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IMEP-38: Determination of total As, Cd, Pb, and Hg in compound feed

Interlaboratory Comparison Report

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IMEP®

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Executive summary

The Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre, a Directorate General of the European Commission, operates the International Measurement Evaluation Programme (IMEP). IMEP organizes proficiency tests (PTs) in support to EU policies. This report presents the results of the PT which focused on the determination of total As, Cd, Pb and Hg in compound 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 commercially available compound feed for cats which after the appropriate processing was spiked, bottled, labelled, numbered accordingly and dispatched to the participants on the 27th of June 2013. Forty-seven laboratories from 24 countries registered to the exercise of which 44 reported results and answered the respective questionnaire. Laboratories were asked to perform two or three independent measurements and to report the mean, the associated uncertainty, the coverage factor of the associated uncertainty and the technique used to perform the measurements.

Three laboratories with demonstrated experience in the field provided results to establish the assigned values (X_{ref}). The standard uncertainties associated to the assigned values (u_{ref}) were calculated according to ISO/IEC Guide 98: 2008 (GUM) and ISO 13528: 2005. Laboratory results were rated using z- and ζ-scores (zeta-scores) in accordance with ISO 13528: 2005. The z-score compares the participant's deviation from the reference value with the target standard deviation for proficiency assessment (σ_p), while the ζ- score states whether the laboratory's result agrees with the assigned value within the respective uncertainty. The standard deviation for proficiency assessment (σ_p), also called target standard deviation, was set to 10 % of the assigned value, for the analysis investigated.

The percentage of satisfactory z-scores ranged from 58 % (total arsenic) to 74 % (total cadmium) and the ζ-scores obtained were lower by 10 to 21%.

1 Introduction

The IMEP-38 exercise was organized aiming to assess the performance of food and feed control laboratories and official control laboratories on the determination of total arsenic, cadmium, lead and mercury in compound feed. This PT was carried out in collaboration with the European Union Reference Laboratory for Heavy Metals (EU-RL-HM), who organised in parallel the PT IMEP-117 for its network of National Reference Laboratories (NRLs), using the same test item. The results submitted to IMEP-117 are not discussed in this report.

Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed [1], describes as "*compound feedingstuffs*" the "*mixtures of feed materials, whether or not containing additives, which are intended for oral animal feeding as complete or complementary feedingstuffs*". The Directive and its amendments set maximum levels for undesirable substances in animal feed (organic and inorganic). Regarding heavy metals, limits are set only for mercury (0.1 mg kg^{-1}) with the exception of mineral feed (0.2 mg kg^{-1}), compound feed for fish (0.2 mg kg^{-1}) and compound feed for dogs, cats and fur animals (0.3 mg kg^{-1}).

The screening of the material that was selected for this exercise (cat feed) revealed very low or no naturally incurred heavy metals and thus a spiking approach was choosen. As a result the test material that was finally distributed to the participants was not compliant with the legislation.

This report summarises and evaluates the outcome of IMEP-38.

2 IMEP support to EU policy

The International Measurement Evaluation Programme is hold by the Joint Research Centre - Institute for Reference Materials and Measurements. IMEP provides support to the European measurement infrastructure in the following ways:

IMEP disseminates metrology from the highest level down to the field laboratories. These laboratories can benchmark their measurement result against the IMEP certified reference value. This value is established according to metrological best practice.

IMEP helps laboratories to assess their estimate of measurement uncertainty. The participants are invited to report the uncertainty on their measurement results. IMEP integrates the estimate into the scoring, and provides assistance for the interpretation.

IMEP supports EU policies by organising interlaboratory comparisons in the frame of specific EU Directives or on request of a specific EC Directorate-General. In the case of IMEP-38 it was organised to support the Directorate General for Health and Consumers (DG SANCO) with the implementation of Directive 2002/32/EC [1]. Furthermore, IMEP-38 provided support to the following stakeholders:

- The **European Cooperation for Accreditation** (EA) in the frame of a Memorandum of Understanding on a number of metrological issues, including the organisation of interlaboratory comparisons. National accreditation bodies were invited to nominate a limited number of laboratories for free participation in IMEP-38. Mrs Alexandra Morazzo from Instituto Português de Acreditação (IPAC) liaised between EA and IMEP for this ILC. This report does not discern the EA nominees from the other participants. Their results are however summarised in a separate report to EA.

- The **Asia Pacific Laboratory Accreditation Cooperation** (APLAC), in the frame of the collaboration with APLAC. Ms Cynthia Chen (APLAC PT Committee) liaised between APLAC and IMEP, announcing the exercise to the accreditation bodies in the APLAC network.

- The **Inter-American Accreditation Cooperation** (IAAC). Mrs. Barbara Belzer liaised between IAAC and IMEP. She was invited to announce the exercise to the accreditation bodies in the IAAC network.

3 Scope and aim

The scope of this PT was to test the competence of the participating laboratories to determine total As, Cd, Pb and Hg in compound feed.

The assessment of the measurement results was undertaken on the basis of requirements laid down in legislation [1], and follows the administrative procedure and logistics of IMEP / IRMM of the European Commission Directorate General Joint Research Centre. IMEP is accredited according to ISO 17043:2010 [2]. The designation of this PT is IMEP-38.

4. Set up of the exercise

4.1 Time frame

The exercise was announced via the IMEP web page on the 3rd of May 2013 (Annex 1). On the same day the exercise was announced to the European Cooperation for Accreditation, to the Asian Pacific Laboratory Accreditation Cooperation and to the Inter-American Accreditation Cooperation, (Annexes 2 - 4).

Registration was opened till the 7th June 2013. The deadline for reporting results was the 30th July 2013. Dispatch was followed by the messenger's parcel tracking system on the internet.

4.2 Confidentiality

The following confidentiality statement was made to EA, IAAC and APLAC: *"Confidentiality of the participants and their results towards third parties is guaranteed".* In the case of EA the following was added: "*However, IMEP will disclose details of the participants that have been nominated by EA to you. The EA accreditation bodies may wish to inform the nominees of this disclosure".*

4.3 Distribution

Samples were dispatched to the participants by IRMM on 27th of June 2013. Each participant received:

- One bottle containing approximately 20 g of powdered compound feed.
- A "Sample accompanying letter" (Annex 5).
- A "Confirmation of receipt form" to be sent back to IRMM after receipt of the test material (Annex 6).

4.4 Instructions to participants

Concrete instructions were given to all participants in the above mentioned letter accompanying the test item. The measurands and matrix were defined as "Total As, Cd,

Pb and Hg in compound feed" following Directive 2002/32/EC on undesirable substances in animal feed".

Laboratories were asked to perform two or three independent measurements and to report the mean, the associated expanded uncertainty, the coverage factor of the associated expanded uncertainty and the technique used to perform the measurements. The measurement results were to be corrected for (i) recovery and (ii) moisture, the latter following the procedure described in the sample accompanying letter. Participants were asked to follow their routine procedures for the analysis and to report results in the same way (e.g. number of significant figures) as they would report to their customers. Likewise they were asked to calculate the moisture content of the test material using the recipe provided in the accompanying letter and to report all data as based on dry-mass.

The results were to be reported in a special on-line form for which each participant received an individual access code. A questionnaire was attached to this on-line form (Annex 7).

The laboratory codes were given randomly and communicated to the participants by e-mail.

5 Test material

5.1 Preparation

The material used as test item was a commercially available feed purchased at a local market in Belgium. The composition reported on the label by the producer is indicated hereafter between brackets:

> Cereals, vegetable proteins, meat and animal sub-products, vegetable sub-products, oil and fats, fish and fish sub-products, yeast, minerals, vegetables.
>
> Nutritional additives in UI Kg^{-1}: Vit. A (12500), Vit. D3 (1000)
>
> in mg Kg^{-1}: Fe (48), I (1.5), Cu (9), Mn(5.1), Zn(67), Se(0.1)
>
> Analytical components: proteins (34.0 %), fat (8.0 %), ash (7.0 %), ash (7.0 %), fibers (4.0 %)

Two bags (4 kg each) of the granular compound feed (cat-food), were emptied in two stainless steel drums which were thereafter immersed in liquid N_2 to cool down the material prior to cryogenic milling. An all-titanium vibrating cryogenic mill was then used to mill the material (Palla VM-KT, Humboldt-Wedag, Köln, Germany).

After milling at temperatures between -196 to -100 °C the material was pre-cooled again and sieved over a 250 µm stainless steel sieve (Russel Finex, London, United Kingdom). Cold sieving was achieved under gentle flow of liquid N_2 to avoid clogging. The resulting powder (7.8 kg, < 250 µm) was placed in an 80 L stainless steel drum in which 32.5 L of tap water were added. The slurry was then mixed, homogenized and spiked with Pb, Hg, As and Cd standard solutions. Pure concentrated standards (Merck, 1000 mg/l ICP standards) with a certified concentration and associated uncertainty were used to obtain the following theoretical concentrations in the final material: 2.36, 0.76, 5.08 and 0.79 mg $kg^{-1}$of As, Cd, Pb and Hg, respectively. The

recipient in which the spike was contained was rinsed once with tap water and added to the slurry to ensure a quantitative transfer. The spiked slurry was stirred for 2 hours using an IKA (Janke- Kunkel, Staufen, Germany) stirrer for further homogenisation.

Approximately 1 L of slurry per tray was placed on the freeze drying trays, (31 trays in total) and placed at -20 °C in a freeze cell over-night. After freeze drying the material was found to be sufficiently dry for the next steps (1.13 ± 0.17 % m/m for n = 2) as measured by Karl Fischer titration (KFT).

The dried slurry formed hard cakes on the trays which were crushed using a Teflon pestle inside a plastic drum. Teflon balls were then added to the drum placed in a 3-dimensional mixer for 1 h (Dynamix CM-200, WAB, Basel, Switzerland). The resulting powder-lump mixture was passed over a 710 µm stainless steel sieve and the lumps were crushed on the sieve using sieve inserts and the scoop. The resulting material was sieved over a 250 µm stainless steel sieve. Crushing of lumps and sieving through 710 and 250 µm sieves was repeated until 4.7 kg of powder was obtained. The powder bulk was then homogenized by placing the drum in the 3-dimensional mixer for 30 minutes.

The top particle size in the final material was 241 µm for X_{90} and 346 µm for X_{99} as measured by laser diffraction. Water content in the final material was 1.52 ± 0.22 % (m/m) as measured by KFT. An oven method was developed to provide equivalent result as obtained by KFT. The drying recipe was provided to the participants of the PT-testing round in order to harmonise the drying protocol.

Amber glass 60-ml bottles with a PE insert were filled with slightly more than 20 g each using a vibrating feeder and a balance. Units of IMEP-117 and IMEP-38 were labeled intermittently. In total 200 bottles were filled and kept at 4 ^{o}C until dispatch.

5.2 Homogeneity and stability

The homogeneity and stability studies were performed by ALS Scandinavia AB (Luleå, Sweden) using inductively coupled plasma sector field mass spectrometry (ICP/SFMS) after microwave digestion with a mixture of HNO_3/H_2O_2.

Homogeneity was evaluated according to ISO 13528: 2005 [3]. The material proved to be adequately homogeneous for all measurands under study.

The stability study was conducted following the isochronous approach [4, 5]. The material proved to be stable for the 5 weeks that elapsed between the dispatch of the samples and the deadline for submission of results, for total As, Cd, Pb and Hg.

The contribution from homogeneity (u_{bb}) and stability (u_{st}) to the uncertainty of the reference value (u_{ref}) was calculated using SoftCRM [6]. The analytical results and the statistical evaluation of the homogeneity and stability studies are presented in Table 1 and Annex 8.

6. Reference values and their uncertainties

6.1 Assigned value X_{ref}

The assigned values for the four measurands investigated were determined by:
LNE – Laboratoire National de Metrologie et d' Essais (Paris, France);
SCK-CEN – Studiecentrum voor Kernenergie (Mol, Belgium); and
VITO – Vlaamse Instelling voor Technologisch Onderzoek (Mol, Belgium).

Experts were asked to use the method of their choice with no further metrological requirements. Experts were also required to report their results together with the associated expanded uncertainty and with a clear and detailed description on how uncertainty was estimated.

LNE used microwave digestion with a mixture of HNO_3/H_2O_2 with double isotope dilution - inductively coupled plasma mass spectrometry (ID-ICP/MS) for the determination of total Cd, Pb and Hg and standard addition method with ICP/MS for total As.

SCK-CEN used neutron activation analysis for the determination of total As and Hg.

VITO used digestion in a high pressure asher using quartz vessels with a mixture of HNO_3/H_2O_2 and inductively coupled plasma atomic emission spectroscopy (ICP/AES) for the determination of total As, Cd and Pb and cold vapour atomic absorption spectrometry (CV-AAS) after thermal decomposition and amalgamation for the determination of total Hg.

For this PT, the mean of the independent means provided by the expert laboratories was used to derive the assigned values (X_{ref}) according to ISO Guide 35 [7].

The assigned values were disclosed to the participants in an e-mail sent on the 11th October 2013.

6.2 Associated uncertainty u_{ref}

The associated uncertainties (u_{ref}) of the assigned values were calculated combining the uncertainty of the characterization (u_{char}) with the contributions for homogeneity (u_{bb}) and stability (u_{st}) in compliance with ISO/IEC Guide 98 (GUM) [8] using Eq.1:

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

In the case of total Pb and Hg the expert laboratories reported values with overlapping expanded uncertainties (Table 1). u_{char} was calculated according to ISO 13528:2005 [3]:

$$u_{char} = \frac{1.25}{p}\sqrt{\sum_1^p u_i^2} \qquad \text{Eq. 2}$$

Where: *p* refers to the number of expert laboratories used to assign the reference value and u_i is the standard associated uncertainty reported by the experts.

For total As and Cd the experts reported values non-overlapping within their respective expanded uncertainties (Table 1). u_{char} was then calculated according to ISO Guide 35 [7]:

$$u_{char} = \frac{s}{\sqrt{p}} \qquad \text{Eq. 3}$$

Where: *s* refers to the standard deviation of the values obtained by the expert laboratories.

