



Belgian National Reference Laboratory for Trace Elements in Food and Feed



# PT-2017-NRL-TE-FASFC

# Determination of As, Asi, Cd, Pb, Cu and Zn in chocolate

Final report on the 2017 Proficiency Test organised by the National Reference Laboratory for Trace Elements in Food and Feed

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----- Scientific research at the service of safe food production and animal health ----

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#### **Summary**

From the 1<sup>st</sup> of January 2008, the laboratory for Trace Elements in the Veterinary and Agrochemical Research Centre (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 chocolate. The results from the PT were treated in CODA-CERVA, Tervuren.

The 2017 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 eleven participated in the exercise.

The test material used in this test was dark chocolate (>50% cacao), bought in a local supermarket. The choice for this matrix was based on the implementation of maximum levels for cadmium in this matrix as from 1 January 2019 (Commission Regulation (EC) No 2015/1006 [1]). The material was spiked with Cd and As<sub>i</sub>, 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<sub>i</sub>), inorganic arsenic (As<sub>i</sub>), cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn).

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, 11 submitted results for As, Pb, Cd, Cu and Zn and four submitted results for As<sub>i</sub>. The laboratories performed excellent with no questionable z-scores. Only three of the calculated  $\zeta$ -scores were unsatisfactory.

It was a successful exercise and the laboratories have proven their competence to measure the concerned trace elements in the matrix.



#### Introduction

Trace elements occur in varying amounts as natural elements in soils, plants and animals, and consequentially in food and feed. Concerning food and feed of plant origin, the characteristics of the soil on which the plants are grown have a considerable influence on the content of trace elements in the plant. The concentration of trace elements in plants is often correlated to the corresponding concentrations in the soil on which they were grown, but also soil texture, soil pH and soil organic matter content influence the trace element content in the plants. To ensure public health, maximum levels for trace elements in foodstuff have been laid down in the Commission Regulation (EC) N° 1881/2006 [2]. 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. Chocolate and cocoa powder sold to the final consumer can contain high levels of cadmium and are an important source of human exposure. They are frequently consumed by children, e.g. chocolate as such or as sweetened cocoa powders used in cocoa beverages. Therefore, the CONTAM opinion resulted in lower maximum limits for Cd in certain matrices [1]. When establishing maximum levels of cadmium, occurrence data for different types of chocolates and for cocoa powders sold to the final consumer were considered. Since cadmium levels in cocoa products are related to their cocoa content, it was appropriate to establish different maximum levels of cadmium for products containing different percentages of cocoa (Figure 1). The Member States and food business operators were allowed time to adapt to the new maximum levels established by this Regulation. The date of application of the maximum levels of cadmium in chocolate is 1 January 2019. There is currently no European legislation regarding As, Pb, Cu or Zn in chocolate.

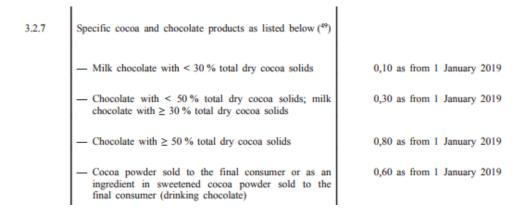
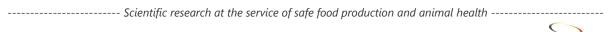


Figure 1: Snapshot of maximum limits of Cd (mg/kg) in chocolate [1].

The scope of this PT was to test the competence of the participating laboratories to determine the total mass fraction of As, As<sub>i</sub>, Cd, Pb, Cu and Zn in chocolate.



#### Time frame, test material and instructions to participants

Invitation letters to this PT were sent to participants in April (Annex 1). The 2017 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 eleven participated in the exercise. One laboratory could not report in time due to technical problems. The samples were dispatched to the participants by the end of May 2017. Reporting deadline was the 23rd of June.

This year the test material was a sample of dark chocolate (> 50% cocoa). The chocolate was purchased in a local supermarket. The chocolate was melted in the laboratory and spiked with a  $Cd(NO_3)_2$  and  $Na_2AsHO_4$  solution. The liquid chocolate was homogenized for 6 h and divided in small portions. The chocolate was stored in the fridge (4°C) to solidify. The chocolate pieces were put in little containers to obtain about 20 g sample per container. These portions were dry and dark stored at room temperature.

