BELGIAN NATIONAL REFERENCE LABORATORY FOR TRACE ELEMENTS IN FOOD AND FEED

PT-2019-NRL-TE-FASFC "Determination of As, Asi, Cd, Pb, Cu, Zn, Cr, Ni in lentils"

FINAL REPORT ON THE 2019 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 lentils. The results from the PT were treated in Sciensano, Tervuren.

The 2019 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). Twelve laboratories registered for and participated in the exercise.

The test material used in this test was dried black lentils, bought in a local supermarket. The choice for this matrix was based on the demand to include also Ni and Cr as elements to analyse. Legumes contain typically elevated concentrations of these elements [1]. The material was homogenized after purchase and divided in small containers. 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), chromium (Cr) and nickel (Ni).

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 12 laboratories that registered for participation, 12 submitted results for As, Pb, Cu and Zn, 11 submitted results for Cd, Ni and Cr and five submitted results for As_i. All but three of the z-scores that were calculated, were satisfactory. Estimation of a correct measurement uncertainty stays a difficult excersize: two of the calculated ζ-scores were questionable, seven of the calculated ζ-scores were unsatisfactory. Not all laboratories met the LOQ criteria for Cd and Pb [2].

No consensus value could be derived for As, Asi and Cd. The measurement of Cr was difficult, but overall the laboratories performed satisfactory. The performance of the laboratories to analyse Pb, Cu, Zn and Ni in this matrix was very succesfull.

CONTENTS

INTRODUCTION

Trace elements occur in varying amounts as natural elements in soils, plants and animals, and consequently 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 [3]. 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. Both Lead (Pb) and Cadmium (Cd) concentrations are regulated in vegetables.

Lead

3.1.12	Cereals and pulses	0,20

Cadmium

3.2.1	Vegetables and fruit, excluding root and tuber vegetables, leaf vegetables, fresh herbs, leafy brassica, stem vegetables, fungi and seaweed ([27])	0,050

Figure 1 : Snapshots of maximum limits of Pb and Cd (mg/kg) in vegetables as published in [4].

There is currently no European legislation regarding Copper (Cu), Zinc (Zn) and Arsenic (As) in legume vegetables.

In addition, there are currently no maximum levels for nickel (Ni) and Chromium (Cr) in food. However, these elements gained interest the last five years. In February 2015, EFSA published a scientific opinion on the risks to human health from Ni in food, particularly in vegetables, and also in drinking water [1]. EFSA set a safe level, known as the tolerable daily intake (TDI), of 2.8 micrograms per kilogram of body weight per day. Based on current mean and high exposures, EFSA's experts concluded that current chronic dietary exposure to Ni is of concern for the general population. In addition, at the current levels of acute dietary exposure to Ni, there is a concern that Ni-sensitized individuals may develop eczematous flare-up skin reactions. The CONTAM Panel noted the need for mechanistic studies to assess the human relevance of the effects on reproduction and development observed in experimental animals and for additional studies on human absorption of Ni from food, for example in combination with duplicate diet studies.

In March 2014, the CONTAM Panel published a scientific opinion on the risk to human health from chromium in food, particularly in vegetables and in bottled drinking water [5]. EFSA's experts established a TDI of 0.3 milligrams per kilogram of body weight per day for Cr(III). Dietary exposure across all age groups is well below the TDI and therefore does not raise concerns for public health. However, to improve the risk assessment, there is a need for data on the content of Cr(III) and Cr(VI) in food.

The 2019 PT emphasized on the abilities of the participating laboratories to determine Ni and Cr in legume vegetables. Lentils were chosen because of the known elevated Ni and Cr concentrations. In addition, this PT tests the competence of the participating laboratories to determine the total mass fraction of As, As_i, Cd, Pb, Cu and Zn in lentils.

TIME FRAME, TEST MATERIAL AND INSTRUCTIONS TO PARTICIPANTS

Invitation letters to this PT were sent to participants in April (Annex 1). The 2019 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). Twelve 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 2019. Reporting deadline was the 21st of June.

This year the test material was a sample of dried, homogenized lentils. The sample was purchased in a local supermarkrt. After purchase, the sample was homogenized and divided in small containers. The samples were stored at ambient temperature.

