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

Total Cd, Pb and Hg and extractable Cd and Pb in feed

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Heavy Metals
in Feed and Food

Report of the third interlaboratory comparison

Total Cd, Pb and Hg and extractable Cd and Pb in feed

IMEP®

January 2007

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Table of Contents

1 Summary

The Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre, a Directorate-General of the European Commission, operates the Community Reference Laboratory for Heavy Metals in Feed and Food (CRL-HM). One of its core tasks is to organise interlaboratory comparisons (ILCs) among appointed National Reference Laboratories (NRLs). This report presents the results of the third ILC of the CRL-HM which focused on the determination of total Cd, Pb and Hg and extractable Cd and Pb in feed according to Directive 2002/32/EC[1] of the European Parliament and of the Council on undesirable substances in animal feed.

The test material used in this exercise was a commercial compound feed for fish provided by the Centro di Referenza Nazionale per la Sorveglianza e il Controllo degli Alimenti per Animali, Istituto Zooprofilattico del Piemonte. The material, naturally contaminated, was processed, bottled, labelled and dispatched by the Reference Materials Unit of the IRMM. The samples were dispatched on the second half of October 2007. Each participant received one bottle containing approximately 20 g of test material. Thirty-one participants from 25 countries registered to the exercise of which 31 submitted results for total Cd and total Pb, 28 for total Hg, 26 for extractable Cd and 24 for extractable Pb.

The assigned values (X_{ref}) were provided by IRMM using isotope dilution-inductively coupled plasma-mass spectrometry (ID-ICP-MS). The analytical uncertainty of X_{ref}, u_{char}, was calculated according to the ISO Guide to the Expression of Uncertainty in Measurement (GUM)[2]. Homogeneity and stability studies were subcontracted to Bundesanstalt für Materialforschung und –Prüfung (BAM). The uncertainties of the respective assigned values, u_{ref}, were calculated combining the analytical uncertainty, u_{char}, with a contribution for the between-bottle homogeneity, u_{bb}, and for the short term stability of the test material, u_{sts}. Participants were invited to report the uncertainty on their measurements. This was done by 29 laboratories for total Cd, 23 for total Pb, 25 for total Hg, 18 for extractable Cd and 13 for extractable Pb.

Laboratory results were rated with z and zeta scores in accordance with ISO 13528[3]. Standard deviation for proficiency assessment (also called target standard deviation) were calculated using the modified Horwitz equation[4] and were 22 % of the assigned value for total Pb and Hg and for extractable Pb and 16 % of the assigned value for total and extractable Cd.

2 Introduction

In humans, about one third of the total cadmium burden originates from animal products. As a result of contaminated animal feed, cadmium is ubiquitous in food of animal origin. The rate of oral uptake in animals exceeds excretion and so accumulation results in some animal tissues, mainly in kidneys and liver. Cadmium-containing dust can increase the concentration of cadmium in plants by resorption via the leaves. The cadmium contents of vegetable feeds vary from 0.005 to 0.1 mg kg^{-1}, and in certain cases up to 1 mg kg^{-1}. Very likely cadmium accumulates in marine organisms[5]. Fresh water fish and shellfish of polluted areas show cadmium levels of about 0.03 mg kg^{-1}. Accumulation of cadmium in animals can be avoided if the feed concentration does not exceed 0.6 mg kg^{-1}, and drinking water for animals should not contain more than 0.05 mg L^{-1} [6].

About half of human lead intake is through food, of which more than half originates from plants. Lead found in food and animal feed comes mainly from external sources. Plant feed have lead contents ranging from less than 0.1 mg kg^{-1} up to 5 to 10 mg kg^{-1} of dry matter. The lead content of animal tissues is caused mainly by the uptake of contaminated feed, only 10 % of the total burden is caused by the inhalation of lead containing dust. To avoid accumulation, animal drinking water should not contain more than 0.1 mg kg^{-1}. Lead does not play a major role in aquatic food chains. Lead levels in fish depend upon the amount of lead pollution in the environmental water and there is remarkable variation in the existing data in particular among different species of fish. In mammals the amount of lead in the organism can be divided into three fractions: blood lead and some rapidly exchanging soft tissues with a half-life of about 19 days, soft tissues and a rapidly exchangeable bone fraction with a half life of about 21 days and bones and the skeleton with a half life of about 10 to 20 years. Similarly, Alves and Wood[7] indicate that bone accumulates the most lead per fish weight in some fish species (rainbow trout). Nevertheless, not a lot of information exists in the literature about the metabolism of lead in fish.

Food and animal feed derived from plants usually have mercury contents between 0.001 and 0.03 mg kg^{-1}. Through industrial usage, mercury is sometimes introduced into bodies of water. Marine organisms are especially able to transform inorganic species of mercury into organic compounds, which make mercury easily transferred through the aquatic food chain. Accumulation factors in the marine food chain are 100 to 1000 compared to 2 to5 in the terrestrial food chain. Thus, seafood is a particular source of mercury burden in man and animals. Mercury passes to domestic animals and animal-derived food products via fish meal which is in particular widely used in the feed for poultry.

To overcome problems associated with a high metal content in feed maximum levels in several commodities have been laid down in Directive 2002/32/EC, and a network has been built up to ensure quality and comparability in official controls throughout the European Union.[8] In March 2006 a footnote was introduced in Directive 2002/32/EC in which it is stated that *"Maximum levels refer to an analytical determination of lead and cadmium, whereby extraction is performed in nitric acid (5 % w/w) for 30 minutes at boiling temperature"*.

The Community Reference Laboratory for Heavy Metals in Feed and Food has organised a proficiency test (PT) exercise for the network of appointed National Reference Laboratories (NRLs) to determine the total Cd, Pb and Hg and extractable Cd and Hg. The two later measurands are to be determined using an extraction procedure agreed upon by the CRL-HM and the network of NRLs, and which is in agreement with the requirements laid down in Directive 2002/32/EC.

3 Scope

As stated in Regulation 882/2004 of the European Parliament and of the Council[8], one of the core duties of the CRL-HM is to organise interlaboratory comparisons for the benefit of staff from National Reference Laboratories. The scope of this ILC is to test the competence of the appointed NRLs to determine the total concentration of Cd, Pb and Hg and of extractable Cd and Pb according to Directive 2002/32/EC.

The assessment of the measurement results is undertaken on the basis of requirements laid down in legislation[1], and follows the administrative and logistic procedures of IMEP[9], the International Measurement Evaluation Programme of the IRMM of the European Commission's Directorate Joint Research Centre. The designation of this ILC is IMEP-103.

4 Time frame

This interlaboratory comparison was agreed upon by the NRLs network at the first CRL-HM workshop held on 25/26 September 2006. Specific details of the exercise were refined during the second CRL-HM workshop held on 24/25 September 2007. Invitation letters were sent to the participants on 5 October 2007. (cf. Annex 1). The samples were dispatched to the participants on 18 October 2007. Reporting deadline was 30 November 2007.

5 Test material

5.1 Preparation

The test material, commercially available feed for fish, was provided by the Centro di Referenza Nazionale per la Sorveglianza e il Controllo degli Alimenti per Animali, Istituto Zooprofilattico del Piemonte. Upon arrival at IRMM the material was processed by the Reference Materials Unit in the following way: the particle size distribution was assessed by laser diffraction and the water content determined by Karl-Fisher titration. Coarse particles were removed sieving through a 500 µm sieve and the water content was reduced to about 5 % by vacuum drying. The material was then homogenised and distributed (vibrating feeder) into amber glass bottles with polyethylene (PE) insert and screw cap lid with crimp film, containing approximately 20 g of test material each. Before and after processing, the material was stored at -20 ºC. The processing took place at room temperature.

5.2 Homogeneity and stability

The measurements for homogeneity and stability studies were performed by Bundesanstalt für Materialforschung und –prüfung (BAM). 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. The material proved to be homogeneous according to the IUPAC International Harmonised Protocol for the five measurands. The test material was also homogeneous for the total content of Hg according to ISO 13528. For the total and extractable Cd the test material was not homogeneous according to ISO13528. Unfortunately, the homogeneity measurements for both total and extractable Cd were not performed in a random way but following the filling sequence, which made it not possible to determine if the observed drift between bottles was due to the filling or to the analytical process. A contribution to the assigned uncertainty, u_{ref}, due to likely heterogeneity of the material, u_{bb}, of 6 % as provided by the Soft CRM software[12] licensed to the Reference Material Unit of IRMM, was taken on board following ISO Guide 35[13]. For total and extractable lead, the material was not homogeneous according to ISO 13528, and in fact even high within-bottle heterogeneity was found. A similar problem has been previously observed at IRMM (data not published) with a fish material. Intrinsic micro-heterogeneity of the material due to the fact that most likely the largest fraction of lead in fish is found in bones, as indicated in the introduction[7], could be an explanation for the obtained results.

The study of the stability of the test material was conducted following the isochronous approach[14]. The evaluation of the stability of the test material was made using the Soft CRM software[15]. The material proved to be stable at 4 ºC for the six weeks that elapsed between the dispatch of the samples and the deadline for submission of results for total Cd and Hg and for extractable Cd. No sound stability statement could be made for total and extractable Pb for the reasons already explained above.

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

5.3 Distribution

One set of material was sent to every participant. The test material was dispatched to the participants by IRMM on 18 October 2007. Each participant received: a) one bottle containing approximately 20 g of test material (one laboratory received two bottles because the method to be used, dry ashing, required the use for 5 g of material per replicate and 20 g would not be enough for the five measurands plus the water content determination), b) and accompanying letter with instructions on sample handling and reporting and with the method to be applied for the determination of extractable Cd and Pb (cf. Annex 3) and c) a form which had to be sent back after receipt of the sample to confirm its arrival (cf. Annex 4).

6 Instructions to participants

Details on 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 (Annex 3). The measurands and matrix were clearly defined as *"**Total** Cd, Pb and Hg and **extractable amounts** of Cd and Pb in feed following Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed"*.

Laboratories were asked to perform two or three independent measurements and report them, together with the mean of the results and its associated uncertainty. Some laboratories reported four independent results. Participants were asked to follow their routine procedures for the determination of total Cd, Pb and Hg and the procedure previously agreed upon for the determination of extractable Cd and Pb. The results were to be reported in the same manner (eg. number of significant figures) as when reporting to customers.

