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CCQM-K43.1

As, Hg, Se and methylmercury content in marine fish (swordfish)

Draft B / Final Report

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ABSTRACT

The CCQM-K43.1; arsenic, mercury, selenium and methylmercury in marine fish (Swordfish) was organized by the inorganic analysis working group (IAWG) of CCQM as a subsequent key comparison of CCQM-K43. National Metrology Institute of Japan (NMIJ) is the coordinating laboratory for this subsequent key comparison.

Nine NMIs and one unofficial institute participated in CCQM-K43.1. The data of unofficial institute were treated as reference data. All participants were allowed to choose the measurands (arsenic, mercury, selenium and methylmercury) of their interest. For measurement arsenic, mercury and selenium, different measurement methods (IDMS, ICP-MS, AAS and INAA) were used and all participants with one exception (NAA) used microwave digestion methods. For measurement methylmercury, all participants used IDMS and different extraction methods (Microwave, Mechanical shaker and Ultrasonic) were used. Most results agreed well with one or two apparent outlier for arsenic and selenium. The agreement of measurement results between NMIs is very good for mercury and methylmercury.

1. INTRODUCTION

The CCQM-K43.1 is Supplementary Key Comparison for Arsenic, Mercury, Selenium and methylmercury in marine fish (Swordfish) for NIM and other interested NMIs to demonstrate and document improvements in measurement capability achieved since CCQM-K43 (As, Hg, Pb, Se and methylmercury in salmon). The CCQM-K43 key comparison, completed in 2005, was a successor to CCQM-P39 (As, Hg, Pb, Se and methylmercury content in tuna fish) study, and was performed to demonstrate and document the capability of interested NMIs to measure As, Hg, Pb, Se and methylmercury content in a fish sample. CCQM-P39 and K43 (parallelized pilot study P39.1) were coordinated by the Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre (JRC) of the European Commission. NIM participated in the pilot study P39.1 but not in K43. NIM wishes to participate in Supplementary Key Comparison to demonstrate improved performance of their analytical capabilities and wants to claim its CMC of the above measurements to CCQM. And for the other NMIs which did not participate in K43, Supplementary Key Comparison is useful. Since NMIJ is in the process of certifying a new marine fish CRM, and since NMIJ successfully participated in both P39 and K43, NMIJ will coordinate CCQM-K43.1. The focused measurands are As, Hg, Se and methylmercury. Because the content of Pb in swordfish is very low, Pb is excluded from the measurand of this comparison.

2. LIST OF PARTICIPANTS

The Supplementary Key Comparison was proposed in October 2006 CCQM/IAWG meeting and approved as CCQM-K43.1. The call for participation was circulated to the NMIs in April 2007. Table 1 contains the full names of all participating NMIs.

Table 1 List of participants:

<i>Participant</i>	<i>Institute Full Name</i>	<i>Country</i>	<i>Contact Person</i>	<i>Chose measurand</i>
BAM	Federal Institute for Materials Research and Testing	Germany	Christian Piechotta	MeHg
CMQ-FC	Centro Metrologia Quimica Fundacion Chile	Chile	Gabriela Massiff	As, Hg
INTI*	Instituto Nacional de Tecnologia Industrial	Argentina	Liliana Valiente	As, Hg, Se, MeHg
KRISS	Korea Research Institute of Standards and Science	Korea	Euijin Hwang	Se
NIM	National Institute of Metrology P.R.China	China	Jun Wang	As, Hg, MeHg
NIST	National Institute for Standards and Technology	USA	Gregory Turk	MeHg
NMIJ	National Metrology Institute of Japan	Japan	Kazumi Inagaki Takayoshi Kuroiwa	As, Hg, Se, MeHg
UME	TUBITAK National Metrology Institute	Turkey	Duran Karakas	As, Hg, Se
VNIIM	All-Russia D.I. Mendeleev Scientific and Research Institute for Metrology	Russia	L.A. Konopelko, Yu.A. Kustikov	As, Hg, Se
CENA/USP**	Centro de Energia Nuclear na Agricultura	Brazil	Elisabete Fernandes	As, Hg, Se

* INTI did not report their result of methylmercury for some reasons of validated the methodology.

**CENA/USP is unofficial participant. Their results were not used for various calculations in this report.

3. TIME SCHEDULE

Deadline for registration: 15 May 2007 the date has been extended to 18 May 2007.

Sample distribution: May – June 2007

Deadline for analysis reporting: 30 September 2007

Distribution:

Each participant received one numbered bottle containing about 10 g sample. The sample number was also the lab register number. The sample had been shipped on 19 June 2007 to the most participants by EMS, as BAM was late to register, the sample sent to them on 29 June.

Table 2 The date of sample sent and the date of report received:

<i>Participant</i>	<i>Sample No.</i>	<i>Date sample sent</i>	<i>Date report received</i>
BAM	10	29 June	16 October
CMQ-FC	9	19 June	29 September and 5 November
INTI	7	19 June	3 October
KRISS	2	19 June	30 September
NIM	1	19 June	30 September
NIST	3	19 June	29 September
UME	6	19 June	1 October
VNIIM	8	19 June	6 October
CENA/USP	5	19 June	28 September

4. THE SWORDFISH SAMPLE

The swordfish sample was prepared for candidate CRM of NMIJ. NMIJ will certify the concentration of some elements, methylmercury and arsenobetaine in this sample in March 2009. We use only results obtained by NMIJ the certified values. The results of the comparison will be mentioned in the certificate.

Sample Preparation:

The material of the swordfish tissue was collected in the Pacific Ocean close to Japan in May-June 2004. The fish was filleted and only muscle tissues were collected. The muscle tissues were cut to convenient size. They were freeze-dried, freeze-pulverized and sieved to yield a fraction with the particle size less than 250 μm . The obtained materials were mixed by V-blender for homogenization. The homogenized powder was packed into clean amber glass bottles (10 g each) and they were sterilized by ^{60}Co gamma radiation (20 kGy). After sterilization, each bottle was vacuum sealed in aluminum-nylon pouch. The material has been prepared 650 bottles.

Homogeneity testing:

The homogeneity of the materials was determined by analyzing 10 bottles selected from the lot of 650 bottles. The content of Hg was measured by thermal decomposition Au-amalgam trap cold vapor atomic absorption spectroscopy. The content of As and Se were measured by ICP-MS, after decomposed by microwave acid digestion. The data were treated with an analysis of variance. The obtained s_{bb} (between-bottle homogeneity standard deviation) are 0.87% for Hg, 0.69% for As and 1.47% for Se. From our experiences of development of other CRMs, we believe that s_{bb} value of methylmercury is at the almost same level. The homogeneity is good enough to be used in this comparison.

5. INSTRUCTIONS TO PARTICIPANTS

The instructions that consisted of a technical protocol and results reporting form sent to the participants by e-mail and with sample distribution.

The technical protocol attached as appendix A instructed participants concerning sample handling and storage, dry-mass correction, uncertainty calculation and reporting of the results. The participants were free to use any suitable analytical method for measurement of As, Hg, Se and methylmercury content provided it was fit for purpose. They had to completely describe their analysis methods in the reporting the results.

6. METHODS OF MEASUREMENT

The methods of measurement were left free to be selected by the participants. This study was expected to enable comparison of different approaches to assay elements in food matrix. Most of participants had dried sample in parallel with analysis according to a method recommended in the technical protocol. Analytical results were calculated on a dry-mass basis. The results of moisture content are shown in Table 3.

The applied analytical method for measurement of As, Hg and Se are summarized in Table 4. All official participants used microwave digestion method and most of them used ICP-MS (CENA used NAA without digestion). The applied analytical method for measurement of MeHg is summarized in Table 5. The extraction method and its condition are different with each participant. Almost of participants applied the IDMS analytical method. GC-ICP-MS, HPLC-ICP-MS, GC-AED and GC-MS were used for measurement.

