BELGIAN NATIONAL REFERENCE LABORATORY FOR TRACE ELEMENTS IN FOOD AND FEED

PT-2020-NRL-TE-FASFC “Determination of As, Cd, Pb and Hg in food supplements”

FINAL REPORT ON THE 2020 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 food supplements. The results from the PT were treated in Sciensano, Tervuren.

The 2020 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 food supplement. The material was separated from the package after purchase and divided in small containers. Each participant received approximately 15 g of test material.

Participants were invited to report the mean value and measurement uncertainty on their results for arsenic (As), cadmium (Cd), lead (Pb) 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, Cd and Pb, 10 submitted results for Hg. All but two of the z-scores that were calculated, were satisfactory. Estimation of a correct measurement uncertainty stays a difficult excersize: five of the calculated ζ-scores were unsatisfactory. Not all laboratories met the LOQ criteria for Hg [1].

No consensus value could be derived for Hg. The measurement of As was difficult, but overall the laboratories performed satisfactory. The performance of the laboratories to analyse Pb, and Cd 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 (including food supplements) have been laid down in the Commission Regulation (EC) Nº 1881/2006 [2].

Both Lead (Pb) ,Cadmium (Cd) and Mercury (Hg) concentrations are regulated in food supplements.

Pb

3.1.22	Food supplements (39)	3,0

Cd

3.2.21	Food supplements (39) excl. food supplements listed in point 3.2.22	1,0
3.2.22	Food supplements (39) consisting exclusively or mainly of dried seaweed, products derived from seaweed, or of dried bivalve molluscs	3,0

Hg

3.3.3	Food supplements (39)	0,10

Figure 1 : Snapshots of maximum limits of Pb, Cd and Hg (mg/kg) in food supplements as published in [3]. (39)The maximum level applies to the product as sold.

There is currently no European legislation regarding Arsenic (As) in food supplments. In Belgium, according to the Royal Decree of June 7th 2014 fixing maximum concentrations of As in some types of dietary supplements (Figure 2), a maximum level of 1 mg kg^{-1} total As was set initially. This limit was revised in 2016 with an extra paragraph allowing the producers to subtract the non toxic aresnobetaine concentration from the total As concentration before comparing with the maximum level (Figure 3)[4].

Artikel 1. Voor de toepassing van dit besluit wordt verstaan onder voedingssupplementen :

— producten in de zin van artikel 2 van het koninklijk besluit van 3 maart 1992 betreffende het in de handel brengen van nutriënten en van voedingsmiddelen waaraan nutriënten werden toegevoegd (nutriënten in voorgedoseerde vorm);

en

— producten in de zin van artikel 4 van het koninklijk besluit van 29 augustus 1997 betreffende de fabricage van en de handel in voedingsmiddelen die uit planten of uit plantenbereidingen samengesteld zijn of deze bevatten (planten en plantenbereidingen in voorgedoseerde vorm).

Article 1er. Pour l'application du présent arrêté, on entend par suppléments alimentaires :

— les produits dans le sens de l'article 2 de l'arrêté royal du 3 mars 1992 concernant la mise dans le commerce de nutriments et de denrées alimentaires auxquelles des nutriments ont été ajoutés (nutriments sous forme prédosée);

et

— les produits dans le sens de l'article 4 de l'arrêté royal du 29 août 1997 relatif à la fabrication et au commerce de denrées alimentaires composées ou contenant des plantes ou préparations de plantes (plantes et préparations de plantes sous forme prédosée).

Figure 2: Snapshot of Royal Decree of 14/06/2002 defining food supplements for this regulation

Artikel 1. Artikel 2 van het koninklijk besluit van 14 juni 2002 tot vaststelling van maximale gehaltes aan contaminanten waaronder zware metalen in voedingssupplementen, vervangen bij het koninklijk besluit van 7 januari 2014, wordt vervangen als volgt:

"Art. 2. § 1. Het is verboden voedingssupplementen in de handel te brengen met gehaltes aan arseen hoger dan 1 mg arseen per kg product.

§ 2. Voor de toepassing van paragraaf 1 wordt onder het gehalte aan arseen verstaan het gehalte aan totaal arseen waarvan het gehalte aan de species arsenobetaïne wordt afgetrokken.