The results were to be reported in a special on line form for which every 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 standard uncertainties

The reference value used, X_{ref}, for this ILC was determined by IRMM using Isotope Dilution Inductively Coupled Plasma Mass Spectrometry (ID-ICP-MS). IRMM has proven its measurement capabilities by successful participation in CCQM key comparisons. No reference value was provided for total and extractable Pb due to the heterogeneity problems also found by BAM.

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

$$u_{ref} = \sqrt{u_{char}^2 + u_{bb}^2 + u_{lts}^2} \qquad \text{Eq. 1}$$

where:

$\mathbf{u}_{ref}$ standard uncertainty associated to the assigned value
$\mathbf{u}_{char}$ standard uncertainty of characterisation
$\mathbf{u}_{bb}$ contribution for the between-bottle homogeneity
$\mathbf{u}_{sts}$ standard uncertainty contribution derived from the short-term-stability study

The values of X_{ref}, u_{char}, u_{bb}, u_{sts}, u_{ref} and the expanded standard uncertainty U_{ref}, are summarised in Table 1.

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

	$\mathbf{X_{ref}}$ [mg kg^{-1}]	$\mathbf{u_{char}}$ [%]	$\mathbf{u_{bb}}$ [%]	$\mathbf{u_{sts*}}$ [%]	$\mathbf{u_{ref}}$ [%]	$\mathbf{U_{ref}}$ [mg kg^{-1}]
Total Pb			10	20.9		
Extract. Pb			12	23.9		
Total Cd	1.100	1.55	6	2.30	6.61	0.145
Extract. Cd	1.100	1.55	6	1.40	6.61	0.145
Total Hg	0.0499	3.51	1	0.60	3.70	0.004

* For six weeks,

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%.

As summarised in Table 1, total digestion and partial extraction of the test material, following the procedure described in the accompanying letter to the participants, provide identical Cd concentrations. This finding is supported by the Youden plot, Figure 1.a, constructed with the results provided by the participants in this exercise. One single cloud of points is observed on both axes around the reference value when total vs extractable Cd is plotted, showing that the results are not dependent of the method applied.

Unfortunately, due to the heterogeneity problems already described no reference value was provided for Pb, total or extractable. The Youden plot is also shown for lead, Figure 1.b, with no indication of method dependence. Nevertheless, more studies are needed before extracting any definitive conclusion on the Pb matter.

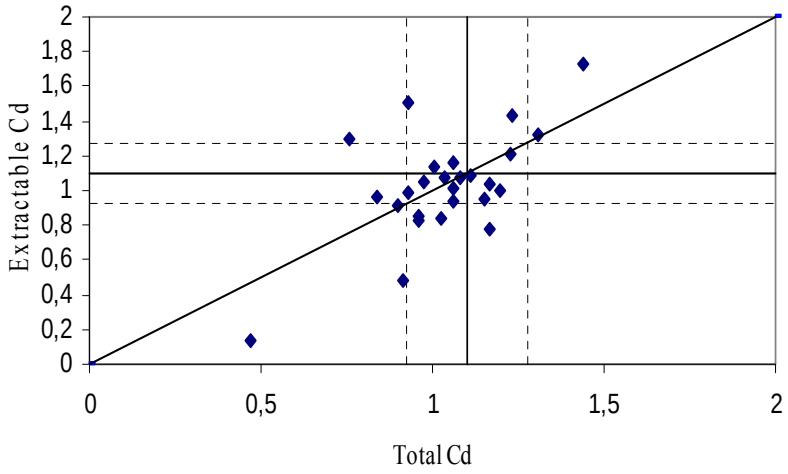

Figure 1.a
Youden plot for total and extractable Cd as obtained from the participants.

in mg kg^{-1}

_____ X_{ref}

---------- Xref ± $\hat{\sigma}$

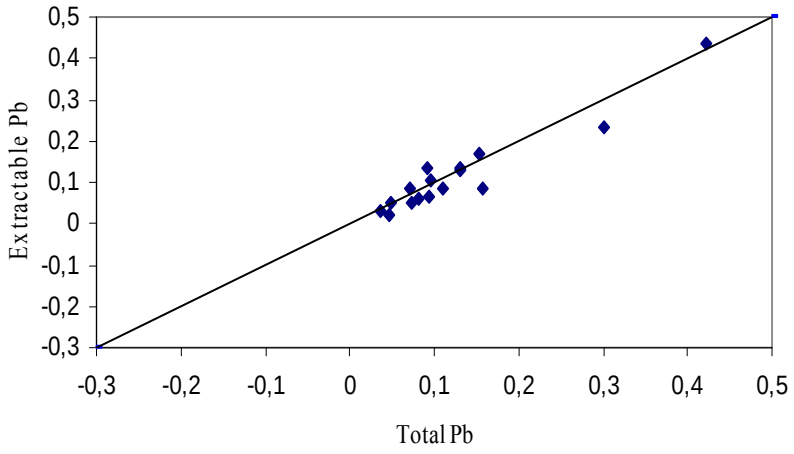

Figure 1.b
Youden plot for total and extractable Pb as obtained from the participants.

in mg kg^{-1}

8 Evaluation of results

8.1 General observations

Thirty-one laboratories from 25 countries registered for participation in this exercise. Thirty-one laboratories submitted results for total Cd and total Pb (5 laboratories reported "< than" for total Pb), 28 for total Hg (two out of the 28 reported "< than "), 26 for extractable Cd and 24 for extractable Pb (7 laboratories out of the 24 reported "< than"). All laboratories reported two or more measurement values. Twenty-nine laboratories reported uncertainty for total Cd, 23 for total Pb, 25 for total Hg, 18 for extractable Cd and 13 for extractable Pb. All laboratories 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[3] and the International Harmonised Protocol[11].

$$z = \frac{x_{lab} - X_{ref}}{\hat{\sigma}} \qquad \text{Eq. 2}$$

$$zeta = \frac{x_{lab} - X_{ref}}{\sqrt{u^2_{ref} + u^2_{lab}}} \qquad \text{Eq. 3}$$

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 $\hat{\sigma}$ is derived from the improved Horwitz equation[4]. The values for $\hat{\sigma}$ obtained for this exercise when applying the improved Horwitz equation were 22 % for total Hg and 16% for total and extractable Cd.

No scoring is provided for total and extractable Pb, in view of the heterogeneity problems. This fact made it not possible even for internationally recognised expert laboratories in the field, such as BAM and IRMM, to provide sound values for total and extractable Pb.

Should participants feel that these $\hat{\sigma}$ values are 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. If reproducibility of 22 % for

total mercury and 16 % for total and extractable Cd are regarded as satisfactory, the z-score can be interpreted as:

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

Zeta score state if the laboratory result agree with the assigned value within the respective uncertainties. The interpretation of the zeta score is similar to the interpretation of the z-score:

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

An unsatisfactory zeta-score might be due to an underestimation of the uncertainty, or to a large error causing a large deviation from the reference value, or to a combination of the two factors. A laboratory with an unsatisfactory zeta-score has an estimation of the uncertainty of its measurements which is not consistent with the laboratory's deviation from the reference value. Zeta-scores were only provided for total Cd and total Hg. It was understood by the organiser that laboratories have had little time for the implementation of the method for the partial extraction of Cd and thus laboratories may have had difficulties for sound calculation of the uncertainty budget of that measurand. For those laboratories which made the effort to provide an uncertainty estimate for the concentration of extractable Cd and additional assessment is given here which aims at giving the laboratory an indication of the plausibility of its uncertainty estimate. The standard uncertainty should fall in a range between a minimal required (u_{min}), and a maximal allowed (u_{max}) reported standard uncertainty. u_{min} is set to the standard uncertainty of the reference value. It is unlikely that a laboratory carrying the analysis on a routine basis is able to measure the measurand with a smaller uncertainty that the reference laboratory itself. u_{max} is set to the standard deviation accepted for the proficiency test, $\hat{\sigma}$. If the standard uncertainty from the laboratory, $u_{lab} < u_{min}$ it is likely that the laboratory has underestimated its uncertainty. If $u_{lab} > u_{max}$, some effort should be made to reduce it because it exceeds the present state-of-the-art in that field of analysis.

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 $\sqrt{3}$, as recommended by Eurachem and CITAC[16].

8.3 Laboratory results and scorings

The results, as reported by the participants, are summarised in Table 2a-e for total Cd, total Pb, total Hg, extractable Cd and extractable Pb, respectively, together with the z- and zeta scores, where appropriate (not for total and extractable Pb). Laboratory codes were given randomly.

Three sets of figures are provided for total Cd, extractable Cd and total Hg (Fig 2-4). Each set includes (a) the Kernel Density plot, (b) individual mean value and associated expanded uncertainty, (c) the z- and zeta scores. For total and extractable Pb (Fig 5-6) only the mean values and associated expanded uncertainties are provided. The solid line represents the

assigned value, the dashed lines delimit the reference interval ($X_{ref} \pm 2u_{ref}$) and the dotted lines delimit the target interval ($X_{ref} \pm 2\sigma$). The Kernel plots were obtained using a software tool developed by AMC[17].

Taking into consideration the z-score, twenty-nine laboratories (94 %) performed well for total Cd against the target standard deviation of 16 %. Two laboratories (6 %) obtained z-scores $3 > |z|$. For extractable Cd, twenty-two (84.5 %) out of the twenty-six laboratories which reported values for this measurand obtained z-scores $|z| \leq 2$. Three laboratories (11.5 %) obtained a z-score $3 > |z|$ and one laboratory (4 %) obtained a z-score $2 < |z| \leq 3$. For the determination of total Hg, twenty-four laboratories (92 %), obtained a satisfactory z-score against the target standard deviation of 22 % and two laboratories (8 %) obtained an unsatisfactory $3 > |z|$, Table 3.

As regards zeta-scores for total Cd, twenty-five laboratories (81 %) obtained satisfactory zeta-score, two laboratories (6 %) obtained a zeta-score $2 < |z| \leq 3$ and four laboratories (13 %) obtained an unsatisfactory zeta-score. For total Hg, eleven laboratories (42 %) out of the twenty-six which reported data obtained a $|z| \leq 2$, five laboratories (19 %) obtained a questionable zeta-score and 10 laboratories (39 %) obtained an unsatisfactory zeta-score $|z| > 3$. One third of the laboratories seem to have problems in making an estimation of the uncertainty of their measurements which is consistent with the laboratory's deviation from the reference value for Hg, Table 3.

