Proficiency test for heavy metals in compound feed

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Project leader: W.C.M. de Nijs

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P.O. Box 230, 6700 AA Wageningen, The Netherlands, T +31 (0)317 48 02 56, E info.RIKILT@wur.nl, www.wur.eu/rikilt. RIKILT is part of Wageningen University & Research.

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Distribution list:
- Twenty-one participating laboratories.

Contents

Summary

The here described proficiency test for heavy metals in compound feed was organised by RIKILT, Wageningen UR in accordance with ISO 17043. RIKILT Wageningen UR has an ISO/IEC 17043 accreditation for proficiency tests of residues in products of animal origin. However, this specific test is not part of the accreditation. The primary goal of this proficiency test was to give laboratories the opportunity to evaluate or demonstrate their competence for the analysis of heavy metals in compound feed.

For this proficiency test, two test materials were dispatched. The heavy metal concentration in each material is given in mg/kg with a moisture content of 12%:
- Chicken feed with a consensus value of 0.37 mg/kg cadmium and 5.2 mg/kg lead (A);
- Chicken feed with a consensus value of 0.62 mg/kg cadmium and 3.0 mg/kg arsenic (B).

The fortified materials were prepared by spiking a slurry of commercially obtained chicken compound feed, followed by extensive mixing and freeze-drying.

Homogeneity assessment showed that all materials were sufficiently homogeneous for proficiency testing. The stability test demonstrated no statistically significant loss of cadmium, lead or arsenic from the materials during the timescale of the proficiency test.

Twenty-one European laboratories participated in this proficiency test and submitted results in time. For test material A, twenty laboratories reported quantitative results and one laboratory reported a screening result for cadmium and for lead, twenty-one laboratories reported quantitative results. For test material B, twenty-one and nineteen laboratories reported quantitative results for respectively cadmium and arsenic. Two laboratories did not report quantitative results for arsenic due to the absence of the compound in their scope.

Fifteen laboratories reported details of the applied extraction method. Twelve of the fifteen laboratories applied comparable digestion methods for sample preparation and in general used ICP-MS (six laboratories) or AAS (four laboratories) for identification and quantification of the heavy metals in the materials.

The results of the proficiency test on heavy metals in feed are summarized in Table 1. Overall, 17 out of 21 participants showed optimal performance by detecting cadmium, lead and arsenic with a correct quantification/qualification in chicken compound feed.

Table 1 *Summarized performance of laboratories reporting results in the proficiency test on heavy metals 2016.*

Compound	# of laboratories	FN[1)]	Used z-score	Correct results %
Cadmium in A	21	0	Z_a	100
Lead in A	21	0	Z_a	90
Cadmium in B	21	0	Z_a	100
Arsenic in B	19	0	Z_a	89

1) FN = false negatives

1 Introduction

Proficiency testing is conducted to provide laboratories with a powerful tool to evaluate and demonstrate the reliability of the data that are produced by the laboratory. Proficiency testing is an important requirement of the EU Additional Measures Directive 93/99/EEC [1] and is demanded by ISO 17025:2005 [2].

The preparation of the test materials, including the homogeneity and stability testing of the test materials, and the evaluation of the quantitative results were carried out in accordance to the guidelines of the ISO/IEC 17043 [3] accreditation of RIKILT Wageningen UR. RIKILT Wageningen UR has an ISO/IEC 17043 accreditation for proficiency tests of residues in products of animal origin. However, matrix-compound used in this Proficiency test is not part of the accreditation.

Maximum levels of the heavy metals, cadmium (Cd), arsenic (As) and lead (Pb) are regulated in the EU in a.o. compound feed. The maximum levels (ML), as regulated in Regulation No 2002/32/EC [4] are: 0.5 mg/kg Cd, 5 mg/kg As and 2 mg/kg Pb, in compound feed with a moisture content of 12%.

The aim of this proficiency test was to give laboratories the opportunity to evaluate or demonstrate their competence for the analysis of heavy metals in compound feed.

2 Material and methods

This proficiency test concerns the heavy metals cadmium (Cd), arsenic (As) and lead (Pb) in compound feed. The target concentrations for this test are presented in Table 1.

2.1 Material preparation

Commercially available chicken compound feed was used to prepare the materials A and B for the proficiency test. Each of the two materials was prepared by grinding the compound feed through a 0.5 mm sieve. Two kilograms of each material were mixed with three litres of water and homogenized using a blender (according to in-house standard operating procedures [5]). The slurry of either material A or B was fortified by adding a 2 - 3% nitric acid solution of cadmium, lead and arsenic, aiming at the levels as presented in Table 2. The fortified slurries were immediately freeze-dried, homogenized and stored at room temperature until use.

Table 2 *Target added concentrations of heavy metals in the proficiency test materials.*

Material	Target concentration (mg/kg)		
	Cadmium	Lead	Arsenic
A	0.25	5	0
B	0.5	0	3

2.2 Sample identification

After freeze-drying and homogenization, the materials were divided into sub-portions of 15 gram and stored in polypropylene, airtight closed containers at room temperature. The samples for the participants were randomly selected and coded using a web application designed for proficiency tests (crlwebshop). The code used was Metals/2015/feed/000, in which the three digit number at the end of the code was automatically generated by the software. One sample set was prepared for each laboratory consisting of one random selected sample of each material A and B. The codes of the samples for each sample set are presented in Annex 1. For homogeneity and stability testing, 56 randomly selected containers of materials A and B were assigned.

2.3 Participants

Laboratories were asked to participate by an email sent to the laboratories present in the RIKILT Wageningen UR database and posting an announcement on the RIKILT Wageningen UR website. Twenty-one laboratories registered for the participation in the proficiency test. All of these laboratories are situated in Europe. Each participant was asked *a priori*, to indicate which compounds were included in the scope of their method. The participants were asked to report the results through a web application designed for proficiency tests.

2.4 Material distribution and instructions

Each of the participating laboratories received a randomly assigned laboratory code, generated by the web application. The sample sets with the corresponding number, consisting of two coded samples (Annex 1) were sent to the participating laboratories on January 18th 2016. The sample sets were packed in a box and were dispatched at room temperature to the participants immediately by courier. The samples were accompanied by a letter describing the requested analysis (Annex 3) and an acknowledgement of receipt form. By e-mail the laboratories received instructions on how to use the web application to report the results.

The laboratories were asked to store the samples according to their own laboratory procedure and to analyse the samples according to their routine method. A single analysis result for cadmium, lead and arsenic in each sample was requested. The deadline for submitting the quantitative results was February 29th 2016, allowing six weeks for the analysis.

