JRC Scientific and Technical Reports

Report of the second interlaboratory comparison organised by the Community Reference Laboratory for Heavy Metals in Feed and Food

Total Cd, Pb and Hg in mineral water

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EUR 22870 EN - 2007

JRC EUROPEAN COMMISSION

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Joint Research Centre
Institute for Reference Materials and Measurements

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JRC7915

EUR 22870 EN
ISBN 978-92-79-06473-9
ISSN 1018-5593

Luxembourg: Office for Official Publications of the European Communities

Cover Photo: Image Source Limited

Printed in Belgium

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Heavy Metals
in Feed and Food

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IMEP®

July 2007

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1 Summary

The institute for Reference Materials and Measurements (IRMM) of the European Commission's Directorate-General Joint Research Centre holds the Community Reference Laboratory for Heavy Metals in Feed and Food (CRL-HM). One of the core tasks is to organise interlaboratory comparisons (ILCs) among appointed National Reference Laboratories (NRLs). This report presents the results of the second ILC of the CRL-HM which focused on the determination of the total Cd, Pb and Hg content in mineral water.

The test material used in this exercise was a commercial mineral water purchased in Belgium at a local supermarket. The material was spiked with Cd, Pb and Hg, rebottled and dispatched by the Reference Material Unit of the IRMM. The samples were dispatched on the first half of May 2007. Each participant received two sets of samples containing one bottle per set. Each bottle contained approximately 100 mL of test material. Twenty participants from 17 countries registered to the exercise of which 20 submitted results for Cd and Pb and 19 for Hg.

The assigned values were those obtained from the gravimetric measurements used to spike the material. The homogeneity and stability studies were subcontracted to the University of Natural Resources and Applied Life Sciences (BOKU) in Vienna.

The uncertainty of the assigned values was calculated combining the uncertainty of the spiking procedure with a contribution for the between-bottle homogeneity and for long-term stability of the test material. Participants were invited to report the uncertainty on their measurements. This was done by all them.

Laboratory results were rated with z and zeta scores in accordance with ISO 13528[1]. Standard deviation for proficiency assessment (also called target standard deviation) for Cd, Pb and Hg was 10% of the assigned value.

2 Introduction

To overcome problems associated with a high metal content in food and feed maximum allowed limits in several commodities have been laid down in the European legislation, a.o. Commission Regulation (EC) 333/2007[2], Commission Directive 2002/32/EC[3] and Commission Regulation (EC) 1881/2006[4].

In order to utilise a result to decide whether it indicates a compliance or non-compliance with a specification or in deciding whether or not two results are in agreement, it is necessary - as recommended in the ISO 17025 standard[5] and the EURACHEM/CITAC Guide[6] - to take into account the measurement uncertainty. Uncertainty, as defined by VIM[7], is a parameter associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measurand.

Due to a general lack of knowledge of metrology among researchers, laboratory practitioners, laboratory managers and legal experts, not enough effort is invested in calculating the uncertainty associated to a certain analytical

measurement. Recently some guidelines on uncertainty have been included in the legislation[2].

The CRL-HM has organised in the first half of 2007 a proficiency test exercise (PT) to evaluate the capability of the NRLs in estimating the measurement uncertainty of the reported results. A simple "mineral water" matrix - not requiring any long and tedious sample treatment - was selected to monitor the accuracy of the reported results.

3 Scope

As stated in Regulation (EC) No 882/2004 of the European Parliament and of the Council[8], two of the core duties of the CRL-HM are to organise interlaboratory comparisons and training for the benefit of staff from national reference laboratories. The scope of this comparison is to test the competence of the appointed NRLs to evaluate the uncertainty budget associated to the determination of total Cd, Pb and Hg in mineral water.

The assessment of the measurement results is undertaken on the basis of requirements laid down in legislation[2,4], and follows the administrative and logistic procedures of IMEP[9], the International Measurement Evaluation Programme of the Institute for Reference Materials and Measurements (IRMM) of the European Commission's Directorate-General Joint Research Centre. The designation of this intercomparison is IMEP-102.

4 Time frame

The interlaboratory comparison was agreed upon by the NRLs network at the first CRL-HM workshop on 25/26 September 2006. Invitation letters were sent to the participants on 27 March 2007 (cf. Annex 1). The samples were dispatched to participants on 8 May 2007. Reporting deadline was 8 June 2007 (which is one week later than what was initially mentioned in the invitation letter due to a delay in the dispatch of the samples).

5 Test material

5.1 Preparation

The mineral water, purchased at a local supermarket, was weighed (12011.62 g) and spiked with Merck standard solutions as follows:
3.656 g of Cd (999 ± 2 mg L^{-1}), having a density of 1.013 g mL^{-1};
2.424 g of Pb (1000 ± 2 mg L^{-1}), with d = 1.02 g mL^{-1} and
6.163 g of Hg (1000 ± 2 mg L^{-1}), with d = 1.054 g mL^{-1}).
After spiking and homogenisation the water was dispensed in polyethylene bottles of approximately 110 mL capacity. Preparation and homogenisation of the test material was done by the Reference Materials Unit of the IRMM.

5.2 Homogeneity and stability

The measurements for homogeneity and stability studies were performed at the University of Natural Resources and Applied Life Sciences (BOKU, Vienna). Homogeneity was evaluated according to the method proposed by Fearn and Thompson[10] (one of the approaches recommended by the IUPAC International Harmonised Protocol[11]) and to the method proposed in the ISO 13528[1]. The test material proved to be homogeneous for the three measurands, total Cd, Pb and Hg, according to the two protocols.

The study of the stability of the test material was conducted following the isochronous approach[12]. The evaluation of the stability of the test material was made using the Soft CRM software[13] licensed to Reference Materials Unit of the IRMM. The material proved to be stable at 18 ºC during fourteen weeks, from production of the material to the deadline for submission of results.

The analytical results and statistical evaluation of the homogeneity and long-term stability studies are provided in Annex 2.

5.3 Distribution

Two sets of material were sent to the participants. The test material was dispatched to the participants by IRMM on 8 May 2007. Each participant received: a) two bottles containing approximately 110 mL of test material, b) an accompanying letter with instructions on sample handling and reporting (cf. Annex 3) and c) a form that had to be sent back after receipt of the sample to confirm its arrival (cf. Annex 4).

6 Instructions to participants

Details of this ILC were discussed with the NRLs at the first workshop. Concrete instructions were given to all participants in a letter that accompanied the samples. The measurands and matrix were clearly defined as "total Cd, Pb and Hg in mineral water".

Laboratories were asked to perform two or three independent measurements and report them, together with the mean of the results and its associated uncertainty. Participants were asked to follow their routine procedures. The results were to be reported in the same manner (e.g., number of significant figures) as when reporting to customers.

The results were to be reported in a special online form for which each participant received an individual access code. A special questionnaire, aiming to collect additional information, was included in the online form. The questionnaire is presented in Annex 5.

7 Reference values and their uncertainties

As described earlier, the test material used in this exercise was mineral water fortified with aliquots of standard solutions of the analytes, gravimetrically measured. The reference value (X_{ref}) for this ILC was calculated using the following equation:

$$C_{water} = \frac{m_{std} * c_{std}}{d_{std} * m_{water}} \quad \text{Eq. 1}$$

where:

C_{water} final concentration of Cd, Pb and Hg in the test material, respectively;
m_{std} mass of the Cd, Pb and Hg standard solution, respectively;
d_{std} density of the Cd, Pb, and Hg standard solutions, respectively;
m_{water} final mass of test material after fortification with the heavy metal standard solutions and acidification with HNO_3.