Additional information was gathered from the questionnaire completed by the participants. Thirteen laboratories have corrected their results for recovery and eighteen did not. Of those which did, nine used a reference material to calculate the recovery. The rest spiked the sample with a known amount of the same analyte to be measured. Of those which did not correct for recovery, four indicated that they do not do it in routine analysis, five replied that their recovery is 100 %, three declared not to have corrected for recovery because they do not have an adequate certified reference material to calculate it, two answered that corrections for recovery are not to be applied in analysis of heavy metals. Various reasons were given by the rest of the laboratories.

When asked about the level of confidence reflected by the reported coverage factor (k), twenty-three reported a level of 95 %, one of 90 %, one did not provide any figure, two gave an answer which did not correspond to the question, one indicated that no statistical analysis is applied, two said not to use coverage factor and one reported "*no idea*".

For uncertainty estimates, various combinations of two or more options (question 3 of the questionnaire shown in Annex 5) were given. Nine laboratories make uncertainty budget calculation according to ISO-GUM. Three use the known uncertainty of the standard method. Twenty-one report the uncertainty as estimated during in-house validation, ten use precision data and one makes a "guesstimate". Fifteen laboratories provide an uncertainty statement to their customers for this type of analysis.

Seventeen laboratories corrected their results for the water content. From those which did not, two indicated that it is not normally done according to their procedures. Four indicated that they did not correct for humidity because it was not requested by the organiser.

Two laboratories indicated to have introduced some modification to the prescribed protocol for the partial extraction of Cd and Pb: L24 took 1 g of test material, added 42.5 mL 5 %

HNO_3 and after extraction diluted to 50 mL. L17 took 1 g in 50 mL Erlenmeyer flask, and used paper filter "Red ribbon".

The thirty-one laboratories participating in this exercise apply total matrix digestion in routine analysis and no one of them applied partial extraction for routine analysis.

Seventeen laboratories analyse the test material following an official method. The information reported by the remaining fourteen laboratories about their method of analysis is summarised in Annex 6.

Twenty-three laboratories carry out this type of analysis on a routine basis. The distribution of them in terms of number of samples analysed per year in shown in Annex 7.

Twenty-nine laboratories have a quality system in place. One out the twenty-nine is accredited according to ISO 9000, the remaining twenty-eight being accredited according to ISO/IEC 17025. Twenty laboratories are accredited for this type of analysis (when applying total digestion of the matrix).

Twenty-five laboratories declared to participate regularly in ILC's for this type of analysis.

Twenty laboratories use a reference material for this type of analysis. Eight out of the twenty use the reference material for calibration purposes and seventeen use it for the validation of the method.

9 Conclusions

The main conclusion that can be extracted out of this study is that the concentration of total and extractable Cd (according to Directive 2002/32/EC) can be considered identical when analysing feed with a high content of organic matter. This implies that laboratories can continue applying total matrix digestion for the determination of Cd in organic feed matrices. Total matrix digestion helps to overcome some drawbacks of the partial extraction such as interferences from the matrix. BAM reported high carbon interferences when partial extraction was applied. Also L31 reported that extractable Pb could not be determined due to interferences.

According to the Youden plot the concentration of total and extractable Pb are also similar. However, due to heterogeneity problems of the test material no definite conclusion can be extracted so far and further studies are needed.

Some attention must be paid to the fact that in general feed containing fish could suffer from a lack of homogeneity with the consequent impact in the results obtained. Nevertheless, further studies on this subject must be carried out before stating final conclusions. Nevertheless, it appears that the homogeneity of a test material may differ considerably for different measurands within the same batch. This shows that it may be insufficient to determine the homogeneity of only one measurand while assuming that the others behave similar.

Although the overall performance of the laboratories is quite satisfactory for total and extractable Cd and for total Hg, it must be pointed out that a number of laboratories have a problem with the estimation of their uncertainty in particular for the determination of total Hg.

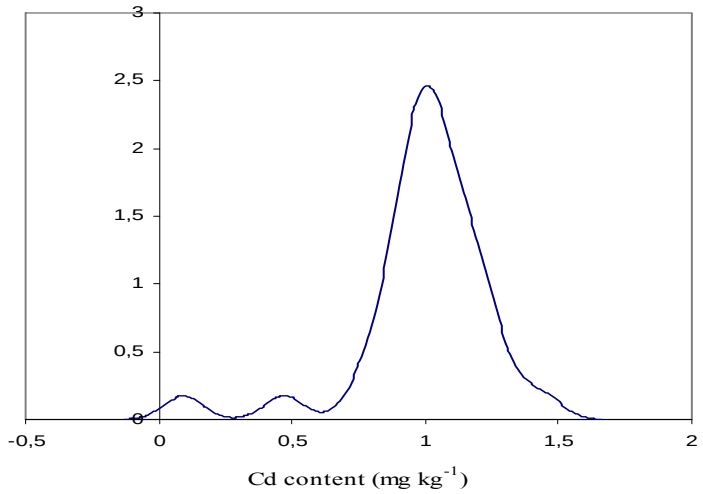

Figure 2a
Total Cd in feed
Kernel density,
All results

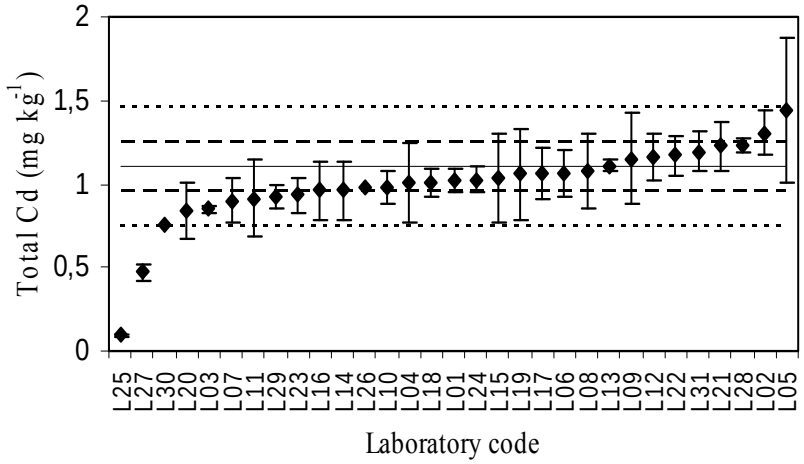

Figure 2b
Reported results and corresponding expanded uncertainties

X_{ref} = 1.100
u_{ref} = 0.017
$\hat{\sigma}$ = 0.176 (16%)

in mg kg^{-1}

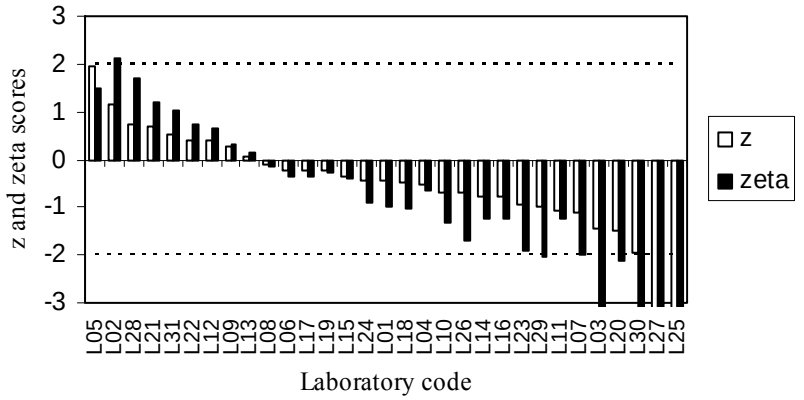

Figure 2c
Performance evaluation
$z = (x_{lab}-X_{ref})/ \hat{\sigma}$
$zeta = (x_{lab}-X_{ref})/ \sqrt{(u_{lab}^2+u_{ref}^2)}$

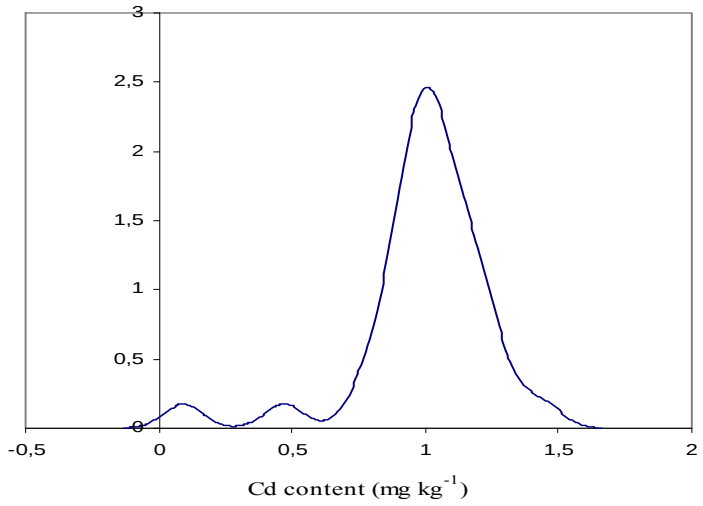

Figure 3a
Extractable Cd in feed
Kernel density,
All results

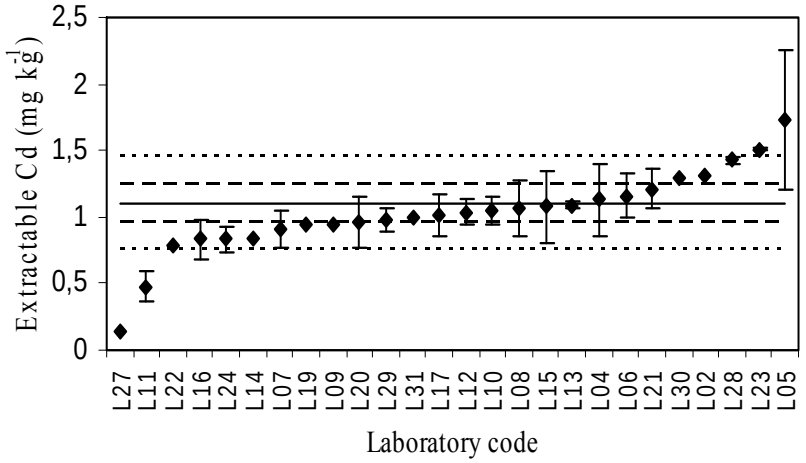

Figure 3b
Reported results and corresponding expanded uncertainties

X_{ref} = 1.100
u_{ref} = 0.017
$\hat{\sigma}$ = 0.176 (16%)