Results should be reported for cadmium, lead and arsenic as mg/kg product with a moisture content of 12%. Participants were asked to provide some information on their analytical method (extraction solvent, clean-up procedure, internal standards used, detection technique, limit of detection, limit of quantification).

2.5 Homogeneity study of the test materials

The homogeneity of the test materials was tested according to The International Harmonized Protocol for Proficiency Testing of Analytical Laboratories [4] and ISO 13528 [6], taking into account the insights discussed by Thompson [7] regarding the Horwitz equation. With this procedure the between-sample standard deviation (s_s) and the within-sample standard deviation (s_w) are compared with the standard deviation for proficiency assessment derived from the Horwitz equation (σ_P, §4.3). The method applied for homogeneity testing is considered suitable if $s_w < 0.5*\sigma_P$ and a material is considered adequately homogeneous if $s_s < 0.3* \sigma_P$.

Ten containers of test materials A and B were analysed in duplicate for cadmium to determine the homogeneity of the materials. The homogeneity of the other compounds in the materials was not tested, since the homogeneity test of cadmium was considered adequate to prove sufficient homogeneity of the materials since the elements added were dissolved in one solution before spiking the matrix. The results of the homogeneity study (grand mean with the corresponding RSD) and their statistical evaluation of materials A and B are presented in Annex 2 and Table 3. Materials A and B demonstrated to be sufficiently homogeneous for use in the proficiency test.

The levels of cadmium in materials A and B were respectively 1.5 and 1.2 times higher than anticipated. This was due to the fact that the blank compound feed used for the preparation of the materials A and B contained traces of cadmium.

Table 3 *Concentration of cadmium in materials A and B obtained during homogeneity testing.*

Material code	Concentration (mg/kg)	RSD (%)
	Cadmium	Cadmium
A	0.37*	1.7
B	0.62*	1.6

* Blank materials contains traces of cadmium

2.6 Stability of the test materials

On January 18th 2016, the day the test materials were distributed to the participants, six randomly selected samples of each test material were stored at <-18 °C. It is assumed that the heavy metals are stable at these storage conditions. Also, six samples of each test material were stored at 4 °C and six at room temperature.

On March 4th 2016, 47 days after distribution of the samples, six samples of materials A and B that were stored at <-18 °C, at 4 °C and at room temperature were analysed for cadmium, lead and arsenic. For each set of test samples, the average of the results and the standard deviation were calculated.

A possible 'consequential instability' of the analytes was determined in the test materials stored at 4 °C or at room temperature [8, 6]. A consequential instability is observed when the average value of an analyte in the test samples stored at 4 °C or stored at room temperature is more than $0.3\sigma_H$ below the average value of the analyte in the samples stored at <-18 °C. If so, the instability has a significant influence on the calculated z-scores. A possible statistically significant instability was determined using a Students t-test [6]. The results and statistical evaluation of the stability test are presented in Annex 4.

For cadmium, lead and arsenic in test materials A and B, no consequential nor statistical significant difference were observed among the samples stored at <-18°C, the samples stored at 4 °C and the samples stored at room temperature. The compounds in the test materials are, therefore, considered stable for the duration of the study.

3 Applied methods of analysis

Twenty-one laboratories carried out quantitative analyses for cadmium and lead and nineteen laboratories carried out quantitative analyses for arsenic. An overview of the information provided by the participants regarding the quantitative methods applied in this proficiency test is presented in Annex 5.

Twelve laboratories applied ICP-MS (inductively coupled plasma mass spectrometry) for the identification and quantification of the heavy metals, two laboratories applied ICP-AES (inductively coupled plasma atomic emission spectroscopy) also referred as inductively coupled plasma optical emission spectrometry (ICP-OES), five laboratories applied AAS (atomic absorption spectroscopy) of which two laboratories used the graphite furnace (GFAAS) and one laboratory used the atomization flame (FAAS). One laboratory applied ICP-HRMS (inductively coupled plasma high resolution mass spectrometry), while one laboratory did not report the detection technique.

Twelve laboratories used microwave digestion for sample preparation and therefore different acid digestion procedures were employed for the determination of elements in compound feed. Six laboratories carried out the acid digestions with a mixture of nitric acid and hydrogen peroxide to bring the sample in the form of a solution in order to introduce it into the analyzer, one laboratory used a mixture of nitric acid, hydrogen peroxide combined with hydrofluoric acid, two laboratories used only nitric acid and three laboratories used microwave digestion but without further specifications. One laboratory ashed the sample dry at 550 °C and hydrochloric acid was used as an ashing acid. One laboratory digested the sample with a mixture of nitric acid and hydrochloric acid and diluted the extract for measurement and one laboratory centrifuge and filtrate the sample without further specifications. Six laboratories provided no details on the sample preparation conditions they used.

Ten laboratories reported the use of one or more internal standards. For arsenic one laboratory used germanium as an internal standard while one laboratory used scandium. For cadmium two laboratories used indium and for lead one laboratory used bismuth and one used indium. Other labs used iridium, beryllium, rhodium, lithium, gold, yttrium and tiberium without further specifications.

One laboratory reported the use of standard addition for quantification of the elements and one laboratory used standard addition only for arsenic.

4 Statistical evaluation

The statistical evaluation was carried out according to the International Harmonized Protocol for the Proficiency Testing of Analytical Laboratories [8], elaborated by ISO, IUPAC and AOAC and ISO 13528 [6] in combination with the insights published by the Analytical Methods Committee [9, 10] regarding robust statistics.

For the evaluation of the quantitative results, the consensus value, the uncertainty of the consensus value, the standard deviation for proficiency assessment and z-scores were calculated.

4.1 Calculation of the consensus value

The consensus value (X) was determined using robust statistics [6, 9, 10]. The advantage of robust statistics is that all values are taken into account: outlying observations are retained, but given less weight. Furthermore, it is not expected to receive normally distributed data in a proficiency test. When using robust statistics, the data do not have to be normally distributed in contrast to conventional outlier elimination methods.

The robust mean of the reported results of all participants, calculated from an iterative process that starts at the median of the reported results using a cut-off value depending on the number of results, was used as the consensus value [6, 10].

4.2 Calculation of the uncertainty of the consensus value

The uncertainty of the consensus value is calculated to determine the influence of this uncertainty on the evaluation of the laboratories. A high uncertainty of the consensus value will lead to a high uncertainty of the calculated participants z_a-scores. If the uncertainty of the consensus value and thus the uncertainty of the z_a-score is high, the evaluation could indicate unsatisfactory method performance without any cause within the laboratory. In other words, illegitimate conclusions could be drawn regarding the performance of the participating laboratories from the calculated z_a-scores if the uncertainty of the consensus value is not taken into account.