Table 1 presents the results reported by the expert laboratories, standard uncertainty contributions, the reference values (X_{ref}, u_{ref} and U_{ref}) and the standard deviation for the PT assessment σ_p.

Table 1 – *Reported values by the expert laboratories, assigned values, their associated expanded uncertainties and target standard deviations for the measurands of this ILC (all values in mg kg^{-1}).*

	total-As	**total-Cd**	**total-Pb**	**total-Hg**
Expert lab 1	*2.61 ± 0.075*	*0.866 ± 0.011*	*5.639 ± 0.085*	*0.787 ± 0.025*
Expert lab 2	*3.02 ± 0.21*	*0.892 ± 0.014*	*5.67 ± 0.37*	*0.815 ± 0.058*
Expert lab 3	*2.84 ± 0.14*			*0.87 ± 0.07*
X_{ref}	**2.823**	**0.879**	**5.655**	**0.824**
u_{char}	0.119	0.013	0.119	0.020
U_{bb}	0.079	0.011	0.040	0.012
u_{st}	0.062	0.008	0.017	0.008
u_{ref}	0.156	0.019	0.126	0.024
U_{ref}	**0.311**	**0.037**	**0.252**	**0.048**
σ_p	0.282	0.088	0.565	0.082
σ_p (%)	10%	10%	10%	10%

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

5.3 Standard deviation of the proficiency test assessment σ_p

On the basis of previous experience for this type of analysis the standard deviation for proficiency assessment σ_p (also called target standard deviation) was set to 10 % of the respective assigned values (Table 1).

7 Evaluation of results

7.1 Scores and evaluation criteria

Individual laboratory performance was expressed in terms of z- and ζ-scores in accordance with ISO 13528: 2005 [3]:

$$z = \frac{x_{lab} - x_{ref}}{\sigma_p} \qquad \text{Eq. 4 and} \qquad \zeta = \frac{x_{lab} - x_{ref}}{\sqrt{u_{ref}^2 + u_{lab}^2}} \qquad \text{Eq. 5}$$

where:
- x_{lab} is the measurement result reported by a participant;
- u_{lab} is the standard uncertainty reported by a participant;
- X_{ref} is the reference value (assigned value);
- u_{ref} is the standard uncertainty of the reference value; and
- σ_p is the standard deviation for proficiency assessment

The interpretation of the z- and ζ-score is done according ISO 17043:2010 [2]:

|score| ≤ 2	satisfactory result	(green in Annexes 7 to 12)
2 < |score| < 3	questionable result	(orange in Annexes 7 to 12)
|score| ≥ 3	unsatisfactory result	(red in in Annexes 7 to 12)

The z-score compares the participant's deviation from the reference value with the target standard deviation for proficiency assessment (σ_p) used as common quality criterion. σ_p is defined by the PT organizer as the maximum acceptable standard uncertainty.

The ζ-score states whether the laboratory's result agrees with the assigned value within the respective uncertainty. The denominator is the combined uncertainty of the assigned value and the measurement uncertainty as stated by the laboratory. The ζ-score is therefore the most relevant evaluation parameter, as it includes all parts of a measurement result, namely the expected value (assigned value), its uncertainty and the unit of the result as well as the uncertainty of the reported values. An unsatisfactory ζ-score can either be caused by an inappropriate estimation of the concentration or of its uncertainty, or both.

The standard uncertainty of the laboratory (u_{lab}) was estimated by 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 [9].

Uncertainty estimation is not trivial, therefore an additional assessment was provided to each laboratory reporting uncertainty, indicating how reasonable their uncertainty estimate is. The standard uncertainty from the laboratory (u_{lab}) is most likely to fall in a range between a minimum uncertainty (u_{min}), and a maximum allowed (u_{max}). u_{min} is set to the standard uncertainty of the reference value. It is unlikely that a laboratory carrying out the analysis on a routine basis would measure the measurand with a smaller uncertainty than the expert laboratories chosen to establish the assigned value. u_{max} is set to the target standard deviation (σ_p) accepted for the PT. If u_{lab} is smaller than u_{min}, (case "b") the laboratory may have underestimated its uncertainty. Such a statement has to be taken with care as each laboratory reported only measurement uncertainty, whereas the uncertainty of the reference value also includes

contributions of homogeneity and stability. If those are large, measurement uncertainties smaller than u_{min} are possible and plausible. If $u_{lab} > u_{max}$, (case "c") the laboratory may have overestimated the uncertainty. An evaluation of this statement can be made when looking at the difference of the reported value and the assigned value: if the difference is small and the uncertainty is large, then overestimation is likely. If, however, the deviation is large but is covered by the uncertainty, then the uncertainty is properly assessed but large. It should be pointed out that u_{max} is only a normative criterion if set down by legislation.

7.2 Laboratory results and scorings

From the 47 laboratories having registered, 44 laboratories (24 countries) submitted results and answered the associated questionnaire (36 for total As, 42 for total Cd, 42 for total Pb and 37 for total Hg).

Annexes 9 to 12 present the reported results as a table and as a graph. Furthermore, the graphs include the corresponding Kernel density plots, obtained using the software available from the Statistical Subcommittee of the Analytical Methods Committee of the UK Royal Society of Chemistry [10].

Those laboratories reporting "less than" and "0" values were not included in the evaluation. However, reported "less than" values were compared with the corresponding $X_{ref} - U_{ref}$ values. If the reported limit value is lower than the corresponding $X_{ref} - U_{ref}$, this statement is considered incorrect, since the laboratory should have detected the respective element. In this exercise laboratory L20 reported incorrectly "less than" 0.1 mg kg^{-1} for total Cd ($X_{ref} - U_{ref}$ = 0.84 mg kg^{-1}) and "less than" 0.5 mg kg^{-1} for total Pb ($X_{ref} - U_{ref}$ = 5.40 mg kg^{-1}) and L39 reported "less than" 0.0005 mg kg^{-1} for total Hg ($X_{ref} - U_{ref}$ = 0.78 mg kg^{-1}).

The overall performance of the participants regarding the z- and ζ-scores, is summarised in Figure 1: 58% to 74% of the participants performed satisfactorily in this exercise for the determination of the target measurands.

In all cases, except for total As for which the number of satisfactory z- and ζ-scores remained the same (54% of the participants), the number of satisfactory ζ-scores are less than that of z-scores. For total Pb the percentage of unsatisfactory ζ-scores is double that of z-scores.

Seventeen out of the 44 reporting participants performed satisfactorily for all the measurands. Six laboratories, acquired unsatisfactory z-scores for all of the measurands for which they reported results. The latter observation could be attributed to the use of inappropriate or inadequate pre-treatment procedures. This is supported by the fact that in most cases these laboratories underestimated or overestimated all of their reported results, indicating a possible carry-over bias from the pre-treatment.

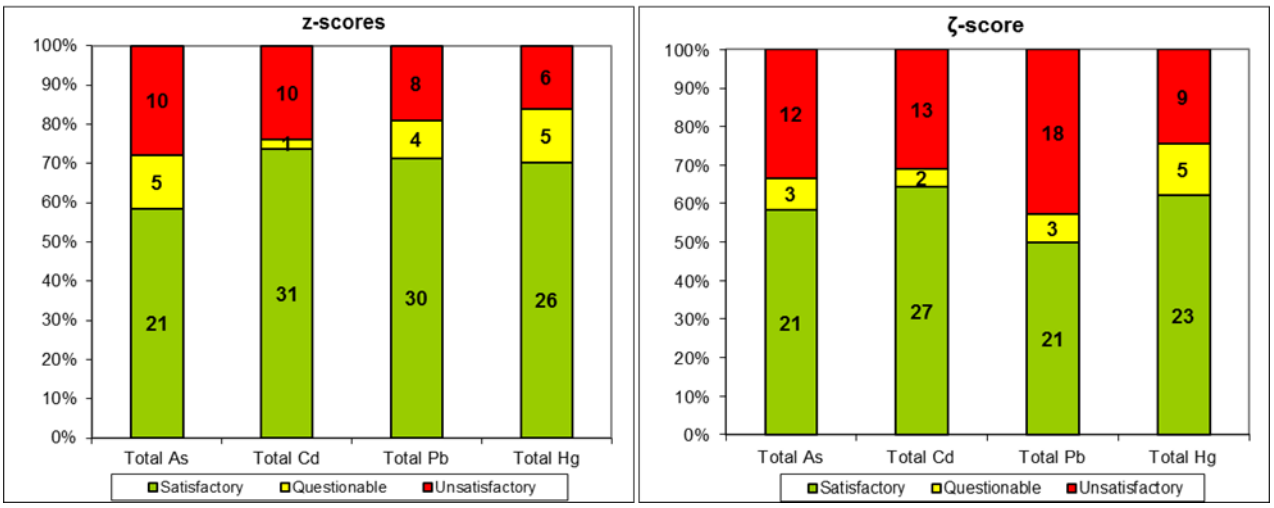


Figure 1: *Number and percentages of laboratories with satisfactory, questionable and unsatisfactory scores. (The numbers on the bars correspond to the exact number of laboratories in a certain scoring category).*

The uncertainty assessment ("a", "b" and "c") is presented in Annexes 9 to 12. In the case of total As, only 2 of the 21 laboratories having performed satisfactorily obtained an "a". The uncertainty assessment was fairly improved in the cases of total Cd, Pd and Hg where 50, 40 and 30 % of the participants reported reasonable uncertainty estimates (case "a"). Underestimated uncertainties (case "b") were reported by 20 laboratories for total As, 13 for total Cd, 18 for total Pb and 12 for total Hg, probably because repeatability results were used as standard uncertainties.

Several approaches were used to evaluate measurement uncertainties (Table 2). Most of the laboratories do not usually report uncertainty to their customers while 14 do although, there was no clear indication that the latter group performed better in terms of uncertainty estimation. Several laboratories estimated satisfactorily the standard uncertainties associated to their results even though they do not report uncertainties to their customers. This information could be correlated to the difficulties faced by the participants to rationally estimate their uncertainty budgets.

Table 2 - *Approaches used by the participants in IMEP-38 to estimate the uncertainty of their measurements.*

Approach followed for uncertainty calculation	Number of labs.
Uncertainty budget (ISO-GUM), validation	11
Uncertainty of the method (in-house)	18
Measurement of replicates (precision)	15
Use of intercomparison data	2
Other: • FAVV procedure: using CRM to estimate bias and precision (1 lab) • by NORDTEST TR 537: ver. 3, 2008 (2 labs)	3

7.3 Discussion on the reported results and on the additional information extracted from the questionnaire

The associated questionnaire was answered by 38 of the participants. The experimental details provided by the laboratories for the methods used, are summarised in Annex 13. Sixteen participants used an official method to perform the analysis. No direct correlation could be found between the methods used and the quality of the reported results for total Cd, Pb and Hg.

However, some interesting observations were made on the results reported for total As: 9 laboratories out of the 36 that reported values for total As obtained unsatisfactory or questionable z-scores due to underestimation. Frequently, underestimations in total As in organic test items are attributed to incomplete mineralisation of some organic species of As, such as arsenobetaine. In those cases, laboratories using ICP-based methods have less problems to perform satisfactorily for total As than those using AAS-based determinations. The high temperatures reached in the plasma make it unnecessary to use high temperatures (> 280 °C) during the mineralisation. This was indeed the case in IMEP-38: 14 out of 20 laboratories that used ICP-based techniques, performed satisfactorily while the majority of the participants that used AAS-based techniques (9 out of 14) obtained unsatisfactory z-scores. However the organisers of IMEP-38 knew that incomplete mineralisation of organic species of arsenic could not be the cause of underestimation in this PT because the test item was not naturally incurred but spiked, and that it had been spiked using only inorganic arsenic. Scrutinising carefully the analytical methods used by the laboratories (Annex 13), it was observed that 6 laboratories (L11, L30, L31, L36, L45 and L46) out of the 9 that received an unsatisfactory or questionable z-score due to underestimation, have used open wet digestion or dry ashing, Figure 2. Inorganic arsenic is volatile and precautions need to be taken to avoid loses due to volatilisation. L04 must have had a general problem during the digestion step because it obtained unsatisfactory and questionable results by underestimation for all measurands despite having used closed microwave digestion.

The majority of the laboratories used ICP-based methods of determination for all the measurands but for total Hg for which the techniques of choice were cold vapour-atomic absorption spectrometry (CV-AAS), gold amalgamation-ultraviolet detection (GAUV) and Direct Mercury Analyser.

Participants were asked to report the LoDs of the methods that they have used for the determination of the four measurands. These LoDs together with the respective techniques used are presented in Annex 14. Large discrepancies were observed even among laboratories that used the same technique.

All participants but five corrected their results for the moisture content, determined using the protocol described in the accompanying letter (Annex 5). The moisture content values reported ranged from 0.4 to 2.1 %. However, no major effect on the performance of these 5 laboratories (L03, L10, L15, L39 and L47) would have taken place should they have corrected their results for moisture.