Table 3 Moisture content:

<i>Participant</i>	<i>n=</i>	<i>Moisture content / %</i>
BAM	3	4.68 ± 0.09
CMQ-FC	3	5.13 ± 0.4
INTI	3	5.58 ± 0.27
KRISS	3	4.3 ± 0.05
NIM	As: 6, Hg: 4, MeHg: 4	As: 4.88 ± 0.29 Hg: 4.88 ± 0.36 MeHg: 5.0 ± 0.3
NIST	6	4.89
NMIJ	10	5.11 ± 0.007
UME	5	2.5 ± 0.1
VNIIM	4	1.50 ± 0.01
CENA/USP	-	Not reported

Table 4 Analytical methods and instrumental techniques in CCQM-K43.1 for As, Hg, Se

<i>Participant</i>	<i>Digestion</i>	<i>Analytical method</i>	<i>Instrumental technique</i>
CMQ-FC	Microwave HNO ₃ , 7 g H ₂ O ₂ , 3 g	external calibration	As: ICP-MS Hg: CVAAS
INTI	Microwave HNO ₃ , 5 mL H ₂ O ₂ , 2 mL-	As, Se: standard addition Hg: external calibration	As, Se: ETAAS Hg: CVAAS
KRISS	Microwave HNO ₃ , 5 mL H ₂ O ₂ , 2 mL H ₂ O, 2 mL	double IDMS	Se: ICP-MS (reaction gas: NH ₃ =0.4 mL/min)

NIM	Microwave HNO ₃ , 5 mL	As: standard addition Hg: Double IDMS	ICP-MS
NMIJ	Microwave As: HNO ₃ , 5 mL H ₂ O ₂ , 2 mL Se: HNO ₃ , 5 mL HClO ₄ , 1 mL Hg: aqua-regia, 6mL	As: external calibration Hg, Se: double IDMS	ICP-MS (As: He=3.0 mL/min & H ₂ =1.0 mL/min, Hg: He=1.5 mL/min, Se: H ₂ =4.0 mL/min)
UME	Microwave HNO ₃ , 10 mL	standard addition	ICP-MS
VNIIM	Microwave HNO ₃ , 10 mL	external calibration	ICP-MS
CENA/USP	-	NAA	k ₀ -INAA

Table 5 Analytical methods and instrumental techniques in CCQM-K43.1 for methylmercury

<i>Participant</i>	<i>Extraction Derivatization</i>	<i>Analytical method</i>	<i>Instrumental technique</i>
BAM	Microwave (C ₆ H ₅) ₄ BNa Hexane	IDMS using ERM-AE670 as a spike and other method	GC-AED and GC-MS (mean of results of two techniques)
NIM	Mechanical shaker KOH CH ₂ Cl ₂ Na ₂ S ₂ O ₃	IDMS using ERM-AE670 as spike	HPLC-ICP-MS (200Hg/202Hg)
NIST	Microwave TMAH CH ₃ COONa NaB(OH) ₄ hexane	Double IDMS using ERM-AE670 as spike	GC-ICP-MS (200Hg/202Hg)
NMIJ	Ultrasonic & Shake KOH HCl NaCl toluene	Double IDMS using ERM-AE670 as spike	GC-ICP-MS (200Hg/202Hg)

7. RESULTS AND DISCUSSION

The CCQM-K43.1 reported results are summarized in Table 6 to Table 9.

Table 6 CCQM-K43.1 results of As

<i>Participant</i>	<i>n=</i>	<i>Results (mg/kg)</i>	<i>U</i>	<i>k</i>
CMQ-FC	6	6.779	0.428	1.96
INTI	6	5.28	0.22	2
NIM	6	6.64	0.32	2
NMIJ	9	6.61	0.17	2
UME	5	6.58	0.3	2
VNIIM	6	4.35	0.24	2
CENA/USP	6	6.642	0.150	2
Average		6.04	SD	1.00

Table 7 CCQM-K43.1 results of Hg

<i>Participant</i>	<i>n=</i>	<i>Results (mg/kg)</i>	<i>U</i>	<i>k</i>
CMQ-FC	8	5.094	0.069	2.18
INTI	6	5.47	0.31	2
NIM	6	5.26	0.15	2
NMIJ	12	5.33	0.13	2
UME	5	5.55	0.41	2
VNIIM	6	5.08	0.40	2
CENA/USP	6	5.435	0.240	2
Average		5.30	SD	0.19

Table 8 CCQM-K43.1 results of Se

<i>Participant</i>	<i>n=</i>	<i>Results (mg/kg)</i>	<i>U</i>	<i>k</i>
INTI	6	1.96	0.31	2
KRISS	4	2.335	0.066	1.96
NMIJ	9	2.18	0.08	2
UME	5	2.72	0.17	2
VNIIM	6	1.32	0.08	2
CENA/USP	6	2.341	0.075	2
Average		2.10	SD	0.52

Table 9 CCQM-K43.1 results of MeHg

<i>Participant</i>	<i>n=</i>	<i>Results (mg/kg)</i>	<i>U</i>	<i>k</i>
BAM	6	5.12	0.18	2.16
NIM	6	4.91	0.16	2
NIST	9	5.08	0.25	2
NMIJ	9	5.00	0.15	2
Average		5.03	SD	0.09

The results that converted the reported results into molar concentration are summarized in Table 10 to Table 13.

Table 10 CCQM-K43.1 results of As

<i>Participant</i>	<i>n=</i>	<i>Results ($\mu\text{mol/kg}$)</i>	<i>U</i>	<i>k</i>
CMQ-FC	6	90.48	5.71	1.96
INTI	6	70.47	2.94	2
NIM	6	88.63	4.27	2
NMIJ	9	88.23	2.27	2
UME	5	87.83	4.00	2
VNIM	6	58.06	3.20	2
CENA/USP	6	88.65	2.00	2
Average		80.62	SD	13.29

Table 11 CCQM-K43.1 results of Hg

<i>Participant</i>	<i>n=</i>	<i>Results ($\mu\text{mol/kg}$)</i>	<i>U</i>	<i>k</i>
CMQ-FC	8	25.40	0.34	2.18
INTI	6	27.27	1.55	2
NIM	6	26.22	0.75	2
NMIJ	12	26.57	0.65	2
UME	5	27.67	2.04	2
VNIM	6	25.33	1.99	2
CENA/USP	6	27.10	1.20	2
Average		26.41	SD	0.96

Table 12 CCQM-K43.1 results of Se

<i>Participant</i>	<i>n=</i>	<i>Results ($\mu\text{mol/kg}$)</i>	<i>U</i>	<i>k</i>
INTI	6	24.82	3.93	2
KRISS	4	29.57	0.84	1.96
NMIJ	9	27.61	1.01	2
UME	5	34.45	2.15	2
VNIM	6	16.72	1.01	2
CENA/USP	6	29.65	0.95	2
Average		26.63	SD	6.56

Table 13 CCQM-K43.1 results of MeHg

<i>Participant</i>	<i>n=</i>	<i>Results (μmol/kg)</i>	<i>U</i>	<i>k</i>
BAM	6	23.74	0.83	2.16
NIM	6	22.77	0.74	2
NIST	9	23.56	1.16	2
NMIJ	9	23.18	0.70	2
Average		23.31	SD	0.43

The figures about the results of K43.1 are followed. In figures, red result points show official participants and blue result point show unofficial participant. The orange line shows the median and the orange dashed line shows the expanded uncertainty of median.