The uncertainty of the consensus value (the robust mean) is calculated from the estimation of the standard deviation of the consensus value and the number of values used for the calculation of the consensus value [6]:

$$u = 1.25 * \frac{\hat{\sigma}}{\sqrt{n}} \qquad \text{Equation I}$$

where:
u = Uncertainty of the consensus value;
n = Number of values used to calculate the consensus value;
$\hat{\sigma}$ = The estimate of the standard deviation of the consensus value resulting from robust statistics.

According to ISO 13528 [6] the uncertainty of the consensus value (u) is negligible and therefore does not have to be included in the statistical evaluation if:

$u \leq 0.3\sigma_P$ Equation II

where:
u = The uncertainty of the consensus value;
σ_P = Standard deviation for proficiency assessment (§4.3).

In case the uncertainty of the consensus value does not comply with this criterion, the uncertainty of the consensus value should be taken into account when evaluating the performance of the participants regarding the accuracy (§4.4). In case the uncertainty is > $0.7\sigma_P$ the calculated z-scores should not be used for evaluation of laboratories performance and are presented for information only.

4.3 Calculation of the standard deviation for proficiency assessment (σ_P)

According to Commission Decision 2002/657/EC [11], the coefficient of variation for the repeated analysis of a reference or fortified material under reproducibility conditions, shall not exceed the level calculated by the Horwitz equation. The Horwitz equation, $\sigma_H = 0.02c^{0.8495}$, presents a useful and widespread applied relation between the expected relative standard deviation of a singular analysis result under reproducibility conditions, and the concentration, c (g/g). It expresses inter-laboratory precision expected in inter-laboratory trials. Therefore, this relation is suitable for calculating the standard deviation for proficiency assessment in proficiency tests (σ_P).

Thompson [7] demonstrated that the Horwitz equation is not applicable to the lower concentration range (<120 µg/kg) as well as to the higher concentration range (>138 g/kg). Therefore a complementary model is suggested:

For analyte concentrations <120 µg/kg:

$\sigma_P = 0.22c$ Equation III

For analyte concentrations >138 g/kg:

$\sigma_P = 0.01c^{0.5}$ Equation IV

where:
σ_P = Expected standard deviation in proficiency tests;
c = Concentration of the analyte (g/g).

4.4 Performance characteristics with regard to the accuracy

For illustrating the performance of the participating laboratories with regard to the accuracy a z_a-score is calculated. For the evaluation of the performance of the laboratories, ISO 13528 [6] is applied. According to these guidelines z_a-scores are classified as presented in Table 4.

Table 4 *Classification of z_a-scores.*

$\lvert z_a \rvert \leq 2$	Satisfactory
$2 < \lvert z_a \rvert < 3$	Questionable
$\lvert z_a \rvert \geq 3$	Unsatisfactory

If the calculated uncertainty of the consensus value complies with the criterion mentioned in §4.2, the uncertainty is negligible. In this case the accuracy z-score is calculated from:

$$z_a = \frac{\overline{x} - X}{\sigma_P}$$ Equation V

where:
z_a = Accuracy z-score;
$\overline{x}$ = The average result of the laboratory;
X = Consensus value;
σ_P = Standard deviation for proficiency assessment.

However, if the uncertainty of the consensus value does not comply with the criterion mentioned in §4.2, it could influence the evaluation of the laboratories. Although, according to ISO 13528 in this case no z-scores can be calculated if a consensus value is used as the consensus value, we feel that evaluation of the participating laboratories is of main importance justifying the participating laboratories' effort. Therefore in this case, the uncertainty is taken into account by calculating the accuracy z-score [6]:

$$z'_a = \frac{\overline{x} - X}{\sqrt{\sigma_P^2 + u^2}}$$ Equation VI

where:
z'_a = Accuracy z-score taking into account the uncertainty of the consensus value;
$\overline{x}$ = The average result of the laboratory;
X = Consensus value;
σ_P = Standard deviation for proficiency assessment;
u = Uncertainty of the consensus value.

If a consequential instability of the proficiency test materials is observed, this can influence the evaluation of the laboratory performance. Therefore, in that case the consequential instability is taken into account when calculating z-scores. Because instability only regards one side of the confidence interval (a decrease of the concentration) this correction only applies to the lower 2s limit and results in an asymmetrical confidence interval.

In the case of a consequential instability the accuracy z-score for the laboratories that reported an amount below the consensus value is corrected for this instability by:

$$z_{ai} = \frac{\overline{x} - X}{\sqrt{\sigma_P^2 + \Delta^2}}$$ Equation VII

where:
z_{ai} = Accuracy z-score taking into account the instability of the consensus value;
$\overline{x}$ = The average result of the laboratory;
X = Consensus value;
σ_P = Standard deviation for proficiency assessment;
Δ = Difference between average concentration of compound stored at <-18 °C, 4 °C and average concentration at room temperature.

In some cases the uncertainty of the consensus value does not comply with the criterion in §4.2 and a consequential instability is observed. In this case the z'_a-score for the laboratories that reported an amount below the consensus value is corrected for this instability by:

$$z'_{ai} = \frac{\bar{x} - X}{\sqrt{\sigma_P^2 + \Delta^2 + u^2}}$$ Equation VIII

where:

z'_{ai} = Accuracy z-score taking into account the uncertainty and instability of the consensus value;

$\bar{x}$ = The average result of the laboratory;

X = Consensus value;

σ_P = Standard deviation for proficiency assessment;

Δ = Difference between average concentration of compound stored at <-18 °C, 4 °C and average concentration at room temperature;

u = Uncertainty of the consensus value.

5 Results

Twenty-one laboratories registered for the participation in the proficiency test and all submitted the results in time. Laboratories PT814 and PT820 did not report quantitative results for arsenic due to the absence of the compound in their scope. The performance of individual laboratories is summarized in Annex 7.

All laboratories reported results for cadmium, lead and arsenic in the test material A and B. However, test material A was not fortified with arsenic and test material B was not fortified with lead. Therefore, these compounds in the respective test materials were not evaluated.

Limits of detection (LODs) reported by the participants ranged from 0.0033 to 0.163 mg/kg for cadmium, from 0.005 to 1 mg/kg for lead and from 0.002 to 0.18 mg/kg for arsenic. Levels of quantification (LOQs) reported by the participants ranged from 0.01 to 0.5 mg/kg for cadmium, from 0.017 to 3 mg/kg for lead and from 0.002 to 0.85 mg/kg for arsenic (Annex 5).

