BELGIAN NATIONAL REFERENCE LABORATORY FOR TRACE ELEMENTS IN FOOD AND FEED

PT-2018-NRL-TE-FASFC
Determination of As, As_i, Cd, Pb, Cu, Zn and Hg in baby food

FINAL REPORT ON THE 2018 PROFICIENCY TEST ORGANISED BY THE NATIONAL REFERENCE LABORATORY FOR TRACE ELEMENTS IN FOOD AND FEED

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Executive summary

From the 1st of January 2008, the laboratory for Trace Elements at Sciensano (former CODA-CERVA), Tervuren, operates as National Reference Laboratory for Trace Elements in Food and Feed (NRL-TE). One of its core tasks is to organise proficiency tests (PTs) among laboratories appointed by the Federal Agency for the Safety of the Food Chain. This report presents the results of the proficiency test organised by the NRL-TE which focused on the determination of trace elements in baby food. The results from the PT were treated in Sciensano, Tervuren.

The 2018 PT was obligatory for all laboratories approved for the analysis of heavy metals in foodstuff by the Federal Agency for the Safety of the Food Chain (FASFC). Eleven laboratories registered for and participated in the exercise.

The test material used in this test was a complete baby food containing spinach, white fish and rice, bought in a local supermarket. The choice for this matrix was based on the existing maximum levels for lead and cadmium in this matrix (Commission Regulation (EC) No 2006/1881 [1]). The material was spiked with Pb, homogenized and used as PT material. Each participant received approximately 20 g of homogenized test material.

Participants were invited to report the mean value and measurement uncertainty on their results for arsenic (As), inorganic arsenic (As_i), cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn) and mercury (Hg).

The assigned values (x_a) and their uncertainty ($u(x_a)$) were determined as the consensus of participant's results. Standard deviations for proficiency assessment were calculated using the modified Horwitz equation.

Of the 11 laboratories that registered for participation, 11 submitted results for As and Cd, 10 submitted results for Pb, Cu, Zn and Hg and five submitted results for As_i. The PT sample was not homogenous for Hg, so these data could not be used. All but one of the z-scores that were calculated, were satisfactory. Only one z-score was questionable and one quantification limit was unsatisfactory. One of the calculated ζ-scores were questionable, one laboratory did not mention their measurement uncertainty, resulting in four unsatisfactory ζ-scores.

The laboratories have proven their competence to measure the concerned trace elements in the matrix but extra attention should be given to formulate a correct compliance statement.

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INTRODUCTION

Trace elements occur in varying amounts as natural elements in soils, plants and animals, and consequentially in food and feed. To ensure public health, maximum levels for trace elements in foodstuff have been laid down in the Commission Regulation (EC) N° 1881/2006 [1]. Scientific opinions of the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM Panel) have led to developments of this commission regulation.

The EFSA CONTAM panel scientific opinion of 2009 concluded that the mean dietary exposures to cadmium in European countries are close to or slightly exceeding the Tolerable Weekly Intake (TWI) of 2,5 µg/kg body weight. Certain subgroups of the population may exceed the TWI by about 2 fold. The CONTAM Panel further concluded that, although adverse effects on kidney function are unlikely to occur for an individual exposed at this level, exposure to cadmium at the population level should be reduced. This opinion resulted in lower maximum limits for Cd in certain matrices [3]. The panel also concluded that processed cereal based foods and other baby foods for infants and young children are an important source of exposure to cadmium for infants and young children. A particular maximum level of cadmium (0.040 mg/kg) was therefore established for processed cereal based and other baby foods for infants and young children (Figure 1). The definition of babyfood is stated in Commission Regulation (EC) N° 609/2013 (Figure 2, [2]).

3.2.20	Processed cereal-based foods and baby foods for infants and young children (3) (29)	0,040 as from 1 January 2015

Figure 1 : Snapshot of maximum limits of Cd (mg/kg) in processed cereal-based foods and baby foods for infants and young children as published in [3].

(f) 'baby food' means food intended to fulfil the particular requirements of infants in good health while they are being weaned, and of young children in good health as a supplement to their diet and/or for their progressive adaptation to ordinary food, excluding:

(i) processed cereal-based food; and

(ii) milk-based drinks and similar products intended for young children;

Figure 2: Snapshot of the defition of babyfood as indicated in [2]

The EFSA CONTAM panel scientific opinion of 2010 identified a need to reduce exposure of Pb due to concern over possible neurodevelopmental effects in young children. This resulted in a specific maximum limit for Pb in processed cereal-based foods and babyfoods for infants and young children of 0.050 mg/kg (Figure 3).

3.1.3	Processed cereal-based foods and baby foods for infants and young children (3) (29) other than 3.1.5	0,050

Figure 3 : Snapshot of maximum limits of Pb (mg/kg) in processed cereal-based foods and baby foods for infants and young children as published in [4].

There is currently no European legislation regarding Cu, Zn, As or Hg in babyfood.

The scope of this PT was to test the competence of the participating laboratories to determine the total mass fraction of As, As_i, Cd, Pb, Cu, Zn and Hg in babyfood.

TIME FRAME, TEST MATERIAL AND INSTRUCTIONS TO PARTICIPANTS

Invitation letters to this PT were sent to participants in April (Annex 1). The 2018 PT was obligatory for all laboratories approved for the analysis of heavy metals in foodstuff by the Federal Agency for the Safety of the Food Chain (FASFC). Eleven laboratories, which were approved for these foodstuffs, registered for and participated in the exercise. The samples were dispatched to the participants by the end of May 2018. Reporting deadline was the 22nd of June.

This year the test material was a sample of complete babyfood containing spinach, white fish and rice. The sample was purchased in a local supermarkrt. The sample was spiked with $Pb(NO_3)_2$ and homogenized. After spiking, the sample was sterilized and divided in small portions, close air-tied. The samples were stored in the fridge (4°C).

The homogeneity of the test materials was tested following the recommended procedure according to IUPAC [5]. The trace elements appeared to be homogeneously distributed in the samples, except Hg (Annex 2). Each participant received the test material samples, an accompanying letter (Annex 3) with instructions on sample handling and reporting (Annex 4), a form that had to be sent after receipt of the samples to confirm their arrival (Annex 5) and a reporting form (Annex 6).