§ 3. Het maximumgehalte uit paragraaf 1 is van toepassing op het product in de vorm waaronder het op de markt werd gebracht en is enkel van toepassing op het eetbaar gedeelte ervan.".

Article 1er. L'article 2 de l'arrêté royal du 14 juin 2002 fixant des teneurs maximales en contaminants comme les métaux lourds dans les suppléments alimentaires, remplacé par l'arrêté royal du 7 janvier 2014, est remplacé par ce qui suit:

« Art. 2. § 1er. Il est interdit de mettre dans le commerce des suppléments alimentaires qui ont des teneurs en arsenic plus élevées que 1 mg d'arsenic par kg de produit.

§ 2. Pour l'application du paragraphe 1er, on entend par teneur en arsenic la teneur totale en arsenic dont la teneur en arsenic présente sous la forme d'arsénobétaïne est soustraite.

§ 3. La teneur maximale visée au paragraphe 1er s'applique au produit tel qu'il a été mis sur le marché et s'applique seulement à la partie comestible. ».

Figure 3: Snapshot of the Royal Decree of 28/10/2016 amending the Royal Decree of 14/06/2002, setting maximum limits for As in food supplements

The 2020 PT emphasized on the abilities of the participating laboratories to determine As, Cd, Pb and Hg in food supplements containing high levels of Molybdenum, a known interferent for Cd analysis.

TIME FRAME, TEST MATERIAL AND INSTRUCTIONS TO PARTICIPANTS

Invitation letters to this PT were sent to participants in April (Annex 1). The 2020 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 samples were dispatched to the participants by the end of May 2020. Reporting deadline was the 24th of June.

This year the test material were some food supplement tablets. The sample was purchased in a local supermarket. After purchase, tablets were separated from the packeges, mixed manually 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 [5]. 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 homogenized according to the laboratoriums procedure. 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 food supplement 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 As (excluding the outliers), Cd and Pb. 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 (the result of a laboratory is the average of the laboratories replicates) 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 Hg only few quantified results were available, no value was assigned for this elements and no 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.

	As [1] **mg/kg**	**Cd mg/kg**	**Pb mg/kg**
n (number of participants with quantifiable result)	8[2]	11	10
Mean	0.056	0.22	0.078
Standard deviation (SD)	0.012	0.023	0.011
Robust mean (median)	0.055	0.22	0.075
Robust SD (nIQR)	0.013	0.013	0.007
Assigned value x_a	**0.055**	**0.22**	**0.075**
Standard uncertainty of the assigned value $u(x_a)$	**0.006**	**0.005**	**0.003**
σ_p	0.012	0.044	0.017

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

$|z| \leq 2$	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)

x_a = **0.055** ± 0.01 mg/kg (k = 2)

Eleven laboratories submitted results for total As concentrations. One value was below the laboratory's limit of quantification, however, the quantification limit was not lower than the corresponding x_a-3 u(x_a) value, so the statement is satisfactory. Two values were identified as outlier (>50% higher than the median value), excluding these values resulted in an symmetric Kernel distribution. 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 for As against the standard deviation accepted for the proficiency test (Table 4, Figure 2). Two laboratories (L09 and L11) obtained unsatisfactory informal z-scores. Seven laboratories obtained satisfactory informal ζ-scores against their stated measurement uncertainty. Two laboratories (L02 and L09) obtained unsatisfactory informal ζ-scores. For L11, the informal ζ-score is not calculated as the extended uncertainty given by the laboratory is unrealistic.

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})	Informal z-scores	Informal ζ-scores
1	0.058	0.041	0.028	<0.05			
2	0.034	0.032		0.033		-1.8	-3.8
3	0.051	0.054	0.051	0.052	0.007	-0.3	-0.4
4	0.057	0.054	0.054	0.055	0.012	0.00	-0.01
5	0.0499	0.0548	0.0607	0.0551	0.0116	0.00	0.01
6	0.055			0.055	0.0165	0.00	0.00
7	0.0606	0.0575	0.0607	0.0606		0.5	0.9
8	0.060	0.056		0.058		0.2	0.5
9	0.099	0.093	0.107	0.100	0.015	3.7	4.7
10	0.07754	0.08040	0.0733	0.077	0.018	1.8	2.0
11	0.496	0.449		0.473	2.240	34.5	