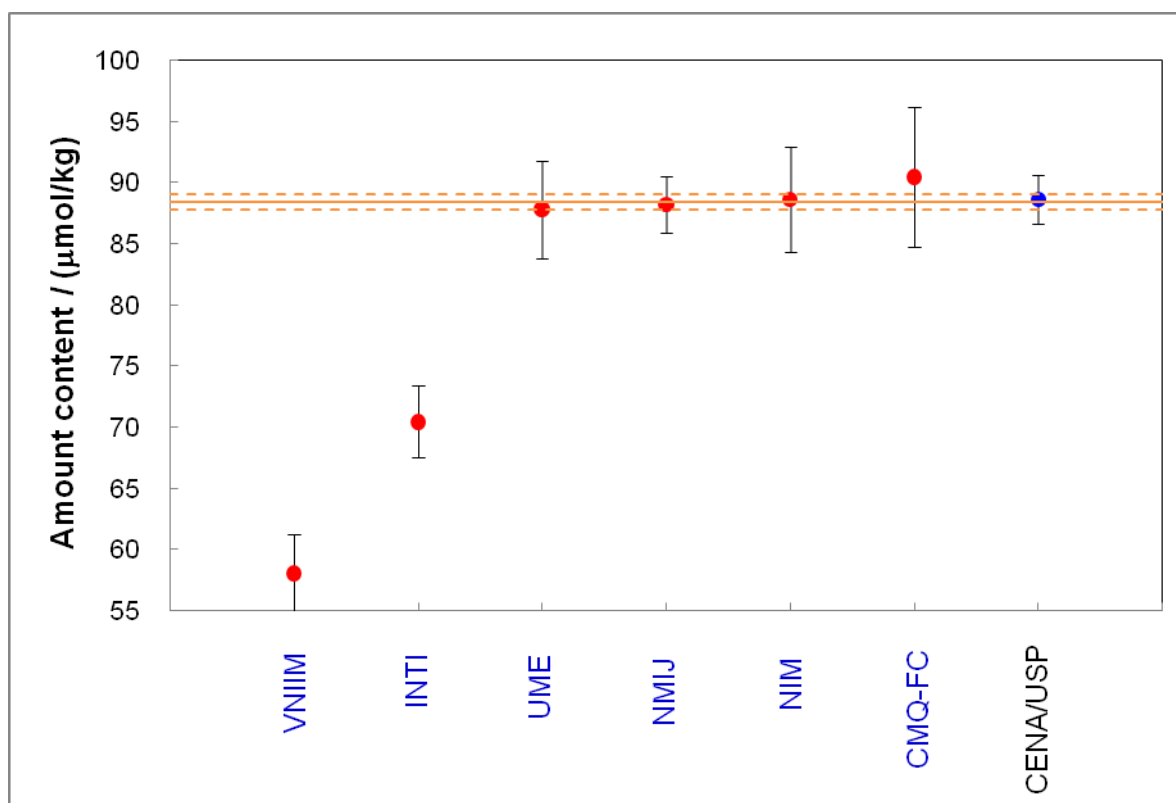


Figure 1 CCQM-K43.1: As in marine fish (swordfish), (The results of CMQ-FC: $k=1.96$)
Median $\pm U$ ($k=2$): 88.4 ± 0.6 $\mu\text{mol/kg}$ (excl. VNIIM & INTI)

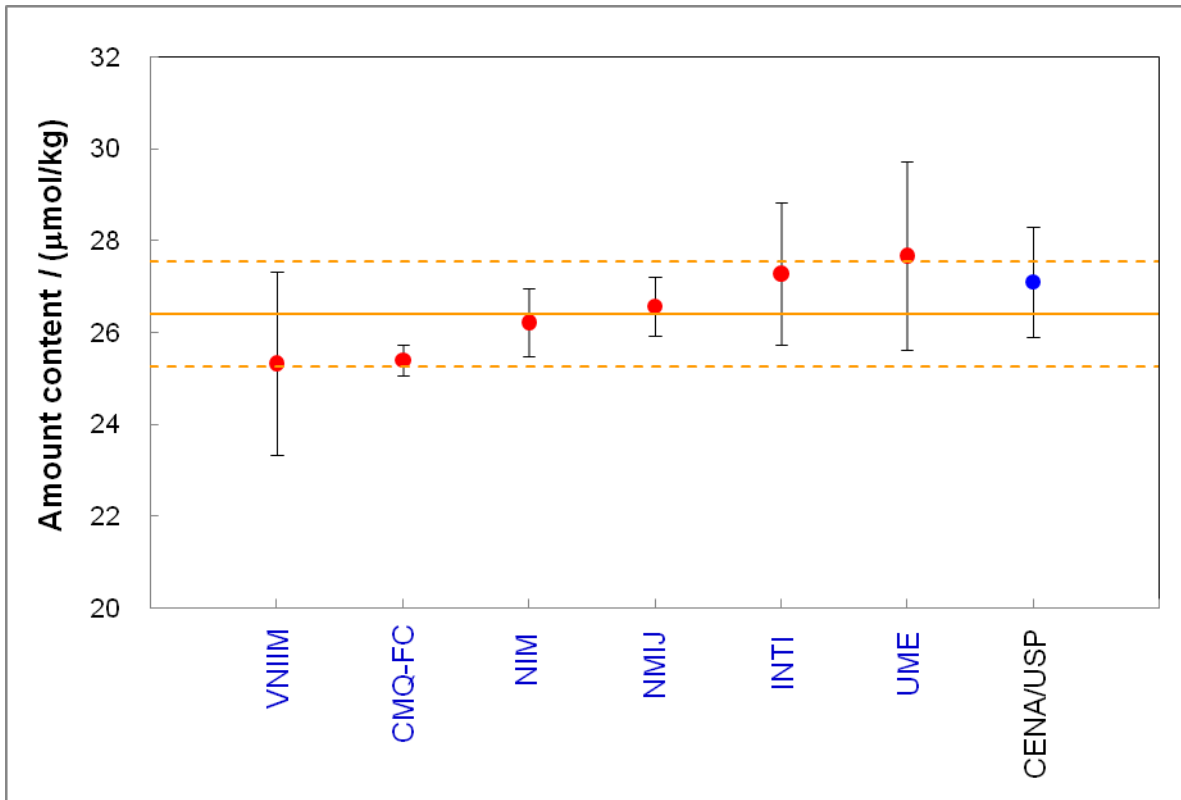


Figure 2 CCQM-K43.1: Hg in marine fish (swordfish), (The results of CMQ-FC: $k=2.18$)
Median $\pm U$ ($k=2$): 26.4 ± 1.1 µmol/kg

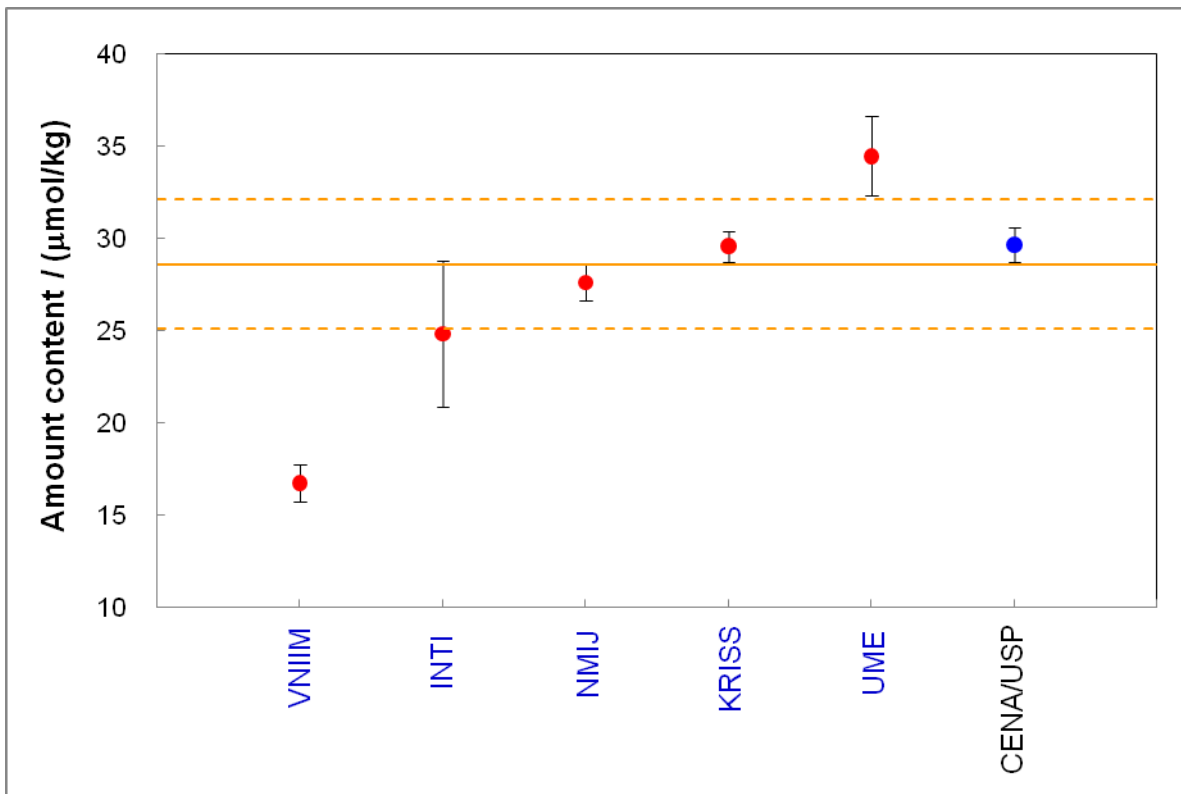


Figure 3 CCQM-K43.1: Se in marine fish (swordfish), (The results of KRISS: $k=1.96$)
Median $\pm U$ ($k=2$): 28.6 ± 3.5 µmol/kg (excl. VNIM)

