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IMEP-27: Total Cd, Pb and As and extractable Cd and Pb in mineral feed

A. Semeraro, M.B. de la Calle, I. Wysocka, C. Quétel, T. Linsinger, H. Emteborg,
F. Cordeiro, I. Verbist, D. Vendelbo, P. Taylor

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Report of IMEP-27

Total Cd, Pb and As, and extractable Cd and Pb in mineral feed

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Antonella Semeraro
María Beatriz de la Calle
Irena Wysocka
Christophe Quétel
Thomas Linsinger
Håkan Emteborg
Fernando Cordeiro
Inge Verbist
Danny Vendelbo
Philip Taylor

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1 Summary

This report presents the results of an Interlaboratory Comparison (ILC) which focused on the determination of total Cd, Pb and As 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 mineral feed provided by AGES, Zentrum Analytik und Mikrobiologie, in Austria. 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 November 2008. Each participant received one bottle containing approximately 30 g of test material. Twenty-four participants from 11 countries registered to the exercise of which 19 submitted results for extractable Pb and Cd and 20 submitted results for total Pb, Cd and As. Three laboratories did not submit results.

The assigned values (X_{ref}) for total and extractable Cd and Pb 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]. The assigned value for total As was provided by the Studiecentrum voor Kernenergie (SCK-CEN) using neutron activation analysis. The analytical uncertainty of X_{ref}, u_{char}, for total As was calculated according to GUM[3]. Homogeneity and stability studies were subcontracted to Bayer Antwerpen. 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 of their measurements. This was done by 17 laboratories for extractable Pb and Cd and by 18 laboratories for total As, Cd and Pb.

The laboratory performance was evaluated using z and zeta scores in accordance with ISO 13528[4]. The standard deviations for proficiency assessment (also called target standard deviation) were calculated using the modified Horwitz equation[5] and were between 15 and 16 % for all the measurands.

2 IMEP support to EU policy

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

- IMEP distributes 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 metrologically best practice.

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

IMEP supports EU policies by organising intercomparisons in the frame of specific EU Directives, or on request of a specific Directorate-General. IMEP-27 provided specific support to the following stakeholders:

- The European Co-operation for Accreditation (EA) in the frame of a formal collaboration on a number of metrological issues, including the organisation of intercomparisons. National accreditation bodies were invited to distribute information about this exercise in the network of the routine control laboratories.

- The Community Reference Laboratory for Heavy Metals in Feed and Food (CRL-HM) in the frame of the support to the National Reference Laboratories (NRLs) of its network. The exercise was announced to the network of NRLs and they were invited to distribute the information between the routine laboratories in their country. The results gathered in IMEP-27 interlaboratory comparison represent the state of the art of the official control laboratories involved in feed analysis in Europe.

3 Introduction

The basic nutrients that animals require for growth, reproduction, and good health include carbohydrates, proteins, fat, vitamins and minerals. Minerals essential for animal life include sodium chloride, calcium, phosphorus, sulphur, potassium, magnesium, manganese, iron, copper, cobalt, iodine, zinc, molybdenum, and selenium. The last six being toxic when provided in excessive amounts.

All farm animals generally need more sodium chloride than is contained in their feed, they are supplied with it regularly. Of the other essential minerals, phosphorus and calcium are most apt to be lacking, because they are heavily drawn upon to produce bones, milk, and egg shells. Phosphorous supplements are bone meal, dicalcium phosphate, and defluorinated phosphates. Egg shells are nearly pure calcium carbonate. Calcium may readily be supplied by ground limestone, ground shells or marl that is high in calcium.
Small amounts of iodine are needed by animals for the formation of thyroxine. A serious deficiency of iodine may cause goitre, a disease which has caused in certain regions heavy losses of newborn pigs, lambs, kids, calves, and foals.

In some areas, soil and forage are deficient in copper and cobalt, which are needed together with iron for the formation of haemoglobin. In these areas, farm animals may suffer from anaemia unless the deficiency is corrected by suitable mineral supplement.

Iron, is amply supplied in most animal feed, except milk. The only practical problem with iron deficiency occurs in young suckling pigs before they start to consume other feed in addition to milk.

Though manganese is essential for animals, the usual rations for all farm animals, except poultry, supply sufficient quantities. A lack of manganese can cause the nutritional disease of chicks and young turkeys called *perosis* and may also cause failure of eggs to hatch.

Normal rations for swine are often deficient in zinc, especially in the presence of excess calcium. Adding 100 parts per million of zinc carbonate cures zinc deficiency symptoms, which include retarded growth and severe scaliness and cracking of the skin.

A trace of selenium is necessary for normal health of animals; excessive amounts found in forages in some regions poison animals may cause death.

To furnish both calcium and phosphorus, livestock may be feed a mixture of 60 % dicalcium phosphate and 40 % sodium chloride.

To overcome problems associated with a high metal content in feed maximum levels for trace elements 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[6]. 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"*.

IMEP organised a proficiency test (PT) exercise for the determination of total Cd, Pb and As and extractable Cd and Pb in mineral feed. This exercise was opened for all laboratories involved in feed analysis and it was carried out in parallel with a PT organised by the CRL-HM for its network of NRLs (IMEP-105). The same test material was used in both exercises.

4 Scope

The scope of this ILC is to test the competence of the participant laboratories to determine the total concentration of Cd, Pb and As 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, the International Measurement Evaluation Program of the IRMM of the European Commission Directorate Joint Research Centre. IMEP is accredited according to ISO Guide 43. The designation of this ILC is IMEP-27.

5 Time frame

During the months September and October 2009 the exercise was publicly announced via the IMEP website[7]. At the same time EA was invited to announce the exercise to its network of accreditation bodies and of accredited laboratories. Furthermore the network of NRLs of the CRL-HM was informed about the exercise during the third workshop organized by the CRL-HM. NRLs were invited to distribute information about this exercise in the network of the routine control laboratories of their representative countries. The registration to the inter-laboratory comparison was opened on 10th October 2008. The samples were dispatched to the participants on 14th November 2008. Reporting deadline was 19th December 2008.

6 Test material

6.1 Preparation

The test material, commercially available mineral feed for piglets, was provided by AGES, Zentrum Analytik und Mikrobiologie. Upon arrival at IRMM the material was processed by the Reference Materials Unit as follows: The material was milled to obtain particles around 500 μm with a Retsch, Heavy Duty mill. 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. The material was then homogenised and distributed using a vibrating feeder into amber glass bottles (60 mL) with polyethylene (PE) insert and screw cap lid with crimp film, containing approximately 30 g of test material each. Before

processing the material was stored at room temperature. After processing the storing temperature was 4 ºC. Processing took place at room temperature.

6.2 Homogeneity and stability

The measurements for the homogeneity and stability studies were performed by the Central Laboratory of Bayer Antwerpen. Homogeneity was evaluated according to ISO 13528 and to the method proposed by Fearn and Thompson[8] (one of the approaches recommended by the IUPAC International Harmonised Protocol[9]). Homogeneity and stability studies were performed for extractable Cd and Pb but not for total Cd and Pb. Our past experience showed that total and extractable Cd and Pb behave the same in terms of homogeneity and stability. The material proved to be homogeneous according to the IUPAC International Harmonised Protocol and to ISO 13528 for total As and for extractable Cd. It was hence assumed that the material was also homogeneous for total Cd. For Extractable Pb, the material was not homogeneous neither according to the IUPAC International Harmonised Protocol nor to ISO 13528, and it was therefore concluded that total Pb was also not homogeneous. Figure 1 shows that the material consisted of a mixture of materials characterised by different colours. It was decided not to mill the material down to finer powder in order to keep the test material similar to real routine samples. For this reason, u_{bb} was set to 10 % as provided by the software SoftCRM[10], and propagated according to GUM.

Figure 1: Picture of the test material used in IMEP-27.

The stability study of the test material was conducted following the isochronous approach[11]. The evaluation of the stability of the test material was made using the software SoftCRM[12]. The material proved to be stable at room temperature for the six weeks that elapsed between the dispatch of the samples and the deadline for submission of results for all the tested measurands. The results for Pb were highly scattered, confirming the lack of homogeneity observed, earlier described. Since the scatter observed was identical to u_{bb}, u_{sts} was set to zero, to avoid overestimation of the uncertainty.

The analytical results and statistical evaluation of the homogeneity and short term stability studies are provided in Annex 1.

6.3 Distribution

One set of material was sent to every participant. The test material was dispatched to the participants by IRMM on 14th November 2008. Each participant received: a) one bottle containing approximately 30 g of test material, b) an 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 2) and c) a form which had to be sent back after receipt of the sample to confirm its arrival (cf. Annex 3).

7 Instructions to participants

Concrete instructions were given to all participants in a letter that accompanied the samples (Annex 2). The measurands and matrix were clearly defined as *"Total Cd, Pb and As and extractable amounts of Cd and Pb in mineral 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 As and the procedure previously agreed upon for the determination of extractable Cd and Pb. The results were to be reported in the same manner (e.g. number of significant figures) as when reporting to customers.

The results were to be reported in a special on line form for which every participant received an individual access code. A special questionnaire, aiming at collecting additional information, was included in the online form. The questionnaire is presented in Annex 4.

8 Reference values and their standard uncertainties

The reference values, X_{ref}, for this ILC for total and extractable Cd and Pb were determined by IRMM using Isotope Dilution Inductively Coupled Plasma (ID-ICP-MS). IRMM has proven its measurement capabilities by successful participation in CCQM key comparisons. For total As the reference value was provided by the Studiecentrum voor Kernenergie (SCK-CEN) using neutron activation analysis. SCK-CEN has participated in key comparisons organised by the Comité Consultative de la Quantité de Matière for the determination of total arsenic in different matrices, with satisfactory results.

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

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

Where:

u_{ref}: standard uncertainty associated to the assigned value

u_{char}: standard uncertainty of characterisation

u_{bb}: standard uncertainty contribution for the between-bottle homogeneity

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 measurands of this ILC.