Participants were instructed to store the materials at 4°C in the dark until analysis. Before starting the analyses, the samples had to be re-homogenized following routine analysis. The procedure followed for the exercise, had to be as close as possible to the method used by the participant in routine sample analysis. Nevertheless participants were instructed to perform three independent measurements per parameter and to report measurement uncertainty. The laboratories were asked to make a compliance statement based on their results.

A questionnaire was attached to the reporting form. The questionnaire was intended to provide further information on the measurements and the laboratories. A copy of the questionnaire is presented in Annex 6.

Laboratory codes were given randomly and communicated confidentially to the corresponding participant.

ASSIGNED VALUES

The assigned values for the different trace elements in the babyfood sample were determined as the consensus of participant's results. The major advantages of consensus values are the straightforward calculation and the fact that none of the participants is accorded higher status. The disadvantages are that the consensus values are not independent of the participant's results and, especially in the current case with 11 participants, that the uncertainty on the consensus (identified as the standard error) may be high and the information content of the z-scores will be correspondingly reduced. However, the IUPAC guide of 2010 on the selection and use of proficiency testing schemes for a limited number of participants [6] states that if the standard uncertainty of the assigned value $u(x_a)$ is insignificant in comparison to the fit-for-intended-use target standard deviation σ_p $(u(x_a)^2 < 0.1 * \sigma_p^2)$, then z-scores can be calculated in a small scheme in the same matter as for a large scheme. This was the case for all elements (except As_i). A minimum of eight quantified results is accepted to calculate z- and ζ-scores (eight is the minimum number to create a Kernel density distribution).

First, it was checked whether the distribution of the reported results was apparently unimodal and roughly symmetric, possible extreme outliers aside. A Kernel distribution with a bandwith of 0.75 σ_p was plotted. It was analysed if this resulted in a unimodal and roughly symmetric kernel density, and if the mode and median were nearly coincident. If this was the case, robust statistics were accepted.

The ISO 13528:2015 guide was followed for the robust statistical analysis. There are many different robust estimators of mean ($\hat{\mu}_{rob}$) and standard deviation ($\hat{\sigma}_{rob}$) [7], [8]. The median and nIQR (normalised InterQuartile Range) were chosen here as robust estimators.

$$\hat{\mu}_{rob} = \text{median } (x)$$

$$\hat{\sigma}_{rob} = nIQR(x) = 0.7413(Q_3(x) - Q_1(x))$$

The standard uncertainty of the assigned value $u(x_a)$ was estimated as:

$$u(x_a) = 1.25 \frac{\hat{\sigma}_{rob}}{\sqrt{n}}$$

With n the number of quantified results.

The factor 1.25 is based on the standard deviation of the median, or the efficiency of the median as an estimate of the mean. This factor has been recommended because proficiency testing results typically are not strictly normally distributed, and contain unknown proportions of results from different distributions.

The modified Horwitz equation was used to establish the standard deviation for proficiency testing (σ_p) [5][9]. It is an exponential relationship between the variability of chemical measurements and concentration. The Horwitz value is widely recognized as a fitness-for-purpose criterion in proficiency testing in food analysis.

For As_i, only two quantified results were available, no value was assigned for this element and no scores are calculated.

The consensus values, their standard uncertainty and some other statistical parameters are summarised in Table 1.

Table 1 : Summary of statistical parameters for the test material.

	As mg/kg	**Cd** mg/kg	**Pb** mg/kg	**Cu** mg/kg	**Zn** mg/kg
n (number of participants with quantifiable result)	11	10	10	8	10
Mean	0.139	0.014	0.076	0.49	2.6
Standard deviation (SD)	0.010	0.002	0.008	0.041	0.31
Robust mean (median)	0.140	0.013	0.074	0.50	2.7
Robust SD (nIQR)	0.008	0.001	0.003	0.020	0.15
Assigned value x_a	**0.14**	**0.013**	**0.074**	**0.50**	**2.7**
Standard uncertainty of the assigned value $u(x_a)$	**0.003**	**0.0004**	**0.001**	**0.009**	**0.06**
σ_p	0.030	0.0029	0.016	0.088	0.37

Assigned value x_a: median of the reported results; σ_p: standard deviation for proficiency assessment.

Scores and Evaluation Criteria

Individual laboratory performances are expressed in terms of z-scores and ζ-scores in accordance with ISO 13528:2015 and the International Harmonised Protocol [5], [8].

$$z = \frac{x_{lab} - x_a}{\sigma_p}$$

$$\zeta = \frac{x_{lab} - x_a}{\sqrt{u^2(x_a) + u^2(x_{lab})}}$$

where:

x_{lab} is the mean of the individual measurement results as reported by the participant

x_a is the assigned value

σ_p is the standard deviation for proficiency assessment

$u(x_a)$ is the standard uncertainty for the assigned value

$u(x_{lab})$ is the reported standard uncertainty on the reported value x_{lab}. When no uncertainty was reported by the laboratory, it was set to zero.

The z-score compares the participant's deviation from the reference value with the standard deviation accepted for the proficiency test, σ_p. Should participants feel that these σ values are not fit for their purpose they can recalculate their scorings with a standard deviation matching their requirements.

The z-score can be interpreted as:

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

The ζ-score states if the laboratory result agrees with the assigned value within the uncertainty claimed by this laboratory (taking due account of the uncertainty on the reference value itself). The interpretation of the ζ-score is similar to the interpretation of the z-score.

$\lvert \zeta \rvert \leq 2$	satisfactory result
$2 < \lvert \zeta \rvert \leq 3$	questionable result
$\lvert \zeta \rvert > 3$	unsatisfactory result

RESULTS

ARSENIC (As)

x_a = **0.14** ± 0.006 mg/kg (k = 2)

Eleven laboratories submitted results for total As concentrations. The median of the 11 results was used as assigned value. All laboratories obtained satisfactory z-scores for As against the standard deviation accepted for the proficiency test (Table 2, Figure 4). In addition, 10 laboratories obtained satisfactory ζ-scores against their stated measurement uncertainty. One laboratory (L08) obtained an unsatisfactory ζ-score, due to the fact that no measurement uncertainty was reported.

