that have been previously forwarded by e-mail.

The first deadline for result reporting was 26th September 2003. After the autumn IAWG meeting in October 2003, at EMPA (St. Gallen, Switzerland), the deadline for MeHg result reporting was extended on the request of the participants to 10th November 2003. At the same meeting only preliminary normalised results were presented.

In December 2003, a summary report was circulated amongst all participants for confirmation of the reported results and applied analytical technique. Final results were presented at the last CCQM-IAWG meeting in Paris in April 2004.

CCQM-P39 information documents

- Letter to the participants pointing out the deadlines
- Scope of the CCQM-P39 pilot study
- General instructions
- Instructions for result reporting and uncertainty evaluation
- Instructions for the dry-mass correction and digestion of the tuna fish
- Information for CCQM-P39 participants measuring methylmercury, including the draft IRMM-670 certificate
- Example of the result report table (excel.xls) to be returned by e-mail
- Example of the questionnaire form (word.doc) to be returned by e-mail

Protocol for dry-mass correction

The determination of the moisture content of the samples is to some extent "operationally defined" [8, 9]. In view of the comparability of the results, the protocol in CCQM-P39 for correction of the moisture was as follows:
"For correction of the measured values to dry-mass, water content measurements should be made on a separate portion of the same material with a minimum mass of 0.5 g. The material should be dried before weighing for a minimum of 24 hours in a ventilated oven at 85 ± 2°C. Cycles of drying and weighing should be repeated until a constant mass is attained. Each weighing has to be carried out after the sample has reached thermal equilibrium at room temperature in an evacuated dessicator. Successive weights should not differ more than 0.001 g. The loss of mass corresponds to the "dry-mass correction factor" that should be applied".
The water content was also determined by Karl-Fischer titration. No significant difference was observed. The reported results in CCQM-P39 for the measured water content in the tuna fish samples are listed in Table 1.

Uncertainty evaluation

The participants were asked to report a complete uncertainty budget with their results. The uncertainty statement should be evaluated and presented according to the principles outlined in, e.g. "ISO/GUM" [10] or the EURACHEM/CITAC Guide [11].

The organising laboratory asked all the participants in CCQM-P39 the following:

- state your measurement equation
- identify all significant sources of uncertainty
- state your input quantities
- quantify uncertainty components and convert them to standard uncertainties
- calculate the combined standard uncertainty u_c
- present an expanded uncertainty U with the coverage factor k=2
- include factors related to sample treatment in your measurement equations
- describe the applied evaluation process and type of assumed distribution for your uncertainty estimation

The complete uncertainty statement should be forwarded to the organising laboratory as an attachment to the result reporting sheets. Before finalising this report, the organising institute contacted for the last time all participants that had not reported a complete uncertainty budget. Although some of the invited expert laboratories were not so familiar with establishing a complete uncertainty budget, 6 of them forwarded an uncertainty budget. BKA-ET reported that their uncertainty was derived from results from method validation. JSI did not forward an uncertainty budget. From the IAWG members, only IAEA did not forward an uncertainty budget for any of the measured analytes.

Table 1 Reported values for water content determination and dry-mass correction

CCQM-P39 Correction for dry-mass		
Participant	Water content in %	Corr. factor
BAM	4.33 ± 1.2 vial 1 2.77 ± 1.2 vial 2	0.96
BKAE-ET	1.85 ± 0.22	0.9815
BNM/LNE - As	1.7	0.983
BNM/LNE - Hg	4.8	0.952
BNM/LNE - Pb	5.3	0.947
BNM/LNE - Se	1.7	0.983
CENA/USP.	1.7 ± 0.2	0.983
ENEA	0.911	0.99
Frontier Geosciences Inc.	4.1	0.959
IAEA_AAS	9.4	0.906
IAEA_ICP-MS	9.4	0.906
IMGC	1.69	0.9831
IRMM	2.30 ± 1.00	0.977 ± 0.010
JSI	2.44	0.9756
KRISS	2.07	0.9793
LGC	2.11	0.9789
NARL	2.03 ± 0.4 - 3.08 ± 0.4 (depending on jar)	1 ± 0.0045
NIST	1.78	0.9822
NMIJ	2.3 ± 0.001	0.977 ± 0.00041
NRC	2.02 ± 0.001	0.9802 ± 0.00031
SP	1.99 ± 0.01	0.9801 ± 0.0051
Trent University	3.9 ± 0.001	0.961
Umeå University	1.99 ± 0.004	0.9803 ± 0.0016
University of Oviedo	1.8	0.982
University of Pau	1.297 ± 0.14	0.98703

Analytical methods and techniques

Different methods and instrumental techniques besides IDMS were applied in CCQM-P39. The methods applied were:

- isotope dilution mass spectrometry (IDMS) using sector field or quadrupole inductively coupled plasma-mass spectrometry (ICP-MS)
- external calibration using time-of flight MS
- atomic absorption spectrometry (AAS)
- atomic fluorescence spectrometry (AFS)
- Instrumental neutron activation analysis (INAA) and k_0-neutron activation analysis (k_0-NAA)
-

Each of the 5 analytes under investigation was measured with at least 3 of these analytical techniques (Table 2, Table 3). In Annex 1 of this report, all the results are graphically presented sorted by analytical technique.

Table 2 Analytical methods and instrumental l techniques in CCQM-P39 for methylmercury

CCQM-P39 analyte: MeHg		
Participant	**Analytical method**	**Instrumental technique**
BKÁE-ÉT	other method than species specific IDMS	SPME-GC-AFS
ENEA	direct IDMS using IRMM-670 as spike solution	GC-MS
Frontier Geosciences Inc.	other method than species specific IDMS	GC-CV-AFS
IAEA	other method than species specific IDMS	CV-AFS
IRMM	direct IDMS using IRMM-670 as spike solution	GC-ICP-MS
JSI	other method than species specific IDMS	GC-CV-AFS
LGC Ltd.	double IDMS using IRMM-670 as spike solution	LC-ICP-MS
NIST	direct IDMS using IRMM-670 as spike solution	GC-MS
NMIJ	double IDMS using IRMM-670 as spike solution (re-characterised)	GC-ICP-MS
NRC	direct IDMS using IRMM-670 as spike solution	SPME-GC-ICP-MS
Trent University	direct IDMS using IRMM-670 as spike solution	GC-ICP-MS
Umeå University	direct IDMS using IRMM-670 as spike solution	GC-ICP-MS
University of Oviedo	double IDMS using IRMM-670 as spike solution (re-characterised)	GC-MS (electron impact)
University of Pau	direct IDMS using IRMM-670 as spike solution	GC-ICP-MS

Table 3 Analytical methods and instrumental techniques in CCQM-P39 for As, Hg, Pb, Se

CCQM-P39 analyte: As, Hg, Pb, Se		
Participant	**Analytical method**	**Instrumental technique**
BAM	NAA	INAA
BKÁE-ÉT	External calibration	As:- ICP-TOF-MS Se:- FI-HG-AFS Hg:- DMA Direct Mercury Analysis
BNM-LNE	As: External calibration with matrix matching (Na - K - Mg - Ca) Hg: simple IDMS Pb, Se: double IDMS	As, Se: ICP-QMS with CCT Hg, Pb: double focussing simple collector ICP-MS (low resolution)
CENA/USP	NAA	INAA
ENEA	external calibration	As, Pb: ICP-MS Se: HG-AAS Hg: DMA Direct Mercury Analysis
Frontier Geosciences Inc.	external calibration	As, Pb, Se: ICP-MS Hg: CV-AFS
IAEA_AAS	Pb, Hg external calibration As, Se standard addition	Pb, Se, As: Furnace Hg: VGA-SnCl2
IAEA_ICP-MS	external calibration	As: ICP-magnetic sector field MS (HR) Se: ICP-magnetic sector field MS Pb: ICP-magnetic sector field MS
IMGC	NAA	INAA
IRMM	IDMS	ICP-QMS
IRMM-SCK	NAA	k_0-NAA
JSI	Standard addition	gold amalgamation CV AAS
KRISS	As: Internal Standardization Hg, Pb, Se: IDMS	ICP-QMS
LGC Ltd.	matching double IDMS	ICP-MS with collision cell
NARL	Exact matching IDMS	ICP-MS magnetic sector field MS
NIST	NAA	INAA
NMIJ	As: external calibration Hg, Pb, Se: IDMS	ICP-MS with an octopole reaction cell
NRC	IDMS	ICP-MS magnetic sector field MS
SP	AAS	DMA Direct Mercury Analysis
Trent University	IDMS	ICP-MS magnetic sector field MS

CCQM-P39 participant's result

The CCQM-P39 participants' results, as reported to the organising institute, are listed in Table 4.

IAEA did not report an expanded uncertainty but experimental reproducibility for their As and Pb measurements with AAS and for their As and Se measurements with ICP-MS.

Table 4 CCQM-P39 participants reported results

REPORTED RESULTS AND EXPANDED UNCERTAINTY (*k*=2) in 10^{-5} mol·kg^{-1}					
Participant	**As**	**Hg**	**MeHg**	**Pb**	**Se**
BAM	6.69 ± 0.48	2.06 ± 0.15	-	-	8.21 ± 0.60
BKÁE-ÉT	6.02 ± 0.38	2.096 ± 0.015	1.80 ± 0.14	-	7.35 ± 0.71
BNM-LNE	6.06 ± 0.13	2.164 ± 0.081	-	0.234 ± 0.014	7.99 ± 0.27
CENA	6.39 ± 0.20	2.080 ± 0.086	-	-	8.03 ± 0.21
ENEA	7.23 ± 0.59	2.136 ± 0.038	2.09 ± 0.36	0.162 ± 0.038	4.87 ± 0.33
Frontier Geosciences	8.56 ± 1.29	2.15 ± 0.11	1.96 ± 0.18	0.290 ± 0.063	11.70 ± 2.29
IAEA_AAS	7.19 ± 0.11*	2.19 ± 0.22	1.92 ± 0.31	0.235 ± 0.028	8.86 ± 0.12*
IAEA_ICP-MS	7.23 ± 0.25*	-	-	0.24 ± 0.017*	8.73 ± 0.61
IMGC	6.41 ± 0.17	-	-	-	7.82 ± 0.17
IRMM	-	2.150 ± 0.037	1.98 ± 0.11	0.2417 ± 0.0030	-
IRMM-SCK	6.55 ± 0.31	-	-	-	8.06 ± 0.38
JSI	-	2.140 ± 0.051	1.140 ± 0.061	-	-
KRISS	7.20 ± 0.22	2.107 ± 0.022	-	-	7.92 ± 0.10
LGC	-	-	1.962 ± 0.079	0.2344 ± 0.0087	8.14 ± 0.12
NARL	-	2.172 ± 0.089	-	-	-
NIST	6.53 ± 0.11	-	2.390 ± 0.074	-	7.73 ± 0.21
NMIJ	6.50 ± 0.27	2.120 ± 0.044	1.983 ± 0.058	0.243 ± 0.013	7.97 ± 0.14
NRC	-	2.048 ± 0.050	2.01 ± 0.11	-	-
SP	-	2.022 ± 0.033	-	-	-
Trent Univ.	-	2.019 ± 0.042	2.00 ± 0.24	-	-
Umeå Univ.	-	-	1.951 ± 0.047	-	-
Univ. Oviedo	-	-	1.893 ± 0.096	-	-
Univ. Pau	-	-	1.993 ± 0.032	-	-

* experimental reproducibility

MM-PDF based summary statistics

At the CCQM meeting in Paris in April 2004, David L. Duewer from NIST presented a robust approach for the determination of CCQM Key Comparison Values and Uncertainties to the IAWG members [12]. He introduced the mixture model probability density function (MM-PDF) for each measurement population as a means of data analysis for key comparisons and pilot studies. MM-PDF based summary statistics enable estimation of the expected performance of the majority of participants of a key comparison or pilot study [13]. The "true value" of a measurand in a given material can be estimated in a robust way even when some of the results are not in accordance with the majority. In this study, the mixture model median (MM-median) and the MM-median based Standard Deviation S(MM-Median) was chosen as robust estimation of the true amount content of the analytes under investigation. The MM-median is closely related to the median. It is robust to outliners, but also accounts for the reported uncertainty of each participant.

In the tables below the MM-median, the mean and the median are listed for all the analytes in CCQM-P39 as $\mu \pm \sigma^* t_s / \sqrt{n}$ which is the 95% confidence interval on μ for *n* reported results. Table 5 lists the summary statistics for all CCQM-P39 participants' results.

Table 6 lists the summary statistics for IAWG members participating in CCQM-P39 not taking into account the results of the expert laboratories. It can be seen that the MM-median gives a reliable robust estimate even for analytes with larger spread of results like As, Pb and MeHg.

Table 5 CCQM-P39 summary statistics for all participants

$\mu \pm \sigma^* t_s / \sqrt{n}$ in 10^{-5} mol·kg^{-1}					
	As	**Hg**	**MeHg**	**Pb**	**Se**
MM-median	**6.59 ± 0.36**	**2.112 ± 0.036**	**1.967 ± 0.054**	**0.2388 ± 0.0089**	**7.99 ± 0.20**
mean	6.81 ± 0.41	2.110 ± 0.030	1.93 ± 0.15	0.235 ± 0.029	8.10 ± 0.80
median	6.55 ± 0.44	2.120 ± 0.033	1.971 ± 0.030	0.2384 ± 0.0051	8.01 ± 0.17

Table 6 CCQM-P39 summary statistics for IAWG members

$\mu \pm \sigma^* t_s / \sqrt{n}$ in 10^{-5} mol·kg^{-1}					
	As	**Hg**	**MeHg**	**Pb**	**Se**
MM-median	**6.55 ± 0.38**	**2.112 ± 0.052**	**1.991 ± 0.082**	**0.2394 ± 0.0078**	**8.02 ± 0.17**
mean	6.67 ± 0.29	2.112 ± 0.041	2.041 ± 0.18	0.2384 ± 0.0045	8.13 ± 0.24
median	6.54 ± 0.16	2.114 ± 0.053	1.982 ± 0.036	0.2384 ± 0.0060	8.03 ± 0.11

The CCQM-P39 graphs

The CCQM-P39 results for As, Hg, Pb and Se are presented graphically in Annex 1 of this report. In the first 4 graphs, all CCQM-P39 results are plotted in ascending order, including the MM-median. In the following 8 graphs, the results are presented according to applied analytical technique for all participants and for IAWG members, separately. On these MM-PDF graphs the MM-median and the spread of the population of results are included. To enable the comparison of the spread of data, the scale of all graphs is ± 50 % deviation with respect to the MM-median. In Annex 2 the results and questionnaire data for MeHg are presented and discussed in more detail.