An overview of the results on the analysis of the compounds for each laboratory is presented in Annex 6.

5.1 Test material A

Nineteen laboratories reported results for arsenic in test material A, which was not fortified with arsenic. Traces of arsenic were detected in test material A by eleven laboratories, which varied from 0.07 – 0.125 mg/kg. Two laboratories reported arsenic respectively < 0.1 and <0.3 mg/kg. Two laboratories reported arsenic as detected (see Annex 6).

5.1.1 Cadmium

Laboratory PT814 did not quantify cadmium in material A, but they reported it as a screening result. They reported cadmium present in the sample below the LOQ of 0.5 mg/kg of their method.

Twenty laboratories reported quantitative results for cadmium. The lowest concentration reported was 0.322 mg/kg and the highest was 0.468 mg/kg (see Annex 6). The consensus value was 0.37 mg/kg with a robust standard deviation of 0.022 mg/kg (resulting in an RSD_R of 6.0%) expressing the reproducibility within this proficiency test. The robust standard deviation of 0.022 mg/kg is 3 times lower than the target standard deviation σ_P of 0.068 mg/kg. Based on the results it is concluded that the quantification of cadmium in compound feed is reported with small ranges in this proficiency test.

The uncertainty of the consensus value was 0.006 mg/kg. Since this value does not exceed $0.3\sigma_P$ (0.020 mg/kg, §4.2), the uncertainty is not taken into account in the evaluation.

No consequential instability was observed for cadmium in the stability test during the storage period of 47 days. Therefore, the z_a-scores using equation V (§4.4), were calculated. With respect to the accuracy all results were satisfactory.

5.1.2 Lead

Twenty-one laboratories reported quantitative results for lead in material A. The lowest concentration reported was 3.45 mg/kg and the highest was 11.1 mg/kg (see Annex 6). The consensus value was 5.2 mg/kg with a robust standard deviation of 0.45 mg/kg (resulting in an RSD_R of 8.6%) expressing the reproducibility within this proficiency test. The robust standard deviation of 0.45 mg/kg is almost 1.5 times lower than the target standard deviation σ_P of 0.64 mg/kg. Based on the results it is

concluded that the quantification of lead in compound feed is reported with small ranges in this proficiency test.

The uncertainty of the consensus value was 0.12 mg/kg. Since this value does not exceed $0.3\sigma_P$ (0.193 mg/kg, §4.2), the uncertainty is not taken into account in the evaluation.

No consequential instability was observed for lead in the stability test during the storage period of 47 days. Therefore, the z_a-scores using equation V (§4.4), were calculated. With respect to the accuracy, laboratory PT812 reported a questionable result and laboratory PT844 produced an unsatisfactory result.

5.2 Test material B

Traces of lead were detected in test material B (not fortified with lead) by thirteen laboratories, which varied from 0.06 – 0.354 mg/kg. Two laboratories reported lead < 0.3 mg/kg. Five laboratories reported lead as not detected and one laboratory as detected (see Appendix 6).

5.2.1 Cadmium

Twenty-one laboratories reported quantitative results for cadmium in material B. The lowest concentration reported was 0.525 mg/kg and the highest was 0.781 mg/kg (see Annex 6). The consensus value was 0.62 mg/kg with a robust standard deviation of 0.043 mg/kg (resulting in an RSDR of 6.9%) expressing the reproducibility within this proficiency test. The robust standard deviation of 0.043 mg/kg is 2.5 times lower than the target standard deviation σ_P of 0.11 mg/kg. Based on the results it is concluded that the quantification of cadmium in compound feed is reported with small ranges in this proficiency test.

The uncertainty of the consensus value was 0.012 mg/kg. Since this value does not exceed σ_P (0.032 mg/kg, §4.2), the uncertainty is not taken into account in the evaluation.

No consequential instability was observed for cadmium in the stability test during the storage period of 47 days. Therefore, the z_a-scores using equation V (§4.4) were calculated. With respect to the accuracy all results were satisfactory.

5.2.2 Arsenic

Laboratories PT814 and PT820 did not report quantitative results for arsenic due to the absence of the compound from their scope.

Nineteen laboratories reported quantitative results for arsenic. The lowest concentration reported was 1.05 mg/kg and the highest was 4.10 mg/kg (see Annex 6). The consensus value was 3.0 mg/kg with a robust standard deviation of 0.31 mg/kg (resulting in an RSDR of 10%) expressing the reproducibility within this proficiency test. The robust standard deviation of 0.31 mg/kg is almost 1.3 times lower than the target standard deviation σ_P of 0.41 mg/kg. Based on the results it is concluded that the quantification of arsenic in compound feed is reported with small ranges in this proficiency test.

The uncertainty of the consensus value was 0.087 mg/kg. Since this value does not exceed $0.3\sigma_P$ (0.122 mg/kg, §4.2), the uncertainty is not taken into account in the evaluation.

No consequential instability was observed for arsenic in the stability test during storage period of 47 days. Therefore, the z_a-scores using equation V (§4.4) were calculated. With respect to the accuracy, laboratory PT818 reported a questionable result and laboratory PT816 produced an unsatisfactory result.

6 Discussion and conclusions

Twenty-one laboratories reported results for the proficiency test on heavy metals in two compound feed samples. The aim of this proficiency test was to give laboratories the possibility to evaluate or demonstrate their competence for the analysis of heavy metals in compound feed. Each participant was asked to indicate *a priori* which compounds were included in their scope. This allowed the evaluation of the results which regard to the laboratories' scope.

Two materials were sent to the participants. Cadmium, lead and arsenic were homogeneously distributed in the materials. An overview of each participant's performance is shown in Annex 7 and a summary of the results is presented in Table 5.

Table 5 *Summarized performance of laboratories reporting results in the proficiency test on heavy metals 2016.*

Compound	# of laboratories	FN[1)]	Used z-score	Correct results %
Cadmium in A	21	0	Z_a	100
Lead in A	21	0	Z_a	90
Cadmium in B	21	0	Z_a	100
Arsenic in B	19	0	Z_a	89

1) FN = false negatives

Of the 21 laboratories that applied a quantitative confirmatory method, seventeen laboratories showed optimal performance by detecting all compounds with sufficient sensitivity, a correct quantification of cadmium, lead and arsenic in the test materials A and B and the absence of false positive and false negative results.