Table 2 : values reported for As (mg/kg) by the participants and scores calculated by the organizer

Lab code	Result 1 (mg kg^{-1})	Result 2 (mg kg^{-1})	Result 3 (mg kg^{-1})	Mean (mg kg^{-1})	Extended uncertainty (k = 2) (u_{lab}; mg kg^{-1})	z-scores	ζ-scores
1	0.15	0.15	0.15	**0.15**	0.040	0.3	0.5
2	0.13	0.15	0.15	**0.14**	0.050	0.0	0.0
3	0.15	0.15	0.13	**0.14**	0.034	0.1	0.2
5	0.13	0.13	0.13	**0.13**	0.031	-0.3	-0.6
6	0.15	0.14	0.15	**0.15**	0.007	0.2	1.1
7	0.14	0.14	0.14	**0.14**	0.022	-0.1	-0.2
8				**0.12**	0.000	-0.7	-7.2
9	0.16	0.14	0.13	**0.15**	0.004	0.2	1.4
10	0.15	0.15	0.15	**0.15**	0.030	0.3	0.7
11	0.14	0.14	0.15	**0.14**	0.030	0.0	0.0
12	0.13	0.13	0.13	**0.13**	0.021	-0.3	-0.9

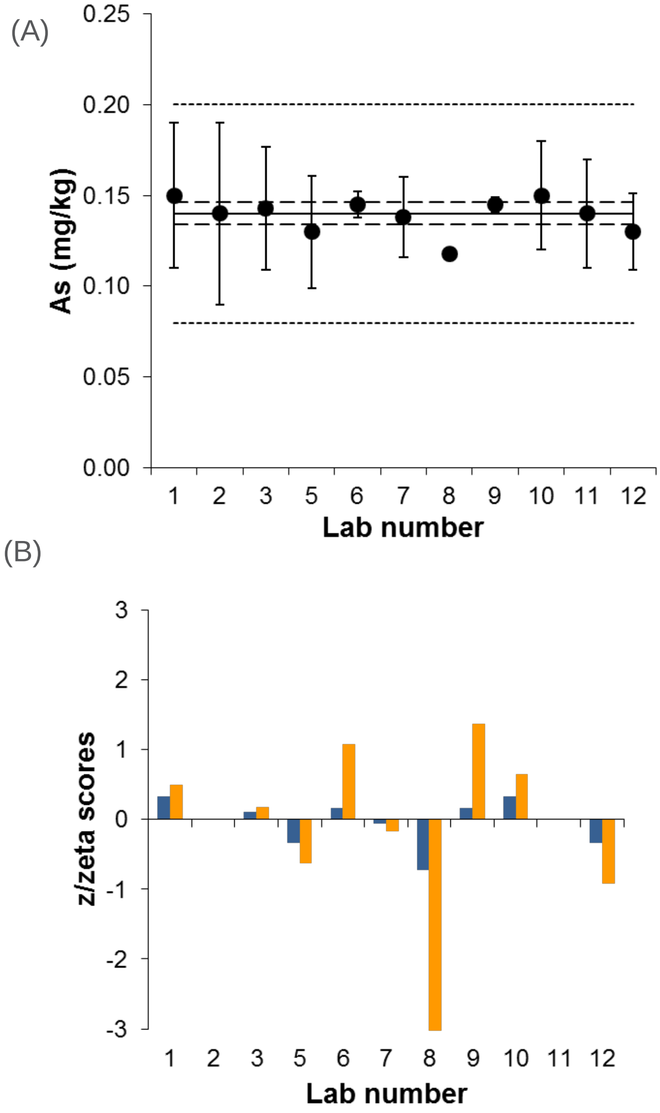

Figure 4 : (a) Results with expanded uncertainty for As, as reported by the participants (dashed lines: $x_a \pm 2\ u(x_a)$, dotted lines: $x_a \pm 2\ \sigma_p$, and (b) z (blue bars) and ζ-scores (orange bars)

INORGANIC ARSENIC (As_i)

Five laboratories submitted results for As_i concentrations, with only two quantified results. No scores were calculated and results were variable. Due to the low concentration range, no conclusions are drawn for this analyte.

Table 3 : values reported for As_i (mg/kg) by the participants.

Lab code	Result 1 (mg kg^{-1})	Result 2 (mg kg^{-1})	Result 3 (mg kg^{-1})	Mean (mg kg^{-1})	Extended uncertainty (k = 2) (u_{lab}; mg kg^{-1})
1	0.002	0.003		**0.003**	0.0005
2	<0.02	<0.02	<0.02	**<0.02**	
5	0.010	0.011	0.010	**0.010**	0.003
7	<0.027	<0.027	<0.027	**<0.027**	
10	<0.02	<0.02	<0.02	**<0.02**	

CADMIUM (Cd)

x_a = **0.013** ± 0.0008 mg/kg (k = 2)

Eleven laboratories submitted results for Cd concentrations. The median of ten results was used as assigned value. One laboratory could not obtain results above their limit of quantification. All ten laboratories obtained satisfactory z-scores for Cd against the standard deviation accepted for the proficiency test (Table 4, Figure 5). Nine laboratories did also obtain good ζ-scores against their stated measurement uncertainty. One laboratory (L08) obtained an unsatisfactory ζ-score due to the fact that no measurement uncertainty was reported. The quantification limits of L06 was not lower than the corresponding x_a-3 $u(x_a)$ value, so the statements is satisfactory.