Table 7 summarises all the CCQM-P39 graphs of Annex 1 and Annex 2.

Table 7 CCQM-P39 graphs

General Graphs - Annex 1	**As, Hg, Pb and Se**
All participants	✓
All participants - Analytical techniques	✓
IAWG members - Analytical techniques	✓
Methylmercury Graphs - Annex 2	**MeHg (CH_3Hg)**
All participants	✓
All participants - Analytical techniques	✓
IAWG members - Analytical techniques	✓
All participants - Results with amended JSI and NIST value	✓

Discussion

As can be seen from Table 4 and the graphs in Annex 1 and Annex 2, the spread of results depended on the analyte as well as on the experience of the laboratories. Amongst the 21 institutes that participated in CCQM-P39 were 13 NMIs and 8 expert laboratories. Those 8 laboratories were invited because of their expertise in Hg and MeHg measurements. This is also reflected in the outcome of this study because the spread of all the reported Hg results of IAWG members and invited expert laboratories together are within 4% deviation from the MM-median.

For MeHg the majority of participants reported results within 10% deviation from the MM-median. As explained in Annex 2, JSI reduced as corrective action the sample intake and NIST changed the extraction reagent. Both institutes remeasured the tuna fish with a result for MeHg amount content in agreement with the other laboratories.

Excluding the initially reported NIST result, the other participating NMIs reported results within 3% deviation from the MM-median for MeHg.

Some invited expert laboratories took the opportunity to compare their measurement results with the NMIs' also for As, Pb and Se measurements, although in some cases the invited laboratory did not have a lot of experience with this kind of analysis. But it was a perfect means for them to assess their measurement capabilities for these analytes. This explains why for As and Se, the results of the IAWG member's fall within a range of ± 10% and for Pb within ± 2% relative to the MM-median. Including the reported results of the invited laboratories the spread of results increases for As, Pb and Se up to 20-40%.

Conclusion

All analytes under investigation in CCQM-P39 have been measured with at least 3 different techniques. This confirms that, for this kind of analysis, reliable measurements of highest metrological quality can be performed with various techniques (IDMS, ICP-MS, AAS, AFS, NAA and k_o-NAA) and are not method dependent.

CCQM-P39 is also a good example of a study involving not only the NMIs, but also expert laboratories of their countries as it is one of the NMIs' major tasks to disseminate metrological principles and good measurement practice to the laboratories in their countries. CCQM pilot studies make it possible for NMIs to appoint expert institutes in their countries for participation in these internationally recognised studies to demonstrate their measurement capabilities.

The main purpose of any Interlaboratory Comparison, thus also of CCQM studies, is to assess capabilities and to discover problems and correct analytical procedures accordingly. In this sense, JSI and NIST immediately benefited from participating in CCQM-P39. The organising laboratory appreciates that JSI and NIST are sharing this experience with all the other participants in the pilot study.

Citation from e-mail communication with NIST *"I know that there has been at least one article pointing to potential problems with using acetic acid for digestion. This was the first sample that I experienced problems with. Recently, I have noticed a similar effect with a fresh frozen fish sample"*.

Citation from e-mail communication with JSI *"In conclusion I may say that this intercomparison was most useful for our laboratory and in particular for staff working on mercury speciation. We learned that the amount of dry sample may significantly influence the results for MeHg. It has been known to us, that such a problem exists with a number of matrices, but with tuna we have never encountered this difficulty. Although the trueness was verified by the analysis of a BCR CRMs Tuna Fish Materials #463 and #464, with perfect concentration and matrix match, the results obtained for MeHg in the CCQM-P39 sample were significantly lower. This indicates that the use of CRMs alone does not guarantee the trueness of the obtained results in the sample".*

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The authors acknowledge very much the input and support of David L. Duewer from NIST. He provided a copy of his PDF_maker macro spreadsheet to enable the calculation of the MM-median in this pilot study. Special thanks also to B. Gawlik from the JRC-IES and Dr. H. Muntau for their support in providing the suitable tuna fish sample for this study and to all the IRMM scientists who contributed to the reprocessing and charcacterisation of the tuna fish sample : - K. H. Grobecker, M. Bickel, F. Ulberth, S. Yazgan, P. Conneely and G. Kramer from IRMM. Furthermore the editorial assistance of C. Harper and S. Bynens is warmly acknowledged and the support of M. Sargent as chairman of the CCQM IAWG.

List of abbreviations

AAS	Atomic Absorption Spectrometry
AFS	Atomic Fluorescence Spectrometry
BIPM	Bureau International des Poids et Mesures (Paris, France)
CCQM	Comité Consultatif pour la Quantité de Matière
CITAC	Co-operation for International Traceability in Analytical Chemistry
CRMs	Certified Reference Materials
EC	European Commission
EU	European Union
EURACHEM	A focus for Analytical Chemistry in Europe
GC-ICP-MS	Gas Chromatography Isotope Dilution Inductively Coupled Plasma Mass Spectrometry
GUM	Guide for expression for Uncertainty in Measurement
IAWG	Inter-Agency Working Group
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
IDMS	Isotope Dilution Mass Spectrometry
IMEP®	International Measurement Evaluation Programme
INAA	Instrumental Neutron Activation Analysis
IRMM	Institute for Reference Materials and Measurements (EC, Geel, Belgium)
ISO	International Organisation for Standardisation
JRC	Joint Research Centre
K_o-NAA	Neutron Activation Analysis
MM-median	Mixture Model Median
MM-PDF	Mixture Model Probability Density Function
NAA	Neutron Activation Analysis
NABs	National Accreditation Bodies
NMIs	National Metrology Institutes
NRLs	National Reference Laboratories
SCF	Scientific Committee on Food
SS-ZAAS	Solid Sample Zeemann Atomic Absorption Spectrometry

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Annex 1 – Graphical presentation