The uncertainty associated to the assigned value (u_{ref}) was calculated as:

$$u_{ref} = \sqrt{u_{char}^2 + u_{bb}^2 + u_{lts}^2} \quad \text{Eq. 2}$$

where:

u_{ref} uncertainty associated to the assigned value
u_{char} standard uncertainty of characterisation
u_{bb} contribution for the between-bottle homogeneity
u_{lts} uncertainty contribution derived from the long-term-stability study

The values of X_{ref}, u_{char}, u_{bb}, u_{lts}, u_{ref} and the expanded uncertainty U_{ref}, are summarised in Table1.

Table 1: assigned values and their uncertainties for the parameters of this ILC.

	X_{ref} [mg kg^{-1}]	**u_{char}** [%]	**u_{bb}** [%]	**u_{lts}** [%]	**u_{ref}** [%]	**U_{ref}** [mg kg^{-1}]
Cd	0.300	0.256	0.13	1.20	1.23	0.0074
Pb	0.198	0.255	0.86	1.30	1.58	0.0062
Hg	0.486	0.248	0.29	0.70	0.80	0.0078

X_{ref} is the certified reference value and u_{ref} the corresponding standard uncertainty; U_{ref} is the estimated expanded uncertainty, with a coverage factor k=2, corresponding to a level of confidence of about 95%.

8 Evaluation of results

8.1 General observations

Twenty laboratories from 17 countries registered for participation in this exercise. Twenty laboratories reported results for Cd and Pb and 19 for Hg. All laboratories reported two or more measurement values. All laboratories except one reported the measurement uncertainty. All participants responded to the questionnaire included in the online reporting form.

8.2 Scores and evaluation criteria

Individual laboratory performance is expressed in terms of z and zeta scores in accordance with ISO 13528[1] and the International Harmonised Protocol[11]

$$z = \frac{x_{lab} - X_{ref}}{\hat{\sigma}} \qquad \text{Eq. 3}$$

$$zeta = \frac{x_{lab} - X_{ref}}{\sqrt{u^2_{ref} + u^2_{lab}}} \qquad \text{Eq. 4}$$

where

x_{lab} is the measurement result reported by a participant
X_{ref} is the certified reference value (assigned value)
u_{ref} is the standard uncertainty of the reference value
u_{lab} is the standard uncertainty reported by a participant
$\hat{\sigma}$ is the standard deviation for proficiency assessment

The z score compares the participant's deviation from the reference value with the standard deviation accepted for the proficiency test, $\hat{\sigma}$. Very frequently, in the area of food and feed σ is derived from the improved Horwitz equation[14]. The values for σ obtained for this exercise when applying the improved Horwitz equation were 19, 20 and 18 % for Cd, Pb and Hg, respectively. Those values were considered not stringent enough taking into consideration the simple matrix and the high concentration levels of the analytes present in the test material. For this reason a standard deviation of 10 % was chosen for the evaluation of the results. Should participants feel that this approach is not fit for their purpose they can recalculate their scorings with a standard deviation matching their requirements. X_{lab} is the mean of the individual measurement results calculated by the ILC organiser. The z-score can be interpreted as:

$|z| \leq 2$ satisfactory result
$2 < |z| \leq 3$ questionable result
$|z| > 3$ unsatisfactory result

The interpretation of the zeta score is similar to the interpretation of the z-score.

The standard uncertainty of the laboratory (u_{lab}) was calculated dividing the reported expanded uncertainty by the reported coverage factor (*k*). When no uncertainty was reported, it was set to zero ($u_{lab} = 0$). When *k* was not specified, the reported expanded uncertainty was considered as the half-width of a rectangular distribution; u_{lab} was then calculated by dividing this half-width by √3, as recommended by Eurachem and CITAC[6].

8.3 Laboratory results and scores

The results, as reported by the participants, are summarised in Table 2a-c for Cd, Pb and Hg, respectively, together with the z- and the zeta scores. Laboratory codes were given randomly.

Three sets of figures are provided for Cd, Pb and Hg (Fig 1-3). Each set includes (a) the Kernel Density plot, (b) individual mean value and associated expanded uncertainty, (c) the z- and zeta scores. The solid line represents the assigned value, the dotted lines delimit the reference interval ($X_{ref} \pm 2u_{ref}$) and the dashed lines delimit the target interval ($X_{ref} \pm 2\sigma$). The Kernel plots were obtained using a software tool developed by AMC[15].

Laboratory L03 most likely made a mistake in reporting the units of the submitted results since the values reported for the three analytes were systematically three orders of magnitude higher than the assigned values. For this reason no scorings were given to L03.

Taking into consideration the z-score, all laboratories performed well against the target standard deviation of 10% for Pb and Hg. For Cd seventeen laboratories (90%) obtained z-scores $|z| \leq 3$, one laboratory (5%) obtained a z-score $2 < |z| \leq 3$ and one laboratory (5%) obtained a z-score $3 > |z|$.

Regarding the zeta-scores for Cd fifteen laboratories (80%) reported satisfactory, one questionable (5 %) and three unsatisfactory (15%). For Pb analysis, sixteen laboratories (84%) scored satisfactory, one (5%) questionable and two (11%) unsatisfactory. For Hg, eleven laboratories (61%) had a satisfactory zeta-score, six (33%) questionable and one (6%) unsatisfactory. L20 most likely used the wrong units to report uncertainty.

As stated in the International Harmonised Protocol[11], "*zeta-score provides an indication of whether the estimate of uncertainty is consistent with the laboratory's deviation from the reference value*". An unsatisfactory zeta-score might be due to an underestimation of the uncertainty, or to a gross error causing a large deviation from the reference value. Thus, a laboratory having a satisfactory z-score and an unsatisfactory zeta-score is likely to have an underestimated uncertainty.

Additional information was gathered from the questionnaire completed by the participants. When asked about the level of confidence reflected by the reported coverage factors (k) sixteen laboratories reported a level of 95%, two did not provide any figure and two gave an answer which did not correspond to the question.

As for uncertainty estimates, various combinations of two or more options (cf: question 3) were selected by several laboratories. Fourteen laboratories reported having made use of precision, four laboratories used intercomparison data, two included a guesstimate, three calculated the uncertainty budget according to ISO-GUM, one laboratory followed ISO 5725-2 and one followed a national standard. Nineteen laboratories reported a coverage factor for their uncertainty. Twelve laboratories declared that they provide regularly an uncertainty

statement to their customers. Participants were also asked about the main component of their uncertainty. The answers are summarised in Annex 6.

Five laboratories analysed the test material following an official method. The information reported by the remaining 15 laboratories about their method of analysis is summarised in Annex 7.

Thirteen laboratories out of the twenty carry out this type of analyses on a routine bases. The distribution of these thirteen in terms of number of samples analysed per year is shown in Annex 8.

Nineteen laboratories have a quality system in place, the nineteen being accredited according to ISO/IEC 17025. However, seven laboratories out of the nineteen are not accredited for this type of samples.

Fifteen laboratories participate regularly in ILCs.