Four laboratories reported questionable or unsatisfactory results. One laboratory reported a correct screening result for the analyte cadmium that was below the LOQ of their method. Two laboratories did not report quantitative results for arsenic due to the absence of the compound in their scope.

Based on the results of this proficiency test it was concluded that:

- None of the laboratories reported false negative results;
- All 21 laboratories showed satisfactory results for the analysis of cadmium in compound feed materials A and B (resp. consensus value of 0.37 mg/kg and 0.62 mg/kg);
- Nineteen out of 21 laboratories showed satisfactory quantitative results (90%) for lead in test material A (consensus value of 5.2 mg/kg);
- Seventeen out of 19 laboratories showed satisfactory quantitative results (89%) for arsenic in test material B (consensus value of 3.0 mg/kg);
- The laboratories applied similar digestion methods for the analysis of the heavy metals, in general using a microwave and the quantitative analysis are mostly performed with ICP-MS and AAS;
- There is a large range regarding the LOQs, varying from 0.01 to 0.5 mg/kg for cadmium, from 0.017 to 3 mg/kg for lead and from 0.002 to 0.85 mg/kg for arsenic

References

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Annex 1 Codification of the samples

Laboratory code	A*	B*
PT735	396	264
PT808	840	705
PT810	834	299
PT811	851	519
PT812	458	167
PT813	498	814
PT814	997	120
PT815	854	957
PT816	546	172
PT817	588	416
PT818	740	237
PT819	556	349
PT820	209	440
PT821	531	147
PT838	206	464
PT839	171	221
PT840	679	381
PT841	735	403
PT842	184	606
PT843	623	449
PT844	173	399

* All sample codes start with Metals/2015/Feed/

Annex 2 Statistical evaluation of homogeneity data of test materials

Statistical evaluation of homogeneity data of test material A for cadmium.

	Cadmium (mg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/A001	0.378	0.379
Hom/A002	0.382	0.378
Hom/A003	0.368	0.370
Hom/A004	0.379	0.367
Hom/A005	0.366	0.371
Hom/A006	0.368	0.366
Hom/A007	0.359	0.367
Hom/A008	0.364	0.363
Hom/A009	0.378	0.371
Hom/A010	0.376	0.372
Grand mean	0.371	
SD	0.006	
RSD (%)	1.68	
Cochran's test		
C	0.438	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	Horwitz: 0.069	
s_x	0.006	
s_w	0.004	
s_s	0.005	
Critical= 0.3 σ_P	0.021	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

RSD = relative standard deviation

Statistical evaluation of homogeneity data of test material B for cadmium.

	Cadmium (mg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/B001	0.623	0.625
Hom/B002	0.627	0.621
Hom/B003	0.620	0.611
Hom/B004	0.615	0.608
Hom/B005	0.605	0.604
Hom/B006	0.627	0.618
Hom/B007	0.598	0.604
Hom/B008	0.616	0.614
Hom/B009	0.621	0.630
Hom/B010	0.608	0.600
Grand mean	0.615	
SD	0.0096	
RSD (%)	1.55%	
Cochran's test		
C	0.203	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	Horwitz: 0.106	
s_x	0.009	
s_w	0.005	
s_s	0.009	
Critical= 0.3 σ_P	0.0318	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

sx = Standard deviation of the sample averages.

sw = Within-sample standard deviation.

ss = Between-sample standard deviation.

RSD = relative standard deviation

Annex 3 Instruction letter

RIKILT
WAGENINGEN UR

For quality of life

P.O. Box 230 | 6700 AE WAGENINGEN | The Netherlands

DATE
January 14, 2016

SUBJECT
Proficiency test heavy metals in coumpound feed

OUR REFERENCE
15/RIK0520

POSTAL ADDRESS
P.O. Box 230
6700 AE WAGENINGEN
The Netherlands

VISITORS' ADDRESS
Wageningen Campus
Building 123
Akkermaalsbos 2
6708 WB WAGENINGEN

INTERNET
www.wageningenUR.nl/rikilt

CoC NUMBER
09098104

HANDLED BY
D.P.K.H. Pereboom - de Fauw

TELEPHONE
+31 (0)317 480 355

EMAIL
pt.rikilt@wur.nl

Dear participant,

Thank you very much for your interest in the proficiency study for the analysis of heavy metals in compound feed. Hereby I send you a parcel containing two samples for RIKILT PT. Each sample consists of approximately 15 grams of test material. The samples may contain cadmium, lead and arsenic.

Please fill out the accompanied 'acknowledgement of receipt form' and return it immediately upon receipt of the samples, preferably by e-mail (pt.rikilt@wur.nl).

Instructions:

- After arrival store the samples according to your laboratory's procedures and treat the test material as if it was a sample for routine analysis.
- Determine the level of Cd, Pb, and As present in the test material, carry out a single analysis for each sample.
- The deadline for submitting test-results for this test is February 29th 2016.
- Please use the web application for entering your results: https://crlwebshop.wur.nl/apex/f?p=307:LOGIN.
- Report all results relative to a feed with a moisture content of 12%.

- Your username is:
- Your password is:
- Your lab code to enter this proficiency test is:

- Please inform us about your applied method and detection technique (preferably via the web application).

Please contact me if you have any questions or need any assistance.

With kind regards,

D. Pereboom

D.P.K.H. Pereboom - de Fauw
pt.rikilt@wur.nl

Wageningen UR (Wageningen University and various research institutes) is specialised in the domain of healthy food and living environment.

RIKILT, part of Wageningen UR, carries out research into the safety, quality and health of food and feed and provides consultancy services to (inter)national governmental authorities. RIKILT is ISO 17025 and ISO 17043 accredited (the accredited tests are described on www.rva.nl (no. L014 and R013).

Annex 4 Statistical evaluation of stability data

Statistical evaluation for cadmium in test material A.

Storage temperature	-20 °C	4 °C	room temperature
Time (days)	0	47	47
Calculated amounts (mg/kg)	0.364	0.353	0.363
	0.370	0.369	0.366
	0.360	0.365	0.366
	0.365	0.365	0.368
	0.365	0.363	0.361
	0.361	0.369	0.366
Average amount (mg/kg)	0.364	0.364	0.365
n	6	6	6
st. dev (mg/kg)	0.004	0.006	0.003
Difference		0.000	-0.001
$0.3*\sigma_H$		0.020	0.020
Consequential difference? Diff < $0.3*\sigma_H$		NO	NO

Statistical evaluation for lead in test material A.