The homogeneity of the test materials was tested following the recommended procedure according to IUPAC [3]. All 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 in a dark and dry place 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.

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**

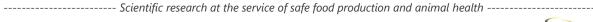
The assigned values for the different trace elements in the chocolate sample were determined as the consensus of participant's results [3]. The major advantages of consensus values are the straightforward calculation and the fact that none of the participants is accorded higher status. The disadvantages 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 [4] 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  $\sigma_p$  ( $u(x_a)^2 < 0.1^*$   $\sigma_p^2$ ), then z-scores can be calculated in a small scheme in the same matter as recommended in IUPAC 2006 for a large scheme. This was the case for all elements (except As<sub>i</sub>). A minimum of eight quantified results is accepted to calculate z- and  $\zeta$ -scores (eight is the minimum number to create a Kernel density distribution).

The <u>robust statistic</u> approach is a convenient modern method of handling results when they are expected to follow a near-normal distribution and it is suspected that they include a small proportion of outliers. There are many different robust estimators of mean and standard deviation [5]. The median and MAD (median absolute difference) were chosen here as robust estimators.

The modified <u>Horwitz equation</u> was used to establish the standard deviation for proficiency testing  $(\sigma_p)$  [3][6]. 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.

- a) Results that were identifiable invalid or extreme outliers were excluded.
- b) A visual presentation of the remaining results was examined. It was checked whether the distribution was apparently unimodal and roughly symmetric, possible outliers aside. If so  $\Rightarrow$  c); else  $\Rightarrow$  d).
- c) The robust mean  $\hat{\mu}_{\text{rob}}$  and standard deviation  $\hat{\sigma}_{\text{rob}}$  of the n results were calculated as  $\hat{\mu}_{\text{rob}}$  = median and  $\hat{\sigma}_{\text{rob}}$  = 1.4826\*MAD. If  $\hat{\sigma}_{\text{rob}}$  was less than about 1.2 $\sigma_{\text{p}}$ , then  $\hat{\mu}_{\text{rob}}$  was used as the assigned value  $x_a$  and  $\hat{\sigma}_{\text{rob}}$ /Vn as its standard uncertainty  $u(x_a)$ .
- d) A Kernel density estimate of the distribution was made using normal kernels with a bandwith h of  $0.75\sigma_p$ . If this resulted in a unimodal and roughly symmetric kernel density, and the mode and median were nearly coincident, then  $\hat{\mu}_{\text{rob}}$  was used as the assigned value and  $\hat{\sigma}_{\text{rob}}$ /Vn as its standard uncertainty; else  $\Rightarrow$ e).
- e) If the minor mode could be safely attributed to an outlying result, then  $\hat{\mu}_{\text{rob}}$  was still used as the assigned value and  $\hat{\sigma}_{\text{rob}}/\sqrt{n}$  as its standard uncertainty; else no consensus value could be derived.

Scheme 1: Scheme followed to calculate the assigned value according to [3]





The scheme that was followed to estimate the consensus and its uncertainty is outlined in Scheme 1. In the current case, steps (a), (d) and (e) were not needed.

For As<sub>i</sub>, only three quantified results were available, no value was assigned for this element and no scores are calculated.

For As, Cd, Pb, Cu and Zn the assigned value was calculated as  $\hat{\mu}_{\rm rob}$  = median and the standard deviation as  $\hat{\sigma}_{\rm rob}$  = 1.4826\*MAD.

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	As <sub>i</sub>	Cd	Pb	Cu	Zn
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
N	10	3	11	8	11	11
Mean	0.095	0.077	0.87	0.042	12.9	21.1
SD	0.008	0.004	0.06	0.008	1.2	1.7
Robust mean (median)	0.099	0.079	0.85	0.043	12.6	21.5
Robust SD ( $\hat{\sigma}_{rob}$ )	0.009	0.002	0.04	0.005	1.3	0.89
Assigned value x <sub>a</sub>	0.099		0.85	0.043	12.6	21.5
Standard uncertainty of the assigned value u(x <sub>a</sub> )	0.003		0.01	0.002	0.4	0.3
$\sigma_{p}$	0.022		0.14	0.009	1.4	2.2

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



#### Scores and evaluation criteria

Individual laboratory performances are expressed in terms of z-scores and  $\zeta$ -scores in accordance with ISO 135283 and the International Harmonised Protocol [3], [7].