Contents

CCQM-P39: As, Hg, Pb, Se and Methylmercury in Tuna Fish

Annex 1 – All participants results

Figure 1

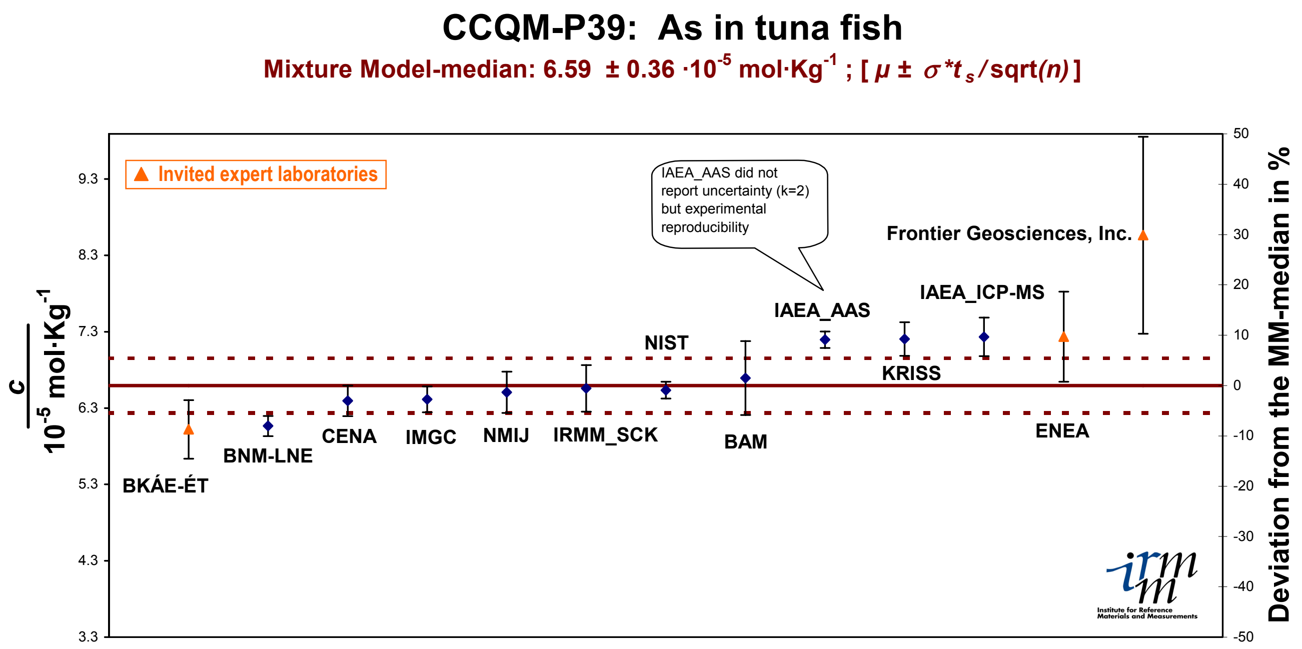


Figure 2

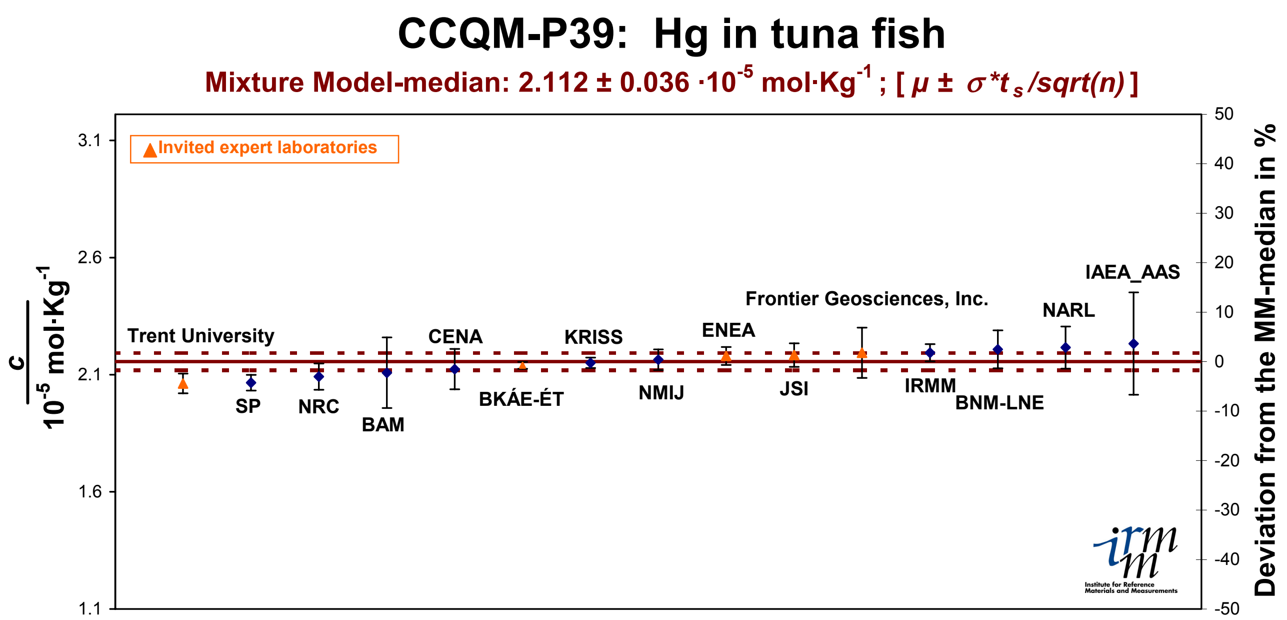


Figure 3

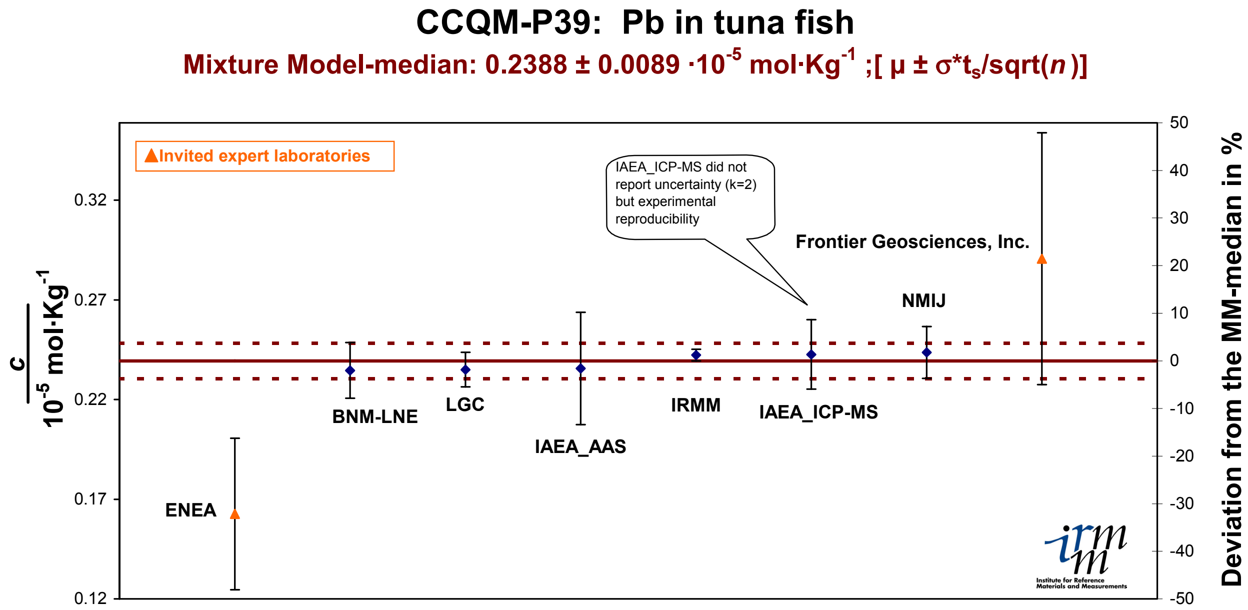
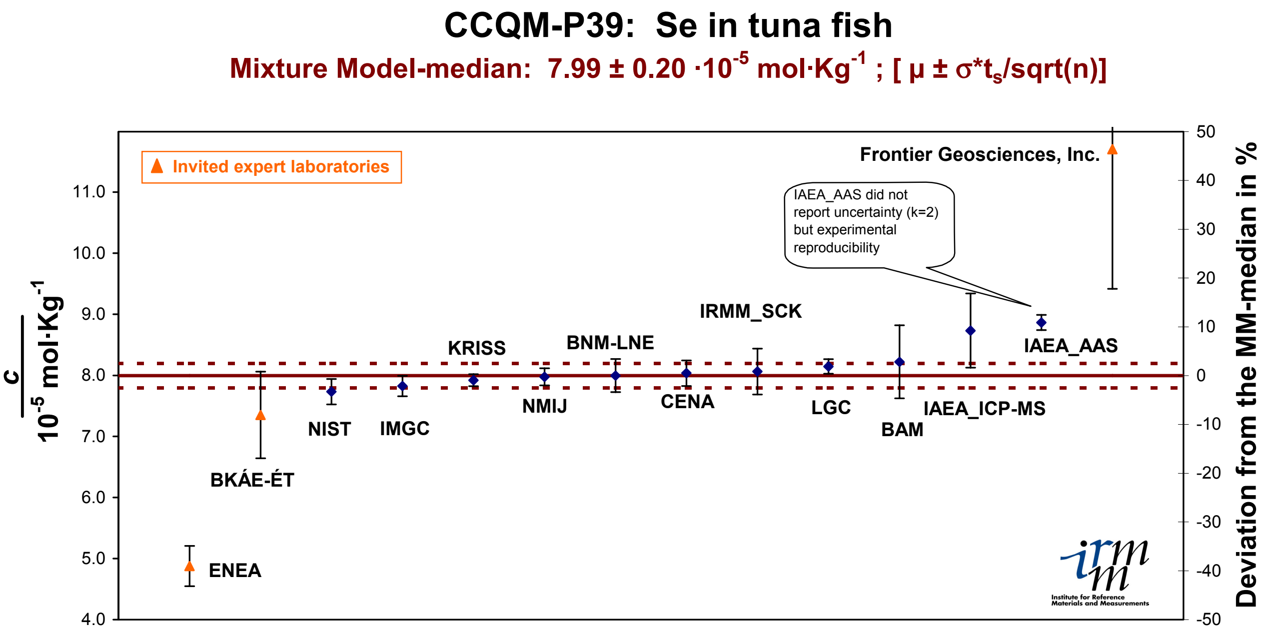


Figure 4



CCQM-P39: As, Hg, Pb, Se and Methylmercury in Tuna Fish

Annex 1 – All participants – Analytical techniques

Figure 5 Results for As from all CCQM-P39 participants sorted by analytical method

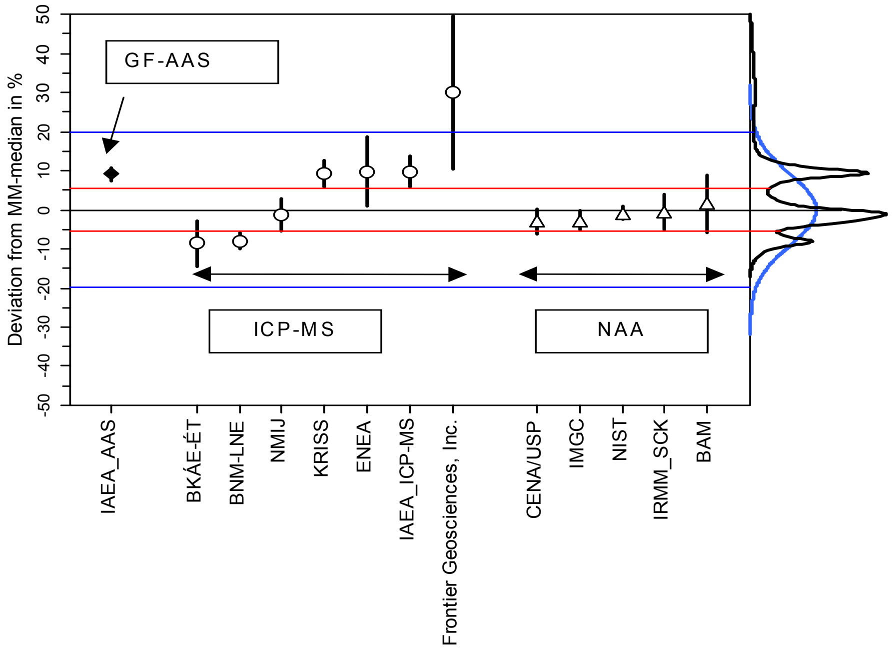


Figure 6 Results for As from IAWG members sorted by analytical method

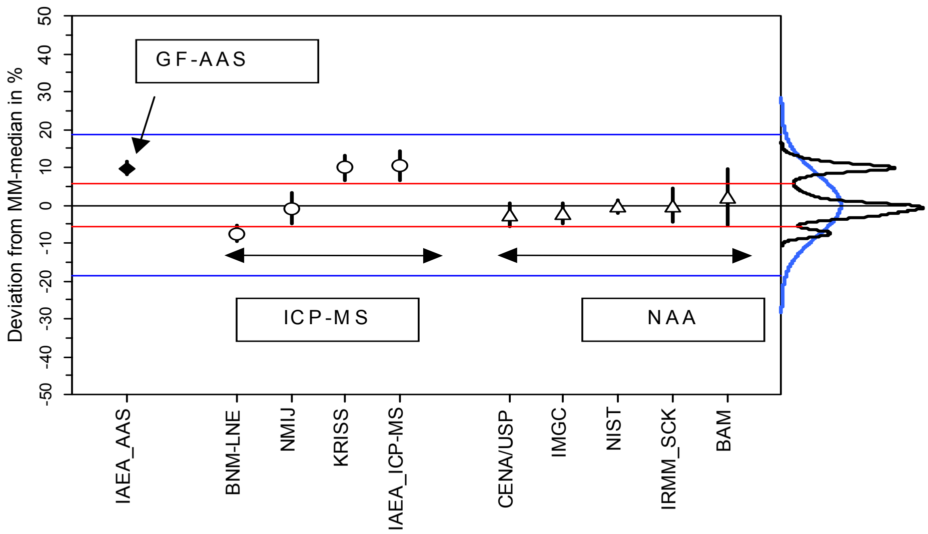


Figure 7 Results for Hg from all CCQM-P39 participants sorted by analytical method

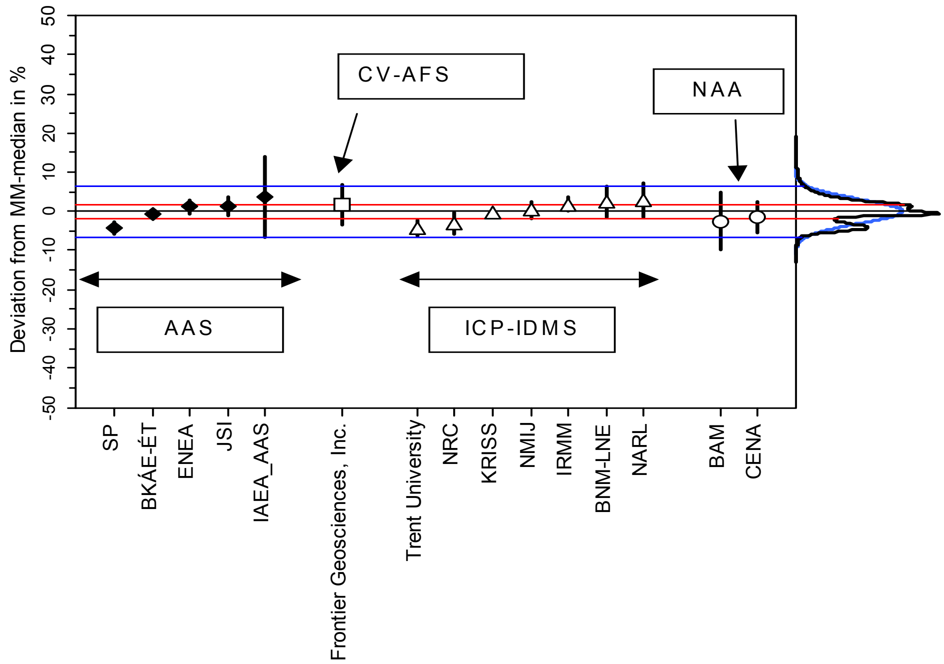


Figure 8 Results for Hg from IAWG members sorted by analytical method

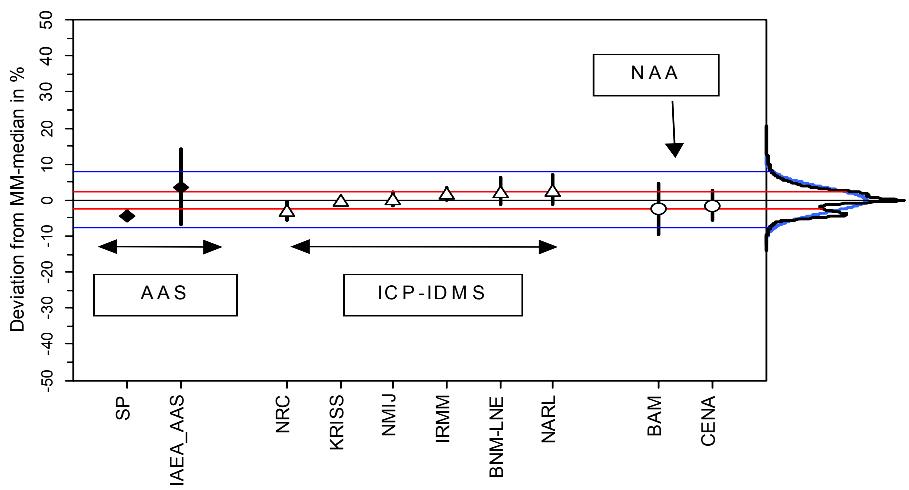


Figure 9 Results for Pb from all CCQM-P39 participants sorted by analytical method

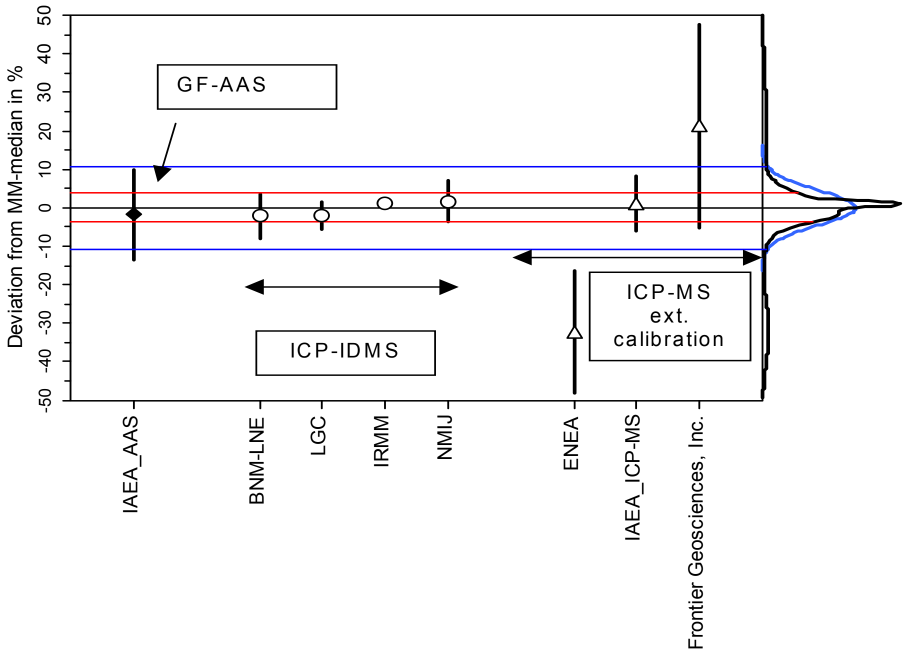


Figure 10 Results for Pb from IAWG members sorted by analytical method

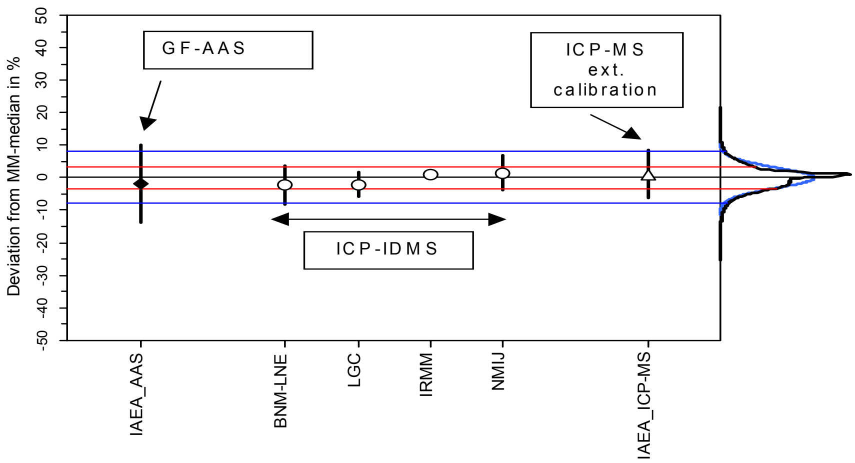


Figure 11 Results for Se from all CCQM-P39 participants sorted by analytical method