The homogeneity of the test materials was tested following the recommended procedure according to IUPAC [6]. The trace elements appeared to be homogeneously distributed in the samples (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 ambient temperature until analysis. Before starting the analyses, the samples had to be re-homogenized. The procedure followed for the exercise, had to be as close as possible to the method used by the participant in routine sample analysis. 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 lentil 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 12 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 [7] 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 Pb, Cu, Zn, Ni, but not for As, Asi, Cd, and Cr. 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 ($\hat{\sigma}_{rob}$) [8], [9]. 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) [6][10]. 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, As_i and Cd, only few quantified results were available, no value was assigned for these elements and no scores were calculated. For Cr, informal scores were 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.

	Pb mg/kg	Cu mg/kg	Zn mg/kg	Ni mg/kg	Cr[1] mg/kg
n (number of participants with quantifiable result)	8[2]	12	12	11	10[3]
Mean	0.025	10.4	50.7	2.06	0.44
Standard deviation (SD)	0.003	0.89	3.4	0.15	0.06
Robust mean (median)	0.023	10.6	50.8	2.05	0.42
Robust SD (nIQR)	0.003	0.76	2.4	0.14	0.08
Assigned value x_a	**0.023**	**10.6**	**50.8**	**2.05**	**0.42**
Standard uncertainty of the assigned value $u(x_a)$	**0.002**	**0.3**	**0.9**	**0.05**	**0.03**
σ_p	0.005	1.2	4.5	0.29	0.08

Assigned value x_a: median of the reported results, excluding outliers; σ_p: standard deviation for proficiency assessment. [1] For Cr an informal consensus value was calculated, [2] one outlier, [3] two outliers

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 [6], [9].

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

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

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:

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

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

RESULTS

ARSENIC (As)

Twelve laboratories submitted results for total As concentrations. However, only five laboratories submitted results above their quantification limit. No scores were calculated, but the quantified results were comparable with a relative standard deviation of the results of only 12%.

The results are listed in Table 2

Table 2 : values reported for As (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	<0.05	<0.05	<0.05	**<0.05**	
2	< 0.125	< 0.125	< 0.125	**<0.125**	
3	0.0128	0.0125	0.0124	**0.0126**	0.007
4	<0.02	<0.02		**<0.02**	
5	<0.03	<0.03	<0.03	**<0.03**	
6	0.013			**0.013**	n.a.
7	<0.025			**<0.025**	
8	0.0155	0.0171	0.0165	**0.0164**	0.004
9	<0.05	<0.05	<0.05	**<0.05**	
10	0.0146	0.0160	0.0160	**0.016**	0.004
11	<0.2	<0.2	<0.2	**<0.2**	
12	0.014	0.016	0.014	**0.015**	0.003

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})
4	<0.02	<0.02		**<0.02**	
5	<0.02	<0.02	<0.02	**<0.02**	
8	0.0127	0.0103	0.0119	**0.012**	0.003
9	<0.027	<0.027	<0.027	**<0.027**	
10	0.0046	0.0047		**0.0047**	0.0011

CADMIUM (Cd)

Eleven laboratories submitted results for Cd concentrations. Only five laboratories reported values higher than the quanification limit (Table 4, Figure 2). Results were higly variable and reported values and quantification limits overlapped. No consensus could be drawn from these values. An explanation can be that a spectral interference caused the higher reported concentrations, as observed in ICP-MS/MS analysis operated at different masses and in different gass modes.

In the framework of the current legislation, the minimum quantification limit of Cd in this matrix should be two fifth of the maximum limit, i.e. maximum 0.020 mg/kg [2]. The laboratories L02 and L11 do not meet this criterium, efforts should be made to lower these LOQ's.

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})
1	<0.01	<0.01	<0.01	**<0.01**	
2	<0.03	<0.03	<0.03	**<0.03**	
3	<0.01	<0.01	<0.01	**<0.01**	
4	0.024	0.031		**0.027**	0.007
5	0.013	0.013	0.012	**0.013**	0.002
6	0.0018			**0.0018**	n.a.
7	0.010			**0.010**	0.002
8	0.013	0.014	0.015	**0.014**	0.003
9	<0.005	<0.005	<0.005	**<0.005**	
11	<0.05	<0.05	<0.05	**<0.05**	
12	<0.0055	<0.0055	<0.0055	**<0.0055**	