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

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

where:

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

 $x_a$  is the assigned value

 $\sigma_p$  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,  $\sigma_p$ . Should participants feel that these  $\sigma$  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:

 $|z| \le 2$  satisfactory result

 $2 < |z| \le 3$  questionable result

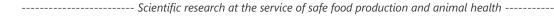
|z| > 3 unsatisfactory result

The  $\zeta$ -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  $\zeta$ -score is similar to the interpretation of the z-score.

 $|\zeta| \le 2$  satisfactory result

 $2 < |\zeta| \le 3$  questionable result

 $|\zeta| > 3$  unsatisfactory result



#### **Results**

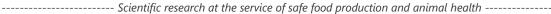
#### Arsenic (As)

 $x_a = 0.099 \pm 0.006 \text{ mg/kg (k = 2)}$ 

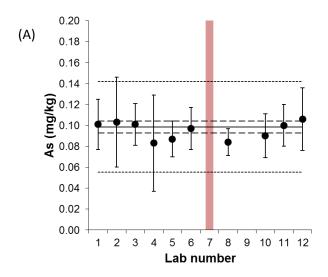
Eleven laboratories submitted results for total As concentrations. The median of 10 results was used as assigned value. All laboratories obtained satisfactory z-scores for As against the standard deviation accepted for the proficiency test (Table 2, Figure 2). In addition, all laboratories obtained satisfactory  $\zeta$ -scores against their stated measurement uncertainty. The quantification limits of L07 was not lower than the corresponding  $x_a$ -3  $u(x_a)$  value, so the statements is satisfactory.

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

Lab code	Result 1 (mg kg <sup>-1</sup> )	Result 2 (mg kg <sup>-1</sup> )	Result 3 (mg kg <sup>-1</sup> )	Mean (mg kg <sup>-1</sup> )	Extended uncertainty (k = 2) $(u_{lab}; mg kg^{-1})$	z-scores	ζ-scores
1	0.113	0.100	0.091	0.101	0.024	0.1	0.2
2	0.104	0.105	0.100	0.103	0.043	0.2	0.2
3	0.097	0.104	0.101	0.101	0.020	0.1	0.2
4	0.083	0.083	0.083	0.083	0.046	-0.7	-0.7
5	0.085	0.088	0.088	0.087	0.017	-0.5	-1.3
6	0.097			0.097	0.020	-0.1	-0.1
7				<0.40			
8	0.089	0.080	0.084	0.084	0.013	-0.7	-2.0
10	0.090	0.088	0.092	0.090	0.021	-0.4	-0.8
11	0.101	0.097	0.103	0.100	0.020	0.1	0.1
12	0.103	0.101	0.114	0.106	0.030	0.3	0.5







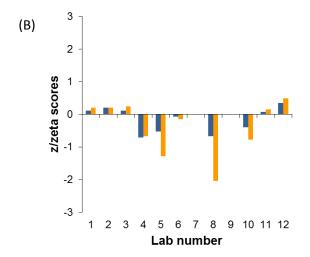


Figure 2 : (a) Results with expanded uncertainty for As, as reported by the participants (dashed lines:  $x_a \pm 2$  u( $x_a$ ), dotted lines:  $x_a \pm 2$   $\sigma_p$ , red bars represent the limits of quantification of the corresponding labs with the y-axis cut-off at 0.2 mg/kg) and (b) z (blue bars) and  $\zeta$ -scores (orange bars)



## Inorganic arsenic (Asi)

Only four laboratories submitted results for  $As_i$  concentrations, with only three quantified results. No scores were calculated, but results were comparable with a relative standard deviation of the result values of only 6%. The results are listed in Table 3.

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

Lab code	Result 1 (mg kg <sup>-1</sup> )	Result 2 (mg kg <sup>-1</sup> )	Result 3 (mg kg <sup>-1</sup> )	Mean (mg kg <sup>-1</sup> )	Extended uncertainty (k = 2) $(u_{lab}; mg kg^{-1})$
2	0.082	0.078	0.077	0.079	0.017
5	<0.08				
10	0.080	0.080	0.081	0.080	0.020
12	0.073	0.072		0.072	0.015





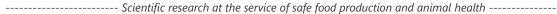
#### Cadmium (Cd)

 $x_a = 0.85 \pm 0.02 \text{ mg/kg (k = 2)}$ 

Eleven laboratories submitted results for Cd concentrations. The median of all results was used as assigned value. All laboratories obtained satisfactory z-scores for Cd against the standard deviation accepted for the proficiency test (Table 4, Figure 3). The laboratories did also obtain good  $\zeta$ -scores against their stated measurement uncertainty.