Storage temperature	-20 °C	4 °C	room temperature
Time (days)	0	47	47
Calculated amounts (mg/kg)	4.55	4.42	4.56
	4.69	4.60	4.61
	4.47	4.60	4.56
	4.56	4.60	4.57
	4.50	4.58	4.57
	4.68	4.65	4.69
Average amount (mg/kg)	4.57	4.58	4.59
n	6	6	6
st. dev (mg/kg)	0.092	0.078	0.052
Difference		-0.001	-0.018
$0.3*\sigma_H$		0.175	0.174
Consequential difference? Diff < $0.3*\sigma_H$		NO	NO

Statistical evaluation for cadmium in test material B.

Storage temperature	-20 °C	4 °C	room temperature
Time (days)	0	47	47
Calculated amounts (mg/kg)	0.631	0.617	0.623
	0.623	0.613	0.630
	0.636	0.616	0.621
	0.618	0.634	0.604
	0.603	0.632	0.636
	0.617	0.625	0.617
Average amount (mg/kg)	0.621	0.623	0.622
n	6	6	6
st. dev (mg/kg)	0.012	0.009	0.011
Difference		-0.002	-0.0005
$0.3*\sigma_H$		0.032	0.032
Consequential difference? Diff < $0.3*\sigma_H$		NO	NO

Statistical evaluation for arsenic in test material B.

Storage temperature	-20 °C	4 °C	room temperature
Time (days)	0	47	47
Calculated amounts (mg/kg)	2.68	2.59	2.58
	2.70	2.61	2.69
	2.66	2.67	2.64
	2.65	2.72	2.64
	2.58	2.69	2.70
	2.60	2.63	2.67
Average amount (mg/kg)	2.65	2.65	2.65
n	6	6	6
st. dev (mg/kg)	0.046	0.049	0.043
Difference		-0.006	-0.008
$0.3*\sigma_H$		0.110	0.110
Consequential difference? Diff < $0.3*\sigma_H$		NO	NO

Annex 5 Overview of the applied methods

Lab	Destruction	Internal standard	LOD (mg/kg)			LOQ (mg/kg)			Detection method
			As	Cd	Pb	As	Cd	Pb	
PT735	Microwave digestion HNO3+H2O2, none clean-up	None				0.6	0.06	0.4	GFAAS
PT808	Microwave digestion 0.5g/50ml + HNO3 and H2O2	External calibration and spike correction				0.3	0.1	0.3	Inductively coupled plasma -Atomic Emission Spectrometer ICP-AES
PT810	Desintegration with conc HNO3 in microwave system					0.1	0.01	0.1	ICP-HRMS
PT811	Micowave destruction					0.02	0.02	0.1	ICP-MS
PT812	Closed microwave digestion (HNO3+H2O2)	Standard addition for As determination and external calibration for Pb, Cd.	0.067	0.0033	0.01	0.2	0.01	0.03	AAS
PT813	Microwave digestion with HNO3+H2O2+HF		0.18	0.075	0.5	0.85	0.25	1.8	AAS with standard addition method of calibration
PT814	not applied	not applied		0.163	1		0.5	3	FAAS
PT815	Mineralisation in open microwave, digestion mixture H2O2+HNO3, 0.5 g of sample, final volume 25ml	Ge (As); In (Cd); Bi (Pb)	0.006	0.006	0.09	0.02	0.02	0.3	ICP-MS,external calibration
PT816	Weight 0.5g sample. Microwave digestion in closed MW system with 5ml HNO3+1ml H2O2. Transfer to 50ml with DDW.		0.06	0.01	0.07	0.45	0.05	0.5	GFAAS AS:Matrix modificator 10ul Pd 500ug/ml; Cd/Pb:matrix modificator 5ul 1%NH4H2PO4+100ug/mg
PT817	Microwave digestion	In	0.002	0.02	0.02	0.002	0.03	0.04	ICP-MS
PT818	Addition of hydrogen peroxide and nitric acid with subsequent microwave digestion.	In/Ge/Ir				0.046	0.021	0.267	ICP-MS
PT819	Microwave digestion: 0.5g of sample + 5 mL HNO3 Final volume: 50 mL; Dilution 1/5; 1/10 or 1/50	In for Pb and Cd in standard mode; Sc for As in collision mode (He)							ICP-MS
PT820	'Verassen bij 550 °C. Koken met 2M HCL' ISO 27085:2009	Beryllium		0.075	0.1		0.15	0.2	ICP-OES
PT821		Scandium	0.1	0.01	0.05	0.2	0.02	0.1	ICP-MS

Lab	Destruction	Internal standard	LOD (mg/kg)			LOQ (mg/kg)			Detection method
PT838	Samples digested in nitric and hydrochloric acid mixture, then diluted for measurement. Arsenic measured using collision cell, helium mode.□Cadmium and lead in no gas mode.	Rhodium and Indium	0.005	0.005	0.005	0.017	0.017	0.017	Inductively coupled plasma mass spectrometry (ICP-MS)
PT839									ICP-MS
PT840									
PT841		based on NEN-EN-ISO 17294 1/2	0.01	0.005	0.01				ICP-MS
PT842	centrifugeren / filtreren	Li, Sc, Ge, Rh, Ir, Au	0.033	0.0033	0.016	0.1	0.01	0.05	ICP-MS
PT843	microgolfontsluiting	Bismut / Lithium / scandium / Yttrium / Indium / Tiberium	0.005	0.005	0.005	0.01	0.01	0.01	ICP-MS (Inductief gekoppeld plasma Massa Spectroscopie).
PT844			0.006	0.006	0.006	0.05	0.01	0.1	ICP-MS

Annex 6 Results

	Arsenic in A	Cadmium in A CV: 0.368 mg/kg u: 0.006 mg/kg σ_p: 0.068 mg/kg robust σ: 0.022 mg/kg		Lead in A CV: 5.15 mg/kg u: 0.121 mg/kg σ_p: 0.643 mg/kg robust σ: 0.445 mg/kg	
Labcode	Results (mg/kg)	Result(mg/kg)	z_a-score	Result (mg/kg)	z_a-score
PT735	nd	0.34	-0.41	5	-0.23
PT808	<0.3	0.37	0.03	5	-0.23
PT810	nd	0.36	-0.12	5.05	-0.15
PT811	0.081	0.359	-0.13	4.74	-0.63
PT812	nd	0.35	-0.26	3.45	**-2.64**
PT813	nd	0.36	-0.12	4.98	-0.26
PT814	nt	detected		5.53	0.60
PT815	0.094	0.346	-0.32	5.13	-0.03
PT816	detected	0.412	0.64	4.976	-0.27
PT817	0.074	0.322	-0.67	4.679	-0.73
PT818	0.116	0.468	1.46	6.216	1.66
PT819	detected	0.357	-0.16	5.38	0.36
PT820	nt	0.3548	-0.19	5.4666	0.50
PT821	0.075	0.36	-0.12	4.9	-0.38
PT838	0.088	0.407	0.57	5.72	0.89
PT839	0.087	0.372	0.06	4.89	-0.40
PT840	0.125	0.365	-0.04	4.576	-0.89
PT841	0.1	0.4	0.47	5.3	0.24
PT842	<0.1	0.381	0.19	5.1	-0.07
PT843	0.1	0.4	0.47	5.75	0.94
PT844	0.07	0.38	0.17	11.1	**9.25**

CV consensus value.

u uncertainty of consensus value.

nd not detected.

nt not tested for

* reported only screening results.