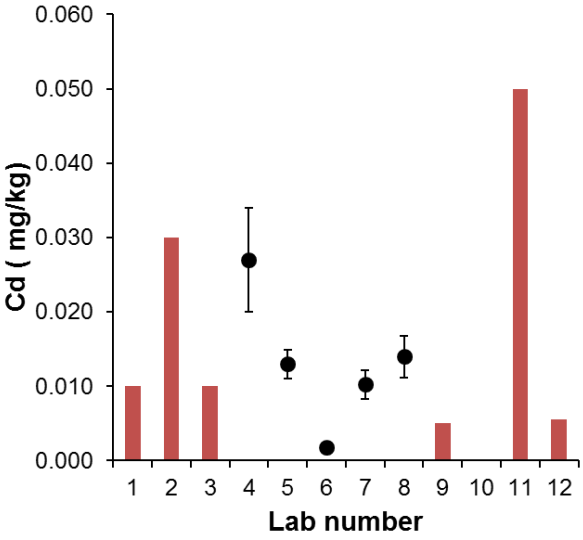


Figure 2 : Results with expanded uncertainty for Cd, as reported by the participants (red bars represent the limits of quantification of the corresponding labs)

LEAD (Pb)

x_a = **0.023** ± 0.002 mg/kg (k = 2)

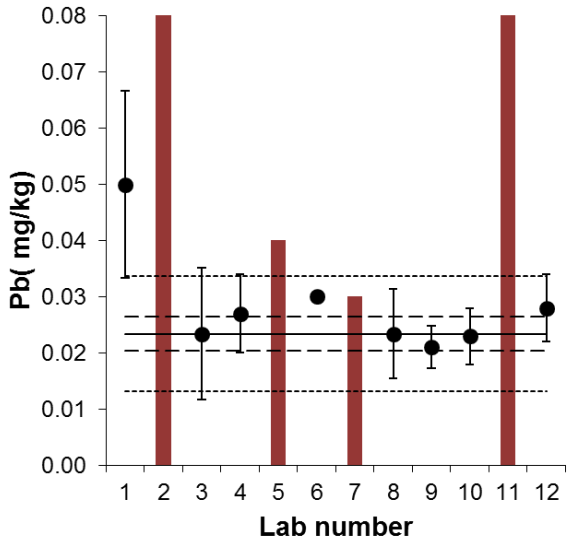
Twelve laboratories submitted results for total Pb concentrations. Eight laboratories reported values higher than their quantification limit. One value was excluded as outlier (>50% higher than the median value), the median of the other seven values was used as assigned value. Seven laboratories obtained satisfactory z-scores for Pb against the standard deviation accepted for the proficiency test (Table 5, Figure 3), one laboratory (L01) had an unsatisfactory result. The quantification limits of L02, L05, L07 and L11 were not lower than the corresponding x_a-3 $u(x_a)$ value, so the statements are satisfactory. However, in the framework of the current legislation the minimum quantification limit of Pb in this matrix should be one fifth of the maximum limit, i.e. maximum 0.040 mg/kg [2]. The laboratories L02 and L11 do not meet this criterium, efforts should be made to lower these LOQ's.

Six laboratories did obtain satisfactory ζ-scores against their stated measurement uncertainty. Two laboratories had unsatisfactory ζ-scores, of which one laboratory did not mention an 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	0.0496	0.0488	0.0515	**0.0500**	0.017	5.2	3.1
2	<0.125	<0.125	<0.125	**<0.125**			
3	0.0238	0.0231	0.0234	**0.0234**	0.012	0.0	0.0
4	0.028	0.025		**0.027**	0.007	0.7	0.9
5	<0.04	<0.04	<0.04	**<0.04**			
6	0.030			**0.030**	n.a.	1.3	4.4
7	<0.03			**<0.03**			
8	0.022	0.023	0.025	**0.023**	0.008	0.0	0.0
9	0.024	0.020	0.020	**0.021**	0.004	-0.5	-1.0
10	0.0230	0.0234	0.0225	**0.023**	0.005	-0.1	-0.1
11	<0.1	<0.1	<0.1	**<0.1**			
12	0.0276	0.0270	0.0302	**0.028**	0.006	0.9	1.4

(A)



(B)

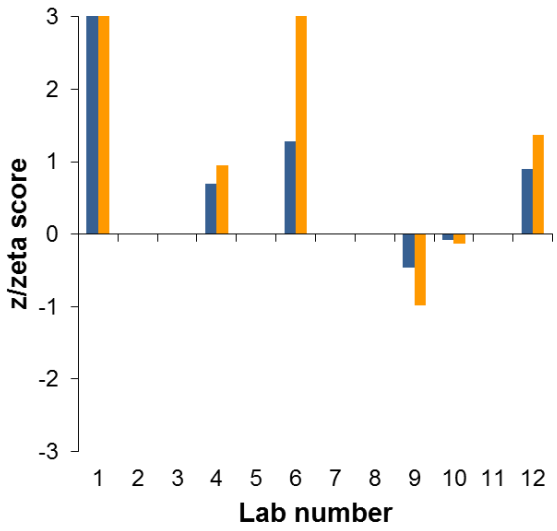


Figure 3 : (a) Results with expanded uncertainty for Pb, as reported by the participants (dashed lines: $x_a \pm 2\ u(x_a)$, dotted lines: $x_a \pm 2\ \sigma_p$), red bars represent the limits of quantification of the corresponding labs with the y-axis cut-off at 0.08 mg/kg and (b) z- (blue bars) and ζ-scores (orange bars)