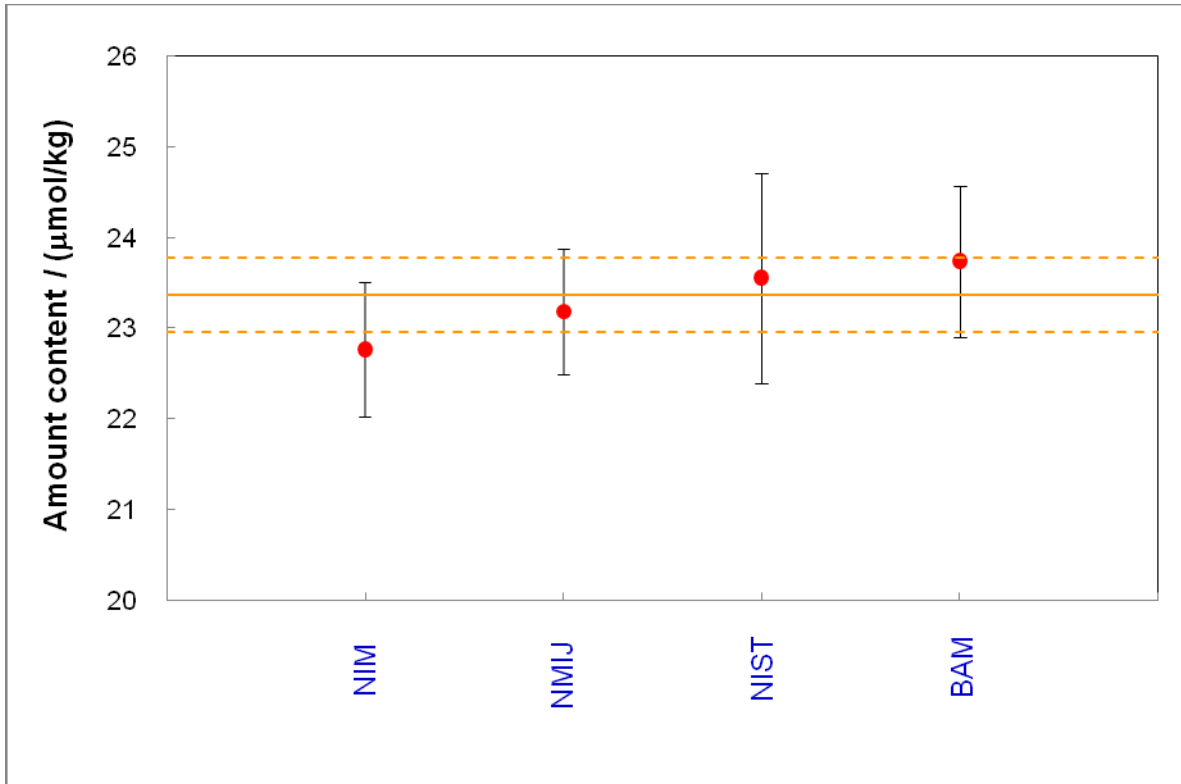


Figure 4 CCQM-K43.1: MeHg in marine fish (swordfish), (The results of BAM: $k=2.16$)
Median $\pm U$ ($k=2$): 23.4 ± 0.4 μmol/kg

Concerning to Hg and methylmercury measurement, all results are very good agreement. Concerning to As and Se measurement, in general results are good agreement with one or two apparent outlier for arsenic and selenium.

8. THE CCQM-K43.1 KCRV

Several approaches to estimate the key comparison reference value (KCRV) were considered.

The difference among the Mean, the median and the weighted mean are compared in Table 14.

In the case of As, the results of VNIIM and INTI are excluded from these calculations for reasons of some technical problems of their results. And in the case of Se, the result of VNIIM is excluded from these calculations for reasons of some technical problems of their result.

INTI analyzed which was their problem in the arsenic determination. As they had employed three CRMs as internal quality control, and they had had a good agreement with all of them, they decided to study the reason for their deviation in this data set. They think, they have a good agreement with the CRM because their As concentration are higher than swordfish, then they have diluted them and decrease the matrix effect which probably cause the interference. The new value obtained using a more dilute solution, is close to the proposed KCRV of other participants. INTI agreed that their result of As is excluded from calculation of the KCRV. VNIIM began to analyze that review of their results of As and Se in order to estimate of possible mistake sources. Their review has not yet finished. But, VNIIM agreed that their results of As and Se are excluded from calculation of the KCRV.

In all measurands, the results of CENA/USP are excluded from these calculations, because they are unofficial participant.

Table 14 Candidates of KCRV of As for CCQM-K43.1

	As (n=4)		Hg (n=6)		Se (n=4)		MeHg (n=4)	
		<i>U</i> (k=2)		<i>U</i> (k=2)		<i>U</i> (k=2)		<i>U</i> (k=2)
Mean*1	88.8	1.2	26.4	0.8	29.1	4.1	23.3	0.4
Median*2	88.4	0.6	26.4	1.1	28.6	3.5	23.4	0.4
Weighted mean*3	88.4	1.1	25.8	0.1	29.1	0.2	23.3	0.2

*1: The expanded uncertainty was based on the standard deviation of the mean.

*2: The uncertainty of the median was based on the estimate from median ($|x_i - \text{median}|$)/0.6745, where x_i is each reported value.

*3: The square of reciprocal of reported uncertainty was used a weight

We proposed that a new KCRV is calculated for this subsequent key comparison rather than link through NMIJ anchor point to the original K43.

Because the significant difference is not found among the mean value, the median value and the weighted mean for all measurands, we proposed that the median for each measurand is reasonable as the KCRV for CCQM-K43.1.

The median of all submitted results (except CENA/USP) is proposed. As and Se results from VNIIM and As result from INTI are excluded from the calculation of the KCRV. These proposals were accepted in IAWG meeting on October, 2008.

9. EQUIVALENCE STATEMENTS

The degree of equivalence and its uncertainty between the result and the KCRV is calculated according to the following equations (the BIPM guidelines):

$$D_i = (x_i - x_R)$$

$$U_i^2 = (k^2 u_i^2 + 2^2 u_R^2)$$

where D_i is the degree of equivalence between the result x_i and the KCRV x_R , and U_i is the expanded uncertainty ($k = 2$; declared ones of some participants were not used.) of D_i calculated by both the combined standard uncertainty u_i of x_i and the standard uncertainty u_R of x_R . The calculation results are shown in Table 15 to Table 18.

Table 15 Equivalence statement for CCQM-K43.1-As

Participant	Content U		k	D_i U_i		D_i U_i	
	/ ($\mu\text{mol/kg}$)			/ ($\mu\text{mol/kg}$)		/ (%)	
KCRV	88.43	0.59	2	-	-	-	-
VNIM*	58.06	3.20	2	-30.37	3.26	-34.34	3.68
INTI*	70.47	2.94	2	-17.95	3.00	-20.30	3.39
UME	87.83	4.00	2	-0.60	4.05	-0.68	4.58
NMIJ	88.23	2.27	2	-0.20	2.35	-0.23	2.65
NIM	88.63	4.27	2	0.20	4.31	0.23	4.88
CMQ-FC	90.48	5.71	1.96	2.06	5.86	2.32	6.63
CENA/USP	88.65	2.00	2	0.23	2.09	0.26	2.36

Table 16 Equivalence statement for CCQM-K43.1-Hg

Participant	Content U		k	D_i U_i		D_i U_i	
	/ ($\mu\text{mol/kg}$)			/ ($\mu\text{mol/kg}$)		/ (%)	
KCRV	26.40	1.13	2	-	-	-	-
VNIM	25.33	1.99	2.18	-1.07	2.29	-4.06	8.69
CMQ-FC	25.40	0.34	2	-1.00	1.18	-3.80	4.46
NIM	26.22	0.75	2	-0.17	1.36	-0.66	5.15
NMIJ	26.57	0.65	2	0.17	1.31	0.66	4.95
INTI	27.27	1.55	2	0.87	1.92	3.31	7.26
UME	27.67	2.04	2	1.27	2.34	4.82	8.86
CENA/USP	27.10	1.20	2	0.70	1.65	2.64	6.25

Table 17 Equivalence statement for CCQM-K43.1-Se

Participant	Content U		k	D_i U_i		D_i U_i	
	/ ($\mu\text{mol/kg}$)			/ ($\mu\text{mol/kg}$)		/ (%)	
KCRV	28.59	3.52	2	-	-	-	-
VNIIIM*	16.72	1.01	2	-11.87	3.66	-41.53	12.81
INTI	24.82	3.93	2	-3.77	5.27	-13.18	18.44
NMIJ	27.61	1.01	2	-0.98	3.66	-3.43	12.81
KRISS	29.57	0.84	1.96	0.98	3.62	3.43	12.67
UME	34.45	2.15	2	5.86	4.13	20.49	14.43
CENA/USP	29.65	0.95	2	1.06	3.65	3.70	12.75

Table 18 Equivalence statement for CCQM-K43.1-MeHg

Participant	Content U		k	D_i U_i		D_i U_i	
	/ ($\mu\text{mol/kg}$)			/ ($\mu\text{mol/kg}$)		/ (%)	
KCRV	23.37	0.41	2	-	-	-	-
NIM	22.77	0.74	2	-0.60	0.85	-2.58	3.63
NMIJ	23.18	0.70	2	-0.19	0.81	-0.79	3.46
NIST	23.56	1.16	2	0.19	1.23	0.79	5.26
BAM	23.74	0.83	2.16	0.37	0.88	1.59	3.75

The figures about the equivalence statements for K43.1 are followed. From Figure 5 to 8 are shown in absolute results, from Figure 9 to 12 are shown in relative results.