in mg kg^{-1}

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

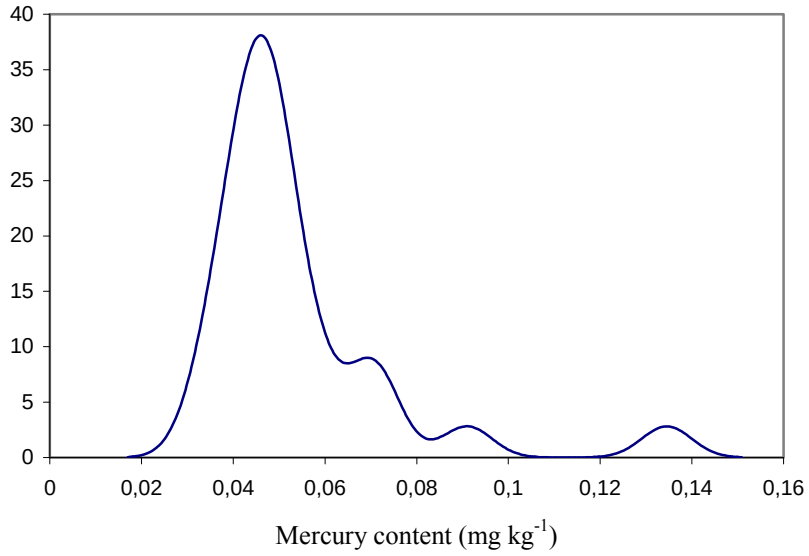

Figure 4a
Total Hg in feed
Kernel density,
All results

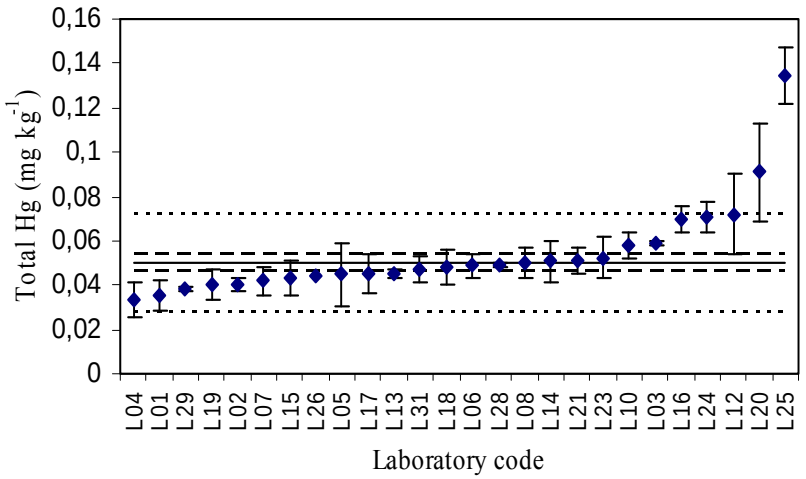

Figure 4b
Reported results and corresponding expanded uncertainties

X_{ref} = 0.0499
u_{ref} = 0.0018
$\hat{\sigma}$ = 0.0110 (22%)
in mg kg^{-1}

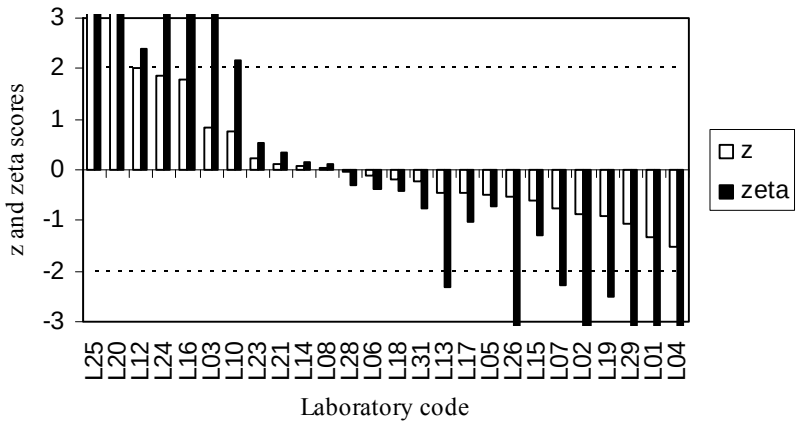

Figure 4c
Performance evaluation
$z = (x_{lab}-X_{ref})/ \hat{\sigma}$
$zeta = (x_{lab}-X_{ref})/ \sqrt{(u_{lab}^2+u_{ref}^2)}$

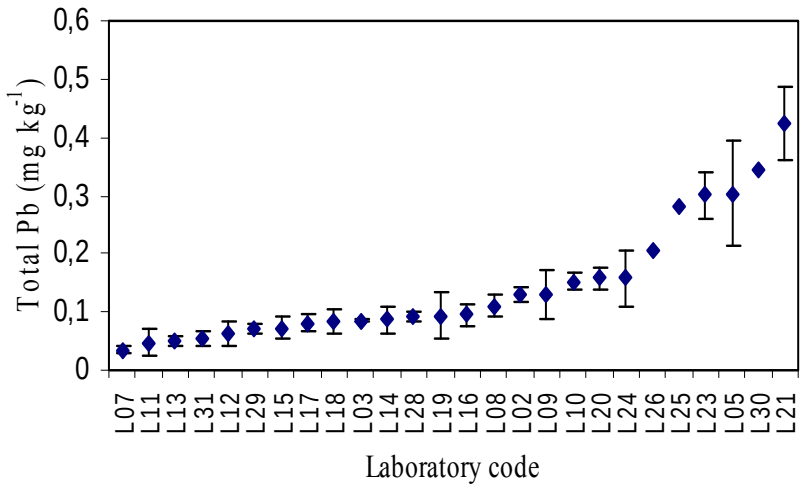

Figure 5
Reported results and corresponding expanded uncertainties for total Pb

in mg kg^{-1}

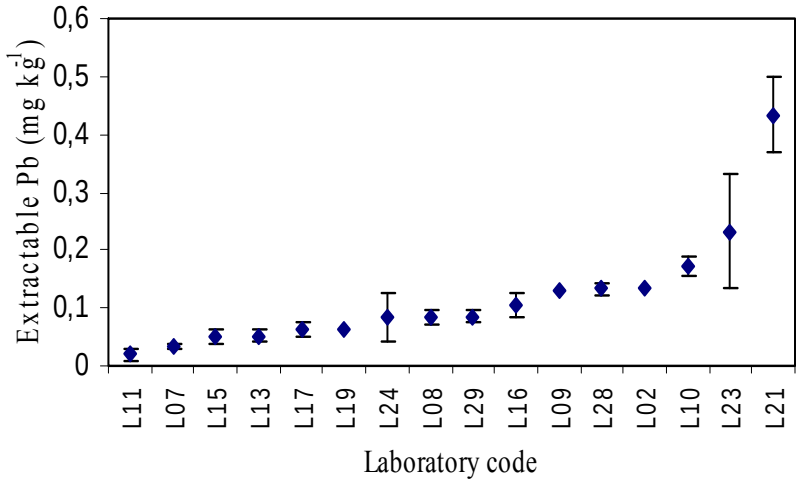

Figure 6
Reported results and corresponding expanded uncertainties for extractable Pb

in mg kg^{-1}

Table 2a: Total Cd, quantitative information reported by participants plus the laboratory scorings provided by the organiser.
Total Cd content: 1.100 ± 0.145 mg kg^{-1}

Lab code	x1	x2	x3	x4	U	k	Technique	Mean-calc	z	zeta
L01	1.01	1.02	1.04		0.07	3	ICP-AES	1.023	-0.4	-1.0
L02	1.41	1.18	1.33		0.131	2	ZETAAS	1.307	1.2	2.1
L03	0.8429	0.8459	0.8522		0.0156	2	AAS	0.847	-1.4	-3.5
L04	1.01	1	1.01		0.242	2	ICP-MS	1.007	-0.5	-0.7
L05	1.5	1.42	1.4		0.43	2	AAS	1.440	1.9	1.5
L06	1.07	1.056			0.14	2	ICP-AES	1.063	-0.2	-0.4
L07	0.904	0.894	0.904		0.135	2	ICP-AES	0.901	-1.1	-2.0
L08	1.07	1.08	1.09		0.22	2	ETAAS	1.080	-0.1	-0.2
L09	1.17	1.13			0.27	2	AAS	1.150	0.3	0.3
L10	1.05	0.95	0.93		0.098	**b**	AAS	0.977	-0.7	-1.3
L11	0.89	0.93	0.92		0.23	**b**	ETAAS	0.913	-1.1	-1.2
L12	1.18	1.25	1.07		0.14	2	AAS	1.167	0.4	0.7
L13	1.115	1.142	1.078		0.032	2	ICP-MS	1.112	0.1	0.2
L14	0.948	0.946	0.991		0.173	2	ETAAS	0.962	-0.8	-1.2
L15	1.03	1.035	1.043		0.26	**b**	ETAAS	1.036	-0.4	-0.4
L16	0.93	0.992			0.173	2		0.961	-0.8	-1.2
L17	1.064	1.06			0.15	2	ICP-MS	1.062	-0.2	-0.4
L18	1.018	0.955	1.011	1.064	0.085	2	ICP-MS	1.012	-0.5	-1.0
L19	1.02	1.1			0.27	2	ETAAS	1.060	-0.2	-0.3
L20	0.929	0.759	0.823		0.172	**b**		0.837	-1.5	-2.1
L21	1.23	1.22			0.15	2	ICP-QMS	1.225	0.7	1.2
L22	1.131	1.205			0.12	2	AAS	1.168	0.4	0.7
L23	0.88	0.981			0.1	2	AAS	0.931	-1.0	-1.9
L24	1.051	1.04	0.981		0.078	**b**	AAS	1.024	-0.4	-0.9
L25	0.08	0.08	0.11	0.1	0.01	**b**	AAS	0.093	-5.7	-13.8
L26	0.976	0.965	0.987	0.972	**a**	2	ICP-MS	0.975	-0.7	-1.7
L27	0.466	0.473	0.471		0.054	2	GF-AAS	0.470	-3.6	-8.1
L28	1.16	1.27	1.26		0.037	**b**	ICP-AES	1.230	0.7	1.7

Lab code	x1	x2	x3	x4	U	k	Technique	Mean-calc	z	zeta
L29	0.927	0.929	0.934		0.07	**b**	ICP-MS	0.930	-1.0	-2.0
L30	0.7892	0.7668	0.7204		**a**	**b**	ICP-OES	0.759	-1.9	-4.7
L31	1.18	1.17	1.22	1.21	0.12	2	AAS	1.195	0.5	1.0

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

a) k not reported; u = U/√3

b) u not reported; set to zero in zeta.