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

Lab code	Result 1 (mg kg <sup>-1</sup> )	Result 2 (mg kg <sup>-1</sup> )	Result 3 (mg kg <sup>-1</sup> )	Mean (mg kg <sup>-1</sup> )	Extended uncertainty (k = 2) $(u_{lab}; mg kg^{-1})$	z-scores	ζ-scores
1	0.82	0.83	0.82	0.82	0.210	-0.2	-0.2
2	0.87	0.82	0.84	0.85	0.313	0.0	0.0
3	0.97	1.01	0.99	0.99	0.200	1.0	1.4
4	0.81	0.82	0.82	0.82	0.229	-0.2	-0.3
5	0.83	0.84	0.89	0.85	0.102	0.0	0.1
6	0.80			0.80	0.256	-0.3	-0.4
7	0.85	0.84	0.83	0.84	0.025	-0.1	-0.4
8	0.88	0.85	0.87	0.87	0.130	0.2	0.3
10	0.84	0.86	0.84	0.85	0.212	0.0	0.0
11	0.86	0.88	0.87	0.87	0.170	0.2	0.3
12	0.95	0.93	1.03	0.97	0.220	0.9	1.1





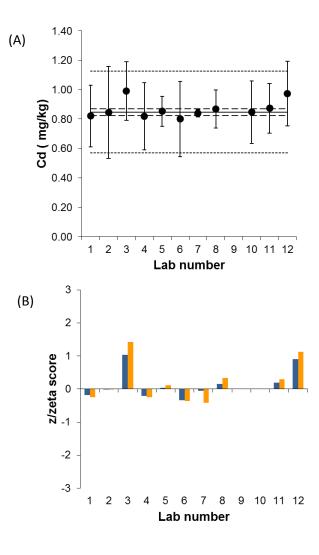


Figure 3 : (a) Results with expanded uncertainty for Cd, as reported by the participants (dashed lines:  $x_a \pm 2 u(x_a)$ , dotted lines:  $x_a \pm 2 \sigma_p$  and (b) z (blue bars) and  $\zeta$ -scores (orange bars)



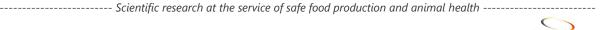
#### Lead (Pb)

 $x_a = 0.043 \pm 0.004 \text{ mg/kg (k = 2)}$ 

Eleven laboratories submitted results for total Pb concentrations. The median of eight results was used as assigned value. Three laboratories could not obtain results above their limit of quantification. All laboratories obtained satisfactory z-scores for Pb against the standard deviation accepted for the proficiency test (Table 5, Figure 4). Seven laboratories did obtain also satisfactory  $\zeta$ -scores against their stated measurement uncertainty. One laboratory (L10) obtained an unsatisfactory  $\zeta$ -score. The quantification limits of L05, L06 and L07 were not lower than the corresponding  $x_a$ -3  $u(x_a)$  value, so the statements is satisfactory.

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

1 Lab code	Result 1 (mg kg <sup>-1</sup> )	Result 2 (mg kg <sup>-1</sup> )	Result 3 (mg kg <sup>-1</sup> )	Mean (mg kg <sup>-1</sup> )	Extended uncertainty (k = 2) $(u_{lab}; mg kg^{-1})$	z-scores	ζ-scores
1	0.055	0.046	0.053	0.051	0.017	0.8	0.92
2	0.042	0.038	0.046	0.042	0.014	-0.1	-0.14
3	0.046	0.053	0.048	0.049	0.010	0.6	1.13
4	0.045	0.0	0.045	0.044	0.018	0.1	0.11
5	<0.04			<0.04			
6	<0.05			<0.05			
7	<0.2			<0.2			
8	0.035	0.037	0.039	0.037	0.007	-0.6	-1.52
10	0.021	0.028	0.03	0.026	0.007	-1.8	-4.30
11	0.039	0.041	0.044	0.042	0.010	-0.1	-0.19
12	0.04	0.047	0.044	0.044	0.011	0.1	0.17