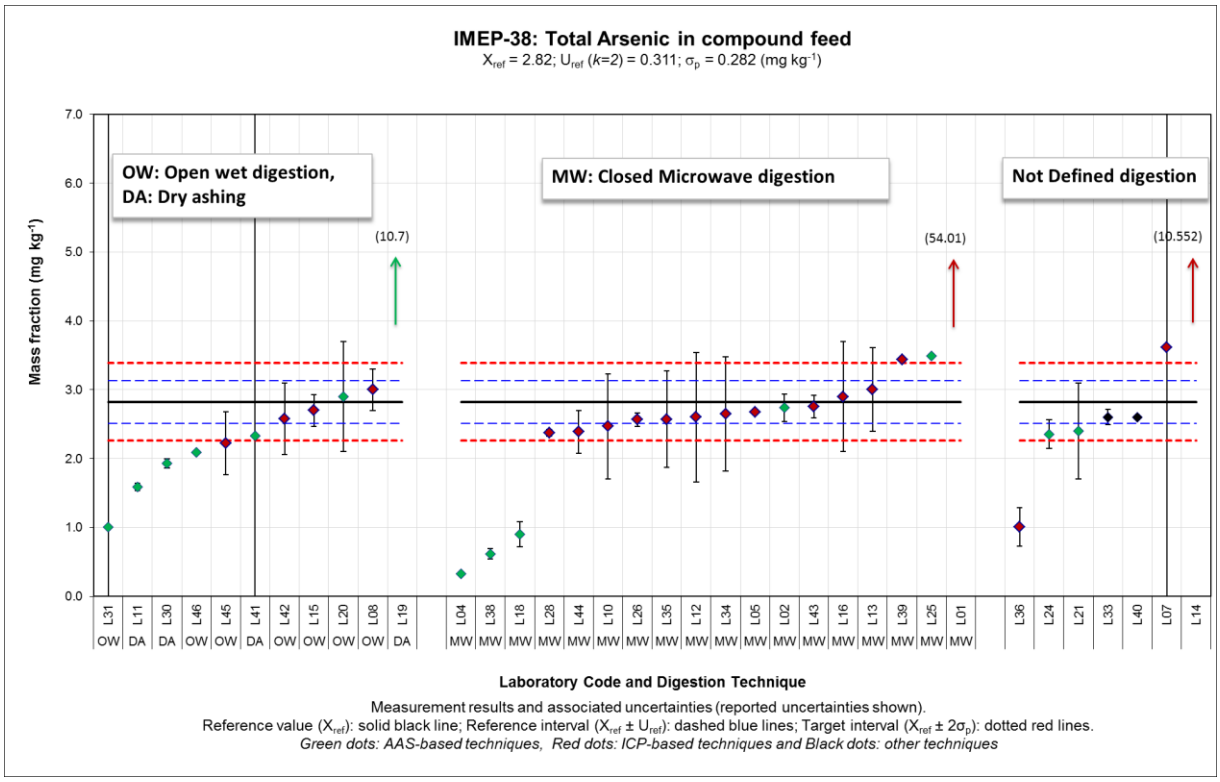


***Figure 2**: Performance overview of the laboratories for the analysis of total As on the basis of the mineralization approach used.*

Thirty laboratories corrected their results for recovery. Twenty-six laboratories applied recoveries in the range 71.05-126.5 %. The remaining four did not provide information about the recovery correction applied. Laboratories that reported recoveries lower than 80 % and higher than 120 % must be aware that such recoveries indicate that the analytical method used is significantly biased and that corrective actions should be undertaken. The 34 participants that reported to have calculated a recovery factor applied one or several of the options shown in Table 3.

All laboratories which answered to the questionnaire have a quality system in place based on ISO 17025. In four cases the quality system is also based on ISO 9000. The majority of the laboratories regularly take part in PTs (29 out 38).

Table 3 - *Methods applied by the laboratories to determine the recovery factors of the exercise.*

How did you determine the recovery factor?		**Number of labs.**
adding a known amount of the same analyte to be measured (spiking)		15
using a certified reference material		12
Other : (labs)	- "Using In-house sample" – (2) - "Use of Interlaboratory Comparison samples" – (2) - "No recovery factor was used" – (4)	10

8 Conclusions

According to the results collected in the frame of IMEP-38 the participating laboratories performed satisfactorily by 58 % for total As, 74 % for total Cd, 71 % for total Pb and 70 % for total Hg. It can be concluded that there is room for improvement taking into account the relatively high concentration levels of the measurands in the spiked test item. This is particularly evident when the outcome of IMEP-38 is compared with that of IMEP-117. The overall rates of satisfactory performance, obtained by the NRLs (expressed as z-scores) were 10 % (for total Pb) to 32 % (for total As) higher than the respective rates in IMEP-38.

Once again the need for an extra effort was identified in the evaluation of uncertainties associated to the results, since the number of questionable and unsatisfactory ζ-scores is systematically higher than those of z-scores for all analytes (excluding total As). Measurement uncertainty is of paramount importance in cases of litigation and so its sound calculation is fundamental for control laboratories.

Significant discrepancies were observed for the limits of detections reported, even for similar analytical methods. There is a clear confusion between the LoD of the method and that of the technique used and on the definition of LoD.

IMEP-38 has shown that PT providers must be extremely careful when trying to evaluate the cause of systematic bias/trends since some parameters such as technique used could hide some underlying sources of error.

9 Acknowledgements

C. Contreras and P. Connely from the Standards for Innovation and Sustainable Development (SID) Unit of the IRMM are acknowledged for their support in the isochronous study and in optimizing the method to measure the moisture content, respectively. F. Ulberth is also acknowledged for revising the manuscript.

The laboratories participating in this exercise, listed in table 4, are kindly acknowledged.

Table - 4: *Laboratories that participated in IMEP-38 and their respective countries of origin.*

Organization	Country
Seibersdorf Labor GmbH	AUSTRIA
FAVV	BELGIUM
Laboratorium ECCA NV	BELGIUM
SGS Belgium	BELGIUM
SGS do Brasil LTDA	BRAZIL
Maxxam Analytics	CANADA
Nutreco Canada	CANADA
SGS Canada Inc.	CANADA
Universidad de Costa Rica	COSTA RICA
ZAVOD ZA JAVNO ZDRAVSTVO ZADAR	CROATIA

Organization	Country
PANKEMI LAB	CYPRUS
GEMANALYSIS	CYPRUS
VitaTrace Nutrition Ltd.	CYPRUS
MVDr. Pavel Mikulas	CZECH REPUBLIC
University Sts Cyril and Methodius, Faculty for veterinary medicine	FYR OF MACEDONIA
PHI Center for Public Health	FYR OF MACEDONIA
Center for public health Bitola	FYR OF MACEDONIA
Intertek Food Services GmbH	GERMANY
AWA-Institut	GERMANY
Blgg Deutschland GmbH	GERMANY
Geo-Chem Laboratories Pvt. Ltd.	INDIA
The Standards Institution of ISRAEL	ISRAEL
Milouda&Migal	ISRAEL
Office National d'Inspection Sanitaire des Produits de la Pêche et de l'Aquaculture	MAURITANIA
Qarshi Research INternational Pvt. Ltd.	PAKISTAN
Diaz Gill Medicina Laboratorial S.A.	PARAGUAY
Instituto Tecnológico de la Producción (ITP)	PERU
World Survey Services Perú SAC	PERU
Okręgowa Stacja Chemiczno-Rolnicza w Warszawie	POLAND
Zakład Higieny Weterynaryjnej	POLAND
Institute of Soil Science and Plant Cultivation - State Research Institute	POLAND
DSVSA-LSVSA Calarasi	ROMANIA
Sanitary Veterinary and Food Safety BRAILA	ROMANIA
Jozef Stefan Institute	SLOVENIA
Eusko Jaurlaritza/Gobierno Vasco	SPAIN
Laboratori Agroalimentari - DAAM (Generalitat de Cataluinya)	SPAIN
TROUW NUTRITION ESPAÑA, S.A.	SPAIN
Laboratorio Agrario Regional de la Junta de Castilla y León	SPAIN
ALS Scandinavia	SWEDEN
Eurofins Environment Testing Sweden AB	SWEDEN
Service de la consommation et des affaires vétérinaires (SCAV)	SWITZERLAND
Livestock Research Institute, Council of Agriculture, Executive Yuan	TAIWAN
Genysis Nutritional Labs	UNITED STATES
Eurofins Frontier Global Sciences	UNITED STATES

10. Abbreviations

AMC	Analytical Methods Committee of the Royal Society of Chemistry
BIPM	Bureau International des Poids et Mesures
CITAC	Co-operation for International Traceability in Analytical Chemistry
CONTAM	Panel on Contaminants in the Food Chain
CV-AAS	Cold Vapour Atomic Absorption Spectrometry
DG SANCO	Directorate General for Health and Consumer Protection
EA	European Co-operation for Accreditation
EFSA	European Food Safety Authority
ETAAS	Electrothermal atomic absorption spectrometry
EU	European Union
EURACHEM	A focus for Analytical Chemistry in Europe
EU-RL-HM	European Union Reference Laboratory for Heavy Metals in Feed and Food
GAUV	
GUM	Guide for the Expression of Uncertainty in Measurement
ID-ICP-MS	Isotope dilution - inductively coupled plasma - mass spectrometry
ILC	Interlaboratory Comparison
IMEP	International Measurement Evaluation Programme
IRMM	Institute for Reference Materials and Measurements
JRC	Joint Research Centre
LoD	Limit of detection
NRL	National Reference Laboratory
PE	Polyethylene
PT	Proficiency Test
RM	Reference material

11 References

1 Commission Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed & Commission Regulation (EU) No 744/2012 of 16 August 2012 amending Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed.

2 ISO 17043:2010 – "Conformity assessment – General requirements for proficiency testing", issued by ISO-Geneva (CH), International Organization for Standardization.

3 ISO 13528:2005 - "Statistical Methods for Use in Proficiency Testing by Interlaboratory Comparisons", issued by ISO-Geneva (CH), International Organization for Standardization.

4 Lamberty A., Schimmel H., Pauwels J. (1998) "The study of the stability of reference materials by isochronous measurements", Fresenius' Journal of Analytical Chemistry 360(3-4): 359-361.

5 Linsinger T. P. J., Pauwels J., Lamberty A., Schimmel H. G., Van Der Veen A. M. H., Siekmann L. (2001) "Estimating the uncertainty of stability for matrix CRMs", Analytical and Bioanalytical Chemistry 370(2-3): 183-188.

6 http://www.eie.gr/iopc/softcrm/index.html, (Accessed at date of publication of this report).

7 ISO Guide 35 Reference Materials – general and statistical principles for certification (2006), issued by ISO-Geneva (CH), ISO-Geneva (CH).

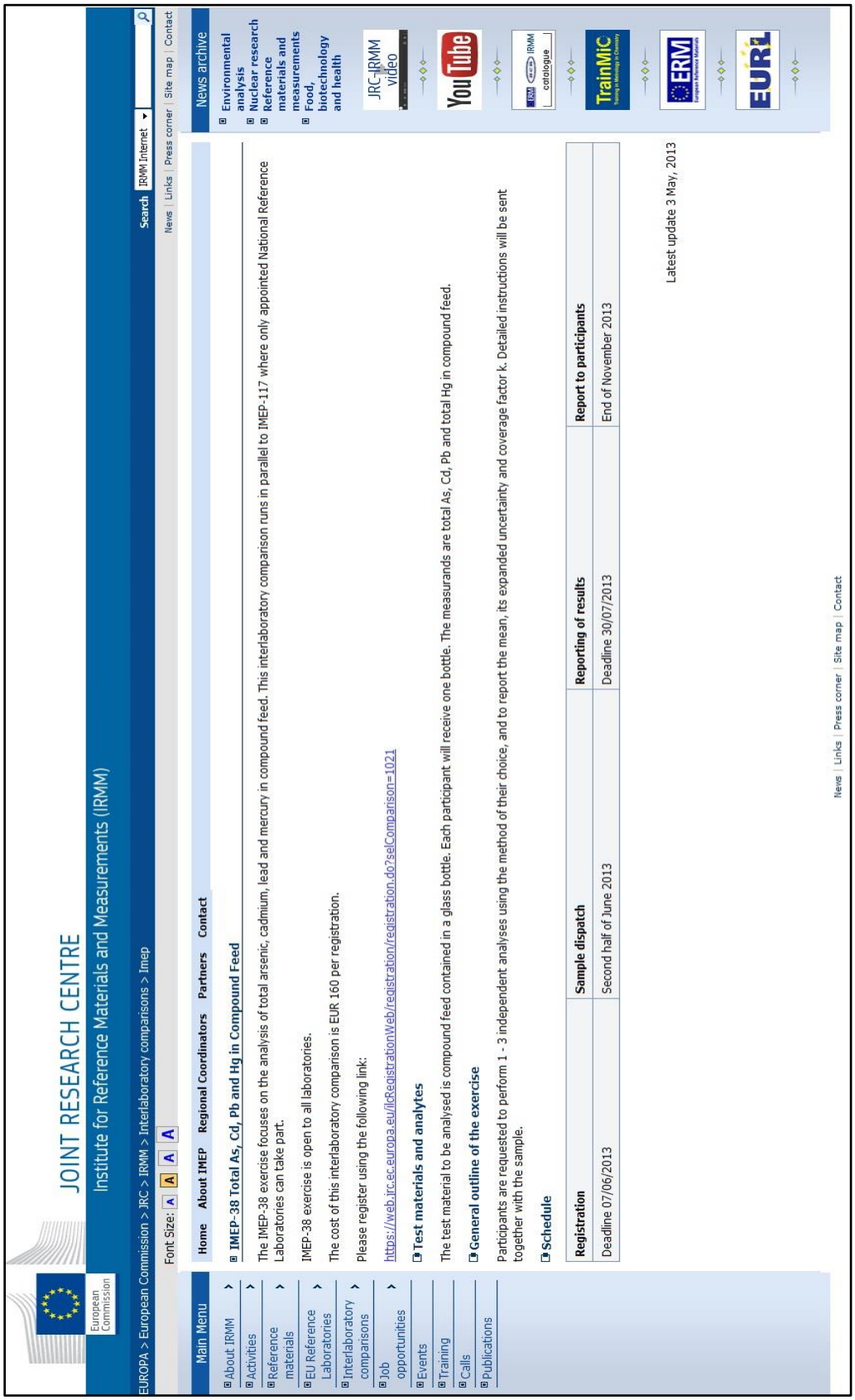
8 ISO/IEC Guide 98:2008, "Uncertainty of measurement - Part 3: Guide to the expression of uncertainty in measurement" (GUM 1995), issued by International Organisation for Standardisation, Geneva.

9 Eurachem/CITAC (2000) "Quantifying Uncertainty in Analytical Measurement", http://www.eurachem.org.

10 AMC/RSC (2006), "Representing data distributions with Kernel density estimates", Issued by the Statistical Subcommittee of the Analytical Methods Committee (AMC) of the Royal Society of Chemistry (RSC), AMC Technical Brief.