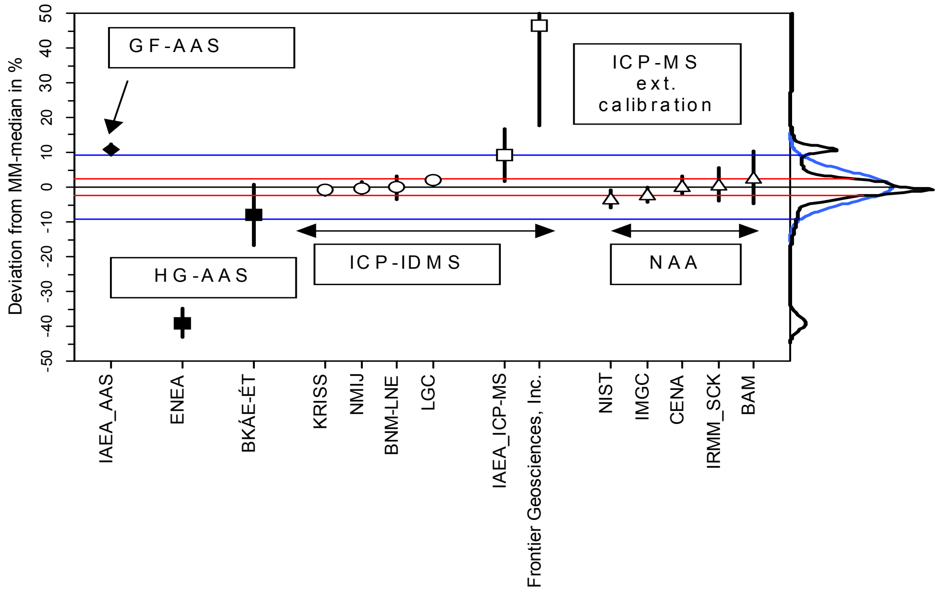
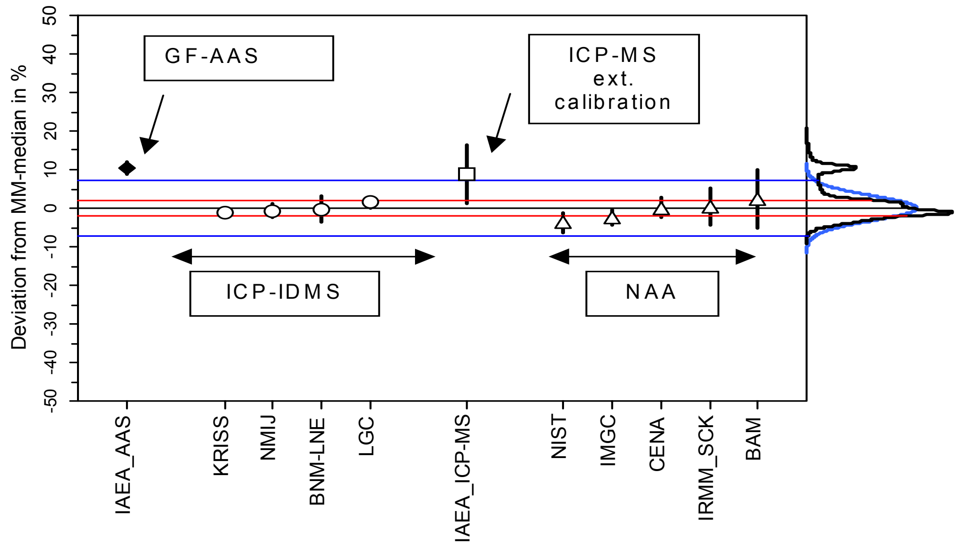


Figure 12 Results for Se from IAWG members sorted by analytical method



Annex 2 – Methylmercury (CH_3Hg)

Contents

CCQM-P39: As, Hg, Pb, Se and Methylmercury in Tuna Fish

CCQM-P39: As, Hg, Pb, Se and Methylmercury in Tuna Fish

Annex 2 – All participants results Methylmercury (CH_3Hg)

Methylmercury measurements

Participants who measured methylmercury received 3 bottles of the tuna fish material and 1 bottle of the BCR-464 CRM. The BCR-464 tuna fish CRM has similar characteristics as the CCQM-P39 tuna fish, which was given to participants in order to, rehearse their analytical methodology. Furthermore, participants received the ^{202}Hg enriched spike solution IRMM-670 with the draft certificate. Participants were entirely free to implement the measurement method of their choice. The IRMM-670 material was intended for use by those willing to apply species specific IDMS as it gave them the possibility of doing it directly without having to re-characterise the material prior to its use. After CCQM-P39, IRMM-670 was renamed ERM®-AE670 [1], and is now distributed under the European Reference Materials programme [2]. 4 CCQM-P39 participants, including 1 NMI, relied on external calibration or the method of standard additions, whereas the other 10 implemented an IDMS approach and chose to use the IRMM-670. 7 of these 'IDMS participants' used the IRMM-670 draft certificate values whereas 3 preferred to re-characterise it. The 4 'non-IDMS participants' used cold vapour atomic fluorescence spectrometry for the detection step, whereas 7 of the others used ICP-MS and the remaining 3 used electron impact MS. All the 8 invited expert laboratories and 5 IAWG members reported measurement results for MeHg in this study. These participants were asked to fill in a separate questionnaire. This Annex summarises the questionnaire data and presents the results from the expert laboratories and the IAWG members graphically.

Discussion

As shown in Figure 1 - Figure 3, results for MeHg are within 10% relative to the MM-median (excluding the results from JSI and NIST). Furthermore, the remaining participating NMIs reported results for MeHg within a spread of 4% relative to the MM-median.

After the CCQM-P39 summary report was distributed to the participants, JSI investigated their measurement protocol thoroughly to find the origin of the bias. They found out that the amount of sample had an influence on the recovery. They repeated the same measurement with a lower sample intake of about 0.1 g applying the same method as initially used in CCQM-P39. The method is based on acid digestion, solvent extraction, ethylation, gas chromatographic separation, pyrolysis and detection by CV-AFS. As a result of these measurements, JSI reported for the MeHg amount content a value of $1.96 \pm 0.21\ 10^{-5}$ mol·kg^{-1} (mean ± 2 STDEV).

NIST also made some further investigations and found out that by changing the extraction reagent the measured MeHg content is in agreement with the other participants. NIST repeated the measurements and used 1M HCl instead of the acetic acid for the acidic digestion. Applying this analytical approach, the NIST value for MeHg dropped to $1.99 \pm 0.03\ 10^{-5}$ mol·kg^{-1} (mean ± 2 STDEV).

Excluding the initially reported NIST MeHg results and taking into account the amended NIST value, the results for MeHg by the NMIs would fall within 2% deviation from the MM-median. A detailed evaluation of the CCQM-P39 results for the MeHg measurements will be published in the near future [3].

This is a very good example how participation in a pilot study results in immediate correction measures, which is the main purpose for participation in Interlaboratory Comparisons. JSI and NIST asked the organising laboratory to also include these values in the CCQM-P39 report (see Figure 4). Furthermore, this example also proves the robustness of the MM-median towards outliers. In Figure 1 and Figure 4 it can be seen that there is only a minimal change in the value for the MM-median. CCQM-P39 also proves that the MM-median is a good way to calculate the KCRV in the future, without having to exclude any reported data as long as there is no scientific explanation for the observed bias.

Figure 1 Results for MeHg from all CCQM-P39 participants

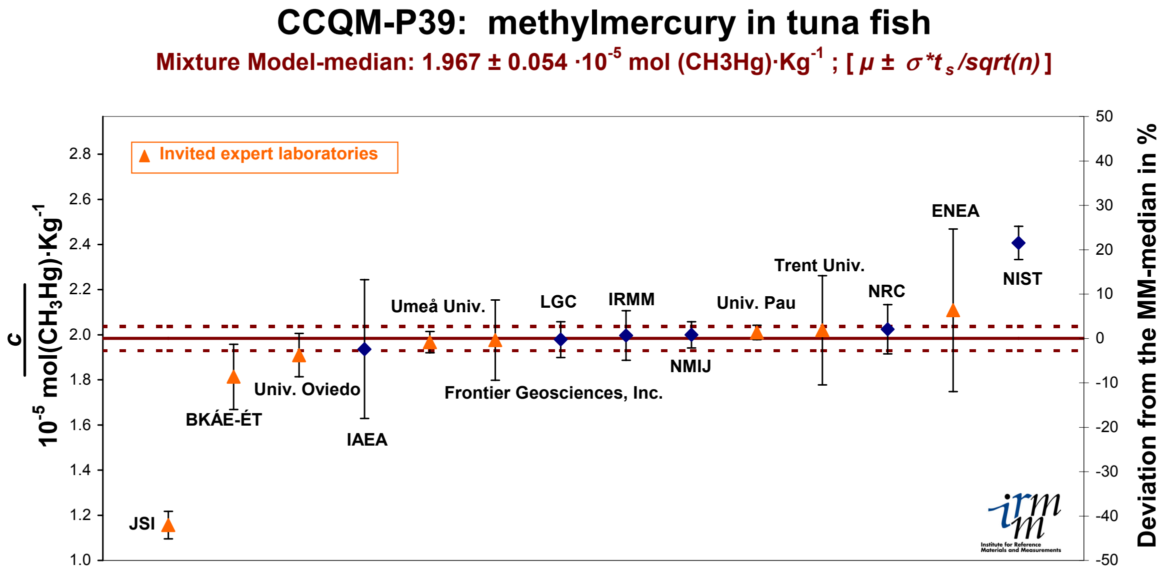


Figure 2 Results for MeHg from all CCQM-P39 participants sorted by analytical method

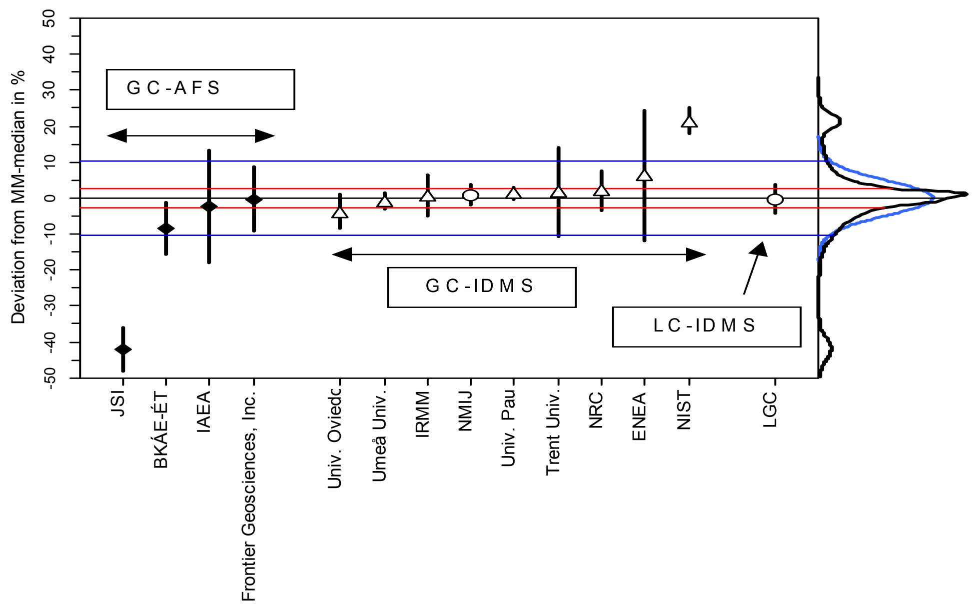


Figure 3 Results for MeHg from IAWG members sorted by analytical method

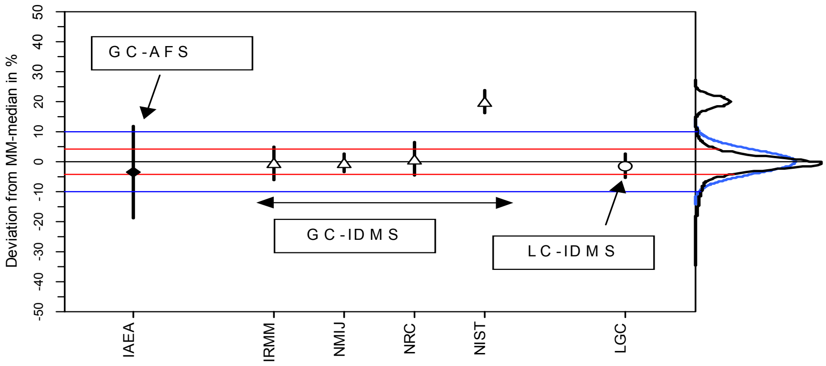
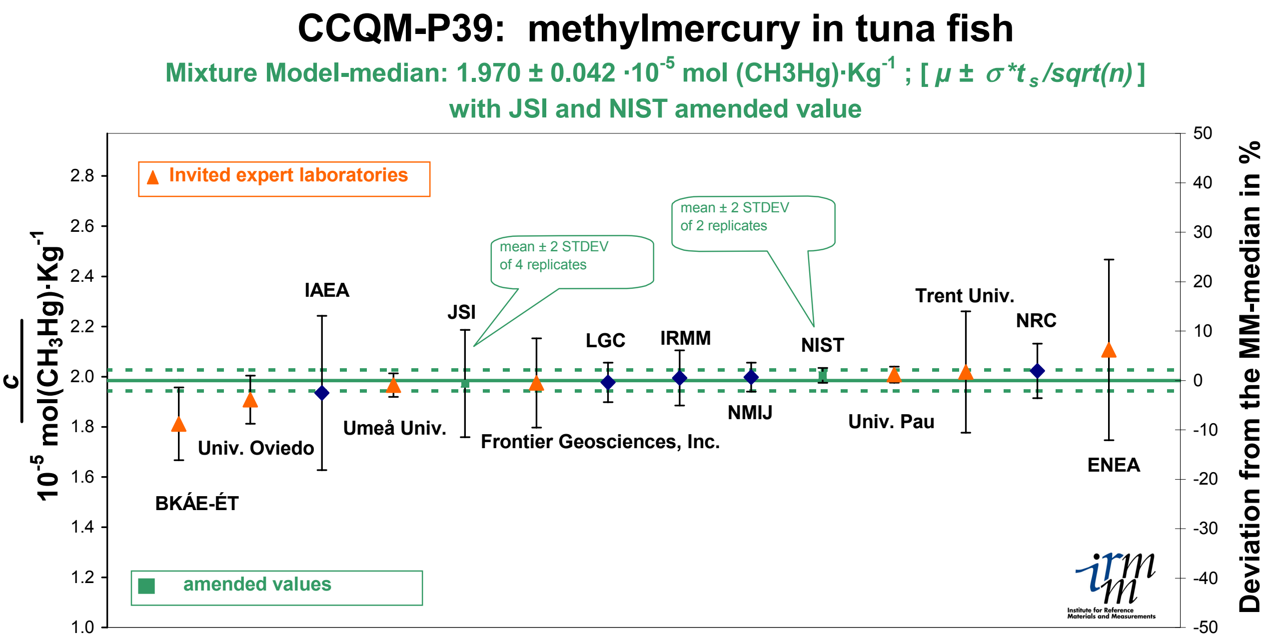