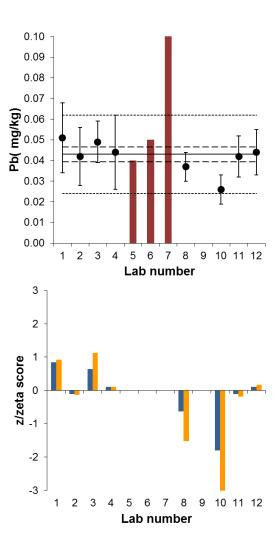


Figure 4 : (a) Results with expanded uncertainty for Pb, as reported by the participants (dashed lines:  $x_a \pm 2$  u( $x_a$ ), dotted lines:  $x_a \pm 2$   $\sigma_p$ , red bars represent the limits of quantification of the corresponding labs with the y-axis cut-off at 0.1 mg/kg) and (b) z (blue bars) and  $\zeta$ -scores (orange bars)



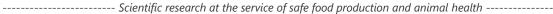
#### Copper (Cu)

 $x_a = 12.6 \pm 0.8 \mu g/kg (k = 2)$ 

Eleven laboratories submitted results for Cu concentrations. The median of all results was used as assigned value. All eleven laboratories obtained satisfactory z-scores for Cu against the standard deviation accepted for the proficiency test (Table 6, Figure 5). Ten laboratories did obtain also satisfactory  $\zeta$ -scores against their stated measurement uncertainty. One laboratory (LO7) obtained an unsatisfactory  $\zeta$ -score.

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

Lab code	Result 1 (mg kg <sup>-1</sup> )	Result 1 (mg kg <sup>-1</sup> )	Result 1 (mg kg <sup>-1</sup> )	Mean (mg kg <sup>-1</sup> )	Extended uncertainty (k=2) ( <i>u<sub>lab</sub>;</i> mg kg <sup>-1</sup> )	z-scores	ζ-scores
1	13.8	13.4	13.4	14.0	2.8	1.0	1.0
2	12.4	12.9	12.6	12.6	3.1	0.0	0.0
3	13.4	14.3	14.0	13.9	2.4	0.9	1.0
4	12.1	12.3	12.2	12.2	2.7	-0.3	-0.3
5	12.0	11.0	11.0	12.0	2.4	-0.4	-0.5
6	11.1			11.1	3.0	-1.1	-1.0
7	15.1	15.4	15.3	15.3	0.3	2.0	6.3
8	12.0	12.0	11.8	11.9	2.4	-0.5	-0.5
10	13.5	13.5	13.9	13.6	4.1	0.7	0.5
11	12.1	12.4	12.2	12.3	2.5	-0.2	-0.3
12	13.2	13.1	14.1	13.5	2.4	0.7	0.7





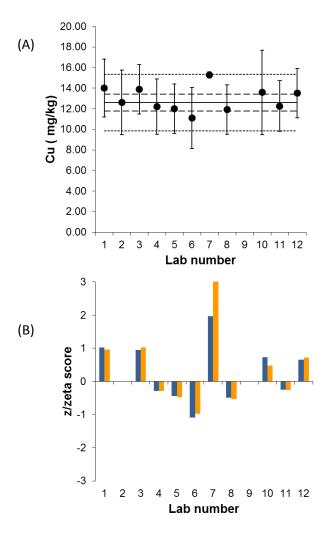


Figure 5 : (a) Results with expanded uncertainty for Cu, as reported by the participants (dashed lines:  $x_a \pm 2$  u( $x_a$ ), dotted lines:  $x_a \pm 2$   $\sigma_p$ , and (b) z (blue bars) and  $\zeta$ -scores (orange bars)



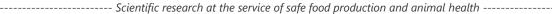
#### Zinc (Zn)

 $x_a = 21.5 \pm 0.5 \text{ mg/kg (k = 2)}$ 

Eleven 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 6). Ten laboratories did obtain also satisfactory  $\zeta$ -scores against their stated measurement uncertainty. One laboratory (LO5) did not report a measurement uncertainty value and obtained an unsatisfactory  $\zeta$ -score.