Nine laboratories use a certified reference material (CRM) for this type of analysis, of which two laboratories use the CRM for calibration of the instrument and all of them during the validation of the method.

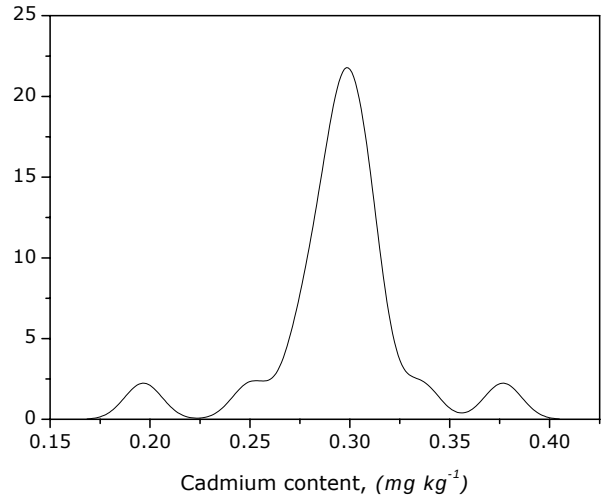

Figure 1a
Cadmium in mineral water
Kernel density,
All results (excl. L03)

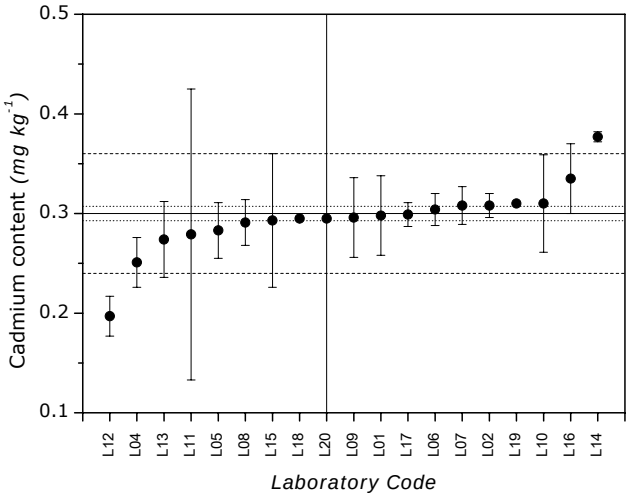

Figure 1b
Reported results and corresponding expanded uncertainties

X_{ref} = 0.300
u_{ref} = 0.004
$\hat{\sigma}$ = 0.030 (10%)
in mg kg^{-1}

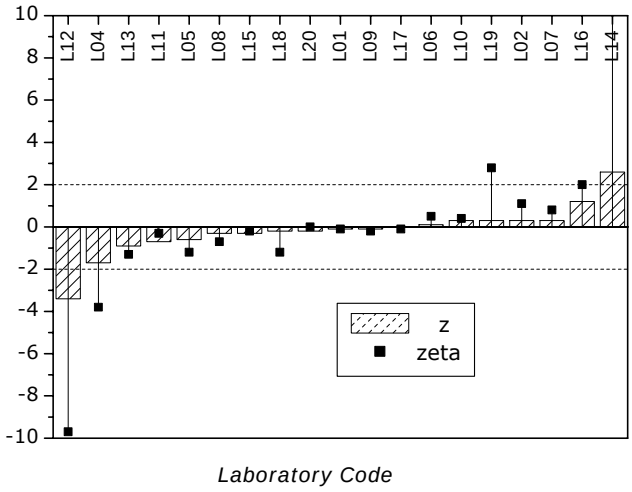

Figure 1c
Performance evaluation
$z = (X_{lab}-X_{ref})/ \hat{\sigma}$
$zeta = (X_{lab}-X_{ref})/ \sqrt{(u_{lab}^2+u_{ref}^2)}$

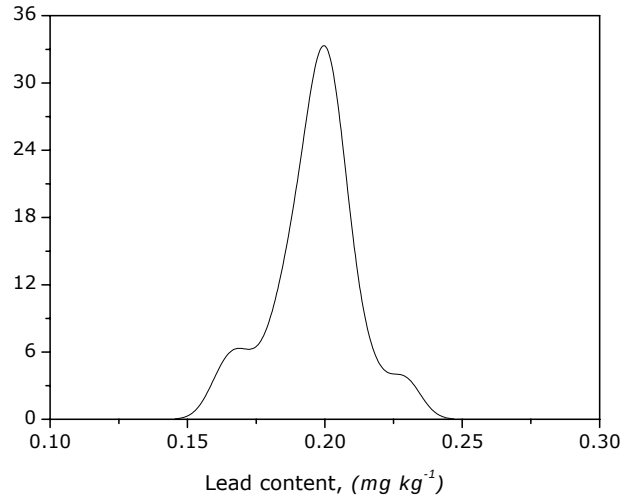

Figure 2a
Lead in mineral water
Kernel density,
All results (excl. L03)

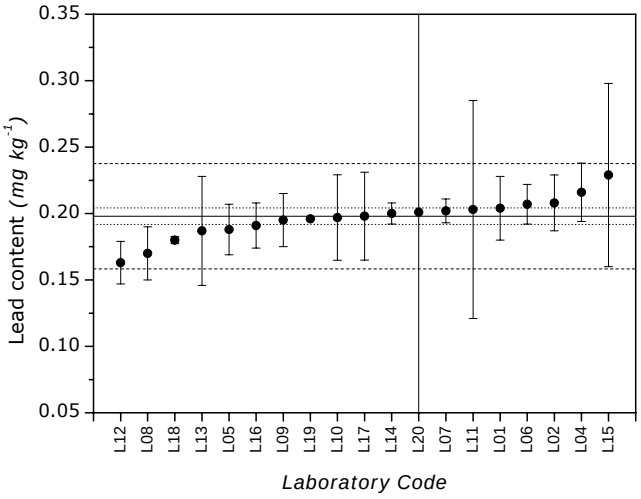

Figure 2b
Reported results and corresponding expanded uncertainties

X_{ref} = 0.198
u_{ref} = 0.003
$\hat{\sigma}$ = 0.020 (10%)
in mg kg^{-1}

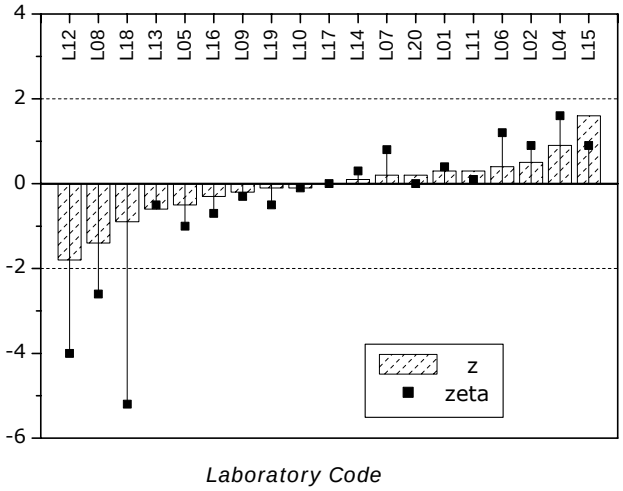

Figure 2c
Performance evaluation
$z = (X_{lab}-X_{ref})/ \hat{\sigma}$
$zeta = (X_{lab}-X_{ref})/ \sqrt{(u_{lab}^2+u_{ref}^2)}$