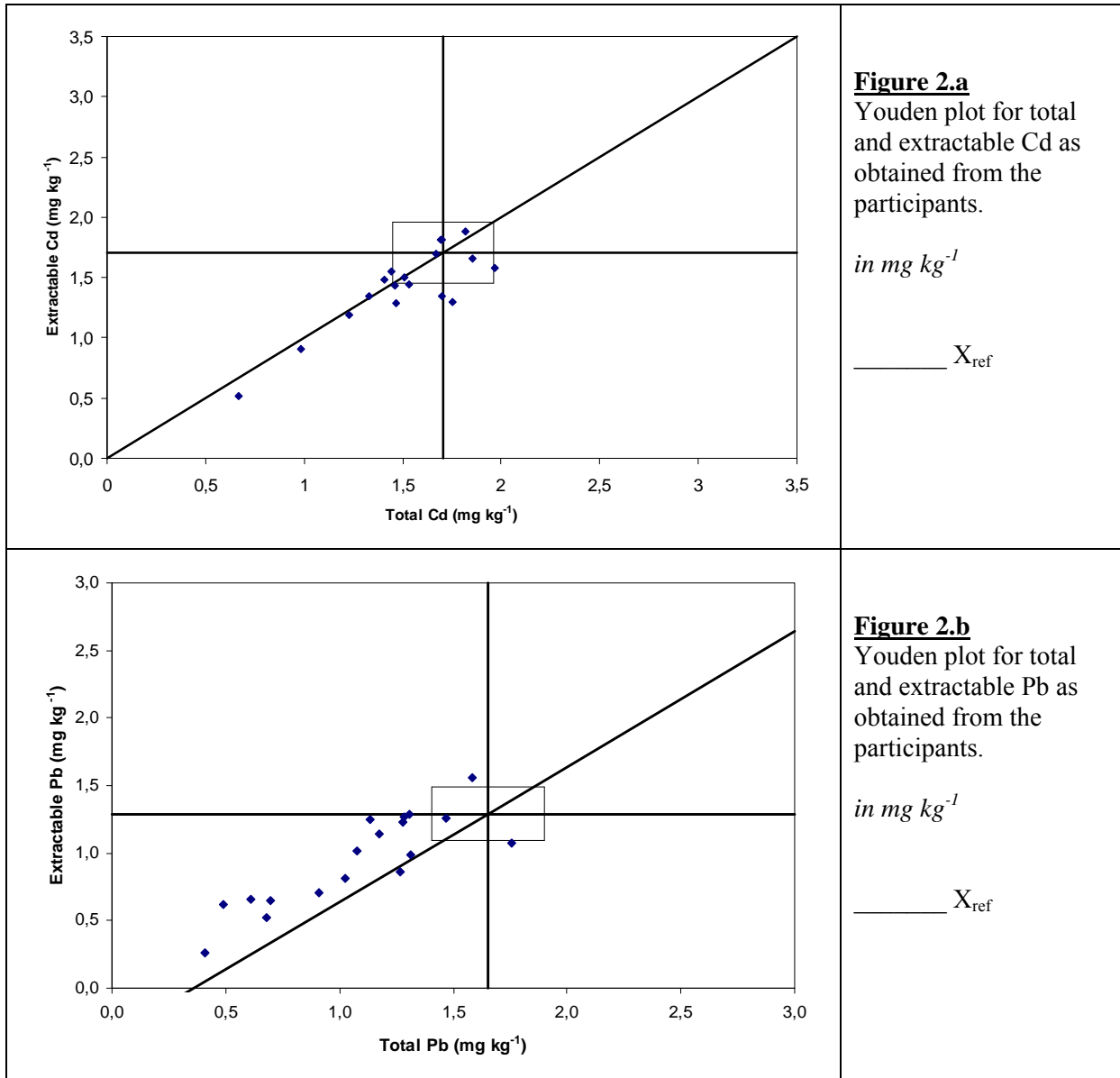|	X_{ref} [mg kg^{-1}]	u_{char} [%]	u_{bb} [%]	u_{sts}* [%]	u_{ref} [%]	U_{ref} [%]
Total Pb	1.65	5.8	10	0 [#]	12	23
Extract. Pb	1.29	5.4	10	0 [#]	11	23
Total Cd	1.71	1.6	2	2.1	3.3	6.7
Extract. Cd	1.71	1.6	2	2.1	3.3	6.7
Total As	1.18	3.8	2	3.8	5.7	12

* For six weeks

[#] See section 5.2

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 2.a, constructed with the results provided by the participants in this exercise. One cloud of points is observed on both axes around the reference value and within the square formed by the reference values ± sigma-hat, when total vs extractable Cd is plotted, showing that the results are not dependent of the method applied. The laboratories that have deviated in their reported values from the reference values show the same bias in both the total and the extractable Cd, and so they are spread along the diagonal of the Youden plot.

In the case of Pb, the reference value obtained by IRMM for extractable Pb is about 80% of the total Pb concentration. In the Youden Plot, Figure 2.b it can be seen that almost all results were negatively biased, and that most laboratories reported concentrations lower than the reference values both for the total and the extractable Pb. Nevertheless, for most laboratories the bias seems to be higher in the results reported for total Pb than for extractable Pb, so that the values are not along the diagonal of the Youden Plot but above it.

Figure 2.a
Youden plot for total and extractable Cd as obtained from the participants.

in mg kg⁻¹

_____ X_{ref}

Figure 2.b
Youden plot for total and extractable Pb as obtained from the participants.

in mg kg⁻¹

_____ X_{ref}

9 Evaluation of results

9.1 General observations

Twenty-four laboratories from 11 countries registered for participation in this exercise. Twenty laboratories reported values for total As, Cd and Pb and nineteen laboratories submitted results for extractable Cd and Pb. Three laboratories did not submit results. Seventeen laboratories reported uncertainty for extractable Pb and Cd and eighteen laboratories for total As, Cd and Pb. All laboratories responded to the questionnaire included in the on-line reporting form.

9.2 Scores and evaluation criteria

Individual laboratory performance is expressed in terms of z and zeta scores in accordance with ISO 13528[4] and the International Harmonised Protocol[9].

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

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

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
σ 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, σ. Usually, in the area of food and feed σ is derived from the improved Horwitz equation[5]. The values for σ obtained for this exercise were 15 % of the assigned value for total and extractable Cd, 15 % for total Pb and 15.5 % for extractable Pb and for total As. If those reproducibilities are considered as satisfactory, the z-score can be interpreted as:

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

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

$|z| \le 2$ satisfactory result
$2 < |z| \le 3$ 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 laboratory's deviation from the reference value. 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, σ. 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. If zeta >2, the results disagree within the expanded uncertainties, if the k-factor is chosen so that the expanded uncertainty is 95 %.

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[13].

Should participants feel that the σ values are not fit for their purpose they can recalculate their scorings with a standard deviation matching their requirements, as recommended in the IUPAC Harmonized Protocol.

9.3 Laboratory results and scorings

The results, as reported by the participants, are summarised in Table 2a-e for total Cd, total Pb, total As, extractable Cd and extractable Pb, respectively, together with the z- and zeta scores. The Annex 6 summarises the z and zeta scores for all the measurands obtained by each laboratory. Laboratory codes were given randomly.

Three sets of figures are provided for total Cd, extractable Cd, total Pb, extractable Pb and total As (Fig 3-7). Each set includes (a) the Kernel Density plot, (b) individual mean value and associated expanded uncertainty, (c) the z-and zeta scores. The solid line represents the assigned value, the 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[14]

Table 2a: Total Cd, quantitative information reported by the participants plus the laboratory scorings provided by the organiser.
Total Cd content: 1.71 ± 0.057 mg kg^{-1}

Code	x1	x2	x3	x4	Ulab	k	Mean	Technique	z	zeta
L02	1,287	1,325	1,384		0,0898	2	1,332	ICP-OES	-1,5	-5,2
L03	1,841	1,836	1,841	1,948	0	√3	1,867	ICP-MS	0,6	2,8
L04	1,22	1,24			11,53	2	1,23	ETAAS	-1,9	-0,1
L05	1,80	1,68	1,78		0,33	2	1,75	HR-ICP-MS	0,2	0,3
L06	1,47	1,47	1,45		0	√3	1,46	ICP-MS	-1,0	-4,3
L07	1,85	1,81	1,9		0,07	2	1,85	ETAAS	0,6	2,2
L08	0,702	0,628			0,100	2	0,665	ICP-MS	-4,1	-13,8
L09	1,840	1,566	1,668		0,169	√3	1,691	GF-AAS	-0,1	-0,1
L10	0,99	0,98	0,99		0,03	√3	0,99	ICP-MS	-2,8	-12,1
L11	1,71	1,61	1,78		0,023	√3	1,70	ICP-MS	0,0	-0,1
L12	1,704	1,687	1,715		0,035	2	1,702	ICP-MS	0,0	-0,1
L13	1,62	1,71	1,68		0,1	√3	1,67	ETAAS	-0,1	-0,5
L14	1,44	1,51	1,57		0,301	2	1,51	ICP-MS	-0,8	-1,3
L15	1,44	1,45			0,252	2	1,445	FAAS	-1,0	-1,9
L19	1,429	1,429	1,358		0,28	100	1,405	ETAAS	-1,2	-5,3
L20	1,98	1,70	1,77		0,15	1	1,82	ETAAS	0,4	0,7
L21	1,424	1,469	1,515		0,091	1	1,469	ICP-OES	-0,9	-2,2
L22	1,98	2,01	1,91		0,250	√3	1,97	GF-AAS	1,0	1,7
L23	1,55	1,57	1,51	1,5	0,1	√3	1,53	ICP-OES	-0,7	-2,2
L24	1,75	1,63	1,8		0,13	2	1,73	ICP-MS	0,1	0,2

All results are expressed in mg kg^{-1}

Table 2b: Total Pb, quantitative information reported by the participants plus the laboratory scorings provided by the organiser.
Total Pb content: 1.65 ± 0.19 mg kg^{-1}

Code	x1	x2	x3	x4	Ulab	k	Mean	Technique	z	zeta
L02	0,9895	1,0652	1,012		0,2503	2	1,022	ICP-OES	-2,5	-2,8
L03	1,811	1,744	1,864	1,620	0	√3	1,760	ICP-MS	0,4	0,6
L04	0,7	0,66			21,58	2	0,68	ETAAS	-3,9	-0,1
L05	1,26	1,34	1,23		0,26	2	1,28	HR-ICP-MS	-1,5	-1,6
L06	1,28	1,26	1,32		0	√3	1,29	ICP-MS	-1,5	-1,9
L07	1,06	1,11	1,06		0,05	2	1,08	ETAAS	-2,3	-3,0
L08	0,884	0,930			0,145	2	0,907	ICP-MS	-3.0	-3,6
L09	0,450	0,444	0,327		0,041	√3	0,407	GF-AAS	-5.0	-6,5
L10	1,23	1,22	1,47		0,61	√3	1,31	ICP-MS	-1,4	-0,9
L11	1,28	1,66	1,46		0,013	√3	1,47	ICP-MS	-0,7	-1,0
L12	1,275	1,241	1,288		0,06	2	1,268	ICP-MS	-1,5	-2,0
L13	1,17	1,11	1,25		0,14	√3	1,18	ICP-MS	-1,9	-2,3
L14	1,39	1,37	1,17		0,26	2	1,31	ICP-MS	-1,4	-1,5
L15	0,596	0,622			0,16	2	0,609	FAAS	-4,2	-5,1
L19	0,499	0,486	0,488		0,100	100	0,491	ETAAS	-4,7	-6,1
L20	1,91	1,50	1,86		0,22	1	1,76	ETAAS	0,4	0,4
L21	1,219	1,341	0,850		0,511	1	1,137	ICP-OES	-2,1	-0,9
L22	0,665	0,842	0,587		0,349	√3	0,698	GF-AAS	-3,8	-3,4
L23	1,59	1,61	1,58	1,55	0,10	√3	1,58	ICP-OES	-0,3	-0,3
L24	1,24	1,47	1,56		0,30	2	1,42	ICP-MS	-0,9	-0,9

All results are expressed in mg kg^{-1}

Table 2c: Total As, quantitative information reported by the participants plus the laboratory scorings provided by the organiser.
Total As content: 1.18 ± 0.068 mg kg^{-1}