COPPER (Cu)

x_a = 10.6 ± 0.5 µg/kg (k = 2)

Twelve laboratories submitted results for Cu concentrations. The median of all results was used as assigned value. All twelve laboratories obtained satisfactory z-scores for Cu against the standard deviation accepted for the proficiency test (Table 6, Figure 4). Nine laboratories also obtained satisfactory ζ-scores against their stated measurement uncertainty. Two laboratories (L02 and L05) obtained a questionable ζ-score. One laboratory (L06) obtained an unsatisfactory ζ-score, partially due to the fact that no measurement uncertainty was reported.

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	9.933	10.868	10.654	**10.485**	2.10	-0.1	-0.1
2	9.90	9.43	9.64	**9.66**	0.69	-0.8	-2.1
3	10.3	10.2	10.2	**10.2**	2.24	-0.3	-0.3
4	11.494	10.563		**11.029**	3.20	0.4	0.3
5	9.1	8.1	8.7	**8.6**	1.72	-1.7	-2.2
6	12.00			**12.00**	n.a.	1.2	5.2
7	9.41			**9.41**	2.01	-1.0	-1.1
8	10.75	10.68	10.75	**10.70**	3.40	0.1	0.1
9	10.01	9.72		**9.87**	1.91	-0.6	-0.7
10	10.6	10.8	11.1	**10.8**	1.9	0.2	0.2
11	11	11	11	**11**	1.40	0.2	0.3
12	10.9	10.7	11.1	**10.9**	1.9	0.3	0.3

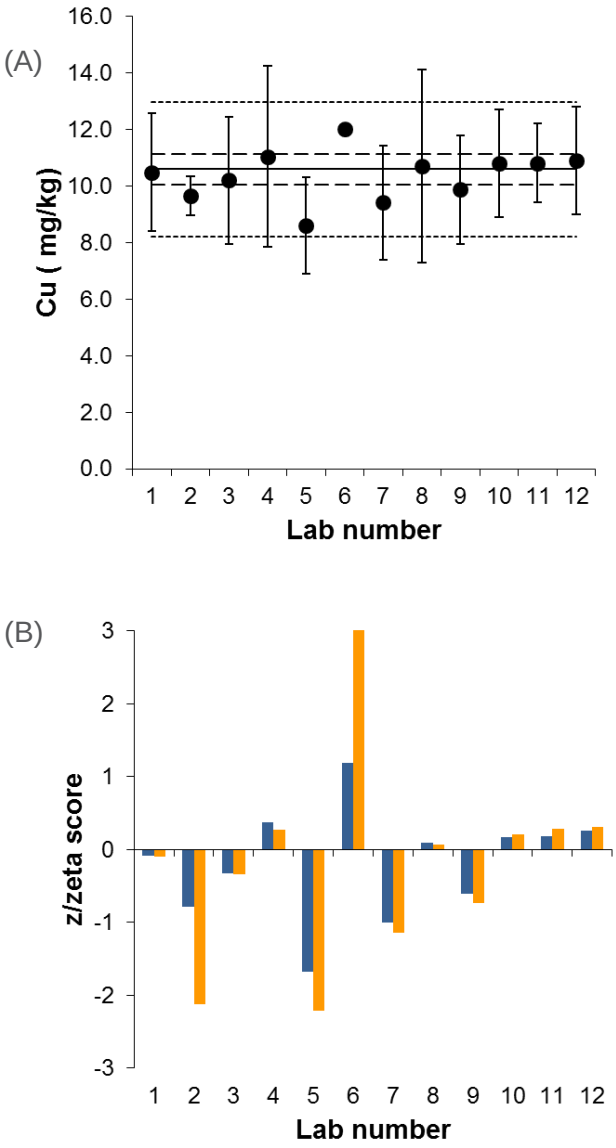


Figure 4 : (a) Results with expanded uncertainty for Cu, as reported by the participants (dashed lines: $x_a \pm 2\ u(x_a)$, dotted lines: $x_a \pm 2\ \sigma_p$) and (b) z- (blue bars) and ζ-scores (orange bars)