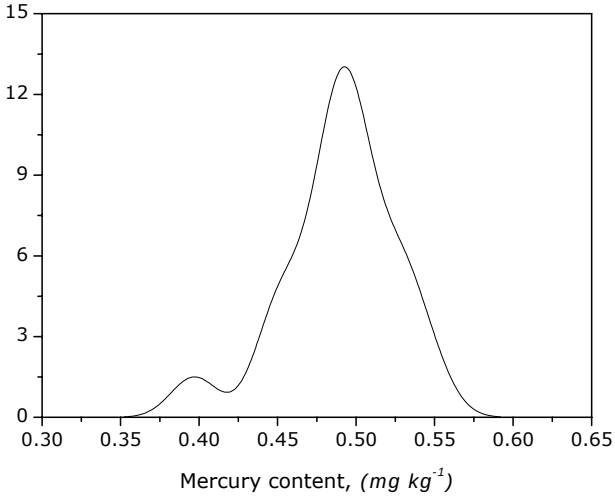

Figure 3a
Mercury in mineral water
Kernel density,
All results (excl. L03)

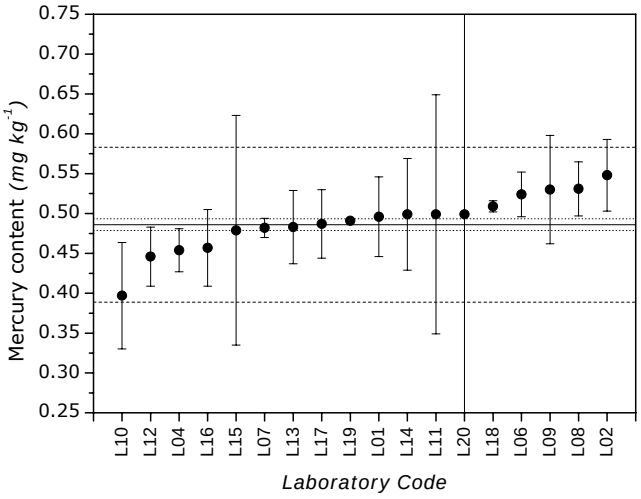

Figure 3b
Reported results and corresponding expanded uncertainties

X_{ref} = 0.486
u_{ref} = 0.004
$\hat{\sigma}$ = 0.049 (10%)
in mg kg^{-1}

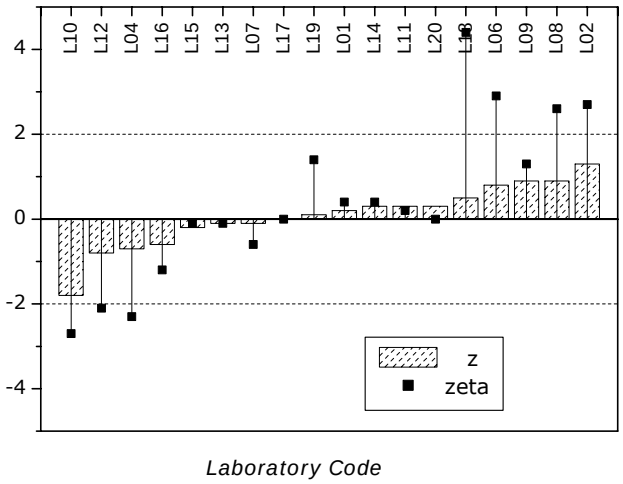

Figure 3c
Performance evaluation
$z = (X_{lab}-X_{ref})/ \hat{\sigma}$
$zeta = (X_{lab}-X_{ref})/ \sqrt{(u_{lab}^2+u_{ref}^2)}$

Table 2a: Cadmium, quantitative information reported by participants plus the laboratory scorings provided by the organiser

Lab Code	x1	x2	x3	"avg"	Uc	k	Technique	avg-calc	Z-score	zeta	
L01	0.299	0.297		0.298	0.04	2	ICP-OES	0.298	-0.1	-0.1	
L02	0.307	0.308			0.012	2	ICP-MS	0.308	0.3	1.1	
L03	390.8	379.75	390.34	386.97	92.9	2	ICP-MS	387	--	--	
L04	0.252	0.249			0.025	2	ICP-MS	0.251	-1.7	-3.8	
L05	0.292	0.273		0.282	0.028	2	GF-AAS	0.283	-0.6	-1.2	
L06	0.313	0.304	0.296	0.304	0.016	2.2	ICP-MS	0.304	0.1	0.5	
L07	0.306	0.312	0.305	0.308	0.019	2	Z-ETAAS	0.308	0.3	0.8	
L08	0.297	0.292	0.284	0.291	0.023	2	GF-AAS	0.291	-0.3	-0.7	
L09	0.294	0.298			0.04	2	ICP-MS	0.296	-0.1	-0.2	
L10	0.3104	0.3085	0.3121	0.3103	0.0489	2	FAAS	0.310	0.3	0.4	
L11	0.265	0.293			0.146	2	ETAAS	0.279	-0.7	-0.3	
L12	0.2	0.19	0.2	0.197	0.02	2	GF-AAS	0.197	-3.4	-9.7	
L13	0.264	0.273	0.286	0.274	0.038	2	ICP-MS	0.274	-0.9	-1.3	
L14	0.379	0.377	0.374	0.377	0.005	2	FAAS	0.377	2.6	17.2	
L15	0.287	0.298		0.293	0.067	2	GF-AAS	0.293	-0.3	-0.2	
L16	0.35	0.322	0.334	0.335	0.035	2	GF-AAS	0.335	1.2	2.0	
L17	0.307	0.291			0.012	--	ICP-OES	0.299	0.0	-0.1	a
L18	0.29	0.298	0.298		0.0014	2	GF-AAS	0.295	-0.2	-1.2	
L19	0.309	0.312	0.31		--	--	ICP-MS	0.310	0.3	2.8	b
L20	0.293	0.293	0.298		5.77	2	GF-AAS	0.295	-0.2	0.0	

All results expressed in (mg kg^{-1})

a) *k* not reported; u_c = *Uc* / √3

b) u_c not reported; set to zero in *zeta*

x-ref =	0.300
u-ref =	0.004
σ =	0.030

Table 2b: Lead, quantitative information reported by participants plus the laboratory scorings provided by the organiser