Code	x1	x2	x3	x4	Ulab	k	Mean	Technique	z	zeta
L02	0,852	0,8813	0,9462		0,1894	2	0,8932	ICP-OES	-1,6	-2,5
L03	1,256	1,322	1,825	1,918	0	√3	1,580	ICP-MS	2.2	5.9
L04	1,11	1,1			14,33	2	1,11	HG- Flame Atomic Fluoresc. Spect.	-0,4	0,0
L05	1,12	1,18	1,16		0,23	2	1,15	HR-ICP-MS	-0,1	-0,2
L06	1,37	1,34	1,35		0	√3	1,35	ICP-MS	0,9	2,6
L07	1,21	1,13	1,17		0,06	2	1,17	HG-AAS	-0,1	-0,1
L08	0,551	0,596			0,063	2	0,574	ICP-MS	-3,3	-8,1
L09	1,262	1,255	1,209		0,286	√3	1,242	HG-AAS	0,3	0,3
L10	0,87	0,85	0,88		0,06	√3	0,87	ICP-MS	-1,7	-4,1
L11	1,16	1,17	1,22		0,03	√3	1,18	ICP-MS	0,0	0,0
L12	1,518	1,496	1,504		0,028	2	1,506	ICP-MS	1,8	4,7
L13	0,974	0,987	1,044		0,08	√3	1,002	HG-AAS	-1,0	-2,2
L14	1,76	1,65	1,64		0,25	2	1,683	ICP-MS	2,8	3,5
L15	1,456	1,418			0,282	2	1,437	HG-AAS	1,4	1,6
L19	1,097	1,084	1,085		0,16	100	1,089	HG-AAS	-0,5	-1,3
L20	1,27	1,31	1,29		0,02	1	1,29	HR-ICP-MS	0,6	1,6
L21	1,045	1,078	1,009		0,069	1	1,044	HG-ICP-MS	-0,7	-1,4
L22	1,07	1,09	1,06		0,50	√3	1,073	HG-AAS	-0,6	-0,4
L23	1,24	1,16	1,18	1,16	0,10	√3	1,19	ICP-OES	0,0	0,1
L24	1,51	1,48	1,71		0,20	2	1,57	ICP-MS	2,1	3,2

All results are expressed in mg kg^{-1}

Table 2d: Extractable Cd, quantitative information reported by the participants plus the laboratory scorings provided by the organiser.
Extractable Cd content: 1.71±0.057 mg kg^{-1}

Code	x1	x2	x3	x4	Ulab	k	Mean	Technique	z	zeta
L02	1,342	1,346	1,349		0,090	2	1,346	ICP-OES	-1,4	-5,0
L04	1,18	1,2			0	√3	1,19	ETAAS	-2,0	-9,1
L05	1,25	1,30	1,34		0,26	2	1,297	HR-ICP-MS	-1,6	-2,9
L06	1,43	1,45	1,42		0	√3	1,43	ICP-MS	-1,1	-4,8
L07	1,66	1,63	1,69		0,05	2	1,66	ETAAS	-0,2	-0,8
L08	0,524	0,509			0,078	2	0,5165	ICP-MS	-4,7	-17,3
L09	1,800	1,820			0,181	√3	1,810	GF-AAS	0,4	0,9
L10	0,89	0,87	0,95		0,18	√3	0,90	ICP-MS	-3,1	-6,8
L11	1,84	1,75	1,85		0,02	√3	1,81	ICP-MS	0,4	1,8
L12	1,363	1,325			0,245	2	1,344	ETAAS	-1,4	-2,7
L13	1,68	1,74	1,66		0,10	√3	1,69	ETAAS	-0,1	-0,2
L14	1,5	1,49	1,52		0,30	2	1,50	ICP-MS	-0,8	-1,3
L15	1,544	1,548			0,318	2	1,546	FAAS	-0,6	-1,0
L17	1,77	1,541	1,5		0,243	√3	1,604	ETAAS	-0,4	-0,7
L19	1,51	1,489	1,449		0,3	100	1,483	ETAAS	-0,9	-4,0
L20	1,90	1,91	1,83		0,04	1	1,88	ETAAS	0,7	2,5
L21	1,395	1,213	1,242		0,195	1	1,283	ICP-OES	-1,7	-2,1
L22	1,56	1,56	1,61		0,25	√3	1,58	GF-AAS	-0,5	-0,8
L23	1,45	1,40	1,48	1,43	0,10	√3	1,44	ICP-OES	-1,0	-3,3

All results are expressed in mg kg^{-1}

Table 2e: Extractable Pb, quantitative information reported by the participants plus the laboratory scorings provided by the organiser.
Extractable Pb content: 1.29±0.15 mg kg

Code	x1	x2	x3	x4	Ulab	k	Mean	Technique	z	zeta
L02	0,8168	0,8375	0,7783		0,1986	2	0,811	ICP-OES	-2,4	-2,7
L04	0,54	0,5			0	√3	0,52	ETAAS	-3,9	-5,2
L05	1,20	1,29	1,20		0,246	2	1,23	HR-ICP-MS	-0,3	-0,3
L06	1,24	1,26	1,3		0	√3	1,27	ICP-MS	-0,1	-0,2
L07	1,03	1,05	0,97		0,07	2	1,02	ETAAS	-1,4	-1,8
L08	0,723	0,684			0,113	2	0,704	ICP-MS	-2,9	-3,7
L09	0,252	0,264			0,026	√3	0,258	GF-AAS	-5,2	-7,0
L10	1,37	1,29	1,19		0,37	√3	1,28	ICP-MS	0,0	-0,0
L11	1,26	1,25	1,25		0,14	√3	1,25	ICP-MS	-0,1	-0,2
L12	0,8282	0,8976			0,4408	2	0,8629	ETAAS	-2,1	-1,6
L13	1,10	1,11	1,22		0,12	√3	1,14	ICP-MS	-0,7	-0,9
L14	1,01	0,973	0,972		0,187	2	0,985	ICP-MS	-1,5	-1,8
L15	0,655	0,656			0,190	2	0,656	FAAS	-3,2	-3,6
L17	1,147	1,047	1,001		0,134	√3	1,065	ETAAS	-1,1	-1,4
L19	0,573	0,722	0,569		0,12	100	0,621	ETAAS	-3,3	-4,6
L20	1,08	0,99	1,16		0,09	1	1,08	ETAAS	-1,1	-1,2
L21	1,41	1,09	1,252		0,320	1	1,251	ICP-OES	-0,2	-0,1
L22	0,641	0,674	0,644		0,327	√3	0,653	GF-AAS	-3,2	-2,7
L23	1,56	1,59	1,57	1,52	0,1	√3	1,56	ICP-OES	1,4	1,7

All results are expressed in mg kg^{-1}
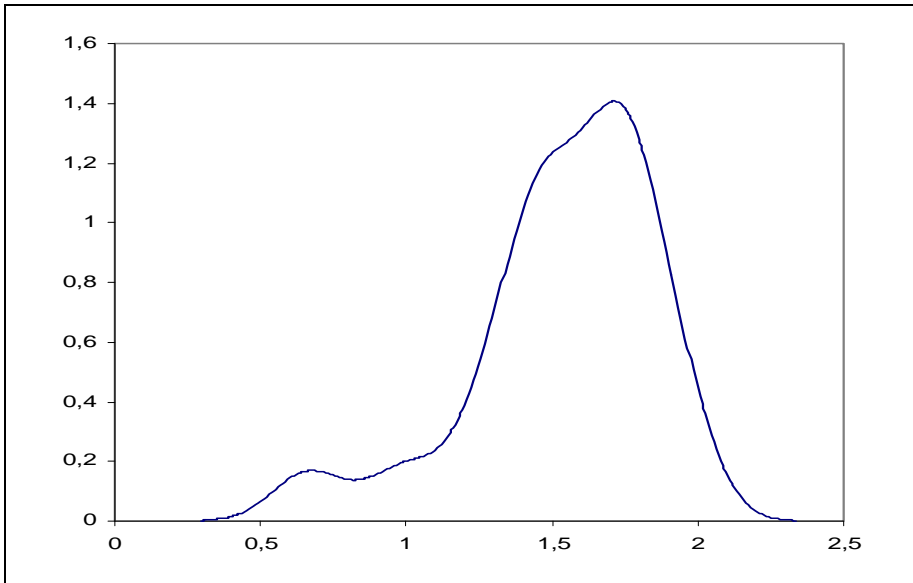
Figure 3a

Total Cd in mineral feed
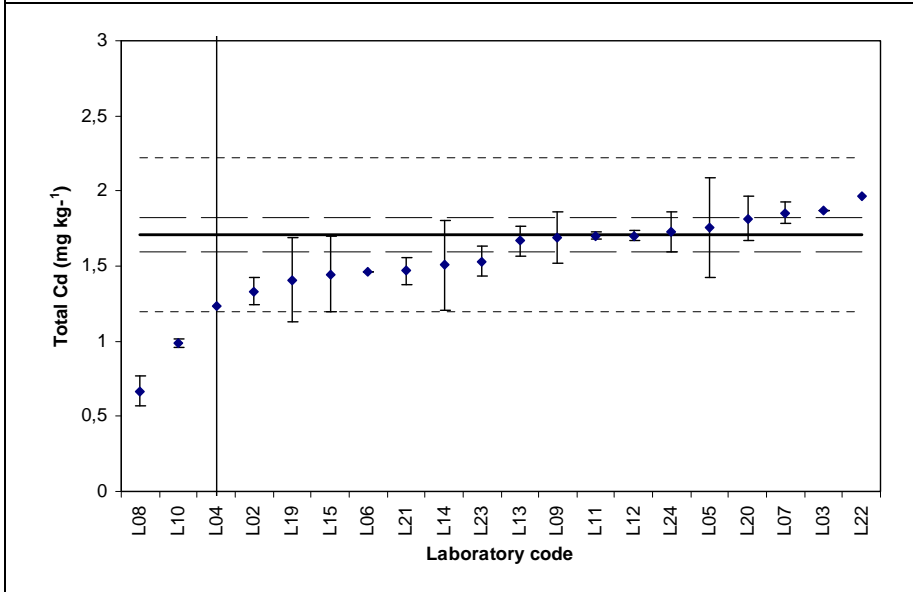Kernel density,
all results