Table 2b: Extractable Cd, quantitative information reported by participants plus the laboratory scorings provided by the organiser.
Extractable Cd content: 1.100 ± 0.145 mg kg^{-1}

Lab code	x1	x2	x3	x4	U	k	Technique	Mean-calc	z
L02	1.31	1.32			**a**	**b**	ZETAAS	1.315	1.2
L04	1.13	1.13	1.13		0.271	2	ICP-MS	1.130	0.2
L05	1.68	1.8	1.72		0.52	2	AAS	1.733	3.6
L06	1.15	1.17			0.16	2	ICP-AES	1.160	0.3
L07	0.916	0.909	0.914		0.137	2	ICP-AES	0.913	-1.1
L08	1.04	1.07	1.1		0.21	2	AAS	1.070	-0.2
L09	0.963	0.932	0.958		**a**	**b**	AAS	0.951	-0.8
L10	1.01	1.05	1.15	0.98	0.098	**b**	AAS	1.048	-0.3
L11	0.47	0.49	0.48		0.12	**b**	ETAAS	0.480	-3.5
L12	1.06	1.04	1.02		0.09	2	AAS	1.040	-0.3
L13	1.078	1.115	1.076		0.022	2	ICP-MS	1.090	-0.1
L14	0.846	0.856	0.84		**a**	**b**		0.847	-1.4
L15	1.063	1.058	1.105		0.27	**b**	ETAAS	1.075	-0.1
L16	0.83	0.831			0.15	2		0.831	-1.5
L17	1.022	1.005			0.15	2	ICP-MS	1.014	-0.5
L19	1.02	0.86			**a**	**b**	ETAAS	0.940	-0.9
L20	0.893	0.924	1.077		0.197	**b**		0.965	-0.8
L21	1.25	1.17			0.15	2	ICP-QMS	1.210	0.6
L22	0.796	0.765			**a**	**b**	AAS	0.781	-1.8
L23	1.511	1.509			0.01	2	AAS	1.510	2.3
L24	0.839	0.895	0.775		0.098	**b**	AAS	0.836	-1.5
L27	0.148	0.128	0.121		**a**	**b**	GF-AAS	0.132	-5.5
L28	1.36	1.42	1.51		0.026	**b**	ICP-AES	1.430	1.9
L29	0.978	0.992	0.977	0.984	0.09	**b**	ICP-MS	0.983	-0.7
L30	1.2575	1.34			**a**	**b**	ICP-OES	1.299	1.1
L31	1.01	1.02	0.98	0.98	**a**	**b**	AAS	0.998	-0.6

All results expressed in (mg kg^{-1}). a) k not reported; u = U/√3, b) u not reported.

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

Total Hg content: 0.0499 ± 0.0037 mg kg^{-1}

Lab code	x1	x2	x3	x4	U	k	Technique	Mean-calc	z	zeta
L01	0.032	0.037	0.037		0.007	3	CV-ICP-AES	0.035	-1.3	-4.9
L02	0.04	0.041	0.04		0.003	2	TDA-AAS	0.040	-0.9	-4.0
L03	0.06	0.0587	0.059	0.0593	0.0009	2	HG-AAS	0.059	0.9	4.9
L04	0.06	0.02	0.02		0.0075	2	ICP-MS	0.033	-1.5	-4.0
L05	0.047	0.042	0.045		0.014	2	AMA 254	0.045	-0.5	-0.7
L06	0.0493	0.0481			0.0051	2	CV-AAS	0.049	-0.1	-0.4
L07	0.0418	0.0399	0.0435		0.0062	2	CV-AAS	0.042	-0.7	-2.3
L08	0.05	0.05	0.051		0.007	2	CV-AAS	0.050	0.0	0.1
L10	0.059	0.061	0.055	0.058	0.0058	**b**	CVAAS	0.058	0.8	2.2
L11	<0.1	<0.1	<0.1				HG-AAS			
L12	0.062	0.066	0.088		0.018	2	AAS	0.072	2.0	2.4
L13	0.047	0.043	0.045		0.002	2	AMA	0.045	-0.4	-2.3
L14	0.051	0.049	0.052		0.009	2	AAS	0.051	0.1	0.2
L15	0.047	0.046	0.037		0.008	**b**	CV-AAS	0.043	-0.6	-1.3
L16	0.066	0.073			0.006	2		0.070	1.8	5.6
L17	0.045	0.045			0.009	2		0.045	-0.4	-1.0
L18	0.045	0.05	0.045	0.052	0.008	2	ICP-MS	0.048	-0.2	-0.4
L19	0.04	0.04			0.007	2	AAS	0.040	-0.9	-2.5
L20	0.119	0.063			0.022	**b**		0.091	3.7	3.1
L21	0.0501	0.0522			0.0061	2	CV-AAS	0.051	0.1	0.4
L22	<0.1						ICP-MS			
L23	0.0569	0.0479			0.009	2	HG-AAS	0.052	0.2	0.5
L24	0.067	0.07	0.074		0.007	**b**	AAS	0.070	1.9	4.5
L25	0.139	0.13			0.013	**b**	AMA 254	0.135	7.7	10.8
L26	0.044	0.0437	0.0444	0.0438	**a**	2	AMA-254	0.044	-0.5	-3.2
L28	0.05	0.05	0.048		0.001	**b**	AFS	0.049	-0.1	-0.3
L29	0.0373	0.037	0.0395	0.0398	0.001	**b**	Combustion in oxygen + CV - AAS	0.038	-1.0	-5.9
L31	0.048	0.047	0.048	0.046	0.006	2	AAS	0.047	-0.2	-0.8

All results expressed in (mg kg^{-1}). a) k not reported; u = U/√3, b) u not reported; set to zero in zeta.

Table 2d: Total Pb, quantitative information reported by participants.

Lab code	x1	x2	x3	x4	U	k	Technique	Mean-calc
L01	< 0.78	< 0.78	< 0.78				ICP-AES	
L02	0.11	0.15	0.13		0.013	2	ZETAAS	0.130
L03	0.0854	0.0876	0.0835		0.0021	2	AAS	0.086
L04	< 0.1	< 0.1	< 0.1				ICP-MS	
L05	0.31	0.25	0.35		0.09	2	AAS	0.303
L06	< 0.5	< 0.5	< 0.5				ICP-AES	
L07	0.0324	0.0357	0.0388		0.0053	2	ETAAS	0.036
L08	0.107	0.117	0.108		0.018	2	ETAAS	0.111
L09	0.13	0.13			0.04	2	AAS	0.130
L10	0.157	0.16	0.15	0.145	0.0145	**b**	AAS	0.153
L11	0.05	0.04	0.05		0.023	**b**	ETAAS	0.047
L12	0.069	0.062	0.057		0.021	2	AAS	0.063
L13	0.058	0.044	0.047		0.007	2	ICP-MS	0.050
L14	0.086	0.084	0.09		0.022	2	ETAAS	0.087
L15	0.086	0.065	0.066		0.018	**b**	ETAAS	0.072
L16	0.084	0.107			0.019	2		0.096
L17	0.083	0.079			0.015	2	ICP-MS	0.081
L18	0.077	0.081	0.089	0.09	0.02	2	ICP-MS	0.084
L19	0.1	0.1	0.08		0.04	2	ETAAS	0.093
L20	0.168	0.154	0.151		0.018	**b**	AAS	0.158
L21	0.411	0.433			0.063	2	ICP-QMS	0.422
L22	< 0.3						AAS	
L23	0.271	0.331			0.04	2	AAS	0.301
L24	0.183	0.135	0.157		0.048	**b**	AAS	0.158
L25	0.28	0.21	0.35		**a**	**b**	AAS	0.280
L26	0.209	0.203	0.204	0.21	**a**	2	ICP-MS 7500c	0.207
L27	< 0.023	< 0.023	< 0.023				GF-AAS	
L28	0.087	0.093	0.094		0.009	**b**		0.091
L29	0.0725	0.068	0.0706	0.0729	0.007	**b**	ICP-MS	0.071

Lab code	x1	x2	x3	x4	U	k	Technique	Mean-calc
L30	0.3822	0.3066	0.3374		**a**	**b**	ICP-OES	0.342
L31	0.052	0.057	0.051		0.012	2	AAS	0.053

All results expressed in (mg kg^{-1}). a) k not reported, b) u not reported.

Table 2e: Extractable Pb, quantitative information reported by participants.

Lab code	x1	x2	x3	x4	U	k	Technique	Mean-calc
L02	0.12	0.15			**a**	**b**	ZETAAS	0.135
L04	< 0.1	< 0.1	< 0.1				ICP-MS	
L05	< 0.2	< 0.2	< 0.2				AAS	
L06	< 0.5	< 0.5					ICP-AES	
L07	0.0283	0.0349	0.0365		0.005	2	ETAAS	0.033
L08	0.073	0.1	0.077		0.014	2	ETAAS	0.083
L09	0.13	0.13			**a**	**b**	AAS	0.130
L10	0.17	0.18	0.16	0.175	0.0175	**b**	Z-ETA-AAS	0.171
L11	0.02	0.02	0.02		0.01	**b**	ETAAS	0.020
L13	0.043	0.062	0.051		0.01	2	ICP-MS	0.052
L14	< 0.005	< 0.005	< 0.005				ETAAS	
L15	0.073	0.042	0.039		0.012	**b**	ETAAS	0.051
L16	0.105	0.107			0.021	2		0.106
L17	0.073	0.05			0.012	2	ICP-MS	0.062
L19	0.062	0.064			**a**	**b**	ETAAS	0.063
L20	< 0.05	< 0.05	< 0.05					
L21	0.447	0.42			0.065	**b**	ICP-QMS	0.434
L22	< 0.3						AAS	
L23	0.285	0.18			0.1	2	AAS	0.233
L24	0.081	0.065	0.103		0.043	**b**	AAS	0.083
L27	< 0.023	< 0.023	< 0.023				GF-AAS	
L28	0.12	0.14	0.14		0.011	**b**	AAS	0.133
L29	0.0859	0.0894	0.083	0.085	0.01	**b**	ICP-MS	0.086

All results expressed in (mg kg^{-1}). a) k not reported, b) u not reported.