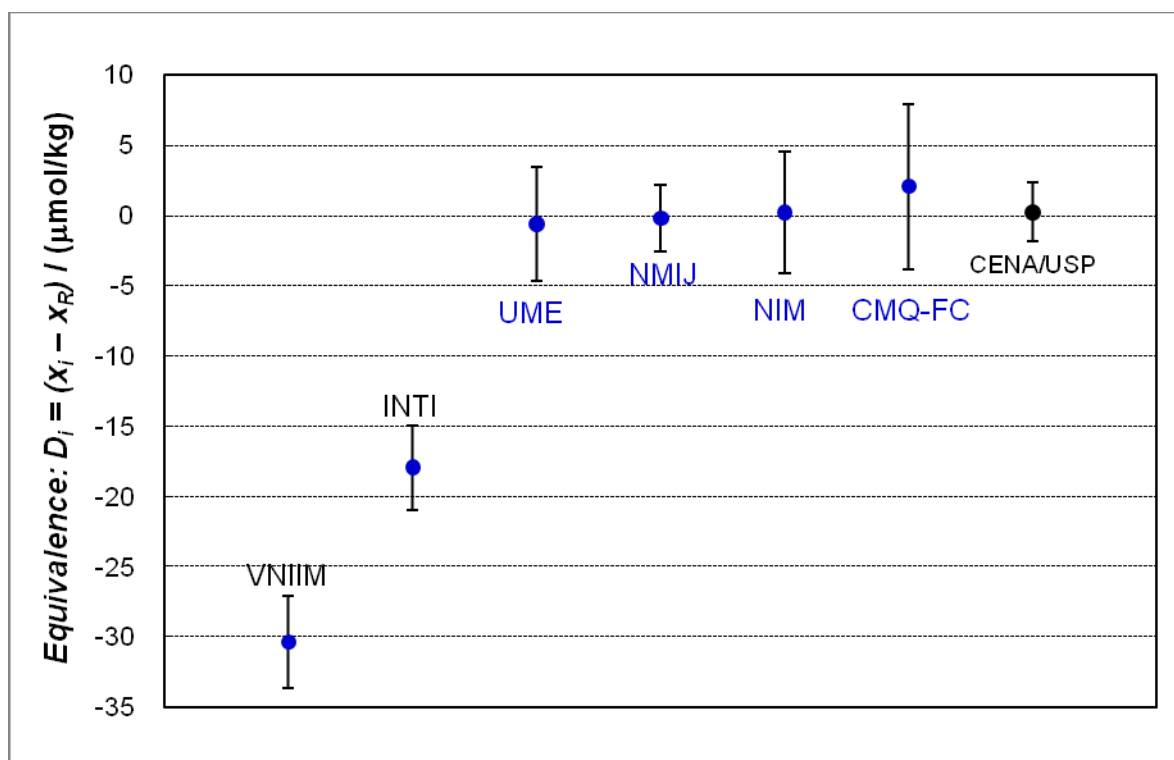


Figure 5 CCQM-K43.1: Degrees of equivalence-As

KCRV_Median $\pm U$ ($k=2$): $88.4 \pm 0.6 \mu\text{mol/kg}$

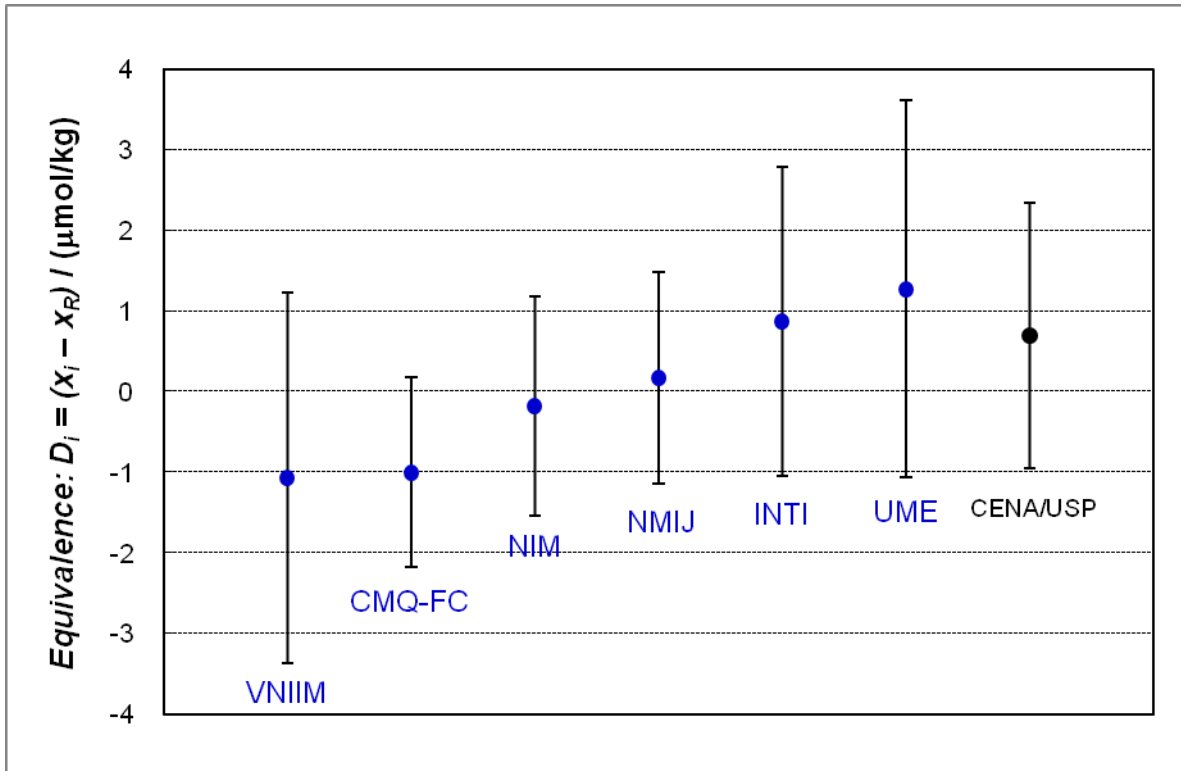


Figure 6 CCQM-K43.1: Degrees of equivalence-Hg

KCRV_Median $\pm U$ ($k=2$): 26.4 ± 1.1 μmol/kg

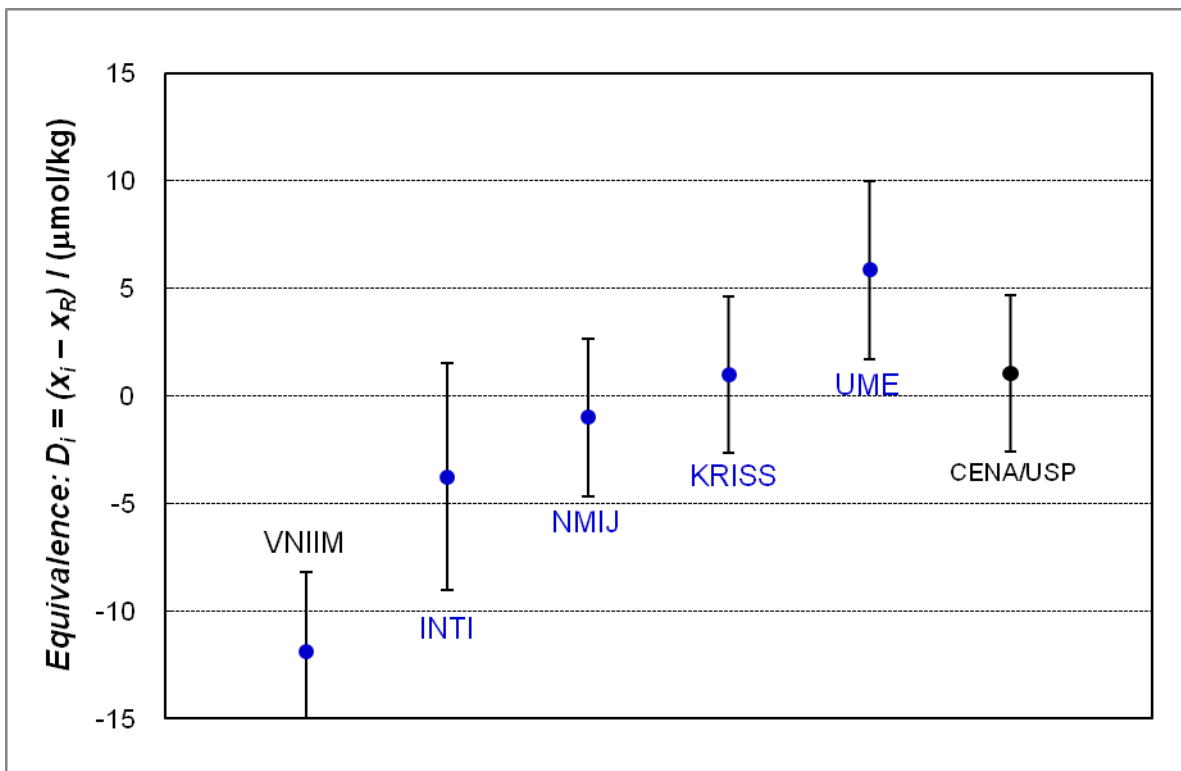


Figure 7 CCQM-K43.1: Degrees of equivalence-Se

KCRV_Median $\pm U$ ($k=2$): 28.6 ± 3.5 μmol/kg

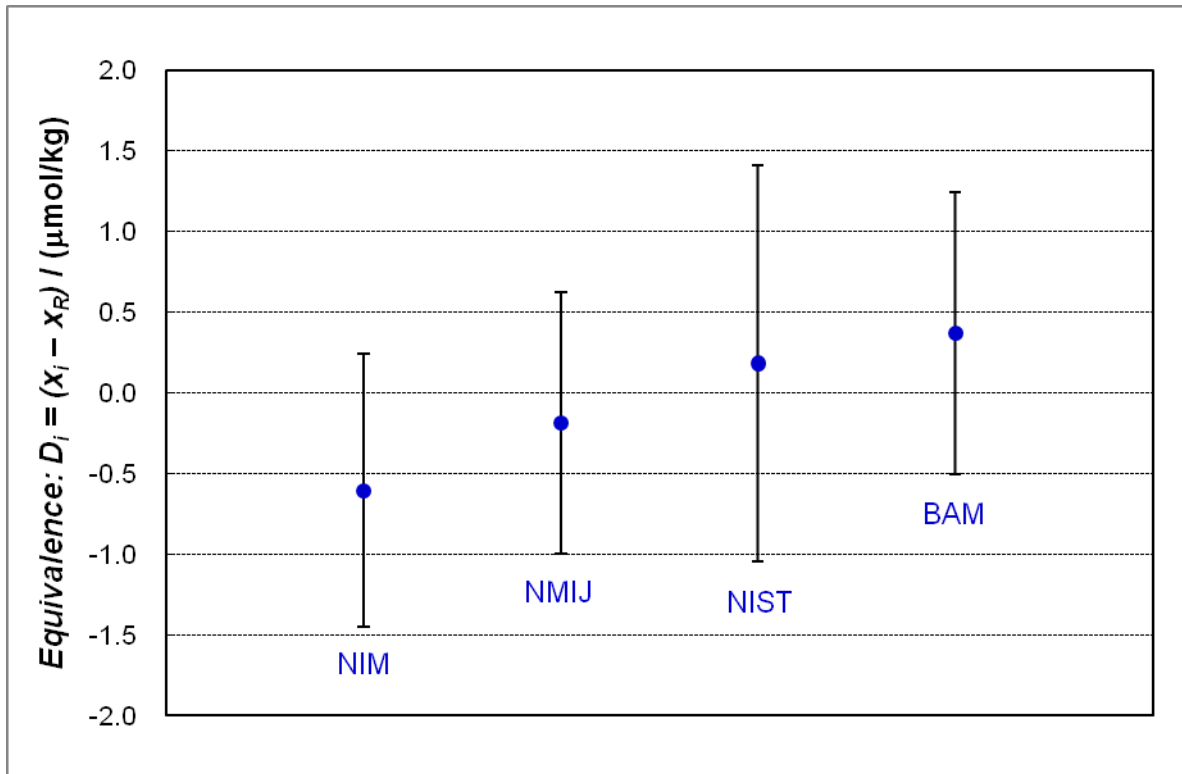


Figure 8 CCQM-K43.1: Degrees of equivalence-MeHg
KCRV_Median \pm U ($k=2$): 22.4 \pm 0.4 $\mu\text{mol/kg}$