Figure 3b

Reported results and
corresponding expanded
uncertainties

$X_{ref} = 1.71$
$u_{ref} = 0.057$
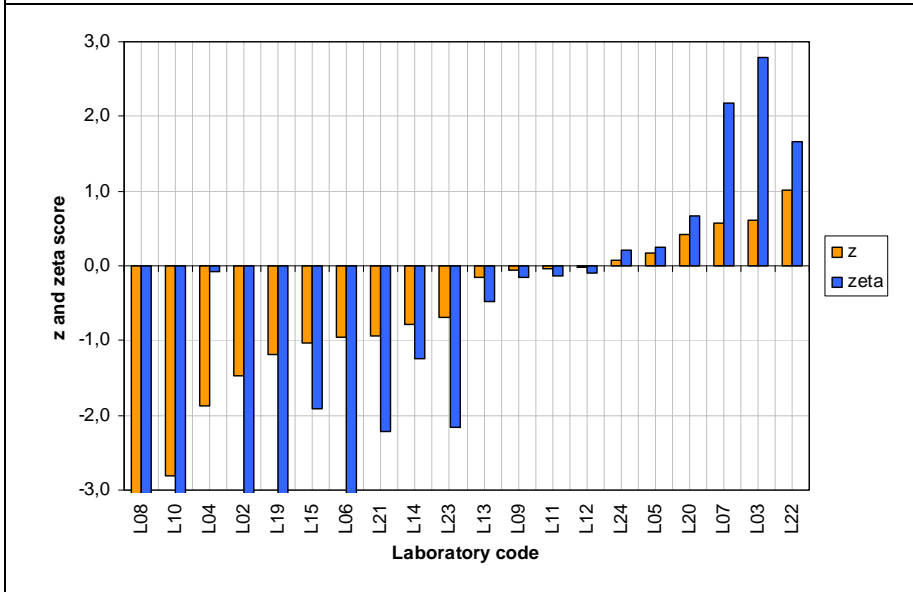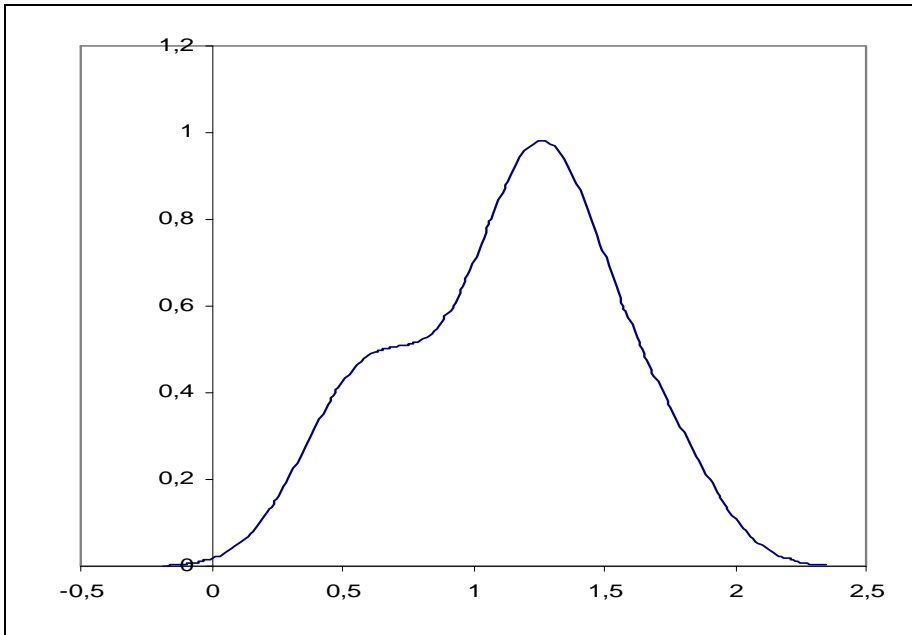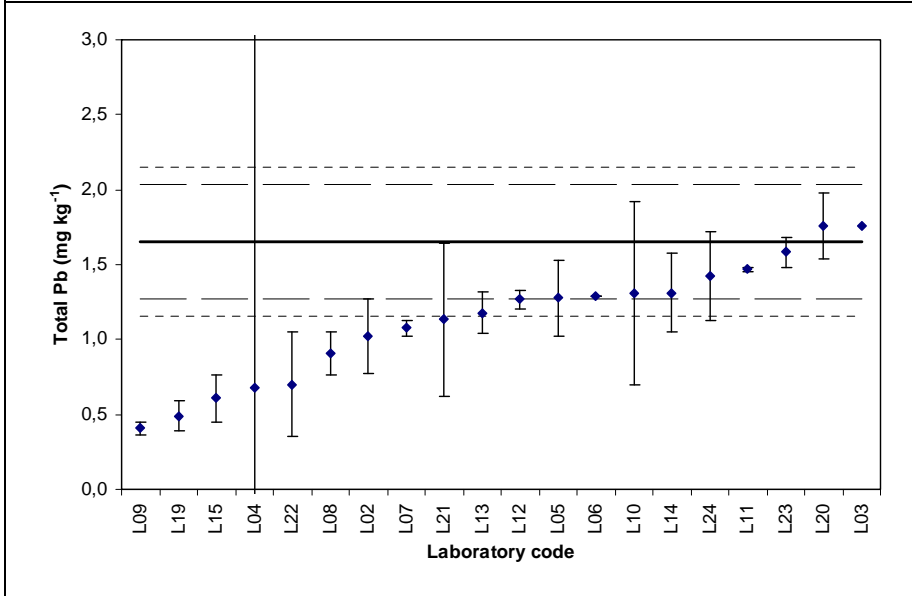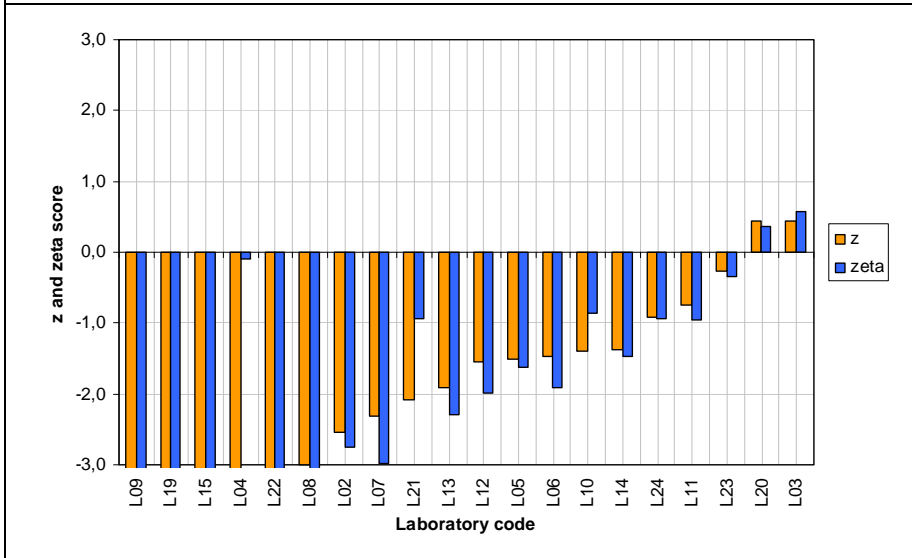$\hat{\sigma} = 0.26$ (15 %)
in mg kg^{-1}

Figure 3c

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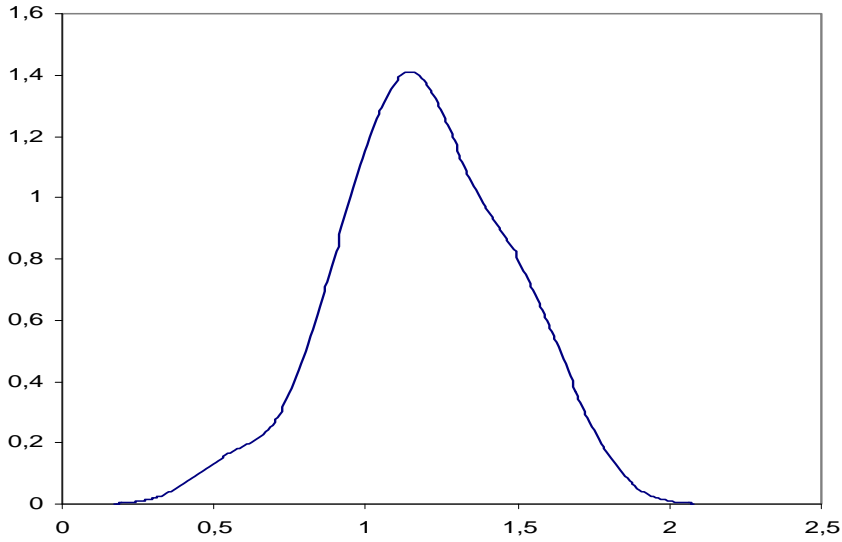zeta $= (x_{lab}-X_{ref})/ \sqrt{(u_{lab}^2+u_{ref}^2)}$

Figure 4a:

Total Pb in mineral feed
Kernel density,
all results

Figure 4b:

Reported results and
corresponding expanded
uncertainties

X_{ref} = 1.65
u_{ref} = 0.19
$\hat{\sigma}$ = 0.25 (15 %)
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 5a:

Total As in mineral feed
Kernel density, all results

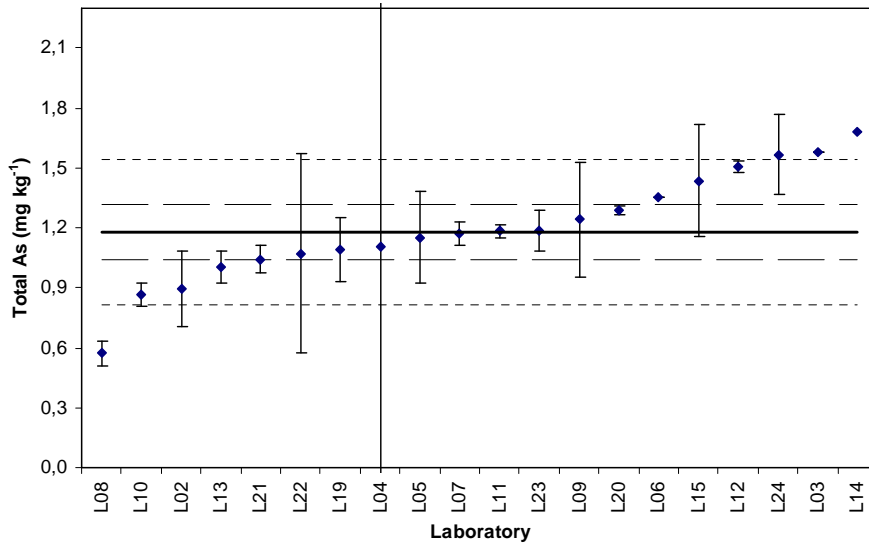**Figure 5b:**

Reported results and corresponding expanded uncertainties

$X_{ref} = 1.18$
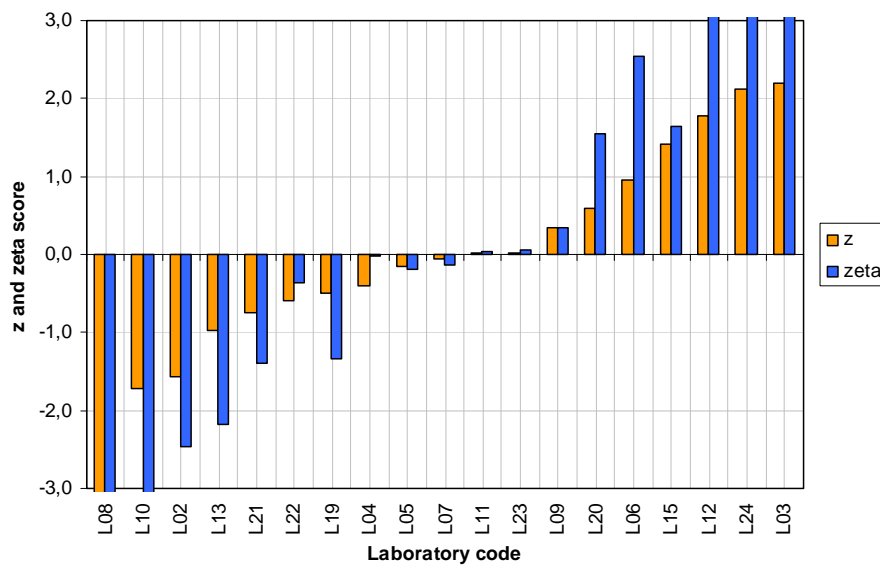$u_{ref} = 0.07$
$\hat{\sigma} = 0.18\ (15.5\ \%)$
in mg kg^{-1}

Figure 5c:

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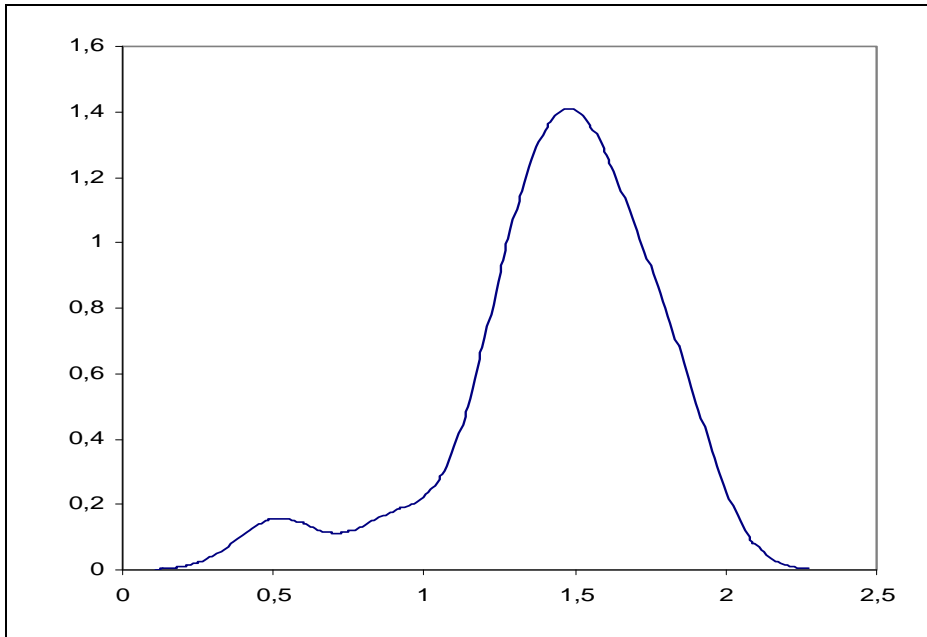$zeta = (x_{lab} - X_{ref})/\sqrt{(u_{lab}^2 + u_{ref}^2)}$

IMEP-27. Total Cd, Pb and As and extractable Cd and Pb in mineral feed

Figure 6a:

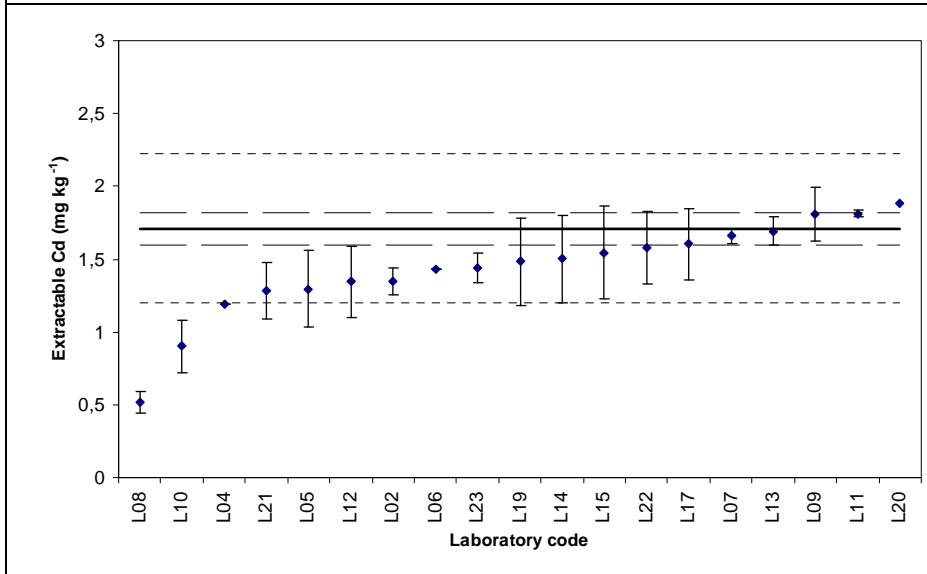Extractable Cd in mineral feed Kernel density, all results

Figure 6b:

Reported results and corresponding expanded uncertainties

X_{ref} = 1.71
u_{ref} = 0.057
$\hat{\sigma}$ = 0.26 (15 %)
in mg kg^{-1}

Figure 6c:

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

Figure 7a:

Extractable Pb in mineral feed Kernel density, all results

Figure 7b:

Reported results and corresponding expanded uncertainties

$X_{ref} = 1.29$
$u_{ref} = 0.14$
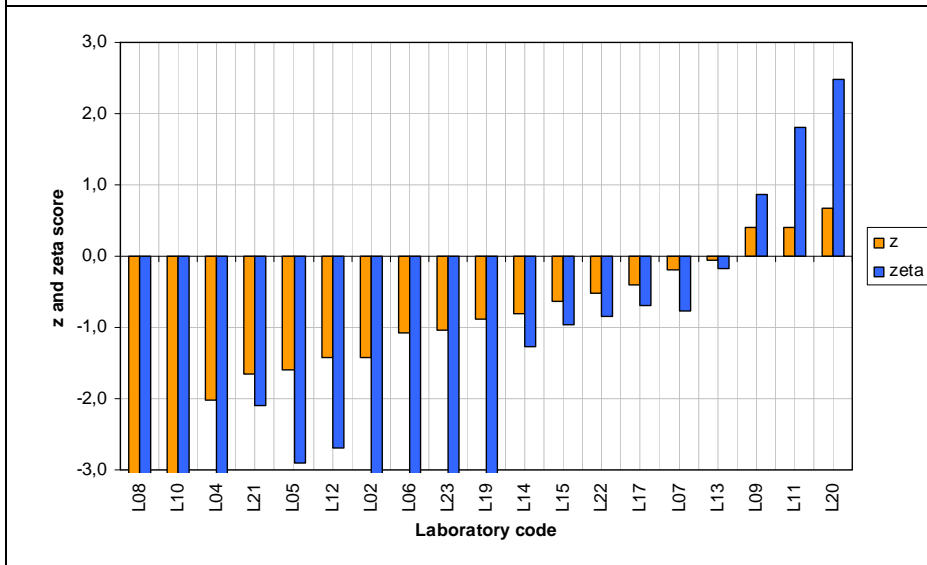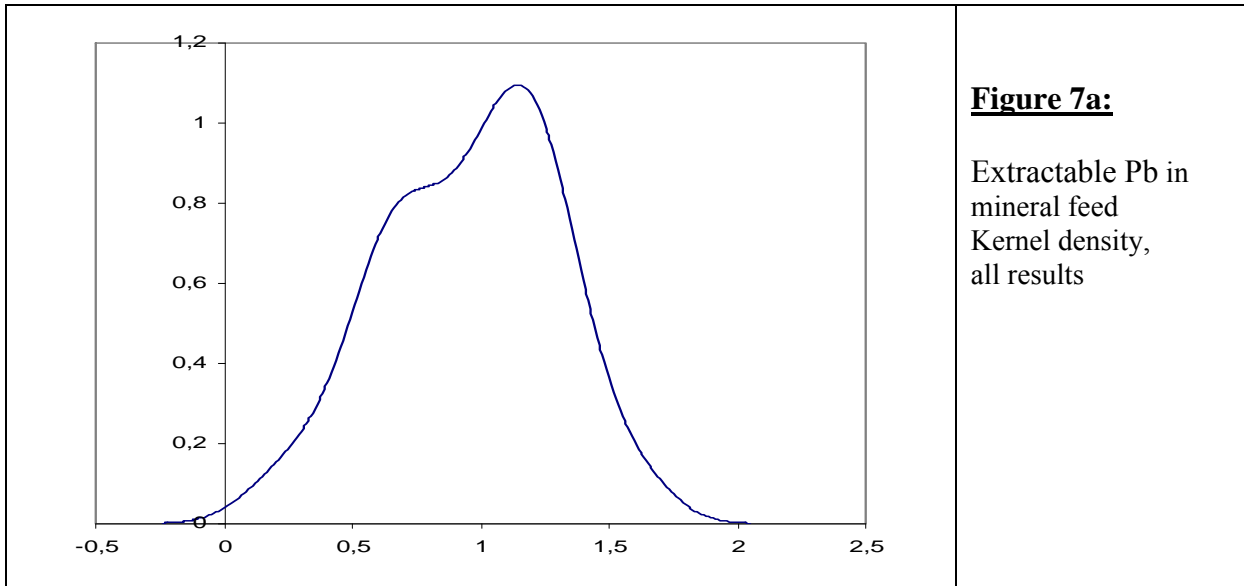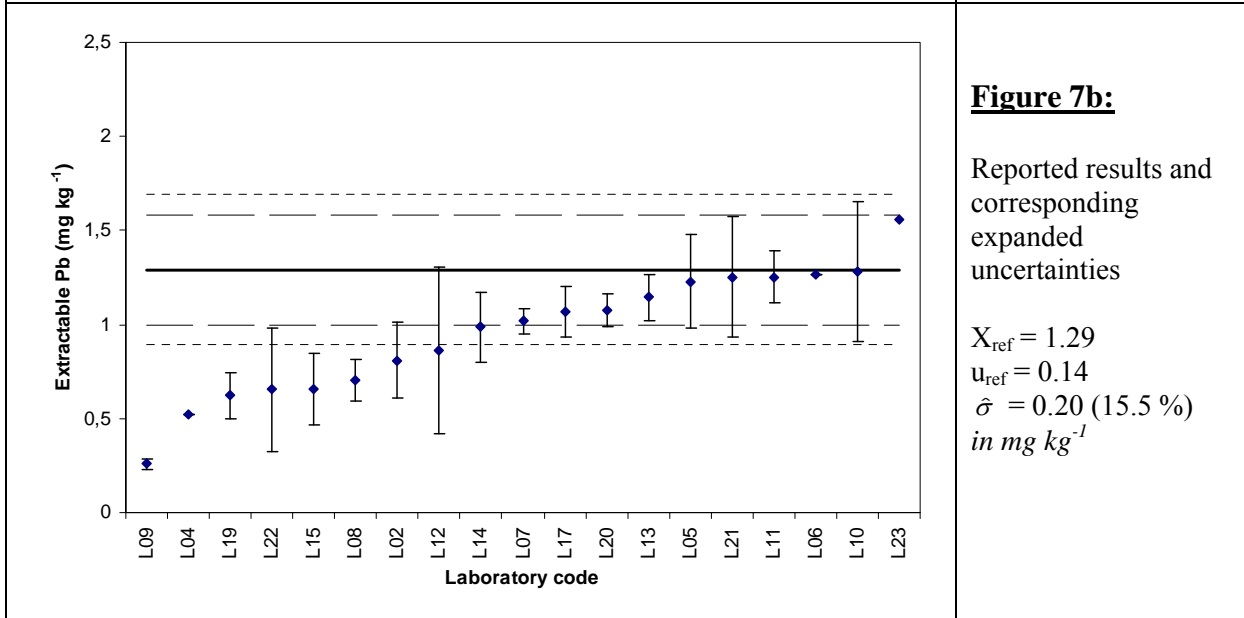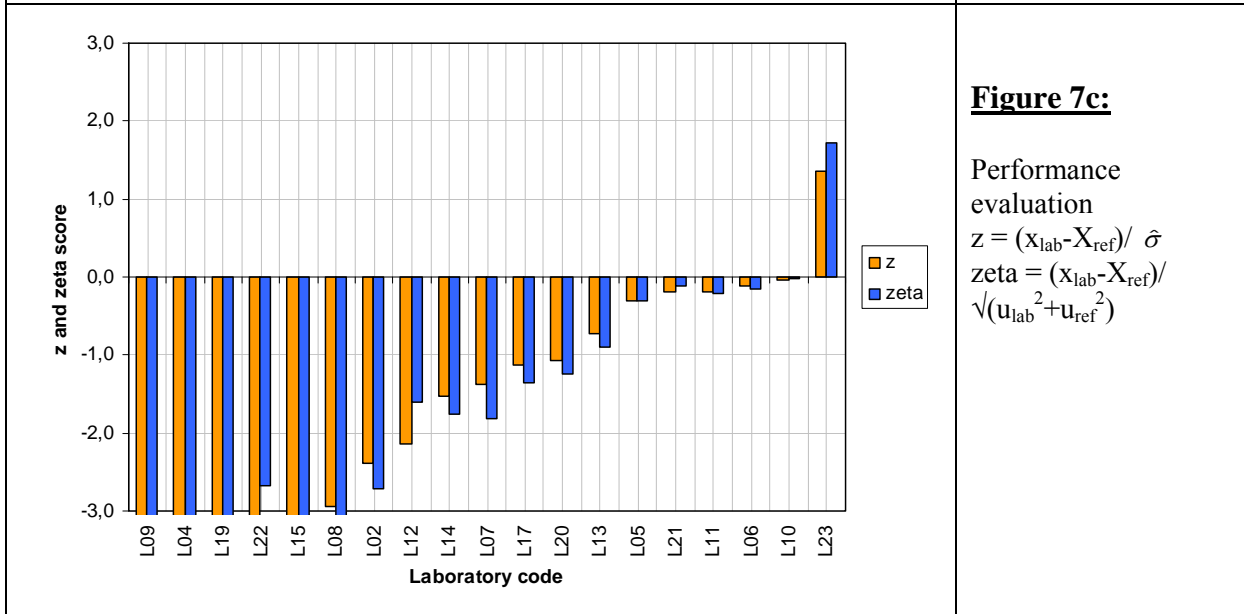$\hat{\sigma} = 0.20$ (15.5 %)
in mg kg^{-1}