ZINC (Zn)

x_a = **50.8** ± 1.7 mg/kg (k = 2)

Twelve laboratories submitted results for Zn concentrations. The median of all results was used as assigned value. All laboratories obtained satisfactory z-scores for Zn against the standard deviation accepted for the proficiency test (Table 7, Figure 5). Eleven laboratories also obtained satisfactory ζ-scores against their stated measurement uncertainty. One laboratory (L06) obtained an unsatisfactory ζ-score partially due to the fact that no measurement uncertainty was reported.

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	49.90	48.78	50.97	**49.89**	10.0	-0.2	-0.2
2	51.2	50.5	50.2	**50.6**	n.a.	0.0	-0.2
3	48.5	47.6	48.4	**48.2**	7.7	-0.6	-0.7
4	61.86	53.84		**57.8**	11.0	1.6	1.9
5	44.3	48.3	48.5	**47.0**	9.4	-0.8	-0.8
6	54.0			**54.0**	n.a.	0.7	3.7
7	44.8			**44.8**	8.7	-1.3	-1.4
8	52.05	52.30	51.88	**52.10**	6.2	0.3	0.4
9	52.038	51.098		**51.568**	7.3	0.2	0.2
10	50.3	50.6	52.4	**51.0**	7.8	0.0	0.0
11	49.33	49.16	48.55	**49.01**	1.1	-0.4	-1.8
12	52.7	51.9	51.4	**52.0**	7.5	0.3	0.3

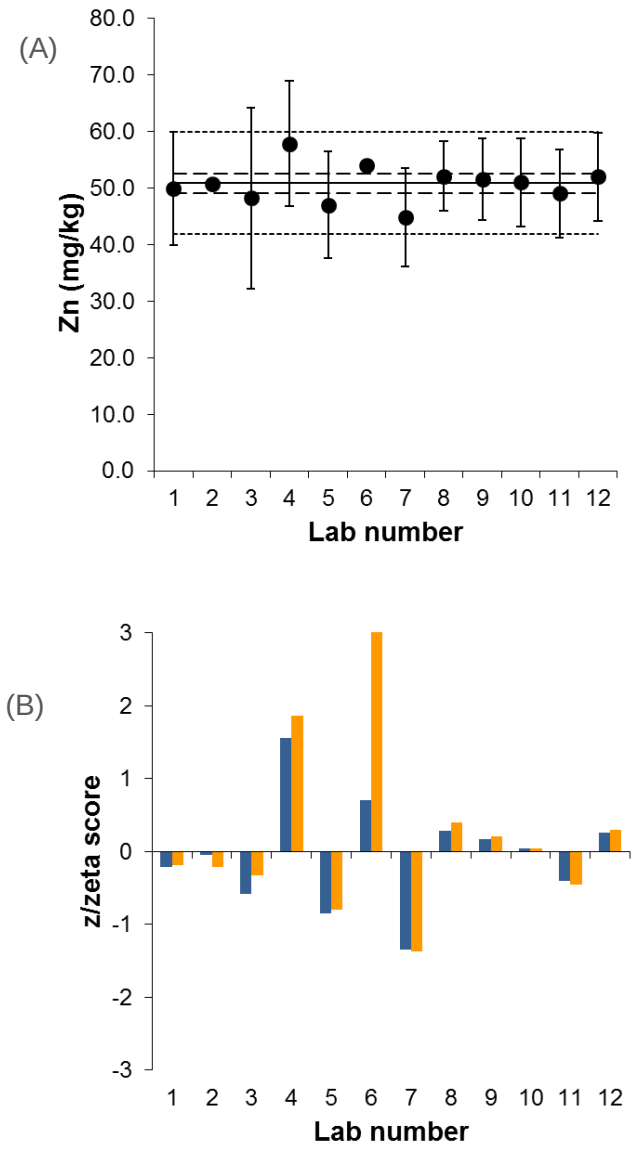


Figure 5 : (a) Results with expanded uncertainty for Zn, as reported by the participants (dashed lines: $x_a \pm 2\ u(x_a)$, dotted lines: $x_a \pm 2\ \sigma_p$) and (b) z- (blue bars) and ζ-scores (orange bars)

NICKEL (NI)

x_a = **2.05** ± 0.11 mg/kg (k = 2)