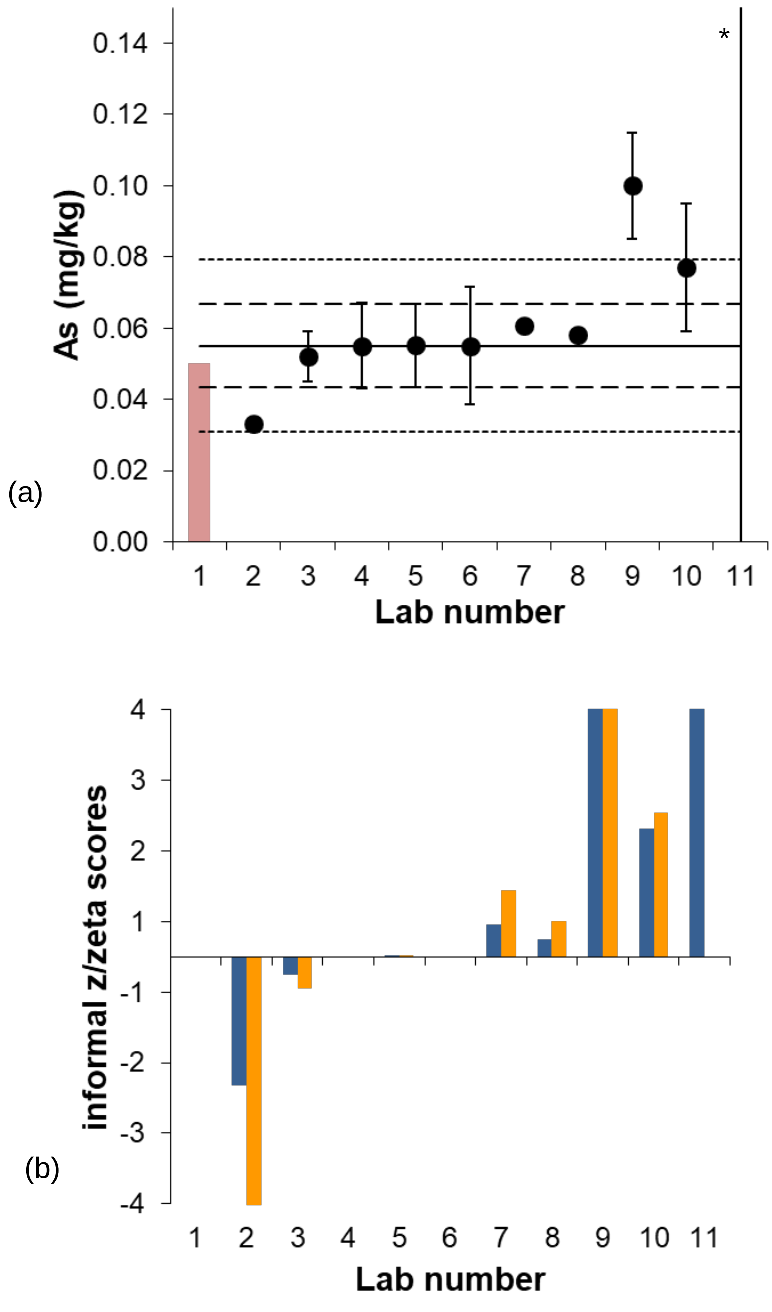


Figure 4: (a) Results with expanded uncertainty for As, as reported by the participants (dashed lines: $x_a \pm 2\ u(x_a)$, dotted lines: $x_a \pm 2\ \sigma_p$), red bars represent the limits of quantification of the corresponding laboratories, the average reported value of L11 was 0.47 mg/kg and (b) informal z- (blue bars) and ζ-scores (orange bars)*

CADMIUM (Cd)

x_a = **0.22** ± 0.01 mg/kg (k = 2)

Eleven laboratories submitted results for Cd concentrations. The median of all results was used as assigned value. All eleven laboratories obtained satisfactory z-scores for Cd against the standard deviation accepted for the proficiency test (Table 3, Figure 3). Eight laboratories also obtained satisfactory ζ-scores against their stated measurement uncertainty. Two laboratories (L02 and L07) obtained an unsatisfactory ζ-score, partially due to the fact that no measurement uncertainty was reported. For L11, the ζ-score is not calculated as the extended uncertainty given by the laboratory is unrealistic.