Figure 7c:

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

The results for z and zeta score are summarised in Table3.

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

	Total Cd		Total Pb		Total As		Extractable Cd		Extractable Pb	
	Numb. Lab.	%	Numb. Lab.	%	Numb. Lab.	%	Numb. Lab.	%	Numb. Lab.	%
z										
Satisf.	18	90	11	55	16	80	17	90	11	58
Quest.	1	5	4	15	3	15	0		3	16
Unsat.	1	5	5	30	1	5	2	10	5	26
zeta										
Satisf.	11	55	12	60	11	55	9	47	12	63
Quest.	3	15	3	15	3	15	3	16	2	11
Unsat.	6	25	5	26	6	30	7	37	5	26

Most of the laboratories obtained a satisfactory z-score for total and extractable Cd and for total As. An improvement is necessary in the determination of total and extractable Pb in which only 60% of the participants obtained a satisfactory z-score. Looking at the zeta scores, around half of the participants obtained results within the range ± 2 for all the measurands. Between 25 and 35% of the laboratories had an unsatisfactory zeta score, which means that they had some difficulties to provide a reliable uncertainty of their measurements.

Participants were asked to complete a questionnaire, the information extracted from it is reported below.

Six laboratories corrected their results for recovery: two of which calculated the recovery using a reference material, three adding a known amount of the same analyte to be measured and one using the two previously mentioned approaches. Fifteen laboratories did not correct the result for the recovery and when asked why, three said that they do not do it on a routine basis and two answered that in this kind of analysis it is not necessary because they digest the samples. One participant replied that reference materials give nearly 100% of recovery, one that they validate the method without using the recovery and one said that the recovery is 80-120%. Several laboratories did not answer to this question.

Participants were asked about the confidence level reflected by the coverage factor (k) reported. Thirteen laboratories reported a level of 95% and three laboratories did not reply to this question. The remaining laboratories misunderstood the question and some of them gave an answer also if they did not provide any measurement uncertainty or coverage factor.
For the uncertainty estimate, several participants gave various combinations of two or more of the following options. Eight use the uncertainty of the method as determined during in-house validation. Ten laboratories estimated the uncertainty using data from measurements of replicates (i.e. precision). Three laboratories applied the ISO-GUM. One used the known uncertainty of the standard method. Four laboratories made use of intercomparison data. One used the expert guesstimate. Twelve laboratories provide an uncertainty estimate to their customer and nine do not.

About the procedure used to perform the analysis. All laboratories used the prescribed protocol for partial extraction without any modification. Thirteen participants analysed the content of Cd, Pb and As following an official procedure. The information reported by the

remaining laboratories about their method of analysis is summarised in Annex 5. Nineteen participants corrected the results for the water content and two laboratories did not.

Nineteen laboratories carry out this type of analysis on a routine basis and two laboratories do not.

Twenty laboratories have a quality system in place. Seventeen laboratories have a quality system based on ISO 17025, one has a quality system based on both ISO17025 and ISO 9000 series, one based on ISO 9000 series and one laboratory did not specify it. Seventeen laboratories are accredited and four laboratories are not although they perform this type of analysis on a routine basis.

Total digestion is the sample treatment used in routine analysis by seventeen laboratories, one laboratory uses partial digestion (according the legislation), two laboratories use both sample treatments and one participant did not answer.

Eighteen laboratories take part in an interlaboratory comparison on a regular basis.

Nineteen laboratories use a reference material for this type of analysis. Eighteen laboratories use the reference material for the validation and six for calibration. Ten laboratories use a reference material of organic composition, and one uses a reference material of mineral origin (L23). Eight laboratories did not specify which reference material they use. Table 4 summarises the CRM's used for the validation of the methods as reported by the participants. Organic matrices are easier to mineralise than matrices of mineral origin, which may easily result in an overestimation of the recovery and thus in an underestimation of the analyte concentration when the same method is applied to a mineral matrix. This hypothesis would explain why according to the Youden plot some participants obtained results for total Pb more biased than those reported for extractable Pb. The concentration of extractable Pb is method dependent and has been obtained by all the laboratories using the same method. When developing and validating a method to analyse heavy metals in mineral feed, it would be more appropriate to use sediment CRM if no mineral feed CRM is available on the market.

Table 4: Certified Reference Materials used by the laboratories taking part in this exercise for the validation of the methods used for the analysis and in some cases for calibration purposes, as reported by the participants.

Lab code	Do you use a CRM?	Is the CRM used for validation?	Is The CRM used for calibration?	Which one(s)?
L02	Yes	Yes	No	Several animal feed (FAPAS, AAFCO)
L03	Yes	Yes	No	
L04	Yes	Yes	No	
L05	Yes	Yes	No	
L06	Yes	Yes	No	Samples from Bonner Enquete or IAG
L07	Yes	Yes	Yes	Bonner Enquete 321 QC; Rice flour 1568 a NIST
L08	Yes	Yes	No	
L09	Yes	Yes	Yes	NIST Wheat Gluten and NIST Wheat Flour
L10	Yes	Yes	No	BCR 482 (trace elements in lichen)
L11	Yes	Yes	No	
L12	Yes	Yes	Yes	NIST CRM, commercial SRM, local RM
L13	Yes	No	No	Oyster tissue, NBS 1566a, Bovine Liver, NBS 1577a
L14	Yes	Yes	No	Nist Durum Wheat Flour 8436, INCT-MPH-2
L15	Yes	Yes	Yes	Standards 1 g/L of As, Cd, Pb
L17	No			
L19	Yes	Yes	No	
L20	Yes	Yes	No	
L21	Yes	Yes	No	
L22	Yes	Yes	Yes	Samples from collaborative studies like Bonner Enquete, ALVA Enquete or IAG Enquete
L23	Yes	Yes	Yes	Different international rock standards (Chinase SRMS, Wageningen monitor standard)
L24	No	No	No	

Figure 8 shows the distribution of the z-scores for total Pb, which was the most critical measurand, as function of the technique used after applying multivariant analysis. Five out of six laboratories that used electrothermal atomic absorption spectroscopy (ETAAS) and graphite furnace atomic absorption spectroscopy (GF-AAS) are grouped together. No other clustering was observed for the other technique used in this exercise.
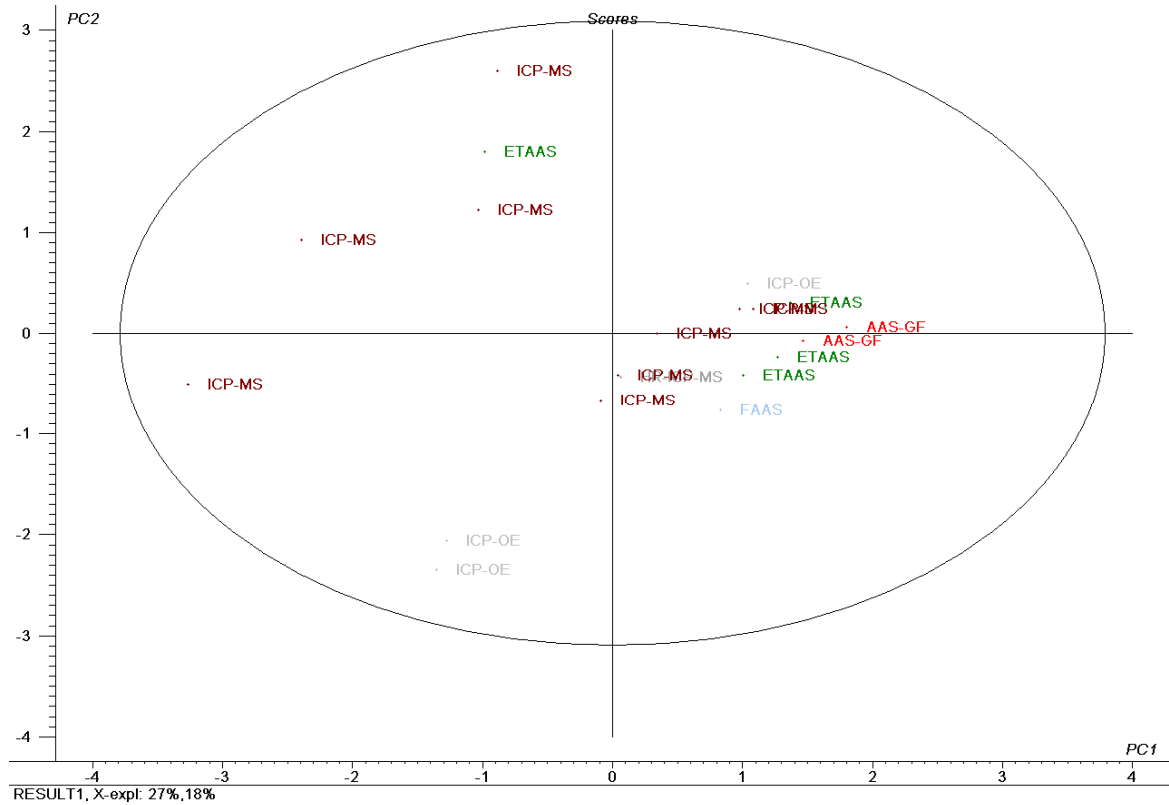

Figure 8: Distribution of z-scores for total Pb in function of the technique used as derived from multivariant analysis (projection to latent structures (PLS) relating the Pb z-score to the answers provided by the laboratories from the questionnaire).

Table 6 describes in detail the method used by IRMM for the determination of total Cd, Pb and As in mineral feed. This method was used to obtain the reference values for the mentioned measurands. Table 6 also contains the method applied by Bayer Antwerpen for the determination of total As in the test material to perform the homogeneity and stability studies. The results obtained by Bayer Antwerpen are in agreement with the reference value provided for total As by SCK-CEN. The method used by SCK is neutron activation analysis, which is not a technique commonly available in routine control laboratories. The methods summarised in Table 6 could be used as starting point for laboratories having problems with the determination of total Cd, Pb and As and that want to improve their performance.