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

Lab code	Result 1 (mg kg <sup>-1</sup> )	Result 1 (mg kg <sup>-1</sup> )	Result 1 (mg kg <sup>-1</sup> )	Mean (mg kg <sup>-1</sup> )	Extended uncertainty (k = 2) $(u_{lab}; mg kg^{-1})$	z-scores	ζ-scores
1	22.4	21.8	21.1	22.0	4.4	0.2	0.2
2	22.0	22.9	23.0	22.6	4.8	0.5	0.5
3	21.2	22.2	22.1	21.8	3.1	0.1	0.2
4	20.1	20.4	20.1	20.2	3.2	-0.6	-0.8
5	20.0	18.0	18.0	19.0	0.0	-1.2	-9.3
6	17.5			17.5	4.3	-1.8	-1.8
7	21.2	21.5	21.5	21.4	0.4	0.0	-0.3
8	21.0	21.2	20.6	20.9	4.0	-0.3	-0.3
10	21.7	22.3	22.0	22.0	3.3	0.2	0.3
11	21.3	22.0	21.3	21.5	3.4	0.0	0.0
12	23.1	22.7	25.1	23.6	3.6	1.0	1.2





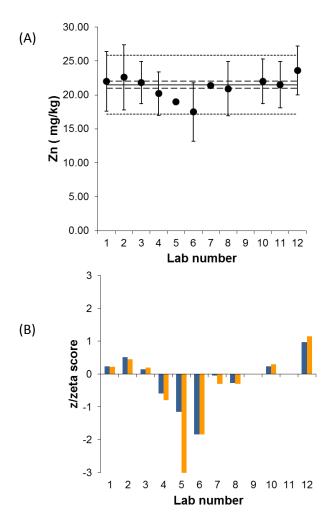


Figure 6 : (a) Results with expanded uncertainty for Zn, as reported by the participants (dashed lines:  $x_a \pm 2 u(x_a)$ , dotted lines:  $x_a \pm 2 \sigma_p$ , and (b) z (blue bars) and  $\zeta$ -scores (orange bars)

#### **Discussion and conclusion**

The only used technique for the analysis of As, Cd and Pb was ICP-MS (Inductively Coupled Plasma-Mass Spectrometry), with one laboratory using a high-resolution ICP-MS and 2 laboratories ICP-MS instruments with a tandem MS/MS. For Cu and Zn six laboratories used ICP-MS, five 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 one laboratory used SPE (Solid Phase Extraction). The latter method could not perform results above their quantification limit.

The laboratories were asked to state if the sample is compliant according to the current legislation. In Commission Regulation (EC) 333/2007 [8] 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 there are only maximum limits for Cd. The maximum Cd limit in chocolate containing >50% cocoa is 0.80 mg/kg (Figure 1). However, this limit enters into force on 1 January 2019. Regarding the current legislation, the samples are compliant. However, most laboratories performed the exercise assuming the limits are already into force. In that case the measured concentration should be compared with the ML taking into account the expanded measurement uncertainty. 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. Three laboratories indicated that the chocolate is not compliant regarding the legislation in 2019. However, for two of them, the results are compliant if the extended measurement uncertainty is subtracted from the measured concentration, which should not be ignored.



Table 8 : Compliance statements for Cd by the participating laboratories (no(n) or yes(y)) with regard to the legislation in 2019, and comparison with the measured values minus the expanded measurement uncertainty  $(x_a-U(x_a))$ .

	x <sub>lab</sub> -U(x <sub>lab</sub> ) (mg/kg)	
	Cd	stated by lab
L01	0.61	У
L02	0.53	n
L03	0.79	У
L04	0.59	У
L05	0.75	У
L06	0.55	n
L07	0.82	n
L08	0.74	
L10	0.64	У
L11	0.70	У
L12	0.75	У
ML	0.8	·

To conclude, this was a very successful exercise. Unfortunately, 2 laboratories gave not a correct compliance statement. However, all the laboratories performed excellent with all satisfactory z-scores.



## **Bibliography**

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- [3] M. Thompson, S. L. R. Ellison, and R. Wood, "The International Harmonized Protocol for the proficiency testing of analytical chemistry laboratories (IUPAC Technical Report)," *Pure Appl. Chem.*, vol. 78, no. 1, pp. 145–196, 2006.
- [4] I. Kuselman and A. Fajgelj, "IUPAC/CITAC Guide: Selection and use of proficiency testing schemes for a limited number of participants—chemical analytical laboratories (IUPAC Technical Report)," *Pure Appl. Chem.*, vol. 82, no. 5, pp. 1099–1135, 2010.
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- [6] M. Thompson, "The amazing Horwitz function," AMC Tech. Br., no. 17, 2004.
- [7] ISO 13528, Statistical methods for use in proficiency testing by interlaboratory comparison, vol. 2015. 2015.
- [8] European Commission, "Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs," *Off. J. Eur. Union*, vol. L88, no. 333, pp. 29–38, 2007.