Table 3: Percentages of laboratories scoring satisfactory, questionable and unsatisfactory.

	Total Cd (%)	Extractable Cd (%)	Total Hg(%)
z-score			
Satisfactory	94	84.5	92
Questionable	0	4	0
Unsatisfactory	6	11.5	8
zeta-score			
Satisfactory	81	--	42
Questionable	6	--	19
Unsatisfactory	13	--	39

10 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. BAM is acknowledged for performing the measurements for the homogeneity and stability studies. The authors thank T. Linsinger (Reference Materials Unit) for his support with the statistical evaluation of the homogeneity and stability data and of the results.
I. Wisocka, E. Vassileva and C. Quétel are acknowledge for the provision of reference values for total Cd and Hg and for extractable Cd.
P. Robouch is acknowledged for fruitful discussions.

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

Organisation	Country
• AGES Zemtrum Analytik und Mikrobiologie	Austria
• AGES Competence Centre of Elements	Austria
• Institute of Public Health	Belgium
• National Veterinary Services	Bulgaria
• The State Veterinary Institute (SVI) in Olomuc	Czech Republic
• State General Laboratory	Cyprus
• The Danish Plant Directorate	Denmark
• The Veterinary and Food Laboratory	Estonia
• Agricultural Research Centre	Estonia
• Finnish Food Safety Authority. Evira	Finland
• SCL. Laboratoire de Bordeaux Pessac	France
• Ministry of Rural Development and Food	Greece
• General Chemical State Laboratory	Greece
• Regional Centre of Magnisia	Greece
• Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL)	Germany
• National Institute for Agricultural Quality Control	Hungary
• Public Analyst's Laboratory	Ireland
• Istituto Zooprofilattico Turin	Italy
• National Veterinary Laboratory	Lithuania

• Food Control Laboratory	Latvia
• Public Health Laboratory	Malta
• Instituut voor voedselveiligheid (RIKILT)	The Netherlands
• Voedsel en Waren Autoriteit (VWA)	The Netherlands
• National Veterinary Research Institute	Poland
• IPIMAR – Instituto de Investigação das Pescas e do Mar	Portugal
• Laboratório Nacional de Investigação Veterinária	Portugal
• HVPHI	Romania
• State Veterinary and Food Institute	Slovakia
• National Veterinary Institute	Slovenia
• Laboratorio Arbitral Agroalimentario	Spain
• National Veterinary Institute	Sweden

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

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[17] 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 described in the AMC Technical Brief "Representing data distributions with Kernel density estimates" (2006), see www.rsc.org/amc.

Annexes

Annex 1: Invitation letter to laboratories

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for reference materials and measurements
Community reference laboratory for heavy metals in feed and food

CRL Heavy Metals in Feed and Food

«Title» «M_1st_name» «last_name»
«Institute»
«Department»
«Address»
«ZIP» «City»
«COUNTRY»

Geel, 5 October 2007
GE/IM/L/74/07
D04-IM(2007)D/23185

Dear «Title» «last_name»,

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 the Proficiency Test (IMEP-103) for the determination of **total** Cd, Pb and Hg and **extractable amounts** of Cd and Pb in feed following Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed.

I would like to remind you that – according to Regulation (EC) No 882/2004 - you have the duty as NRL to participate in PTs organised by the CRL if you hold a mandate for the type of matrix investigated.

Please register electronically for this inter-laboratory comparison using the following link: http://www.irmm.jrc.be/imepapp/registerForComparison.action?comparison=90

Your participation is free of charge.

Once you have submitted your registration electronically, please follow the procedure indicated: a) print your registration form; b) sign it; and c) fax it to us. **Your fax is the confirmation of your participation.**

The **deadline for registration is 12 October 2007**. Samples will be sent to participants during the second half of October. The deadline for submission of results is 30 November 2007.

As I am the project leader for this inter-laboratory comparison, please do not hesitate to contact me in case of questions/doubts.

Yours sincerely,

Dr M.B. de la Calle
Deputy-Operating Manager CRL-HM

Cc: Philip Taylor, Piotr Robouch

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 252. Fax: (32-14) 571 865.

E-mail: jrc-irmm-crl-heavy-metals@ec.europa.eu

IMEP

Annex 2: Results of the homogeneity study

1a. Homogeneity data for total Cd in feed.

According to the IUPAC International Harmonised Protocol[11]

	Cd (µg kg^{-1})	
Bottle ID	**Replicate 1**	**Replicate 2**
15	880,16	889,94
32	869,04	876,81
52	827,85	834,54
80	824,08	815,92
96	811,63	790,05
112	790,85	773,39
138	784,69	758,19
142	767,36	769,05
175	754,31	754,91
195	733,05	734,5
Mean, n	**802,016**	**20**
Target RSD %	16	
S_{an}^2	87,27398	
S_{sam}^2	2453,812114	
σ_{all}^2	38,9779776	
Critical	2944,398267	
S_{sam}^2<critical?	ACCEPT	

According to ISO 13528[3]

0.3σ	38,9779776
s_x	49,97448453
s_w	9,342054378
s_s	49,53596789
$s_s \leq 0.3\ \sigma$	NOT ACCEPT

1b. Stability data for total Cd in feed.

As computed by SOFT CRM

Total Cd at 4°C

	Time in Weeks			
samples	0	3	5	8
1	914,2	824,1	812,5	787,8
2	836	815,5	804,2	801,9

Calculation of u_{sts}
Given Xshelf = 6 Weeks
u_{sts} = 19,186
u_{sts} [%] = 2,3%

Slope = -9,778
SE Slope = 3,198
Intercept = 863,637
SE Intercept = 15,828
Correlation Coefficient =0,609
Slope of the linear regression significantly <> 0 (95%) :Yes
Slope of the linear regression significantly <> 0 (99%)
:No

2. Homogeneity data for total Pb in feed.

According to the IUPAC International Harmonised Protocol[11]

	Pb (µg kg^{-1})	
Bottle ID	**Replicate 1**	**Replicate 2**
15	75,04	78,05
32	69,3	70,61
52	113,09	76,89
80	76,94	99,85
96	149,79	96,96
112	106,79	81,42
138	131,78	81,54
142	78,95	83,41
175	83,9	88,81
195	81,58	83,98
Mean, n	**90,434**	**20**
Target RSD %	22	
S_{an}^2	392,72737	
S_{sam}^2	43,62916944	
σ_{all}^2	35,6247112	
Critical	463,6291008	
S_{sam}^2<critical?	ACCEPT	

According to ISO 13528[3]

0.3σ	5,968644
s_x	15,49170276
s_w	19,81735023
s_s	6,605238031
$s_s \leq 0.3\ \sigma$	NOT ACCEPT

2b. Stability data for total Pb in feed.

As computed by SOFT CRM

Total Pb at 4°C

	Time in Weeks			
samples	0	3	5	8
1	111,3	68,5	74	128,7
2	79,2	68	93,2	56,8

Calculation of u_{sts}
Given Xshelf = 6 Weeks
u_{sts} = 17,780
u_{sts} [%] = 20,9%

Slope = 0,157
SE Slope = 3,200
Intercept = 84,333
SE Intercept = 15,839
Correlation Coefficient =0,000
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 feed.

According to the IUPAC International Harmonised Protocol[11]

	Hg (µg kg^{-1})	
Bottle ID	**Replicate 1**	**Replicate 2**
112	45,68	45,2
138	44,35	45,75
175	45,4	45,72
32	45,68	45,15
80	45,13	45,66
142	45,73	45,3
96	45,54	45,44
195	44,81	46,17
52	46,14	45,76
15	45,07	44,67
Mean, n	**45,4175**	**20**
Target RSD %	22	
S_{an}^2	0,260175	
S_{sam}^2	-0,045675	
σ_{all}^2	8,985335978	
Critical	17,15520839	
S_{sam}^2<critical?	ACCEPT	

According to ISO 13528[3]

0.3σ	2,997555
s_x	0,290538294
s_w	0,510073524
s_s	---
$s_s \leq 0.3\ \sigma$	ACCEPT

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

As computed by SOFT CRM

Total Hg at 4°C

	Time in Weeks			
samples	0	3	5	8
1	45,51	45,36	45,93	45,5
2	45,27	45,33	44,77	46,03

Calculation of u_{sts}
Given Xshelf = 6 Weeks
u_{sts} = 0,287
u_{sts}[%] = 0,6%

Slope = 0,044
SE Slope = 0,048
Intercept = 45,285
SE Intercept = 0,240
Correlation Coefficient =0,122
Slope of the linear regression significantly <> 0 (95%)
:No
Slope of the linear regression significantly <> 0 (99%)
:No

4a. Homogeneity data for extractable Cd in feed.
According to the IUPAC International Harmonised Protocol[11]

	Cd (µg kg^{-1})	
Bottle ID	**Replicate 1**	**Replicate 2**
15	924,4	1024,73
32	1004,46	1037,49
52	975,56	968,12
80	957,06	944,77
96	932,97	912,99
112	908,83	884,52
138	877,01	878
142	866,88	862,1
175	866,56	869,25
195	857,74	856,71
Mean, n	**920,5075**	**20**
Target RSD %	16	
S_{an}^2	619,289475	
S_{sam}^2	2902,416142	
σ_{all}^2	2001,369151	
Critical	4388,056373	
S_{sam}^2<critical?	ACCEPT	

According to ISO 13528[3]

0.3σ	44,7366645
s_x	56,67504635
s_w	24,88552742
s_s	53,87407671
$s_s \leq 0.3\ \sigma$	NOT ACCEPT

4b. Stability data for extractable Cd in feed.
As computed by SOFT CRM

Extractable Cd at 4°C

	Time in Weeks			
samples	0	3	5	8
1	835,27	850,48	822,76	819,86
2	839,78	804,15	823,26	809,11

Calculation of u_{sts}
Given Xshelf = 6 Weeks
u_{sts} = 11,313
u_{sts} [%] = 1,4%

Slope = -2,837
SE Slope = 1,675
Intercept = 836,933
SE Intercept = 8,291
Correlation Coefficient =0,323
Slope of the linear regression significantly <> 0 (95%)
:No
Slope of the linear regression significantly <> 0 (99%)
:No

5. Homogeneity data for extractable Pb in feed.

According to the IUPAC International Harmonised Protocol[11]

	Pb (µg kg^{-1})	
Bottle ID	**Replicate 1**	**Replicate 2**
15	61,05	113,7
32	68,53	71,21
52	62,98	74,75
80	71,16	69,52
96	109,3	103,08
112	133,28	78,5
138	87,31	149,78
142	94,68	79,63
175	73,22	71,86
195	77,89	64,95
Mean, n	**85,819**	**20**
Target RSD %	22	
S_{an}^2	512,91304	
S_{sam}^2	83,33136778	
σ_{all}^2	32,08150771	
Critical	578,3554049	
S_{sam}^2<critical?	ACCEPT	

According to ISO 13528[3]

0.3σ	5,664054
s_x	18,43333632
s_w	22,64758354
s_s	9,128601633
$s_s \leq 0.3\ \sigma$	NOT ACCEPT

5b. Stability data for extractable Pb in feed.