Annexes

Annex 1: IRMM – IMEP web announcement



Annex 2: Invitation letter to EA

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Reference Materials and Measurements
International Measurement Evaluation Program

Mrs Alexandra Morazzo
IPAC - Instituto Português de Acreditação
Rua António Gião, 2-5º
2829-513 Caparica
PORTUGAL

IMEP-38: Interlaboratory comparison exercise for the determination of total As, Cd, Pb and Hg in compound feed

Dear Mrs Morazzo,

The Institute for Reference Materials and Measurements (IRMM) organises an interlaboratory comparison named **"IMEP-38: Determination of total As, Cd, Pb and Hg in compound feed"** in support to the *Directive No 2002/32/EC of the European Parliament and the Council on undesirable substances in animal feed*.

In the frame of the EA-IRMM collaboration agreement, IRMM kindly invites EA to nominate laboratories for free participation. They should hold (or be in the process of obtaining) an accreditation for this type of measurement.

I suggest that you forward this invitation to the national EA accreditation bodies for its consideration. There is a limited number of samples at your disposal and the number of nominees should not exceed 2-3 laboratories per country.

Confidentiality of the participants and their results towards third parties is guaranteed. However, IMEP will disclose details of the participants that have been nominated by EA to you. The EA accreditation bodies may wish to inform the nominees of this disclosure.

The registration page for laboratories appointed by EA is open until **07 June 2013**. Distribution of the samples is foreseen for the second half of June 2013. The deadline for submission of results is **30 July 2013**.

In order to register, laboratories must:

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211
Telephone: direct line (32-14) 571 273. Fax: (32-14) 571 865

E-mail: jrc-irmm-imep@ec.europa.eu
Web site: http://irmm.jrc.ec.europa.eu

1. **Enter** their details online:

https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=1021

2. **Print** the completed form when the system asks to do so.

3. **Clearly indicate on the printed form that they have been appointed by the European Cooperation for Accreditation** to take part in this exercise **otherwise the laboratory will be invoiced 160 € for participation** as charged to the non-appointed laboratories.

4. **Send** the printout to both the IMEP-38 and the EA-IMEP-38 coordinators:

IMEP-38 coordinator	**EA-IMEP-38 coordinator**
Dr. Ioannis Fiamegkos	Mrs Alexandra Morazzo
Fax +32 14 571865	Fax ++351 21 2948202
E-mail: jrc-irmm-imep@ec.europa.eu	E-mail: amorazzo@ipac.pt

Please contact me if you have any questions or comments. We are looking forward to our cooperation!

With kind regards

Ioannis Fiamegkos
IMEP-38 Coordinator

Annex 3: Invitation letter to APLAC

Ref. Ares(2013)1003391 - 03/05/2013

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Reference Materials and Measurements
International Measurement Evaluation Program

To: Ms Cynthia Chen
APLAC PT Committee

Interlaboratory comparison exercise for the determination of total Arsenic, cadmium, lead and mercury in compound feed.

Dear Ms Chen,

The Institute for Reference Materials and Measurements (IRMM) organises an interlaboratory comparison named **"IMEP-38: Determination of total As, Cd, Pb and Hg in compound feed".**

IRMM kindly invites APLAC to nominate 10 laboratories for free participation. However, they should hold (or be in the process of obtaining) an accreditation for this type of measurement. I suggest that you forward this invitation to a selection of specialised laboratories in this area.

In addition to the 10 laboratories above mentioned, other laboratories may take part in IMEP-38 paying a registration fee of 160 €.

Confidentiality of the participants and their results towards third parties is guaranteed.

Registration of participants is open until **7 June 2013**. Distribution of the samples is foreseen for the second half of June 2013, and the deadline for submission of results is **30 July 2013**.

In order to register, laboratories must:

1. **Enter** their details online:

https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=1021

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211
Telephone: direct line (32-14) 571 273. Fax: (32-14) 571 865

E-mail: jrc-irmm-imep@ec.europa.eu
Web site: http://irmm.jrc.ec.europa.eu

2. **Print** the completed form when the system asks to do so.

3. **Clearly indicate on the printed form that they have been appointed by APLAC** to take part in this exercise **otherwise the laboratory will be invoiced 160 € for participation** normally applied for non-appointed laboratories.

4. **Send** the printout to both the IMEP-38 and the APLAC coordinators:

IMEP-38 coordinator	**APLAC coordinator**
Ioannis Fiamegkos	Cynthia Chen
Fax +32 14 571 865	
E-mail: jrc-irmm-imep@ec.europa.eu	E-mail: cynthia_chen@taftw.org

Please contact me if you have any questions or comments. We are looking forward to our cooperation!

With kind regards

Dr. Ioannis Fiamegkos
IMEP-38 Coordinator

Annex 4: Invitation letter to IAAC

Ref. Ares(2013)1003400 - 03/05/2013

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Reference Materials and Measurements
International Measurement Evaluation Program

To: Barbara Belzer
IAAC Lab Committee

IMEP-38: Interlaboratory comparison exercise for the determination of total arsenic, cadmium, lead and mercury in compound feed.

Dear Mrs. Belzer,

The Institute for Reference Materials and Measurements (IRMM) organises an interlaboratory comparison named **"IMEP-38: Determination of total As, Cd, Pb and Hg in compound feed".**

IRMM kindly invites IAAC to nominate 10 laboratories for free participation. However, they should hold (or be in the process of obtaining) an accreditation for this type of measurement. I suggest that you forward this invitation to a selection of specialised laboratories in this area.

In addition to the 10 laboratories above mentioned, other laboratories may take part in IMEP-38 paying a registration fee of 160 €.

Confidentiality of the participants and their results towards third parties is guaranteed.

Registration of participants is open until **07 June 2013**. Distribution of the samples is foreseen for the second half of June 2013, and the deadline for submission of results is **30 July 2013**.

In order to register, laboratories must:

1. **Enter** their details online:

https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=1021

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211
Telephone: direct line (32-14) 571 273. Fax: (32-14) 571 865

E-mail: jrc-irmm-imep@ec.europa.eu
Web site: http://irmm.jrc.ec.europa.eu

2. **Print** the completed form when the system asks to do so.

3. **Clearly indicate on the printed form that they have been appointed by IAAC** to take part in this exercise **otherwise the laboratory will be invoiced 160 € for participation** normally applied for non-appointed laboratories.

4. **Send** the printout to both the IMEP-38 and the IAAC coordinators:

IMEP-38 coordinator	**IAAC coordinator**
Ioannis Fiamegkos	Barbara Belzer
Fax +32 14 571 865	
E-mail: jrc-irmm-imep@ec.europa.eu	E-mail: barbara.belzer@nist.gov

Please contact me if you have any questions or comments. We are looking forward to our cooperation!

With kind regards

Dr. Ioannis Fiamegkos
IMEP-38 Coordinator

Annex 5: Sample accompanying letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Reference Materials and Measurements
International Measurement Evaluation Program

Geel, 27 May 2013
JRC.D5/IF/acs/

«Title» «Firstname» «Surname»
«Organisation»
«Department»
«Address»
«Address2»
«Zip» «Town»
«Country»

Participation in IMEP-38, a proficiency test exercise for the determination of total arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) in compound feed.

Dear «Title» «Surname»,

Thank you for participating in the IMEP-38 proficiency test for the determination of total As, Cd, Pb and Hg in compound feed. This exercise takes place in support to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed.

Please keep this letter. You need it to report your results.

This parcel contains:
a) One 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, send the "Confirmation of receipt" form back (fax: +32-14-571865, e-mail: jrc-irmm-imep@ec.europa.eu). You should store the sample in a dark place at ≤4 C until analysis.

The measurands are total As, Cd, Pb and Hg in compound feed.

The procedure used for the analyses should resemble as closely as possible the one that you use in routine analyses.

The results are to be reported with correction for moisture (in dry mass).

To calculate the **moisture content** in the test material, please apply the following procedure:

Retieseweg 111, B-2440 Geel - Belgium. Telephone: +32-(0)14-571 211.
Telephone: direct line +32-(0)14-571 374, Fax: +32-(0)14-571 865.

E-mail: jrc-irmm-imep@ec.europa.eu
Web site: http://irmm.jrc.ec.europa.eu

1. Weigh accurately 1 g of test material in a glass container of 5-7 cm diameter. Preferably with a lid because when the prescribed drying time has passed, the glass container must cool down about 30 minutes in a desiccator before weighing.

2. Place it in a checked and calibrated drying oven for 1 h ± 5 min at 105 ± 1 °C.

3. Place the glass container covered with a lid in a desiccator and wait 30 min before weighing the test material again.

4. Calculate the average mass loss from the dried material in percentage of the initial mass.

Note that this drying method is devised to result in a mass loss that corresponds to the water content in % (m/m) as measured by Karl Fischer titration which is specific for water. Therefore it is not necessary to dry and continue weighing until constant mass. Keeping the material longer than one hour in the oven will result in an excessive mass loss and an erroneous dry-mass correction.

Note : do not use for the heavy metal determinations the aliquots of test material that you have used for the water content determination!

Reporting of results

Perform two or three independent measurements, correct the measurements results for recovery and for the moisture content and report on the reporting website:

- the **mean** of your two or three measurement results (mg kg^{-1}, as dry mass)
- the associated expanded **uncertainty** (mg kg^{-1}),
- the **coverage factor** and
- the **technique** you used.

The results should be reported in the same form (e.g. number of significant figures) as those normally reported to the customer.

The reporting website is https://irmm.jrc.ec.europa.eu/ilc/ilcReporting.do

To access the webpage you need a personal password key, which is: «Part_key». The system will guide you through the reporting procedure. After entering your results, please complete also 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 last definitive confirmation.

Retieseweg 111, B-2440 Geel - Belgium. Telephone: +32-(0)14-571 211.
Telephone: direct line +32-(0)14-571 374, Fax: +32-(0)14-571 865.

E-mail: jrc-irmm-imep@ec.europa.eu
Web site: http://irmm.jrc.ec.europa.eu

The **deadline** for submission of results is 30/07/2013.

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-imep@ec.europa.eu

With kind regards,

Ioannis Fiamegkos (PhD)

IMEP-38 Coordinator

Cc: F. Ulberth (SFB HoU)

Annex 6: Confirmation of receipt form

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Reference Materials and Measurements
International Measurement Evaluation Program

«Title» «Firstname» «Surname»
«Organisation»
«Address»
«Address2»
«Zip» «Town»
«Country»

IMEP-38

Total arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) in compound 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:

Ioannis Fiamegkos

IMEP-38 Coordinator
EC-JRC-IRMM
Retieseweg 111
B-2440 GEEL, Belgium

Fax : +32-14-571865

e-mail : JRC-IRMM-IMEP@ec.europa.eu

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211
Telephone: direct line (32-14) 571 374, Fax: (32-14) 571 865

E-mail: jrc-irmm-imep@ec.europa.eu
Web site: http://irmm.jrc.ec.europa.eu

Annex 7: Questionnaire

Comparison for IMEP-38

Please fill in this questionnaire

Submission Form

1. **How did you determine the recovery factor? By:**

- ☐ a) adding a known amount of the same analyte to be measured (spiking)
- ☐ b) using a certified reference material
- ☐ c) other

1.1. If "Other" please specify

1.2. Please enter the correction factors used and the LODs of your methods

Analytical recovery (in %) and limit of detection (LoD in mg / Kg)

Questions/Response table	Total As	Total Cd	Total Pb	Total Hg
Recovery %				
LODs (mg/Kg)				

2. **Experimental details for the analysis**

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

- ○ a) Yes
- ○ b) No

2.1.1. If "Yes" which one?

2.1.2. The reference material was used for: (multiple answers are possible)

- ☐ the calibration of instruments
- ☐ the validation of the procedure

2.2. Did you use an official method?

- ○ a) Yes
- ○ b) No

2.2.1. If "Yes", Which one(s)? Please refer to the help button (?) for an example

2.3. Which type of digestion did you use for each element?

Questions/Response table	**Closed Microwave Dig.**	**Dry Ashing**	**Open Microwave Dig.**	**Open Wet Dig.**	**Pressure Bomb Dig.**	*Info*
Total As	○	○	○	○	○	
Total Pb	○	○	○	○	○	
Total Cd	○	○	○	○	○	
Total Hg	○	○	○	○	○	

2.4. What kind of digestion mixture did you use for each element? (Multiple selections are possible)

Questions/Response table	**H2O2**	**H2SO4**	**HCl**	**HClO4**	**HF**	**HNO3**	*Info*
Total As	☐	☐	☐	☐	☐	☐	
Total Pb	☐	☐	☐	☐	☐	☐	
Total Cd	☐	☐	☐	☐	☐	☐	
Total Hg	☐	☐	☐	☐	☐	☐	

2.5. Does your laboratory carry out ths type of analysis on a regular basis? (samples per year)

Questions/Response table	**a) Never**	**b) 0-50**	**c) 50-250**	**d) 250-1000**	**e) more than 1000**	*Info*
Total As	○	○	○	○	○	
Total Pb	○	○	○	○	○	
Total Cd	○	○	○	○	○	
Total Hg	○	○	○	○	○	

2.6. Additional remarks/comments on the method(s) of analysis.

3. **Did you correct for the moisture content of the sample?**

- ○ a) Yes
- ○ b) No

3.1. If "Yes", what is the moisture content of the sample (in % of the sample mass)?

3.2. If "no", what was the reason not to do this?

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

- ☐ a) Uncertainty budget (ISO-GUM)
- ☐ b) Known uncertainty of the standard method (ISO 21748)
- ☐ c) Uncertainty of the method (in-house validation)
- ☐ d) Measurement of replicates (precision)
- ☐ e) Estimation based on judgemnt
- ☐ f) Use of intercomparison data
- ☐ g) Other

4.1. If other, please specify

5. **What is the level of confidence (in %) reflected by the coverage (k) assigned to your reported uncertainty**

6. **Do you usually provide an uncertainty statement to your customers for this type of analysis?**

- ○ a) Yes
- ○ b) No

7. **Does your laboratory have a quality system in place?**

- ○ a) Yes
- ○ b) No

7.1. If "Yes", which:

- ☐ a) ISO 17025
- ☐ b) ISO 9000 series
- ☐ c) Other

7.1.1. If other, please specify

8. **Does your laboratory take part in interlaboratory comparison scheme for this type of analysis?**