Lab Code	x1	x2	x3	"avg"	Uc	k	Technique	avg-calc	Z-score	zeta	
L01	0.199	0.208		0.204	0.024	2	ICP-OES	0.204	0.3	0.4	
L02	0.206	0.21			0.021	2	ICP-MS	0.208	0.5	0.9	
L03	324.16	298.39	296.24	306.26	94.9	2	ICP-MS	306	--	--	
L04	0.211	0.221			0.022	2	ICP-MS	0.216	0.9	1.6	
L05	0.189	0.186		0.188	0.019	2	GF-AAS	0.188	-0.5	-1.0	
L06	0.211	0.206	0.203	0.207	0.015	2.2	ICP-MS	0.207	0.4	1.2	
L07	0.201	0.202	0.204	0.202	0.009	2	Z-ETAAS	0.202	0.2	0.8	
L08	0.173	0.172	0.166	0.17	0.02	2	GF-AAS	0.170	-1.4	-2.6	
L09	0.194	0.196			0.02	2	ICP-MS	0.195	-0.2	-0.3	
L10	0.1962	0.1926	0.2015	0.1968	0.0322	2	FAAS	0.197	-0.1	-0.1	
L11	0.205	0.201			0.082	2	ETAAS	0.203	0.3	0.1	
L12	0.17	0.17	0.15	0.163	0.016	2	GF-AAS	0.163	-1.8	-4.0	
L13	0.186	0.189	0.185	0.187	0.041	2	ICP-MS	0.187	-0.6	-0.5	
L14	0.201	0.195	0.203	0.2	0.008	2	FAAS	0.200	0.1	0.3	
L15						2	GF-AAS	0.229	1.6	0.9	
L16	0.184	0.189	0.201	0.191	0.017	2	GF-AAS	0.191	-0.3	-0.7	
L17	0.204	0.191			0.033	--	ICP-OES	0.198	0.0	0.0	a
L18	0.181	0.18	0.18		0.0027	2	GF-AAS	0.180	-0.9	-5.2	
L19	0.192	0.196	0.201		--	--	ICP-MS	0.196	-0.1	-0.5	b
L20	0.199	0.199	0.205		7.13	2	GF-AAS	0.201	0.2	0.0	

All results expressed in (mg kg^{-1})

a) *k* not reported; u_c = *Uc* / $\sqrt{3}$

b) u_c not reported; set to zero in *zeta*

x-ref =	0.198
u-ref =	0.003
σ =	0.020

Table 2c: Mercury, quantitative information reported by participants plus the laboratory scorings provided by the organiser

Lab Code	x1	x2	x3	"avg"	Uc	k	Technique	avg-calc	Z-score	zeta	
L01	0.509	0.482		0.496	0.05	2	CV-AAS	0.496	0.2	0.4	
L02	0.558	0.537			0.045	2	ICP-MS	0.548	1.3	2.7	
L03	501.7	495.18	508.75	501.88	125.5	2	ICP-MS	502	--	--	
L04	0.453	0.455			0.027	2	CV-AAS	0.454	-0.7	-2.3	
L05											
L06	0.526	0.527	0.519	0.524	0.028	2.2	ICP-MS	0.524	0.8	2.9	
L07	0.48	0.49	0.476	0.48	0.012	2	AMA	0.482	-0.1	-0.6	
L08	0.521	0.528	0.543	0.531	0.034	2	CV-AAS	0.531	0.9	2.6	
L09	0.525	0.534			0.068	2	AAS-DMA	0.530	0.9	1.3	
L10	0.4017	0.4056	0.3833	0.3969	0.0667	2	CV-AAS	0.397	-1.8	-2.7	
L11	0.498	0.505	0.493		0.15	2	Mercury Analyse	0.499	0.3	0.2	
L12	0.453	0.435	0.449	0.446	0.037	2	TDA-AAS, Therm	0.446	-0.8	-2.1	
L13	0.494	0.474	0.48	0.483	0.046	2	ICP-MS	0.483	-0.1	-0.1	
L14	0.54	0.481	0.475	0.5	0.07	2	ICP-MS	0.499	0.3	0.4	
L15	0.473	0.504	0.459	0.479	0.144	2	Hg-analyzer	0.479	-0.2	-0.1	
L16	0.445	0.47	0.455		0.048	2	CV-AAS	0.457	-0.6	-1.2	
L17	0.486	0.488			0.043	--	AFS / Atomic-Flu	0.487	0.0	0.0	a
L18	0.499	0.525	0.502		0.007	2	CV-AAS	0.509	0.5	4.4	
L19	0.482	0.487	0.505		--	--	ICP-MS	0.491	0.1	1.4	b
L20	0.482	0.507	0.508		2.5	2	FIMS	0.499	0.3	0.0	

All results expressed in (mg kg^{-1})

a) *k* not reported; u_c = *Uc* / √3

b) u_c not reported; set to zero in *zeta*

x-ref =	0.486
u-ref =	0.004
σ =	0.049

9 Acknowledgements

H. Emteborg, C. Contreras and A. Lamberty from the Reference Materials Unit are acknowledged for their support in the processing of the test material. BOKU is acknowledged for performing the measurements for the homogeneity and stability studies. The authors thank T. Linsinger (Reference Materials Unit) for his support in the evaluation of the homogeneity and stability data.

The NRLs participating in this exercise, listed below are kindly acknowledged.

Organisation	Country
• Institute of Public Health	Belgium
• The State Veterinary Institute (SVI) in Olomuc	Czech Republic
• Danish Food Institute (DTU)	Denmark
• The Danish Plant Directorate	Denmark
• The Veterinary and Food Laboratory	Estonia
• Finnish Customs Laboratory	Finland
• Agence Française de Sécurité Sanitaire des Aliments (AFSSA), Laboratoire d'Études et de Recherches sur la Qualité des Aliments et des Procédés Agro-Alimentaires (LERQAP)	France
• Laboratoire de la Direction Général de la Concurrence, de la Consommation et de la Répression des frauds (DGCCRF)	France
• Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL)	Germany
• Public Analyst's Laboratory	Ireland
• Istituto Zooprofilattico	Italy
• Public Health Laboratory	Malta
• Instituut voor voedselveiligheid (RIKILT)	Netherlands
• Voedsel en Waren Autoriteit (VWA)	Netherlands
• National Institute of Hygiene	Poland
• State Veterinary and Food Institute	Slovakia
• National Veterinary Institute	Slovenia
• Laboratorio Arbitral Agroalimentario	Spain
• National Food Administration	Sweden
• UK OLC - Chemical contaminants	United Kingdom

Countries not appearing of the above list did not register to this interlaboratory comparison.

10 References

1 ISO 13528:2005; Statistical Methods for Use in Proficiency Testing by Interlaboratory Comparisons.

2 Commission Regulation No 333/2007 of 28 March 2007 laying down the sampling methods of analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a) pyrene in foodstuffs.

3 Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed.

4 Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

5 ISO/IEC/EN 17025:2005, General Requirement for the Competence of Calibration and Testing Laboratories.

6 Eurachem/CITAC guide "Quantifying Uncertainty in Analytical Measurement" (2000), see www.eurachem.ul.pt.

7 International Vocabulary of basic and general terms in metrology. ISO, Geneva, Switzerland, 1993 (ISBN 92-67-10175-1).

8 Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.

9 IMEP report "Trace elements, PCBs, PAHs in sewage sludge. Report to participants", see http://www.irmm.jrc.be/html/interlaboratory_comparisons/imep/index.htm.

10 T. Fearn, M. Thompson, *Analyst*, (2001), **126**, 1414-1417.

11 M. Thompson, S.L.R. Ellison, R. Wood, *Pure Appl. Chem.*, (2006), **78(1)**, 145-196.

12 A. Lamberty, H. Schimmel, J. Pauwels, *Fresenius J Anal. Chem.*, (1998), **360**, 359–361.

13 See www.**softcrm**.com.

14 M. Thompson, *Analyst*, (2002), **125**, 385-386.

15 The software to calculate Kernel densities is provided by the Statistical Subcommittee of the Analytical Methods Committee (AMC) of the Royal Society of Chemistry and descrived in the AMC Technical Bried "Representing data distributions with Kernel density estimates" (2006), see www.rsc.org/amc.