Table 4 : values reported for Cd (mg/kg) by the participants and scores calculated by the organizer

Lab code	**Result 1 (mg kg^{-1})**	**Result 2 (mg kg^{-1})**	**Result 3 (mg kg^{-1})**	**Mean (mg kg^{-1})**	**Extended uncertainty (k = 2) (*u_{lab}*; mg kg^{-1})**	**z-scores**	**ζ-scores**
1	0.014	0.015	0.015	**0.015**	0.003	0.7	1.3
2	0.013	0.014	0.013	**0.013**	0.004	0.0	0.0
3	0.018	0.018	0.014	**0.017**	0.004	1.4	2.0
5	0.013	0.012	0.013	**0.013**	0.003	0.0	0.0
6				**<0.15**			
7	0.013	0.013	0.013	**0.013**	0.002	0.0	0.0
8				**0.011**	0.000	-0.7	-4.6
9	0.013	0.013	0.012	**0.013**	0.003	0.0	0.0
10	0.013	0.013	0.013	**0.013**	0.002	0.0	0.0
11	0.015	0.013	0.016	**0.015**	0.003	0.7	1.3
12	0.013	0.013	0.014	**0.013**	0.003	0.0	0.0

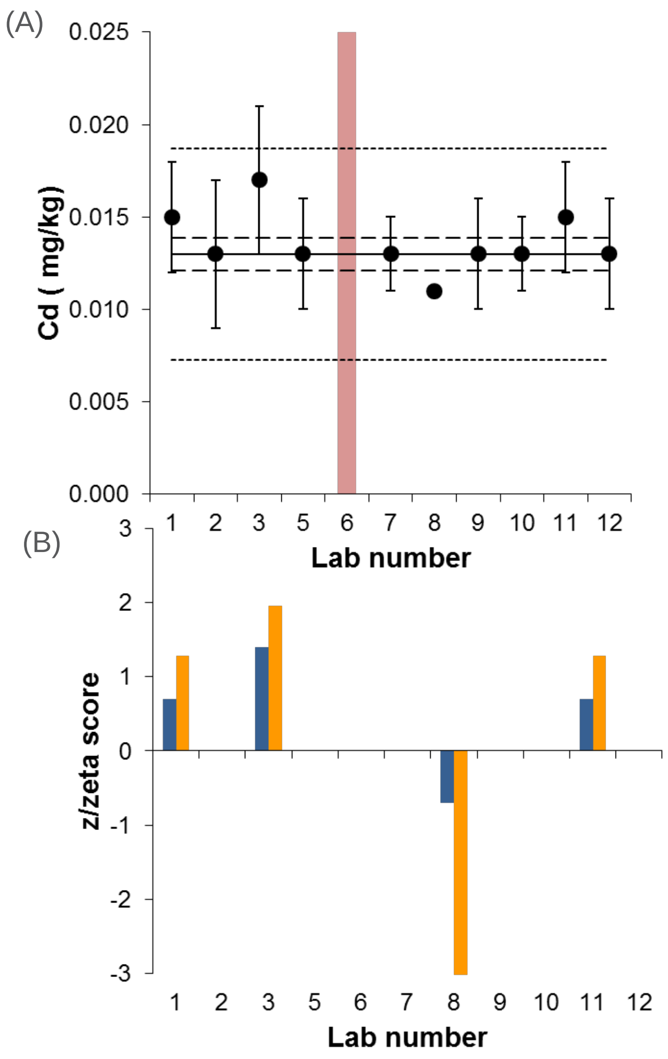

Figure 5 : (a) Results with expanded uncertainty for Cd, as reported by the participants (dashed lines: $x_a \pm 2\ u(x_a)$, dotted lines: $x_a \pm 2\ \sigma_p$, red bars represent the limits of quantification of the corresponding labs with the y-axis cut-off at 0.025 mg/kg) and (b) z (blue bars) and ζ-scores (orange bars)

LEAD (Pb)

x_a = **0.074** ± 0.002 mg/kg (k = 2)

Ten laboratories submitted results for total Pb concentrations. The median of all results was used as assigned value. All laboratories obtained satisfactory z-scores for Pb against the standard deviation accepted for the proficiency test (Table 5, Figure 6). All laboratories did obtain also satisfactory ζ-scores against their stated measurement uncertainty.

Table 5 : values reported for Pb (mg/kg) in by the participants and scores calculated by the organizer

Lab code	Result 1 (mg kg^{-1})	Result 2 (mg kg^{-1})	Result 3 (mg kg^{-1})	Mean (mg kg^{-1})	Extended uncertainty (k = 2) (u_{lab}; mg kg^{-1})	z-scores	ζ-scores
1	0.079	0.078	0.080	**0.079**	0.019	0.3	0.6
2	0.072	0.072	0.076	**0.074**	0.026	0.0	0.0
3	0.098	0.097	0.100	**0.099**	0.033	1.6	1.5
5	0.076	0.080	0.070	**0.075**	0.026	0.1	0.1
6							
7	0.072	0.074	0.071	**0.073**	0.014	0.0	-0.1
8				**0.072**	0.000	-0.1	-1.2
9	0.077	0.067	0.066	**0.070**	0.015	-0.2	-0.5
10	0.074	0.070	0.069	**0.071**	0.011	-0.2	-0.4
11	0.079	0.070	0.082	**0.077**	0.016	0.2	0.4
12	0.077	0.068	0.074	**0.073**	0.015	0.0	-0.1