As computed by SOFT CRM

Extractable Pb at 4°C

	Time in Weeks			
samples	0	3	5	8
1	100,59	158,58	94,58	100,68
2	74,86	75,24	174,69	114,67

Calculation of u_{sts}
Given Xshelf = 6 Weeks
u_{sts} = 26,661
u_{sts} [%] = 23,9%

Slope = 2,868
SE Slope = 4,655
Intercept = 100,263
SE Intercept = 23,039
Correlation Coefficient =0,060
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
JOINT RESEARCH CENTRE

Institute for reference materials and measurements
Community reference laboratory for heavy metals in feed and food

CRL Heavy Metals in Feed and Food

Geel, 16 October 2007
GE/IM/L/79/07
D04-IM(2007)BdlC/ive/D/24334

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

Participation to IMEP-103, a proficiency test exercise for the determination of total Cd, Pb and Hg and extractable amounts of Cd and Pb in feed

Dear «TITLE» «SURNAME»,

Thank you for participating in the IMEP-103 intercomparison for the determination of **total** Cd, Pb and Hg and **extractable amounts** of Cd and Pb in feed. This exercise takes place in the frame of the CRL Heavy Metals in Feed and Food.

This parcel contains:
a) One glass bottle containing approximately 20 g of the test material
b) A "Confirmation of Receipt" form
c) This accompanying letter

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 (4 °C) until analysis.

The measurands are: **total** Cd, Pb and Hg and **extractable** amounts of Cd and Pb according to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed, in a feed matrix of fish origin.

As agreed upon during the workshop held in September, the determination of the **extractable** amounts of Cd and Pb shall be carried out by strictly applying the following procedure:

«PARTKEY»

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 252. Fax: (32-14) 571 865.

E-mail: jrc-irmm-crl-heavy-metals@ec.europa.eu

Protocol for the partial extraction of Cd and Pb in feed (IMEP-103)

1. *Weigh about 2 g of the prepared test sample to the nearest 1 mg into a 250 mL beaker.*
2. *Add 85 mL of a 5 % (w/w) HNO_3 solution (see note for the preparation of the HNO_3 solution).*
3. *Cover the beaker with a watch-glass and boil for 30 min on a hot plate (make sure that the plate warms up homogeneously all over the surface).*
4. *Allow to cool. Decant the liquid into a 100 mL volumetric flask, rinsing the beaker and the watch-glass several times with 5 % (w/w) HNO_3.*
5. *Dilute to the mark with 5 % (w/w) HNO_3.*
6. *After homogenising, filter through a fry folded filter paper into a dry container. Use the first portion of the filtrate to rinse the glassware and discard that part. If the determination is not carried out immediately, the container with filtrate shall be stoppered.*
7. *Carry out a blank test at the same time as the extraction, with only the reagents and follow the same procedure as for the samples.*

To construct the calibration curve dilute the standards in 5 % (w/w) HNO_3.

NOTE: To prepare 1 kg stock of 5 % (w/w) HNO_3 (density ~ 1.0257 kg/l): mix 77 g of 65 % (w/w) HNO_3 with 923 g water. Use a balance of two digits for the weighing.

For the determination of the **total** content of Cd, Pb and Hg the procedure that you use should resemble as closely as possible the one that you use in routine sample analysis.

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 results should be 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 4". After entering all results, please also complete the relating questionnaire. Do not forget to submit and confirm always when required.

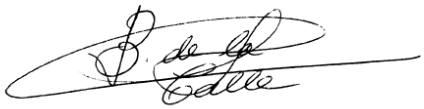
Directly after submitting your results and the questionnaire information online, you will be prompted to print the completed report form. Please do so, sign the paper version and return it to IRMM by fax (at +32-14-571-865) or by e-mail. Check your results carefully for any errors before submission,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

The deadline for submission of results is 30/11/2007.

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-103 Co-ordinator

Enclosures: 1) one glass bottle containing the test material; 2) confirmation of receipt form; 3) Accompanying letter.

Cc: P. Taylor

Annex 4: Sample receipt confirmation form

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for reference materials and measurements
Community reference laboratory for heavy metals in feed and food

CRL Heavy Metals in Feed and Food

Annex to D04-IM(2007)BdlC/ive/D/24334

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

CRL-HM-03 / IMEP-103

Total Cd, Pb and Hg and extractable amounts of Cd and Pb in feed

Confirmation of receipt of the samples

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-103 Coordinator
EC-JRC-IRMM
Retieseweg 111
B-2440 GEEL, Belgium

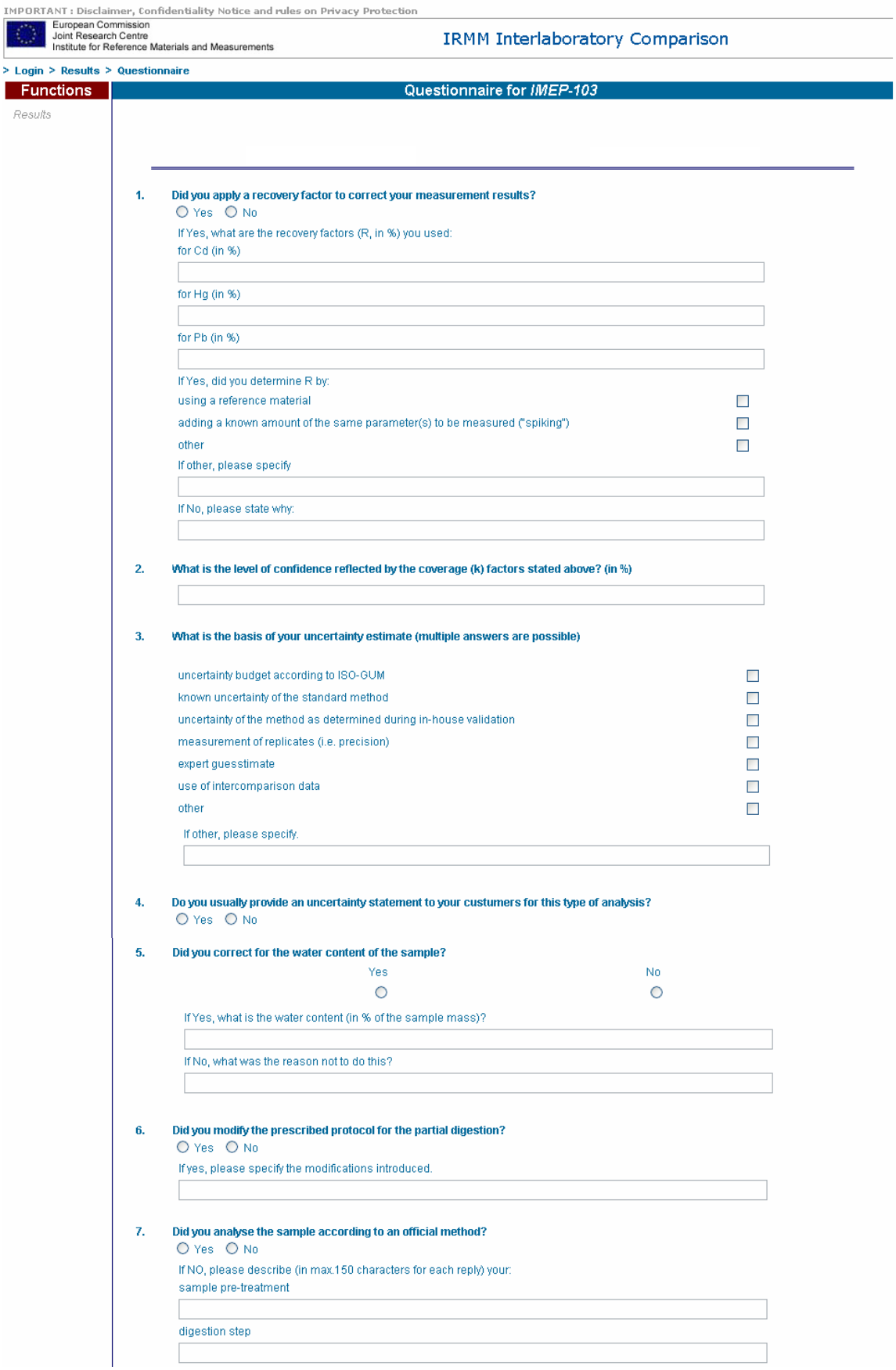
Fax : +32-14-571865
e-mail : JRC-IRMM-CRL-HEAVY-METALS@ec.europa.eu

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 252. Fax: (32-14) 571 865.

E-mail: jrc-irmm-crl-heavy-metals@ec.europa.eu

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-103*

1. **Did you apply a recovery factor to correct your measurement results?**
   ○ Yes ○ No
   If Yes, what are the recovery factors (R, in %) you used:
   for Cd (in %)
   for Hg (in %)
   for Pb (in %)
   If Yes, did you determine R by:
   using a reference material ☐
   adding a known amount of the same parameter(s) to be measured ("spiking") ☐
   other ☐
   If other, please specify
   If No, please state why:

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. **Did you correct for the water content of the sample?**
   Yes ○ No ○
   If Yes, what is the water content (in % of the sample mass)?
   If No, what was the reason not to do this?