#### Annexes

#### Annex 1: Invitation letter to laboratories







#### 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 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 2017 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-2017-NRL-TE-FASFC "Determination of As, iAs, Cd, Pb, Cu and Zn in chocolate"

Closing date for the inscription: 28<sup>th</sup> of April 2017 (week 17)
 Shipment of the samples: 22<sup>nd</sup> of May 2017 (week 21)
 Submission of the test results: 23<sup>rd</sup> of June 2017 (week 25)
 Draft report: 4<sup>th</sup> of September 2017 (week 36)

If your laboratory is approved by the FASFC for trace elements in foodstuffs, participation to the PT-2017-NRL-TE-FASFC "Determination of As, iAs, Cd, Pb, Cu and Zn in chocolate" is mandatory for all accredited elements and the costs for this PT (€ 229.9) 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.

29th of September 2017 (week 39)

If your laboratory is not approved by the FASFC for trace elements in foodstuffs, participation to the PT-2017-NRL-TE-FASFC "Determination of As, iAs, Cd, Pb, Cu and Zn in chocolate" is voluntary and the costs for the PT, € 229.9 + shipment costs, will be billed by CODA-CERVA. The results will not be disclosed to the FASEC.

You can receive more information about our PT programme by contacting karlien.chevns@coda-cerva.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 28th of April 2017. 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,

Final report:

Dr ir Karlien Cheyns and Dr ir Nadia Waegeneers Belgian National Reference Laboratory for Trace Elements in Food and Feed CODA-CERVA

------ Scientific research at the service of safe food production and animal health

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Kerklaan 68 B-1830 Machelen Tel. +32 (0)2 251 33 26 Fax. +32 (0)2 251 00 12

Experimental centre

e-mail info@coda-cerva.be website : http://www.coda-cerva.be

.be



**Annex 2: Results of the homogeneity studies** 

	As	Cd	Pb	Cu	Zn			
Cochran test for variance outliers								
Cochran test statistic         0.541         0.584         0.301         0.326         0.55								
Critical (99%)	0.718	0.718	0.718	0.718	0.718			
Cochran < critical	use complete dataset							
	Test	t for sufficient ho	omogeneity					
S <sub>an</sub> <sup>2</sup>	51	2789	17	92126	383044			
S <sub>sam</sub> <sup>2</sup>	21	987.6	12	36683	349006			
$\sigma_{all}^2$	47	2501	8.2	129445	513679			
F1	1.88	1.88	1.88	1.88	1.88			
F2	1.01	1.01	1.01	1.01	1.01			
Critical	139	7518	32.4	336404	1352591			
S <sub>sam</sub> <sup>2</sup> < critical?	accept	accept	accept	accept	accept			



#### Annex 3: Letter accompanying the sample







May 22nd 2017

Dear colleague,

Following your subscription for the proficiency test (PT-2017-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 (<a href="mailto:karlien.chevns@coda-cerva.be">karlien.chevns@coda-cerva.be</a>). The time schedule of the PT is given below:

PT-2017-NRL-TE-FASFC "Determination of As, Asi, Cd, Pb, Cu and Zn in chocolate"

Closing date for the inscription: 28<sup>th</sup> of April 2017 (week 17)
 Shipment of the samples: 22<sup>nd</sup> of May 2017 (week 21)
 Submission of the test results: 23<sup>rd</sup> of June 2017 (week 25)
 Draft report: 4<sup>th</sup> of September 2017 (week 36)
 Final report: 29<sup>th</sup> of September 2017 (week 39)

We expect the results of the analysis the latest by the end of week 25 (the 23<sup>rd</sup> 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-2017-NRL-TE-FASFC "Determination of As, As<sub>i</sub>, Cd, Pb, Cu and Zn in chocolate" is mandatory for all accredited elements and the costs for PT-2017-NRL-TE-FASFC (€ 229.9) 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.chevns@coda-cerva.be

Kind regards,

Dr ir Karlien Cheyns and Dr ir Nadia Waegeneers

Belgian National Reference Laboratory for Trace Elements in Food and Feed CODA-CERVA