Eleven laboratories submitted results for Ni concentrations. The median of all results was used as assigned value. All laboratories obtained satisfactory z-scores for Ni against the standard deviation accepted for the proficiency test (Table 7, Figure 5). In addition, all laboratories did obtain also satisfactory ζ-scores against their stated measurement uncertainty

Table 8 : values reported for Ni (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	2.097	2.351	2.149	**2.199**	0.440	0.5	0.7
2	2.04	2.00	1.97	**2.00**	0.096	-0.2	-0.7
3	2.05	2.02	2.01	**2.03**	0.609	-0.1	-0.1
4	2.274	2.039		**2.157**	0.841	0.4	0.3
5	2.01	1.88	1.80	**1.90**	0.48	-0.5	-0.6
6	2.10			**2.10**	n.a.	0.2	0.9
7	1.766			**1.766**	0.284	-1.0	-1.9
8	2.053	2.001	2.091	**2.050**	0.820	0.0	0.0
9	1.929	2.003		**1.966**	0.316	-0.3	-0.5
11	2.33	2.31	2.16	**2.270**	0.499	0.7	0.9
12	2.41	2.15	2.16	**2.2**	0.3	0.5	0.9

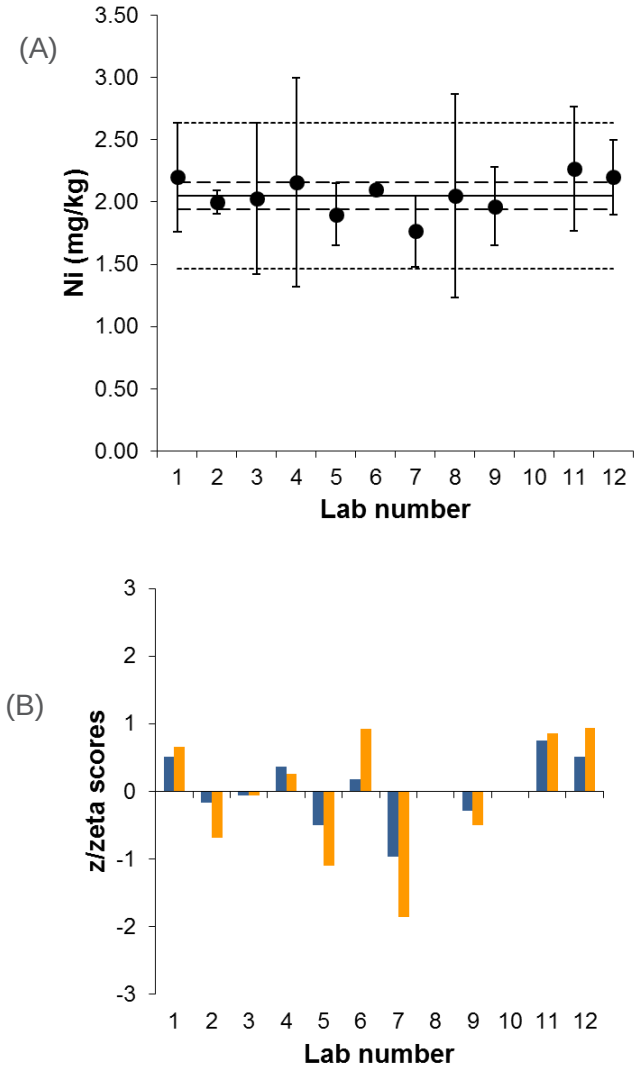


Figure 6 : (a) Results with expanded uncertainty for Ni, as reported by the participants (dashed lines: x_a ± 2 $u(x_a)$, dotted lines: x_a ± 2 σ_p, and (b) z- (blue bars) and ζ-scores (orange bars)

CHROMIUM (CR)

x_a = **0.42** ± 0.06 mg/kg (k = 2)

Eleven laboratories submitted results for Cr concentrations, of which ten results were above their quantification limits. Two values were excluded as outliers (>50% lower than the median value), the remaining eight values did not meet the criterium of $u(x_a)^2 < 0.1 * \sigma_p^2$. However, the median of these eight results was used as informal consensus value. Using this informal consensus value, eight laboratories obtained satisfactory informal z-scores against the standard deviation accepted for the proficiency test (Table 7, Figure 5). Two (L05 and L09) laboratories obtained unsatisfactory informal z-scores. The quantification limit of L03 was not lower than the corresponding x_a-3 $u(x_a)$ value, so the statement is satisfactory.