Table 3 : 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	0.23	0.22	0.21	0.22	0.033	0.0	0.1
2	0.25	0.31		0.28		1.4	13.2
3	0.22	0.22	0.21	0.21	0.04	-0.2	-0.4
4	0.22	0.22	0.23	0.23	0.05	0.3	0.5
5	0.2036	0.1997	0.2002	0.2012	0.04	-0.4	-0.8
6	0.199			0.199	0.06	-0.4	-0.6
7	0.1971	0.1944	0.2001	0.1971		-0.5	-4.5
8	0.21	0.22		0.22		0.0	0.4
9	0.229	0.208	0.218	0.218	0.037	0.0	0.0
10	0.2113	0.2194	0.2115	0.21	0.04	-0.2	-0.4
11	0.221	0.215		0.218	2.52	0.0	

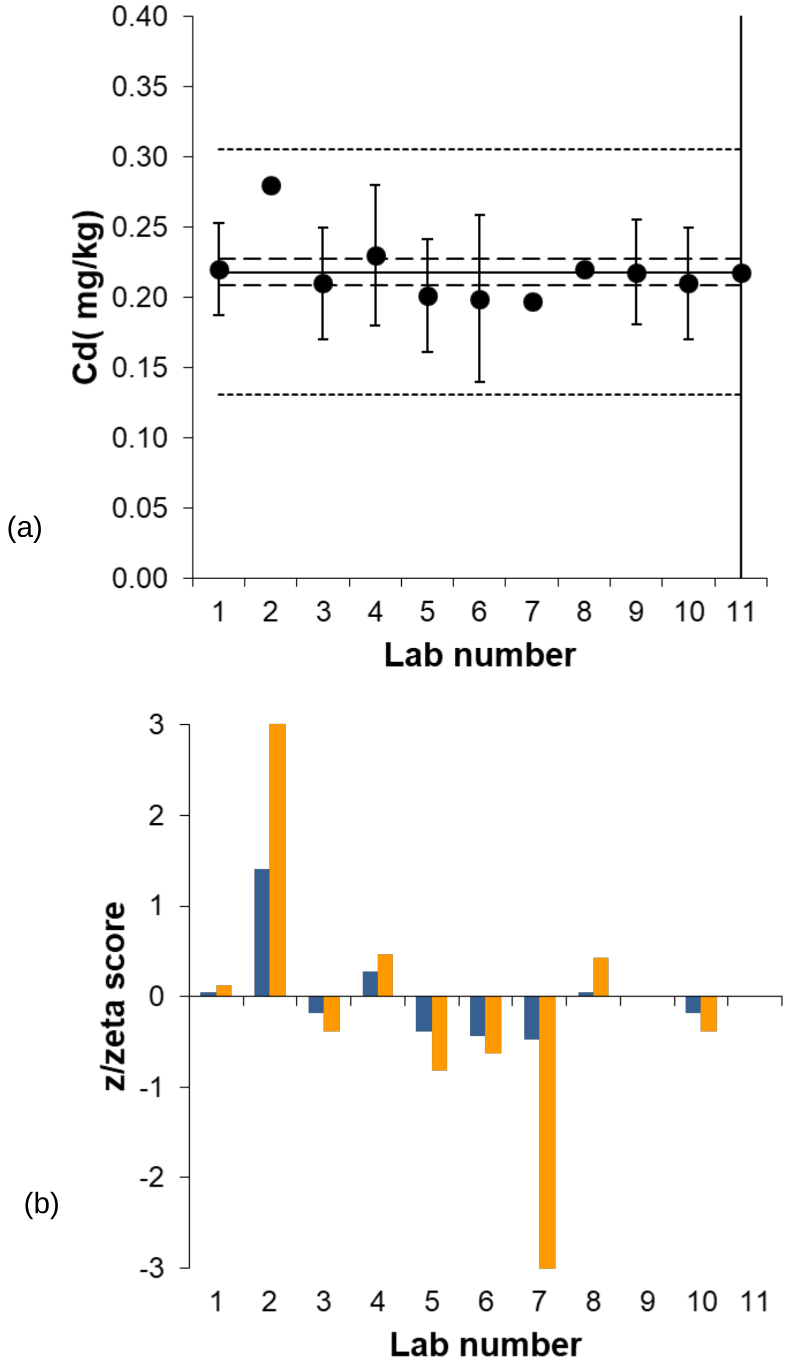


Figure 5: (a) Results with expanded uncertainty for Cd, as reported by the participants (dashed lines: $x_a \pm 2\ u(x_a)$, dotted lines: $x_a \pm 2\ \sigma_p$) and (b) z- (blue bars) and ζ-scores (orange bars)

LEAD (Pb)

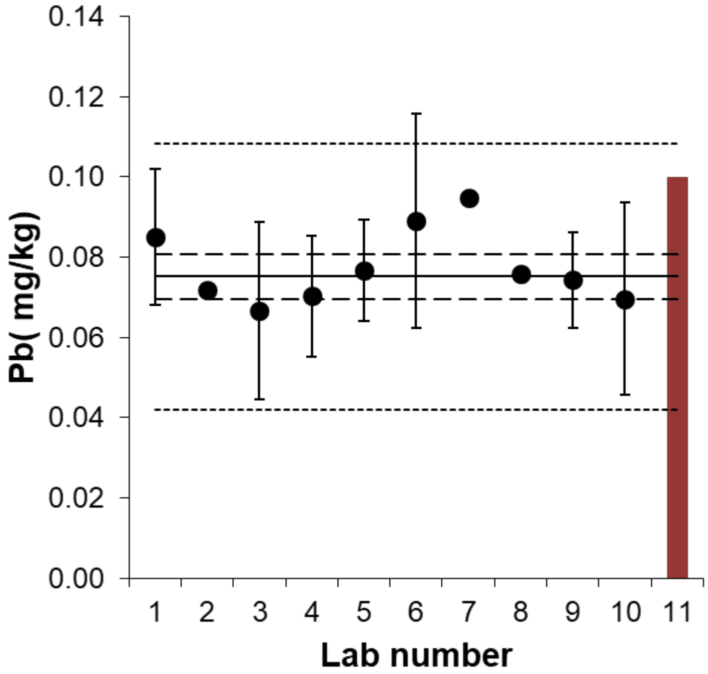
x_a = **0.075** ± 0.006 mg/kg (k = 2)

Twelve laboratories submitted results for total Pb concentrations. Eleven laboratories reported values higher than their quantification limit. The median of the eleven values was used as assigned value. All eleven laboratories obtained satisfactory z-scores for Pb against the standard deviation accepted for the proficiency test (Table 4, Figure 4). The quantification limit of L11 was not lower than the corresponding x_a-3 $u(x_a)$ value, so the statements are satisfactory. Ten laboratories did obtain satisfactory ζ-scores against their stated measurement uncertainty. One laboratory had an unsatisfactory ζ-score, due to a lack of a stated measurement uncertainty.

Table 4 : 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	0.085	0.083	0.087	0.085	0.017	0.6	1.1
2	0.071	0.073		0.072		-0.2	-1.0
3	0.074	0.063	0.063	0.067	0.022	-0.5	-0.7
4	0.070	0.072	0.069	0.070	0.015	-0.3	-0.6
5	0.087	0.072	0.072	0.0767	0.013	0.1	0.2
6	0.089			0.089	0.027	0.8	1.0
7	0.0813	0.0992	0.1037	0.1037		1.7	10.0
8	0.075	0.077		0.076		0.1	0.3
9	0.074	0.070	0.079	0.074	0.012	-0.1	-0.2
10	0.0747	0.0689	0.0654	0.070	0.024	-0.3	-0.4
11				<0.1			

(a)



(b)