Table 6: Summary of methods that have been used for the determination of total Cd and Pb by IRMM, and of total As by Bayer Antwerpen, on the test material used in this exercise.

Method	Procedure
Method used by IRMM to determine total Cd and Pb.	About 0.2 g of feed sample were weighed into a microwave Teflon boat. 4 mL of HNO_3 (65%), 2 mL of HCl (36%) and 1 mL HF (49%) were added to the microwave vessel and left for 2 h for the pre-digestion of the samples. The digestion in the microwave was done applying the following programme: **1)** 2 min at 250 W, **2)** 2 min at 0 W, **3)** 6 min at 250 W, **4)** 2 min at 0 W, **5)** 5 min at 650 W, **6)** 2 min at 0 W, **7)** 5 min at 650 W, **8)** 20 min at 0 W. After finishing the microwave decomposition, the Teflon vessels were put into a heating block and the sample digests are evaporated almost to dryness. The digest and reference materials dilutions were made with 2% HNO_3. The procedural blank samples followed exactly the same digestion procedure as the feed samples.
Method used by Bayer Antwerpen to determine total As	Sample preparation: Extraction with reflux boiling with concentrated HCl

1. Weigh 600 mg of the prepared test sample to the nearest mg into a 100 mL flask.
2. Add 25 mL concentrated HCl (p.a. Baker) and 25 mL milli-Q-water and place a reflux cooler on the flask.
3. Put the flask and cooler on a hot plate and let the solution boil for a total time of one hour.
4. After 20 minutes, add dropwise 10 mL H_2O_2 (w/w) (p.a. Baker) via the dosing capillary.
5. Wait 20 minutes before flushing the capillary with 10 mL milli-Q-water.
6. Leave the flask and the cooler on the hot plate for another 20 minutes.
7. Remove the flask and the cooler from the hot plate, allow to cool down and rinse the cooler with milli-Q-water.
8. Add 2 ml of KI solution and dilute to the mark with milli-Q-water.
9. Leave the solution to stand for another 50 minutes before starting the measurements.

Measurement:
HG-AAS on Varian SpectrAA-300 with VGA-76 hydride generator
Wavelength: 193,7 nm
Dilution: the extraction solution (8) was measured undiluted
Calibration: 5, 10, 15 µg/L
Quality Control: samples spiked with 5 µg/L As |

10 Conclusions

In order to obtain a good evaluation of the systematic errors, the reference material used to estimate the trueness of the analysis procedure should be similar to the matrix of real samples. As shown as outcome of this exercise, using reference materials that do not match the type of matrix of the test material some of the measurands could have a significant bias. This was the case of total Pb in this exercise, which was underestimated by most of the participants.

There seems to be some confusion among the participants on whether results are to be corrected for recovery or not. According to Commission Regulation (EC) No 333/2007: *"If an extraction step is applied in the analytical method, the analytical result shall be corrected for recovery. In this case the level of recovery must be reported. In case no extraction step is applied in the analytical method (e.g. in case of metals), the result may be reported uncorrected for recovery if evidence is provided by ideally making use of suitable certified reference material that the certified concentration allowing for the measurement uncertainty is achieved (i.e. high accuracy of the measurement). In case the result is reported uncorrected for recovery this shall be mentioned".* This means that during the validation of a method the laboratory must perform studies to evaluate the accuracy of the method, ideally using CRMs when available. When the result is not biased and it falls in the concentration range provided by the CRM (taking into consideration the uncertainty of the certified value), or when there is an overlap of the concentration range obtained by the laboratory (taking into consideration the uncertainty of the measurement as evaluated by the lab), then there is no need to correct for recovery and so it must be reported to the customer, as indicated in the legislation. Otherwise, the results are to be corrected for recovery. Analysts must keep in mind that extractions with organic solvents are not the only steps in an analytical procedure that could introduce a low recovery. Precipitation, volatilisation, incomplete digestion and adsorption are, among others, possible sources of biased results.

The questionnaires show that half of the participant laboratories estimate their uncertainty using data from measurements replicates. A reliable uncertainty estimate must take into account all the significant source of uncertainty and they can not be covered only by a precision study. This kind of approach is responsible of an underestimation of the uncertainty of measurements and could explain why there is a consistent gap between z and zeta score.

11 Acknowledgements

C. Contreras and A. Lamberty from the Reference Materials Unit are acknowledged for their support in the processing of the test material. The Central Laboratory of Bayer Antwerpen is acknowledged for performing the measurements for the homogeneity and stability studies. The authors thank the Studiecentrum voor Kernenergie (SCK-CEN) and in particular P. Vermarke, for providing the reference value for total As. P. Robouch is acknowledged for the support in the construction and interpretation of the Youden plots.

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

Organisation	Country
State Veterinary Institute Jihlava	CZECH REPUBLIC
Evira	FINLAND
IDAC	FRANCE
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit	GERMANY
Landesamt für Landwirtschaft Forsten und Gartenbau SA	GERMANY
Landesbetrieb Hessisches Landeslabor	GERMANY
Lower Saxony State Office for Consumer Protection and Food Safety	GERMANY
LTZ Augustenberg	GERMANY
Staatliches Veterinäruntersuchungsamt	GERMANY
Thüringer Landesanstalt für Landwirtschaft Jena	GERMANY
Technische Universität München	GERMANY
Geological Institute of Hungary	HUNGARY

Organisation	Country
The State Laboratory	IRELAND
Istituto Zooprofilattico Sperimentale-Puglia e Basilicata	ITALY
Analysesenteret	NORWAY
Department of Hygiene Veterinary	POLAND
Junta de Castilla y León	SPAIN
Laboratorio Agrario y Fitopatológico de Galicia. Xunta de Galicia	SPAIN
Laboratorio Agroalimentario y de Sanidad Animal	SPAIN
ALS Scandinavia AB	SWEDEN
Eurofins Food & Agro Sweden AB	SWEDEN

11 References

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

[2] ISO Guide to Expression of Uncertainty in Measurement, (1993).

[3] P. Robouch, G. Arana, M. Eguskiza, S. Pommé and N. Etxebarria, *Journal of Radioanalytical and Nuclear Chemistry*, (2000), **245**, 195-197

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

[5] M. Thompson, *Analyst*, (2002), **125**, 385-386.

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

[7] http://irmm.jrc.ec.europa.eu/html/interlaboratory_comparisons/

[8] T. Fearn, M. Thompson, *Analyst*, (2001), **126**, 1414-1416.

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

[10] See www.softCRM.com

[11] A. Lamberty, H. Schimmel, J. Pawels, *Fresenius J. Anal. Chem.,* (1998), **360**, 359-361.

[12] T.P.J. Linsinger, J. Pawels, A. Lamberty, H.G. Schimmel, A.M.H. van der Veen, L. Siekmann, *Anal. Bioanal. Chem.*, (2001), **370**, 183-188.

[13] Eurachem/CITAC guide "Quantifying Uncertainty in Analytical Measurements" (2000), see www.eurachem.ul.pt

[14] 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

IMEP-27. Total Cd, Pb and As and extractable Cd and Pb in feed

Annexes

Annex 1: Results of the homogeneity and stability studies

1a. Homogeneity data for total As in mineral feed

According to the IUPAC International Harmonised Protocol[8]

Bottle ID	As (mg kg^{-1})	
	Replicate 1	Replicate 2
13	1,03	1,13
53	1,01	1,17
77	1,16	1,15
120	1,09	1,12
145	1,13	1,11
170	1,13	1,11
208	1,11	1,13
214	1,13	1,1
264	1,15	1,18
293	1,15	1,11
Mean, n	**1.12**	**20**
Target RSD %	16	
S_{an}^2	0.00206	
S_{sam}^2	-0,000352222	
σ_{all}^2	0,002782774	
Critical	0,007312214	
S_{sam}^2<critical?	ACCEPT	

According to ISO 13528[3]

0.3σ	0,052752
s_x	0,026034166
s_w	0,045387223
s_s	#NUM!
$s_s \leq 0.3\ \sigma$	ACCEPT

1.b Stability data for total As in mineral feed
As computed by SOFT CRM

TEMPERATURE = 18°C

samples	Time in Weeks			
	0	3	5	8
1	1,11	1,15	1,05	1,22
2	1,2	1,18	1,14	1,23

CALCULATION OF u_{sts} for given Xshelf
Given Xshelf = 6 Weeks
U_b =0,007

u_{sts} = 0,044
u_{sts}[%] = 3,8%

Slope = 0,006
SE Slope = 0,008
Intercept = 1,135
SE Intercept = 0,037
Correlation Coefficient =0,101

Slope of the linear regression significantly <> 0 (95%) :No
Slope of the linear regression significantly <> 0 (99%) :No

2a. Homogeneity data for extractable Cd in mineral feed

According to the IUPAC International Harmonised Protocol[8]

	Cd (mg kg^{-1})	
Bottle ID	**Replicate 1**	**Replicate 2**
13	1,73	1,74
53	1,69	1,73
77	1,78	1,78
120	1,77	1,7
145	1,77	1,76
170	1,76	1,71
208	1,76	1,81
214	1,69	1,67
264	1,66	1,69
293	1,82	1,75
Mean, n	**1,7385**	**20**
Target RSD %	15	
S_{an}^2	0,000895	
S_{sam}^2	0,001225	
σ_{all}^2	0,005877959	
Critical	0,011954513	
S_{sam}^2<critical?	ACCEPT	

According to ISO 13528[3]

0.3σ	0,07666785
s_x	0,04089621
s_w	0,029916551
s_s	0,035
$s_s \leq 0.3\,\sigma$	ACCEPT

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

TEMPERATURE = 18°C

samples	**Time in Weeks**			
	0	3	5	8
1	1,66	1,75	1,78	1,72
2	1,76	1,77	1,8	1,82

CALCULATION OF u_{sts} for given Xshelf
Given Xshelf = 6 Weeks
U_b =0,006

u_{sts} = 0,036
u_{sts}[%] = 2,1%

Slope = 0,008
SE Slope = 0,006
Intercept = 1,726
SE Intercept = 0,028
Correlation Coefficient =0,247

Slope of the linear regression significantly <> 0 (95%) :No
Slope of the linear regression significantly <> 0 (99%) :No

3a. Homogeneity data for extractable Pb in mineral feed

According to the IUPAC International Harmonised Protocol[8]

Bottle ID	Pb (mg kg^{-1})	
	Replicate 1	Replicate 2
13	1,27	1,35
53	1,44	1,36
77	1,08	1,13
120	1,19	1,09
145	1,17	1,07
170	1,18	1,39
208	1,31	1,16
214	1,43	1,55
264	1,2	1,12
293	1,07	1,05
Mean, n	**1,2305**	**20**
Target RSD %	15.5	
S_{an}^{2}	0,006155	
S_{sam}^{2}	0,016430556	
σ_{all}^{2}	0,003273928	
Critical	0,012371535	
S_{sam}^{2}<critical?	NOT ACCEPT	

According to ISO 13528[3]

0.3σ	0,05721825
s_x	0,139671241
s_w	0,078453808
s_s	0,128181729
$s_s \leq 0.3\ \sigma$	NOT ACCEPT

3.b Stability data for extractable Pb in mineral feed
As computed by SOFT CRM

TEMPERATURE = 18°C

		Time in Weeks			
samples		0	3	5	8
	1	1,04	1,46	1,26	1,39
	2	1,12	1,19	1,12	1,42

CALCULATION OF u_{sts} for given Xshelf
Given Xshelf = 6 Weeks
U_b =0,019

u_{sts} = 0,115
u_{sts}[%] = 9,2%

Slope =   0,034
SE Slope =   0,015
Intercept =   1,113
SE Intercept =   0,075
Correlation Coefficient =0,458

Slope of the linear regression significantly <> 0 (95%) :No
Slope of the linear regression significantly <> 0 (99%) :No

Annex 2: 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**

IMEP®

Geel, 12 November 2008
D04-IM(2008)BdlC/ive/D/29335

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

**Participation to IMEP-27, a proficiency test exercise for the determination of
total Cd, Pb and As and extractable amounts of Cd and Pb in mineral feed**

Dear «TITLE» «SURNAME»,

Thank you for participating in the IMEP-27 intercomparison for the determination of
total Cd, Pb and As and **extractable amounts** of Cd and Pb in mineral 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 (not more than 18 ℃) until
analysis.