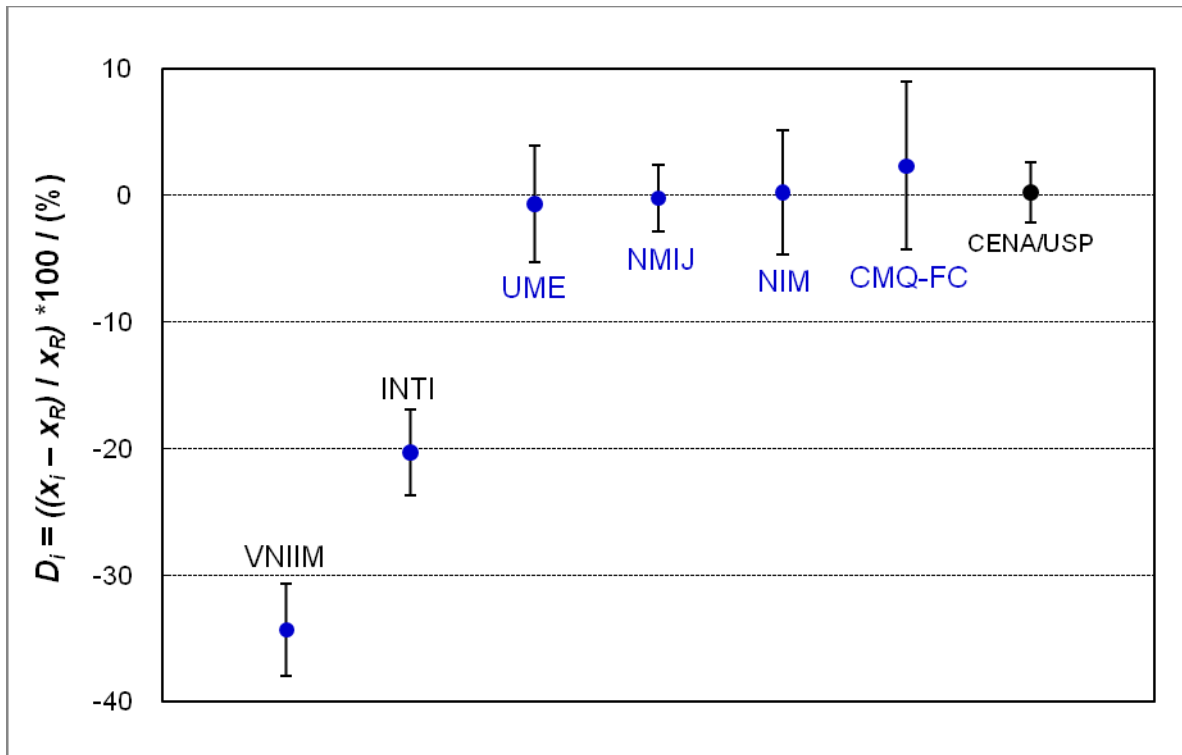


Figure 9 CCQM-K43.1: Degrees of equivalence-As

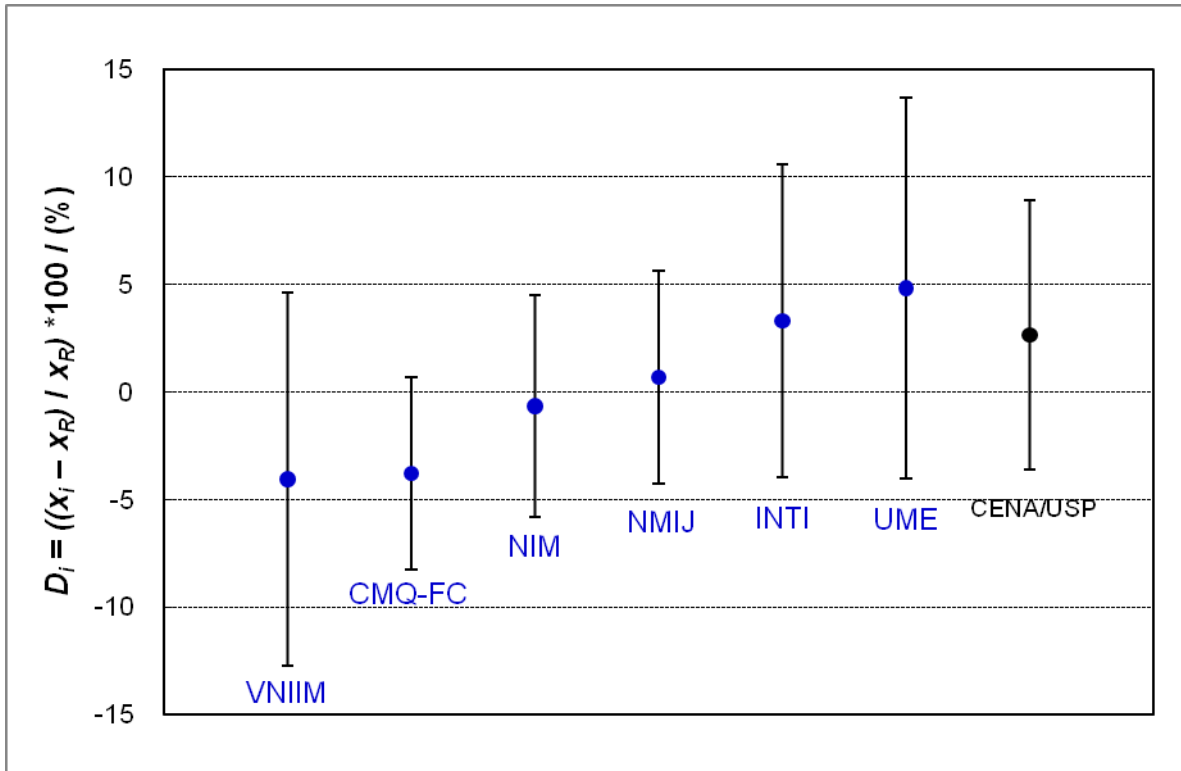


Figure 10 CCQM-K43.1: Degrees of equivalence-Hg

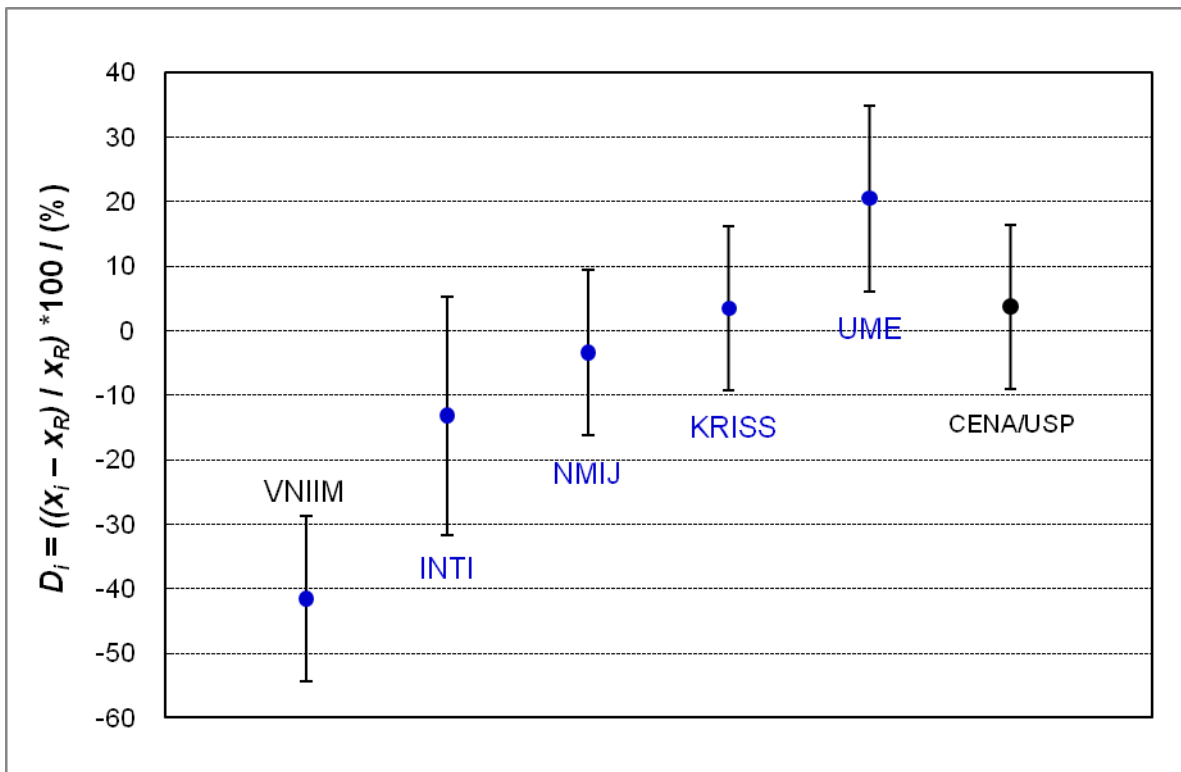


Figure 11 CCQM-K43.1: Degrees of equivalence-Se

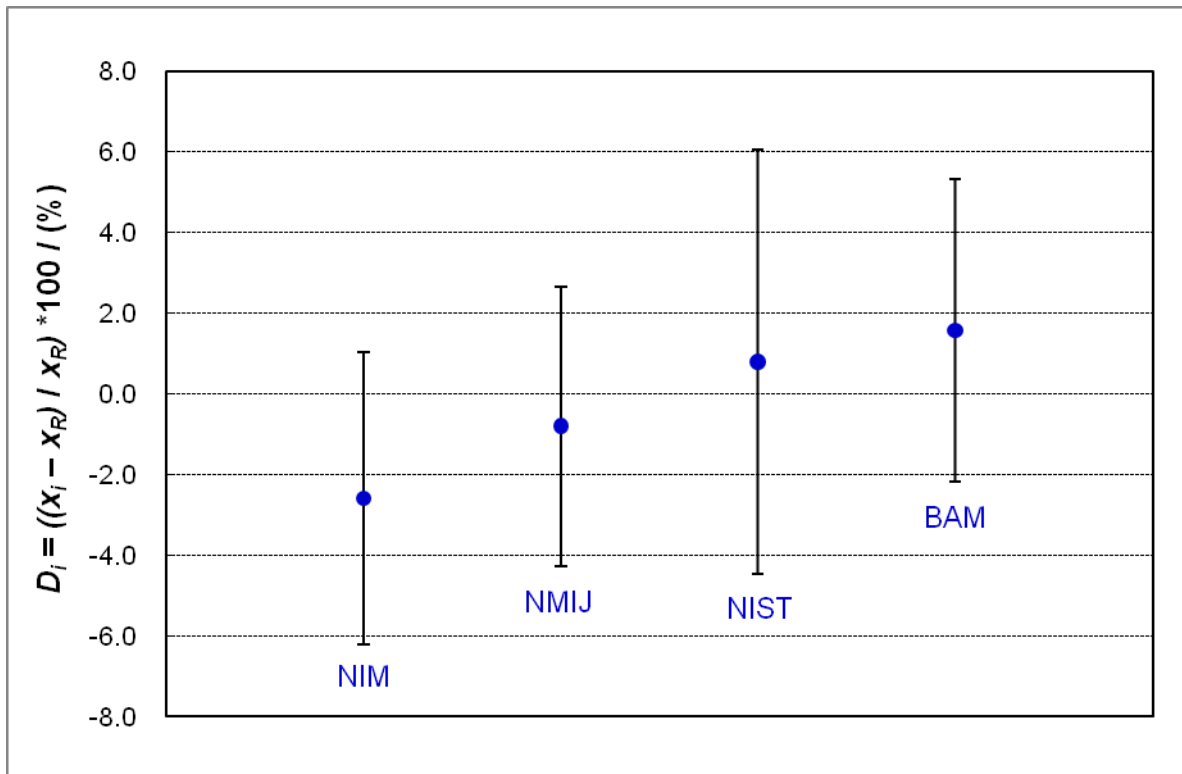


Figure 12 CCQM-K43.1: Degrees of equivalence-MeHg

10. CONCLUSION

The CCQM-K43.1 is a successful subsequent key comparison. Most results overlap within the expanded uncertainty. This key comparison shows the reliable analytical capabilities of the participated NMIs.

Concerning to Hg and methylmercury measurement, all results are very good agreement. Concerning to As and Se measurement, in general results are good agreement. But, the results of VNIIM and INTI have some technical problems. In the case of As, the results of VNIIM and INTI are excluded from these calculations for reasons of some technical problems of their results. And in the case of Se, the result of VNIIM is excluded from these calculations for reasons of some technical problems of their result.