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#### **Annex 4: Instructions to participants**







# PRO/2.5/06/DOC03/V02: INSTRUCTIONS TO THE PARTICIPANTS PRO/2.5/06/DOC03/V02: INSTRUCTIES AAN DE DEELNEMERS PRO/2.5/06/DOC03/V02: INSTRUCTIONS AUX PARTICIPANTS

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

PT-2017-NRL-TE-FASFC "Determination of As, As, Cd, Pb, Cu and Zn in chocolate"

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

As, As, Cd, Pb, Cu, Zn

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

Dark chocolate containing ≥ 50 % cocoa

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

One small container, containing about 20 g sample

Storage method / Wijze van bewaring / Mode de conservation :

Ambient, dry, 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.chevns@coda-cerva.be
(an electronic version of the reporting form will be sent by e-mail). Fax: +32 2 769 2305; Address: Coda-Cerva,
Leuvensesteenweg 17, 3080 Tervuren

Deadline for sending the results to the OD-CSF / Datum (deadline) waarop de resultaten moeten opgestuurd worden naar de OD-CVV / Date (deadline) à laquelle les résultats doivent être envoyés à la DO-SCA :

23/06/2017

Specific instructions / Specifieke Intructies / Instructions spécifiques :

- · 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

Reminder : for the Belgian official control labs, the results are communicated to the FASFC (FAVV-AFSCA) Herinnering : Voor de Belgische erkende labo's worden de resultaten aan het FAVV meegedeeld Rappel : Pour les laboratoires belges agréés, les résultats sont communiqués à l'AFSCA



## **Annex 5: Materials receipt form**







# PRO/2.5/06/DOC04/V02: PROFICIENCY TESTING MATERIALS RECEIPT FORM FORMULIER VAN BEVESTIGING VAN ONTVANGST VAN HET MATERIAAL FORMULAIRE DE CONFIRMATION DE RÉCEPTION DU MATÉRIEL PT-2017-NRL-TE-FASFC "Determination of As, Asi, Cd, Pb, Cu and Zn in chocolate" NAME ORGANISATION (LAB) / NAAM ORGANISATIE (LABO) / NOM ORGANISATION (LABO): CONTACT PERSON / CONTACTPERSOON / PERSONNE DE CONTACT : TEL: FAX: 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 : OPEN / OPEN / OUVERT BAD (specify) / SLECHT (specificeren) / MAUVAIS (à préciser) REMARKS / OPMERKINGEN / REMARQUES : SIGNATURE / HANDTEKENING / SIGNATURE DATE / DATUM / DATE :



## Annex 6: Reporting form and questionnaire







May 22nd 2017

National Reference Laboratory for Trace Elements in Food and Feed

# PT-2017-NRL-TE-FASFC "Determination of As, As, Cd, Pb, Cu and Zn in chocolate"

#### RESULTS REPORTING FORM

	NESOLIS REPORTING FORM								
Lat	b code:								
1.	Does your laboratory carry out this typ As regards to:	e of analysis on a routi	ine basis?						
	The parameters	The matrix	The used method						
	☐ Yes ☐ No	□ Yes □ No	□ Yes □ No						
2.	• · · · · · · · · · · · · · · · · · · ·								
	Matrix	_	Element						
	□ Terrestrial vegetable origin	□ A	As .						
	<ul> <li>Aquatic vegetable origin</li> </ul>	□ A	As <sub>i</sub>						
	☐ Terrestrial animal origin		Cd .						
	<ul> <li>Aquatic animal origin</li> </ul>	□ P	Pb .						
	□ Drinks	□ H	<del>l</del> g						
	□ Processed food		Cu						
	□ Feed	□ Z	žn						
	<b>-</b>	. 🗆 N	Ni						
		_ c	Or .						

1/2



Extended

Element	<u>Iechnique used*</u>	<u>Units</u>	Replicate 1	Replicate 2	Replicate 3	Mea
---------	------------------------	--------------	-------------	-------------	-------------	-----

Element	<u>Technique used*</u>	<u>Units</u>	Replicate 1	Replicate 2	Replicate 3	<u>Mean value</u>	uncertainty (k=2)
As		mg/kg					
As <sub>i</sub>	•	mg/kg					
Cd		mg/kg					
Pb		mg/kg					
Cu		mg/kg					

*please specify separation and quantification techniqu
--

Lab Code: .....

2/2