6. **Did you modify the prescribed protocol for the partial digestion?**
   ○ Yes ○ No
   If yes, please specify the modifications introduced.

7. **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

extraction / separation step

instrument calibration step

If Yes, which:

8. Does your laboratory carry out this type of analysis (as regards the parameters, matrix and methods) on a routine basis?

○ Yes ○ No

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

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

○ Yes ○ No

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

If Other, please specify.

10. Which type of sample treatment do you routinely use for such samples?

Total digestion ☐

Partial digestion (according to the legislation) ☐

11. Is your laboratory accredited for the sample treatment that you specify in question 10?

12. Does your laboratory take part in an interlaboratory comparison for this type of analysis on a regular basis?

○ Yes ○ No

If yes, which one(s):

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

○ Yes ○ No

	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):

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

Clear

Submit questionnaire

IMEP version 1.5.1 : This site is managed by the Informatics Unit, IRMM. Last update : 16/11/2007

Annex 6: Experimental details

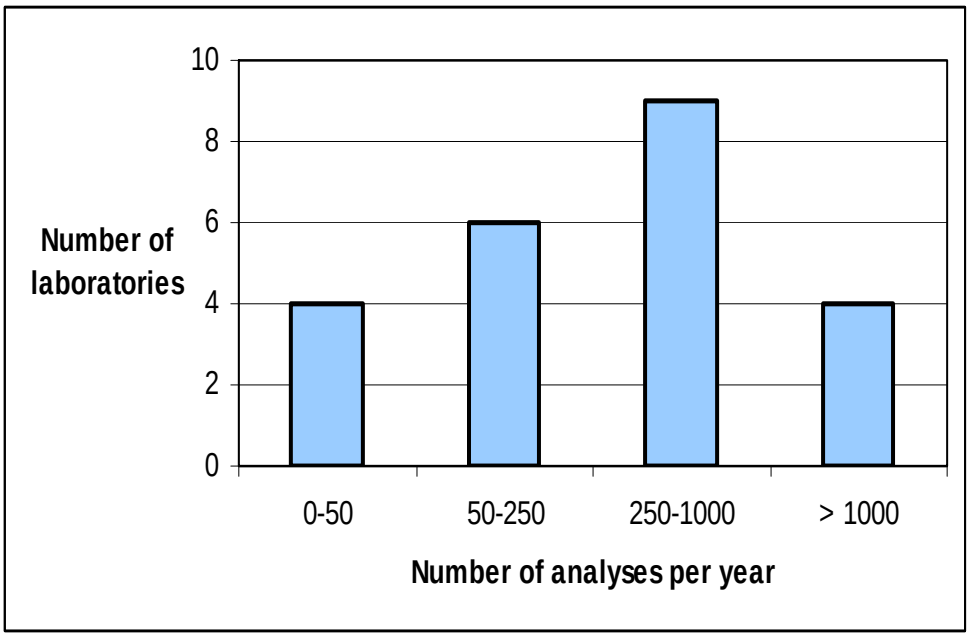
Lab Code	SOP?	Which SOP?	Sample pre-treatment	Digestion step	Extraction / separation	Instrument calibration
L01	No		Milling if necessary	Bomb digestion. 0.4 dry material is digested with 3 ml of conc. nitric acid for 3 h at 160 degrees in an oven	The acid residue is diluted to 10 ml with ultra-pure water (sample solution)	Calibration without standard addition Five standards 0, 0.05 0.10, 0.25 and 0.50 µg/ml with the same acid conc as in the sample solution.
L02	No		None	Digestion with H_2O_2(30%), HF and HNO_3 conc. by microwave high pressure (for Pb,Cd)	None	Add method; std solution Cd: 2 ppb; Pb: 50 ppb. Hg: square calibration with std solution 25-50-100-150-300 ppb 1-2-5 ppm
L03	No		Add 2 ml 65% HNO_3	microwave	dilution	4 step
L04	Yes	AOAC official method 999.10				
L05	No	EN 15550	No	HNO_3 25%	No	0 to 100 µg/l
L06	No		No pretreatment	Pb/Cd: Ashing at 470 C Hg: Wet digestion w. concentrated HNO_3/HCl/H_2O_2	Pb/Cd: Extraction with 37% HCl and evaporation followed by extraction with 2% HCl. Hg: No extraction step	External calibration with correction for spike recovery.
L07	Yes					
L08	No	SR EN 13806/2003, SR EN 14082/2003, R (EC)18/2007, R (EC)1881/2006	We weigh approximately 0.5g of the sample and we add 10 ml of HNO_3 65% suprapur	We digest the sample in a microwave oven	After the digestion we dilute the sample up to 20 ml	we calibrate the instrument using standard solutions of Cd (0.2 -2.0 ng/ml), of Pb(5.0-50.0ng/ml)
L09	Yes					
L10	Yes					

Lab Code	SOP?	Which SOP?	Sample pre-treatment	Digestion step	Extraction / separation	Instrument calibration
L11	Yes	National Feed Codex				
L12	Yes					
L13	No		After shaking and mixing for the homogeneity, one weighs of about 0.5 g of sample was placed in PFA tube	Wet digestion; HNO_3 + H_2O_2 at 180°C	none	External calibration
L14	No	AOAC	Weigh, dreing, disolving	dry in oven, digestet in muffle furnace, disolved in 1N HCl, Hg - AMA 254	Extraction	Calibration curve for Pb (10-60 ug/l), calibration curve for Cd (1-10 ug/l), Hg (0.05-5ug/ml
L15	Yes	EU- Guideline; VdLUFA				
L16	Yes	ASU L00.00-19/3				
L17	No	EN 14084 for Cd and Pb; EPA 7473 for Hg	Microwave	5 % NO_3H	No	ICP-MS
L18	No	ANSWER	None	Microwave digestion at 200°C for 25 min with HNO_3 and H_2O_2	None	ICP calibration with known solution. Calibration curves (5 concentrations) were used for each metal
L19	Yes					
L20	Yes	AOAC 999.10 (2005), AOAC 974.14 (2000), EN 13806 (2002)				
L21	Yes	NMKL				

Lab Code	SOP?	Which SOP?	Sample pre-treatment	Digestion step	Extraction / separation	Instrument calibration
L22	No	AOAC Official Method 999.10, AOAC Official Methods of Analysis, 2000, Chapter 9, p16-19.	Dry ashing	dissolving the ash in diluted hydrochloric acid	Complexes of Pb and Cd with DDC are extracted into MIBK.	Matrix matched calibration curve
L23	No		None	0.5g sample with 5ml HNO_3 microwave digestion at 200 celsius for15minutes	none	yes
L24	Yes	Cd, Pb - AOAC 999.11, Hg AOAC 971.21				
L25	Yes	ANSWER				
L26	Yes	ISO 17294-2,2005				
L27	Yes	EN 14082				
L28	Yes	4 point calibration with similar matrix				
L29	No		None	microwave assisted digestion of 0,2 g dry sample with 1 ml H_2O, 3,5 ml HNO_3 and 1 ml H_2O_2 in the pressure bomb, made up to 10 g	None	linear through zero; 0,5 - 1- 2- 5 - 10 ppb in solution; corrected to added In-115 as internal standard
L30	No	SOP based on CEN method	we balanced 0,5 gr of the sample, we added 10 ml HNO_3	microwave	No	No

Lab Code	SOP?	Which SOP?	Sample pre-treatment	Digestion step	Extraction / separation	Instrument calibration
L31	Yes	EN 15510:2007				

Annex 7: Number of samples analysed per year



European Commission

EUR 23236 EN – Joint Research Centre – Institute for Reference Materials and Measurements
Title: Report of the third interlaboratory comparison organised by the Community Reference Laboratory for Heavy Metals in Feed and Food. Total Cd, Pb and Hg and extractable Cd and Pb in feed.
Author(s): M.B. de la Calle, J. van de Kreeke, I. Verbist, S. Bynens, P. Taylor
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DOI 10.2787/1332

Abstract
The Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre, a Directorate-General of the European Commission, operates the Community Reference Laboratory for Heavy Metals in Feed and Food (CRL-HM). One of its core tasks is to organise interlaboratory comparisons (ILCs) among appointed National Reference Laboratories (NRLs). This report presents the results of the third ILC of the CRL-HM which focused on the determination of total Cd, Pb and Hg and extractable Cd and Pb in feed according to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed.

The test material used in this exercise was a commercial compound feed for fish provided by the Centro di Referenza Nazionale per la Sorveglianza e il Controllo degli Alimenti per Animali, Istituto Zooprofilattico del Piemonte. The material, naturally contaminated, was processed, bottled, labelled and dispatched by the Reference Materials Unit of the IRMM. The samples were dispatched on the second half of October 2007. Each participant received one bottle containing approximately 20 g of test material. Thirty-one participants from 25 countries registered to the exercise of which 31 submitted results for total Cd and total Pb, 28 for total Hg, 26 for extractable Cd and 24 for extractable Pb.

The assigned values (X_{ref}) were provided by IRMM using isotope dilution-inductively coupled plasma-mass spectrometry (ID-ICP-MS). The analytical uncertainty of X_{ref}, u_{char}, was calculated according to the ISO Guide to the Expression of Uncertainty in Measurement (GUM). Homogeneity and stability studies were subcontracted to Bundesanstalt für Materialforschung und –Prüfung (BAM). The uncertainties of the respective assigned values, u_{ref}, were calculated combining the analytical uncertainty, u_{char}, with a contribution for the between-bottle homogeneity, u_{bb}, and for the short term stability of the test material, u_{sts}. Participants were invited to report the uncertainty on their measurements. This was done by 29 laboratories for total Cd, 23 for total Pb, 25 for total Hg, 18 for extractable Cd and 13 for extractable Pb.

Laboratory results were rated with z and zeta scores in accordance with ISO 13528. Standard deviation for proficiency assessment (also called target standard deviation) were calculated using the modified Horwitz equation and were 22 % of the assigned value for total Pb and Hg and for extractable Pb and 16 % of the assigned value for total and extractable Cd.

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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