- ○ a) Yes
- ○ b) No

8.1. If "yes", which one(s)?

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

Annex 8: Homogeneity and stability studies

8.1 Homogeneity studies

	Total As		Total Cd		Total Pb		TotalHg	
Bottle ID	*R1*	*R2*	*R1*	*R2*	*R1*	*R2*	*R1*	*R2*
46	2.71	2.72	0.906	0.890	5.92	5.92	0.819	0.808
9	2.56	2.63	0.871	0.887	5.83	5.82	0.787	0.816
37	2.76	2.74	0.884	0.891	5.76	5.92	0.795	0.818
72	2.73	2.89	0.868	0.894	5.72	5.99	0.773	0.81
16	2.68	2.70	0.872	0.869	5.92	5.72	0.784	0.795
117	2.72	2.77	0.873	0.901	5.80	5.84	0.812	0.827
57	2.84	2.88	0.894	0.928	5.95	6.04	0.82	0.837
70	2.73	2.82	0.917	0.912	5.91	6.04	0.834	0.841
97	2.64	2.64	0.885	0.890	5.91	5.97	0.819	0.794
23	2.64	2.62	0.890	0.871	5.93	5.90	0.813	0.809
Mean	2.72		0.890		5.89		0.811	
σ_p	0.28		0.088		0.57		0.082	
0.3* σ_p	0.08		0.026		0.17		0.025	
Critical value	0.015		0.0015		0.067		0.0013	
s_x	0.08		0.014		0.07		0.015	
s_w	0.05		0.013		0.09		0.014	
s_s	0.08		0.010		0.01		0.011	
$s_s \leq 0.3 * s_p$	Pass		Pass		Pass		Pass	
$s_s^2 <$ critical	Pass		Pass		Pass		Pass	

Where σ_p is the standard deviation for the PT assessment,

s_x is the standard deviation of the sample averages,

s_w is the within-sample standard deviation,

s_s is the between-sample standard deviation,

8.2 Stability studies

	Time in Weeks				u_{st}
	0	**3**	**5**	**8**	
As	2.7	2.67	2.45	2.64	**2.2%**
	2.7	2.5	2.67	2.57	
Cd	0.852	0.862	0.872	0.837	**0.9%**
	0.861	0.842	0.865	0.848	
Pb	5.82	5.81	5.75	5.69	**0.3%**
	5.86	5.8	5.82	5.7	
Hg	0.8	0.805	0.771	0.772	**1.0%**
	0.83	0.784	0.782	0.775	

Annex 9: Results for total As

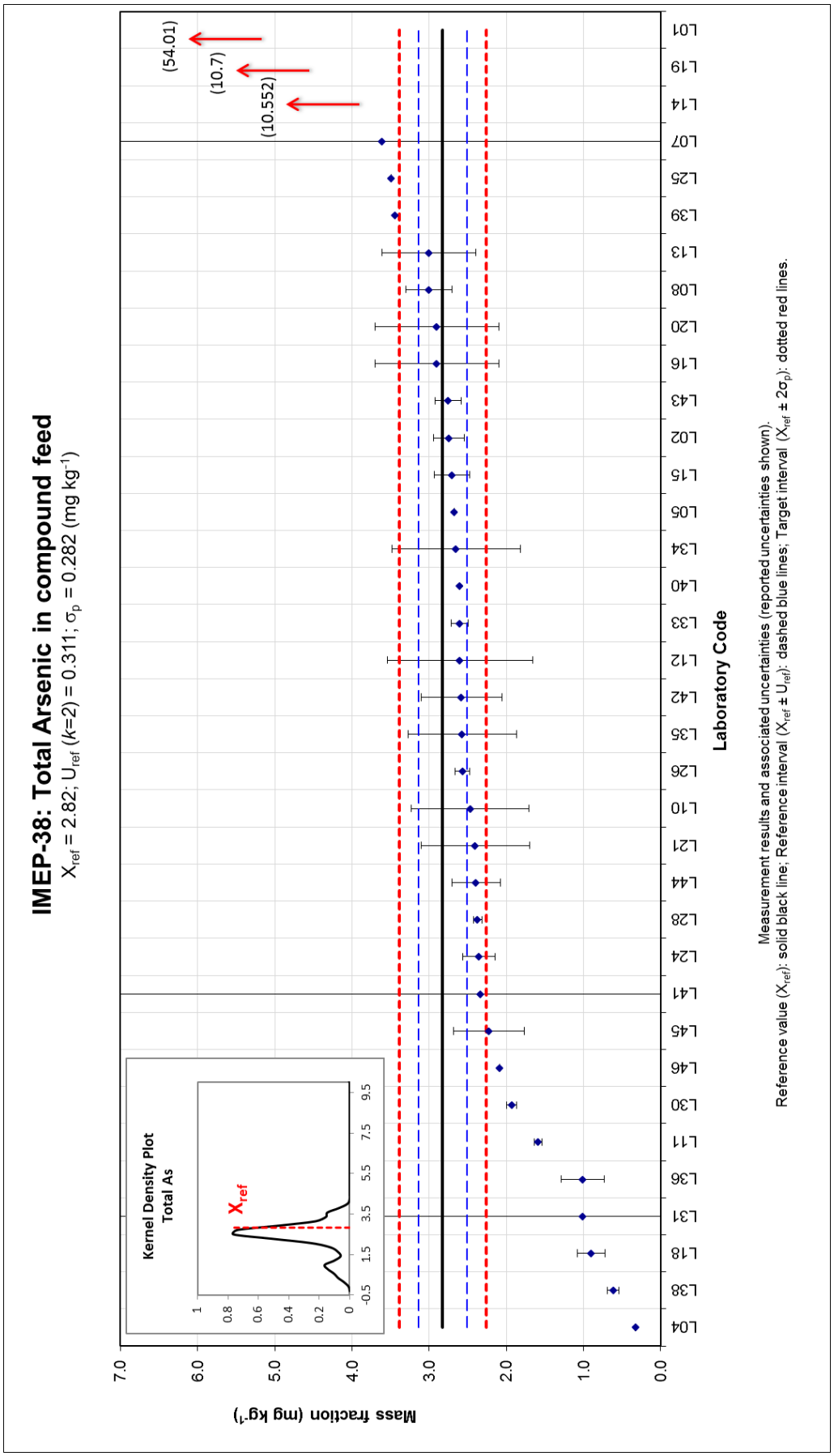
Assigned range: X_{ref} = 2.82; U_{Ref} (*k=2*) = 0.311; σ_p = 0.282 (all values in mg kg^{-1})

Lab Code	X_{lab}	U_{lab}	k[a]	technique	u_{lab}	z-score[b]	ζ-score[b]	uncert.[c]
L01	54.01	3.50	2	ICP-MS	1.75	181.3	29.1	c
L02	2.74	0.2	2	HG-AAS	0.1	-0.3	-0.5	b
L04	0.326	0.007	2	ET-AAS	0.0035	-8.8	-16.1	b
L05	2.677			ICP-MS	0	-0.5	-0.9	b
L07	3.611	15	2	ICP-MS	7.5	2.8	0.1	c
L08	3.0	0.3	2	ICP-MS	0.15	0.6	0.8	b
L10	2.468	0.7650	2	ICP-OES	0.3825	-1.3	-0.9	c
L11	1.588	0.05	2	HG-AAS	0.025	-4.4	-7.8	b
L12	2.60	0.94	2	ICP-MS	0.47	-0.8	-0.5	c
L13	3.003	0.604	2	ICP-MS	0.302	0.6	0.5	c
L14	10.552			ICP-AES	0	27.4	49.7	b
L15	2.7	0.23	2	ICP-MS	0.115	-0.4	-0.6	b
L16	2.9	0.8	2	ICP-AES	0.4	0.3	0.2	c
L18	0.9	0.18	2	ET-AAS	0.09	-6.8	-10.7	b
L19	10.7	2.4	2	HG-AAS	1.2	27.9	6.5	c
L20	2.9	0.8	2	FAAS	0.4	0.3	0.2	c
L21	2.4	0.7	2	HG-AAS	0.35	-1.5	-1.1	c
L24	2.352	0.209	2	FAAS-MHS	0.1045	-1.7	-2.5	b
L25	3.49			ET-AAS	0	2.4	4.3	b
L26	2.565	0.095	2	ICP-QMS	0.0475	-0.9	-1.6	b
L28	2.37	0.05	√3	ICP-AES	0.028868	-1.6	-2.9	b
L30	1.93	0.066	0.9221	HG-AAS	0.071576	-3.2	-5.2	b
L31	1.005	10.11	√3	AAS	5.837011	-6.4	-0.3	c
L33	2.60	0.11	1	NAA	0.11	-0.8	-1.2	b
L34	2.65	0.83	2	ICP-MS	0.415	-0.6	-0.4	c
L35	2.57	0.70	2	ICP-MS	0.35	-0.9	-0.7	c
L36	1.01	0.28	1.96	ICP-AES	0.142857	-6.4	-8.6	b
L38	0.614	0.074	2	ET-AAS	0.037	-7.8	-13.8	b
L39	3.44			ICP-MS	0	2.2	4.0	b
L40	2.6				0	-0.8	-1.4	b
L41	2.33	12.1	2	HG-AAS	6.05	-1.7	-0.1	c
L42	2.58	0.52	2	ICP-AES	0.26	-0.9	-0.8	a
L43	2.754	0.166	2	ICP-MS	0.083	-0.2	-0.4	b
L44	2.39	0.31	√3	ICP-AES	0.178979	-1.5	-1.8	a
L45	2.223	0.459	2	ICP-QMS	0.2295	-2.1	-2.2	a
L46	2.09			HG-AAS	0	-2.6	-4.7	b

[a] √3 is set by the ILC coordinator when no expansion factor *k* is reported. The reported uncertainty was assumed to have a rectangular distribution with *k*=√3.

[b] **Satisfactory, Questionable, Unsatisfactory**

[c] **a** : $u_{min} \leq u_{lab} \leq u_{max}$; **b** : $u_{lab} < u_{min}$; and **c** : $u_{lab} > u_{max}$


IMEP-38: Total Arsenic in compound feed
X_{ref} = 2.82; U_{ref} (k=2) = 0.311; σ_p = 0.282 (mg kg^{-1})
(54.01)
(10.7)
(10.552)
Kernel Density Plot
Total As
X_{ref}
Mass fraction (mg kg^{-1})
Laboratory Code
Measurement results and associated uncertainties (reported uncertainties shown).
Reference value (X_{ref}): solid black line; Reference interval ($X_{ref} \pm U_{ref}$): dashed blue lines; Target interval ($X_{ref} \pm 2\sigma_p$): dotted red lines.

Annex 10: Results for total Cd

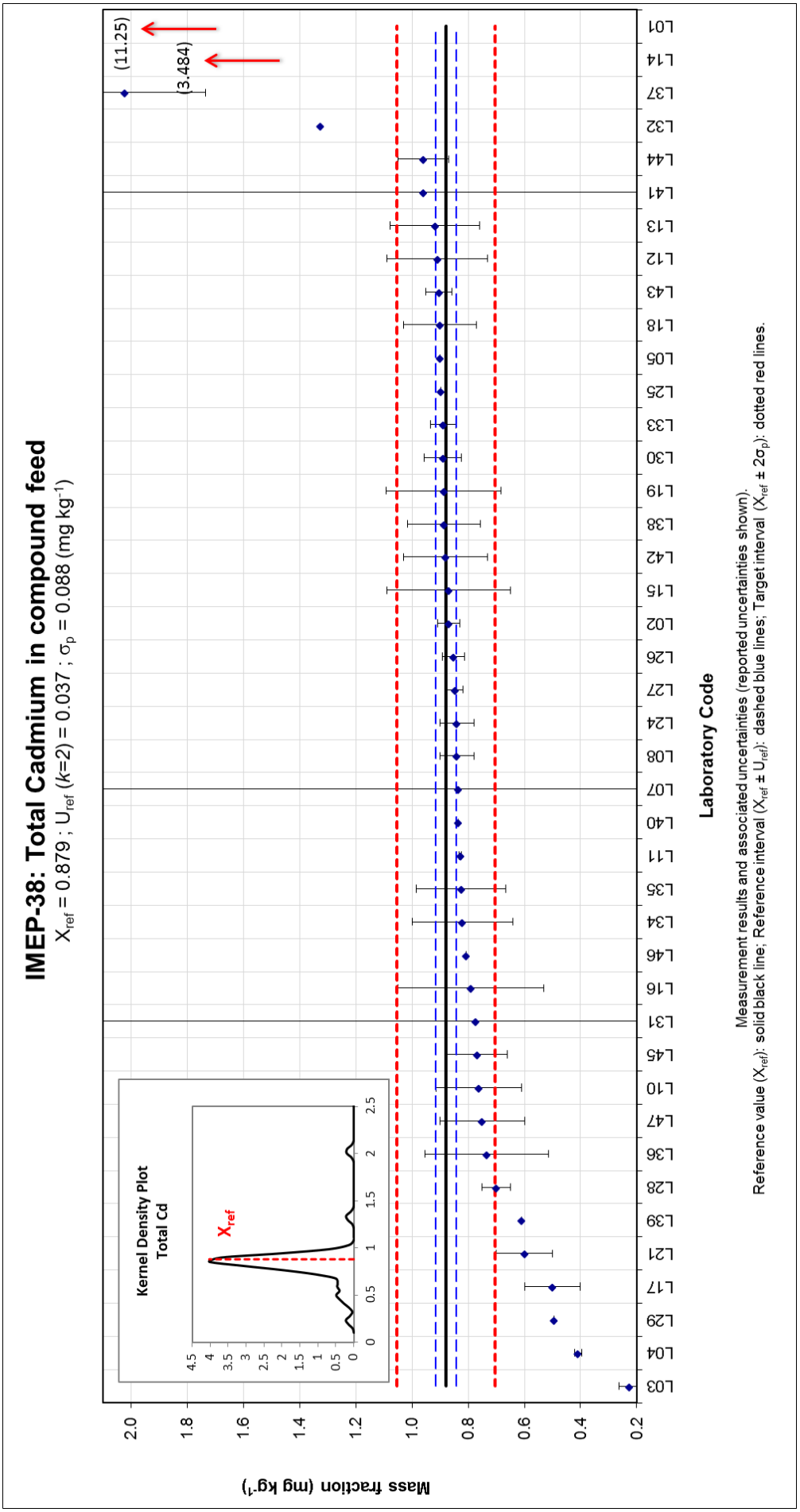
Assigned range: X_{ref} = 0.879 ; U_{ref} (*k=2*) = 0.037 ; s_p = 0.088 (all values in mg kg^{-1})