From this key comparison, the capability of the NMIs which participate in K43.1 has been testified in determining elements and methylmercury in food matrix materials field.

ACKNOWLEDGEMENTS

The work of the subsequent key comparison was done by the contributions from many scientists as well as the contact persons and analyst: Christian Piechotta and T. Sommerfeld (BAM); Gabriela Massiff and Monica Espinoza (CMQ-FC); Liliana Valiente, Margarita Piccinna and Lorena Iribarren (INTI); Euijin Hwang, Yong-Hyeon Yim, Yongran Lim and Kyeong Seok Lee (KRISS); Jun Wang, Liandi Ma, Chao Wei and Chao Zhang (NIM); Gregory Turk and W. Clay Davis (NIST); Duran Karakas (UME); L.A. Konopelko, Yu.A. Kustikov, Maksakova I.B., Barkalina U.I. and Fursachik E.V. (VNIIM); Elisabete Fernandes, Cláudio L. Gonzaga and Márcio A. Bacchi (CENA/USP) and Mike Sargent (chairman of CCQM-IAWG).



National Metrology Institute of Japan (NMIJ),
National Institute of Advanced Industrial Science and Technology (AIST),
Tsukuba, JAPAN.

Appendix A

Technical Protocol

CCQM-K43.1

April 12, 2007

**CCQM-K43.1 (Subsequent Key Comparison of CCQM-K43)
As, Hg, Se and methylmercury in marine fish (Swordfish)
Technical Protocol
(Coordinated by NIM and NMIJ)**

CCQM-K43.1 is Supplementary Key Comparison for Arsenic, Mercury, Selenium and methylmercury in marine fish (Swordfish) for NIM and other interested NMIs to demonstrate and document improvements in measurement capability achieved since CCQM-K43 (As, Hg, Pb, Se and methylmercury in salmon). The CCQM-K43 key comparison, completed in 2005, was a successor to CCQM-P39 (As, Hg, Pb, Se and methylmercury content in tuna fish) study, and was performed to demonstrate and document the capability of interested NMIs to measure As, Hg, Pb, Se and methylmercury content in a fish sample. CCQM-P39 and K43 (parallelized pilot study P39.1) were coordinated by the Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre (JRC) of the European Commission. NIM participated in the pilot study P39.1 but not in K43. NIM wishes to participate in Supplementary Key Comparison to demonstrate improved performance of their analytical capabilities and wants to claim its CMC of the above measurements to CCQM. Since NMIJ is in the process of certifying a new marine fish CRM, and since NMIJ successfully participated in both P39 and K43, NMIJ will coordinate CCQM-K43.1. Because the content of Pb in swordfish is very low, we excluded it from the measurand of this comparison.

Time schedule

- Call for participation: April 2007
- Deadline for registration: 15th May 2007
- Sample distribution: May-June 2007
- Deadline for analysis reporting: 30th September 2007

Preparation of swordfish sample

The material of the swordfish tissue was collected in the Pacific Ocean close to Japan in May-June 2004. The fish was filleted and only muscle tissues were collected. The muscle tissues were cut to convenient size. They were freeze-dried, freeze-pulverized and sieved to yield a fraction with the particle size less than 250 μm . The obtained materials were mixed by V-blender for homogenization. The homogenized powder was packed into clean amber glass bottles (10 g each) and they were sterilized by ^{60}Co gamma radiation (20 kGy). After sterilization, each bottle was vacuum sealed in aluminum-nylon pouch. The material has been prepared 650 bottles.

Homogeneity testing

The homogeneity of the materials was determined by analyzing 10 bottles selected from the lot of 650 bottles. The content of Hg was measured by thermal decomposition Au-amalgam trap cold vapor atomic absorption spectroscopy. The content of As and Se were measured by ICP-MS, after decomposed by microwave acid digestion. The data were treated with an analysis of variance. The obtained s_{bb} (between-bottle homogeneity standard deviation) are 0.87% for Hg, 0.69% for As and

1.47% for Se. From our experiences of development of other CRMs, we believe that s_{bb} value of methylmercury is at the almost same level. The homogeneity is good enough to be used in this comparison.

Distribution

Each participant will receive one bottle of 10 g-sample. Participants are required to confirm the receipt of the sealed samples, and send the return receipt to us by e-mail or fax. If any damage will occur, please contact us immediately and NMIJ will send another one.

Nominal value

The content amounts of total As, Hg, Se and methylmercury in the swordfish are approximately in the range of 1 – 10 mg/kg.

Handling and storing

To avoid any decomposition, the samples and arsenobetaine CRMs should be kept sealed until they are used. They should be stored at room temperature in its original bottle, tightly capped and not exposed to intense direct light and ultraviolet radiation. The sample and arsenobetaine CRM bottles should be opened carefully and not left for long to avoid contamination.

Dry-mass correction

Moisture assessment should be carried out in parallel with analysis. Moisture content should be assessed by taking a portion (approximately 0.3 g) of the material and drying it in an oven at $102 \pm 2^\circ\text{C}$ for 6 hours. Analytical results must be calculated on a dry-mass basis.

Sample preparation and measurement method

The participant is free to use any suitable analytical method for measurement of As, Hg, Se and methylmercury content provided it is fit for purpose. Please include a full description of your method of analysis when reporting the results. If IDMS method is used, please report the source of isotopically labelled spike material used.

Reporting

A suggestion for a summary report table will be enclosed (Excel sheet). The report should be sent to the coordinating laboratory (NMIJ) by e-mail **before 30th September 2007**. NMIJ will confirm the receipt of each report.

In order to allow a sufficient evaluation of the comparison, the report must include,

- ◆ A final results and uncertainty summary. The results will be reported as mass fraction [mg/kg]. At least six determinations should be performed (if applicable). If the final result has been calculated from more than one method, the individual results from the contributing methods must also be reported.
- ◆ A detailed description of the applied method of measurement. If more than one method is applied, a detailed description must be given for each method.
- ◆ Information about sample digestion (used acid and quantity, digestion program and so on), extraction (used solvent and quantity, extraction program and so on) and preparation.

- ◆ Information about the reference material used for calibration (origin, standard value and standard uncertainty) or other materials used in the analytical procedure.
- ◆ Calculation of the uncertainty expressed as a combined standard uncertainty and an expanded uncertainty at 95% confidence. The uncertainty should be evaluated according to the principals outlined in, e.g. “ISO/GUM” or the Eurachem/CITAC Guide. It must include:
 - The complete specification of the measurement equation including corrections
 - The identification and quantification of all significant uncertainty sources
 - Calculate the combined standard uncertainty U_c
 - The values for the coverage factor (suggested to $k=2$) and the expanded uncertainty U

Participants

Participation is open to all CCQM members and official observers.

Participants are allowed to choose the measurands of their interest in the four measurands.

Candidate CRM

This sample was prepared for candidate CRM of NMIJ. We will certificate the concentration of methylmercury, arsenobetaine and some elements in swordfish tissue in the near future. We use only results obtained by NMIJ for the certified values. The results of this comparison will mention in the certificate document.

Coordinating laboratory and contact persons

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kk-chiba@aist.go.jp

REGISTRATION FORM

CCQM-K43.1

As, Hg, Se and methylmercury in marine fish (Swordfish)

We would like to register for the determination of the following measurand;

As	Hg	Se	Methyl-Hg

Name (contact person): _____

Institute: _____

Address: _____

Telephone: _____

Fax: _____

E-mail: _____

Signature: _____

Date: _____

Please return this sheet by e-mail or fax no later than 15 May 2007 to:

Takayoshi Kuroiwa
National Metrology Institute of Japan (NMIJ), AIST
Tsukuba Central 3-10, 1-1-1 Umezono,
Tsukuba, Ibaraki, 305-8563, Japan
Fax & Fax: +81-29-861-6889
E-mail: t-kuroiwa@aist.go.jp



RETURN RECEIPT

CCQM-K43.1

As, Hg, Se and methylmercury in marine fish (Swordfish)

Name (contact person): _____

Institute: _____

Address: _____

Tel: _____

Fax: _____

E-mail: _____

Bottle No.: _____

Has any damage occurred? : Yes No _____

Receipt Date: _____

When you received the sample, please return this sheet to:

Takayoshi Kuroiwa

National Metrology Institute of Japan (NMIJ), AIST

Tsukuba Central 3-10, 1-1-1 Umezono,

Tsukuba, Ibaraki, 305-8563, Japan

Tel: +81-29-861-6889 Fax: +81-29-861-6889

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