Annexes

Annex 1: Invitation letter to laboratories

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

CRL
Heavy Metals
in Feed and Food

Geel, 27 March 2007
IM/L/24/07
D04-IM(2007)D/7687

Dear Madam / Sir,

Intercomparison for CRL Heavy Metals in Feed and Food

On behalf of the CRL Heavy Metals in Feed and Food, I would like to invite you to participate in a Proficiency Test (PT) exercise for the determination of total Cd, Pb and Hg in mineral water which will take place in the next months. The aim of this study is to evaluate the capabilities of the NRL network to calculate the uncertainty associated to their measurements. For this reason we encourage all of you (regardless you have a mandate for feed or food) to participate in this exercise.

I would like to remind you that it is a duty for you as an NRL to participate in the PTs organised by the CRL if you hold a mandate for this type of matrix. There is no charge for participation.

Please register electronically for this intercomparison at
http://www.irmm.jrc.be/imepapp/registerForComparison.action?comparison=84

Once you have submitted your registration electronically, please follow the further steps on your screen. These steps include printing and signing your registration, and then **sending it to us by fax**. That fax is your confirmation of participation.

The **deadline for registration is 13 April 2007**. Samples will be sent to the participants during the second half of April. The deadline for submission of results is 31 May 2007.

If you have any questions please contact the responsible for this intercomparison: Dr. Beatriz de la Calle (JRC-IRMM-CRL-HEAVY-METALS@ec.europa.eu), phone +32-14-571252 or fax +32-14-571865.

Yours sincerely,

Dr. M.B. de la Calle
Project leader of the CRL

Cc: Philip Taylor, Piotr Robouch

irmm Institute for Reference Materials and Measurements
Retieseweg 111, B-2440 Geel, Belgium
Tel.: +32-(0)14-571 252 • Fax: +32-(0)14-571 865
JRC-IRMM-CRL-HEAVY-METALS@ec.europa.eu • http://www.irmm.jrc.be
IMEP

Annex 2: Results of the homogeneity study

1a. Homogeneity data for total Cd in mineral water.

According to the IUPAC International Harmonised Protocol[9]

	Cd (µg kg^{-1})	
Bottle ID	**Replicate 1**	**Replicate 2**
3	295	292,1
13	291,6	291,2
21	292,2	292,9
35	292,6	292,8
43	292,1	292,9
51	293,1	292,9
64	292,8	292,1
76	291,9	291,6
82	293,3	292,9
92	293,5	292
Mean, n	**292,6**	**20**
Target RSD %	10	
S_{an}^2	0,6385	
S_{sam}^2	0,073666667	
σ_{all}^2	77,04011756	
Critical	145,480306	
S_{sam}^2<critical?	ACCEPT	

According to ISO 13528[1]

0.3σ	8,778
s_x	0,626830652
s_w	0,79906195
s_s	0,27141604
$s_s \leq 0.3\ \sigma$	ACCEPT

1b. Stability data for total Cd in mineral water.

As computed by SOFT CRM

Cadmium	18oC

samples	0	3	5	8
1	283,5	284,1	283,9	281,9
2	284,5	283	285,4	276,9
3	284,1			
4	283,6			

Calculation of Ults	
Xshelf =	14 Weeks
Ults =	3,417
Ults[%] =	1,20%

Slope =	-0,435
SE Slope =	0,208
Intercept =	284,481
SE Intercept =	0,922
Correlation Coefficient =	0,352
Slope of the linear regression significantly <> 0 (95%) :	No
Slope of the linear regression significantly <> 0 (99%) :	No

2. Homogeneity data for total Pb in mineral water.

According to the IUPAC International Harmonised Protocol[9]

	Pb (µg kg^{-1})	
Bottle ID	**Replicate 1**	**Replicate 2**
3	209,3	206,2
13	212	211,7
21	210,1	208,6
35	208,7	208,7
43	213,9	213,1
51	212,2	210,8
64	213,3	211,6
76	210,6	213,2
82	211,9	213,4
92	212,4	214,8
Mean, n	**211,3**	**20**
Target RSD %	10	
S_{an}^2	1,6105	
S_{sam}^2	3,321	
σ_{all}^2	40,19243006	
Critical	77,18837352	
S_{sam}^2<critical?	ACCEPT	

According to ISO 13528[1]

0.3σ	6,339
s_x	2,031317307
s_w	1,269054766
s_s	1,822361106
$s_s \leq 0.3\ \sigma$	ACCEPT

2b. Stability data for total Pb in mineral water.

As computed by SOFT CRM

Lead	18oC

samples	0	3	5	8
1	197,8	198	199,3	195,2
2	197,8	201,8	197,8	200,7
3	197,8			
4	198,6			

Calculation of Ults

Xshelf =	14 Weeks
Ults =	2,619
Ults[%] =	1,30%

Slope =	0,008
SE Slope =	0,198
Intercept =	198,455
SE Intercept =	0,878
Correlation Coefficient =	0
Slope of the linear regression significantly <> 0 (95%) :	No
Slope of the linear regression significantly <> 0 (99%) :	No

3. Homogeneity data for total Hg in mineral water.

According to the IUPAC International Harmonised Protocol[9]

	Hg (µg kg^{-1})	
Bottle ID	**Replicate 1**	**Replicate 2**
3	451,8	451,6
13	448,2	451,7
21	446,3	449,3
35	449,9	448,5
43	454,8	450,6
51	450,8	452,7
64	450,8	450,5
76	450,9	450,1
82	451,9	451,2
92	449,1	444,9
Mean, n	**450,3**	**20**
Target RSD %	10	
S_{an}^2	3,168	
S_{sam}^2	1,751111111	
σ_{all}^2	182,4768706	
Critical	346,2561967	
S_{sam}^2<critical?	ACCEPT	

According to ISO 13528[1]

0.3σ	13,509
s_x	1,826228658
s_w	1,779887637
s_s	1,323295549
$s_s \leq 0.3\ \sigma$	ACCEPT

3b. Stability data for total Hg in mineral water.

As computed by SOFT CRM

Mercury	18oC

samples	0	3	5	8
1	539,1	534,1	532,8	533,6
2	537,1	530,9	534,9	535,6
3	537,8			
4	538			

Calculation of Ults	
Xshelf =	14 Weeks
Ults =	3,777
Ults[%] =	0,70%

Slope =	-0,485
SE Slope =	0,229
Intercept =	536,941
SE Intercept =	1,015
Correlation Coefficient =	0,359
Slope of the linear regression significantly <> 0 (95%) :	No
Slope of the linear regression significantly <> 0 (99%) :	No

Annex 3: Letter accompanying the sample

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

CRL
Heavy Metals
in Feed and Food

Geel, 3 May 2007
GE/IM/L/34/07
D04-IM(2007)D/10750

«TITLE» «FIRSTNAME» «SURNAME»
«ORGANISATION»
«DEPARTMENT»
«ADDRESS»
«ADDRESS2»
«ADDRESS3»
«ADDRESS4»
«ZIP» «TOWN»
«COUNTRY»

Participation to IMEP-102, a proficiency test exercise for the determination of total Cd, Pb and Hg in mineral water, 2nd mailing of sample

Dear **«TITLE» «SURNAME»,**

Thank you for participating in the IMEP-102 intercomparison for the determination of total Cd, Pb and Hg in mineral water. This exercise takes place in the frame of the CRL Heavy Metals in Feed and Food.