Lab Code	X_{lab}	U_{lab}	k[a]	technique	u_{lab}	z-score[b]	ζ-score[b]	uncert.[c]
L01	11.25	0.95	2	ICP-MS	0.475	118.0	21.8	c
L02	0.87	0.04	2	FAAS	0.020	-0.1	-0.3	a
L03	0.227	0.035	2	ET-AAS	0.018	-7.4	-25.6	b
L04	0.409	0.013	2	ET-AAS	0.007	-5.3	-24.0	b
L05	0.899			ICP-MS	0	0.2	1.1	b
L07	0.8362	20	2	ICP-MS	10.000	-0.5	0.0	c
L08	0.84	0.06	2	ICP-MS	0.030	-0.4	-1.1	a
L10	0.7609	0.1521	2	ICP-AES	0.076	-1.3	-1.5	a
L11	0.828	0.005	2	FAAS	0.003	-0.6	-2.7	b
L12	0.91	0.18	2	ICP-AES	0.090	0.4	0.3	c
L13	0.918	0.160	2	ICP-MS	0.080	0.4	0.5	a
L14	3.484			ICP-AES	0	29.6	140.7	b
L15	0.87	0.22	2	ICP-MS	0.110	-0.1	-0.1	c
L16	0.79	0.26	2	ICP-AES	0.130	-1.01	-0.68	c
L17	0.5	0.1	2	ETAAS	0.050	-4.3	-7.1	a
L18	0.9	0.13	2	ET-AAS	0.065	0.2	0.3	a
L19	0.887	0.204	2	ET-AAS	0.102	0.1	0.1	c
L20	<0.1			FAAS				
L21	0.6	0.1	2	ET-AAS	0.050	-3.2	-5.2	a
L24	0.84	0.06	2	FAAS	0.030	-0.4	-1.1	a
L25	0.897			ET-AAS	0	0.2	1.0	b
L26	0.852	0.04	2	ET-AAS	0.020	-0.3	-1.0	a
L27	0.848	0.03	2	ICP	0.015	-0.4	-1.3	b
L28	0.7	0.05	√3	ICP-AES	0.029	-2.0	-5.2	a
L29	0.493			FAAS	0	-4.4	-20.8	b
L30	0.89	0.066	0.8674	ICP-AES	0.076	0.1	0.1	a
L31	0.774	28.09	√3	AAS	16.218	-1.2	0.0	c
L32	1.326				0	5.1	24.1	b
L33	0.890	0.045	1	ICP-MS	0.045	0.13	0.2	a
L34	0.82	0.18	2	ICP-MS	0.090	-0.7	-0.6	c
L35	0.825	0.16	2	ICP-MS	0.080	-0.6	-0.7	a
L36	0.735	0.22	1.96	ICP-AES	0.112	-1.6	-1.3	c
L37	2.023	0.288	√3	ET-AAS	0.166	13.0	6.8	c
L38	0.886	0.129	2	ET-AAS	0.065	0.1	0.1	a
L39	0.61			ICP-MS	0	-3.1	-14.5	b
L40	0.8354				0	-0.5	-2.4	b
L41	0.96	7.7	2	FAAS	3.850	0.9	0.0	c
L42	0.88	0.15	2	ICP-AES	0.075	0.0	0.0	a
L43	0.904	0.047	2	ICP-MS	0.024	0.3	0.8	a
L44	0.96	0.09	√3	ICP-AES	0.052	0.9	1.5	a
L45	0.769	0.109	2	ICP-QMS	0.055	-1.3	-1.9	a
L46	0.807			ET-AAS	0	-0.8	-3.9	b
L47	0.75	0.15	94	FAAS	0.002	-1.5	-6.9	b

[a] √3 is set by the ILC coordinator when no expansion factor *k* is reported. The reported uncertainty was assumed to have a rectangular distribution with *k*=√3.

[b] **Satisfactory, Questionable, Unsatisfactory**

[c] **a** : $u_{min} \leq u_{lab} \leq u_{max}$; **b** : $u_{lab} < u_{min}$; and **c** : $u_{lab} > u_{max}$


IMEP-38: Total Cadmium in compound feed
X_{ref} = 0.879 ; U_{ref} (k=2) = 0.037 ; σ_p = 0.088 (mg kg^{-1})
(11.25)
(3.484)
Kernel Density Plot
Total Cd
X_{ref}
Mass fraction (mg kg^{-1})
Laboratory Code
Measurement results and associated uncertainties (reported uncertainties shown).
Reference value (X_{ref}): solid black line; Reference interval ($X_{ref} \pm U_{ref}$): dashed blue lines; Target interval ($X_{ref} \pm 2\sigma_p$): dotted red lines.

Annex 11: Results for total Pb

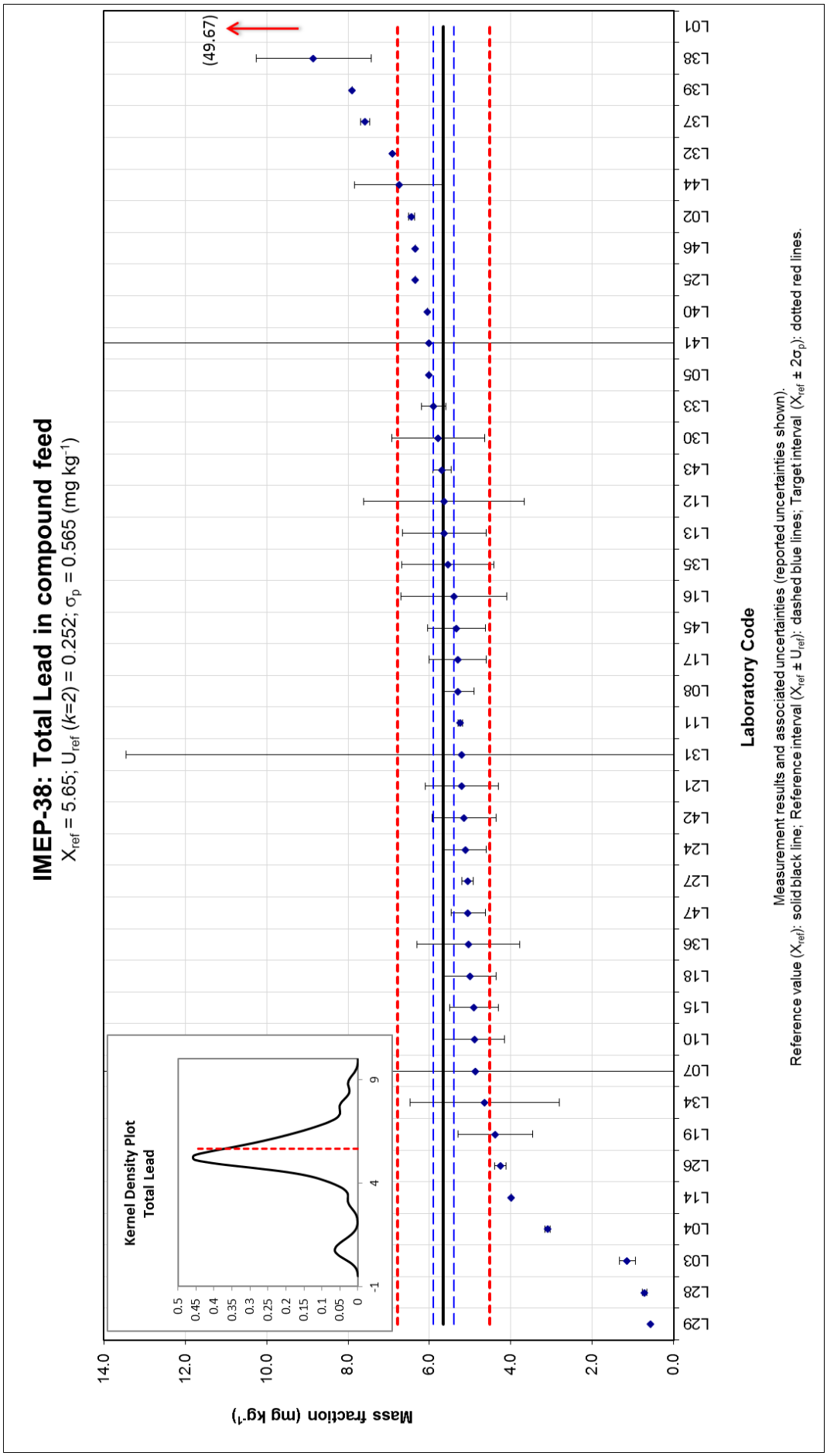
Assigned range: X_{ref} = 5.65; U_{ref} (k=2) = 0.252; σ_p = 0.565 (all values in mg kg^{-1})

Lab Code	X_{lab}	U_{lab}	k[a]	technique	u_{lab}	z-score[b]	ζ-score[b]	uncert.[c]
L01	49.67	4.32	2	ICP-MS	2.160	77.84	20.34	c
L02	6.44	0.08	2	FAAS	0.040	1.39	5.93	b
L03	1.141	0.2	2	ET-AAS	0.100	-7.98	-28.03	b
L04	3.102	0.063	2	ET-AAS	0.032	-4.51	-19.62	b
L05	6.0			ICP-MS	0	0.61	2.74	b
L07	4.86	20	2	ICP-MS	10.000	-1.41	-0.08	c
L08	5.3	0.4	2	ICP-MS	0.200	-0.63	-1.50	a
L10	4.896	0.7344	2	ICP-AES	0.367	-1.34	-1.95	a
L11	5.242	0.05	2	ET-AAS	0.025	-0.73	-3.21	b
L12	5.64	1.97	2	ICP-AES	0.985	-0.03	-0.01	c
L13	5.637	1.026	2	ICP-MS	0.513	-0.03	-0.03	a
L14	3.982			ICP-AES	0	-2.96	-13.25	b
L15	4.9	0.6	2	ICP-MS	0.300	-1.33	-2.32	a
L16	5.4	1.3	2	ICP-AES	0.650	-0.45	-0.38	c
L17	5.3	0.7	2	ETAAS	0.350	-0.63	-0.95	a
L18	5.0	0.64	2	ET-AAS	0.320	-1.16	-1.90	a
L19	4.38	0.92	2	ET-AAS	0.460	-2.25	-2.67	a
L20	<0.5			FAAS				
L21	5.2	0.9	2	ET-AAS	0.450	-0.80	-0.97	a
L24	5.12	0.51	2	FAAS	0.255	-0.95	-1.88	a
L25	6.34			ET-AAS	0	1.21	5.43	b
L26	4.255	0.143	2	ET-AAS	0.072	-2.48	-9.65	b
L27	5.06	0.14	2	ICP	0.070	-1.05	-4.12	b
L28	0.72	0.05	√3	ICP-AES	0.029	-8.73	-38.11	b
L29	0.56			FAAS	0	-9.01	-40.37	b
L30	5.78	1.141	0.8139	ICP-AES	1.402	0.22	0.09	c
L31	5.212	8.23	√3	AAS	4.752	-0.78	-0.09	c
L32	6.908				0	2.22	9.93	b
L33	5.89	0.30	1	ICP-MS	0.300	0.42	0.72	a
L34	4.64	1.83	2	ICP-MS	0.915	-1.79	-1.10	c
L35	5.55	1.13	2	ICP-MS	0.565	-0.18	-0.18	a
L36	5.04	1.26	1.96	ICP-AES	0.643	-1.09	-0.94	c
L37	7.58	0.113	√3	GF-AAS	0.065	3.41	13.55	b
L38	8.844	1.414	2	GF-AAS	0.707	5.64	4.44	c
L39	7.9			ICP-MS	0	3.97	17.79	b
L40	6.043				0	0.69	3.08	b
L41	6.00	8.2	2	FAAS	4.100	0.61	0.08	c
L42	5.15	0.79	2	ICP-AES	0.395	-0.89	-1.22	a
L43	5.693	0.226	2	ICP-MS	0.113	0.07	0.23	b
L44	6.74	1.10	√3	ICP-AES	0.635	1.92	1.68	c
L45	5.335	0.714	2	ICP-QMS	0.357	-0.57	-0.84	a
L46	6.35			ET-AAS	0	1.23	5.51	b
L47	5.05	0.42	97.6	FAAS	0.004	-1.07	-4.79	b

[a] √3 is set by the ILC coordinator when no expansion factor k is reported. The reported uncertainty was assumed to have a rectangular distribution with k=√3.

[b] **Satisfactory, Questionable, Unsatisfactory**

[c] **a** : $u_{min} \leq u_{lab} \leq u_{max}$; **b** : $u_{lab} < u_{min}$; and **c** : $u_{lab} > u_{max}$


IMEP-38: Total Lead in compound feed
X_{ref} = 5.65; U_{ref} (k=2) = 0.252; σ_p = 0.565 (mg kg^{-1})
(49.67)
Kernel Density Plot
Total Lead
Mass fraction (mg kg^{-1})
Laboratory Code
Measurement results and associated uncertainties (reported uncertainties shown).
Reference value (X_{ref}): solid black line; Reference interval ($X_{ref} \pm U_{ref}$): dashed blue lines; Target interval ($X_{ref} \pm 2\sigma_p$): dotted red lines.