Seven laboratories also obtained satisfactory informal ζ-scores against their stated measurement uncertainty. Three laboratories (L02, L05 and L09) obtained unsatisfactory informal ζ-scores.

Table 9 : values reported for Cr (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})	Informal z-scores	Informal ζ-scores
1	0.4745	0.5170	0.4637	**0.4850**	0.1213	0.8	1.0
2	0.54	0.55	0.50	**0.53**	n.a.	1.4	3.5
3	0.408	0.411	0.412	**0.410**	0.205	-0.1	-0.1
4	<3.44	<3.71					
5	0.13	0.13	0.11	**0.12**	0.018	-3.9	-9.2
6	0.40			**0.40**	n.a.	-0.3	-0.6
7	0.372			**0.372**	0.118	-0.6	-0.7
8	0.348	0.366	0.388	**0.367**	0.165	-0.7	-0.6
9	0.131	0.135		**0.133**	0.024	-3.7	-8.5
11	0.47	0.4	0.430	**0.430**	0.095	0.1	0.2
12	0.544	0.496	0.475	**0.50**	0.090	1.0	1.5

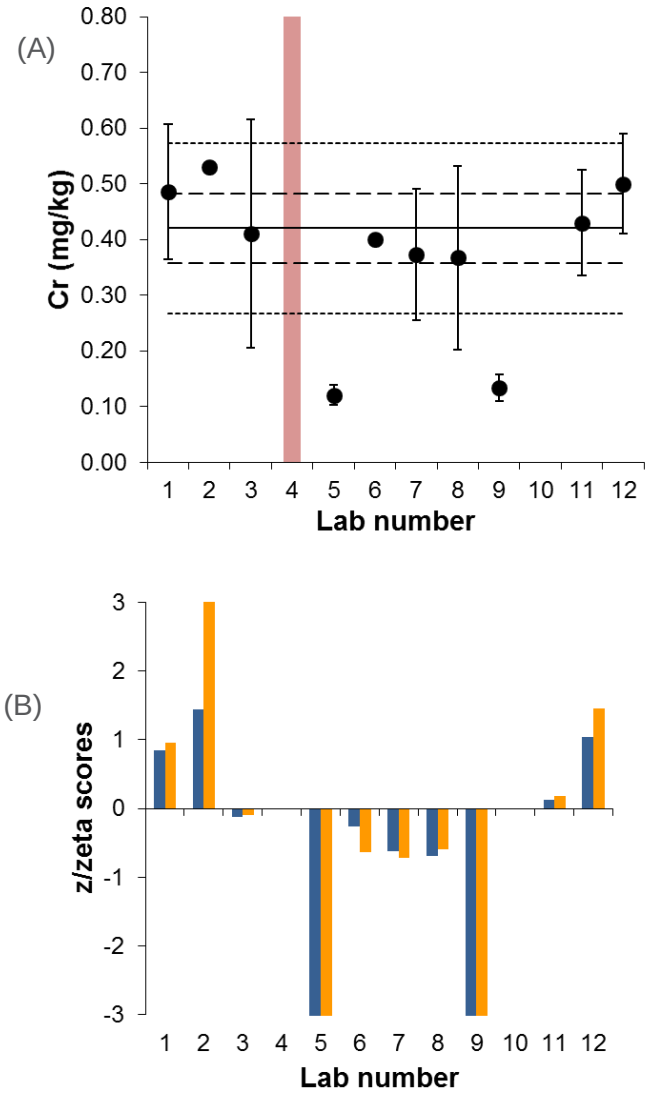


Figure 7 : (a) Results with expanded uncertainty for Cr, as reported by the participants (dashed lines: x_a ± 2 $u(x_a)$, dotted lines: x_a ± 2 σ_p), red bars represent the limits of quantification of the corresponding labs with the y-axis cut-off at 3 mg/kg and (b) z- (blue bars) and ζ-scores (orange bars)

DISCUSSION AND CONCLUSION

The most commonly used technique for the analysis of As, Cd, Pb, Cu, Zn, Ni and Cr was ICP-MS (Inductively Coupled Plasma-Mass Spectrometry). For Cu, Zn and Cr some laboratories used ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry).

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."*

As for the current matrix, no maximum levels of Pb or Cd were exceeded, all laboratories stated the sample correctly as compliant. Unfortunately, not all laboraties do meet the LOQ criteria for Pb and Cd analysis in the matrix lentils, efforts should be done to improve these quantification limits.