Figure 4 Results for MeHg from all CCQM-P39 participants-with JSI and NIST amended values



Questionnaire Data

Table 1 Measurement strategy

Participant	Direct IDMS with IRMM-670	Double IDMS with IRMM-670	Other method	Calibration with external matrix matched standards	Without an internal standard	Method of standard addition (SA) calibration
BKÁE-ÉT			SPME-GC-AFS	✓	✓	✓
ENEA	✓					
Frontier Geosciences Inc.			GC-CV-AFS	✓	✓	
IAEA			GC-CV-AFS	✓	✓	
IRMM	✓					
JSI			GC-CV-AFS	✓		✓
LGC		✓				
NIST	✓					
NMIJ		✓				
NRC	✓					
Trent Univ.	✓					
Umeå Univ.	✓					
Univ. Oviedo		✓				
Univ. Pau	✓					

Table 2 Results for double IDMS by re-characterising the IRMM-670 solution and by using certified values of IRMM-670

REPORTED QUESTIONNAIRE DATA FOR METHYLMERCURY				
	MeHg results obtained using values from re-characterisation of IRMM-670		MeHg results obtained using certified values of IRMM-670	
Participant	Amount content in mol(CH_3Hg)·Kg^{-1}	Uncertainty (*k*=2) mol(CH_3Hg)·Kg^{-1}	Amount content in mol(CH_3Hg)·Kg^{-1}	Uncertainty (*k*=2) mol(CH_3Hg)·Kg^{-1}
NMIJ	1.98E-05	5.80E-07	-	-
Univ. Oviedo	1.89E-05	9.60E-07	1.92E-05	8.50E-07

Table 3 Timing of the spike addition for ID or SA & ref. isotope used for IDMS

REPORTED QUESTIONNAIRE DATA FOR METHYLMERCURY					
Participant	**Some hours prior to MeHg extraction**	**Immediately before MeHg extraction**	**After MeHg extraction, prior to measurement**	**Reference isotope used for IDMS**	**Number of replicate blend samples**
BKÁE-ÉT			✓		
ENEA		✓		202	4
Frontier Geosciences Inc.					
IAEA					
IRMM	✓			202	3
JSI		✓			
LGC	✓			202	6
NIST		✓		202	6
NMIJ	✓			202	6
NRC				201	6
Trent Univ.		✓		200	1 per replicate
Umeå Univ.		✓		200	4
Univ. Oviedo		✓		200	12
Univ. Pau		✓		200	6

Table 4 Measurement process

REPORTED QUESTIONNAIRE DATA FOR METHYLMERCURY						
Participant	**Sample mass (g)**	**Extraction reagents**	**Extraction apparatus**	**Derivatisation reagents**	**Species separation apparatus**	**Detector**
BKÁE-ÉT	0.25	25% methanolic KOH	ultrasound bath at 70 C	NaBPh4	J&W, DB-1, 15m 0.53 mm (megabore) 1.5 um film thickness (carrier : Ar)	AFS
ENEA	0.2	HCl	ultrasound bath	NaB(C6H6)4	GC	MS
Frontier Geosciences Inc.	0.4g	KOH, Methanol	Hot Plate	Sodium Tetraethyl Borate	GC	CV-AFS
IAEA	0.2g	KOH in Methanol (25%)	Oven 75 C	NaB(C2H5)4	Tenax trap, desorb at 200 C, separation on SPB-5 (30m x 75mmID x 1.00 µm df) under Argon	CVAFS after pyrolyse at 800 C
IRMM	from ~ 0.140 g to ~ 0.220 g	1.2 mL HCl, buffer with NaOH sol., complexing with 1.2 mL of 1 M DDDC sol., 2 mL of toluene	centrifugation for phase separation	Grignard 2 M BuMgCl in tetrahydrofurane	Capillary GC, Supelco SPB-1, helium 4 mL/minute	ICP-MS
JSI	0.4g	H2SO4, KBr, CuSO4		NaB (C2H5)4	GC	CV AFS
LGC	0.4g	0.04g Protease Type XIV, 15ml 0.1M ammonium phosphate buffer with 0.01% (vol/vol) mercaptoethanol, 0.04g trypsin	Rotating incubator (at 37 C)	N/A	LC (Agilent 110), Allure PFP Propyl 5um 150x2.1mm (RESTEK CORP), mobile phase : 5% (vol/vol) methanol, 0.3% (vol/vol) mercaptoethanol	ICP-MS (Agilent 7500s)
NIST	0.4	acetic acid	microwave	sodium tetraphenyl borate	GC	Quadru-pole MS

REPORTED QUESTIONNAIRE DATA FOR METHYLMERCURY						
Participant	**Sample mass (g)**	**Extraction reagents**	**Extraction apparatus**	**Derivatisation reagents**	**Species separation apparatus**	**Detector**
NMIJ	around 0.25g	20% TMAH	Ultrasound bath	NaBPh4	GC, HP-5 (30m * 0.32mm * 0.25um)	ICP-MS
NRC	0.4g	KOH/methan ol	Mechanical shaking at room temperature	Sodium tetrapropyl borate + see specifications	GC, DB-5MS 30mx0.25mm id column ; He carrier	ICP-MS, sector field, Element 2
Trent Univ.	+/- 0.02	20% KOH/MeOH	Hot plate 60C	NaBEt4	GC, packed column OV-3 on chromosorb W, AW, DCMS, Ar carrier gas	ICP-MS
Umeå Univ.	0.3g	20% (w/w) TMAH in H_2O complexing with DDTC 2ml toluene	Mechanical shaking at ambient temperature	Butylation by Grignard reagent (2.0M bytulmagnesium chloride in THF	GC, SPB-1 15m x 0.53mm id 1.5micron film thickness, He carrier gas	ICP-MS
Univ. Oviedo	0.4-0.5	NaCl-HCl	Mechanical shaking at room temperature	NaB(C3H7)4	GC, HP5MS, He	MS (electron impact)
Univ. Pau	about 400 mg	TMAH	Open focused μwave, 3min at 40W	NaBPr4 1% in Na-Acetate/acetic acid buffer 0,1 mol/L at pH 3,9	GC Focus, MXT-1 (Restek), 0,53 mm id, 30m, 25mL He/min	ICP-MS

Table 5 Experimental reproducibility from the different sample aliquots

REPORTED QUESTIONNAIRE DATA FOR METHYLMERCURY	
	Experimental reproducibility
BKÁE-ÉT	3.60%
ENEA	1.52%
Frontier Geosciences Inc.	4.5%
IAEA	8%
IRMM	1.39 % (n = 3)
JSI	-
LGC	1.10%
NIST	2.53%
NMIJ	2.5% (SD, n=6)
NRC	1.90%
Trent Univ.	RSD = 4.9% (n=9)
Umeå Univ.	1.66%
Univ. Oviedo	"RSD in % for each six injections of the same sample blend; BCR1: 1,655%; BCR2: 3,265 %; CCQM 1-1: 2,434 %; CCQM 1-2: 1,737 %; CCQM 2-1: 2,444 %; CCQM 2-2: 3,849 %, CCQM 3-1: 2,294%; CCQM 3-2: 3,008 %"
Univ. Pau	1.6% (n=6)

References

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2 http://www.erm-crm.org

3 Determination of methylmercury in tuna: Comité Consultatif pour la Quantité de Matière (CCQM) pilot study P-39 international intercomparison. C. R. Quételet al., 2004, in prep.

Annex 3 – Questionnaire data As, Hg, Pb, Se

Contents

CCQM-P39: As, Hg, Pb, Se and Methylmercury in Tuna Fish

Annex 3 – Questionnaire data

Table 1 Digestion method and acid mixture

REPORTED QUESTIONNAIRE DATA				
Participant	**As**	**Hg**	**Pb**	**Se**
BAM	-	-	-	-
BKÁE-ÉT	Pressure cooker H_2O_2, HNO_3 (1:1)	Direct input via autosampler		H_2O_2, HNO_3 (1:1)
BNM-LNE	Microwave digestion H_2O_2, HNO_3, HF	Microwave digestion Aq. regia, H_2O_2, HF	Microwave digestion H_2O_2, HNO_3, HF	Microwave digestion H_2O_2, HNO_3, HF
CENA	-	-	-	-
ENEA	Microwave digestion H_2O_2, HNO_3 (5:2)	Direct analysis	Microwave digestion H_2O_2, HNO_3 (5:2)	Microwave digestion H_2O_2, HNO_3 (5:2)
Frontier Geosciences Inc.	hot plate digestion HNO_3		hot plate digestion HNO_3	hot plate digestion HNO_3
IAEA_AAS	Microwave digestion HNO_3	Microwave digestion HNO_3	Microwave digestion HNO_3	Microwave digestion HNO_3
IAEA_ICP-MS	Microwave digestion HNO_3	Microwave digestion HNO_3	Microwave digestion HNO_3	Microwave digestion HNO_3
IMGC	-	-	-	-
IRMM		Microwave digestion H_2O_2, HNO_3	Microwave digestion H_2O_2, HNO_3	
IRMM-SCK	-	-	-	-
JSI		wet digestion HNO_3, H_2SO_4		
KRISS	Microwave digestion H_2O_2, HNO_3	Microwave digestion H_2O_2, HNO_3		Microwave digestion H_2O_2, HNO_3
LGC			Microwave digestion H_2O_2, HNO_3 (1:2)	Microwave digestion H_2O_2, HNO_3 (1:2)
NARL		Microwave digestion H_2O_2, HNO_3 (1:4) HNO_3, HCl (4:1)		
NIST	-	-	-	-
NMIJ	Microwave digestion HNO_3 $HClO_4$	Microwave digestion HNO_3:HCl (1:3)	Microwave digestion HNO_3 $HClO_4$	Microwave digestion HNO_3 $HClO_4$
NRC		Microwave digestion H_2O_2, HNO_3 (1:35)		
SP		Direct input via autosampler		
Trent Univ.	-	wet digestion HNO_3, H_2SO_4	-	-

Table 2 Reference isotope for IDMS

REPORTED QUESTIONNAIRE DATA				
Participant	**As**	**Hg**	**Pb**	**Se**
BAM	Not applicable	-	-	-
BKÁE-ÉT	Not applicable	-	-	-
BNM-LNE	Not applicable	202/200	208/206	80/82
CENA	Not applicable	-	-	-
ENEA	Not applicable	-	-	-
Frontier Geosciences Inc.	Not applicable	-	-	-
IAEA_AAS	Not applicable	-	-	-
IAEA_ICP-MS	Not applicable	-	-	-
IMGC	Not applicable	-	-	-
IRMM	Not applicable	202	206	-
IRMM-SCK	Not applicable	-	-	-
JSI	Not applicable	-	-	-
KRISS	Not applicable	202/200	208/206	82/78
LGC	Not applicable	-	206	77
NARL	Not applicable	201Hg + specification	-	-
NIST	Not applicable	-	-	-
NMIJ	Not applicable	202/200	208/206	80/78
NRC	Not applicable	202	-	-
SP	Not applicable	-	-	-
Trent Univ.	Not applicable	-	-	-

Table 3 Number of blends

REPORTED QUESTIONNAIRE DATA				
Participant	**As**	**Hg**	**Pb**	**Se**
BAM	Not applicable	-	-	-
BKÁE-ÉT	Not applicable	-	-	-
BNM-LNE	Not applicable	4	4	4
CENA	Not applicable	-	-	-
ENEA	Not applicable	-	-	-
Frontier Geosciences Inc.	Not applicable	-	-	-
IAEA_AAS	Not applicable	-	-	-
IAEA_ICP-MS	Not applicable	-	-	-
IMGC	Not applicable	-	-	-
IRMM	Not applicable	4	3	-
IRMM-SCK	Not applicable	-	-	-
JSI	Not applicable	-	-	-
KRISS	Not applicable	4	4	4
LGC	Not applicable	-	6	6
NARL	Not applicable	10	-	-
NIST	Not applicable	-	-	-
NMIJ	Not applicable	6	6	6
NRC	Not applicable	6	-	-
SP	Not applicable	-	-	-
Trent Univ.	Not applicable	3	-	-