Annex 12: Results for total Hg

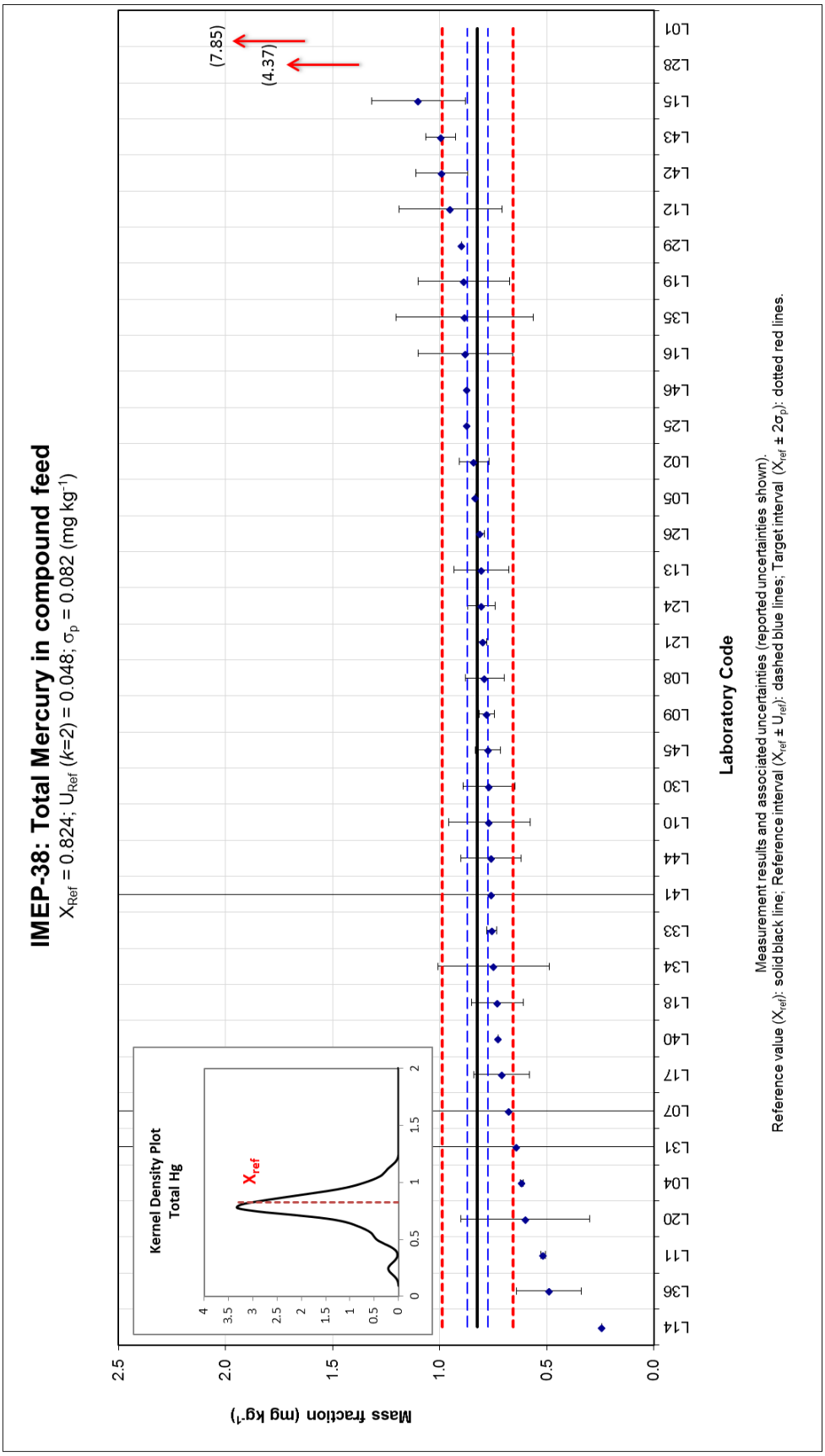
Assigned range: X_{ref} = 0.824; U_{ref} (*k=2*) = 0.048; σ_p = 0.082 (al values in mg kg^{-1})

Lab Code	X_{lab}	U_{lab}	k[a]	technique	u_{lab}	z-score[b]	ζ-score[b]	uncert.[c]
L01	7.85	1.15	2	ICP-MS	0.575	85.3	12.2	c
L02	0.84	0.07	2	CV-AFS	0.035	0.2	0.4	a
L04	0.617	0.008	2	HG-AAS	0.004	-2.5	-8.4	b
L05	0.833			ICP-MS	0	0.1	0.4	b
L07	0.6759	30	2	CV-AFS	15	-1.8	0.0	c
L08	0.79	0.09	2	CV-AAS	0.045	-0.4	-0.7	a
L09	0.78	0.035	2	Combustion-Amalgamation-Cold Vapor-AAS (AMA 254)	0.0175	-0.5	-1.5	b
L10	0.7681	0.1920	2	ICP-AES	0.096	-0.7	-0.6	c
L11	0.518	0.01	2	CV-AAS	0.005	-3.7	-12.4	b
L12	0.95	0.24	2	GAUV	0.12	1.5	1.0	c
L13	0.806	0.129	2	GAUV	0.0645	-0.2	-0.3	a
L14	0.242			ICP-AES	0	-7.1	-24.0	b
L15	1.1	0.219	2	CV-AAS	0.1095	3.3	2.5	c
L16	0.88	0.22	2	GAUV	0.11	0.7	0.5	c
L17	0.71	0.13	2	GAUV	0.065	-1.4	-1.6	a
L18	0.73	0.12	2	CV-AAS	0.06	-1.1	-1.5	a
L19	0.887	0.213	2	automatic analyzer of mercury - AMA 254	0.1065	0.8	0.6	c
L20	0.6	0.3	2	CV-AAS	0.15	-2.7	-1.5	c
L21	0.8	0.02	2	GAUV	0.01	-0.3	-0.9	b
L24	0.804	0.064	2	Advanced Mercury Analyser AMA254	0.032	-0.2	-0.5	a
L25	0.874			CV-AAS	0	0.6	2.1	b
L26	0.812	0.02	2	GAUV	0.01	-0.1	-0.5	b
L28	4.37	0.05	√3	ICP-AES	0.028868	43.0	94.1	a
L29	0.898			CV-AAS	0	0.9	3.1	b
L30	0.77	0.1215	0.9973	FAAS-MHS	0.121829	-0.7	-0.4	c
L31	0.641	9.23	√3	CV AAS	5.328943	-2.2	0.0	c
L33	0.756	0.023	1	CV-AAS	0.023	-0.8	-2.0	b
L34	0.75	0.26	2	ICP-MS	0.13	-0.9	-0.6	c
L35	0.885	0.32	2	ICP-MS	0.16	0.7	0.4	c
L36	0.49	0.15	1.96	ICP-AES	0.076531	-4.1	-4.2	a
L39	<0.0005			ICP-MS	0			
L40	0.7275				0	-1.2	-4.0	b
L41	0.76	17.9	2	CV-AAS	8.95	-0.8	0.0	c
L42	0.99	0.12	2	GAUV	0.06	2.0	2.6	a
L43	0.995	0.069	2	ICP-MS	0.0345	2.1	4.1	a
L44	0.76	0.14	√3	CV-AAS	0.080829	-0.8	-0.8	a
L45	0.775	0.060	2	CV-AAS	0.03	-0.6	-1.3	a
L46	0.874			CV-AAS	0	0.6	2.1	b

[a] √3 is set by the ILC coordinator when no expansion factor *k* is reported. The reported uncertainty was assumed to have a rectangular distribution with *k*=√3.

[b] **Satisfactory, Questionable, Unsatisfactory**

[c] **a** : $u_{min} \leq u_{lab} \leq u_{max}$; **b** : $u_{lab} < u_{min}$; and **c** : $u_{lab} > u_{max}$


IMEP-38: Total Mercury in compound feed
X_{Ref} = 0.824; U_{Ref} (k=2) = 0.048; σ_p = 0.082 (mg kg^{-1})
(7.85)
(4.37)
Kernel Density Plot
Total Hg
X_{ref}
Mass fraction (mg kg^{-1})
Laboratory Code
Measurement results and associated uncertainties (reported uncertainties shown).
Reference value (X_{ref}): solid black line; Reference interval ($X_{ref} \pm U_{ref}$): dashed blue lines; Target interval ($X_{ref} \pm 2\sigma_p$): dotted red lines.

Annex 13: Experimental details and scoring

Lab. Code	Official method	Reference Material	Digestion type	Digestion mix	technique	z-scores
L01	b) No	Inorganic Ventures standard solution / the validation of the procedure	Closed Microwave Dig.	H_2O_2, HNO_3	ICP-MS	Total As
			Closed Microwave Dig.	H_2O_2, HNO_3	ICP-MS	Total Cd
			Closed Microwave Dig.	H_2O_2, HNO_3	ICP-MS	Total Hg
			Closed Microwave Dig.	H_2O_2, HNO_3	ICP-MS	Total Pb
L02	b) No	Standard Reference Material 1566b Oyster Tissue NIST, / the validation of the procedure	Closed Microwave Dig.	HCl, HNO_3	HG-AAS	Total As
			Dry Ashing	HCl	FAAS	Total Cd
			Closed Microwave Dig.	HCl, HNO_3	CV-AFS	Total Hg
			Dry Ashing	HCl	FAAS	Total Pb
L03	SR EN 14082/2003	Certified reference material GBW10011 / the validation of the procedure	Closed Microwave Dig.	HCl, HNO_3	ET-AAS	Total Cd
			Closed Microwave Dig.	HCl, HNO_3	ET-AAS	Total Pb
L04	J. AOAC. Int. 83:1189-1203	LGC Standards/AAFCO check sample / the calibration of instruments and the validation of the procedure	Closed Microwave Dig.	H_2O_2, HNO_3	ET-AAS	Total As
			Closed Microwave Dig.	H_2O_2, HNO_3	ET-AAS	Total Cd
			Closed Microwave Dig.	H_2O_2, HNO_3	HG-AAS	Total Hg
			Closed Microwave Dig.	H_2O_2, HNO_3	ET-AAS	Total Pb
L05	SS-EN 13805	In house / the validation of the procedure	Closed Microwave Dig.	H_2O_2, HF, HNO_3	ICP-MS	Total As
			Closed Microwave Dig.	H_2O_2, HF, HNO_3	ICP-MS	Total Cd
			Closed Microwave Dig.	H_2O_2, HF, HNO_3	ICP-MS	Total Hg
			Closed Microwave Dig.	H_2O_2, HF, HNO_3	ICP-MS	Total Pb
L07					ICP-MS	Total As
					ICP-MS	Total Cd
					CV-AFS	Total Hg
					ICP-MS	Total Pb
L08	EN ISO 17294, EN1483	NIST 1547 / the validation of the procedure	Open Wet Dig.	H_2O_2, HNO_3	ICP-MS	Total As
			Open Wet Dig.	H_2O_2, HNO_3	ICP-MS	Total Cd
				HNO3	CV-AAS	Total Hg
			H_2O_2, HNO_3	H_2O_2, HNO_3	ICP-MS	Total Pb
L09	b) No	BCR 281 and Samples of PT exercises / the validation of the procedure	Dry Ashing		(AMA 254)	Total Hg
L10	EN 15510	Merc, High purity,BAM / the calibration of instruments, the validation of the procedure	Closed Microwave Dig.	H_2O_2, HCl, HNO_3	ICP-AES	Total As
			Closed Microwave Dig.	H_2O_2, HCl, HNO_3	ICP-AES	Total Cd
			Closed Microwave Dig.	H_2O_2, HCl, HNO_3	ICP-AES	Total Hg
			Closed Microwave Dig.	H_2O_2, HCl, HNO_3	ICP-AES	Total Pb
L11	b) No	FisherChemicals / the calibration of instruments	Dry Ashing	HCl, HNO_3	HG-AAS	Total As
			Dry Ashing	HCl, HNO_3	FAAS	Total Cd
			Pressure Bomb Dig.	H_2SO_4, HNO_3	CV-AAS	Total Hg
			Dry Ashing	HCl, HNO_3	ET-AAS	Total Pb
L12	b) No	Bipea ring test samples / the validation of the procedure	Closed Microwave Dig.	H_2O_2, HNO_3	ICP-MS	Total As
			Closed Microwave Dig.	H_2O_2, HNO_3	ICP-AES	Total Cd
			Dry Ashing		GAUV	Total Hg
			Closed Microwave Dig.	H_2O_2, HNO_3	ICP-AES	Total Pb
L13	AOAC 991.14, EPA 7473	Dorm-4, Fish Protein Certified Reference Material for Trace	Closed Microwave Dig.	HNO_3	ICP-MS	Total As
			Closed Microwave Dig.	HNO_3	ICP-MS	Total Cd
					GAUV	Total Hg