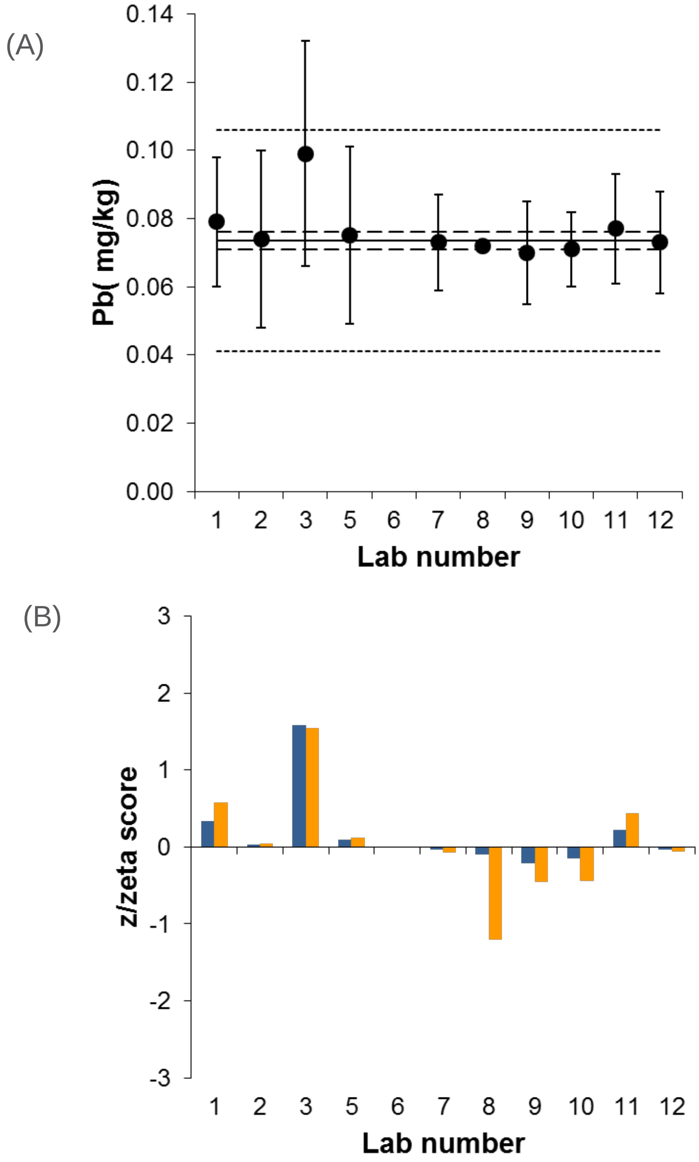

Figure 6 : (a) Results with expanded uncertainty for Pb, as reported by the participants (dashed lines: $x_a \pm 2\ u(x_a)$, dotted lines: $x_a \pm 2\ \sigma_p$) and (b) z (blue bars) and ζ-scores (orange bars)

COPPER (Cu)

x_a = 0.50 ± 0.018 µg/kg (k = 2)

Ten laboratories submitted results for Cu concentrations. Two laboratories could not obtain results above their limit of quantification. The median of the other eight results was used as assigned value. All eight laboratories obtained satisfactory z-scores for Cu against the standard deviation accepted for the proficiency test (Table 6, Figure 7). Six laboratories did obtain also satisfactory ζ-scores against their stated measurement uncertainty. One laboratory (L09) obtained a questionable ζ-score. One laboratory (L08) obtained an unsatisfactory ζ-score due to the fact that no measurement uncertainty was reported. The quantification limit of L02 was not lower than the corresponding x_a-3 $u(x_a)$ value, so the statements is satisfactory. However, the quantification limit of L06 was much lower than the corresponding x_a-3 $u(x_a)$ value, this results is unsatisfactory.

Table 6 : values reported for Cu (mg/kg) by the participants and scores calculated by the organizer

Lab code	Result 1 (mg kg^{-1})	Result 1 (mg kg^{-1})	Result 1 (mg kg^{-1})	Mean (mg kg^{-1})	Extended uncertainty (k=2) (u_{lab}; mg kg^{-1})	z-scores	ζ-scores
1	0.52	0.53	0.53	**0.53**	0.09	0.4	0.8
2	<1	<1	<1	**<1**			
3	0.52	0.52	0.46	**0.50**	0.10	0.1	0.1
5	0.48	0.47	0.53	**0.49**	0.16	-0.1	-0.1
6				**<0.006**			
7	0.50	0.51	0.51	**0.50**	0.10	0.1	0.1
8				**0.41**	0.00	-1.0	-9.7
9	0.48	0.54	0.55	**0.52**	0.01	0.3	2.4
10	0.45	0.43	0.43	**0.44**	0.09	-0.6	-1.2
11	0.47	0.49	0.50	**0.49**	0.09	-0.1	-0.1
12							

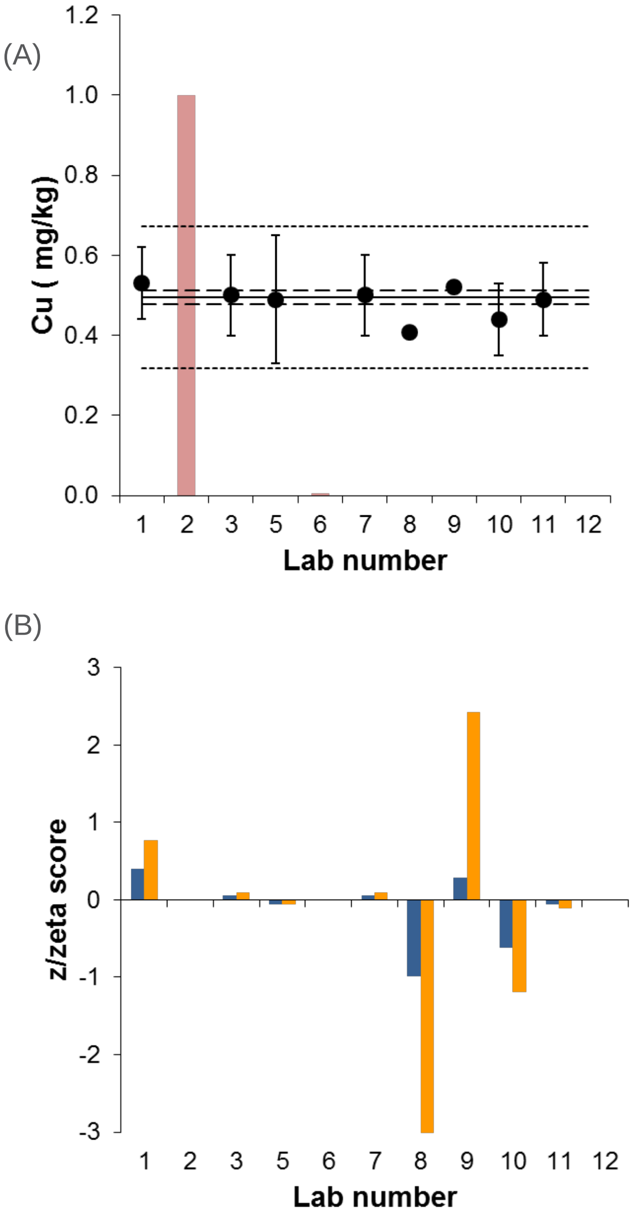

Figure 7 : (a) Results with expanded uncertainty for Cu, as reported by the participants (dashed lines: $x_a \pm 2\ u(x_a)$, dotted lines: $x_a \pm 2\ \sigma_p$, red bars represent the limits of quantification of the corresponding labs) and (b) z (blue bars) and ζ-scores (orange bars)