Table 4 Experimental reproducibility

REPORTED QUESTIONNAIRE DATA				
Participant	**As**	**Hg**	**Pb**	**Se**
BAM	2.66 %	3.10 %	-	1.4 %
BKÁE-ÉT	-	-	-	-
BNM-LNE	1.0076 %	0.16 %	5.9 %	1.16 %
CENA	-	-	-	-
ENEA	3.8%	0.67%	11.6%	2.7%
Frontier Geosciences Inc.	7.5 %	2.5 %	10.9 %	9.8 %
IAEA_AAS	1.5 %	2.9 %	3.9 %	1.4 %
IAEA_ICP-MS	3.55 %	-	7.19 %	1.98 %
IMGC	1.12 %	-	-	2.59 %
IRMM	-	0.46 %	1.51 %	-
IRMM-SCK	1.7 %	-	-	1.9 %
JSI	-	-	-	-
KRISS	-	0.45 %	4.7 %	1.17 %
LGC	-	-	3.6 %	0.33 %
NARL	-	1.6 %	-	-
NIST	0.94 %	-	-	1.87 %
NMIJ	1.4 %	1.6 %	6.6 %	1 %
NRC	-	1.4 %	-	-
SP	-	0.67 %	-	-
Trent Univ.	-	1.03%	-	-

Table 5 (Isotopic) reference materials used for calibration

REPORTED QUESTIONNAIRE DATA				
Participant	**As**	**Hg**	**Pb**	**Se**
BAM	-	-	-	-
BKÁE-ÉT	-	-	-	-
BNM-LNE	Arsenic powder, As2O5, from JM, 99.9%	CRM IRMM 639 and 640	High purity standard of Pb 99.9999% - SRM 981-982-991	high purity Se powder JM 99.999% - spike solution
CENA	-	-	-	-
ENEA	-	-	-	-
Frontier Geosciences Inc.	-	-	-	-
IAEA_AAS	-	-	-	-
IAEA_ICP-MS	-	-	-	-
IMGC	-	-	-	-
IRMM	-	IRMM-640 isotopic CRM	NIST SRM-991	-
IRMM-SCK	no calibration needed, but Alfa ICP standard solutions were used	-	-	no calibration needed, but Alfa ICP standard solutions were used
JSI	-	-	-	-
KRISS	-	IRMM 639	NIST 981, NIST 982	Oak Ridge Nat'1 Lab. 82Se
LGC	-	-	206Pb NIST SRM983 92.1497%	77Se AEA Technology 68.69%
NARL	-	NIST SRM3133	-	-
NIST	High purity metals plus NIST SRMs	-	-	High purity metals plus NIST SRMs
NMIJ	-	-	NIST SRM 981	-
NRC	-	201 Hg Oak ridge	-	-
SP	-	-	-	-
Trent Univ.		201 Hg Trace Sciences		

Table 6 Number of tuna fish vials used for the analysis and Use of square root of *n* for type A uncertainty contributions

REPORTED QUESTIONNAIRE DATA		
Participant	**Number of vials**	**Sqrt (*n*)**
BAM	2	YES
BKÁE-ÉT	3	NO
BNM-LNE	1 per analyte	YES
CENA	1	YES
ENEA	1	NO
Frontier Geosciences Inc.	1	NO
IAEA_AAS	1	NO
IAEA_ICP-MS	1	NO
IMGC	1	YES
IRMM	2	NO
IRMM-SCK	3	YES
JSI	6	NO
KRISS	2	YES
LGC	2	YES
NARL	5	YES
NIST	1	YES
NMIJ	2	YES
NRC	3	YES
SP	1	YES
Trent Univ.	1	YES

Annex 4 – Documentation

Contents

CCQM-P39: As, Hg, Pb, Se and Methylmercury in Tuna Fish

Documentation

EUROPEAN COMMISSION
DIRECTORATE GENERAL JRC
JOINT RESEARCH CENTRE
IRMM
Institute for Reference Materials and Measurements

25th April 2003

CCQM-P39
Hg, Pb, Se, As and Methylmercury in Tuna Fish

Information Package

For further information, please contact:

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CCQM-P39 Co-ordinator
Isotope Measurement Unit
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fax: +32 (0)14 571 865
e-mail: yetunde.aregbe@irmm.jrc.be

Institute for Reference Materials and Measurements (IRMM)
European Commission – JRC
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Distribution list

Registered CCQM_IWG participants:

J. Vogl	BAM
A. Berger	BAM
E. De Nadai-Fernandes	CENA
S. De Mora	IAEA
M. Gallorini	IMGC
C. Quétel	IRMM
P. Robouch	IRMM
E. Hwang	KRISS
R. Hearn	LGC
G. Labarraque	LNE
R. Myors	NARL
R. Greenberg	NIST
K. Inagaki	NMIJ
R.E. Sturgeon	NRC
J. Wang	NRCCRM
B. Magnusson	SP

Registered invited participants to CCQM-P39:

R. Morabito	ENEA
J. Mitchell	FGI
M. Horvat	IJS
O.Donard/E. Krupp	LABEC
J.I. Garcia-Alonso	Oviedo Univ.
P. Fodor	Szent Istvan Univ.
H. Hintelmann	Trent Univ.
W. Frech	UMEA

CCQM and RMO delegates for info:

R. Kaarls	President CCQM
R. Wielgosz	CCQM exec. Secretary
M. Sargent	Inorg. Anal. WG chair
H. Semerjian	CCQM KC WG chair
E. de Leer	CCQM Gas Anal. WG chair
W. Richter	CCQM pH WG chair
É. Deák	EUROMET METCHEM
F. Hengstberger	SADCMET

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Institute for Reference Materials and Measurements
IRMM

Geel, 25th April 2003
L/IM/34/03

To: CCQM-P39 registered participants
Subject: CCQM-P39 “*Hg, Pb, Se, As and Methylmercury in Tuna Fish*”

Dear CCQM-P39 participant,

As proposed, discussed and decided in previous Inorganic Analysis Working Group and CCQM meetings (Paris, Ottawa 2002), IRMM will act as the pilot laboratory of the CCQM-P39 pilot study, *“Hg, Pb, Se, As and Methylmercury in Tuna Fish”.*

The CCQM-P39 sample is a freeze dried and ground tuna fish powder bottled in amber glass vials each one containing ~ 4 g of material. The tuna fish originates from the Mediterranean Sea close to Messina and was taken off the market due to its elevated amount content of Hg. The material is tested for stability and homogeneity. It is appropriate for the needs of this comparison.

The deadline for reporting the CCQM-P39 results is **26th September 2003**. Subsequently the preliminary results can be presented and discussed at the CCQM WG meeting 13th –15th October 2003 at EMPA in Switzerland.

The information package for CCQM-P39 includes:

- This letter
- Scope of the CCQM-P39 pilot study
- General instructions
- Instructions for result reporting and uncertainty evaluation
- Instructions for the dry mass correction and digestion of the tuna fish
- Information for CCQM-P39 participants measuring methylmercury
- Example of result report table (excel.xls to be returned by email to yetunde.aregbe@irmm.jrc.be)
- Example of questionnaire form (word.doc to be returned by email to yetunde.aregbe@irmm.jrc.be)

If any further information is required, please do not hesitate to contact us.

Yours sincerely,

Dr Y. Aregbe
CCQM-P39 co-ordinator
IRMM, IM Unit

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Geel, 25th April 2003

CCQM-P39
"Hg, Pb, Se, As and Methylmercury in Tuna Fish"

Scope of the pilot study

The CCQM-P39 was agreed upon in previous Inorganic Analysis Working Group and CCQM meetings (Paris, Ottawa 2002). IRMM was designated as the pilot laboratory in this pilot study.

In order to protect public health it is essential to keep contaminants at levels, which are toxicologically acceptable, thus surveillance measures were taken regarding the presence of contaminants in foodstuff, including fish.

Mercury is a potential environmental toxicant. The main source of human intake of mercury contaminants originates from methylmercury in fish and fishery products. Methylmercury is particularly interesting due to its high toxicity compared to inorganic mercury and its high proportion among organomercury species in the environment. Mercury species, may induce alterations in the normal development of the brain of infants and may induce neurological changes in adults. To protect public health, maximum levels of mercury in fishery products are laid down in relevant regulations.

Lead may induce reduced cognitive development in children and increased blood pressure and cardiovascular diseases in adults. The EC Directive 2001/22 describes the Community methods for the sampling, the sample preparation and the analysis of Hg and Pb in fish. Very recently another EC Regulation (466/2001) was introduced that endorses officially the threshold value of 1 mg $Hg \cdot Kg^{-1}$ in tuna fish [1].

Selenium is an essential trace element for human beings. Seafood is an important source of Se intake for people in some regions. Certain forms of cancer and cardiovascular diseases have also been associated with Se deficiency. On the other hand Se is counted among the most important elements in terms of food-chain contamination. Se has the narrowest plateau between concentrations that show deficiency and toxic effects, respectively. Recently the European Commission has requested the Scientific Committee on Food (SCF) to review the upper level of daily intake of individual vitamins and minerals, amongst them Se, and provide the basis for the establishment of safety factors [2].

Arsenic is a mononuclidic toxic element and cannot be determined by IDMS. Including also total As measurements in CCQM-P39 can be seen as a contribution to broaden the scope and degree of difficulty of measurements addressed by the CCQM Inorganic Working Group.

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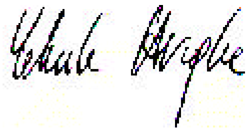
From a metrological point of view, the measurement of the amount content of mercury in tuna fish is representative for many similar measurements in fish and other food matrices. Measurements of MeHg, which is the main mercury species present in this tuna fish material would demonstrate the measurement capabilities of the participating institutes in this important field and broaden the scope of CCQM Inorganic Working Group activities.

Laboratories who demonstrate their capability of measuring the Hg, Pb, Se and As amount content in the CCQM-P39 tuna fish samples, are likely to have the capability, knowledge and skills to measure the amount content of other elements at similar levels in other food matrices which require similar sample preparation.



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CCQM-P39 co-ordinator
IRMM, IM Unit

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1 Commission Regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuff, Official Journal of the European Communities L077, 16/03/2001, 0001-0013

2 Guidelines of the Scientific Committee on Food for the development of tolerable upper intake levels for vitamins, minerals and trace elements SCF/CS/NUT/UPPLEV/11 Final, Nov/2000

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Geel, 25th April 2003

CCQM-P39
Hg, Pb, Se, As and Methylmercury in Tuna Fish

General instructions and guidelines

- On arrival, it is recommended to store the glass vials containing the tuna fish material refrigerated at 4 °C.
- Please inform by email (yetunde.aregbe@irmm.jrc.be) of day of receipt and condition of the vials received.
- The amount content of the measurands in CCQM-P39 in the tuna fish are approximately in the range of 1-10 ppm.
- In view of the limited number of tuna fish samples available, please plan carefully your measurements as far as the amount of sample needed is concerned.
- The participant is free to choose the analytical procedure for measurements in the tuna fish sample provided it is fit for purpose.
- However, since the majority of the laboratories participating in CCQM-P39 will use IDMS, a few specific points for this analytical method are highlighted although most of the participants have considerable experience in isotope dilution measurements.
    - minimise contamination (work in closed systems or class 100 clean bench, check reagents and labware used)
    - Prepare the blends and dilutions gravimetrically. Avoid weighing of too small aliquots of solids or liquids in order to minimise the weighing uncertainty.
    - correct sample weighing for dry mass (see specific instructions)
    - spike the tuna fish prior to the digestion
    - make sure that the tuna fish digestion is complete (see specific instructions)
    - possible isobaric interferences for the Hg, Pb, As and Se isotopes should be investigated and treated accordingly
    - the correction factors for mass discrimination in the ratio measurements should be measured repeatedly using materials of known isotopic composition and ratios similar to those in the blends or samples
    - participants measuring MeHg, please consult the attached information letter “certification of MeHg in tuna fish”

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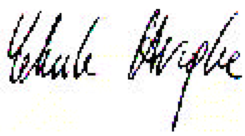
Instructions for result reporting and uncertainty evaluation

- Please report your results by email to: yetunde.aregbe@irmm.jrc.be Enclosed in this information package are examples of the result report sheet and the questionnaire form. The whole information package including these two files for result reporting will also be sent to you via email. Please do not in any case report results hand-written!!!
- The uncertainty statement should be evaluated and presented according to the principles outlined in, e.g. "ISO/GUM" [1] or the Eurachem/CITAC Guide [2].
  This implies that you
  1) state your measurement equation
  2) identify all significant sources of uncertainty
  3) state your input quantities
  4) include factors related to sample treatment in your measurement equations
  5) describe the applied evaluation process and type of assumed distribution for your uncertainty estimation
  6) quantify uncertainty components and convert them to standard uncertainties
  7) calculate the combined standard uncertainty u_c
  8) present an expanded uncertainty U with the coverage factor k=2

  The complete uncertainty statement can be forwarded as attachment by email to yetunde.aregbe@irmm.jrc.be or by FAX to: +32 14 571 865

If you require further information or assistance, do not hesitate to contact us. Please address directly Dr. Christophe Quétel (christophe.quetel@irmm.jrc.be) in case you need more specific information related to the MeHg measurements.



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CCQM-P39 co-ordinator
IRMM, IM Unit

1. International Organisation for Standardisation, "Guide to the Expression of Uncertainty in Measurement", ©ISO, ISBN 92-67-10188-9, Geneva, Switzerland, 1993.
2. Eurachem/CITAC Guide *Quantifying uncertainty in analytical measurement* (2nd ed. 2000), www.eurachem.bam.de.