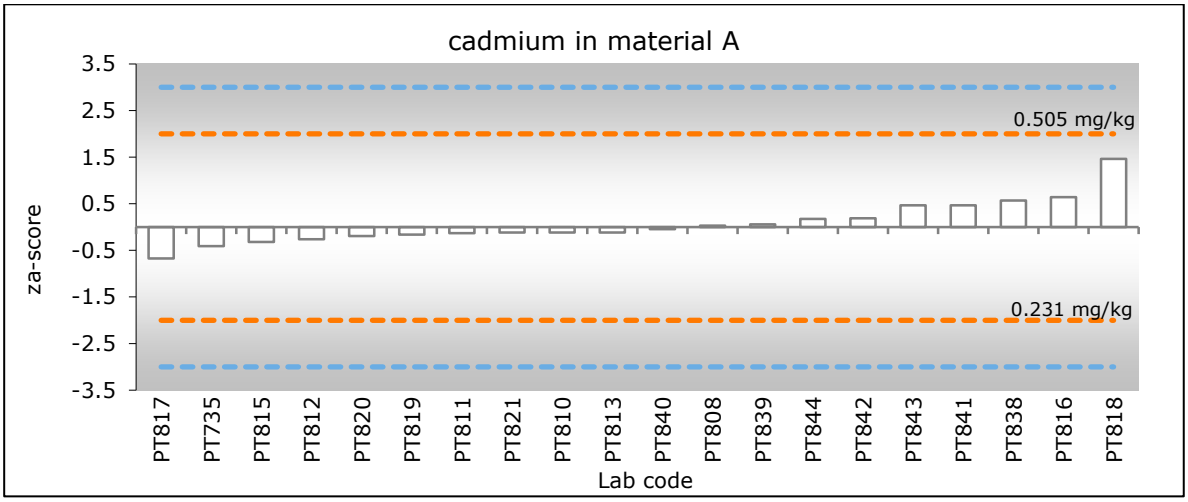

Figure a *Graphical representation of the z_a-scores. The X ± 2σ_P lines (dotted) are calculated according to equation V in §4.4.*

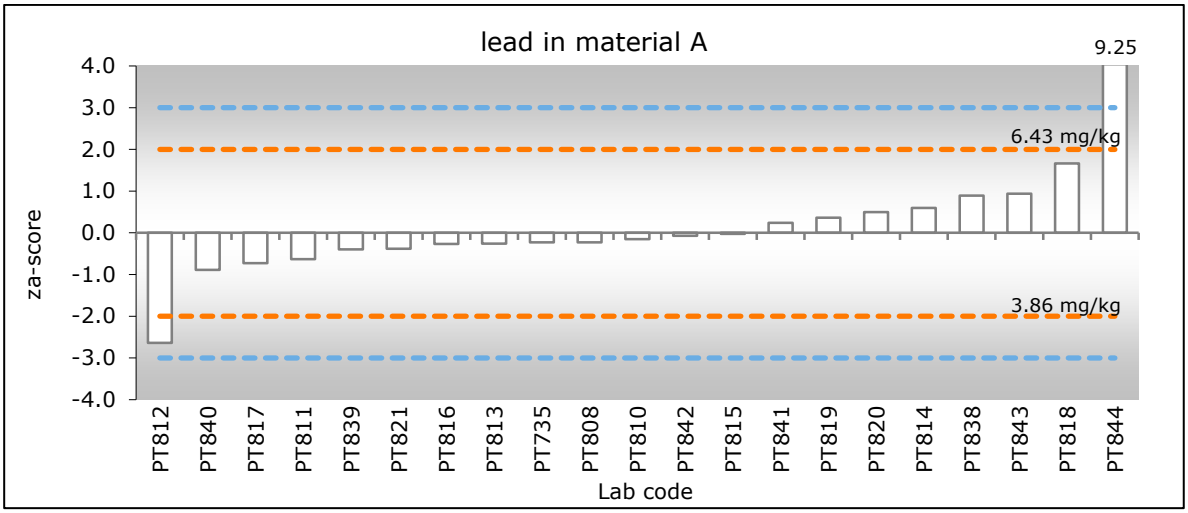

Figure b *Graphical representation of the z_a-scores. The X ± 2σ_P lines (dotted) are calculated according to equation V in §4.4.*

	Lead in B	Cadmium in B CV: 0.623 mg/kg u: 0.012 mg/kg σ_p: 0.107 mg/kg robust σ: 0.043 mg/kg		Arsenic in B CV: 3.01 mg/kg u: 0.087 mg/kg σ_p: 0.407 mg/kg robust σ: 0.305 mg/kg	
Labcode	Results (mg/kg)	Results(mg/kg)	Z_a-score	Results (mg/kg)	z_a-score
PT735	nd	0.64	0.16	3.1	0.23
PT808	<0.3	0.68	0.54	3	-0.01
PT810	nd	0.61	-0.12	3.05	0.11
PT811	nd	0.607	-0.15	2.99	-0.04
PT812	0.062	0.61	-0.12	2.64	-0.90
PT813	nd	0.59	-0.30	2.81	-0.48
PT814	nd	0.669	0.43	nt	
PT815	<0.3	0.569	-0.50	2.99	-0.04
PT816	detected	0.643	0.19	1.046	**-4.81**
PT817	0.06	0.525	-0.91	2.846	-0.39
PT818	0.086	0.781	1.48	4.097	**2.68**
PT819	0.075	0.548	-0.70	2.5	-1.24
PT820	0.0145	0.5962	-0.25	nt	
PT821	0.068	0.59	-0.30	2.98	-0.06
PT838	0.078	0.68	0.54	3.19	0.45
PT839	0.067	0.624	0.01	3.028	0.05
PT840	0.072	0.611	-0.11	3.287	0.69
PT841	0.07	0.69	0.63	3.4	0.97
PT842	0.055	0.631	0.08	3.52	1.26
PT843	0.07	0.67	0.44	3.12	0.28
PT844	0.354	0.615	-0.07	2.68	-0.80

CV consensus value.

u uncertainty of consensus value.

nd not detected.