This parcel contains:

a) One polyethylene jar containing approximately 110 mL of the test material
b) A "Confirmation of Receipt" form

Please keep in mind the test material is acidified with nitric acid up to a concentration of approx. 2 % m/m. The concentration of the three measurands in the test material lays in the range 100-1000 µg L^{-1}.

Please check whether the bottle containing the test material remained undamaged during transport. Then fax (at +32-14-571865) or send the "Confirmation of receipt" form back. You should store the samples in a dark and cold place (not more than 18 ºC) until analysis.

The measurands are: total Cd, Pb and Hg in a food matrix of plant origin. Please perform two or three independent measurements per parameter. Correct the measurement results for recovery, and report the corrected values, plus their mean on the reporting website. The procedure you follow for this exercise should resemble as closely as possible those that you use in routine sample analysis. The results should reported in the same form (e.g., number of significant figures) as those normally reported to the customer.

You can find the reporting website at www.irmm.jrc.be/imepapp/jsp/loginResult.jsp To access this webpage you need a personal password key, which is: «PARTKEY»
. The system will guide you through the reporting procedure. Please enter for each parameter the two or three measurement results plus the technique you used, but do not report the uncertainty for each individual measurement. In addition, please report the mean of the results with technique and with uncertainty information in the allocated space for "measurement. After entering all results, please also complete the relating questionnaire. Do not forget to submit and confirm always when required.

irmm Institute for Reference Materials and Measurements

Retieseweg 111, B-2440 Geel, Belgium
Tel.: +32-14-571252 • Fax: +32-14-571865
jrc-irmm-crl-heavy-metals@ec.europa.eu • http://www.irmm.jrc.be

IMEP

Directly after submitting your results and the questionnaire information online, you will be prompted to print the completed report form. Please do so, sign the paper version and return it to IRMM by fax (at +32-14-571-865) or by e-mail. Check your results carefully for any errors before submission, since this is your definitive confirmation.

We would appreciate to receive the results by 08/06/2007, the latest.

Please keep in mind that collusion is contrary to professional scientific conduct and serves only to nullify the benefits of proficiency tests to customers, accreditation bodies and analysts alike.

Your participation in this project is greatly appreciated. If you have any remaining questions, please contact me by e-mail: JRC-IRMM-CRL-HEAVY-METALS@ec.europa.eu

With kind regards

Dr. M.B. de la Calle
IMEP-102 Co-ordinator

Enclosures: 1) test material in polyethylene jar; 2) confirmation of receipt form

Cc: P. Robouch, P. Taylor, L. Van Nevel

Institute for Reference Materials and Measurements

Retieseweg 111, B-2440 Geel, Belgium
Tel.: +32-14-571252 • Fax: +32-14-571865
jrc-irmm-crl-heavy-metals@ec.europa.eu • http://www.irmm.jrc.be

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Annex 4: Sample receipt confirmation form

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

CRL
Heavy Metals
in Feed and Food

Annex to D04/IM(2007)D/10750

«TITLE» «FIRSTNAME» «SURNAME»
«ORGANISATION»
«DEPARTMENT»
«ADDRESS»
«ADDRESS2»
«ADDRESS3»
«ADDRESS4»
«ZIP» «TOWN»
«COUNTRY»

CRL-HM-02 / IMEP-102
Cd, Hg and Pb in mineral water

Confirmation of receipt of the 2nd sample

Please return this form at your earliest convenience.
This confirms that the sample package arrived.
In case the package is damaged,
please state this on the form and contact us immediately.

ANY REMARKS …….
…….

Date of package arrival …….

Signature …….

Please return this form to:
Dr. Beatriz de la Calle
IMEP-102 Coordinator
EC-JRC-IRMM
Retieseweg 111
B-2440 GEEL, Belgium

Fax : +32-14-571865
e-mail : JRC-IRMM-CRL-HEAVY-METALS@ec.europa.eu

irmm Institute for Reference Materials and Measurements

Retieseweg 111, B-2440 Geel, Belgium
Tel.: +32-14-571252 • Fax: +32-14-571865
jrc-irmm-crl-heavy-metals@ec.europa.eu • http://www.irmm.jrc.be

IMEP

Annex 5: Questionnaire

IMPORTANT : Disclaimer, Confidentiality Notice and rules on Privacy Protection

European Commission
Joint Research Centre
Institute for Reference Materials and Measurements

IRMM Interlaboratory Comparison

> Login > Results > Questionnaire

Functions

Results

Questionnaire for *IMEP-102*

Dr. Questionnaire Example **IRMM BELGIUM**

1. **Indicate the code of the bottle you analysed.**

2. **What is the level of confidence reflected by the coverage (k) factors stated above? (in %)**

3. **What is the basis of your uncertainty estimate (multiple answers are possible)**

uncertainty budget according to ISO-GUM
known uncertainty of the standard method
uncertainty of the method as determined during in-house validation
measurement of replicates (i.e. precision)
expert guesstimate
use of intercomparison data
other

If other, please specify.

4. **Do you usually provide an uncertainty statement to your custumers for this type of analysis**
○ Yes ○ No

5. **According to you which is the main component of your uncertainty?**

6. **Did you analyse the sample according to an official method?**
○ Yes ○ No

If NO, please describe (in max.150 characters for each reply) your:
sample pre-treatment

digestion step, plus the acid(s) used (if applicable)

extraction / separation step

instrument calibration step

If Yes, which:

7. Does your laboratory carry out this type of analysis (as regards the parameters, matrix and basis?

○ Yes ○ No

	0-50 samples per year	50-250 samples per year	250-1000 samples per year
If Yes, please estimate the number of samples (Cd, Hg, Pb measurements together):	○	○	○

8. Does your laboratory have a quality system in place?

○ Yes ○ No

	ISO 9000 series	ISO/IEC 17025
If Yes, wich:	☐	☐

If Other, please specify.

9. Is your laboratory accredited for this type of analysis?

Yes	No
○	○

comment:

10. Does your laboratory take part in an interlaboratory comparison for this type of analysis on

○ Yes ○ No

If yes, which one(s):

11. Does your laboratory use a reference material for this type of analysis?

○ Yes ○ No

If YES, is the material used for the validation of procedures?

If YES, is the material used for calibration of instruments?