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Geel, 25th April 2003

Instructions for determination of the dry-mass correction and the digestion of the tuna fish sample in the CCQM-P39 pilot study

There are two potential problems in measurements performed on matrices like the tuna fish material used in this pilot study.
Firstly, and most important, the water content of the powdered tuna fish will affect the tuna fish mass, and a correction for that is needed. From recent work in that area [1, 2] it seems that this correction is very important because depending on the method used a different result can be obtained. This means that the "measurand" is to some extent "operationally defined". In order to achieve comparability of results the protocol as given below, must be followed by the CCQM-P39 participants.
Secondly, the digestion of the tuna fish material (if needed for the procedure that you are following) is never a trivial exercise.
This document intends to give guidance on the above matters.

1. Dry-mass correction

The tuna fish will absorb ambient moisture at typical laboratory temperature and humidity conditions. Therefore the sample bottle should only be opened immediately before weighing aliquots for the IDMS blend preparation.

For correction of the measured values to dry mass, water content measurements should be made on a separate portion of the same material with a minimum mass of 0.5g. The material should be dried before weighing for a minimum of 24 hours in a ventilated oven at 85 ± 2°C. Cycles of drying and weighing should be repeated until a constant mass is attained. Each weighing has to be carried out after the sample has reached thermal equilibrium at room temperature in an evacuated dessicator. Successive weights should not differ more than 0.001 g. The loss of mass corresponds to the "dry-mass correction factor" that should be applied

2. Digestion of the tuna fish (only in case the method used requires digestion)

There are a variety of digestion methods, employing various combinations of acids and for which different instruments are used.

- use minimum sample mass of 0.4 g.
- aim is to digest the tuna fish material completely.
- should this fail, Hg, Pb, As and Se content in the residue should be measured in order to estimate the correction needed and its uncertainty contribution.

If you require any further assistance or information, please do not hesitate to contact us.

Dr Y. Aregbe
CCQM-P39 co-ordinator
IRMM, IM Unit

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1. S. Rückold et. al. Water as a source of errors in reference materials, Fresenius J Anal Chem (2001) 370: 189 - 193
2. S. Rückold et. al. Determination of the contents of water and moisture in milk powder, Fresenius J Anal Chem (2000) 368: 522 - 527

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Geel, 25 April 2003
IM/L/33/03

CCQM-P39
Certification of MeHg in Tuna

Dear colleague,

You have registered for the CCQM-P39 intercomparison on the determination of the MeHg amount content in tuna. In total, there will be 17 participants including 9 from CCQM and 8 invited experts from worldwide.

With the present letter you must have received
- 3 bottles of test material (~ 4 g of powder in each),
- 1 bottle of the BCR-464 CRM (~ 15 g of powder).

Additionally, in the beginning of July 2003, 1 sealed quartz ampoule containing, in 3% ethanol solution, some ^{202}Hg enriched MeHg will be shipped to you on dry ice.

We synthesised this MeHg from the same ^{202}Hg enriched solid HgO material we used originally to produce the IRMM-640 ^{202}Hg enriched inorganic isotopic reference material (cf. Certificate attached). We are currently completing the characterisation of the MeHg material, including a comprehensive assessment of its stability over a one year period.

The tuna material in the BCR-464 CRM (which main characteristics are very close to those of the CCQM-P39 test samples) should help you rehearsing your analytical methodology and investigating specific issues such as the extraction efficiency of the MeHg compound.

The provision with an ampoule of ^{202}Hg enriched MeHg solution is for the participants intending to apply the isotope dilution mass spectrometry (IDMS) method (the very large majority of participants). However, the use of this material (and of the accompanying certified values for the MeHg content and Hg isotopic composition) is only recommended but is by no mean mandatory.
It is expected that the participants will provide experimental results according to one of the 5 approaches described below.

1- Apply a direct IDMS (equation 1) approach using the ^{202}Hg enriched MeHg IRMM solution.
2- Apply a direct IDMS approach using own characterized spike solution.
3- Apply a double IDMS (equation 2) approach using own non-characterized spike solution.
4- Apply a double IDMS approach using the ^{202}Hg enriched MeHg IRMM solution (and thus chose to re-characterize it).
5- Apply another method than IDMS.

Considering that the characterization of the MeHg content in the IDMS spike material is a crucial step, we invite all the ‘IDMS participants’ to apply scenario 1 by making maximum use of the IRMM ^{202}Hg enriched MeHg spike solution. This way, by minimizing the number of potential sources of measurement biases between participants, our intention is to try to minimize the difficulty for interpreting, at the last stage, the differences that could be observed between the results of all the participants.

Participants following scenario 4 will be asked to provide, on top of their original set of results and only for the sake of an additional comparison with the results from the participants following scenario 1, calculation results from the simulation of scenario 1 (with exactly the same set of experimental results and without the need for any extra measurements).

Again, it is emphasized that the participants should remain entirely free to apply the scenario they like best and/or judge most appropriate.

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Equation 1.

$$c_X = c_Y \cdot \frac{m_Y}{m_X} \cdot \frac{R_Y - K_{R_B} \cdot R_B}{K_{R_B} \cdot R_B - R_X} \cdot \frac{\sum (R_i)_X}{\sum (R_i)_Y}$$

Equation 2.

$$C_X = C_Z \cdot \frac{m_Z}{m_X} \cdot \frac{m_Y}{m_Y^{'}} \cdot \frac{R_Y - R_B}{R_B - R_X} \cdot \frac{R_Z - R_B^{'}}{R_B^{'} - R_Y}$$

Detailed information about the IRMM ^{202}Hg isotopically enriched MeHg certified material will be provided to the participants together with the solution itself in July 2003. In the meanwhile, I shall be happy to answer to any questions that could remain regarding these issues.

Yours sincerely,

Dr Christophe Quétel
Isotope Measurements Unit
EC-JRC-IRMM
Email: christope.quetel@irmm.jrc.be

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CERTIFICATE
SPIKE ISOTOPIC REFERENCE MATERIAL IRMM-640

$1.471(11) \cdot 10^{-8}$ mol (^{202}Hg) · g^{-1} (solution)

The Spike Isotopic Reference Material is supplied with an isotope amount content of ^{202}Hg certified as above.

The amount of other mercury isotopes present are related to the ^{202}Hg content through the following certified amount ratios:

$n(^{196}Hg)/n(^{202}Hg)$:	**0.000 018 09(38)**
$n(^{198}Hg)/n(^{202}Hg)$:	**0.000 623(11)**
$n(^{199}Hg)/n(^{202}Hg)$:	**0.001 603(16)**
$n(^{200}Hg)/n(^{202}Hg)$:	**0.005 499(34)**
$n(^{201}Hg)/n(^{202}Hg)$:	**0.013 351(52)**
$n(^{204}Hg)/n(^{202}Hg)$:	**0.002 595(21)**

This corresponds to an isotopic composition with the following abundances:

amount fraction (·100)		mass fraction (·100)	
$n(^{196}Hg)/n(Hg)$	0.001 76 7(37)	$m(^{196}Hg)/m(Hg)$	0.001 71 5(36)
$n(^{198}Hg)/n(Hg)$	0.060 8(11)	$m(^{198}Hg)/m(Hg)$	0.059 6(11)
$n(^{199}Hg)/n(Hg)$	0.156 6(16)	$m(^{199}Hg)/m(Hg)$	0.154 3(16)
$n(^{200}Hg)/n(Hg)$	0.537 1(33)	$m(^{200}Hg)/m(Hg)$	0.531 9(33)
$n(^{201}Hg)/n(Hg)$	1.304 2(50)	$m(^{201}Hg)/m(Hg)$	1.297 9(50)
$n(^{202}Hg)/n(Hg)$	97.685 9(68)	$m(^{202}Hg)/m(Hg)$	97.698 5(68)
$n(^{204}Hg)/n(Hg)$	0.253 5(20)	$m(^{204}Hg)/m(Hg)$	0.256 0(21)

The molar mass of mercury in this sample is 201.944 66(13) $g \cdot mol^{-1}$

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From the certified values, the following amount and mass contents are derived:

$1.506(11) \cdot 10^{-8}$	mol (Hg) · g^{-1} (solution)
$2.971(23) \cdot 10^{-6}$	g (^{202}Hg) · g^{-1} (solution)
$3.040(23) \cdot 10^{-6}$	g (Hg) · g^{-1} (solution)

NOTES

1. This Spike Isotopic Reference Material is traceable to the SI in the shortest possible way. The values of the Hg isotope ratios were measured at IRMM and are traceable to the SI via the values of the Tl isotope ratios of the isotopic reference material NIST SRM 997. The latter material was used for calibration of the IRMM measurements. The values for the amount content are also traceable to values of IRMM-639. Measurements calibrated against this Isotopic Reference Material have therefore the potential of being traceable to the SI.

2. All uncertainties indicated are expanded uncertainties $U = k \cdot u_c$ where u_c is the combined standard uncertainty estimated following the ISO/BIPM Guide to the Expression of Uncertainty in Measurement. They are given in parentheses and include a coverage factor k=2. They apply to the last two digits of the value. The values certified are traceable to the SI.

3. The IRMM-640 has been prepared by dissolution of HgO, which was enriched in ^{202}Hg, in HCl. Potassium dichromate was added as a stabilizer to the solution to achieve a final concentration of 0.05% (m/v) with respect to the dichromate.

4. The Spike Isotopic Reference Material IRMM-640 comes in a flame-sealed quartz ampoule containing about 75 nmol mercury in 5 mL of a chemically stable hydrochloric acid solution. The molarity is about 0.5 M HCl.

5. The atomic masses, used in the calculations, are [1]

^{196}Hg : 195.965 814(8) g·mol^{-1}
^{198}Hg : 197.966 752(6) g·mol^{-1}
^{199}Hg : 198.968 262(6) g·mol^{-1}
^{200}Hg : 199.968 309(6) g·mol^{-1}
^{201}Hg : 200.970 285(6) g·mol^{-1}
^{202}Hg : 201.970 625(6) g·mol^{-1}
^{204}Hg : 203.973 475(6) g·mol^{-1}

6. Using this Spike Isotopic Reference Material, the Hg content in an unknown sample can be determined by Isotope Dilution, through a measurement of the isotope amount ratio $R(B) = n(^{200}\text{Hg})/n(^{202}\text{Hg})$ in a blend. It should be computed with the aid of the following equation which enables an easy quantification of the uncertainty sources in the procedure:

[1] G. Audi and A.H. Wapstra, The 1993 atomic mass evaluation, Nucl Phys A565 (1993) 1-65.

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$$c(Hg, X) = \frac{R(Y) - R(B)}{R(B) - R(X)} \cdot \frac{\sum R_i(X)}{\sum R_i(Y)} \cdot \frac{m(Y)}{m(X)} \cdot c(Hg, Y)$$

where:

$R(X)$	=	amount ratio $n(^{200}Hg)/n(^{202}Hg)$ in the unkwown sample material X
$R(Y)$	=	amount ratio $n(^{200}Hg)/n(^{202}Hg)$ in the spike material Y
$\sum R_i(X)$	=	sum of all amount ratios in the unknown sample material X
$\sum R_i(Y)$	=	sum of all amount ratios in the spike material Y
$m(X)$	=	mass of unknown sample used in the measurement
$m(Y)$	=	mass of the sample of spike solution used in the measurement
$c(Hg, X)$	=	amount content of Hg · g^{-1} sample material
$c(Hg, Y)$	=	amount content of Hg · g^{-1} spike solution.

7. Full details on the certification procedure can be found in IRMM Internal Report GE/R/SIM/49/97[2] and GE/R/IM/40/99[3].

The isotopic measurements were performed by J Vogl by means of Inductively Coupled Plasma Mass Spectrometry. Chemical preparation of the samples for isotopic measurements was performed by J Vogl. Impurity measurements were performed by A Dobney in the starting material and by S Nelms in the final solution.

Metrological weighings required in the preparation and certification were performed by B Dyckmans. The ampoulation of this Spike Isotopic Reference Material was accomplished by G Van Baelen, A Dobney and A Held. A Verbruggen was responsible for the preparation and issuance of the certificate.

The overall co-ordination leading to the establishment, certification and issuance of this Isotopic Reference Material was performed by C Quétel.

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May 2002

Dr P Taylor
Head
IRMM Isotope Measurements

[2] A. Dobney, Preparation of a mercury spike reference material (IRMM-640) (1997)
[3] J. Vogl, A. Dobney, C. Quétel and P. Taylor, Summary of the measurement results for the certification of the Hg candidate CRMs IRMM-639 and IRMM-640 (1999)

CCQM-P39
Hg, Pb, Se, As and Methylmercury in Tuna Fish

RESULT REPORT FORM

Please return this report form together with the questionnaire by 26th September 2003 by email to:
yetunde.aregbe@irmm.jrc.be

Lab identification :

Name	
Institute	
Address	
Country	
E-mail	
Tel. number	
Fax number	

DATE

Please report all your results and uncertainties in the unit mol·kg^{-1}.
The measurement uncertainty should be evaluated according to ISO/GUM[1] , Eurachem/CITAC[2] or similar and have a coverage factor k=2

[1]International Organisation for Standardisation, “Guide to the Expression of Uncertainty in Measurement”, ©ISO, ISBN 92-67-10188-9, Geneva, Switzerland, 1993.
[2]Eurachem/CITAC Guide Quantifying uncertainty in analytical measurement (2nd ed. 2000), www.eurachem.bam.de.

measurand	**amount content in mol·kg^{-1}**	**Uncertainty (k=2)**
As		
Hg		
Pb		
Se		

measurand	**amount content in mol(CH_3Hg)·kg^{-1}**	**Uncertainty (k=2)**
Methylmercury CH_3Hg		

CCQM-P39

Questionnaire for measurements of:

Hg, Pb, Se, As in Tuna Fish

(a separate questionnaire for MeHg measurements will be distributed together with the ^{202}Hg enriched MeHg beginning July 2003)

Please return the questionnaire together with the result report-form by email to:
yetunde.aregbe@irmm.jrc.be

Lab identification : *Name* : *Date*:
Institute :

This information is needed for better recording and interpretation of the reported results and uncertainty statements (if more space is needed for your answers due to the number of measurands, please use an additional copy of this page or separate pages):

1) Analytical method used:…………………………………………………………….