Lab. Code	Official method	Reference Material	Digestion type	Digestion mix	technique	z-scores
		Metals / the validation of the procedure	Closed Microwave Dig.	HNO_3	ICP-MS	Total Pb
L14					ICP-AES	Total As
					ICP-AES	Total Cd
					ICP-AES	Total Hg
					ICP-AES	Total Pb
L15	b) No	Tomato leaves NIST 1573a / the validation of the procedure	Open Wet Dig.	H_2O_2, HNO_3	ICP-MS	Total As
			Open Wet Dig.	H_2O_2, HNO_3	ICP-MS	Total Cd
			Open Wet Dig.	H_2O_2, HCl, HNO_3	CV-AAS	Total Hg
			Open Wet Dig.	H_2O_2, HNO_3	ICP-MS	Total Pb
L16	b) No	Feed material from Ministery of Agriculture	Closed Microwave Dig.	H_2O_2, HCl, HNO3	ICP-AES	Total As
			Closed Microwave Dig.	H_2O_2, HCl, HNO_3	ICP-AES	Total Cd
			Dry Ashing		GAUV	Total Hg
			Closed Microwave Dig.	H_2O_2, HCl, HNO_3	ICP-AES	Total Pb
L17					ETAAS	Total Cd
					GAUV	Total Hg
					ETAAS	Total Pb
L18	b) No	FAPAS Soya Flower / the validation of the procedure	Closed Microwave Dig.	H_2O_2, HNO_3	ET-AAS	Total As
			Closed Microwave Dig.	H_2O_2, HNO_3	ET-AAS	Total Cd
			Closed Microwave Dig.	H_2O_2, HNO_3	CV-AAS	Total Hg
			Closed Microwave Dig.	H_2O_2, HNO_3	ET-AAS	Total Pb
L19	b) No	Mixed herbs INCT-MPH-2	Dry Ashing		HG-AAS	Total As
			Dry Ashing		ET-AAS	Total Cd
			Dry Ashing		automatic analyzer of mercury - AMA 254	Total Hg
			Dry Ashing		ET-AAS	Total Pb
L20	b) No	NIST traceable / the calibration of instruments	Open Wet Dig.	HCl, HNO_3	FAAS	Total As
			Open Wet Dig.	HCl, HNO_3	FAAS	Less than
			Open Wet Dig.	HCl, HNO_3	CV-AAS	Total Hg
			Open Wet Dig.	HCl, HNO_3	FAAS	Less than
L21					ET-AAS	Total As
					ET-AAS	Total Cd
					GAUV	Total Hg
					ET-AAS	Total Pb
L24					FAAS-MHS	Total As
					FAAS	Total Cd
					Advanced Mercury Analyser AMA254	Total Hg
					FAAS	Total Pb
L25	b) No	b) No	Closed Microwave Dig.	HNO_3	ET-AAS	Total As
			Closed Microwave Dig.	HNO_3	ET-AAS	Total Cd
			Closed Microwave Dig.	HNO_3	CV-AAS	Total Hg
			Closed Microwave Dig.	HNO_3	ET-AAS	Total Pb
L26	b) No	b) No	Closed Microwave Dig.	H_2O_2, HNO_3	ICP-QMS	Total As
			Closed Microwave Dig.	H_2O_2, HNO_3	ET-AAS	Total Cd
			Dry Ashing		GAUV	Total Hg
			Closed Microwave Dig.	H_2O_2, HNO_3	ET-AAS	Total Pb
L27	b) No	AAFCO / the validation of the procedure	Dry Ashing	HCl, HNO_3	ICP	Total Cd
			Dry Ashing	HCl, HNO_3	ICP	Total Pb
L28	b) No	CRM - LPCS 01-1 / the validation of the procedure	Closed Microwave Dig.	H_2O_2, HCl, HNO_3	ICP-AES	Total As
			Closed Microwave Dig.	H_2O_2, HCl,	ICP-AES	Total Cd

Lab. Code	Official method	Reference Material	Digestion type	Digestion mix	technique	z-scores
				HNO_3		
			Closed Microwave Dig.	H_2O_2, HCl, HNO_3	ICP-AES	Total Hg
			Closed Microwave Dig.	H_2O_2, HCl, HNO_3	ICP-AES	Total Pb
L29	a) Yes	a) Yes	Dry Ashing	HNO_3	FAAS	Total Cd
			Closed Microwave Dig.		CV-AAS	Total Hg
			Dry Ashing	HNO_3	FAAS	Total Pb
L30	a) Yes	b) No	Dry Ashing	H_2O_2, HCl, HNO_3	HG-AAS	Total As
			Dry Ashing	H_2O_2, HCl, HNO_3	ICP-AES	Total Cd
			Open Wet Dig.	H_2SO_4, HCl, HNO_3	FAAS-MHS	Total Hg
			Dry Ashing	H_2O_2, HCl, HNO_3	ICP-AES	Total Pb
L31	a) Yes	b) No	Open Wet Dig.	H_2O_2, HNO_3	AAS	Total As
			Dry Ashing	HCl	AAS	Total Cd
			Open Wet Dig.	H_2O_2, HNO_3	CV AAS	Total Hg
			Dry Ashing	HCl	AAS	Total Pb
L32						Total Cd
						Total Pb
L33	b) No	NIST SRM 1547, NIST SRM 1570a / the validation of the procedure			NAA	Total As
			Closed Microwave Dig.	H_2O_2, HNO_3	ICP-MS	Total Cd
			Open Wet Dig.	H_2SO_4, $HClO_4$, HNO_3	CV-AAS	Total Hg
			Closed Microwave Dig.	H_2O_2, HNO_3	ICP-MS	Total Pb
L34	b) No	NIST1643E, NIST1570A, NIST1568A / the validation of the procedure	Closed Microwave Dig.	HCl, HNO_3	ICP-MS	Total As
			Closed Microwave Dig.	HCl, HNO_3	ICP-MS	Total Cd
			Closed Microwave Dig.	HCl, HNO_3	ICP-MS	Total Hg
			Closed Microwave Dig.	HCl, HNO_3	ICP-MS	Total Pb
L35	AOAC 993.14	b) No	Closed Microwave Dig.	HNO_3	ICP-MS	Total As
			Closed Microwave Dig.	HNO_3	ICP-MS	Total Cd
			Closed Microwave Dig.	HNO_3	ICP-MS	Total Hg
			Closed Microwave Dig.	HNO_3	ICP-MS	Total Pb
L36	b) No	b) No		H_2O_2, HNO_3	ICP-AES	Total As
			Open Wet Dig.	H_2O_2, HNO_3	ICP-AES	Total Cd
				H_2O_2, HNO_3	ICP-AES	Total Hg
			Open Wet Dig.	H_2O_2, HNO_3	ICP-AES	Total Pb
L37	EN 14082:2003	Sigma-Aldrich / the calibration of instruments	Dry Ashing	HNO_3	ET-AAS	Total Cd
			Dry Ashing	HNO_3	ET-AAS	Total Pb
L38		b) No	Closed Microwave Dig.	H_2O_2, HNO_3	ET-AAS	Total As
			Closed Microwave Dig.	H_2O_2, HNO_3	ET-AAS	Total Cd
			Closed Microwave Dig.	H_2O_2, HNO_3	ET-AAS	Total Pb
L39	EPA 3052	b) No	Closed Microwave Dig.	H_2O_2, HNO_3	ICP-MS	Total As
			Closed Microwave Dig.	H_2O_2, HNO_3	ICP-MS	Total Cd
			Closed Microwave Dig.	H_2O_2, HNO_3	ICP-MS	Less than
			Closed Microwave Dig.	H_2O_2, HNO_3	ICP-MS	Total Pb
L40	b) No	b) No	Closed Microwave Dig.	H_2O_2, HNO_3		Total As
			Closed Microwave Dig.	H_2O_2, HNO_3		Total Cd
			Closed Microwave Dig.	H_2O_2, HNO_3		Total Hg
			Closed Microwave Dig.	H_2O_2, HNO_3		Total Pb
L41	b) No	the calibration of instruments, the validation of the procedure	Dry Ashing	HCl	HG-AAS	Total As
			Dry Ashing	HNO_3	FAAS	Total Cd
			Dry Ashing		CV-AAS	Total Hg
			Dry Ashing	HNO_3	FAAS	Total Pb

Lab. Code	Official method	Reference Material	Digestion type	Digestion mix	technique	z-scores
L42	b) No	IPE from WEPAL / the calibration of instruments, the validation of the procedure	Open Wet Dig.	HNO_3	ICP-AES	Total As
			Open Wet Dig.	HNO_3	ICP-AES	Total Cd
			Dry Ashing		GAUV	Total Hg
			Open Wet Dig.	HNO_3	ICP-AES	Total Pb
L43	b) No	b) No	Closed Microwave Dig.	H_2O_2, HNO_3	ICP-MS	Total As
			Closed Microwave Dig.	H_2O_2, HNO_3	ICP-MS	Total Cd
			Closed Microwave Dig.	H_2O_2, HNO_3	ICP-MS	Total Hg
			Closed Microwave Dig.	H_2O_2, HNO_3	ICP-MS	Total Pb
L44	EN 15510:2007, DIN EN 12485:2010	b) No	Closed Microwave Dig.	HNO_3	ICP-AES	Total As
			Closed Microwave Dig.	HNO_3	ICP-AES	Total Cd
			Open Wet Dig.	HCl, HNO_3	CV-AAS	Total Hg
			Closed Microwave Dig.	HNO_3	ICP-AES	Total Pb
L45	As, Cd, Pb: EN 15510	b) No	Open Wet Dig.	HNO_3	ICP-QMS	Total As
			Open Wet Dig.	HNO_3	ICP-QMS	Total Cd
			Closed Microwave Dig.	H_2O_2, HNO_3	CV-AAS	Total Hg
			Open Wet Dig.	HNO_3	ICP-QMS	Total Pb
L46	a) Yes	b) No	Open Wet Dig.	HCl, HNO_3	HG-AAS	Total As
			Open Wet Dig.	HCl, HNO_3	ET-AAS	Total Cd
			Open Wet Dig.	HCl, HNO_3	CV-AAS	Total Hg
			Open Wet Dig.	HCl, HNO_3	ET-AAS	Total Pb
L47	Cd, Pb - EN 14082:2003	b) No	Dry Ashing	HCl	FAAS	Total Cd
			Dry Ashing	HCl	FAAS	Total Pb

Annex 14: Techniques used and the respective LoDs

Lab ID	Total As		Total Cd		Total Pb		Total Hg	
	Technique	LODs (mg Kg^{-1})	Technique	LODs (mg Kg^{-1})	Technique	LODs (mg Kg^{-1})	Technique	LODs (mg Kg^{-1})
L01	ICP-MS	5	ICP-MS	2.5	ICP-MS	5	ICP-MS	1
L02	HG-AAS	0.06	FAAS	0.003	FAAS	0.03	CV-AFS	0.01
L03			GF-AAS	0.001	GF-AAS	0.004		
L04	GF-AAS	0.05	GF-AAS	0.005	GF-AAS	0.02	HG-AAS	0.005
L05	ICP-MS	0.005	ICP-MS	0.002	ICP-MS	0.01	ICP-MS	0.005
L07	ICP-MS		ICP-MS		ICP-MS		CV-AFS	
L08	ICP-MS	0.2	ICP-MS	0.05	ICP-MS	0.05	CV-AAS	0.005
L09							(AMA 254)	1 µg/Kg
L10	ICP-OES	0.141	ICP-OES	0.01	ICP-OES	0.048	ICP-OES	0,075
L11	HG-AAS	0.03	FAAS	0.001	GF-AAS	0.03	CV-AAS	0.001
L12	ICP-MS	0.2	ICP-OES	0.1	ICP-OES	0.5	GAUV	0.01
L13	ICP-MS	0.014	ICP-MS	0.001	ICP-MS	0.005	GAUV	0.001
L14	ICP-OES		ICP-OES		ICP-OES		ICP-OES	
L15	ICP-MS	0.0023	ICP-MS	0.0023	ICP-MS	0.0029	CV-AAS	0.0017
L16	ICP-AES	0.003	ICP-AES	0.0002	ICP-AES	0.001	GAUV	0.002
L17			ETAAS		ETAAS		GAUV	
L18	GF-AAS	0.6	GF-AAS	0.06	GF-AAS	0.4	CV-AAS	0.05
L19	HG-AAS	0.01	GF-AAS	0.01	GF-AAS	0.05	AMA 254	0.001
L20	FAAS	0.005	FAAS	0.1	FAAS	0.5	CV-AAS	0.005
L21	HG-AAS		GF-AAS		GF-AAS		GAUV	
L24	FAAS-MHS		FAAS		FAAS		AMA254	
L25	GF-AAS	0.4	GF-AAS	0.008	GF-AAS	0.06	CV-AAS	0.024
L26	ICP-QMS	0.0001	GF-AAS	0.002	GF-AAS	0.01	GAUV	0.000005
L27			ICP	0.03	ICP	0.03		
L28	ICP-AES	0.1	ICP-AES	0.05	ICP-AES	0.1	ICP-AES	0.02
L29			FAAS		FAAS		CV-AAS	
L30	HG-AAS	0.005	ICP-OES	0.0013	ICP-OES	0.003	FAAS-MHS	0.002
L31	AAS	0.005	AAS	0.002	AAS	0.003	CV AAS	0.001
L33	NAA	0.17	ICP-MS	0.005	ICP-MS	0.015	CV-AAS	
L34	ICP-MS	0.05	ICP-MS	0.01	ICP-MS	0.01	ICP-MS	0.0002
L35	ICP-MS	0.007	ICP-MS	0.001	ICP-MS	0.002	ICP-MS	0.05
L36	ICP-AES	0.05	ICP-AES	0.05	ICP-AES	0.05	ICP-AES	0.03
L37			GF-AAS	0.8	GF-AAS	0.7		0.05
L38	GF-AAS	0.074	GF-AAS	0.129	GF-AAS	1.414		
L39	ICP-MS	0.0004	ICP-MS	0,0002	ICP-MS	0.0002	ICP-MS	
L40		0.05		0.05		0.05		
L41	HG-AAS	0.01	FAAS	0.014	FAAS	0.1	CV-AAS	0.0005
L42	ICP-OES	0.05	ICP-OES	0.01	ICP-OES	0.05	GAUV	0.05
L43	ICP-MS	0.001	ICP-MS	0.001	ICP-MS	0.001	ICP-MS	0.001
L44	ICP-OES	0.56	ICP-OES	0.33	ICP-OES	0.39	CV-AAS	0.001
L45	ICP-QMS	0.1	ICP-QMS	0.05	ICP-QMS	0.05	CV-AAS	0.001
L46	HG-AAS		GF-AAS		GF-AAS		CV-AAS	0.05
L47			FAAS	0.01	FAAS	0.1		0.05

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Title: **IMEP-38: Determination of total As, Cd, Pb, and Hg in compound feed - Interlaboratory Comparison Report**

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Abstract
The Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre, a Directorate General of the European Commission, operates the International Measurement Evaluation Programme (IMEP). IMEP organizes interlaboratory comparisons (ILCs) in support to EU policies. This report presents the results of the proficiency test (PT) which focused on the determination of total As, Cd, Pb and Hg in compound feed according to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed.
The IMEP-38 exercise was organized aiming to assess the performance of food and feed control laboratories and official control laboratories. The test material used in this exercise was commercially available compound feed for cats which after the appropriate processing was spiked, bottled, labelled, numbered accordingly and dispatched to the participants on the 27th of June 2013. Forty-seven laboratories from 24 countries registered to the exercise of which 44 reported results and answered the respective questionnaire.
The percentage of satisfactory z-scores ranged from 58 % (total arsenic) to 74 % (total cadmium) and the ζ-scores obtained were lower by 10 to 21%.

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