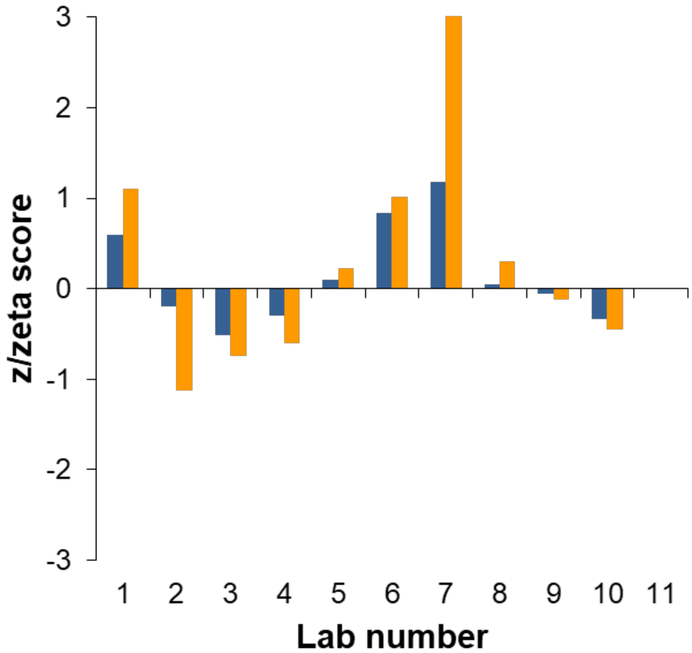


Figure 6 : (a) Results with expanded uncertainty for Pb, as reported by the participants (dashed lines: $x_a \pm 2\ u(x_a)$, dotted lines: $x_a \pm 2\ \sigma_p$), red bars represent the limits of quantification of the corresponding laboratory and (b) z- (blue bars) and ζ-scores (orange bars)

MERCURY (Hg)

Ten laboratories submitted results for total Hg concentrations. However, only four laboratories submitted results above their quantification limit. No scores were calculated and results were variable. Due to the low concentration range, no conclusions are drawn for this analyte.

In the framework of the current legislation, the minimum quantification limit of Hg in this matrix should be two fifth of the maximum limit, i.e. maximum 0.020 mg/kg [1]. Laboratory L01 does not meet this criterium, efforts should be made to lower this LOQ.

Table 5 : values reported for Hg (mg/kg) by the participants

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})
1				**<0.05**	
2				**<0.01**	
3	0.0018	0.0016	0.0014	**0.0016**	0.0004
5				**<0.004**	
6				**<0.02**	
7	0.0157	0.0211	0.0097	**0.0157**	
8	0.003	0.003		**0.003**	
9				**<0.005**	
10	0.0010	0.0013	0.0022	**0.0020**	0.0005
11				**<0.02**	

DISCUSSION AND CONCLUSION

The only used technique for the analysis of As, Cd, Pb was ICP-MS (Inductively Coupled Plasma-Mass Spectrometry). For Hg different techniques are used: CV-AAS (Cold Vapor-Atomic Absoprtion Spectroscopy), ICP-MS and AMA/DMA (Advanced/direct Mercury Analyser).

The laboratories were asked to state if the sample is compliant according to the current legislation. In Commission Regulation (EC) 333/2007 [1] 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, maximum levels were not exceeded, all laboratories stated the sample correctly as compliant. Unfortunately, not all laboraties do meet the LOQ criteria for Hg analysis in the matrix lentils, efforts should be done to improve these quantification limits.

As and Hg concentrations were too low to draw conclusions about the laboratories performance to analyse this matrix. However, the performance to determine Pb and Cd was excellent. Even the elevated concentrations of Molybdenum in the matrix did not influence the Cd results. This shows that laboratories use appropriate instrumentation.

Estimation of a correct measurement uncertainty remains a difficult excersize, resulting in five unsatifactory ζ-scores.

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ANNEXES

ANNEX 1: INVITATION LETTER TO LABORATORIES

Concern: PT-2020-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 2020 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-2020-NRL-TE-FASFC "Determination of As, Cd, Pb and Hg in food supplements"

• Closing date for the inscription:	30th of April 2020 (week 18)
• Shipment of the samples:	25th of May 2020 (week 22)
• Submission of the test results:	24th of June 2020 (week 26)
• Draft report:	4th of September 2020 (week 36)
• Final report:	27th of September 2020 (week 39)

If your laboratory is **approved by the FASFC** for trace elements in foodstuffs, participation to the PT-2020-NRL-TE-FASFC "Determination of As, Cd, Pb and Hg in food supplements" is **mandatory** for all accredited elements and the costs for this PT (€ 236,69) 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-2020-NRL-TE-FASFC is voluntary and the costs for the PT, € 236.69 + 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 30th of April 2020.