2) Instrumental technique used:…………………………………………………….

3) Description of the measurement procedure including sample pre-treatment (please use separate sheets): ……………………………………………………..

4) Correction for dry mass (% of the weighted):………………………………………

5) Reference isotope (for IDMS):.…………………………………………………….

6) Number of blends (for IDMS):……….…………………………………………….

7) Number of vials from which the tuna fish was analysed:......................................

8) Use of square root of n for type A uncertainty contributions: YES/NO..…………

9) Experimental reproducibility of c_x from different sample aliquots in % (blends in case IDMS) (as standard deviation and not the standard deviation of the mean):
………………………………………………………………………………………

10) (Isotopic) reference materials used for calibration purposes:…………..
………………………………………………………………………………………

11) (For IDMS), please indicate the calibrated isotope ratios for Hg, Pb, Se and As that you determined (including an uncertainty statement, *k*=2) in the tuna fish:
………………………………………………………………………………………
………………………………………………………………………………………
………………………………………………………………………………………

CCQM-P39

Methylmercury in Tuna Fish

Questionnaire

(Please return the questionnaire by email to yetunde.aregbe@irmm.jrc.be)

Lab identification: *Name* : *Date*:

Institute :

This information is needed for better recording and interpretation of the reported results and uncertainty statements (if more space is needed for your answers please use additional pages):

a) **What was the correction for moisture content (% of the weighed sample)?**

b) **Which measurement strategy was used?**

☐ Approach 1: direct IDMS using the ^{202}Hg enriched MeHg IRMM-670 as a spike solution.

☐ Approach 2: direct IDMS using own characterized spike solution.

☐ Approach 3: double IDMS using own non-characterized spike solution.

☐ Approach 4: double IDMS using the ^{202}Hg enriched MeHg IRMM-670 as a spike solution (and thus chose to re-characterize it).

☐ Approach 5: another method than species specific IDMS

if so: ☐ Calibration with external matrix matched standards

☐ With an internal standard, please specify: --------

☐ Without an internal standard

☐ Method of standard addition (SA) calibration

☐ Other, please specify: --------

c) **In case '<u>Approach 4</u>' as described in question b) was applied, please report your results for the determination of the MeHg content calculated using your own values for the IRMM-670 solution and using the values provided by IRMM for the IRMM-670 solution**

☐ Non applicable

☐ Applicable

	Results obtained using own values for the IRMM-670 solution		**Results obtained using the IRMM values for the IRMM-670 solution**	
	amount content in mol(CH_3Hg)·kg^{-1}	Uncertainty (k=2) mol(CH_3Hg)·kg^{-1}	amount content in mol(CH_3Hg)·kg^{-1}	Uncertainty (k=2) mol(CH_3Hg)·kg^{-1}
CH_3Hg				

d) **If a measurement strategy based on SA or ID was used which of the options below most closely fits the timing of the spike addition in your method? Otherwise, please tick in 'Non applicable'**

☐ Non applicable

☐ Some hours prior to MeHg extraction

☐ Immediately prior to MeHg extraction

☐ After MeHg extraction, prior to measurement

e) **If the measurement strategy applied followed the '<u>Approach 2</u>' or the '<u>Approach 3</u>' described in question b), (i.e. another MeHg spike material than IRMM-670 was used), please complete the following questions. Otherwise, please tick in 'Non applicable'**

☐ Non applicable

e-1. Was the methylmercury in the spike material

☐ in-house synthesised?

☐ supplied from outside?

e-2. If known, which method was used for methylmercury synthesis?

e-3. Which enriched isotope was used?

e-4. In the case 'Approach 2' was applied, how was the methylmercury concentration of the spike evaluated

☐ Non applicable

☐ Reference to an external document

☐ Measured in-house, please give brief details of the method: --------

f) **If the measurement strategy applied involved species specific IDMS, '<u>Approaches 1 to 4</u>' described in question b), please answer the following questions. Otherwise, please tick in 'Non applicable'**

☐ Non applicable

☐ Reference isotope used during the measurements

☐ Number of replicate blend samples prepared

g) **Please briefly describe your measurement process by listing the types of reagents and apparatus used as requested in the following fields. If applicable, name the reagents for the extraction and derivatisation of MeHg in the sample.**

g-1. Sample mass (g)

g-2. Extraction reagents (eg. HCl, TMAH etc.)

g-3. Extraction apparatus (eg. microwave, ultrasound bath etc.)

g-4. Derivatisation reagents (eg. $NaB(C_2H_5)_4$, Grignard etc.)

g-5. Species separation apparatus (eg. GC, LC, column type, carrier)

g-6. Detector (eg. CVAAS, ICP-MS etc.)

h) **Were Hg species other than methyl and oxidised inorganic Hg observed in the sample with the measurement method applied?**

☐ NO

☐ YES

if so: ☐ elemental mercury

☐ dimethylmercury

☐ ethylmercury

☐ Other, please specify: --------

i) **Experimental reproducibility of c_x from the different sample aliquots measured in % (blends in case of IDMS) (as standard deviation and <u>not</u> the standard deviation of the mean)?**

IRMM

Institute for Reference Materials and Measurements

CERTIFICATE
SPIKE ISOTOPIC REFERENCE MATERIAL IRMM-670

0.171 1 (27) · 10^{-6} mol (^{202}Hg as $CH_3(^{202}Hg)Cl$) · g^{-1} (solution)

The Spike Isotopic Reference Material is supplied with an isotope amount content of ^{202}Hg as $CH_3(^{202}Hg)Cl$ certified as above.

The amount of other mercury isotopes present under the form of CH_3HgCl are related to the ^{202}Hg content as $CH_3(^{202}Hg)Cl$ through the following certified amount ratios:

$n(^{196}Hg)/n(^{202}Hg)$ in CH_3HgCl:	**0.000 02 (1)**
$n(^{198}Hg)/n(^{202}Hg)$ in CH_3HgCl:	**0.000 623 (62)**
$n(^{199}Hg)/n(^{202}Hg)$ in CH_3HgCl:	**0.001 60 (10)**
$n(^{200}Hg)/n(^{202}Hg)$ in CH_3HgCl:	**0.005 50 (33)**
$n(^{201}Hg)/n(^{202}Hg)$ in CH_3HgCl:	**0.013 35 (80)**
$n(^{204}Hg)/n(^{202}Hg)$ in CH_3HgCl:	**0.002 59 (36)**

This corresponds to an isotopic composition as CH_3HgCl with the following abundances:

amount fraction (·100)		mass fraction (·100)	
$n(^{196}Hg)/n(Hg)$ in CH_3HgCl	0.001 95 (98)	$m(^{196}Hg)/m(Hg)$ in CH_3HgCl	0.001 90 (95)
$n(^{198}Hg)/n(Hg)$ in CH_3HgCl	0.060 9 (61)	$m(^{198}Hg)/m(Hg)$ in CH_3HgCl	0.059 7 (59)
$n(^{199}Hg)/n(Hg)$ in CH_3HgCl	0.156 3(98)	$m(^{199}Hg)/m(Hg)$ in CH_3HgCl	0.154 0 (96)
$n(^{200}Hg)/n(Hg)$ in CH_3HgCl	0.537 (32)	$m(^{200}Hg)/m(Hg)$ in CH_3HgCl	0.532 (32)
$n(^{201}Hg)/n(Hg)$ in CH_3HgCl	1.304 (77)	$m(^{201}Hg)/m(Hg)$ in CH_3HgCl	1.298 (77)
$n(^{202}Hg)/n(Hg)$ in CH_3HgCl	97.686 (90)	$m(^{202}Hg)/m(Hg)$ in CH_3HgCl	97.699 (90)
$n(^{204}Hg)/n(Hg)$ in CH_3HgCl	0.253 (35)	$m(^{204}Hg)/m(Hg)$ in CH_3HgCl	0.256 (35)

The molar mass of mercury present as CH_3HgCl in this sample is 201.944 6 (13) g·mol^{-1}

From the certified values, the following amount and mass contents are derived:

$0.175\,2\,(28) \cdot 10^{-6}$	mol (Hg as CH_3HgCl) $\cdot$ g^{-1} (solution)
$34.57\,(55) \cdot 10^{-6}$	g (^{202}Hg as $CH_3(^{202}Hg)Cl$) $\cdot$ g^{-1} (solution)
$35.38\,(56) \cdot 10^{-6}$	g (Hg as CH_3HgCl) $\cdot$ g^{-1} (solution)

The definitive version of the certificate will include the overall mercury isotopic composition, as well as the overall mercury amount and mass contents.

NOTES

1. This Spike Isotopic Reference Material is traceable to the SI in the shortest possible way. The values reported in this certificate result from measurements performed at IRMM and are traceable to the SI via the values of the isotopic reference material IRMM-639. The latter material was used for calibration of the IRMM measurements. The values of the Hg isotope ratios in the IRMM-639 material are traceable to the SI via the values of the Tl isotope ratios of the isotopic reference material NIST SRM 997. The Hg content in the IRMM-639 material is traceable to IRMM Hg amount content measurements based on gravimetry (and correction for impurities experimentally determined at IRMM). Measurements calibrated against the IRMM-639 Isotopic Reference Material have therefore the potential of being traceable to the SI.

2. All uncertainties indicated are expanded uncertainties $U = k \cdot u_c$ where u_c is the combined standard uncertainty estimated following the ISO/BIPM Guide to the Expression of Uncertainty in Measurement. They are given in parentheses and include a coverage factor k=2. They apply to the last two digits of the value. The values certified are traceable to the SI.

3. Mercury oxide enriched in ^{202}Hg was used for the preparation of a solution of ^{202}Hg enriched $(CH_3)HgCl$ in IRMM-670. The same starting material had previously been employed for the preparation of IRMM-640, a ^{202}Hg enriched inorganic mercury isotopic reference material. The $(CH_3)HgCl$ was synthesised by reaction with a Grignard reagent and a subsequent comproportionation reaction between $(CH_3)_2Hg$ and inorganic Hg. The process was optimised to give a high yield of the product, with additional steps to minimise contamination with natural Hg or other Hg species. Using GC-ICP-MS, only MeHg and inorganic Hg was detectable in the finished reference material with inorganic Hg in <2 % of the total amount.

4. The Spike Isotopic Reference Material IRMM-670 comes in a flame-sealed quartz ampoule containing about 0.2 micro-mol mercury in 5 mL of a 1% ethanol/water solution, and should be stored below –20 °C.

5. A possible change of the $(CH_3)HgCl$ content during the next 2 years from July 2003 on is covered by the uncertainty. This duration was evaluated from the results of a year stability study (isochronous measurement scheme).

6. The atomic masses, used in the calculations, are [1]

^{196}Hg : 195.965 814(8) g·mol^{-1}
^{198}Hg : 197.966 752(6) g·mol^{-1}
^{199}Hg : 198.968 262(6) g·mol^{-1}
^{200}Hg : 199.968 309(6) g·mol^{-1}
^{201}Hg : 200.970 285(6) g·mol^{-1}
^{202}Hg : 201.970 625(6) g·mol^{-1}
^{204}Hg : 203.973 475(6) g·mol^{-1}

[1] G. Audi and A.H. Wapstra, The 1993 atomic mass evaluation, Nucl Phys A565 (1993) 1-65.

7. Using this Spike Isotopic Reference Material, the methyl mercury content in an unknown sample can be determined by Isotope Dilution, through a measurement of the methyl mercury isotope amount ratio $R(B) = n(^{200}Hg)/n(^{202}Hg)$ in a blend. It should be computed with the aid of the following equation which enables an easy quantification of the uncertainty sources in the procedure:

$$c(Hg,X) = \frac{R(Y) - R(B)}{R(B) - R(X)} \cdot \frac{\sum R_i(X)}{\sum R_i(Y)} \cdot \frac{m(Y)}{m(X)} \cdot c(Hg,Y)$$

where:

$R(X)$	=	amount ratio $n(^{200}Hg)/n(^{202}Hg)$ in the unkwown sample material X
$R(Y)$	=	amount ratio $n(^{200}Hg)/n(^{202}Hg)$ in the spike material Y
$\sum R_i(X)$	=	sum of all amount ratios in the unknown sample material X
$\sum R_i(Y)$	=	sum of all amount ratios in the spike material Y
$m(X)$	=	mass of unknown sample used in the measurement
$m(Y)$	=	mass of the sample of spike solution used in the measurement
$c(Hg,X)$	=	amount content of Hg · g^{-1} sample material
$c(Hg,Y)$	=	amount content of Hg · g^{-1} spike solution.

8. Full details on the certification procedure can be found in IRMM Internal Report GE/R/IM/??/03[2].

Chemical synthesis of the $(CH_3)HgCl$ compound was performed by J. Snell and T. Larsson at Umeå University (Umeå, Sweden). Stability tests were carried by T. Larsson at Umeå University and by J. Snell at IRMM. The isotopic measurements for certification were performed by J. Snell at IRMM by means of Inductively Coupled Plasma Mass Spectrometry. Chemical preparation of the samples for isotopic measurements was performed by J. Snell at IRMM.

Metrological weighings required in the preparation and certification were performed by S Hendrickx at IRMM. The ampoulation of this Spike Isotopic Reference Material was accomplished by T. Larsson and J. Snell at Umeå University.

The overall co-ordination leading to the establishment, certification and issuance of this Isotopic Reference Material was performed by C Quételʼ.

B-2440 GEEL
July 2003

Dr P Taylor
Head
IRMM Isotope Measurements

[2] J. Snell, C.R. Quetel, Preparation of a 202Hg enriched methyl mercury spike isotopic reference material (IRMM-670) (2003)