ZINC (Zn)

x_a = **2.7** ± 0.12 mg/kg (k = 2)

Ten laboratories submitted results for Zn concentrations. The median of all results was used as assigned value. Nine laboratories obtained satisfactory z-scores for Zn against the standard deviation accepted for the proficiency test (Table 7, Figure 8). The same nine laboratories did obtain also satisfactory ζ-scores against their stated measurement uncertainty. One laboratory (L08) obtained an questionable z-score. The same laboratory did not report a measurement uncertainty value and obtained an unsatisfactory ζ-score.

Table 7 : values reported for Zn (mg/kg) by the participants and scores calculated by the organizer

Lab code	Result 1 (mg kg^{-1})	Result 1 (mg kg^{-1})	Result 1 (mg kg^{-1})	Mean (mg kg^{-1})	Extended uncertainty (k = 2) (u_{lab}; mg kg^{-1})	z-scores	ζ-scores
1	2.9	3.0	2.9	**3.0**	0.4	0.8	1.4
2	2.8	2.8	2.9	**2.8**	0.5	0.3	0.4
3	2.8	3.0	2.9	**2.9**	0.6	0.5	0.7
5	2.7	2.5	2.8	**2.7**	0.3	0.0	0.0
6	2.6	2.7	2.8	**2.7**	0.2	0.0	0.0
7	2.6	2.6	2.7	**2.6**	0.4	-0.3	-0.5
8				**1.9**	0.0	-2.1	-13.1
9	2.4	2.4	2.3	**2.3**	1.8	-1.1	-0.4
10	2.6	2.6	2.5	**2.6**	0.8	-0.3	-0.2
11	2.8	2.6	2.9	**2.8**	0.4	0.3	0.5
12							

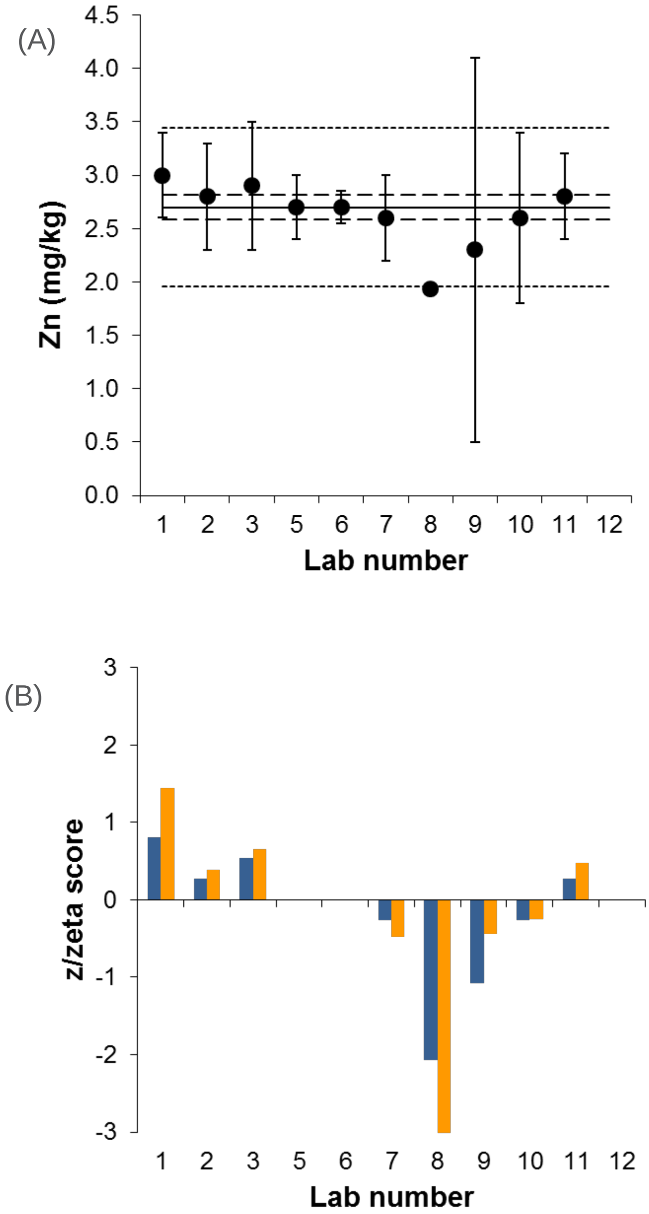

Figure 8 : (a) Results with expanded uncertainty for Zn, as reported by the participants (dashed lines: $x_a \pm 2\ u(x_a)$, dotted lines: $x_a \pm 2\ \sigma_p$, and (b) z (blue bars) and ζ-scores (orange bars)

DISCUSSION AND CONCLUSION

The most commonly used technique for the analysis of As, Cd, Pb, Cu and Zn was ICP-MS (Inductively Coupled Plasma-Mass Spectrometry). Only one exception was noticed by a lab which uses INAA (Instrumental Neutral Activation Analysis). For Cu and/or Zn some laboratories used ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry). No scores were calculated for mercury as the samples were not homogenous for this element.

As for inorganic As, the samples were all analysed by ICP-MS. Three laboratories used HPLC (High Performance Liquid Chromatography), coupled to the ICP-MS, as separation method and two laboratories used SPE (Solid Phase Extraction).