Presumably, there is an interfering element for the measurement of Cd in this matrix which was not seen by all instruments. This demonstrates one of the possible pitfalls of ICP-MS measurements.

The performance of the laboratories to analyse Pb, Cu, Zn and Ni in this matrix was very succesfull. The measurement of Cr was more difficul, but overall the laboratories performed satisfactory. Estimation of a correct measurement uncertainty stays a difficult excersize, resulting in nine questionable or unsatifactory ζ-scores.

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ANNEXES

ANNEX 1: INVITATION LETTER TO LABORATORIES

Concern: PT-2019-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. 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 2019 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-2019-NRL-TE-FASFC "Determination of As, Asi, Cd, Pb, Cu, Zn, Cr, Ni in lentils"

- Closing date for the inscription:	26th of April 2019 (week 17)
- Shipment of the samples:	20th of May 2019 (week 21)
- Submission of the test results:	21st of June 2019 (week 25)
- Draft report:	6th of September 2019 (week 36)
- Final report:	27th of September 2019 (week 39)

If your laboratory is **approved by the FASFC** for trace elements in foodstuffs, participation to the PT-2019-NRL-TE-FASFC "Determination of As, Asi, Cd, Pb, Cu, Zn, Cr, Ni in lentils" is **mandatory** for all accredited elements and the costs for this PT (€ 234.81) 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-2019-NRL-TE-FASFC is voluntary and the costs for the PT, € 234.81 + 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 26th of April 2019. 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	Cr	Ni
Cochran test for variance outliers							
Cochran test statistic	0.564	0.393	0.291	0.354	0.263	0.339	0.281
Critical (95%)	0.602	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	use complete dataset
Test for sufficient homogeneity							
S_{an}^2	0.54	0.32	4.67	15227	208489	1430	2974
S_{sam}^2	0.34	-0.16	1.5	120499	1307965	331	3182
σ_{all}^2	0.99	0.8	2.59	165548	1967076	248	8810
F1	1.88	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	1.01
Critical	2.41	1.8	9.6	326610	3908677	1910	19567
S_{sam}^2 < critical?	accept	accept	accept	accept	accept	accept	accept

ANNEX 3: LETTER ACCOMPANYING THE SAMPLE

Concern: Shipment of sample PT-2019-NRL-TE-FASFC

Dear colleague,

Following your subscription for the proficiency test (PT-2019-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-2019-NRL-TE-FASFC "Determination of As, As_i, Cd, Pb, Cu, Zn, Cr, Ni in lentils"

• Closing date for the inscription:	26th of April 2019 (week 17)
• Shipment of the samples:	20th of May 2019 (week 21)
• Submission of the test results:	21st of June 2019 (week 25)
• Draft report:	6th of September 2019 (week 36)
• Final report:	27th of September 2019 (week 39)

We expect the results of the analysis the latest by the end of week 25 (the **21st 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-2019-NRL-TE-FASFC "Determination of As, Asi, Cd, Pb, Cu, Zn, Cr, Ni in lentils" is **mandatory** for all accredited elements and the costs for this PT (€ 234.81) 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

INSTRUCTIONS TO THE PARTICIPANTS

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

PT-2019-NRL-TE-FASFC "Determination of As, Asi, Cd, Pb, Cu, Zn, Cr, Ni in lentils"

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

As, Asi , Cd, Pb, Cu, Zn, Cr, Ni

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

Black lentils

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

One small container, containing about 25 g sample

Storage method / Wijze van bewaring / Mode de conservation :

Ambient, 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:

21/06/2019

Specific instructions / Specifieke Intructies / Instructions spécifiques :

- *Store the samples dry upon arrival*
- *Homogenize the samples before analysis*
- *Follow as close as possible the analysis method you use in routine sample analysis*
- *Report the extended uncertainty*

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ANNEX 5: MATERIALS RECEIPT FORM

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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-2019-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 :

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ANNEX 6: REPORTING FORM AND QUESTIONNAIRE

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NRL NATIONAL REFERENCE LABORATORIES

PT-2019-NRL-TE-FASFC
"Determination of As, As_i, Cd, Pb, Cu, Zn, Cr, Ni in lentils"

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 lentils
   ☐ As
   ☐ Cd
   ☐ Pb
   ☐ Zn
   ☐ Ni
   ☐ Cr
   ☐ Cu
   ☐ 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: .....

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					
Cr		mg/kg					
Ni		mg/kg					

**please specify separation and quantification techniques*

Is this sample compliant regarding current legislation? Y / N .....