If YES, which one(s):

12. Do you have any comments? Please let us know: ...

Clear

Submit questionnaire

Annex 6: Main component of the uncertainty budget

CODE	Main component of the uncertainty budget
L01	Between-days variations from technicians, calibrations etc
L02	Standard deviation based on in house reproducibility conditions (several analysts at different days) and recovery/trueness
L03	Sample Recovery
L04	Sub-sampling in laboratory, preparation of calibration standards, measurement conditions
L05	Calibration
L06	Calibration
L07	?? MU=2*SRW (SRW: Within-lab Reproducibility)
L08	Recovery
L09	Experimental standard deviation. type A
L10	Uncertainty of laboratory equipment
L11	The main component come from analysis method
L12	Reference materials uncertainty
L13	Sample pre-treatment
L14	Extraction into MIBK
L15	Precision
L16	Error due to instrument variation.
L17	No comment
L18	Repeatability of AA measurement reading
L19	Sampling
L20	The sample water was already acidified therefore no exclusion was needed. The main component in the uncertainty is the measurement

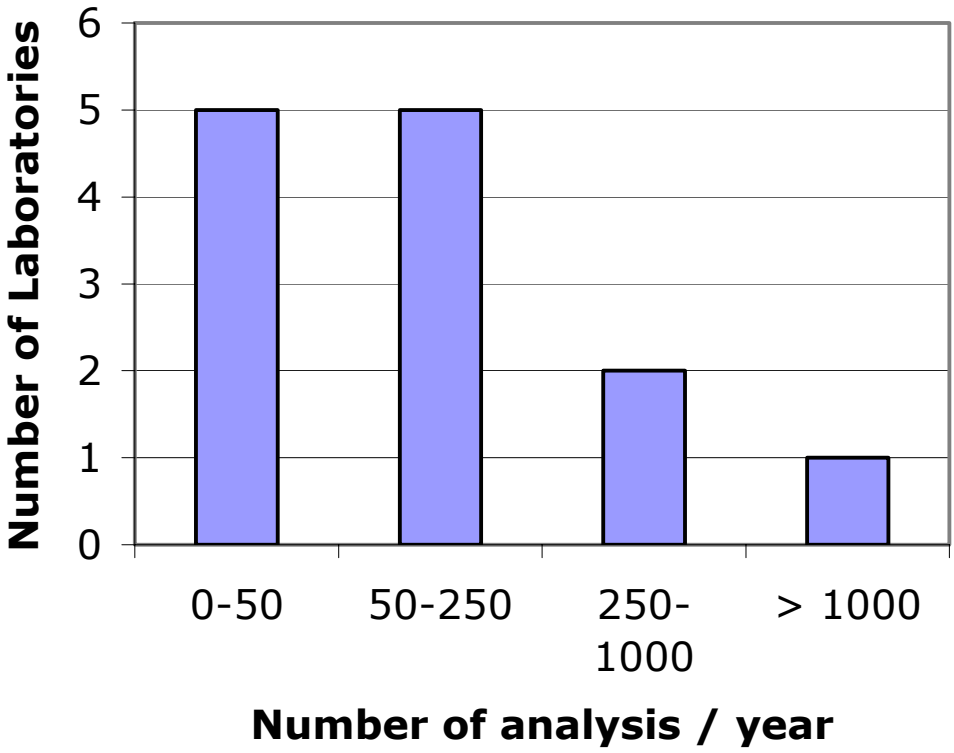
Annex 7: Experimental details

LabCode	SOP ?	Which SOP?	sample pre-treatment	digestion step & acid(s) used	extraction/separation	instrument calibration
L01	No		none	Hg: Digestion with HNO3/HCl/H2O2 for 2 hours. Cd/Pb: No digestion, only 2-5x dilution of sample with 0.5 M HCl		
L02	No		none	no digestion, samples were diluted using: 6 ml concentrated HNO3 and 2 ml 6% H2O2 per 100 ml.	none	ICP-MS was calibrated using a calibration standard with same concentrations of acids. Instrument calibration needs to fullfill specific requirements.
L03	No		Spiking water sample with Indium internal standard	No digestion step required	No extraction step required	5 Point calibration
L04	Yes	EPA Method 200.7				
L05	No		Dilution	No digestion	No extraction	External calibration
L06	No		homogenisation by shaking by hand	no digestion - only dilution with 2% HNO3	no	yes
L07	No		No treatment	No	none	Externe Standard Calibration
L08	Yes	EN 14082			none	External calibration with Hg, Pb and Cd single-element standards
L09	No		no	no	no	Yes

Annex 7: Experimental details *(continued)*

LabCode	SOP ?	Which SOP?	sample pre-treatment	digestion step & acid(s) used	extraction/separation	instrument calibration
L10	No		in-house method according to standard method and methods of the instrument manufacturer	microwave digestion	-	calibration curve - linear, standard addition
L11	Yes	Cd (µg/L) : 0; 2.5; 5; 10 Pb (µg/L) : 0; 10; 26; 50; 100 Hg (ng) : 0; 0.1; 0.3; 1; 2; 3; 10; 20; 29				
L12	No		none	none	none	yes
L13	Yes	NMKL 8.3.14				
L14	No		/	diluted HCl	Complexes of Pb and Cd with DDDC are extracted into MIBK	matrix matched calibration curve
L15	No		no pre-treatment	-	-	-
L16	No		Dilution	Addition of nitric acid	none	4 point calibration
L17	Yes	Amtliche Sammlung von Untersuchungsverfahren (§ 64 Lebensmittel- und Futtermittelgesetzbuch)				
L18	No		None	Pb,Cd, evaporate with acid (0.5% HNO3 for Pb, 2% HCl for Cd) Hg- add HCl/HNO3 (3:1) digest at 95 deg C	Make back to original volume: No extraction step	Standards: 0-75 microg/l Pb : 0-200 microg/l Cd (4 STds) : 0-20 microg/l Hg (4 stds) External standards
L19	No		none	none	none	linear calibration with internal standard
L20	No		Sample was diluted with acidified water	no digestion neeeded	no extraction neede	standards were prepared in acidified water

Annex 8: Number of samples analysed per year



European Commission

EUR 22870 EN – Joint Research Centre – Institute for Reference Materials and Measurements
Title: Report of the second interlaboratory comparison organised by the Community Reference Laboratory Heavy Metals in Feed and Food: Total Cd, Pb and Hg in mineral water.
Author(s): M.B. de la Calle, P. Robouch, S. Bynens, J. van de Kreeke, P. Taylor
Luxembourg: Office for Official Publications of the European Communities
2007 – 32 pp. – 21 x 29.7 cm
EUR – Scientific and Technical Research series – ISSN 1018-5593
ISBN 978-92-79-06473-9

Abstract
The institute for Reference Materials and Measurements (IRMM) of the European Commission's Directorate-General Joint Research Centre holds the Community Reference Laboratory for Heavy Metals in Feed and Food (CRL-HM). One of the core tasks is to organise interlaboratory comparisons (ILCs) among appointed National Reference Laboratories (NRLs). This report presents the results of the second ILC of the CRL-HM which focused on the determination of the total Cd, Pb and Hg content in mineral water.

The test material used in this exercise was a commercial mineral water purchased in Belgium at a local supermarket. The material was spiked with Cd, Pb and Hg, rebottled and dispatched by the Reference Material Unit (RM) of the IRMM. The samples were dispatched on the first half of May 2007. Each participant received two sets of samples containing one bottle each set. The content of the bottle was approximately 100 mL of the test material. Twenty participants from 17 countries registered to the exercise of which 20 submitted results for Cd and Pb and 19 for Hg.

The assigned values were those obtained from the gravimetric measurements used to spike the material. The homogeneity and stability studies were subcontracted to the University of Natural Resources and Applied Life Sciences (BOKU) in Vienna.

The uncertainty of the assigned values was calculated by combining the uncertainty of the spiking procedure with a contribution for the between-bottle homogeneity and a contribution for the stability of the test material. Participants were invited to report the uncertainty on their measurements. This was done by all the participants.

Laboratory results were rated with z and zeta scores in accordance with ISO 13528[i]. Standard deviation for proficiency assessment (also called target standard deviation) for Cd, Pb and Hg was 10% of the assigned value.

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.

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LA-NA-22870-EN-C

ISBN 978-92-79-06473-9

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