The laboratories were asked to state if the sample is compliant according to the current legislation. In Commission Regulation (EC) 333/2007 [10] it is described when a sample is accepted:

> *"The lot or sublot is accepted if the analytical result of the laboratory sample does* ***not exceed*** *the respective maximum level as laid down in Regulation (EC) No 1881/2006* ***taking into account the expanded measurement uncertainty*** *and correction of the result for recovery if an extraction step has been applied in the analytical method used. The lot or sublot is rejected if the analytical result of the laboratory sample* ***exceeds beyond reasonable doubt*** *the respective maximum level as laid down in Regulation (EC) No 1881/2006* ***taking into account the expanded measurement uncertainty*** *and correction of the result for recovery if an extraction step has been applied in the analytical method used."*

For the concerned matrix babyfood there are maximum limits for Cd (ML=0.040 mg/kg) and Pb (ML=0.050 mg/kg) (Table 8). The laboratories were asked to give their compliance statement for their measurement. The measured concentration should be compared with the ML taking into account the expanded measurement uncertainty on the measured concentration. This means that a sample is non-compliant if x_{lab}-U(x_{lab})>ML. Table 8 shows this exercise for the participants. Compliance statements are indicated as well. One laboratory stated that they could not make a compliance statement due to lack of data. Three laboratories stated the sample as compliant, however, the concentration of Pb minus their measurement uncertainty (x_{lab}-U(x_{lab}) was higer than the ML. Seven laboratories stated that the sample was not compliant. Here one laboratory did not take into account their measurement uncertainty, the sample should be compliant.

Table 8 : Compliance statements for the PT sample of babyfood by the participating laboratories (no(n) or yes(y)) with regard to the current legislation, and comparison with the measured values minus the expanded measurement uncertainty (x_a-$U(x_a)$).

	x_{lab}-$U(x_{lab})$ (mg/kg)	x_{lab}-$U(x_{lab})$ (mg/kg)	
	Cd	Pb	stated by lab
L01	0.012	0.060	n
L02	0.009	0.048	n
L03	0.013	0.066	y
L05	0.010	0.049	y
L06			
L07	0.011	0.059	n
L08	0.011	0.072	y
L09	0.010	0.055	n
L10	0.011	0.060	n
L11	0.012	0.061	n
L12	0.010	0.0580	n
ML	**0.040**	**0.050**	

To conclude, overall this was a successful exercise. Unfortunately, measurement uncertainty was not always given and 3 laboratories gave not a correct compliance statement. Again we want to point out that a correct estimation of the measurement uncertainty is indispensable to make a correct compliance statement.

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ANNEXES

ANNEX 1: INVITATION LETTER TO LABORATORIES

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NRL NATIONAL REFERENCE LABORATORIES

Concern: PT-2018-NRL-TE-FASFC

Dear colleague,

It is our pleasure to invite you to participate in the proficiency test (PT) for the detection of trace elements in food, organized by the National Reference Laboratory (NRL) for trace elements in food and feed at Sciensano (former CODA-CERVA). The goal of the PT is to determine the performance of individual laboratories for specific tests. The PT is organised according to the ISO/IEC 17043 norm: 2010 Conformity assessment – General requirements for proficiency testing.

The following PT will be organized by the NRL for trace elements in food and feed in 2018 for the laboratories involved in the official control program of the Federal Agency for the Safety of the Food Chain (FASFC) and other interested laboratories:
PT-2018-NRL-TE-FASFC "Determination of As, Cd, Pb, Cu, Zn, Hg and inorganic As in baby food"

- Closing date for the inscription:	27th of April 2018 (week 17)
- Shipment of the samples:	22nd of May 2018 (week 21)
- Submission of the test results:	22nd of June 2018 (week 25)
- Draft report:	7th of September 2018 (week 36)
- Final report:	28th of September 2018 (week 39)

If your laboratory is **approved by the FASFC** for trace elements in foodstuffs, participation to the PT-2018-NRL-TE-FASFC "Determination of As, Cd, Pb, Cu, Zn, Hg and inorganic As in baby food" is **mandatory** for all accredited elements and the costs for this PT (€ 229.42) will be billed directly by the Federal Agency for the Safety of the Food Chain (FASFC). The individual results of the laboratories approved by the FASFC will be disclosed to the FASFC.

If your laboratory is not approved by the FASFC for trace elements in foodstuffs, participation to the PT-2018-NRL-TE-FASFC is voluntary and the costs for the PT, € 229.42 + shipment costs, will be billed by Sciensano. The results will not be disclosed to the FASFC.

You can receive more information about our PT programme by contacting karlien.cheyns@sciensano.be

We hope you will find this a useful tool to support your laboratory's Quality Assurance system and look forward to receiving your registration before the 27th of April 2018. If you are not the correct contact person for this message or if you know other colleagues that might be interested, please feel free to forward this invitation to your own colleagues or colleagues from other institutes.

Kind regards,

Dr Karlien Cheyns and Dr Nadia Waegeneers

Belgian National Reference Laboratory for Trace Elements in Food and Feed
Service Trace elements and Nanomaterials
Sciensano

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ANNEX 2: RESULTS OF THE HOMOGENEITY STUDIES

	As	Cd	Pb	Cu	Zn	Hg
Cochran test for variance outliers						
Cochran test statistic	0.562	0.458	0.583	0.300	0.264	0.166
Critical (95%)	0.602	0.602	0.602	0.602	0.602	0.602
Cochran < critical	use complete dataset	use complete dataset	use complete dataset	use complete dataset	use complete dataset	use complete dataset
Test for sufficient homogeneity						
S_{an}^2	32	1.2	25	517	16901	0.30
S_{sam}^2	60	0.82	26	521	24112	17.39
σ_{all}^2	106	1.2	20	889	14938	1.53
F1	1.88	1.88	1.88	1.88	1.88	1.88
F2	1.01	1.01	1.01	1.01	1.01	1.01
Critical	237	3.5	93	2194	45154	3.19
S_{sam}^2 < critical?	accept	accept	accept	accept	accept	no homogenity

ANNEX 3: LETTER ACCOMPANYING THE SAMPLE

Concern: Shipment of sample PT-2018-NRL-TE-FASFC

Dear colleague,

Following your subscription for the proficiency test (PT-2018-NRL-TE-FASFC) for the detection of trace elements in food, we ship you the PT sample. You can find your unique lab code on the sample.