* reported only screening results.

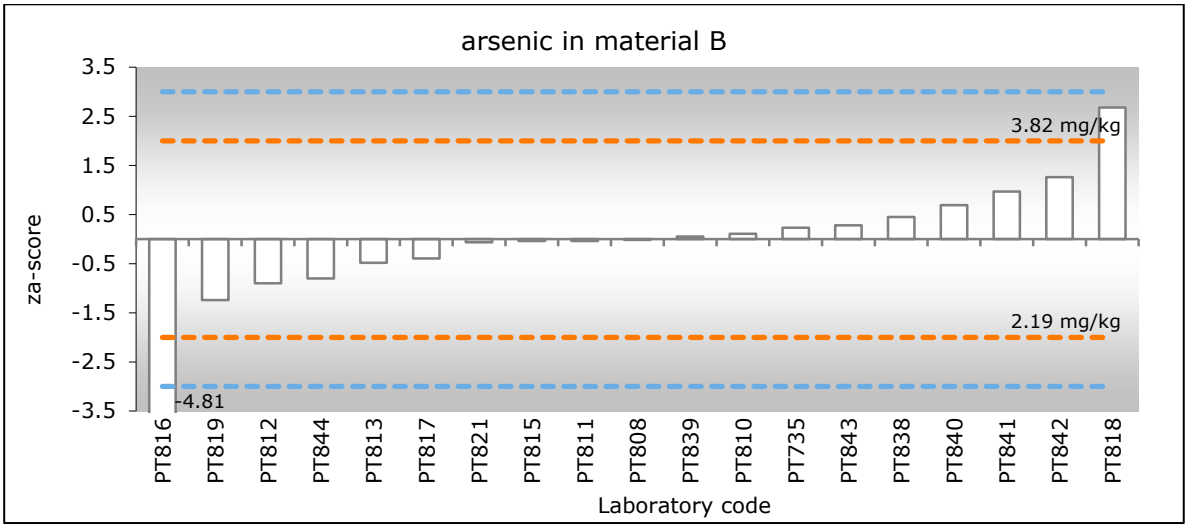

Figure c *Graphical representation of the z_a-scores. The X ± 2σ_P lines (dotted) are calculated according to equation V in §4.4.*

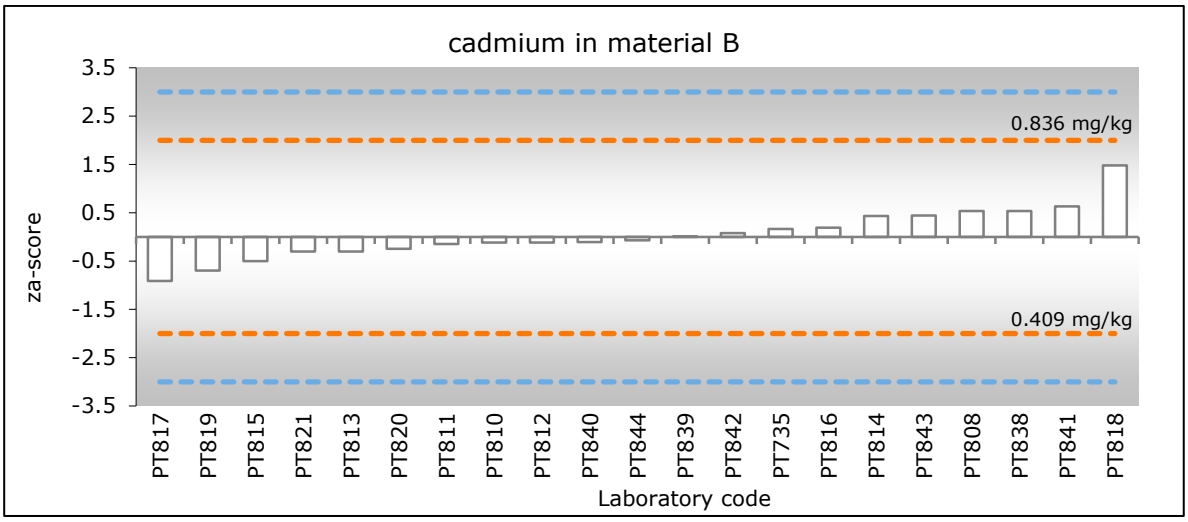

Figure d *Graphical representation of the z_a-scores. The X ± 2σ_P lines (dotted) are calculated according to equation V in §4.4.*

Annex 7 Overview performance per laboratory

Laboratory code	# of z-scores	Remarks
PT735	4	**Optimal performance**
PT808	4	**Optimal performance**
PT810	4	**Optimal performance**
PT811	4	**Optimal performance**
PT812	4	1 questionable z-score
PT813	4	**Optimal performance**
PT814	3	**Optimal performance**
PT815	4	**Optimal performance**
PT816	4	1 unsatisfactory z-score
PT817	4	**Optimal performance**
PT818	4	1 questionable z-score
PT819	4	**Optimal performance**
PT820	3	**Optimal performance**
PT821	4	**Optimal performance**
PT838	4	**Optimal performance**
PT839	4	**Optimal performance**
PT840	4	**Optimal performance**
PT841	4	**Optimal performance**
PT842	4	**Optimal performance**
PT843	4	**Optimal performance**
PT844	4	1 unsatisfactory z-score

RIKILT Wageningen University & Research
P.O. Box 230
6700 AE Wageningen
The Netherlands
T +31 (0)317 48 02 56
www.wur.eu/rikilt

RIKILT report 2016.015

To explore the potential of nature to improve the quality of life

RIKILT Wageningen University & Research
P.O. Box 230
6700 AE Wageningen
The Netherlands
T +31 (0)317 48 02 56
www.wur.eu/rikilt

Report 2016.015

The mission of Wageningen University and Research is "To explore the potential of nature to improve the quality of life". Under the banner Wageningen University & Research, Wageningen University and the specialised research institutes of the Wageningen Research Foundation have joined forces in contributing to finding solutions to important questions in the domain of healthy food and living environment. With its roughly 30 branches, 5,000 employees and 10,000 students, Wageningen University & Research is one of the leading organisations in its domain. The unique Wageningen approach lies in its integrated approach to issues and the collaboration between different disciplines.