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

ANNEX 2: RESULTS OF THE HOMOGENEITY STUDIES

	As	Cd	Pb	Hg
Cochran test for variance outliers				
Cochran test statistic	0.384	0.379	0.435	0.456
Critical (95%)	0.602	0.602	0.602	0.602
Cochran < critical	use complete dataset	use complete dataset	use complete dataset	use complete dataset
Test for sufficient homogeneity				
s_{an}^2	2.38	18.2	9.44	0.031
s_{sam}^2	3.39	45.53	3.7	-0.045
σ_{all}^2	13.97	169.1	11.37	0.008
F1	1.88	1.88	1.88	1.88
F2	1.01	1.01	1.01	1.01
Critical	28.66	346.4	30.9	0.047
s_{sam}^2 < critical?	**accept**	**accept**	**accept**	**accept**

ANNEX 3: LETTER ACCOMPANYING THE SAMPLE

Concern: Shipment of sample PT-2020-NRL-TE-FASFC

Dear colleague,

Following your subscription for the proficiency test (PT-2020-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-2020-NRL-TE-FASFC "Determination of As, Cd, Pb and Hg in food supplements"

• Closing date for the inscription:	30th of April 2020 (week 18)
• Shipment of the samples:	25th of May 2020 (week 22)
• Submission of the test results:	24th of June 2020 (week 26)
• Draft report:	4th of September 2020 (week 36)
• Final report:	27th of September 2020 (week 39)

We expect the results of the analysis the latest by the end of week 26 (the **24th 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-2020-NRL-TE-FASFC is **mandatory** for all accredited elements and the costs for this PT (€ 236,69) 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

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

ANNEX 4: INSTRUCTIONS TO PARTICIPANTS

FORM/25/06/DOC03/V04

Subject: PT-2020-NRL-TE-FASFC: INSTRUCTIONS TO THE PARTICIPANTS

Type of Proficiency test: **PT-2020-NRL-TE-FASFC "Determination of As, Cd, Pb, Hg in food supplements"**

Analytes :

1	As
2	Cd
3	Pb
4	Hg

Matrix : *Food Supplement*

Number of materials sent : 1 package

Test material : *1 pack of ca. 10 tablets: the sample should be treated as a sample for routine analysis including your laboratory's homogenization procedure*

Storage: *Store the test material in dark and dry conditions at ambient temperature*

Receipt form:

Please check if the received package is in agreement with what's specified on your registration and receipt form
Complete the receipt form, sign it and send it to karlien.cheyns@sciensano.be

Data to be sent and to whom:

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 submitting the results : 24/06/2020

Specific instructions:

- *Follow as close as possible the analysis method you use in routine sample analysis*
- *Report the extended uncertainty*

ANNEX 5: MATERIALS RECEIPT FORM

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NRL NATIONAL REFERENCE LABORATORIES

FORM 25/06/DOC04/V05:

PT-2020-NRL-TE-FASFC "DETERMINATION OF As, Cd, Pb, Hg IN FOOD SUPPLEMENTS"
PROFICIENCY TESTING MATERIAL RECEIPT FORM

LIST OF CONTENTS

Material :	**1 pack (with *ca.* 10 tablets)**
Documents :	**Shipment letter** **Receipt form** **Instructions to the participants**

NAME ORGANISATION (LAB) :

CONTACT PERSON

TEL :

E-MAIL : LAB NUMBER (see sample) :

DATE OF THE RECEIPT:

STATE OF MATERIALS RECEIVED

- O GOOD
- O OPEN
- O NOT GOOD (specify) :

REMARKS :

DATE NAME & SIGNATURE

Please complete and sign this form and send it back to: karlien.cheyns@sciensano.be

ANNEX 6: REPORTING FORM AND QUESTIONNAIRE

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NRL NATIONAL REFERENCE LABORATORIES

PT-2020-NRL-TE-FASFC
"Determination of As, Cd, Pb, Hg in food supplements"

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
☐ 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: ....

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healthy all life long

.be

Lab code: L ....

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

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