The measurands are: **total** Cd, Pb and As 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 mineral feed matrix.

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 mineral feed

(IMEP-27)

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 As 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 measurand. 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.

The results are to be reported referring to dry mass and thus corrected for humidity. To calculate the water content in the test material, please apply the following procedure:

Weigh 2 g of test material and dry it at 103 ± 2 °C for 4 hours in triplicate

You can find the reporting website at https://irmm.jrc.ec.europa.eu/ilc/ilcReporting.do
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 save, 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.
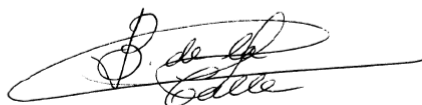
The deadline for submission of results is <u>19/12/2008</u>.

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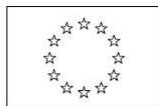JRC-IRMM-IMEP@ec.europa.eu

With kind regards

Dr. M.B. de la Calle
IMEP-27 Co-ordinator

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

Cc: P. Taylor

Annex 3: Sample receipt confirmation form

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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**Community reference laboratory for
heavy metals in feed and food**

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Annex to D04-IM(2008)BCa/ive/D/29335

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

IMEP-27

total Cd, Pb and As and **extractable amounts** of Cd and Pb in mineral 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-27 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. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 252. Fax: (32-14) 571 865.

E-mail: jrc-irmm-imep@ec.europa.eu

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Annex 4: Questionnaire

Please fill in the questionnaire

Submission Form

1. Did you apply a recovery factor to correct your measurement results?

○ no
○ yes

1.1. If Yes, what are the recovery factors (R, in %) you used:

1.1.1. for Cd (in %)

1.1.2. for Pb (in %)

1.1.3. for As (in %)

1.2. If Yes, did you determine R by:

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

1.2.1. If other, please specify.

1.3. 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)

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

3.1. If other, please specify.

4. Do you usually provide an uncertainty statement to your custumers for this type of analysis?

○ no
○ yes

5. Did you correct for the water content of the sample?

O No

O Yes

5.1. If Yes, what is the water content (in % of the sample mass)?

[ ]

5.2. If No, what was the reason not to do this?

[ ]

6. Did you modify the prescribed protocol for the partial digestion?

O no

O yes

6.1. If yes, please specify the modifications introduced.

[ ]

7. Did you analyse the sample according to an official method?

O no

O yes

7.1. If No, please describe (in max. 150 characters for each reply) your:

7.1.1. sample pre-treatment

[ ]

7.1.2. digestion step

[ ]

7.1.3. extraction / separation step

[ ]

7.1.4. instrument calibration step

[ ]

7.2. If Yes, which:

[ ]

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

O no

O yes

8.1. If Yes, please estimate the number of samples (As, Cd, Pb measurements together):

☐ a) 0-50 samples per year
☐ b) 50-250 samples per year
☐ c) 250-1000 samples per year
☐ d) more than 1000 samples per year

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

O no

O yes

9.1. If Yes, which:

☐ e) ISO 17025
☐ b) ISO 9000 series
☐ c) Other

9.1.1. If other, please specify.

[ ]

9.2. If yes, are you
accredited?

    O No

    O
    Yes

    9.2.1. If yes, by which Accreditation Body have you been
    accredited?

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

    ☐ Partial digestion (according to the
legislation)

    ☐ Total digestion

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

  O No

  O
  Yes

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

  O no

  O
  yes

    12.1. If yes, which one
    (s):

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

  O no

  O
  yes

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

        O no

        O
        yes

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

        O no

        O
        yes

    13.3. If yes, which one
    (s)

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

Annex 5: Experimental details

Lab code	Official standard method?	Which official standard method?	Sample treatment	Digestion step	Extraction/separation step	Instrument calibration
L02	No		None	Microwave HNO_3-H_2O_2	None	4 standards: 12,5, 50, 200 and 800 ppb
L03						
L04	Yes					
L05		EPA 200.8	Determination of Dry substances SS 028113	The sample is digested with nitric acid (5 ml) and hydrogen peroxide (0.5 ml) in a closed microwave digestion system		
L06	No	VDLUFA MB VII 2.2.2.5		total: Microwave (Ultra Clave)		Icp-MS, Vudlufa MB VII 2.2.2.5
L07	Yes	DIN EN 14546				
L08	No		homogenization	high pressure microwave digestion, nitric acid and hydrogen peroxide		external calibration
L09	Yes	EN 15550				
L10	No		Drying according to protocol; weighing in ca. 0.3 g sample (accurately)	wet digestion with 6 ml conc. nitric acid in microwave oven		external calibration with internal standardization (Re, Y, Ga)
L11	Yes					
L12	Yes	ICP-MS (DIN EN ISO 17294-2), ETAAS (DIN ISO 11047)				

Lab code	Official standard method?	Which official standard method?	Sample treatment	Digestion step	Extraction/separation step	Instrument calibration
L13	Yes	according to § 64 LFGB, these are German prescriptions for the measurement of foodstuff				
L14	Yes	NMKL 161 mod, 0.5 g sample 8 ml HNO_3 2 ml H_2O_2, microvawe 190 deg celcius, 2 point calibration on ICP-MS				
L15	Yes					
L17	Yes					
L19	Yes	VDLUFA Method VII 2.1.3				
L20	Yes	NS-EN ISO 15586, NS4780 for Pb and Cd, NS-En ISO 17294-2 for As. Minor differences made render the method as				
L21	No		None	Wet system digestion (hotblock) - regale water	filtration	OES-ICP and hydrure generation ICP (As)
L22	Yes	VDLUFA Methodenbuch Band III respectively for Pb and Cd: DIN EN 15550	Microwave digestion using 65% HNO_3% H_2O_2	For As pre-reduction with ascorbic acid and potassiunm iodide		
L23	Yes					
L24	No		We made a pre-digestion step in mineralizing solution for about 30 minutes	Microwave digestion in teflon vessels (Nitric Acid and Hydrogen Peroxide), for 0.5 g of sample	we followed the method indicated in your procedure	We used an external

Annex 6: z and zeta scores for all measurand

Lab code	Total Cd		Total Pb		Total As		extractable Cd		extractable Pb	
	z	zeta	z	zeta	z	zeta	z	zeta	z	zeta
L02	-1,5	-5,2	-2,5	-2,8	-1,6	-2,5	-1,4	-5,0	-2,4	-2,7
L03	0,6	2,8	0,4	0,6	2,2	5,9				
L04	-1,9	-0,1	-3,9	-0,1	-0,4	0,0	-2,0	-9,1	-3,9	-5,2
L05	0,2	0,3	-1,5	-1,6	-0,1	-0,2	-1,6	-2,9	-0,3	-0,3
L06	-1,0	-4,3	-1,5	-1,9	0,9	2,6	-1,1	-4,8	-0,1	-0,2
L07	0,6	2,2	-2,3	-3,0	-0,1	-0,1	-0,2	-0,8	-1,4	-1,8
L08	-4,1	-13,8	-3,0	-3,6	-3,3	-8,1	-4,7	-17,3	-2,9	-3,7
L09	-0,1	-0,1	-5,0	-6,5	0,3	0,3	0,4	0,9	-5,2	-7,0
L10	-2,8	-12,1	-1,4	-0,9	-1,7	-4,1	-3,1	-6,8	0,0	0,0
L11	0,0	-0,1	-0,7	-1,0	0,0	0,0	0,4	1,8	-0,2	-0,2
L12	0,0	-0,1	-1,5	-2,0	1,8	4,7	-1,4	-2,7	-2,1	-1,6
L13	-0,1	-0,5	-1,9	-2,3	-1,0	-2,2	-0,1	-0,2	-0,7	-0,9
L14	-0,8	-1,3	-1,4	-1,5	2,8	3,5	-0,8	-1,3	-1,5	-1,8
L15	-1,0	-1,9	-4,2	-5,1	1,4	1,6	-0,6	-1,0	-3,2	-3,6
L17							-0,4	-0,7	-1,1	-1,4
L19	-1,2	-5,3	-4,7	-6,1	-0,5	-1,3	-0,9	-4,0	-3,3	-4,6
L20	0,4	0,7	0,4	0,4	0,6	1,6	0,7	2,5	-1,1	-1,2
L21	-0,9	-2,2	-2,1	-0,9	-0,7	-1,4	-1,7	-2,1	-0,2	-0,1
L22	1,0	1,7	-3,8	-3,4	-0,6	-0,4	-0,5	-0,8	-3,2	-2,7
L23	-0,7	-2,2	-0,3	-0,3	0,0	0,1	-1,0	-3,3	1,4	1,7
L24	0,1	0,2	-0,9	-0,9	2,1	3,2				

Abstract
This report presents the results of an Interlaboratory Comparison (ILC) which focused on the determination of total Cd, Pb and As 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 mineral feed provided by AGES, Zentrum Analytik und Mikrobiologie, in Austria. 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 November 2008. Each participant received one bottle containing approximately 30 g of test material. Twenty-four participants from 11 countries registered to the exercise of which 19 submitted results for extractable Pb and Cd and 20 submitted results for total Pb, Cd and As. Three laboratories did not submit results.

The assigned values (Xref) for total and extractable Cd and Pb were provided by IRMM using isotope dilution-inductively coupled plasma-mass spectrometry (ID-ICP-MS). The analytical uncertainty of Xref, uchar, was calculated according to the ISO Guide to the Expression of Uncertainty in Measurement (GUM). The assigned value for total As was provided by the Studiecentrum voor Kernenergie (SCK-CEN) using neutron activation analysis. The analytical uncertainty of Xref, uchar, for total As was calculated according to GUM. Homogeneity and stability studies were subcontracted to Bayer Antwerpen. The uncertainties of the respective assigned values, uref, were calculated combining the analytical uncertainty, uchar, with a contribution for the between-bottle homogeneity, ubb, and for the short term stability of the test material, usts. Participants were invited to report the uncertainty of their measurements. This was done by 17 laboratories for extractable Pb and Cd and by 18 laboratories for total As, Cd and Pb.

The laboratory performance was evaluated using z and zeta scores in accordance with ISO 13528. The standard deviations for proficiency assessment (also called target standard deviation) were calculated using the modified Horwitz equation and were between 15 and 16 % for all the measurands.

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.

LA-NA-23776-EN-C

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