Enclosed you can find the instructions to the participants with a reporting form. In addition, a receipt form is added, please retour this by e-mail (karlien.cheyns@sciensano.be). The time schedule of the PT is given below:

PT-2018-NRL-TE-FASFC "Determination of As, Cd, Pb, Cu, Zn, Hg and inorganic As in baby food"

- Closing date for the inscription:	27th of April 2018 (week 17)
- Shipment of the samples:	22nd of May 2018 (week 21)
- Submission of the test results:	22nd of June 2018 (week 25)
- Draft report:	7th of September 2018 (week 36)
- Final report:	28th of September 2018 (week 39)

We expect the results of the analysis the latest by the end of week 25 (the **22nd of June**).

We would like to remind you that if your laboratory is **approved by the FASFC** for trace elements in foodstuffs, participation to the PT-2018-NRL-TE-FASFC "Determination of As, Cd, Pb, Cu, Zn, Hg and inorganic As in baby food" is **mandatory** for all accredited elements and the costs for this PT (€ 229.42) will be billed directly by the Federal Agency for the Safety of the Food Chain (FASFC). The individual results of the laboratories approved by the FASFC will be disclosed to the FASFC.

For any information about our PT programme you can contact karlien.cheyns@sciensano.be

Kind regards,

Dr Karlien Cheyns and Dr Nadia Waegeneers

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ANNEX 4: INSTRUCTIONS TO PARTICIPANTS

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NRL NATIONAL REFERENCE LABORATORIES

INSTRUCTIONS TO THE PARTICIPANTS

Type of proficiency test / Type proficiency test / Type d'essai d'aptitude :

PT-2018-NRL-TE-FASFC "Determination of As, Cd, Pb, Cu, Zn, Hg and inorganic As in baby food"

Analyte(s) / Analyt(en) / Analyte(s) :

As, As_i , Cd, Pb, Cu, Zn, Hg

Matrix(-ces) / Matrix(-ces) / Matrice(s) :

Baby food containing spinach, rice and white fish

Number of materials sent / Aantal verstuurde materialen / Nombre de matériaux envoyés :

One small container, containing about 30 g sample

Storage method / Wijze van bewaring / Mode de conservation :

Cold (4°C), dark conservation

Data to be sent and to whom/ Gegevens die moeten opgestuurd worden en aan wie/ Données à envoyer et à qui:

See 'results reporting form', to be transmitted to Karlien Cheyns, preferably by e-mail: karlien.cheyns@sciensano.be (an electronic version of the reporting form will be sent by e-mail). Address: Sciensano, Leuvensesteenweg 17, 3080 Tervuren

Deadline for sending the results/ Datum (deadline) waarop de resultaten moeten opgestuurd worden/ Date (deadline) à laquelle les résultats doivent être envoyés:

22/06/2018

Specific instructions / Specifieke Intructies / Instructions spécifiques :

- *Store the samples refrigerated upon arrival*
- *Homogenize the samples before analysis*
- *Follow as close as possible the analysis method you use in routine sample analysis*
- *The analysis should be performed in **triplicate***
- *Report the extended uncertainty*

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ANNEX 5: MATERIALS RECEIPT FORM

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NRL NATIONAL REFERENCE LABORATORIES

PROFICIENCY TESTING MATERIALS RECEIPT FORM

PROFICIENCY TESTING MATERIALS RECEIPT FORM
FORMULIER VAN BEVESTIGING VAN ONTVANGST VAN HET MATERIAAL
FORMULAIRE DE CONFIRMATION DE RÉCEPTION DU MATÉRIEL
PT-2018-NRL-TE-FASFC

NAME ORGANISATION (LAB) / NAAM ORGANISATIE (LABO) / NOM ORGANISATION (LABO) :

CONTACT PERSON / CONTACTPERSOON / PERSONNE DE CONTACT :

TEL :

E-MAIL :

DATE OF THE RECEIPT / DATUM ONTVANGST VAN HET MATERIAAL / DATE DE RECEPTION DU MATERIEL :

STATE OF MATERIALS RECEIVED / STAAT BIJ ONTVANGST / ETAT A LA RECEPTION :

- O GOOD / GOED / BON
- O OPEN / OPEN / OUVERT
- O BAD (specify) / SLECHT (specificeren) / MAUVAIS (à préciser) :

REMARKS / OPMERKINGEN / REMARQUES :

DATE / DATUM / DATE :

SIGNATURE / HANDTEKENING / SIGNATURE :

ANNEX 6: REPORTING FORM AND QUESTIONNAIRE

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NRL NATIONAL REFERENCE LABORATORIES

PT-2018-NRL-TE-FASFC "Determination of As, Cd, Pb, Cu, Zn, Hg and inorganic As in baby food"

RESULTS REPORTING FORM

Lab code: L

1. Does your laboratory carry out this type of analysis on a routine basis?
   As regards to:

   ☐ The matrix baby food
   ☐ As
   ☐ Cd
   ☐ Pb
   ☐ Zn
   ☐ Hg
   ☐ Asi

2. Which matrices/elements would be interesting for your laboratory for future PT's?

MATRICES:
- ☐ Terrestrial vegetable origin
- ☐ Aquatic vegetable origin
- ☐ Terrestrial animal origin
- ☐ Aquatic animal origin
- ☐ Drinks
- ☐ Processed food
- ☐ Feed
- ☐ Other:

ELEMENTS:
- ☐ As
- ☐ As_i
- ☐ Cd
- ☐ Pb
- ☐ Hg
- ☐ Cu
- ☐ Zn
- ☐ Ni
- ☐ Cr
- ☐ Other:

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Lab code: L

Element	Technique used*	Units	Replicate 1	Replicate 2	Replicate 3	Mean value	Extended uncertainty (k=2)
As		mg/kg					
As_i	*	mg/kg					
Cd		mg/kg					
Pb		mg/kg					
Cu		mg/kg					
Zn		mg/kg					
Hg		mg/kg					

***please specify separation and quantification techniques**

Is this sample compliant regarding current legislation?